EXFLASKING HIGH HEALTH DAPHNE PLANTLETS

C. B. CHRISTIE AND W. BRASCAMP
Department of Horticultural Science
Massey University
Palmerston North

Abstract. Experiments were aimed to improve *in vivo* rooting and survival of *Daphne odora* 'Leucanthe' and *Daphne odora* 'Rubra' shoots produced *in vitro*. The results indicated that daphne microcuttings were competent to form roots regardless of their size and weight. The quick-dip method of treatment with IBA or NAA did not promote root formation. Soaking the cutting bases for 2 or 5 days prior to transplanting, in a solution containing 5ppm IBA, 5ppm NAA, and 50ppm hydroxyquinoline citrate (HQC) resulted in 74.9% rooting.

REVIEW OF LITERATURE

Daphne has been a popular garden plant in many parts of the world, especially in the Northern Hemisphere where many of the 70 plus species of the genus originate. Our experience with daphne in New Zealand is comparatively limited, as only *Daphne* × *burkwoodii*, *D. cneorum*, *D. genkwa*, *D. mezereum*, and *D. odora* are generally available commercially. The most important species in cultivation is the evergreen *Daphne odora* and its cultivars, particularly *D. odora* 'Leucanthe' and *D. odora* 'Rubra'. This species was being grown extensively in New Zealand and being exported to Australia by Kingsbeers' Nursery in Palmerston North prior to 1930 (9).

Most daphne grown in New Zealand are comparatively short-lived as garden plants. They seldom exceed 10 years and many survive for much less time. The rapid collapse of apparently healthy plants was thought to be caused by a virus. Viruses were first detected in daphne in 1941 by Chamberlain and Mathews (4), following a rejected shipment of daphne plants to Australia in 1938. These plants had mottled leaves, but at the time this was not considered to be the cause of the short plant life. It is now known that all cultivars of D. odora have become badly infected with a complex of viruses that cause mottling and streaking of the normally dark green leaves. This causes premature senescence of mature leaves and new leaves become narrower and are clustered at the shoot tips. As these symptoms develop nursery people were concerned about the short life of daphne plants and daphne plants were considered a difficult plant to propagate (3) although this view was not held universally (8).

Prior to the 1970's only three viruses were reported in *D. odora*, but by 1974 eleven viruses had been detected by Forster and Milne (7). A survey conducted about this time revealed that these viruses were widespread. This was considered an economically important problem as approximately 50,000 *D. odora* plants could be sold each

year in New Zealand alone without considering the export potential. A joint programme between Massey University and the Plant Physiology Division of The Department of Scientific and Industrial Research, (DSIR), Palmerston North, was initiated to eliminate the viruses by thermotherapy and plant tissue culture techniques. Clean plants from this program were repeatedly indexed and when proven to be consistently free of known viruses the plant material was passed onto the New Zealand Nursery Research Centre. Plants were then bulked-up and maintained in an insect proof screenhouse. Distribution of "high health" daphne material to licensed propagators in New Zealand commenced in 1979 and plants were released to the retail trade in 1983.

Consumer interest in "high health" daphne has been good and demand for plant material has often exceeded the supply of conventionally propagated plant material. At the same time, commercial interest was developing in a micropropagation technique (5) for rapid multiplication and maintenance of "high health" plant material.

The early reports with limited trials did not suggest there were any major difficulties in the transfer of plantlets from tissue culture back to the greenhouse environment (5). Feedback from commercial laboratories however, suggested that the process was not straightforward and that the hardening-off step was limiting further utilisation of this system. Micropropagation would be more viable if the variable *in vitro* rooting step could be moved to the greenhouse. In many other kinds of plants microcuttings can be hardened-off and rooted at the same time which reduces the need for one of the costly steps in tissue culture (6,11,12).

MATERIALS AND METHODS

Three experiments have been conducted with unrooted micropropagated daphne shoots provided by the Plant Physiology Division, DSIR. Experiments were carried out in the autumn through to early summer in a greenhouse at the Plant Growth Unit at Massey University. They were aimed to improve the rooting and survival of plant material during the hardening-off stage. A range of auxin treatments was used (1).

Experiment 1.

Treatments:

(i) control: 5 sec. dip in water
(ii) 5 sec. dip in 500 ppm IBA
(iii) 5 sec. dip in 500 ppm NAA
(iv) 5 sec dip in IBA+NAA (250 ppm+250 ppm)

The length and number of leaves of 384 microcuttings of *D. odora* 'Leucanthe' and *D. odora* 'Rubra' were recorded. In each treatment 96 cuttings of each cultivar were used. Following treatment

they were planted in punnets (8 per punnet) filled with peat/pumice (50/50). The punnets were placed in 4 randomized blocks, on a capillary mat with bottom heat (20°C.) within a high humidity tent. The rooting of the cuttings was assessed after 12 weeks.

Experiment 2.

Treatments:

(v) control(1) : spray with water
(vi) control(2) : 5 sec. dip in water
(vii) 5 sec. dip in 500 ppm IBA
(viii) spray with 500 ppm IBA

The procedures used in the second experiment were similar to Experiment one except leaf number was not recorded, but weight was measured. Observations were made after 12 weeks.

In both experiments the response of the microcuttings was too variable to detect any differences. In contrast to other reports with other genera (6,11), these experiments showed that successful rooting and survival were poorly correlated with size or weight of the microcuttings (data not shown). Plantlets were very sensitive to moisture stress and some leaf damage was apparent 2 to 3 days after transplanting. Although many shoots failed to root they were photosynthetically active and persisted in the moist medium.

Experiment 3.

Zimmerman and Fordham (12) described an efficient system for rooting apple shoots *in vitro*. In our experiment a similar system was developed to pretreat cutting bases *in vivo* to determine if there was a critical exposure time for chemical treatments. Both auxins and phloridzin have been reported to stimulate rooting (10).

In each treatment 16 micro cuttings were given a basal soak in microtitre plates. Microcuttings were maintained in high humidity with a 16 hr photoperiod for 2 or 5 days. Each solution contained 50 ppm 8 hydroxyquinoline citrate (8HQC) as a microbial inhibitor. Cuttings were planted out in punnets of 70/30 pumice/peat, covered with white plastic and placed on a capillary mat in a high humidity tent. Plants were assessed after 8 weeks.

Results are shown in Tables 1, 2, and 3.

RESULTS AND DISCUSSION

The period of preplant soaking did not influence rooting in either cultivar. Auxin combinations improved rooting more than the single auxin treatments. HQC and high auxin (5.0ppm) levels promoted the best rooting.

The poly sheet laid over the plantlets in the polythene tent appeared to reduce moisture stress in microcuttings and improve survival. This supports observations made in Europe where polythene tents and fogging are used successfully to wean daphne plantlets back to the greenhouse environment.

Plantlet rooting was improved by treatments used in this experiment but the proportion that remained unrooted was unacceptably high for immediate commercial application.

Table 1. The percentages of rooted, unrooted, and dead cuttings of *Daphne odora* 'Leucanthe' and D. 'Rubra' 12 weeks after treatment with IBA(500ppm), NAA(500ppm) or a combination of IBA and NAA(250ppm + 250ppm).

5 sec. dip	*D. odora* 'Leucanthe'			*D. odora* 'Rubra'		
treatment	rooted	unrooted	dead	rooted	unrooted	dead
control	10.4%	69.8%	19.9%	2.1%	66.7%	31.2%
IBA	4.2	64.6	31.3	8.3	63.5	28.1
NAA	2.1	46.9	51.0	1.0	58.3	40.6
IBA + NAA	7.3	49.0	43.8	0.0	59.4	40.6

Table 2. The percentages of rooted, unrooted, and dead cuttings of *Daphne odora* 'Leucanthe' and *D.* 'Rubra' 12 weeks after treatment with 500 ppm IBA applied as either a dip or a spray.

	D. odora 'Leucanthe'			*D. odora* 'Rubra'		
Treatment	rooted	unrooted	dead	rooted	unrooted	dead
control 1	16.7%	57.3%	26.0%	2.1%	72.9%	25.0%
control 2	16.7	59.4	24.0	3.2	75.0	21.9
IBA dip	7.3	50.0	42.7	4.2	79.2	16.7
IBA spray	30.2	49.0	20.8	4.2	78.1	17.7

Table 3. Cuttings of both *D. odora* 'Leucanthe' and *D.* 'Rubra' were left for 2 or 5 days with their bases in the following solutions: (Combined treatment means are included.)

Treatment	Percentage Rooting
1. water + 50ppm 8HQC	4.7a
2. 1% sugar + 50ppm 8HQC	14.6ab
3. 0.1 ppm IBA + 50ppm 8HQC	25.0b
4. 1 ppm IBA + 50ppm 8HQC	14.1ab
5. 10 ppm IBA + 50ppm 8HQC	9.4ab
6. 0.1 ppm NAA + 50ppm 8HQC	12.5ab
7. 0.5 ppm IBA + 0.5ppm NAA + 50ppm 8HQC	19.0b
8. 0.5 ppm NAA + 0.5 ppm IBA + 2 ppm phloridzin + 50ppm 8HQC	65.2cd
9. 0.5 ppm NAA + 0.5 ppm IBA + 10 ppm phloridzin + 50ppm 8HQC	56.3cd
10. 5 ppm NAA + 5 ppm IBA + 50 ppm 8HQC	74.9d
11. Control, no pretreatment	9.4ab

Means with similar letters are not significantly different (P=0.05).

CONCLUSIONS

Daphne microcuttings were competent to form roots and responded to auxin treatments even after prolonged *in vitro* culture. The best treatments suggested that the combination of IBA and NAA could promote rooting in 74.9% of the microcuttings. The results suggest that further improvement in rooting may be obtained by using higher concentrations of the growth regulators. Regulation of moisture stress was a significant factor determining shoot survival and should be examined more fully in further experiments.

LITERATURE CITED

1. Anon. 1977. Root initiation studies on *Daphne odora* 'Leucanthe'. New Zealand Nursery Research Centre Ann. Rep. p. 43–45.
2. Brickell C. D. and B. Mathew. 1976. *Daphne:* The genus in wild and cultivation. *Alpine Garden Society.* 194 p.
3. Carter, A. R. 1979. Discussion group report. Daphne propagation. *Proc. Inter. Plant Prop. Soc.* 29:248–251.
4. Chamberlain E. E. and R. E. F. Mathews. 1941. A virus disease of cultivated daphne. *N.Z. Jour. Sci. Tech.* 23A:254–256.
5. Cohen D. and P. M. Le Gal. 1976. Micropropagation of *Daphne* spp. New Zealand Nursery Research Centre Ann. Rep. p. 5–6.
6. Conner A. J. and M. B. Thomas. 1981. Re-establishing plantlets from tissue culture: a review. *Proc. Inter. Plant Prop. Soc.* 31:342–357.
7. Forster R. L. S. and K. S. Milne. 1975. Survey of virus infecting daphne in New Zealand. *N.Z. Jour. Agr. Res.* 18:391–398.
8. Haggo B. 1973. Propagation of daphne. *Proc. Inter. Plant Prop. Soc.* 23:372–373.
9. Hart W. L. 1988. Personal communication.
10. Li Z. and Y. Chen. 1986. Some factors which enhance the rapid propagation of *Daphne odora* Thunb. *in vitro. Acta Hort.* Sinica. 13:276–280.
11. Maene L. M. and P. C. Debergh. 1983. Rooting of tissue-cultured plants under *in vivo* conditions. *Acta Hort.* 131:201–208.
12. Zimmerman R. H. and I. Fordham. 1985. Simplified method for rooting apple cultivars *in vitro. Jour. Amer. Soc. Hort. Sci.* 110:34–38.