HNS/10

Hardy Ornamental Nursery Stock

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

Part I

T.R. Marks, HRI - East Malling

Effect of explant position upon culture development *in vitro*

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SUMMARY

Studies of plants with contrasting growth habits showed that the developmental potential of explants *in vitro* is determined by both the nature of stockplant growth and the precise position on the plant from which explants are selected. In *Betula pendula* 'Dalecarlica' EM85, where initial vigorous apical growth of the main stem is followed by lateral development from axillary buds, three explant sources were identified which performed well *in vitro*. Two were derived from sites of apparent maximum growth potential on vigorous shoots, and one on weak basal shoots where correlative inhibition had prevented the expression of growth potential in the intact stockplant. In *Daphne odora* 'Aureomarginata' and *D. cneorum*, both characterised by apically dominant non-branching shoots, maximum growth potential *in vitro* resided in explants from the distal section of each shoot, and was lost progressively in buds towards the proximal region. Rooting potential of *Betula* and *D. cneorum* shoots derived from these explant culture-lines exhibited the same trends as their overall growth *in vitro*, showing differences both between explant sources along individual shoots and between shoot-types. Selection of *Betula* explants at different developmental stages during the growing season revealed that the control of *in vitro* growth was influenced by the developmental phase of the whole plant, as well as that of individual shoots. Implications for the selection of explants and control of variability arising from micropropagation are discussed.

INTRODUCTION

Culture initiation is arguably the most critical stage of micropropagation, because the potential for multiple shoot generation lies within single buds. The problems imposed at this stage by microorganism contamination (Debergh and Maine, 1981), phenolic oxidation (Marks and Simpson, 1990) and tissue maturity (Bonga, 1982) are well described in the literature, and many techniques have been developed to overcome them. The rationale determining which bud is used as an explant to initiate a culture has received less attention. The most common approach is to use the apical bud from a vigorous shoot, often growing at the top of the plant. This ignores other apical buds located on lower shoots, axillary buds on all shoots, and the innate growth characteristics of the plant.

Explant origin has been reported to affect culture growth *in vitro*. Webster and Jones (1989) produced culture-lines from two apical buds of the apple rootstock M.9 each with different shoot growth and rooting capabilities *in vitro*, which remained

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

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FORWARD

This project has been concerned with the identification and control of variation arising in plants produced by micropropagation. Part one of this final report concentrates upon sources of variation which are derived from the (explant) stockplant. Some of this information has been summarised in a simpler form in previous issues of the Project News supplement, but here it is presented in greater detail in the form of a scientific paper as submitted to the Journal of Horticultural Science. This was considered the most appropriate vehicle for the dissemination of technical information to those within the industry, namely the micropropagation companies, who would most likely wish to evaluate its potential commercial practise. Salient findings of the work presented in this paper are summarised below.

OBJECTIVE

The purpose of this work was to determine whether it is possible to increase production efficiency in the early stages of micropropagation by identifying sources of variation among explants in terms of their survival, culture growth and subsequent rooting potential. Given that this is possible, the practical objective would be to maximise the productivity of culture initiation and minimise the variation among the plants which nurserymen buy to grow-on.

RESULTS AND RECOMMENDATIONS

Unexpectedly large differences were discovered in the ability of explants to survive and grow in culture. These related to the different growth habits of specific plants, the location of shoots, and to the position of buds on individual shoots; general rules as to the suitability of explant sources can be proposed.

1. Explants from shoots where growth is apically dominant, such as in *Daphne*, should only be taken from near the **apex**, because it is here that both **maximum and even growth potential** exist. Explants from lower down these shoots fail to survive in culture.

2. In plants such as *Betula*, where a greater range of shoot-types occur, buds from the following positions are recommended as explants:

(i) The **mid-to-bottom zone of vigorous upright shoots** if these buds have high growth potential in the intact tree, as indicated by their ability to grow out as laterals when released from apical dominance in mid-summer.

(ii) Buds from the **upper part of less vigorous, less upright shoots**, which do not produce laterals from lower buds, and where growth in the intact tree continues from the shoot-tip.

(iii) Buds from **anywhere on weak sub-ordinate shoots** at the bottom of the tree. In the intact plant these are dormant, but all grow equally well in culture once released from the dominating effect of the shoots above.

It is important to note that these effects in both *Daphne* and *Betula* are not obvious during early stages *in vitro*, and the potentially better performing explant sources cannot be distinguished until about the third sub-culture, when their growth becomes much more rapid.

High rooting potential is identified with rapidly growing cultures, and follows the same trends for the various shoot-types and bud locations.

The selection of explants on the basis of either their growth potential on the intact tree, or that achieved *in vitro* following the release of apical dominance, will, in appropriate subjects;

(i) Increase the numbers of explant locations available, allowing more efficient use of stockplants.

(ii) Maximise shoot production *in vitro*.

(iii) Maximise rooting potential.

(iv) Reduce within batch variation.

apparent over twenty-one months in culture. Norton and Norton (1986) showed that when apical explants were taken at different positions throughout the canopy of *Prunus cerasifera* and *Spirea* x *bumalda* plants, those cultures originating from the top of the branch system produced more shoots than those from the bottom. Welander (1988) isolated both apical and axillary bud explants of *Betula pendula*, and showed that these displayed different cultural and biochemical characteristics *in vitro* as determined by their position on the plant.

No attention has yet been given to the relationship between the growth potential of apical and of axillary buds from along the length of individual shoots, nor to how *in vitro* development is affected by the growth habit of the shoots from which they are taken. This paper describes the results of such investigations in plant species of diverse growth habit, with the aim of identifying sites of maximum culture growth potential and of reducing variation among propagules.

MATERIALS AND METHODS

Plant material

To reduce microbial contamination, all experimental plants were grown under glass in pots of 75% peat: 25% grit compost incorporating Osmocote 18:11:10 controlled release fertiliser (2.9 kg m^{-3}), magnesium limestone (2.4 kg m^{-3}),lime (1.1 kg m^{-3}) and fritted trace elements (WM 255, 2.9 g m^{-3}). Plants used were: a) Two-year-old *Betula pendula* 'Dalecarlica' EM85, grown from rooted cuttings, where the previous year's shoots were cut back to the proximal bud to produce shoots for experimental use. b) Four-year-old plants of *Daphne odora* 'Aureomarginata' produced from rooted cuttings.
c) Commercially produced *D. cneorum* plants with *D. mezereum* rootstocks, and in their fifth flush of growth subsequent to grafting. Neither *Daphne* source was pruned, and both were capable of flowering.

Explant selection

Betula: Three shoot-types of distinctive growth habit were identified on the stockplants. Those near the top of the plant were upright and vigorous, and produced lateral shoots from proximal axillary buds (Type - I). Shoots further down the main framework were less upright and less vigorous, and produced no laterals (Type - II). Weak, horizontal shoots from near the base of the plant, which did not produce laterals and which set a terminal bud early in the season (Type - III).

Explants consisted of individual apical or axillary buds from along the whole length of a shoot together with approximately 5 mm of shoot tissue either side of the bud. Where an axillary bud had developed into a lateral, the most proximal bud on the new lateral shoot was taken, together with a 5mm section of tissue from the main shoot. Explants were either surface sterilized or aseptically dissected.

D. odora: Seasonal shoot growth took the form of either extension growth from a vegetative apical bud, or lateral

outgrowth from an axillary bud below a floral apical bud. No laterals grew on the current seasons' growth. The apical bud, and sequential axillary buds together with a section of stem tissue (approx. 5mm), were taken from along vegetative shoots and surface sterilized.

D. cneorum: Growth of these plants also took the form of apically dominant shoot elongation with no lateral outgrowths during the growing season. This is a dwarf species with very short internodes and preliminary experiments showed that individually separated axillary buds did not establish in culture. Explants therefore comprised multi-nodal sections from both the distal and proximal regions of these short shoots. Each section contained approximately six axillary buds, and was also surface sterilized.

Culture initiation

To ensure the subsequent identification of the relative positions of axillary buds, entire shoots of *Betula* and *D. odora* were surface sterilized in stoppered measuring cylinders before individual explants were excised. Entire shoots of D.cneorum were small enough to be sterilized in 250 cm^3 flasks, before distal and proximal sections were separated. Both species of *Daphne* shoots were surface sterilized for 15 minutes in 10% v/v 'Domestos'; a proprietary brand of sodium hypochlorite (approx. 0.7% w/v available chlorine plus surfactant), followed by three rinses in sterile deionized water (SDW). *Betula* explants were surface sterilized with 0.5% w/v mercuric chloride plus Tween 20 for 5 minutes, followed by a wash in SDW, and then in 5% w/v filtered calcium hypochlorite plus Tween 20 for 15 minutes, followed by three washes in SDW. Individual apical and axillary buds, and multi-nodal sections where appropriate, were aseptically cut from these shoots and any bleached tissue removed before planting in appropriate medium. The aseptic dissection of *Betula* buds was performed under a Leitz M7 dissecting microscope in a laminar flow cabinet. Only outer bud scales were removed, such that the explant transferred to the medium contained young unfurled leaves and stem tissue along with the apical or axillary meristem itself.

In vitro culture

Cultural requirements for these species were determined in associated studies and are summarised here. *Betula* explants were initiated and cultured on Woody Plant Medium (WPM - Lloyd and McCown, 1980) supplemented with 2.5 mmol m^{-3} BAP, initially in Coulter counter dilution vials, then in 300 cm^3 glass (honey) jars. Both *Daphne* species were initiated on Linsmaier and Skoog (1965 - LS) medium containing 5 mmol m^{-3} 6-benzylaminopurine (BAP) in Coulter counter dilution vials (*D. cneorum*) or tubes (*D. odora*), each containing 10 cm^3 of medium. After the first sub-culture BAP was reduced to 0.5 mmol m^{-3} for *D. cneorum* and 1 mmol m^{-3} for *D. odora*, and shoots transferred to 300 cm^3 glass jars each containing 50 cm^3 of medium. Cultures were transferred to fresh medium every four weeks.

Two methods were used for rooting shoots. For *Betula*, entire shoots were transferred to hormone-free (HF) WPM in dilution vials, whereas for *D. cneorum* 2 cm shoots were cultured on LS medium with half-strength Murashige and Skoog (1962 - MS) macro-elements, supplemented with 33 mmol m^{-3} indolebutyric acid (IBA) for seven days before transfer to dilution vials containing 10 cm^3 HF LS medium with 1/4 MS macro-elements.

Media for both shoot growth and rooting also contained 100 mmol m^{-3} ferric sodium ethylenediaminotetraacetic acid and 87.6 mol m^{-3} sucrose, and were adjusted to pH 5.0 (*Betula*) and pH 5.2 (*Daphne*) prior to the addition of 6 kg m^{-3} agar (Oxoid, purified) and autoclaving.

Experimental design

Betula: Explants were taken from the shoots of *Betula* plants on three dates in the growing season (1989) reflecting different developmental stages. In April (Experiment 1), explants were taken from the apex and all sequential axillary buds along the shoots of types I, II and III on four replicate plants. In May (Experiment 2), when laterals were developing in shoot-type I, explants from the apex and axillary buds from shoot-types I and III on the same four plants were taken. In the more basal axillary bud positions in shoot-type I, the base of the new lateral was sampled to include its most proximal axillary bud as the explant. Finally, in November (Experiment 3), explants were taken from the apex and alternate axillary buds from along shoot-types I, II and III from three of the previously used plants.

Cultures produced both axillary and adventitious shoots and their development was determined by measuring the fresh weight of each culture-line replicate at each sub-culture. To describe the relationship between culture fresh weight and shoot production, eighty 1 cm shoots were each placed in dilution vials of WPM supplemented with BAP. Culture fresh weight and the number of shoots and buds produced were measured after four weeks.

D. odora: In June (1988), prior to the termination of shoot elongation, explants were taken from the apex and sequential axillary buds from along the entire length of six similar sized shoots. Each shoot came from a different plant.

D. cneorum: In July (1988) the distal and proximal sections of twenty flushing shoots were removed from a single plant, their paired identity was retained throughout subsequent culture *in vitro*.

The growth and development of cultures initiated from these explants were recorded over three to five sub-cultures, during which time a record of their original position on the stockplants was retained.

Rooting: In *Betula* between twelve and twenty-four shoots were taken from six ten-month-old cultures-lines from Experiment 2. From shoot-type I these originated from the apex, and the

eighth and nineteenth axillary bud positions. In shoot-type III, shoots related to explants from the apex, and the eighth and twelfth axillary bud positions. These corresponded with the top, middle and bottom of the respective shoot-types. After transfer to HF medium, the appearance of roots was monitored over a twenty-three day period.

In *D.cneorum* twenty shoots were taken from nineteen-month-old culture-lines derived from a pair of proximal and distal explants from the same stockplant shoot. After root induction and transfer to HF medium, root expression was monitored over a further thirty-three days.

Analysis of results

Two types of experiment were performed, which were concerned with; a) explant-derived growth potential over a series of sub-cultures, and b) determination of rooting potential in shoots from these culture-lines.

In *Betula*, the analysis of culture-line development was based upon differences in fresh weight (log transformed) increment, using both correlation and regression analyses depending upon the complexity of the data. For the purpose of describing treatments represented by replicate shoots differing slightly in length, the relative explant positions on different sized shoots was identified by describing each explant in terms of the percentage distance along the original shoot occupied by the original bud, measuring from the first distal axillary bud (= 0%). In most shoots sampled the apical bud was still growing, whilst the axillary buds were dormant. To exclude any effect of this physiological difference, values for the apex were omitted from formal analyses.

Shoots sampled from *D. odora* all contained very similar numbers of buds, so the actual positions were used as points of reference. Also, because of the similarity in developmental potential of the apex and the more distal axillary bud explants from these shoots, the apex was included in analyses. Only fresh weight (log transformed) was considered in the first two sub-cultures, and trends were assessed by quadratic and linear regression analyses. In the second and third sub-cultures differences in fresh weight and shoot numbers were determined by analyses of variance. This was also used for the analysis of *D. cneorum* data where only two source sites were considered on replicate shoots.

Comparison of percentage rooting in different culture-lines of *Betula* and *D. cneorum* was based on chi-squared tests and mean roots per rooted cutting on analysis of variance.

RESULTS

Betula cultures develop through a mixture of axillary and adventitious shoot growth, the latter arising from basal callus. Many of these shoots are too small to be easily recorded at sub-culture, but there is a proportional relationship ($P<0.001$) determined by linear regression analysis of fresh weight ($\log_{10}$ transformed to make the variance approximately constant) on the number of shoots and buds in a culture (Figure 1), allowing fresh weight to be used as a measure of culture growth.

Betula experiment 1

Differences in growth *in vitro* of explant-derived culture-lines of *Betula* were apparent over all five sub-cultures. Each shoot-type showed a distinctive growth response. The correlation coefficients of log transformed fresh weight on relative position along each shoot were mostly positive for shoot-type I, mostly negative for shoot-type II, and showed no significant trends in shoot-type III (Table I). These indicate that culture growth potential was greatest from proximal explants on shoot-type I, distal explants in shoot-type II, and showed no significant differences in shoot-type III.

Betula Experiment 2

By May shoot-types I and II had elongated forming many more axillary buds, while shoot-type III had set a terminal bud. In addition, lateral shoot growth had been expressed from the more proximal axillary buds on shoot-type I. Only shoot-types I and III were sampled in this experiment. Two distinctive trends in growth were identified in culture-lines from the two shoot-types. The quadratic curves fitted to fresh weight values from shoot-type I show the greater growth in culture-lines derived from proximal buds seen in Experiment 1 still to be present. In addition, growth was also greater in culture-lines from the more distal axillary buds; a growth response more similar to that previously observed in cultures from shoot-type II. As also observed in Experiment 1, developmental trends took several sub-cultures to stabilize. This is seen in the change in shape of the quadratic curves to a consistent form in sub-cultures 3-5; for each of these curves, the quadratic term is significant (Figure 2). In shoot-type III, none of the fitted lines was significant, with the exception of the first sub-culture, although they also displayed a change in growth trends over the first three sub-cultures. Growth of many of these cultures, however, was as good as those from shoot-type I (Figure 3). In both cases lines were fitted to values from bud positions located between 10 and 90% along the length of the shoot. This avoided both distal and proximal buds, where spatial separation between nodes was minimal. To avoid confusion in the figures, data values are only displayed for the apices.

Betula Experiment 3

Explants taken in November were from dormant plants, and a more technically-involved aseptic dissection was necessary to obtain sterile explants. As a consequence only the apical and alternate axillary buds were sampled between the apex and the most proximal bud on each shoot. Only three sub-cultures were recorded because extensive necrosis occurred in the fourth reducing culture replication to a level which precluded statistical analysis. The surviving cultures, however, grew vigorously. All three shoot-types were sampled, but the distinctive trends in growth potential observed from shoot-types I and II in Experiments 1 and 2 were not apparent in any of the sub-cultures. Neither were any observed from shoot-type III (Table II). Despite their initiation from dormant buds,

many explants produced abundant growth during these three sub-cultures, as did those which survived the fourth sub-culture.

D. odora

Immediately following initiation, growth in *D. odora* was limited to shoot elongation, and further growth from axillary buds did not occur until the end of the second sub-culture. Fresh weight increments during the first sub-culture showed a significant quadratic effect of explant position, with a maximum at position six (Figure 4). Whereas during the second sub-culture this had changed to a linear response, with the greater growth at the apex. The new lateral shoots produced from the apex and first four axillary bud sources were too few in number to allow any formal statistical analysis. Further lateral shoots were produced in the third and fourth sub-cultures, but with the exception of a single culture-line derived from axillary position eight, all new lateral growth was restricted to culture-lines from either the apices or the first three axillary buds (Table III). Neither the fresh weight nor the number of shoots produced from these varied significantly with bud position, except that values for the culture-line from axillary bud eight were always lower than the others.

D. cneorum

Not all the axillary buds developed on either the proximal or distal multi-nodal shoot sections. However, over the three sub-cultures both culture fresh weight and the number of axillary shoots produced were significantly greater from the distal than from the proximal shoot sections (Table IV).

Rooting

Betula: Roots on shoots from each of the six culture-lines were first observed seven or eight days after transfer to the HF medium, although the rate of their subsequent appearance was dependent upon their origin. Culture-line I 19 gave the highest percentage rooting, significantly ($P<0.01$) higher than I a and I 8, and the shoot-type III sources ($P<0.05$). Percentage rooting did not differ between these, but from day thirteen onwards they were significantly ($P<0.05$) greater than in I a and I 8 (Figure 5). The mean number of roots per rooted cutting differed significantly between sources at the end of the experiment, and reflected the same trends as percentage rooting. The three shoot-type III sources gave fewer roots than source I 19, but more than sources I a and I 8 (Table V).

D. cneorum: Rooting first occurred nine days after transfer to HF medium. In shoots derived from the distal shoot section, the proportion of rooted shoots rose to a maximum of 80% over the next 24 days. Whereas rooting in shoots derived from the proximal section was slower, only rising to a maximum of 40%. The differences between the two sources was statistically significant ($P<0.05$) from day thirty onwards (Figure 6). Measured at the end of the experiment, the mean number of roots per rooted cutting did not differ significantly between

the two explant sources.

DISCUSSION

The conventional rationale for selecting an explant for culture initiation assumes that a bud growing actively on the stockplant will also establish and develop quickly *in vitro*. Making such an assumption implies that factors controlling growth potential *in vitro* directly parallel those *in vivo*. This is not necessarily so. Isolating either an apical or axillary bud at culture initiation, and applying plant growth regulators (PGR) *in vitro*, interferes with the relationship between individual buds which would otherwise determine their particular growth habits on the stockplant. This work has revealed that the potential for optimal growth does not always reside in the apex of the most vigorous shoot. Two factors, both affected by the morphological development of the stockplant, were shown to affect performance *in vitro;* (i) the location of the explant along a growing shoot, and (ii), the location of that shoot on the stockplant. The three subjects used in this study each produced shoot-types where clear differences in explant performance could be identified.

Trends of growth *in vitro* are most noticeable in culture-lines of *Betula*, and support the hypothesis that during the growing season the potential to grow is controlled by the original spatial distribution of the buds on the stockplant. Depending upon their position they are subject to the influence of endogenous PGR's, mainly from growing shoot apices or the root system. The relative effect of either of these can be seen to change as the shoots on the plant develop. For example, in *Betula* shoot-type I, the proximal buds, both before and after the outgrowth of laterals, develop rapidly *in vitro*. However, the differences in culture growth between these and the distal buds decreased as the shoot elongated (Experiment 2, figure 2), causing the development of the distal portion to be similar to that of shoot-type II. In all three subjects, apical dominance during the growing season was sufficient to maintain axillary buds on this shoot-type in a quiescent state. When isolated from the stockplant and cultured *in vitro* the level of growth inhibition was revealed. In *Betula* and *D. cneorum* this was partial and growth occurred, but in proximal buds of *D. odora* it was absolute, despite favourable conditions *in vitro*. The extreme differences in growth potential between the two species of *Daphne* may have been induced by the use of multi-nodal explants, or by the more dwarfing habit of *D. cneorum*, either of which would have reduced the relative spatial separation of axillary buds and presumptive differential response to endogenous PGR's.

With the expression of bud growth on the stockplant being under endogenous PGR control, it might seem likely that the exposure to exogenous PGR's *in vitro* would overcome preconditioned responses carried-over from the stockplant (Srivastava and Steinhauer, 1982). This did not occur in *Betula* and *Daphne* cultures during the period of this study, as demonstrated by their persistent growth trends. The recalcitrance of proximal buds in *D. odora*, which failed to

develop at all despite exposure to cytokinin (BAP) over two sub-cultures *in vitro* demonstrates this further. In fact, the typical trends observed in Experiments 1 and 2 (*Betula*) showed a stronger correlation of fresh weight to bud position with increasing sub-culture. Also, the results of Experiment 3 showed that the developmental potential of buds on a shoot was dynamic, and without an actively growing apex the pattern of individual bud responsiveness was lost. This may also account for the lack of trends in shoot-type III in the first two experiments, where active apical growth terminated early. Buds on shoot-type III are subordinate to all other positions, and consequently growth of that shoot-type was weak and of limited duration. Once these inhibitory factors were removed, however, the buds could fully express their potential *in vitro*. The ability to grow rapidly from an apparently weak source may be due to their proximity to the root system and either a direct or indirect effect of cytokinin. Welander (1988) also reported stronger growth *in vitro* from both resting buds on dormant plants, and from those nearer the base of actively growing plants. This was attributed to the accumulation of starch and soluble carbohydrates in these buds. These results, however, are in disagreement with those of Norton and Norton (1986), where growth of apical explants of *Spiraea x bulmalda in vitro* diminished on shoots located progressively towards the base of the plant, although they used active and not dormant apical buds as explants.

The ability of certain culture-lines of *Betula* and *D. cneorum* to root better than others can be attributed to the fact that they were derived from culture sources with the highest growth rates. Although not directly measured over the intervening period of time, this explant-derived factor was still affecting *in vitro* development in *Betula* and *D. cneorum* some ten and nineteen months after initiation, respectively. That these differences were still apparent after so long would suggest that once established, the growth rates of different culture-lines are determined by their bud origin (Webster and Jones, 1989) and are not readily altered even if cytokinin levels *in vitro* are changed (Norton and Norton, 1986). The possibility that mutations have occurred during the course of *Betula* and *Daphne* sub-cultures cannot be entirely overlooked, especially when shoots have an adventitious origin (*Betula*). However, the trends described in Experiments 1 and 2 are based upon the growth of culture-lines derived from up to one hundred separate buds per shoot-type. It is unlikely that all would have undergone genetic changes to produce the significant effects observed. In *Daphne* species, where shoots arose from axillary buds, this would have been even less likely to occur.

An important aspect of this work is that the differences in explant development described in *Betula* and *Daphne* are displayed by tissues growing in the same season, which are those most commonly used to initiate cultures. They are not from different growth phases (Bonga, 1982), or from different flushes of growth (Favre and Juncker, 1987), which can affect both shoot growth rate and rooting. The importance of

selecting the right explants at initiation can be easily overlooked, dramatically altering the progress of a micropropagation protocol, especially when at least three sub-cultures are needed to establish trends. Firstly, cultures can be established from seemingly similar explants, such as the apices in shoot-types I and II in *Betula*, with the result that multiple culture-lines are formed with different rates of shoot and root production. Alternatively, good explant sources are not recognised because their selection initially appears inappropriate on the stockplant. These would include the distal axillary buds in *D. odora*, which have very similar growth characteristics to the apex, as well as all the buds on shoot-type III in *Betula*. It is therefore necessary at culture initiation to be aware of the growth habit displayed by the stockplant, such that explant selections may be made from sites of similar developmental potential, whether these are expressed on the stockplant or not.

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TABLE I

Correlation coefficients between log transformed fresh weight and relative position of buds along shoots of Betula pendula 'Dalecarlica' EM85. Explants sampled prior to the expression of proximal lateral shoots on shoot-type I (Experiment I)

Sub-culture	Shoot position I		Shoot position II		Shoot position III	
1	0.49 *	(17)	0.67 **	(16)	0.03	(14)
2	-0.11	(16)	-0.36	(16)	-0.19	(15)
3	0.25	(13)	-0.58 *	(14)	-0.12	(16)
4	0.51 *	(13)	-0.69 **	(13)	-0.21	(16)
5	0.46	(11)	-0.72 **	(10)	0.46	(12)

Level of statistical significance, *=P<0.05, **=P<0.01 (d.f.)

TABLE II

Correlation coefficients between log transformed fresh weight and relative position of buds along shoots of Betula pendula 'Dalecarlica' EM85. Explants sampled from dormant shoots (Experiment III)

Sub-culture	Shoot position I		II		III	
1	0.38	(22)	-0.29	(24)	-0.13	(12)
2	-0.02	(22)	0.09	(24)	-0.26	(12)
3	-0.27	(20)	0.06	(23)	0.28	(12)

No values are statistically significant. (d.f.).

TABLE III

Fresh weight and lateral shoot production from viable culture lines of Daphne odora 'Aureomarginata' after the third and forth sub-cultures

	3rd Sub-culture		4th Sub-culture	
Explant	Fresh weight (mg)	Lateral shoots	Fresh weight (mg)	Lateral shoots
Apex	819	3.6	3287	10.0
1st axillary	781	3.8	2534	12.6
2nd axillary	894	4.8	2864	9.3
3rd axillary	838	4.7	2715	10.0
s.e.d. (d.f.)	269 (9)	1.5 (9)	1132 (8)	3.2 (8)
8th ∓ axillary	230	1.0	845	5.0

∓ Excluded from statistical analysis.

TABLE IV

Fresh weight and shoot numbers of distal and proximal derived culture lines of Daphne cneorum measured over three sub-cultures

Sub-culture	Distal source	Proximal source	s.e.d.
Fresh weight (mg)			
1st	330	183	37.7 ***
2nd	3074	1265	472.3 ***
3rd	6500	3177	1095.9 **
Number of shoots			
1st	3.8	2.1	0.43 ***
2nd	4.1	2.4	0.39 ***
3rd	2.2	1.5	0.50

Level of statistical significance between proximal and distal sources; **=P<0.01, ***=P<0.001.

TABLE V

Mean number of roots produced by cuttings derived from culture-lines of shoot-types I and III in Betula pendula 'Dalecarlica' EM85 after 23 days on hormone-free medium

Culture-line source	No. of rooted cuttings	Roots per rooted cutting
I a	7	2.9
I 8	7	2.1
I 19	23	5.1
III a	16	3.5
III 8	18	4.5
III 12	16	3.7

(Approximate s.e.d. = 1.08, 81 d.f.)

Figure 1.

The proportional relationship between log10 fresh weight and the number of shoots and buds produced in shoot cultures of *Betula pendula* 'Dalecarlica' EM85.

Figure 2.

Quadratic curves fitted to values of explant-derived culture-line fresh weight (log10) proportionally distributed along shoot-type I in *Betula pendula* 'Dalecarlica' EM85 over five sub-cultures (Experiment 2). Lines fitted between 10 and 90% positions. Means for the apex-derived culture-lines are shown by point values. Sub-culture 1 (△ —), 2 (□ ---), 3 (● ···), 4 (▲ ---) and 5 (■ -·-).

Figure 3.

Quadratic curves fitted to values of explant-derived culture-line fresh weight (log10) proportionally distributed along shoot-type III in *Betula pendula* 'Dalecarlica' EM85 over five sub-cultures (Experiment 2). Lines fitted between 10 and 90% positions. Means for the apex-derived culture-lines are shown by point values. Sub-culture 1 (△ —), 2 (□ ---), 3 (● ···), 4 (▲ ---) and 5 (■ -·-).

Figure 4.

Quadratic and linear regression lines fitted to log 10 fresh weight of Daphne odora 'Aureomarginata' culture-lines for the first (□ ___), and second (△ ---), compared with the third (■) and fourth (▲) sub-cultures. Point values are the means from bud positions from six replicate shoots.

Figure 5.

Rooting response of six ten-month-old culture-lines of *Betula pendula* 'Dalecarlica' EM85 derived from shoot-types I and III, assessed over a 23 day period (Shoots from Experiment 2). Culture-line I apex (I A ···), I 8th '32%' axillary (I 8 ---), I 19th '76%' axillary (I 19 —), III apex (III A ---), III 8th '57%' axillary (III 8 -·-), III 12th '86%' axillary (III 12 -·-).

Figure 6.

Rooting response of nineteen-month-old distal and proximal culture-lines of *Daphne cneorum* assessed over a 40 day period. Distal-section (—) and proximal-section (---) culture-line derived shoots.

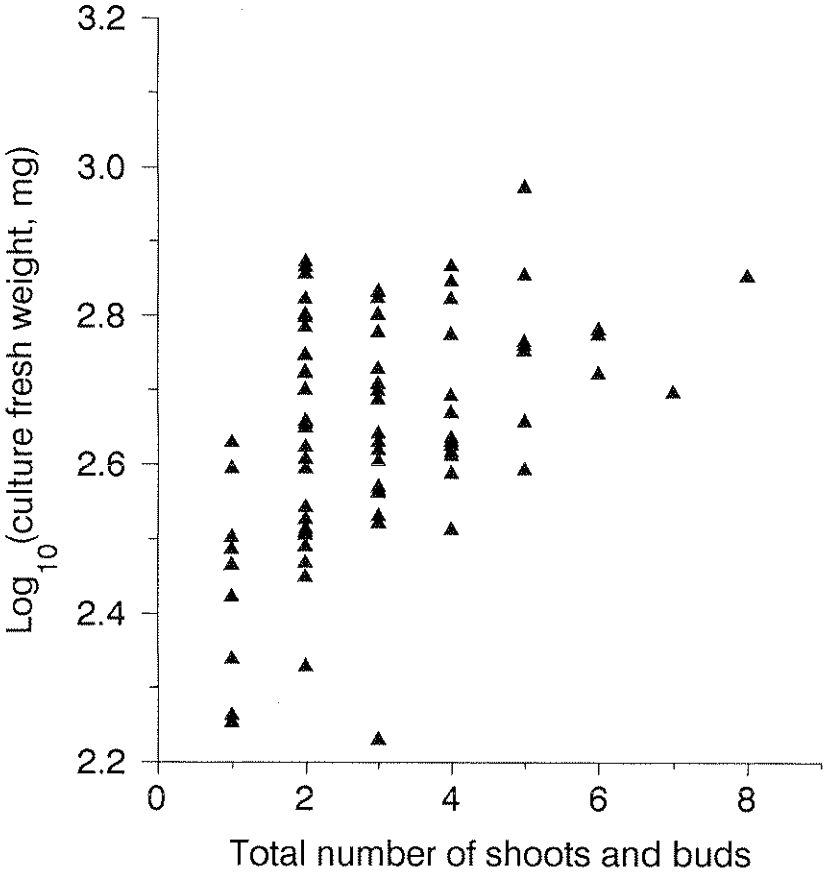

3.2
3.0
2.8
2.6
2.4
2.2
Log10(culture fresh weight, mg)
0
2
4
6
8
Total number of shoots and buds

Figure 1.

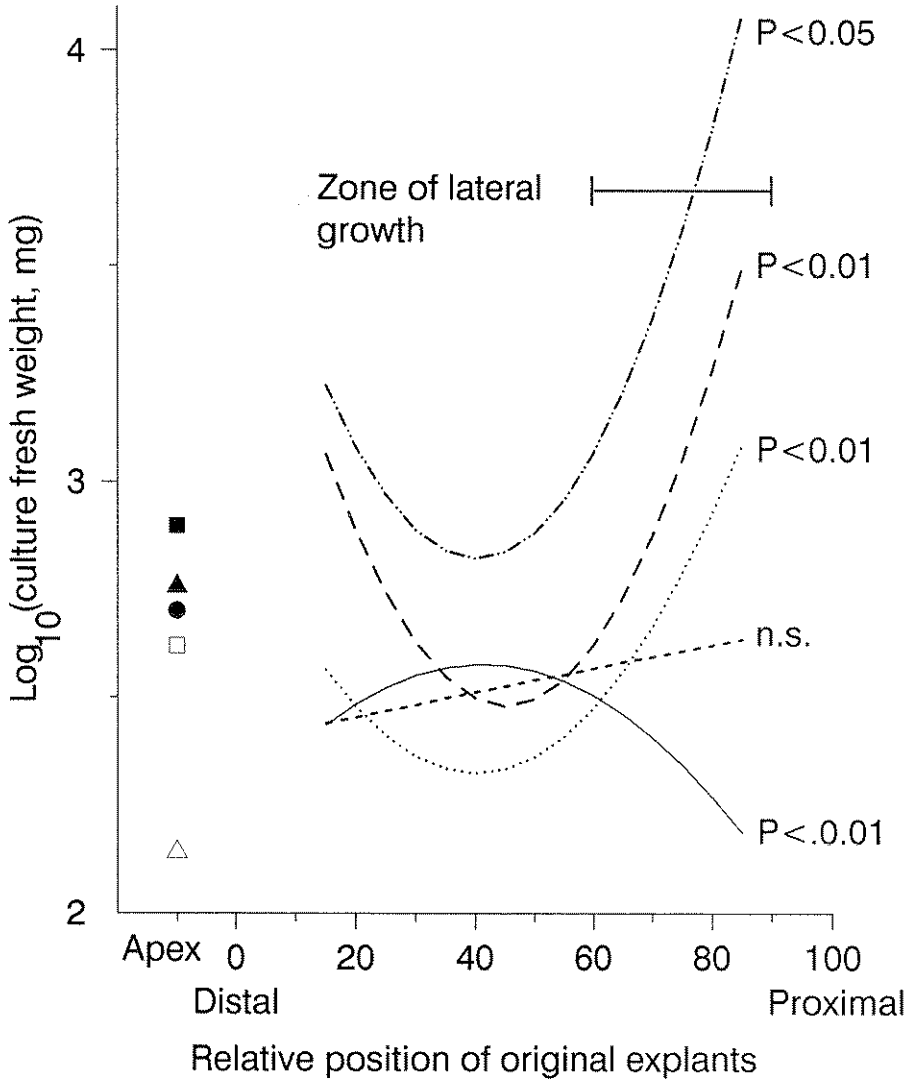

4
3
2
Log10(culture fresh weight, mg)
Zone of lateral growth
P<0.05
P<0.01
P<0.01
n.s.
P<.0.01
Apex
0
20
40
60
80
100
Distal
Proximal
Relative position of original explants

Figure 2.

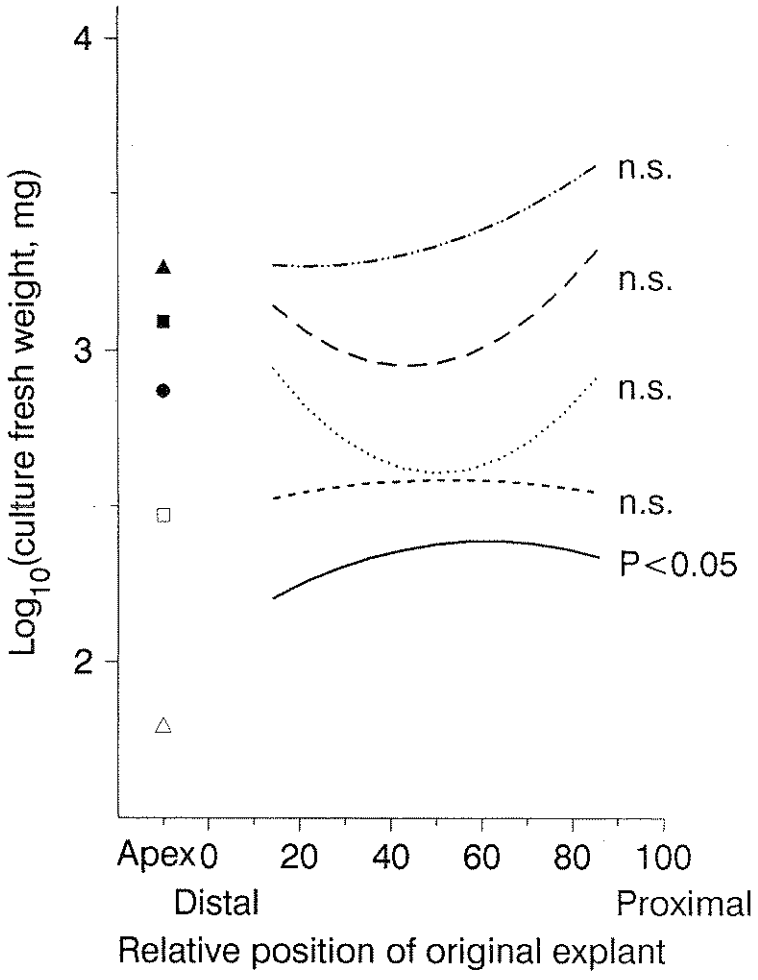

4
3
2
Log10(culture fresh weight, mg)
n.s.
n.s.
n.s.
n.s.
P<0.05
Apex
0
20
40
60
80
100
Distal
Proximal
Relative position of original explant

Figure 3.

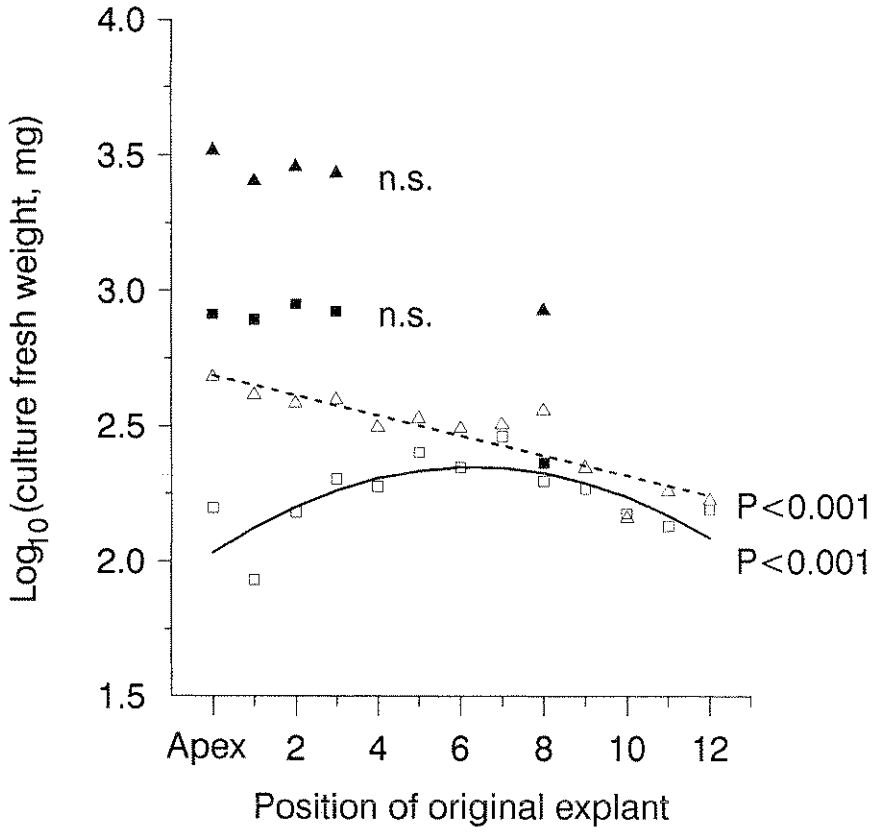

4.0
3.5
3.0
2.5
2.0
1.5
Log10(culture fresh weight, mg)
n.s.
n.s.
P<0.001
P<0.001
Apex
2
4
6
8
10
12
Position of original explant

Figure 4.

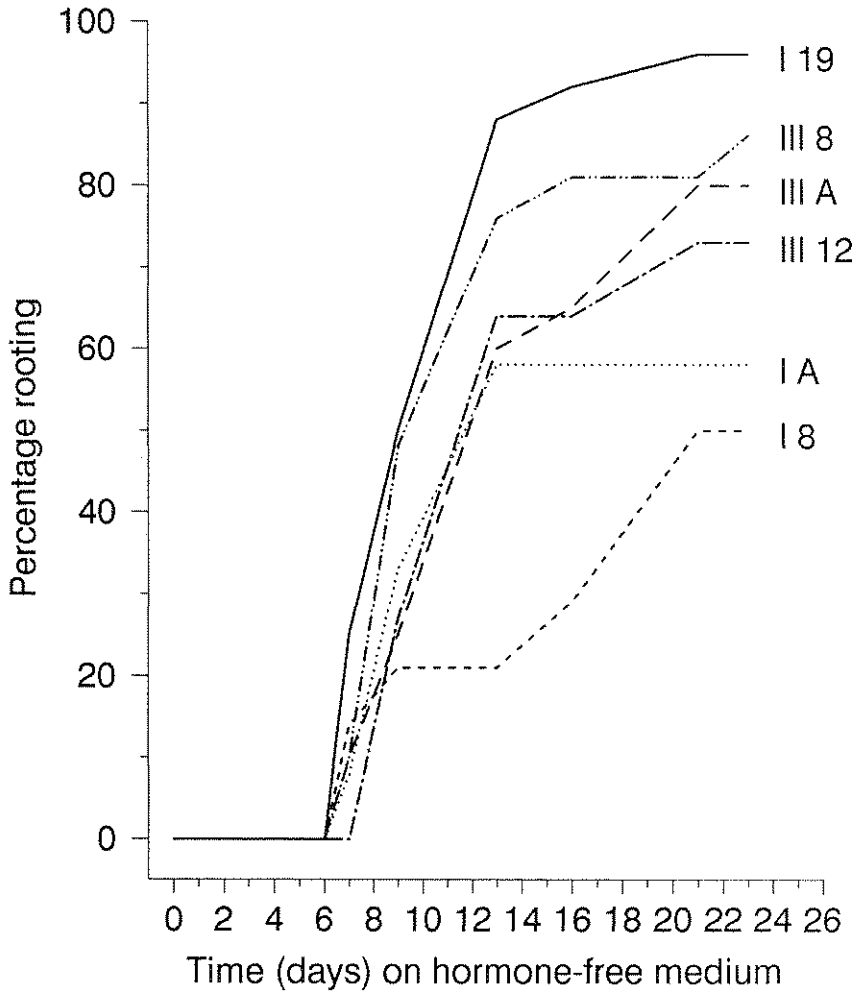
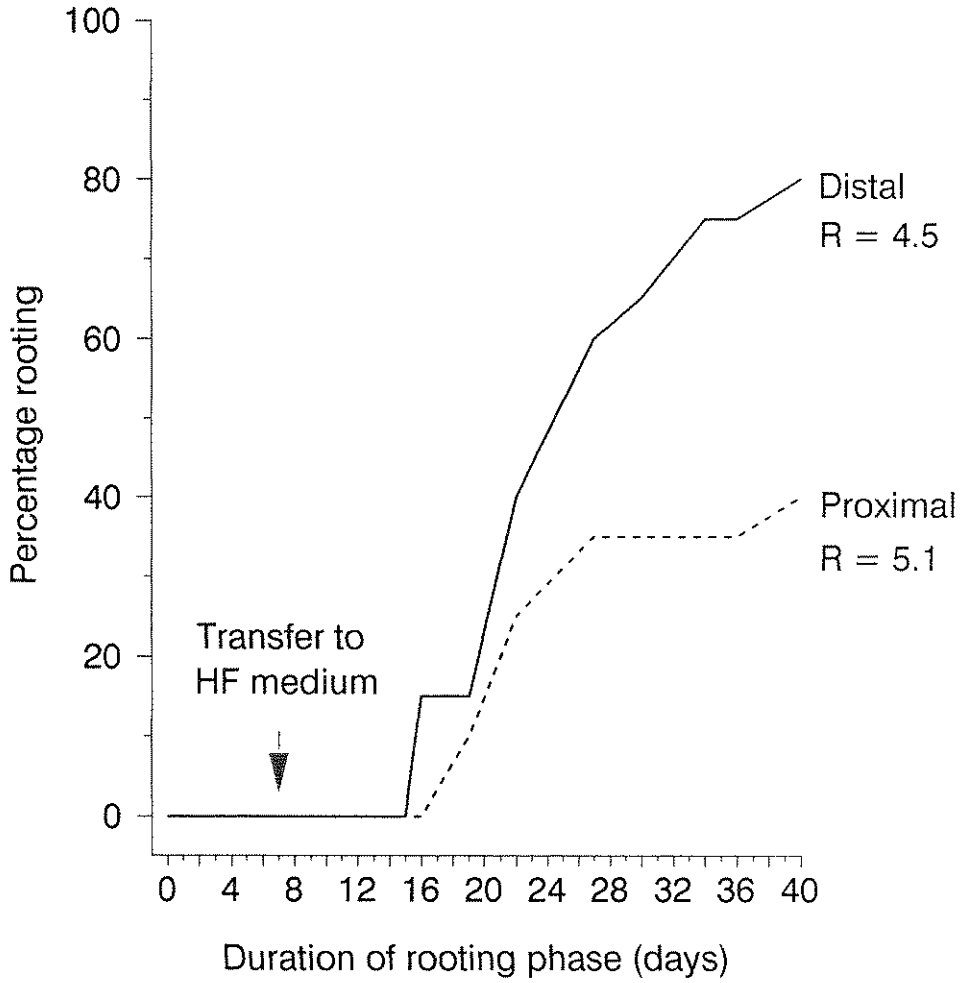

100
80
60
40
20
0
Percentage rooting
0 2 4 6 8 10 12 14 16 18 20 22 24 26
Time (days) on hormone-free medium
I 19
III 8
III A
III 12
I A
I 8

Figure 5.



Mean roots (R) per rooted cutting; s.e.d. = 1.37, 22 d.f.

Figure 6.