HNS/10

Hardy Ornamental Nursery Stock

Final report for 'The identification and control of variability in *in vitro* culture'

Part II

T.R. MARKS, HRI - East Malling

CONTENTS

PROJECT SUMMARY

HNS/10

The identification and control of variability in *in vitro* culture

FORWARD

This is the second part of the final report on project HNS/10 concerned with the identification and control of variability in *in vitro* culture. Part I of the report was concerned with sources of variability derived from the stockplant (explant). Part II concentrates upon variability which arises both during *in vitro* culture, and that which can be induced by particular selection or manipulation strategies adopted during shoot transfer (subculture). Most of the information is presented in the form of a scientific paper which is to be published in The Journal of Horticultural Science. This was considered to be the most appropriate vehicle for the dissemination of technical information to those within the industry directly involved with micropropagation, who would most likely wish to evaluate its commercial potential. Key findings of the work presented in the paper are summarised here, and recommendations on how they may be accommodated are made.

OBJECTIVES

The purpose of this second stage of the project was to identify and control variability which arose either from the development of shoot clusters in culture, or from particular strategies adopted at subculture, in an attempt to alter their patterns of growth. The practical objective was to use these findings to minimise the variability in the micropropagated plants grown-on by nurserymen.

RESULTS AND RECOMMENDATIONS

The protocols used to select and subculture shoots of nursery stock species determines production *in vitro*, rooting capability and the growth habit of the grown-on plant.

Type of shoot: Where very clear differences exist in the type of shoot generated *in vitro*, their subsequent development, and that of the plant derived from them, can be very different (eg. *Daphne odora* - Figure 1). **The subculture of these shoot types must therefore be continued separate lines to avoid unwanted variability in a particular batch.** Where differences are less marked, such separation is not necessary (eg. *D.cneorum* - Tables I).

Part of shoot: Various protocols for subculture use shoot sections rather than the entire shoot. Subsequent growth rates from these can differ markedly. This has an effect upon production rate predictions *in vitro*, and also affects rooting

potential. **If shoot sections are used, the same portion of the shoot must be used at each subculture to ensure uniform growth in the resulting batch** (Table V).

Length of shoot: Shoots within a cluster can grow to different lengths by the end of a subculture. Although of a common source, these will give rise to different rates of shoot production and shoots of different sizes. Rooting potential is also affected, with the likelihood that growth *ex vitro* will be altered as well. **A common approach at subculture must be adopted to reduce variability. This is very important for the rooting stage**, as it is here that many losses are incurred, possibly as a result of shoots being at different developmental stages, which makes the efficient management of root induction, weaning and growing-on difficult (Tables VI and VII) resulting in reduced and variable production.

Physical removal of apical dominance: This is also a common practise used at subculture to induce more axillary buds to break. Its effectiveness, however, is dependent upon the growth habit of the species to which it is applied. **Where subsequent habit is strongly determined by shoot type (*Daphne odora*) shoot tip removal has little effect.** It is more effective in species where shoot responses are inherently more uniform.

Chemical removal of apical dominance: The application of plant growth regulators (PGR's, chiefly cytokinins) to shoot cultures *in vitro* is a classical method of controlling shoot productivity and length. PGR's (plant hormones) may be effective through either stimulating axillary bud growth or by suppressing apical dominance. However, subsequent **growth is dependent upon the type of material selected for transfer and its innate physiology** (Tables IX and X).

Considering the opportunities for selection and manipulation of shoot clusters at subculture, it is **essential that a common and consistent approach is adopted to reduce innate variability.** Once the *in vitro* growth habit of the plant is understood it becomes possible to manipulate production to achieve higher levels of efficiency and batch uniformity.

Combined with the results of Part I of this final project report, information is now available to help the micropropagator understand where variability can arise during micropropagation, and how it may be either controlled or avoided.

The experimental work for this project was performed by Mrs. Pauline Myers of the Propagation Science Section at HRI - East Malling. I would also like to acknowledge the support of Dr. Peter Harper who acted as Project co-ordinator for this work.

Physiological variability arising from *in vitro* culture is induced by shoot selection and manipulation strategies.

By T.R. MARKS and PAULINE E. MYERS
Horticulture Research International, East Malling, West Malling, Kent, ME19 6BJ, UK

ABSTRACT

Decisions made during the selection of shoot-types *in vitro*,and their manipulation at subculture, affect subsequent performance and uniformity of micropropagated plants. Two shoot-types were identified during the *in vitro* culture of *Daphne cneorum*, *D.odora* 'Aureomarginata' and *Kalmia latifolia*. The central apical-types, produced directly by elongation of the transferred shoots, and the lateral-types, produced from axillary buds on the apical shoots. These shoot-types differed in their capacity for growth and rooting *in vitro*, and were most variable in *D.odora*, where the growth habit of weaned plants was also affected by earlier shoot selection. Within these shoot-types variability was further increased by the selection of shoots according to length. Longer shoots of *D.cneorum* and *Kalmia* produced both more and longer axillary shoots, while longer shoots of *D.cneorum* also rooted better. Within-shoot variability occurred in *Kalmia*, where proximal sections produced a greater number of axillary shoots than did distal sections, and the location of buds which grew also varied according to the section from which they originated. The extent to which growth could be modified by either apex removal, or growth regulator application, was dependent upon the type of shoot selected. To reduce variability in micropropagated plants, shoot-types or shoot parts with different developmental potential must be subcultured separately, and uniform physical and chemical treatments applied. The causes of variable growth due to different subculture strategies are discussed.

INTRODUCTION

Unwanted variability can arise during the micropropagation of many woody plant species. This manifests itself during both the *in vitro* phase and in the weaned plantlets. Initial explants can account for some of this variability, because buds from different locations on the stockplant can affect both shoot growth and rooting (Bressan *et al*, 1982; Norton and Norton, 1986; Webster and Jones, 1989; Marks and Myers, 1992).

Establishment of explants in culture and successful micropropagation depend on the correct combination of physical and chemical conditions (Debergh and Read, 1991), but even when these requirements are met, variability in shoot growth and rooting can still be introduced during sequential subcultures (Economou and Read, 1986; Webster and Jones, 1989). Under otherwise uniform culture conditions, morphogenic responses may be altered by manipulating the shoots at

subculture. Shoot orientation (Hidebrandt and Harney, 1983; San-José *et al*, 1988), removal of the apex (Bressan *et al*, 1982; Norton and Norton, 1986), location within the shoot cluster (Vanderschaeghe and DeBergh, 1988), or use of a particular part of the shoot (San-José *et al*, 1988; Vanderschaeghe and Debergh, 1988), can all affect subsequent axillary shoot development. Rooting can also be affected by shoot size (Hildebrandt and Harney, 1983).

This paper describes the sources of variability in shoot growth, rooting and growth habit of the weaned plantlet, as a result of selection and manipulation strategies adopted at subculture, and shows how these may be modified to increase culture and plantlet uniformity.

METHODOLOGY

Plant material

Three woody shrub species were used: Mature flowering plants of *Daphne cneorum* grafted onto *D.mezereum* rootstocks, and mature flowering plants of *D.odora* 'Aureomarginata', growing on their own roots. Both of these had been in culture for approximately one year. Also, three-year-old non-flowering *Kalmia latifolia* plants of seedling origin were used, which had been in culture for four years.

In vitro *shoot culture*

Cultures of *D.odora* were derived from the apical and the three most distal buds on vegetative shoots. These were sterilized together, then aseptically separated and planted individually on appropriate media. *D.cneorum* cultures were derived from multi-bud explants from two locations on the same shoot. These comprised either the apex and adjoining distal axillary buds (a), or a proximal section of the same shoot (b). Each *D.cneorum* explant contained approximately six buds and was sampled from two stockplant shoots, designated 5 and 6. Both species were sterilized in sodium hypochlorite ('Domestos', 0.7% w/v available chlorine) for 15 minutes, followed by three washes in sterile deionized water (SDW). Entire lengths (approx. 8 cm) of *Kalmia* shoots were collected at the end of a growth flush. These were momentarily immersed in 70% ethanol plus 0.05% Tween 20, then in 5% filtered calcium hypochlorite (approx. 1% w/v available chlorine) for 15 minutes, followed by three washes in SDW.

D.odora explants were grown on Linsmaeier and Skoog (LS - 1965) medium containing 1 mmol m^{-3} 6-benzylaminopurine (BAP), initially in Coulter counter dilution vials containing 10 cm^3 of medium, then in 500 cm^3 glass jars containing 100 cm^3 of medium as shoots developed. *D.cneorum* explants were grown on LS medium containing 5 mmol m^{-3} BAP for one subculture in dilution vials, then subsequently with 0.1 mmol m^{-3} BAP in 300 cm^3 glass jars containing 50 cm^3 of medium. For both species

the medium was adjusted to pH 5.2 before the addition of agar (Oxoid, Purified 6.5 kg m^{-3}), and autoclaving. *Kalmia* explants were placed in 100 cm^3 flasks each containing 25 cm^3 of liquid (agitated) Woody Plant medium (WPM - Lloyd and McCown, 1980) adjusted to pH 5.0 before autoclaving. This contained 5 mmol m^{-3} isopentenyladenine (2iP), and was changed every day for one week. Explants were then placed vertically in 125 x 25 mm pyrex test tubes containing 20 cm^3 of liquid (stationary) WPM, which was changed every three weeks. When axillary buds had grown to approx. 5 mm, they were aseptically removed and placed on WPM solidified with 6.5 kg m^{-3} agar. *D.cneorum* and *D.odora* were subcultured every fourth week by excising and replanting single shoots. *Kalmia* was subcultured every eighth week as a basal cluster of shoots with all apices removed. These cultures constituted the stock material grown on standard medium for subsequent experiments. The identity of the different explant sources of *D.cneorum* was maintained separately throughout. Cultures were grown at 25°C under a 16 h photoperiod. Both *Daphne* species were grown under 'Thorn-EMI GroLux' fluorescent tubes with a photon flux density of 48 μmol m^{-2} s^{-1} (photosynthetically active radiation, PAR; 400-700 nm), while the *Kalmia* were grown under 'Philips colour 84' fluorescent tubes with a photon flux density of 57 μmol m^{-2} s^{-1} (PAR) at culture level.

Rooting

D.cneorum shoots were rooted *in vitro*. Excised shoots were placed on a medium with half-strength Murashige and Skoog (MS - 1962) macro-elements (1/2 LS) containing 30 mmol m^{-3} indolebutyric acid (IBA) for seven days, then transferred to hormone-free medium with a quarter MS macro-elements (1/4 LS). Both *D.odora* and *Kalmia* were direct-rooted in peat-based compost (Fisons F1). Shoots were excised from culture, washed in deionized water (DW), immersed in 0.1% w/v Benlate and allowed partially to dry. Shoot bases were dipped in 0.2% w/w IBA in talc (incorporating 10% w/w Captan) and planted in module trays (80 cm^3 cell volume) placed on 5 cm deep heated (21°C) sand beds under 'dry fog' (Lucas Dawes Sonicore; operated at 0.6°C wet bulb depression). Rooting was assessed after four weeks. *D.odora* plantlets were weaned by moving rooted shoots to closed cases on sand beds. Initially the relative humidity was held at >90% for seven days, followed by 60-80% for a further seven days, and then plantlets were moved out to an open sandbed in the glasshouse. Shading allowing 20% transmission of ambient light was used throughout.

Shoot selection

At each transfer particular individual shoots, or shoot parts, were selected for transfer to initiate new subcultures. The selection criteria were; (1) shoot-type, defined as apical- and lateral-types; (2) multi-nodal shoot sections; (3) shoot length.

1. Type: It was possible to recognise two distinct shoot-types at the end of subcultures of *D.cneorum*, *D.odora* and *Kalmia*. In the *Daphne* species these were defined as apical and lateral, to denote that they were either extensions of the intact transferred shoot (apical), or were axillary shoots (lateral) arising from these. In *Kalmia*, the same development occurred, but the extension growth actually originated from an axillary bud on the transferred basal cluster. For each species these two shoot-types were selected and removed from shoot clusters and either cultured further on the same LS or WPM as previously specified, or rooted. From both apical and lateral shoot-types the following was used. For *D.cneorum* 20 x 2 cm long shoots were used to assess shoot growth and 40 x 2 cm long shoots for rooting potential. For *D.odora* 45 x 1.5 cm long shoots were used to assess shoot growth and 40 x 2-4 cm long shoots for rooting. For *Kalmia* 40 x 1 cm long shoots were used to assess shoot growth and 25 x 1.5 cm long shoots for rooting. *D.odora* plantlets derived from apical and lateral shoot-types were grown-on, and their growth and branching habit measured over 18 months. Six weeks after weaning, all shoots were pinched back to a common height of 1 cm, and the area of the top five unfurled leaves (from 9-15 shoots) of each shoot-type was measured using a leaf area meter (Delta-T, Cambridge).

Differences in growth habit of apical and lateral shoots were most extreme in *D.odora*. To determine the ability of these shoot-types to change their branching habit, 15 x 2 cm long shoots of each type were grown over three subcultures, and approximately 2 cm of the central shoot (the extended transferred shoot) was replanted at each transfer.

2. Shoot section: Thirty-five shoots of *Kalmia*, each longer than 2 cm, were removed from culture and the distal (after apex removal) and proximal 1 cm sections were cultured on standard medium.

3. Length: Shoots of different length of *D.cneorum* and *Kalmia* were selected using different criteria. Entire shoots of *D.cneorum* were selected, with 24 shoots in each of three length classes (6-15, 16-25 and 26-35 mm). The apex was removed from *Kalmia* shoots and 25 distal sections were each cut to lengths of 5, 10 and 20 mm. Both species were cultured on their respective media. The same criteria and length classes of *D.cneorum* were used for rooting, except that distal (a) and proximal (b) culture lines from two stockplant shoots (5 and 6) were used (Marks and Myers, 1992). Twenty-four shoots were used for each length class.

Shoot manipulation

The subsequent growth habit of shoots may be additionally influenced by both physical and chemical treatments at subculture. In an attempt to determine the processes controlling variability in *Daphne* and *Kalmia* shoots the

following treatments were applied; (1) removal of the shoot apex; (2) changing the plant growth regulator regime in the medium.

1. Apex removal: To determine the role of apical dominance upon subsequent axillary shoot growth, the apices were removed from 30 lateral-type *D.cneorum* shoots and their growth was compared to an equal number of intact shoots. Both shoot-types were 1 cm long. Apices were removed from 15 apical and 25 lateral shoot-types of *D.odora*, and compared with an equal number of intact shoots on standard medium. Both shoot-types were 1.5 cm long.

2. Plant growth regulators: In an attempt to modify typical growth responses various concentrations of BAP and 2,3,5-triiodobenzoic acid (TIBA) were added to the medium of *D.odora* and *Kalmia* respectively. Ten x 1.5 cm long apical and 21 x 2 cm long lateral shoots of *D.odora* were each cultured on LS medium containing 0, 1 or 5 mmol m^{-3} BAP. Twenty-four x 1 cm long distal and proximal shoot sections of *Kalmia* were each cultured on WPM containing both 5 mmol m^{-3} 2iP and either 0, 1 or 5 mmol m^{-3} TIBA (aqueous solution added by filter sterilization after autoclaving).

Analysis of results

Replication has been described for individual experiments, and each experiment was fully randomised. Data for culture fresh weight, number of shoots, shoot length, number of roots and leaf area were compared by analysis of variance. Numbers of rooted cuttings were assumed to be binomially distributed and were compared using analysis of deviance. Because of the binomial assumption, there were no degrees of freedom associated with the approximate standard errors of differences for percentage rooting in Tables I and VII. A chi-squared test was used to compare numbers of shoots forming on plantlets derived from the apical and lateral shoot-types of *D.odora* in their final assessment.

RESULTS

Shoot selection

The differences in further shoot development and rooting between the two shoot-types was dependent on species. This was most marked in *D.odora* where the number of new axillary shoots, and their lengths were greater than for the apical-type ($P<0.001$ - Table I). More apical-type shoots rooted than did lateral-types, and they also produced more roots ($P<0.01$). In *Kalmia* apical-type shoots also produced more axillary shoots than did the lateral-types ($P=0.06$), although neither their length, nor subsequent rooting differed. Elongation of both of the shoot-types occurred during the subculture. In *Kalmia* the amount of growth did not differ, whereas in *D.odora*

the apical-type grew the most ($P<0.001$), while conversely in *D.cneorum* the lateral-type grew the most ($P<0.01$).

The growth and branching habit of plants derived from apical and lateral selections of *D.odora* were measured over an 18-month period. Before pruning, the only measurable difference was the larger leaf area of the apical-derived plants (18.0 cm^2) compared to the lateral-derived ones (12.0 cm^2 - $P<0.01$). Pinching induced secondary branches in February 1989 in some of the apical selections, but none in the lateral selection. By June, 29% (data not presented) of the apical-types had produced secondary branches, but none had grown on the lateral-types, although the primary branch continued to extend and were significantly ($P<0.01$) longer than the apical-types by May 1990 (Table II). Branching continued in the apical-type, but was first observed in the lateral-type in May 1990 following flowering, although differences in the number of plants branching were not significant. However, only the apical-types exhibited any tertiary branching (Table III). Secondary branches were now present on many of the lateral-types, while those which failed to branch tended to produce very vigorous primary shoots (Table II, Figure 1).

Axillary branching of lateral-type shoots of *D.odora* could be improved by the repeated transfer of the same shoot tips. Over three subcultures the ability of the lateral-type shoots to produce further axillary shoots progressively rose from 46% to 79% of the production of the apical-types (Table IV).

Both the number of axillary shoots ($P<0.001$) and their length ($P<0.01$) were significantly greater in the proximal than in the distal shoot sections of *Kalmia* (Table V). Despite the removal of apical dominance in both shoot section types, shoots only arose from the more basal buds on the distal sections, whereas they arose from buds along the whole length of the proximal sections.

In *D.cneorum* increasing the size of shoot transferred onto cytokinin-rich media progressively increased both the number of axillary shoots ($P<0.05$) and their length ($P<0.001$). Larger distal shoot sections of *Kalmia* also produced significantly more axillary shoots ($P<0.01$), although the similar progressive increase in shoot lengths were not significantly different (Table VI). The same size classes of *D.cneorum* shoots were examined for rooting within four culture lines. Within each culture line, percentage rooting significantly increased with increasing shoot length, and overall the distal-explant derived culture lines rooted better than the proximal-derived ones. The number of roots also significantly increased with size, but the differences between culture lines were not significant (Table VII).

Shoot manipulation

The removal of the apex from lateral-type shoots of *D.cneorum* reduced both the number of new axillary shoots ($P<0.05$) and their length ($P<0.05$). Removing the apex from apical and lateral shoot-types of *D.odora* had no effect on either the number of axillary shoots formed or their length in each shoot-type, although overall growth remained greater ($P<0.001$) in the apical shoot-type. Axillary shoot lengths on the apical shoot-types were longer ($P<0.001$), and apex removal did not alter this (Table VIII).

Increasing the BAP concentration supplied to apical and lateral shoot-types of *D.odora* increased the number of axillary shoots ($P<0.01$), but the relative difference between the two shoot-types was maintained at both concentrations ($P<0.01$). In the absence of BAP no axillary shoots were formed, and at higher concentrations (no data presented) extensive vitrification was observed. Both the extension of transferred shoots and axillary shoot length decreased ($P<0.001$) with increasing BAP concentration (Table IX).

The application of two concentrations of TIBA, in addition to 2iP, to proximal and distal shoot sections of *Kalmia* had an effect similar to BAP in *D.odora*. As TIBA concentration was raised, the number of axillary shoots was increased in both shoot sections ($P<0.05$), but changes in axillary shoot length and fresh weight were not significant. However, the relative differences in growth between the two shoot section types was maintained ($P<0.001$, Table X).

DISCUSSION

The experiments reported in this paper have identified some of the factors operating during subculture which give rise to variability in shoot cultures, and how their effects can be reduced by selection or manipulative procedures *in vitro*.

Shoots chosen from a shoot cluster at subculture do not always develop from axillary buds with the same developmental potential. The apical- and lateral-types described in this paper had distinctive growth characteristics, which were species-specific and showed the most marked response in *D.odora*. This divergence in growth potential between shoot-types has also been observed in *Fagus sylvatica* 'Atropurpurea' (Vanderschaeghe and Debergh, 1988). In both *Daphne* species these shoot-types can be separated at subculture because of their different positions within the shoot cluster. This difference is less easy to see in *Kalmia*, and probably in other species, where shoots are very similar after an extended period of growth. If these differences between shoot-types are not taken into account at subculture, succeeding cultures will have mixed growth and different shoot production rates, and will not produce uniform responses during other physiological

events such as rooting. In *D.odora* these differences extend into the morphological development of weaned plantlets, where shoot vigour and branching habit follow a similar pattern to that exhibited by the two shoot-types *in vitro*. The apical-type lends itself to earlier branching with a more compact and commercially desirable habit (Figure 1). Although apical and lateral shoot growth was most extreme in *D.odora*, the lateral shoot habit may be altered by re-subculturing its apex on cytokinin-containing medium. This change is most likely due either to a progressive localised accumulation of cytokinin, or increasing sensitivity to its morphogenetic effects, producing a response similar to exposing the shoots to higher concentrations of BAP. The recalcitrance of the axillary buds on these lateral shoots to grow is similar to the lack of growth of proximal axillary bud explants in *D.odora* at culture initiation (Marks and Myers, 1992), suggesting that these events share a common control mechanism.

That the greater growth potential lies within the more proximal sections of *Kalmia* shoots may well be due in part to the proximity of axillary buds to cytokinin-containing culture medium, but also to the residual effects of apical dominance in the distal sections prior to subculture. That the effect of apical dominance still persists over subordinate bud development can be further seen by the restriction of axillary shoot growth to the basal portion of the distal sections, whereas axillary shoots grew from most buds on the proximal sections.

Increased shoot production would be expected from the culture of larger shoots with more axillary buds. An increase in the length of axillary shoots with shoot size suggests a mutually beneficial effect of multiple axillary buds, even when apical dominance is still operative within the shoot (*D.cneorum*). The same mechanism appears to operate in *Kalmia* shoot sections even though they no longer experience apical dominance. The effect of size upon growth extends to rooting potential, with similar trends being exhibited by four culture lines of *D.cneorum*. The largest shoots consistently rooted better, and this accords with the results of Hildebrandt and Harney (1983) on *Syringa vulgaris* 'Vesper'. Because entire shoots of *D.cneorum* were removed from culture clusters, the better rooting could reflect either stronger shoot growth or an extended period of growth in the shoot cluster, compared with the shorter shoots. This is supported by results indicating that enhanced rooting in distal cultures lines correlated with better shoot culture growth (Marks and Myers, 1992).

Variability can be further induced at subculture by the introduction of procedures that either physically or chemically modify shoot growth response. The removal of apical dominance by excising the shoot apex potentially increases the number of axillary buds that may grow, although this is dependent upon medium cytokinin concentration (Norton and Norton, 1986). In *Kalmia*, the removal of apical dominance

released growth in subordinate buds, although the location of the shoot section on the original shoot determined the amount of growth that occurred. In neither *Daphne* species was growth enhanced by apex removal, and it was inhibited in *D.cneorum*. Both these observations support the hypothesis that axillary bud growth potential was predetermined (Marks and Myers, 1992). However, the gradual change in shoot growth habit following repeated subcultures of the *D.odora* lateral shoot-type, or of increased growth at higher BAP concentrations, suggests that this recalcitrance of buds to develop may be overcome. The increase in numbers of axillary shoots in *Kalmia* in the presence of TIBA would suggest that it is antagonising endogenous auxin (Depta *et al*, 1983). As the differential response of the transferred shoot sections were still apparent, it is probable that like *D.odora*, there is a hierarchy of responsiveness between the most distal and proximal buds on the shoot.

This work has shown that it is possible to identify components of subculture strategies that can lead to different growth during subsequent cytokinin-directed shoot growth, auxin induced rooting, or plantlet development. Also, once identified, it is possible to modify these processes to reduce variability, but only if the growth potential of different shoot-types is recognised, and the appropriate procedures are then followed. Widely practised treatments at subculture, such as apex removal aimed at increasing axillary growth, are not universally applicable to modifying bud hierarchy-dependent growth. Responses are likely to be dependent upon species, and the degree to which growth will be affected by the location of a shoot within a shoot cluster, or of a section within a shoot will thus vary. The modification of growth regulator regimes may enhance growth; BAP and TIBA in this instance, but these will still remain subject to the underlying potential growth habit of the various micro-propagules described.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support of the Horticultural Development Council for the funding of this study. We are also grateful to Mr. M.S. Ridout of HRI, East Malling, for the statistical analyses.

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Table I

The effects of selecting apical and lateral shoot-types from cultures of Daphne cneorum, D.odora *'Aureomarginata' and* Kalmia latifolia *upon subsequent axillary shoot growth and rooting*

Species	Shoot-type	Final length of transferred shoot (mm)	Number of axillary shoots	Mean axillary shoot length (mm)	Percentage rooting	Roots per rooted shoot
D.cneorum	Apical	23.7	4.4	15.7	69	4.1
	Lateral	30.2	3.4	16.1	50	4.0
	s.e.d.	0.97	0.60	1.87	16.1	1.51
	(d.f.)	(35)	(31)	(35)	-	(19)
D.odora	Apical	14.5	2.7	14.7	65	6.0
	Lateral	19.3	0.5	10.0	48	3.9
	s.e.d.	1.01	0.25	1.48	10.9	0.74
	(d.f.)	(88)	(88)	(57)	-	(43)
Kalmia	Apical	15.0	1.4	4.5	64	1.9
	Lateral	15.4	0.9	4.3	76	2.2
	s.e.d.	0.66	0.22	0.97	12.8	0.35
	(d.f.)	(78)	(78)	(57)	-	(33)

Table II

Length of primary shoots produced on plants derived from apical and lateral shoot-types of Daphne odora *'Aureomarginata' measured over eighteen months*

Date	Apical (mm)	Lateral (mm)	s.e.d. (41 d.f.)
Dec. 1988[+]	62.1	55.3	3.52
Feb. 1989	13.2	11.4	1.81
Jun. 1989	29.6	36.9	4.52
May 1990	122.5	157.3	11.22

[+] After measuring height the shoots were all pruned to a uniform 1 cm and area measurements made of the top five leaves.

Table III
Distribution of branch categories in Daphne odora *'Aureomarginata' plants derived from apical and lateral shoot-types measured in May 1990*

Branch category	Apical	Lateral
Primary shoot only	16%	21%
Primary and secondary shoots only	71%	79%
Primary, secondary and tertiary shoots	13%	0

Table IV

The effect upon subsequent axillary shoot growth of reculturing the apices of apical- and lateral-type shoots of D.odora *'Aureomarginata'*

	Number of laterals		Mean axillary shoot length (mm)	
Subculture	Apical	Lateral	Apical	Lateral
First	4.1	1.9	-	-
Second	5.2	3.1	25.7	32.0
Third	5.3	4.2	25.4	31.3
s.e.d. (d.f.)	1.04 (80)		3.09 (48)	

Table V

The effect of selecting distal and proximal shoot sections at subculture upon subsequent axillary shoot growth in Kalmia latifolia

Shoot section	Number of Axillary shoots	Mean axillary shoot length (mm)
Distal	2.1	4.8
Proximal	4.0	6.2
s.e.d. (66 d.f.)	0.32	0.46

Table VI
The effect of selecting entire shoots or distal shoot sections of various size upon subsequent axillary shoot growth in D.cneorum *and* Kalmia latifolia *respectively*

Species and length classes (mm)	Number of lateral shoots	Mean axillary shoot length (mm)
D.cneorum (whole shoots)		
6-15 (11.0)[+]	2.2	9.5
16-25 (21.2)	3.3	11.1
26-35 (30.3)	3.7	16.8
s.e.d. (53 d.f.)	0.58	1.73
Kalmia (shoot sections)		
5	2.1	4.8
10	2.6	5.2
20	3.0	6.3
s.e.d. (72 d.f.)	0.26	0.77

[+] Values of shoot length in brackets are the means of initial shoot sizes within each class in *D.cneorum*.

Table VII

The effect of selecting entire shoots of various sizes upon subsequent rooting in different culture lines of D.cneorum

Culture line	Shoot length classes (mm)	Percentage rooting	Roots per rooted shoot
5a - Distal	6-15 (10.0)	65	5.5
	16-25 (20.5)	86	7.4
	26-35 (30.5)	86	12.7
		(79)	(8.5)
5b - Proximal	6-15 (11.3)	33	2.9
	16-25 (21.6)	25	9.0
	26-35 (33.4)	54	8.6
		(37)	(6.8)
6a - Distal	6-15 (10.0)	50	3.2
	16-25 (20.5)	73	6.1
	26-35 (30.6)	96	7.6
		(73)	(5.6)
6b - Proximal	6-15 (9.3)	29	4.9
	16-25 (21.3)	52	7.8
	26-35 (31.9)	78	7.4
		(53)	(6.7)
	s.e.d. (d.f.)	14.2	2.43 (133)

Shoot length figures in brackets represent the mean length within that class. Percentages and root number values in brackets are the means for each culture line.

Table VIII

The effect of removing the shoot apex upon subsequent axillary shoot growth in D.cneorum *and* D.odora *'Aureomarginata'*

Species	Shoot -type	Shoot apex	Number of axillary shoots	Mean axillary shoot length (mm)
D. cneorum				
	Lateral	Intact	7.3	6.1
		Removed	5.6	5.1
		s.e.d.	0.67	0.40
		(d.f.)	(56)	(56)
D. odora				
	Apical	Intact	3.6	18.7
		Removed	3.2	24.0
				12.8
	Lateral	Intact	1.3	9.8
		Removed	1.6	2.78
		s.e.d.	0.60	(51)
		(d.f.)	(76)	

Table IX

The modification of axillary shoot growth by BAP in apical and lateral shoot-types selected from cultures of D.odora *'Aureomarginata'*

		BAP (mmol m^{-3})		
	Shoot-type	0	1	5
Final length of transferred shoot (mm)	Apical	27.6	22.9	16.0
	Lateral	26.7	24.3	19.2
	s.e.d. 2.89 (87 d.f.)			
Number of axillary shoots	Apical	0+	5.2	10.7
	Lateral	0+	2.9	5.5
	s.e.d. 1.67 (58 d.f.)			
Mean axillary shoot length (mm)	Apical	-	28.2	12.9
	Lateral	-	20.3	13.9
	s.e.d. 2.84 (51 d.f.)			

+ Not included in statistical analysis.

Table X

The modification of axillary shoot growth by TIBA in distal and proximal shoots sections selected from cultures of Kalmia latifolia

		TIBA (mmol m^{-3})		
	Shoot type	0	1	5
Fresh weight (mg)	Distal	39	35	36
	Proximal	65	56	59
	s.e.d. 6.5 (137 d.f.)			
Number of axillary shoots	Distal	1.7	1.9	2.7
	Proximal	3.1	3.2	4.0
	s.e.d. 0.58 (137 d.f.)			
Mean axillary shoot length (mm)	Distal	3.5	3.7	3.2
	Proximal	5.8	5.0	4.6
	s.e.d. 0.48 (137 d.f.)			

Figure 1.
Eighteen-month-old plants of *Daphne odora* 'Aureomarginata' derived from apical (left) and lateral (right) shoot-types displaying divergent growth habits.

Figure 1.
Eighteen-month-old plants of *Daphne odora* 'Aureomarginata' derived from apical (left) and lateral (right) shoot-types displaying divergent growth habits.