ETIOLATION AS AN AID TO ROOTING[1]

WILBUR C. ANDERSON

Northwestern Washington Research and Extension Unit
Washington State University
1468 Memorial Highway
Mount Vernon, Washington 98273

Abstract. Delicious apple cultures of 'Supreme Red' and 'Wellspur' incubated in a shoot multiplication medium were etiolated by placing them in darkness under culture room conditions for 2 weeks. Etiolated and non-etiolated cultures were exposed to light culture room conditions for 0, 1, 2, 4, 8, and 16 days regreening prior to reincubation on a rooting medium. The treatments were incubated 3 weeks on this medium before rooting was evaluated. Average rooting percentages from etiolated treatments were 53% for 'Supreme Red' and 82% for 'Wellspur'; from non-etiolated treatments 2% and 10%, respectively. Treatments of 0, 1, and 2 days of regreening resulted in many cultures callusing and subsequent loss of the apical shoot tip.

The clonal propagation of self-rooted apple trees is a desirable technique as this would reduce time in producing trees and eliminate the costly step of either budding or grafting scion cultivars on rootstocks. *In vitro* production of self-rooted scion cultivars has been reported by several researchers including Boxus and Quoirin (2), Lane (5), Jones, Pontikis, and Hopgood (4), Zimmerman (6), and Zimmerman and Broome (7). It is recognized that there is variation in rooting difficulty between scion cultivars. The 'Red Delicious' group must be considered one of the most difficult to root cultivars. I have attempted rooting 'Red Delicious' and cultivars derived from it with the objective of determining the genetic stability and growth characteristics of the various cultivars.

We have conducted many experiments in my laboratory attempting to establish the appropriate conditions for rooting. These have included testing auxin concentrations in a range of 0 to 4.5 mg per liter IAA, IBA, and NAA. None of these treatments resulted in significant rooting. Concentrations of GA_3 were tested in a range of 0 to 15 mg per liter with no effect. Activated charcoal in concentrations up to 600 mg per liter has not been effective. Phloroglucinol, both autoclaved and cold sterilized, in concentrations of up to 325 g per liter, which is 2x the strength suggested by Jones (3) was not effective. The concentration ranges of inorganics have also been studied with minimal effect on root initiation.

A common practice used in commercial apple rootstock layering beds has been to etiolate the stems as a necessary

---

[1] The following abbreviations have been used: IAA (indole-3-acetic acid); IBA (indole-3-butyric acid); NAA (B-naphthaleneacetic acid), BA (N_6-benzyl adenine) and GA_3 (gibberellin A_3).

practice for successful rooting. A natural extension of our research effort therefore turned to exploring the use of darkness for root enhancement of Red Delicious shoots propagated in culture.

METHODS AND MATERIALS

Two 'Delicious' apple cultures, 'Supreme Red' and 'Wellspur', were chosen for this experiment. These were cultures that were maintained on shoot multiplication media. The cultures were allowed to reach their climax multiplication by incubating them 3 weeks and then dividing the stocks between etiolated and non-etiolated treatments. These treatments were incubated for 2 more weeks in the multiplication medium. The etiolated cultures were placed in a light tight box for 2 weeks and maintained at normal culture room conditions of 20°C. The lighting in the room was cool white light with 16 hours duration per day and 1000 lux.

Table 1. Apple culture medium composition.

	Quantity per liter	
	Shoot Multiplication	Rooting
Sucrose	30 g	30 g
Inorganics - Anderson's (1)	1 x	0.5 x
Organics		
1 inositol	100 mg	100 mg
Adenine sulfate-dehydrate	80 mg	80 mg
Thiamine-HCl	0.4 mg	0.4 mg
Growth regulators		
IBA	0.1 mg	0.1 mg
BA	1.0 mg	
Agar	8 g	8 g
pH adjusted prior to addition of agar at:	5.7	5 7

In a previous experiment we observed that transferring etiolated shoots directly to a rooting medium resulted in many shoot tips forming a callus layer a few millimeters below the apex and, subsequently, the apex would die. The treatments of this experiment consisted of allowing the etiolated shoots to regreen in the shoot multiplication medium prior to reculturing onto the rooting medium. The regreening periods were 0, 1, 2, 4, 8, and 16 days before the shoots were recultured onto rooting media. The cultures were incubated in the rooting medium 3 weeks before evaluating the root and shoot development.

Each treatment consisted of 10 replicate recultures (1 shoot per 25 × 150 mm culture tube). The harvested shoots were evaluated for percentage of the cultures rooted and the rooting index. The rooting index was: 1 = no roots; 2 = root initials and up to 1 to 2 extended roots; 3 = numerous ex-

tended roots. The rooting index is an average of the 10 replicate shoots in each treatment. Each culture was rated for the percentage of intact shoot tips.

RESULTS

The etiolation treatments compared to the non-etiolated control improved the average percentage of rooting with both Red Delicious cultivars, 51% for 'Supreme Red' and 72% for 'Wellspur' (Table 2). 'Wellspur' consistently had a higher percentage of rooting than 'Supreme Red'. The average rooting index was greater in the etiolated treatments but there was a decline in the average quantity of cultures with intact shoots.

Table 2. Apple rooting percentages of etiolated and non-etiolated shoots and the effect of regreening treatments on maintenance of intact shoot tips.

		ETIOLATED			NON-ETIOLATED		
Cultivar	Days of Regreening	Percent rooted	Rooting[a] Index	Plants with intact shoot tips	Percent rooted	Rooting Index	Plants with intact shoot tips
Supreme Red	0	70%	1 7	30	0%	1 0	100
	1	70	1 7	30	0	1 0	100
	2	60	1 7	70	0	1 1	100
	4	60	2 2	90	0	1 1	100
	8	40	1 4	100	10	1.1	100
	16	20	1 3	100	0	1 0	100
	Average	53	1 7	70	2	1 0	100
Wellspur	0	60	1 7	60	10	1 1	100
	1	80	1 8	60	10	1.1	100
	2	100	2 0	70	50	1 5	100
	4	100	1 6	100	10	1 1	100
	8	80	2 0	100	0	1 0	100
	16	90	2 2	100	10	1 0	100
	Average	82	1 9	82	10	1 2	100

[a] The rooting index is an average of all the cultures in each treatment The rooting index 1 = no roots, 2 = root initials and up to 1-2 extended roots, 3 = numerous extended roots

Regreening of etiolated shoots from 0 to 4 days duration had no effect on the percent rooting. There was a reduction in rooting of 'Supreme Red' for 8 and 16 days regreening but this was not observed with 'Wellspur'. The major concern with the 0 to 2 day regreening was a significant number of plants forming callus and losing their shoot tip; with the 4 day regreening period this was of no concern.

DISCUSSION

Etiolation as a continuation of the shoot multiplication stage resulted in substantial improvement of root initiation. However, a regreening step must be added to reduce the shoot tip callusing and a subsequent loss of the shoot apex. Regreening for 4 days reduced this problem. These results suggest that the addition of the cytokinin BA in the shoot multiplication medium has a significant effect on maintaining integrity of the apical shoot tip in the regreening process.

The use of darkness in root initiation on difficult-to-root apple cultivars appears to have merit. The major problem with the system described is the general low vigor of plantlets established and the low survival rate in the planting out step. Plantlets that are rooted but have lost their apical tip frequently are established in soil but do not grow as rapidly as the plants with intact apical shoots. The dark treatment may not require conditions that cause true etiolation of the shoots. Etiolated shoots seem to be weakened and are difficult to acclimate the plants in the greenhouse. It may only require a short duration of darkness of less than 1 week to change the endogenous growth regulator balances to favor root initiation.

LITERATURE CITED

1. Anderson, W.C. 1980. Tissue culture propagation of red raspberries. Proceedings of the Conference on Nursery Production of Fruit Plants through Tissue Culture — Applications and Feasibility. USDA/SEA ARR-NE-11 pp. 27-34
2. Boxus, P.H and M. Quoirin. 1977 Comportement en pepincire d'arbes fruitiers issus de culture "in vitro". *Acta Hortic.* 78:373-379.
3 Jones, O.P 1976. Effect of phloridzin and phloroglucinol on apple shoots. *Nature* 262:392-393
4. _____, C A Pontikis, M.E Hopgood 1979. Propagation *in vitro* of five apple scion cultivars. *J. Hort. Science* 54:155-158.
5. Lane, W.D. 1978. Regeneration of apple plants from shoot meristem-tips. *Plant Science Letters* 13:281-285.
6 Zimmerman, R.H. 1978. Tissue culture of fruit trees and other fruit plants. *Proc. Inter. Plant Prop. Soc.* 28:539-545.
7 _____ and O.C Broome. 1980. Apple cultivar micro propagation. Proceedings of the Conferences on Nursery Production of Fruit Plants through Tissue Culture — Applications and Feasibility. USDA/SEA ARR-NE-11. pp 54-58

CUTTING PROPAGATION OF *JUNIPERUS SCOPULORUM* CULTIVARS

RODGER A. DUER

Monrovia Nursery Company
Azusa, California 91702

Several *Juniperus scopulorum* cultivars have been grown commercially for many years. These handsome landscape plants, some with striking blue-gray foliage, are used throughout North America as both accent and background plants. Because of their hardiness (Zone 3), they are used quite extensively in the Northern United States and in Canada.

Due to the great difficulty in rooting cuttings of *Juniperus*