SUMMARY

This, then, is a brief sketch of how we grow some of our primulas and violets at Careby. The old-fashioned versions of these popular spring plants are easy to propagate conventionally. As many of them hybridise so readily, it is safest to use vegetative means if stocks are to be produced which are true to name.

INFLUENCE OF THE ENVIRONMENT ON ROOTING *DAPHNE ODORA* CUTTINGS

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Abstract. Analysis of the environmental influence on volume rooting of Daphne odora revealed that irradiance and night temperature requirements must be fulfilled for successful *in vitro* rooting to take place.

INTRODUCTION

Within the commercial micropropagation industry competition demands that all efforts be made to optimise the productivity of the laboratory and the quality of the propagule. The very high cost of research has meant that work at this laboratory on specific difficulties has been done on high value ornamental plants from the sale of which some rapid return on investment can be achieved. The work on *Daphne odora* was done to identify some of the causes of inconsistent rooting response which have been observed in these and other woody plants at this laboratory.

From work on *Daphne* and other species it had been observed that a rooting treatment successful to a high percentage at one point in time might not yield a similar result even when repeated only a month later. From various experiments it appeared that neither the genotype of the subject nor human error in media manufacture or culture handling was responsible for the major part of rooting inconsistencies. Rather, variations in response appeared to be related to variations within the environment of the growth room itself.

It was decided, therefore, that a more detailed examination of the growth room environment was required to examine temperature and irradiance influences on rooting. For this purpose, production growth room rooting responses were compared to rooting

within growth cabinets providing small measured temperature and irradiance variations.

D. odora is described by Brickell and Matthews (3) as a shrub which grows up to 4 feet in height. It is a flowering evergreen and is hardy in many parts of Britain.

MATERIALS AND METHODS

Shoot tips up to 10cm in length were taken from containerised glasshouse-grown stock plants. These shoots were trimmed of leaves and cut into sections 2cm in length which included one or two buds. The sections were sterilized in a 15% solution of Domestos, a proprietory bleach, for 15 minutes before being rinsed in sterile water. Following sterilization, bleach-damaged tissue was removed in a sterile laminar air flow cabinet, and the explants were placed on multiplication media. Culture vessels were clear plastic disposable tubs (Neo Plants Ltd) 7.8cm in diameter and 5.5cm in height and contained 50ml multiplication media consisting of the salts and organics of Lloyd and McCown (6) woody plant medium (WPM) and sucrose (30 gl^{-1}), agar (7.5 gl^{-1}) and benzylaminopurine (BAP) (0.5 mgl^{-1}). The pH was adjusted to 5.7 before autoclaving using dilute NaOH. Media was sterilized by heating to 121°C for 15 minutes, in ½L or 1L bottles and was poured into tubs shortly before setting.

Following introduction to culture the shoots were routinely subcultured every 4 weeks for more than 6 months. Rooting experiments began when enough shoots were available. For rooting work, shoot tips 1cm in length were taken from multiplying cultures and placed on rooting media. All multiplying cultures and cultures rooted in the growth room were provided with a 16 hour day with temperatures between 19° and 26°C and with 40 to 55 $\mu em^{-2}s^{-1}$ irradiance measured inside the culture from warm white fluorescent tubes.

Dark-treated shoots were transferred to rooting media and placed in continuous dark for 4 days before being moved to 16 hour days.

Table 1. Rooting of *Daphne odora* cuttings on F14 Medium in four different production scale runs.

	Batch 1	Batch 2	Batch 3	Batch 4
Number of tubs	191	103	9	70
Number of shoots	3125	1680	106	1390
Total rooted	1250	543	78	1122
Total percent rooted	40	32	73	80

Rooting of *D. odora* cuttings was under as similar conditions as possible. Differences among treatments are the time rooting transfer was made and the area of the growth room used.

For the rooting experiments analysed in Tables 2 to 4 two Leec

growth cabinets were used (Leec Laboratory and Electrical Engineering Company, Nottingham NG4 2AJ, UK).

These provided temperature and daylength control and, with warm white fluorescent tubes mounted vertically at the rear of the cabinets, provided irradiance that ranged from 2 to 64 $\mu em^{-2}s^{-1}$ at different points within the cabinet. Shoots given dark treatment were placed in darkness immediately following transfer to rooting medium at a temperature of 22–26°C.

Irradiance was measured with a unit from Lambda (λ) Instrument Corporation, supplied by T. J. Crump, Scientific Instruments, Wickford, Essex, Model L1/185 in units of $\mu em^{-2}s^{-1}$. Temperature, which was measured by an Edale Multiprobe Thermometer loaned by the local branch of the Agricultural Development and Advisory Service (ADAS), also varied from point to point within the cabinet; from 16.5°C to 22°C at night and 16.5°C to 25°C in daytime, but was found to be constant (± 0.5°C) throughout the experiment in any particular position. Day temperatures, night temperatures, and irradiance were measured within all culture vessels for the experiments analysed in Tables 2 to 4.

Table 2. Combined analysis of variance for irradiance, day and night temperature, on the time to 10% rooting (T10) of *Daphne odora* cuttings.

Terms	DF	Mean Change	VR	
Modifications to model				
+ °C N	1	591.671	88.03	$p = 0.001$
+ °C D	1	77.614	11.55	$p = 0.01$
+ IRR	1	8.257	1.23	NS
Residual	136	6.721		

Table 3. Combined analysis of variance for the effects of irradiance, day temperature and night temperature, on rooting *Daphne odora* cuttings at day 24.

Terms	DF	Mean Change	VR	
Modifications to model				
+ °C Night	1	51.470	13.44	$p<0.001$
+ °C Day	1	12.608	3.29	$p>0.05$ NS
+ Irradiance	1	35.912	9.38	$p<0.01$
Residual	136	3.831		

Shoots taken from multiplying cultures were placed in a rooting medium containing ½ strength salts of WPM with full organics, sucrose (20 gl^{-1}), agar (7.5 gl^{-1}), and the plant growth substances naphthaleneacetic acid (NAA, and/or indolebutyric acid (IBA). The rooting media was also adjusted to a pH of 5.7 and sterilized before pouring into clear plastic culture tubs. All statistical analyses were carried out using the Genstat package developed at Rothamstead Research Station.

RESULTS

During preliminary experiments a rooting percentage of 100 was achieved in growth room conditions providing a 16 hour day. In subsequent unreported work the cause of variable responses seen in Table 1 was shown not be to due to human error.

Tables 2 to 4 are taken from an experiment to identify the most likely cause of variable rooting, assuming no human error or genotype variation. Using growth cabinets (see Materials and Methods), 140 culture tubs containing 22 shoots each were treated to conditions of measured day temperature, night temperature, and irradiance. Rooting was assessed daily in each tub from the first sign of root emergence to the end of the experiment at day 24.

In analysing the results the proportions rooted were transferred into a logit scale and regressed against time. The time to 10% rooting was estimated for each tub from these regressions to give an indication of start time. Both this and the slopes of the lines were regressed against day temperature, night temperature, and irradiance. Day 24 was used as a measure of the final response and the results were analysed with respect to day temperature, night temperature, and irradiance. The complete analysis is shown in Tables 2 to 4, inclusive.

The analysis of the results identifies some of the primary causes of rooting variation as being due to night temperature and irradiance. Whilst reducing the time to 10% rooting (Table 2), higher night temperatures also very significantly influenced the final percentage rooted at Day 24 ($p<0.001$, Table 3). Conversely, irradiance played no part in the time to 10% rooting, but increasing irradiance increased the final rooting percentage($p<0.01$, Table 3).

Table 4. Combined analysis of variance for irradiance, day temperature and night temperature, on rooting rate of *Daphne odora* cuttings.

Terms	DF	Mean Change	VR	
Modifications to model				
°C Night	1	0.7681	3.39	NS
°C Day	1	0.3847	1.7	NS
Irradiance	1	0.1529	0.68	NS
Residual	136	0.2263		

DISCUSSION

From earlier work it was known that *D. odora* could be rooted to 100% in culture. Also clear was that subsequent attempts to produce the crop (Table 1) showed widely different rooting responses to the same chemical recipe. Some conditions provided to the cultures had caused a reduction in rooting response.

After human error had been ruled out as a major cause of variable response, first darkness and then general conditions within the growth room, were examined.

General environmental conditions analysed in Tables 2 to 4 give good evidence of the role of environment in rooting. The analyses (summarised in Table 5) clearly show that high night temperatures account for a significant proportion of variation in rooting response.

Table 5. A summary of the results showing the components of the environment that influenced the rooting of *Daphne odora* cuttings.

Effect	Environmental Factor (Cause)		
	Day Temp.	Night Temp.	Irradiance
Start time (T10)	/	//	X
Rate of rooting	X	X	X
Final rooting Percentage	X	//	/

This table shows the factors of the environment and areas of influence on rooting. X = no effect; / = a significant effect; // = a major effect.

Irradiance was also shown to influence the final rooting response. From the analysis in Table 3 it is apparent that darkness reduces rooting and that high night temperatures do likewise (Table 3). Light is required for successful rooting and, as irradiance increases, so also does rooting percentage (Table 3). Higher temperatures were shown in Table 2 to reduce the time to achieve 10% rooting.

The importance of these results, which are specifically relevant only to *D. odora*, is in the implication of how relatively small changes in laboratory conditions combine to influence the success of the system. Similar work to examine shoot multiplication or quality might prove to be as revealing. The combined effect of growth environment could seriously upset the efficiency of production. The same is likely to be true for conventional propagators, more so because the diversity of variable environment is so much greater.

There are many reports in the literature in which some environmental influence is described. Dark treatment before and during rooting has been reported to influence rooting in a number of species, both in conventional propagation systems (1, 4, 5, 8) and in *in vitro* systems (2, 7, 9, 10). Most of the publications report improved rooting due to darkness but Rowell (8) and Norton and Boe (7) also reported species which either had no preferential response due to darkness or which showed inhibition of rooting in the dark.

One advantage of research into rooting using *in vitro* systems is that a reasonably uniform group of one clone may be exposed equally to a manipulated environment. Zimmerman (10) increased the temperature during dark treatment whilst attempting to root

Malus and improved the rooting percentage as a result. It may be that some species respond to auxin differently in light and dark. There may be two systems which do not lead to the same result. Plants whose rooting is promoted by darkness might reasonably be expected to do better at higher temperatures, the response to auxin perhaps being temperature dependent within certain limits. Plants whose rooting response is not favoured by darkness, such as *Cotinus coggyria* (8), *Chaenomeles japonica* (7) and *D. odora* might be expected to show greater inhibition to rooting by increasing night temperature and might also be more effectively propagated in 24 hour days with supplementary lighting.

Although the results indicate that *D. odora* has rooting requirements for low night temperature and high irradiance, it is likely that these two factors are part of the environmental conditions required to cause the preferred response to auxin which, if tested, might be best provided for by 24 hour days.

Within a system that is sensitive to so many potential causes of variation the interpretation of results is hazardous. The analysis here shows that within the conditions tested, night temperature and irradiance influence rooting. A basis must be established which provides constant conditions for two of the variables and allows the proper study of the third. High quality growth rooms which can provide these conditions and maintain stable conditions might also allow research into light quality, and gaseous phase influences on plant growth.

SIGNIFICANCE TO THE NURSERY INDUSTRY

Understanding the contributing factors that control rooting in response to auxin is very important, particularly to micropropagators, but also to conventional plant producers. It is only really cost effective to produce a crop when, out of 100 cuttings taken upwards of 95 root and grow away. Equally important is that all 95 out of 100 cuttings should root as nearly simultaneously as possible so that resultant uniformity leads to a reduction in the labour costs required for grading and other maintenance work, and thus the cost of production is reduced. For all producers the time spent by a crop in propagation areas must be as short and effective as possible, providing heat and light only when required.

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CONTAINER COMPOST pH AND ITS EFFECT ON PLANT GROWTH

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An experimental trial was set up using seven cultivars, each in a different genus. One was an Ericaceous plant. Each of the cultivars was potted into four composts which had varying amounts of dolomitic limestone added to them, except the Ericaceous plants which were potted into only three composts, and with the addition of dolomitic limestone not being at such great extremes.

From the six cultivars used in the trial, quite remarkable differences in growth rates appeared due to varying the amount of dolomitic limestone added to the compost. With this indicating that the compost's pH is critical in obtaining maximum plant growth, pH control can also be used for restricting growth.

With the one Ericaceous cultivar, no difference in growth rates were noticeable, as the addition of dolomitic limestone to the three trial composts was not at great enough extremes to appreciably alter the pH.