there was very little development.

Temperature. Greater prolification and growth was achieved at 30°C than either 21° or 24°.

Multiplication of cultures. The combination 10 mg/liter PBA and 2 mg/liter IAA was used for the further multiplication of the cultures (Fig. 1A). After 6 weeks 8 shoots developed per culture. The optimum subculture period was 6 weeks.

Development of plants. The development of whole plants from the cultures was examined in experiments with PBA (0, 0.02, 0.2 mg/liter) alone and in combination with 2 mg/liter IAA. These low PBA concentrations were used since the earlier results showed that by using concentrations higher than 0.2 mg/liter more than 1 shoot developed in each culture and the root development was suppressed.

Number and length of shoots, leaves, and roots, was not influenced by low PBA concentration but was increased by 2 mg/liter IAA. This suggests that a medium with 2 mg/liter IAA as the only hormone, will be preferable for development of plants *in vitro* (Fig. 1B).

Establishment in soil. Whole plants with leaves and roots were successfully transplanted to soil (Fig. 1C). Plant morphology of these plants was similar to the original clones.

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In Vitro Micrografting of Apple Shoot Tips[1]

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Abstract. **Micrografting of apple (*Malus domestica* Borkh.) was achieved by placing a 0.1 to 0.2 mm scion explant derived either from field-grown or *in vitro*-grown shoot apices on the hypocotyl of a decapitated 15 day-old seedling. Under asceptic conditons, the scion and hypocotyl unite to form a complete plant which produces 4 to 6 leaves after 6 weeks. These plants were transplanted to sterile vermiculite and conditioned to ambient humidity for 1 week and then transplanted to soil. This technique was used to obtain virus-free plants from virus-infected plants.**

The apple stem grooving virus (SGV) and the symptoms it causes to sensitive herbaceous hosts were described by Lister (8). The virus causes a disease in apple and is not eliminated by thermotherapy (H.W. Guengerich, unpublished data). Most cultivars of apple infected with SGV show no symptoms but the sensitivity of other cultivars has resulted in interstate and international quarantines against the sale of trees that are not certified as free from SGV. Since all clones of the 'Griffith' cultivar[3] were infected with SGV, we initiated a shoot tip culture program as a method of obtaining healthy plants. A satisfactory medium (4) containing the Murashige-Skoog (MS) mineral salts (11) and the vitamins of Gamborg et al. (3) plus 2% sucrose, 0.5 μM 6-benzylamino purine and 0.1 μM gibberellin A_3 was developed but all attempts to promote rooting failed.

The *in vitro* grafting of apple using shoots has been described in a preliminary report (9) and in more detail (7, 13). We have been successful with micrografting of apple (5, 6) as has been described for citrus (10). Here we examined some of the variables which influence successful grafting and determined the efficacy of this method for freeing plants of SGV.

Seedlings of open-pollinated 'Golden Delicious' apple were used as rootstocks. Two media were required, one (MSA) is used for germinating the embryos and a second (MSB) is used to promote growth of the tip meristem sicon. MSA is a solid medium containing the MS inorganics and 0.8% agar. MSB is a liquid medium containing the MS inorganics, B-5 vitamins (3) and 7% sucrose, pH 5.7. Seeds were cold stratified; seed coats removed; and embryos disinfected by a 10 min immersion in 0.5% sodium hypochlorite (10% Clorox) followed by 3 rinses in sterile distilled water. the embryos were placed on MSA and moved to an incubator for 15 days of continuous darkness at 25°C. Seedlings then were removed from the agar and the epicotyls and cotyledons excised. The decapitated seedlings were transferred to flat bottom tubes (2.5 x 11 cm) with a hole in the center in the form of an inverted "U." The radicle of the decapitated seedling was pushed through the hole in the filter paper bridge and a tip meristem scion, either obtained from a 'Griffith' shoot cultured *in vitro* or removed aseptically from shoots of field grown trees, was moistened with MSB then placed in contact with the vascular elements of the exposed hypocotyl. The graft was then exposed to 4 klx of light (10% incandescent, 90% fluorescent) for 16 hr at 27° and 8 hr darkness at 23°. After 1 week, callus was produced on both the hypocotyl and scion and union was achieved. After 6 weeks the scion had developed a shoot with 4 to 6 leaves and the plant was transplanted to a sterilized 11 cm clay pot containing sterile vermiculite. It was then irrigated with MSA and covered with a 400 ml beaker to maintain proper humidity. The plant was irrigated thereafter as needed and the beaker lifted 0.5 cm per day from the vermiculite surface for 6 days. On the 7th day, the beaker was removed and after 2 days the plant was transplanted to a larger sterilized clay pot containing pasteurized soil, then moved to a greenhouse bench.

The probability of successful graft unions increased with the size of the scion piece (Table 1). This was determined from 20 grafts in which the scion consisted of the apical dome only and 20 each of increasingly larger scions (apical dome plus increasing numbers of leaf primordia). When the scion consisted of the dome only, 15% of the graft unions were successful as compared to 65% when the scion included 2 leaf primordia. Since the probability of obtaining the virus-free plants is inversely related to size (10) and since meristems containing the apical dome and 2 leaf primordia will develop into plants 65% of the time, scion explants consisting of the dome and 2 leaf primordia were used in subsequent studies.

The time of year when field grown tips are used as a source of scion explants influenced the success of grafting (Fig.

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[2]Research Assistant and Professor, respectively.

[3]Sold commercially as 'Blushing Gold.'

Table 1. Influence of size of explant[z] on *in vitro* grafting of apple.

Components	No. of explants	Size (mm)	No. of successful grafts
Apical dome	20	0.5 – 0.08	3
Apical dome and 2 leaf primordia	20	0.1 – 0.2	13
Apical dome and 4 leaf primordia	20	0.3 – 0.4	15
Apical dome and 6 leaf primordia	20	0.6 – 0.8	18

[z]Explants were obtained from field-grown trees during the period of May and June.

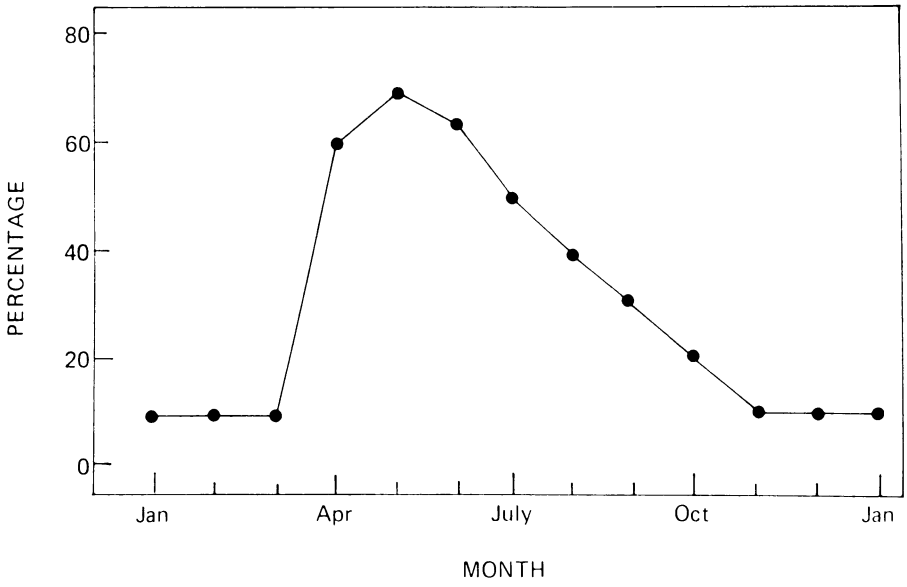

Fig. 1. Influence of time of year when explants are excised from shoots of field grown apple on successful *in vitro* grafts of apple.

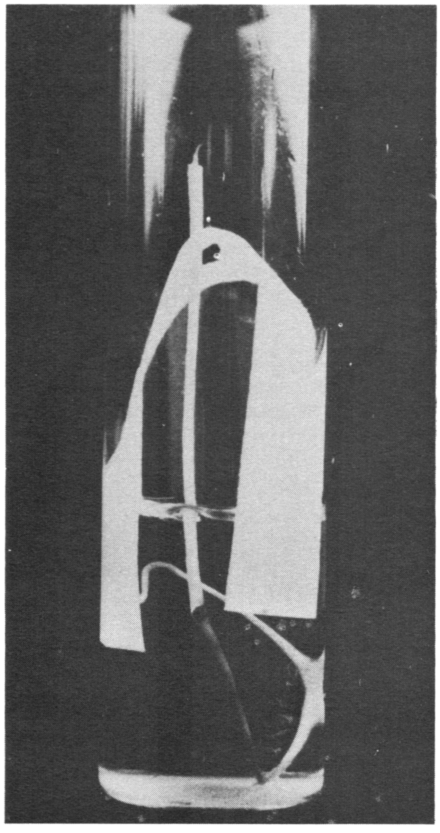

Fig. 2. Apple seedling hypocotyl with shoot tip meristem scion 1 week after grafting.

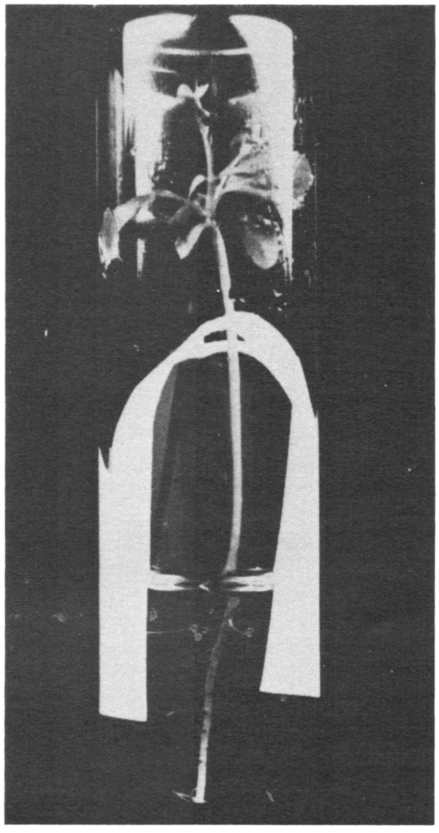

Fig. 3. Grafted seedling and shoot tip meristem scion 6 weeks after grafting.

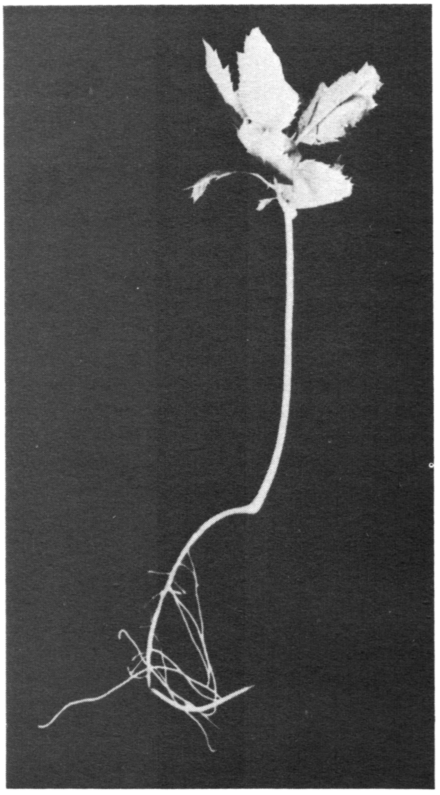

Fig. 4. Grafted seedling and shoot tip meristem scion 8 weeks after grafting. Grafted plant is ready for transplanting.

1). During the growing season the shoots were cut at biweekly intervals from leafing out in early April through leaf cast in November. During the December-March period, the scion explants were excised from shoots cut in early December and stored in the refrigerator until used. Twenty scion explants were grafted monthly throughout a full calender year, November 1, 1977-October 31, 1978. Our results showed that 10% of the grafted explant scions developed into plants during the November-March period, increasing to 70% in May but decreasing about 10% per month from June through October. When scion explants from the *in vitro* produced shoots were used, 60% united with the seedling hypocotyl to form plants regardless of the month or season.

Twelve plants, each originating from the scion explants of two SGV-infected clones, 'Griffith' and PI 344548, were tested for the presence of SGV. Inoculum consisted of leaf tissue from each of these 12 plants macerated separately in 0.05 M phosphate buffer, pH 7.0. Buffer only and buffer extracted juice of leaves from one of the infected parent cultivars served as controls. All inocula included celite as an abrasive. Three plants of *Chenopodium quinoa* Wild. were inoculated with each inoculum and the experiment run on 2 different dates.

None of the *C. quinoa* rubbed with the buffer or buffer extracted juice from leaves of the plants produced from the

scion explants showed any symptoms. Seven to 10 days after inoculation, the leaves rubbed with juice from the SGV infected source showed symptoms described by Lister (8) for SGV infection on *C. quinoa*. They include an initial development of local lesions on the inoculated leaves followed by an epinasty of the growing point, tip distortion, and a mottle on the leaves.

A similar technique of micrografting of apple has been described by Alskieff and Villemur (2). In their study they grafted the scion explants to the epicotyl, just above the cotyledons whereas our technique involves adventitious shoot development and increases the percentage of successful grafting (23 vs 65%). *In vitro* micrografting of apple is suggested as a technique to produce virus-free trees from some explants of infected cultivars whose cultured shoots are difficult to root.

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Effect of Growth Regulators on the Development of 'Delicious' Apples[1]

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Additional index words. Malus domestica, butanedioic acid mono-(2,2-dimethylhydrazide), (2-chloroethyl)phosphonic acid, gibberellin A_4A_7, 6-benzylamino purine, α-(2,4,5-trichlorophenoxy)propionic acid

Abstract. **The color and shape of 'Delicious' apples were improved with a combination treatment of (2-chlorethyl)phosphonic acid + α-(2,4,5-trichlorophenoxy)propionic acid (ethephon/fenoprop) and gibberellin A_4A_7 plus N-(phenylmethyl) 1H-purin-6-amine ($GA_{4,7}$/BA). All ethephon/fenoprop treatments improved red color development. The length/diameter (L/D) ratio of fruit treated with $GA_{4,7}$/BA, $GA_{4,7}$/BA plus ethephon/2,4,5-TP, and $GA_{4,7}$/BA plus ethephon/2,4,5-TP plus daminozide was greater than untreated fruit. The L/D ratio of fruit treated with $GA_{4,7}$/BA plus daminozide did not differ from those not treated. The length, diameter, and weight of fruit treated with daminozide alone was less than for untreated fruit. Diameter of fruit treated with $GA_{4,7}$/BA + daminozide or daminozide + $GA_{4,7}$/BA + ethephon/fenoprop was less than for untreated fruit.**

Poor fruit shape and color development limit the market of 'Delicious' apples. High post-bloom temperatures in the Southeast result in 'Delicious' apples being flatter in shape and lacking calyx points typical of fruit grown in the Northwest (14). A propriatory mixture of $GA_{4,7}$/BA, marketed as Promalin by Abbott Laboratories, North Chicago, Illinois, has been used to promote development of the calyx points, and increase fruit length, L/D ratio, and weight without altering fruit diameter or color (9, 11, 12, 15). Applications during bloom were more effective than pre- or postbloom sprays (11, 14).

Foliar sprays of ethephon and daminozide, alone or in combination, have been used to influence red color development, maturity, and quality of apples. Ethephon (generally combined with a suitable auxinic "stop-drop" agent) enhanced red color development (1, 3, 4, 6, 8, 10, 12), percent soluble solids (3, 7, 8, 13), and hastened maturity (6, 10), but decreased firmness (1, 3, 8, 10, 12, 13). Daminozide enhanced red color development, but not to the same extent as ethephon (2, 8), increased fruit firmness (2, 5, 7, 8, 15). When daminozide and ethephon were combined, greater red color development occurred than when either was used alone (1, 8, 10, 12) and the combination resulted in partial cancellation of their individual effects on fruit firmness and fruit drop (8, 10).

This experiment was conducted to determine the effect of $GA_{4,7}$/BA, daminozide and ethephon/fenoprop, applied alone or in combination on fruit shape, quality, and color development of 'Delicious' apples grown in central Alabama.

$GA_{4,7}$/BA (50 ppm of each) was applied on April 5, at the "king" blossom stage. Daminozide (1500 ppm) was applied on July 1, 6 weeks prior to the anticipated harvest date. Ethephon (300 ppm)/fenoprop (20 ppm) was applied on August 6, 12 days prior to the anticipated harvest date. Each growth regulator or growth regulator mixture was applied alone and in all possible combinations as separate sprays at the appropriate time. Each treatment was replicated 8 times in a randomized complete block design using whole tree plots of 14-year-old 'Miller's Sturdee Spur Delicious' on Malling-merton (MM) 106 rootstock.

Twenty fruit were randomly harvested from each treatment replication at normal harvest maturity (August 18). These fruits were assessed for: fruit firmness, percent soluble solids, length, diameter, L/D ratio, weight, percent of fruit surface with red color development and percent of fruit surface with a solid red blush and seed color. Ten fruit were harvested from each replicate on August 14, 18, and 21 to determine the intensity of red color.

Firmness was measured by use of an Instron Model 1122 Universal Testing Instrument with a 11 mm plunger. Soluble solids were measured by use of a hand refractometer. The percentages of the fruit surface with some red color development and solid red blush were subjectively rated. The intensity of red color on the red side as well as on the green side of the fruit was measured with a Hunter Color Difference Meter D25 D2 by placing the fruit surface directly on the glass cover of the port.

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