ANNUAL REPORT

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HDC Project HNS 138
Annual Report 2007

Manipulation of the root environment in HNS production

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September 2007

Commercial - In Confidence

Grower Summary

HNS 138

MANIPLUATION OF THE ROOT ENVIRONMENT IN HNS PRODUCTION

Annual Report 2007

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HDC project number:	HNS 138
Project leader:	Dr Debbie Fuller, Warwick HRI, Wellesbourne, Warwickshire, CV35 9EF
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Signed on behalf of: **Warwick HRI**

Signature: *Simon Bright*
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Name: Professor Simon Bright
(Director and Head of Department)

Date: 29 November 2007

The results and conclusions in this report are based on an investigation conducted over one year. The conditions under which the experiment was carried out and the results obtained have been reported with detail and accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results especially if they are used as the basis for commercial product recommendations.

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Headline

To date, when grown in a sand based system, pH alone has not influenced adventitious rooting of a number of HNS species.

Background and expected deliverables

It is well known by nurserymen that achieving complete success when rooting hardy nursery stock cuttings is extremely difficult. Harrison-Murray (2003) estimated a 25% failure rate for the 200 million cuttings that are rooted annually. Previous studies have examined how the aerial environment may be manipulated to minimise stress whilst roots develop. Work has also assessed how the balance of air and water within the growing medium influences rooting (Harrison-Murray, 2003). Whilst it is widely accepted that adventitious roots are initiated by plant hormones, chemical factors within the root environment such as pH and concentration of nutrients can also influence the rooting process (Anderson, 1986). Anecdotal evidence from the industry has also suggested that the influence of pH on rooting would warrant further investigation and this project set about to do this.

Summary of the project and main conclusions

In the first year of this project, methods were developed to examine the responses of unrooted cuttings to pH. This had to be achieved whilst minimising the complication of interaction with nutrition or changes in other parameters that can result in altering the pH of more conventional substrates used for rooting. Whilst root development was successful in initial pilot studies, rooting of four different heather species in the main experiments was slow with three of the four heather species dying back rather than rooting. This corresponded with extreme high summer temperatures and it was assumed that this stress was at least partly responsible for the die back observed. Species such as Rosemary, Fuchsia and Ceanothus rooted successfully and had slight pH preferences but responses appeared mild considering the wide range of pH levels examined (pH 4 to pH 8). Experiments were therefore modified in year 2 with changes designed to increase aeration within the rooting medium.

For experiments in year 2, commercially sourced cuttings were struck between October and January when environmental stresses could be expected to be minimal. They were struck in sand filled modules placed on top of sand filled troughs. This was designed to increase drainage. Sand was washed with pH adjusted water to pre-condition it to the desired pH treatment level. Each trough had an independent ebb and flood irrigation system and all troughs were covered with a polythene tent to maintain high relative humidity (RH). Eight species were tested as summarised in the following table:

Species / cultivar	**Date struck**	**Module size**
Erica carnea / Pink Spangles	25/10/07	9 ml
Erica vagans / Cornish Cream	25/10/07	9 ml
Erica tetralix / Riko	25/10/07	9 ml
Calluna vulgaris / H.E.Beale	25/10/07	9 ml
Camellia japonica / Adolphe Audusson	25/10/07	40 ml
Daphne odora / Aureomarginata	25/10/07	40 ml
Rhododendron / Sneezy	14/12/07	40 ml
Pieris / Purity	14/12/07	40 ml
Fuchsia / Dollar Princess	05/01/07	9 ml

Assessments were made on rooting progress at regular intervals according to the following qualitative scoring system:

Score	**Description**
0	Dead and/or decaying .
1	Sound cut surface, no decay.
2	Thickening of stem at cut surface or lenticels or development of a distinct callus.
3	Root initials - one or several up to 5mm in length.
4	Root development, extension, roots 5-10mm but not branching.
5	Good root development with branching, sufficient to support growth after potting on.

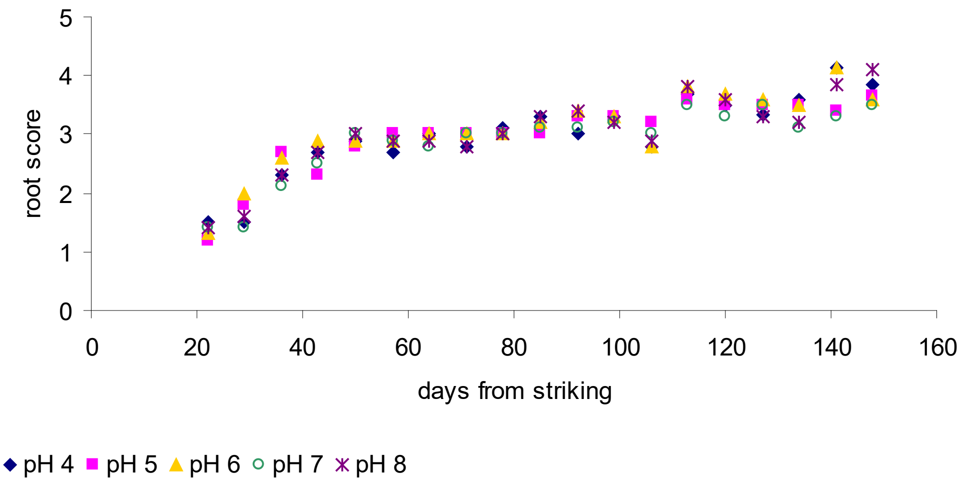
A final assessment was also made in all treatments and this was carried out on all cuttings once those in one replicate treatment reached the maximum score. This was designed to discriminate between treatments in terms of speed of rooting since poorer treatments would be expected to produce fewer roots in the same time frame as more favourable treatments.

In the year 2 experiments, rooting was more successful with cuttings surviving, and rooting occurring without the die back seen in year 1, as illustrated in the table below:

Species / cultivar	Time to first signs of rooting (days)*	Time to first treatment 'rooted' (days)
Erica carnea / Pink Spangles	22	78
Erica vagans / Cornish Cream	22	78
Erica tetralix / Riko	22	148
Calluna vulgaris / H.E.Beale	22	99
Camellia japonica / Adolphe Audusson	43	148
Daphne odora / Aureomarginata	43	124
Rhododendron / Sneezy	38	*
Pieris / Purity	31	101
Fuchsia / Dollar Princess	10	24

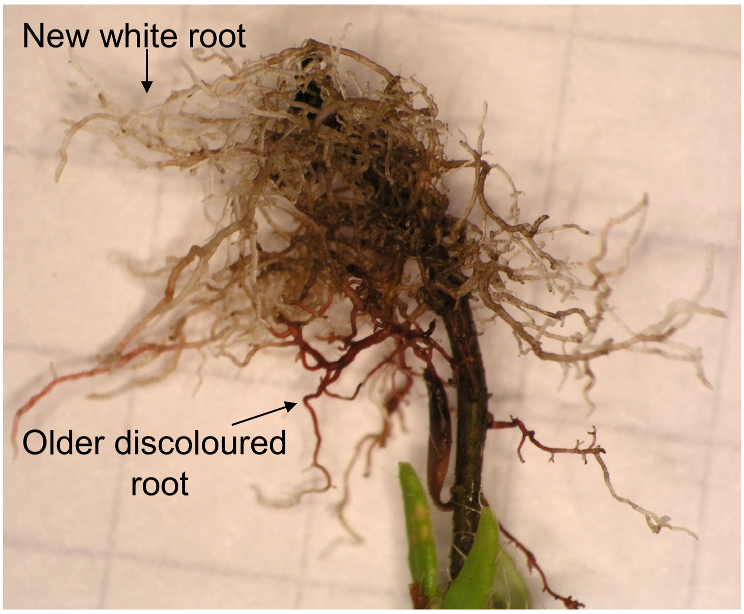
* when the first root initials and/or callus was seen (score 2/3).

There were however few significant differences due to pH despite the wide range of treatments assessed. Rooting was also slow for the heathers, and for *Erica* tetralix in particular. It was noted from the interim assessments that initial root development looked promising overall but after this initial progress, rooting appeared to stall for a period of time between the development of callus/ initial root development stage and subsequent root extension and branching growth. The length of this stalling in root development was most severe in *Erica tetralix* where root score remained static for around 8 weeks before subsequent development occurred as illustrated in the graph below.



The influence of pH on rooting score over time for Erica tetralix Riko.

It was observed in the interim inspections that the initial roots produced tended to darken in colour during the period that root development became suspended, with the new root growth contributing to progression to root scores of 4 and 5 clearly visible as a whiter colour. These two types of root are illustrated in the figure on the following page.



If rooting progress had not been delayed and stalled as described above, it could be assumed that the results of the year 2 experiments confirm those of the year 1 experiments, whereby pH within the range 4 to 8 has little effect on the early stages of adventitious rooting, for the species assessed in a sand based systems where interaction with nutrition is minimised. There are examples within the scientific literature that both agree and disagree with these findings depending on the methods used to alter pH, the species tested and whether the plants are grown *in vitro* or in more conventional media (perlite, vermiculite and peat based substrates).

Since rooting of some species was slow however, questions remain as to whether the factors responsible for the slow rooting may also have masked any response to pH. Reasons for the year 2 findings are discussed in more detail in the main report alongside an overview as to how year 3 experiments will be designed with these factors in mind.

Financial benefits

- To date, pH treatments have not influenced adventitious rooting and so can't yet be evaluated in financial terms.

Action points for growers

- No action points can be recommended to growers at this stage.

SCIENCE SECTION

INTRODUCTION

Two main issues are being addressed in this project. The first of these is the problems encountered with rooting HNS cuttings with an estimated 25% failure rate for the 200 million cuttings rooted annually (Harrison-Murray, 2003). Problems with plant uniformity are also closely associated with rooting performance of cuttings. Work has examined how factors of the aerial environment such as RH, temperature and light influence rooting (Anon, 1999). The effects of media type on the balance of air and water in the growing medium has also been investigated for HNS species (Harrison-Murray, 2003). Reports in the scientific literature suggest how the chemical environment and specifically pH may influence rooting of explants in vitro (e.g. Pierick et al; 1975, Rahman et al; 1992) with preferred levels determined by species. Anecdotal evidence from a commercial heather grower suggests that manipulation of pH in conventional growing systems may encourage rooting of heathers and may merit investigation in other HNS species.

The second issue addresses the position within the industry with regards to control over the root environment. HNS production in the UK relies heavily on the use of CRFs for nutrition which provides a manageable solution to the wide range of species and growth stages likely to be encountered on one nursery. However the benefits of greater control over the root environment have been clearly demonstrated in other sectors of the industry e.g. the significant increases in yield achieved by adopting hydroponics for tomato crops. Specialised HNS nurseries may similarly benefit from the increased control over the root environment that might be achieved using liquid feeding systems. Rooting cuttings is one production phase in which the benefits of this technology could be investigated.

The project was designed to examine responses to pH initially (as covered in this report) with later work focusing on either pH or nutrition depending on initial progress. In year 1 (HNS 138 annual report 2006), pilot studies were carried out to devise a suitable experimental system for testing responses of unrooted cuttings to pH whilst minimising interactions with nutrient availability. This system comprised of ebb and flood troughs supplied by a pH adjusted solution containing a low level (50 mg/l N) of nutrients designed to buffer solution pH. As cuttings had failed to root reliably in the absence of a supporting substrate, several inert media were tested to provide physical support for the cuttings whilst minimising interactions with pH. Sand was chosen as a medium in which the extremes of the wet and dry cycles of the ebb and flood system would be buffered and also in which cuttings were able to root. Sand also had the advantage that cuttings could also be readily removed and inspected with minimal root disturbance for accurate assessment and easy replacement when required. Cuttings were struck directly into the sand substrate. Experiments were carried out with four species of heather, Ceanothus, Fuchsia and Rosemary struck between April and May. Response to a wide pH range (pH 4 to 8) was either small or not significant from these species; however rooting was often slower than expected and cuttings died back in some species. Extreme high temperatures in 2006 may have contributed to the death of cuttings but changes in system design were agreed with the project steering group in order to progress with further work to investigate pH effects on rooting in 2006/07.

MATERIALS AND METHODS

System design and maintenance

Experiments were carried out using the ebb and flood system developed in year 1. Each replicate system consisted of an independent trough system irrigated via a pump on a timer twice a day with a 2 minute flood cycle each time (flooding the sand just to the surface or around 3 cm in depth). The system was modified to address problems encountered in the first main experiment described previously. A fresh sand supply was used with the aim of improving the air/water balance in the root zone. The change in particle size distribution is summarised in table 1. The new sand was composed of 20% less particles of the smallest size grade (<0.05mm) and an increase in proportion in the 0.5 – 1.0 grade and the larger size grades (3.2+).

Table 1. Particle size distribution of sand used in the first and second years of the project.

Size grade (mm)	% sand by weight	
	Year 1	Year 2
>7.0	0.0	0.1
6.0 - 7.0	0.0	1.5
5.0 - 6.0	0.0	3.4
4.0 - 5.0	0.5	6.4
3.15 - 4.0	2.9	3.9
2.0 - 3.15	11.2	7.1
1.0 - 2.0	9.1	8.5
0.5 - 1.0	5.4	18.4
<0.5	70.9	50.6

Experiments were carried out over the autumn/winter period of 2006/07 to try to minimise the risk of unfavourable environmental conditions. The main ebb and flood troughs were filled with sand as in year 1 with each trough having an independent tank supply, but cuttings were struck in sand filled modules placed on top of the ebb and flood systems in order to increase height above the surface of the trough and increase drainage and therefore aeration. The trough systems were covered with a sealed polythene tent to maintain high humidity. Glasshouse temperature was set to 7°C heating and venting was set to 18°C with forced ventilation (via fans) at 20°C. Shade screens were also set to close at external light intensities above 500 W/m². Cuttings were sourced from commercial suppliers who were also asked to send material prepared for striking and to minimise variability in the initial plant material. The method of imposing treatments was the same as year 1. That is, sand was pre-treated with a pH adjusted solution in order to pre-condition it to the relevant pH. After soaking with agitation for 48 hours, the drained sand was used to fill the ebb and flood trough. The pre-treated sand was also used to fill modules that were then stood on top of the sand in the troughs and these modules were used for striking the cuttings to increase drainage compared with that achieved in year 1. Dilute sulphuric acid was used to lower solution pH and sodium hydroxide was used to raise solution pH. A low level of nutrients was also added to the solution in order to try and minimise the drift of solution pH over time. Average concentrations of this dilute nutrient solution were 11mg/l N : 2 mg/l P : 26 mg/l K. Five levels of pH were tested from pH 4 to pH 8 in increments of 1 pH unit, with two replicate systems for each level of pH. Drift of solution pH required checking and adjustment of tanks three times a week, with tanks adjusted whenever the pH drifted more than 0.2 units away from the target level. Testing was carried out by stirring the reservoir and testing pH using a hand held meter with further stirring and testing where acid or alkali was needed to adjust pH. Tanks were routinely topped up with fresh solution every 4 weeks to compensate for evaporation.

Plant material

Species tested in these experiments are summarised in table 2 along with dates of striking and module size used.

Table 2. Summary of species tested in year 2 experiments.

Species / cultivar	Date struck	Module size
Erica carnea / Pink Spangles	25/10/07	9 ml
Erica vagans / Cornish Cream	25/10/07	9 ml
Erica tetralix / Riko	25/10/07	9 ml
Calluna vulgaris / H.E.Beale	25/10/07	9 ml
Camellia japonica / Adolphe Audusson	25/10/07	40 ml
Daphne odora / Aureomarginata	25/10/07	40 ml
Rhododendron / Sneezy	14/12/07	40 ml
Pieris / Purity	14/12/07	40 ml
Fuchsia / Dollar Princess	05/01/07	9 ml

Cuttings were divided into two groups in each pH treatment. One group were designated for regular inspection in order to keep track of rooting progress. This group consisted of rows of 5 cuttings with one of each of these rows to be removed and inspected each week before moving on to inspect the next row the following week. The number of rows of cuttings for interim inspection varied according to the anticipated time for rooting; hence for Fuchsia, 3 rows were used because this species was expected to root rapidly and therefore require few interim inspections. For heathers however, 5 rows of cuttings were used to allow for the longer rooting period and therefore higher number of interim inspections. This system was designed to minimise disturbance of the cuttings and initial aims were to only replace inspected cuttings if they were either unrooted or had only developed callus. In practice many species were slow to root and cuttings with developing roots were replaced and re-assessed at a later stage but with careful handling. The other group consisted of 10 cuttings which were not removed for interim inspections and were used to provide undisturbed material suitable for assessment at final sampling. Cuttings were struck directly into the sand in the modules with holes dibbed for the larger cutting types to minimise damage during striking. Rooting homone (Seradix) was used to treat *Daphne odora*, *Rhododenron* and *Pieris.* Cuttings of *Pieris* and *Rhododendron* were also wounded in preparation for striking using respectively either a small scrape or a larger slice.

Assessments

Cuttings were assessed for rooting progress at regular intervals according to speed of rooting. A qualitative score was assigned to each cutting according to the following scale during the interim assessments.

Score	Description
0	Dead and/or decaying .
1	Sound cut surface, no decay.
2	Thickening of stem at cut surface or lenticels or development of a distinct callus.
3	Root initials, one or several up to 5mm in length.
4	Root development, extension, roots 5-10mm but not branching.
5	Good root development with branching, sufficient to support growth after potting on.

Examples of these scores are given in Appendix 1.

Final rooting assessments were carried out for each species independently. The timing of final assessments was determined by the progress recorded during interim assessments. Hence when all cuttings removed from a replicate treatment reached a score of 5 the final assessments were carried out. Cuttings were assigned a score for rooting as described above.

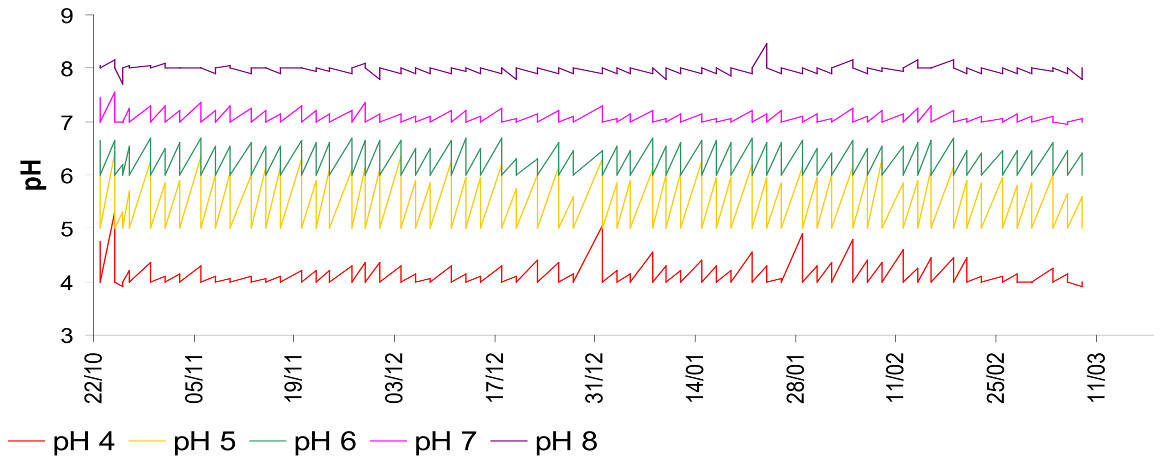
Data collected were analysed using analysis of variance on each interim and final assessment date. The effects of disturbing cuttings was also assessed by comparing scores of cuttings that had been used for interim inspection with cuttings that remained in situ throughout the experiment.

RESULTS

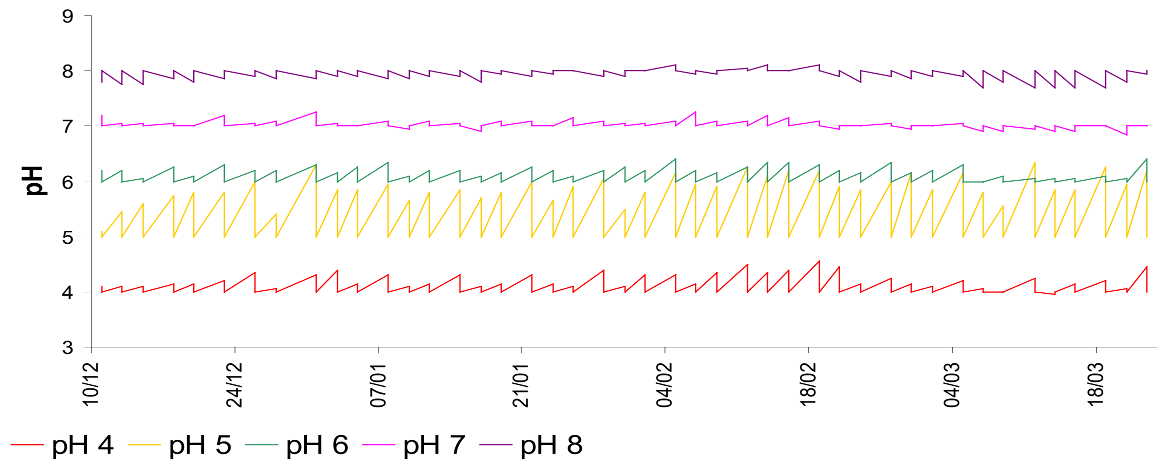
Maintenance of treatment pH

pH of the solution in treatment tanks drifted over time and therefore required frequent adjustment to maintain desired treatments. The amount of drift between subsequent tank adjustments varied with the pH level set (figure 1). In the first experiment struck on 25th October, solutions with a target pH of 5 had the greatest amount of drift which was on average 1.2 units. Solutions with targets of pH 4 and 6 drifted by an average of 0.5 units whilst drift was minimal for pHs 7 and 8 at an average of 0.1 to 0.3 units. Similar patterns of drift were noted for the two later experiments struck on 14th December and 5th January with the pH 5 treatment drifting the most and pHs 7 and 8 drifting the least although the amount of drift was smaller for these two later experiments.

a) pH monitoring for experiment struck 25/10/06



b) pH monitoring for experiment struck 14/12/06



c) pH monitoring for experiment struck 05/01/07

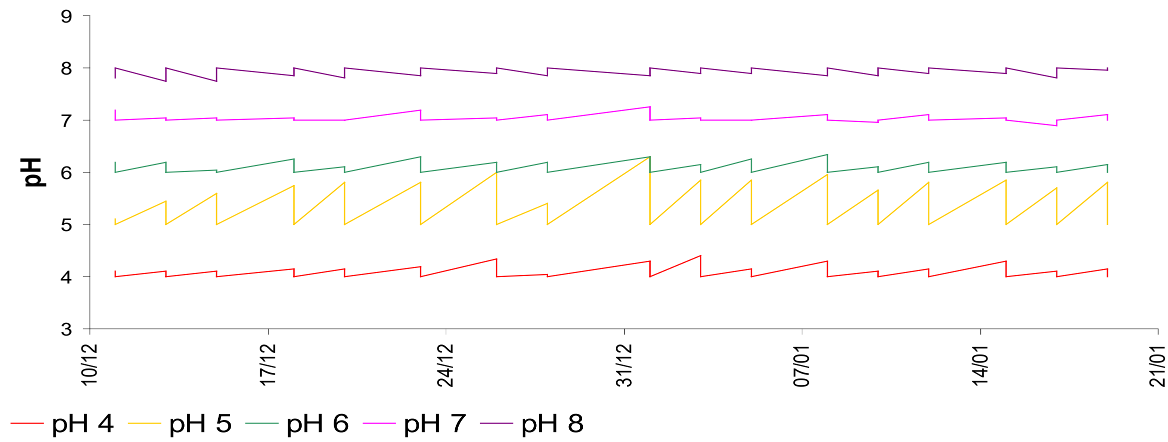


Figure 1. *Solution pH for the five pH treatments for experiments struck on a) 25th October, b) 14th December and c) 5th January.*

Rooting assessments

Seven species were tested in year 2 experiments and results are summarised in the following by species. Data was analysed by analysis of variance. Statistical terms used are defined below.

NS	Not significant
l.s.d.	Least significant difference (differences between treatments must exceed this value to be considered significant at the given probability level)
p<0.05	The probability of this result occurring by chance is equal to or less than 1 in 20 (0.05 = 5%)
p<0.01	The probability of this result occurring by chance is equal to or less than 1 in 100 (0.01 = 1%)
p<0.001	The probability of this result occurring by chance is equal to or less than 1 in 1000 (0.001 = 0.1%)

Erica carnea (Pink Spangles)

Initial signs of rooting (i.e. score 2 or more) were first observed 22 days from striking cuttings and cuttings from at least one trough were considered to be rooted 78 days from striking (11 weeks). There were no significant main pH effects over this period (figure 2). The apparent dip in rooting at 50 days from striking for the pH 4, 5 and 8 treatments reflects the use of a different row of plants at each assessment and hence the cutting to cutting variability in root development.

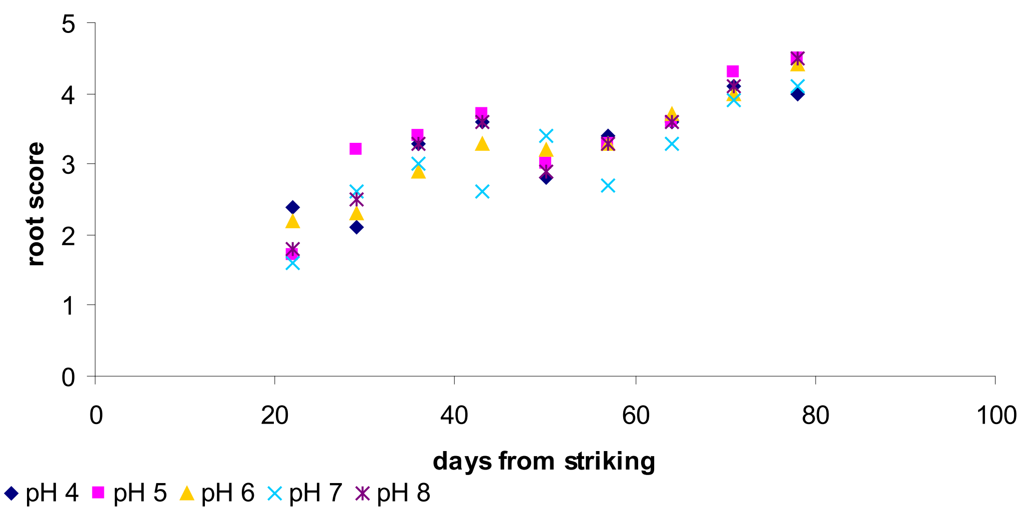


Figure 2. *The influence of pH on rooting score over time for Erica carnea Pink Spangles.*

As with the interim rooting scores, there were no significant differences in rooting score for the final assessment on undisturbed plants due to pH (figure 3). Root scores assessed on the plants removed from the substrate and replaced after interim assessment were compared with those from plants left undisturbed until the final assessment after 78 days and no significant differences were found in the response of these two sets to pH.

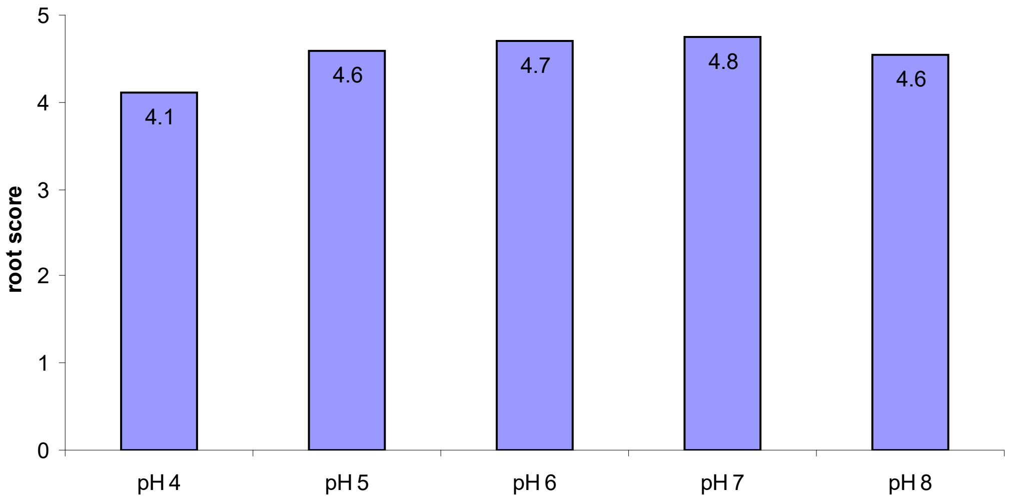


Figure 3. *The influence of pH on final rooting score for Erica carnea Pink Spangles (78 days from striking).*

Rooting was more successful in the current experiment with *E. carnea* than in the experiment in year 1 which was struck in May where many cuttings failed to root and eventually died off. Rooting period was slow at 11 weeks compared with the expected rooting period on commercial nurseries.

Erica vagans Cornish Cream

As with *E. carnea*, the first signs of rooting were recorded 22 days from striking and cuttings were considered sufficiently rooted for final sampling 78 days from striking (figure 4). There were no significant differences due to pH between any of the samples taken. Rooting progress appeared to plateau for 3 weeks when cuttings had reached a score of around 2.5 and 3.2 (indicating early root initials only) before continuing to develop to score 4 and 5.

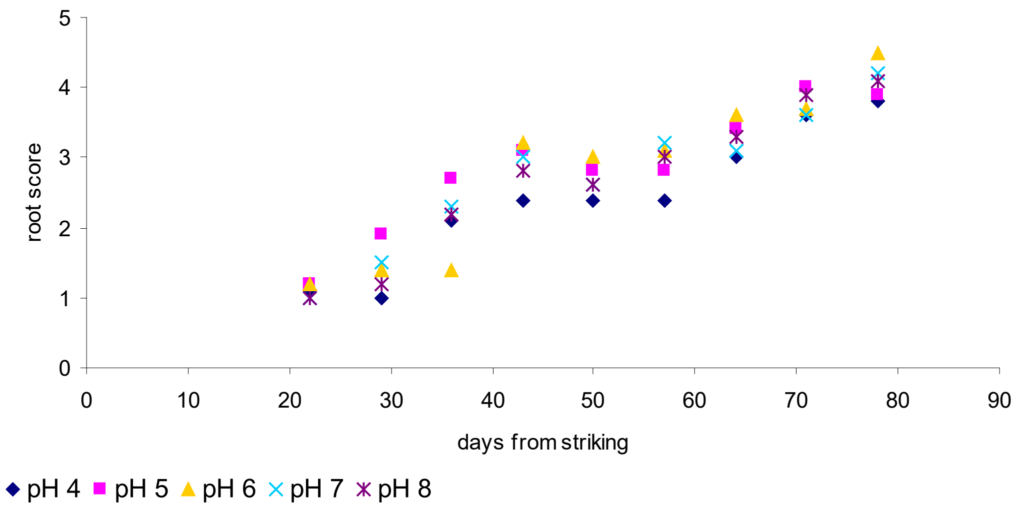


Figure 4. *The influence of pH on rooting score over time for Erica vagans Cornish Cream.*

Average score for the undisturbed cuttings of *E. vagans* at between 3.1 and 4.3 was lower than for *E. carnea* suggesting slower root development in a sand based ebb and flood system at least (figure 5). Final root score was not significantly influenced by pH however and there were also no significant differences between the cuttings that were removed and replaced and those that remained undisturbed throughout the experiment.

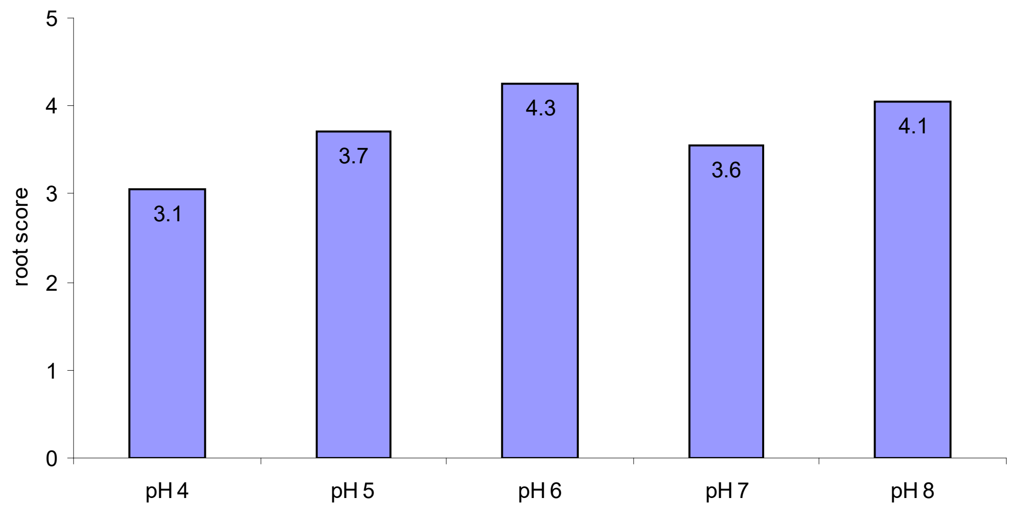


Figure 5. *The influence of pH on final rooting score for Erica vagans Cornish Cream (78 days from striking).*

Erica vagans also rooted better in the year 2 experiments than in year 1 (struck in May) where cuttings failed to root and eventually died off.

<u>*Erica tetralix* Riko</u>

As for the previous two species of *Erica* presented above, *Erica tetralix* showed the first signs of rooting around 22 days from striking (figure 6). Progression to score 3 was also in line with that previously seen but there was then an extended plateau in root score spanning 8 weeks before progress towards score 5 began and the experiment had to be finished 148 days after striking. There were no significant pH effects between any of the weekly assessments made.

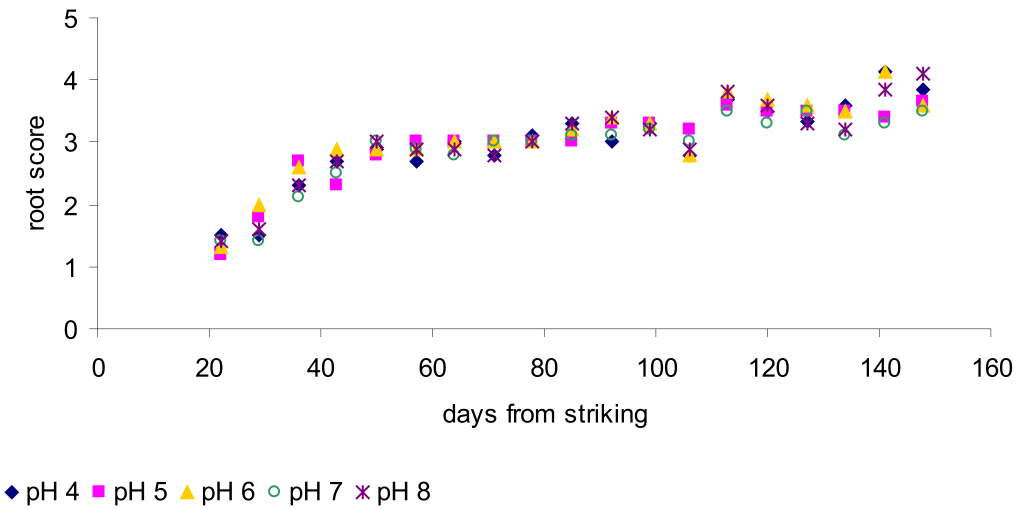


Figure 6. *The influence of pH on rooting score over time for Erica tetralix Riko.*

There were again no significant differences between pH treatments at final assessments between cuttings that had remained undisturbed throughout the experiment (figure 7). Final scores were comparable with those for *Erica carnea* and higher than those for *Erica vagans.*

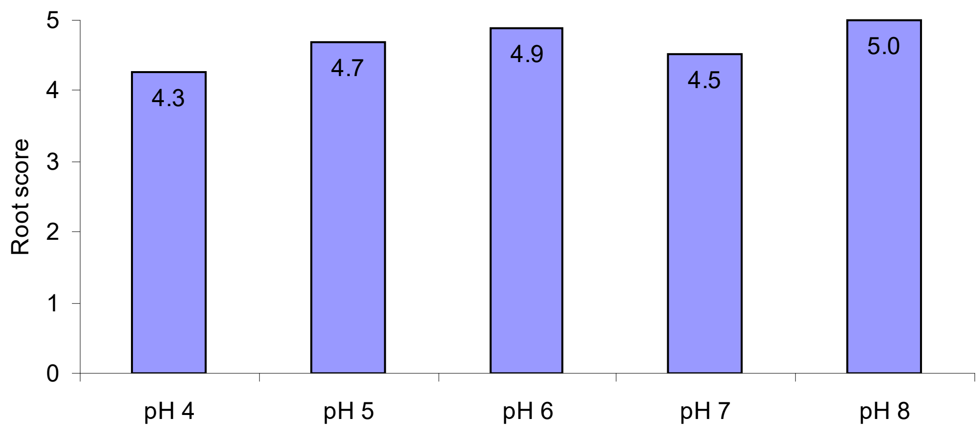


Figure 7. *The influence of pH on final rooting score for Erica tetralix Riko (148 days from striking).*

Whilst *Erica tetralix* achieved high root scores by the end of the experiment, the length of time taken to achieve these scores at 148 days is much longer than would be expected. In this case, rooting was therefore less successful than the experiment in year 1 which was assessed 42 days after striking. It was observed in the interim inspections that the initial roots produced tended to darken in colour during the period that root development became suspended, with the new root growth contributing to progression to root scores of 4 and 5 clearly visible as a whiter colour. These two types of root are illustrated in the figure below.

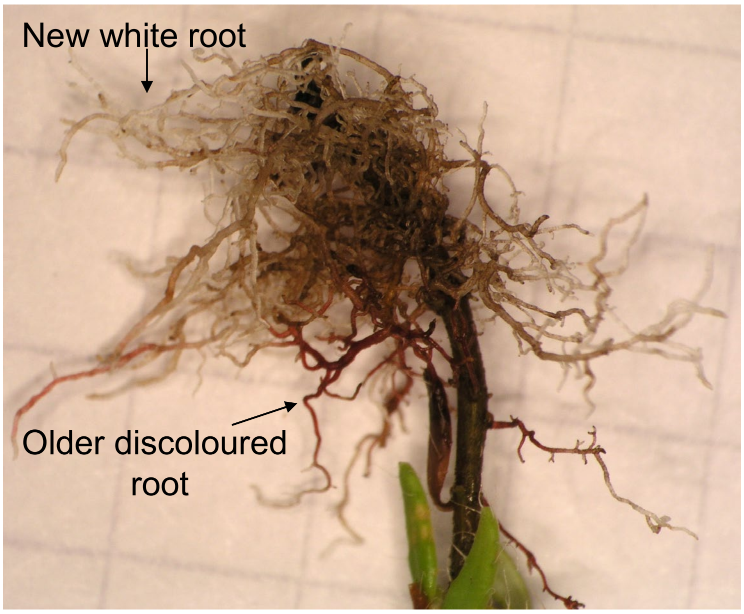


Figure 8. *Root system of Erica tetralix 'Riko' at final assessment showing older discoloured roots and newer white roots..*

<u>*Calluna vulgaris* H.E.Beale</u>

The first rooting progress was recorded 22 days from striking and cuttings in at least one replicate were considered to be rooted after 99 days from striking (figure 9). As noted with *Erica tetralix* and to a lesser extent *Erica vagans*, there was a plateau in the progression in root score at around score 2.5 to 3 which lasted around 5 weeks before rooting progress was re-established. pH treatments 4 and 8 had significantly ($P<0.05$) lower root scores to pHs 5 and 7 from the first assessment 22 days from striking (l.s.d. = 0.62). This result appears inconsistent as no further differences were found in the data in any of the later assessments.

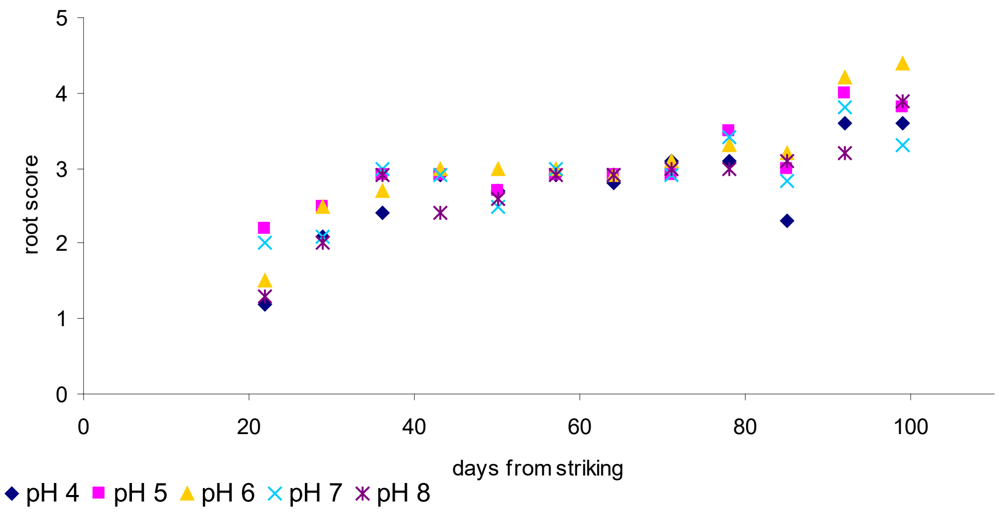


Figure 9. *The influence of pH on rooting score over time for Calluna vulgaris H.E.Beale.*

Overall final rooting score was low for *Calluna* vulgaris and although all cuttings used for interim assessments in one of the troughs had reached a score 5 when final samples were taken, the highest average score for undisturbed cuttings was 4.0 (figure 10). There was no significant effect of pH on final rooting score.

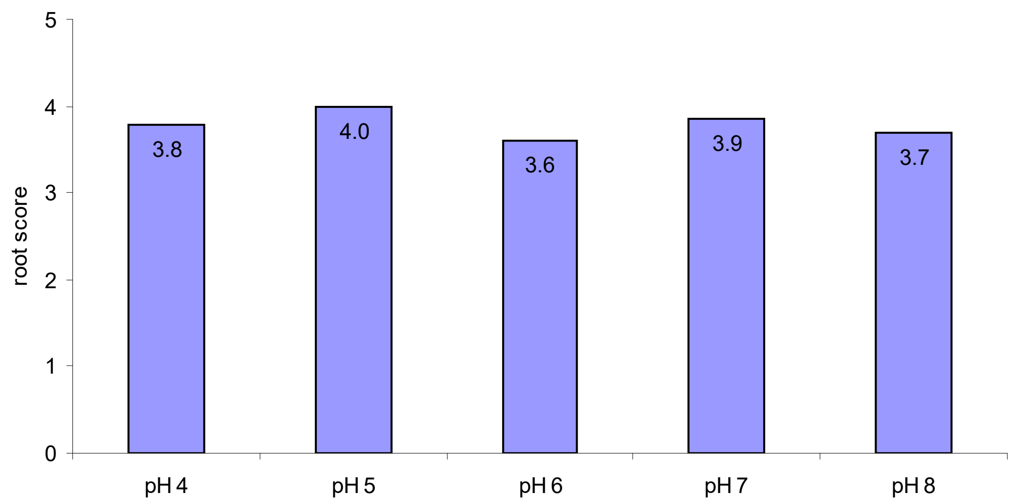


Figure 10. *The influence of pH on final rooting score for Calluna vulgaris H.E.Beale (99 days from striking).*

As observed with the *Erica sp* previously, rooting of *Calluna vulgaris* was slow overall compared to commercial expectations. Rooting in the experiment reported here which was struck in October did not suffer the high levels of root death experienced with the experiment struck in May as reported in the Year 1 report.

Camellia japonica Adolphe Audusson

The first signs of root development took much longer to appear for Camellia compared with the heathers presented previously. The first signs of callus development were noted 43 days from striking and subsequent increases in score over the next 42 days were related to an increase in the number of cuttings showing callus rather than progression to the appearance of initials (figure 11). After 92 days from striking, the first signs of root initials were noted. There was a significant ($P<0.05$) effect of pH on root development when roots were scored 92 days from striking with pH 8 giving a higher root scores than the remaining levels of pH (l.s.d. = 0.17) but there were no significant pH effects for the remaining interim assessments, although pH 8 appears to have given consistently higher scores over the last 4 weeks of assessments. The variability in rooting score from cutting to cutting is highlighted in the change in scores over time and the apparent decline in score from time to time. There were five rows of cuttings dedicated to the interim sampling procedure and so it was 5 weeks before the first row of cuttings were disturbed and re-assessed. This coincides with the dip in average rooting score noted at 78 and 113 days from striking. Final assessment (when one of the replicates scored 100% 5s) was taken at 148 days from striking.

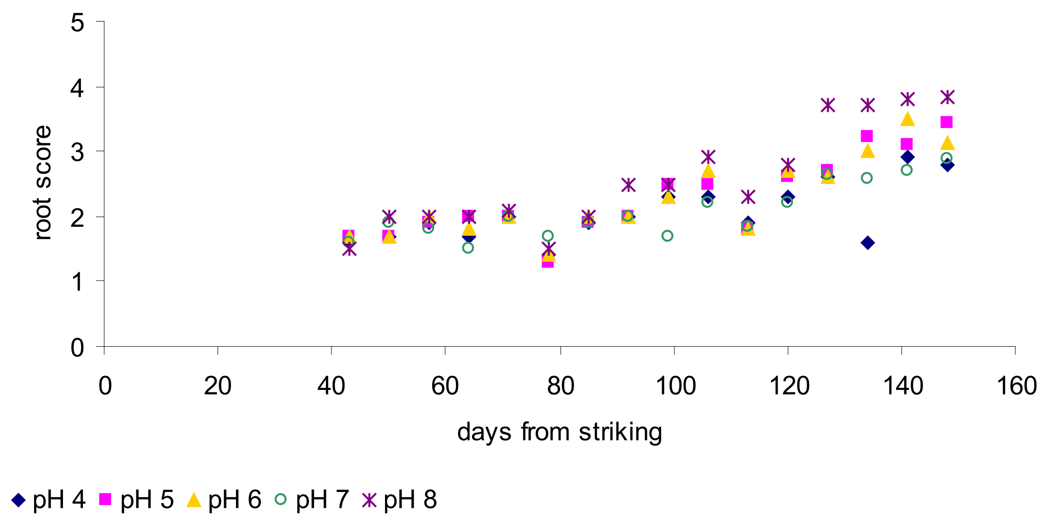


Figure 11. *The influence of pH on rooting score over time for Camellia japonica* Adolphe Audusson.

Average scores at final assessment were low at 2.8 to 3.8 across all treatments, despite at least one of the replicate systems producing cuttings with sufficient root to be scored as a 5 in the interim assessments (figure 12). Cuttings from the pH 8 treatment had a higher average root score than from the other pH levels tested which coincides with the observation made above for the interim assessment scores; however no significant differences were found relating to pH level. There were also no significant differences in the comparison of final scores from disturbed and undisturbed plants. Hence whilst the dip in scores was noted above at five week intervals for the interim assessment data, there is no suggestion that this was a result of disturbing the plants for inspection.

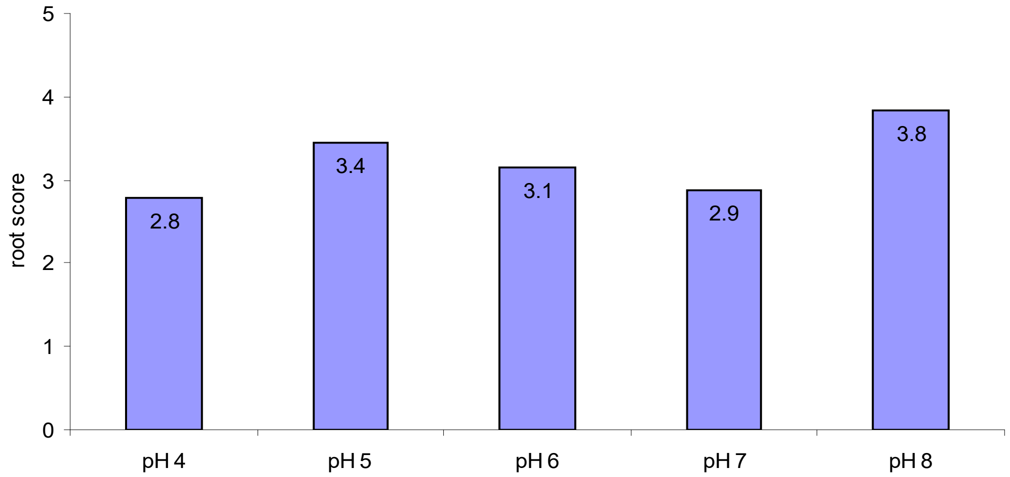


Figure 12. *The influence of pH on final rooting score for Camellia japonica* Adolphe Audusson (148 days from striking).

Daphne odora Aureomarginata

The first signs of callus were recorded 43 days from striking cuttings and rooting score gradually progressed until the final score taken 124 days from striking when all the cuttings in one set scored 5, although clearly when both replicates of treatments were averaged, root score was below 5 at the final of the interim assessments (figure 13). There were no significant effects of pH on root score over time.

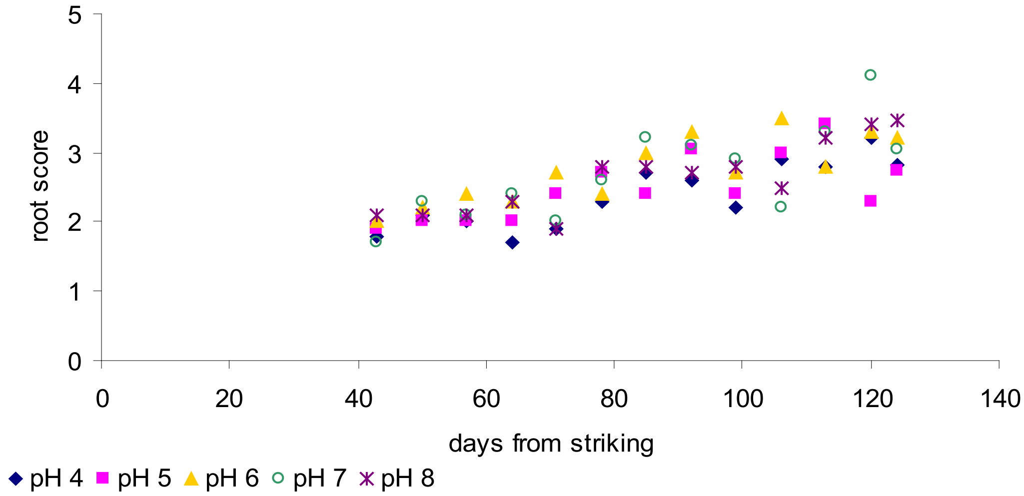


Figure 13. *The influence of pH on rooting score over time for Daphne odora Aureomarginata.*

Average rooting score at final assessment was low overall, peaking at 3.6 (between root initials first emerging and root extension to 5 to 10mm but with little branching), despite timing the final assessment to coincide with at least one complete row of cuttings achieving a score 5 (figure 14). It was noted however that once roots started to grow away from the callus stage, progression to score 5 was rapid, hence at final score a high proportion of cuttings (40-60%) from the interim set, were at score 2 with 20-40% at score 5 and fewer at the scores between these two stages. It is assumed that this rapid change is not apparent from the interim scores data shown above because cuttings were assessed when the first row achieved score 5 rather than waiting for all treatments to reach this stage. Average rooting score varied between treatments but no significant differences were found in relation to pH level.

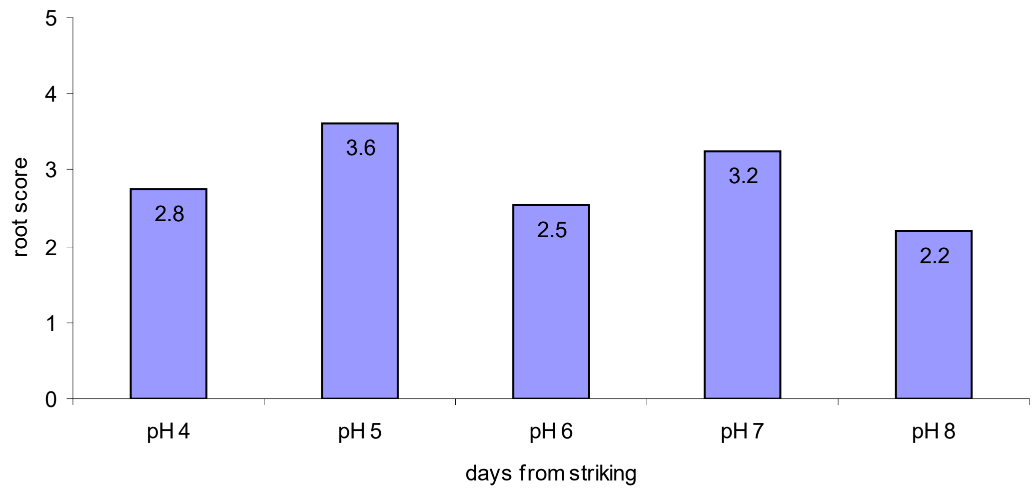


Figure 14. *The influence of pH on final rooting score for Daphne odora Aureomarginata (124 days from striking).*

Rhododendron Sneezy

Callus development on the *Rhododendron* cuttings was recorded from 38 days from striking cuttings across all pH treatments (figure 15). Subsequent progression to root initials however did not occur within 102 days from striking when the experiment had to finish.

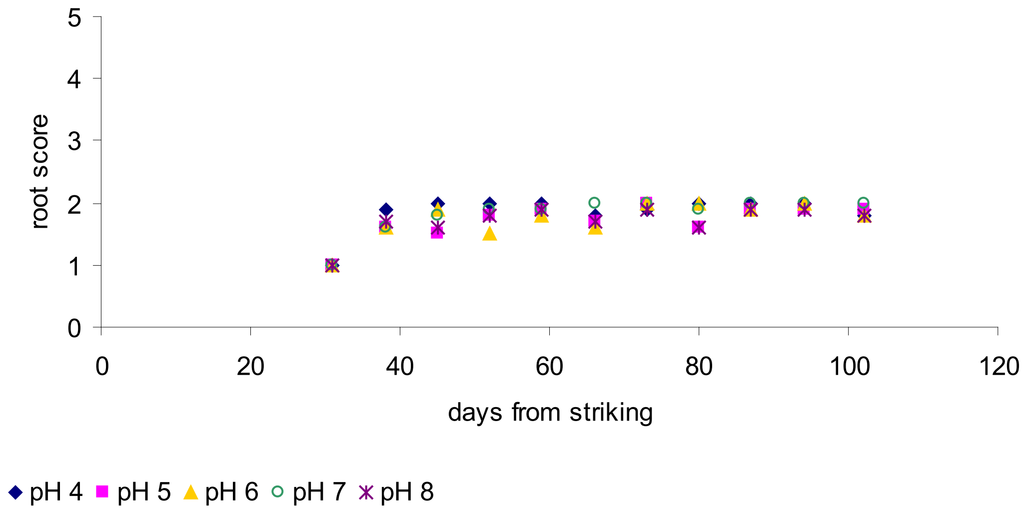


Figure 15. *The influence of pH on rooting score over time for Rhododendron* Sneezy.

Pieris Purity

Rooting of *Pieris* commenced 31 days from striking and final assessments were made 101 days from striking (figure 16). There was a steady increase in average root score until 59 days after which root development appeared to plateau until 87 days from striking when scores began to increase again. pH had no significant effect on weekly root scores.

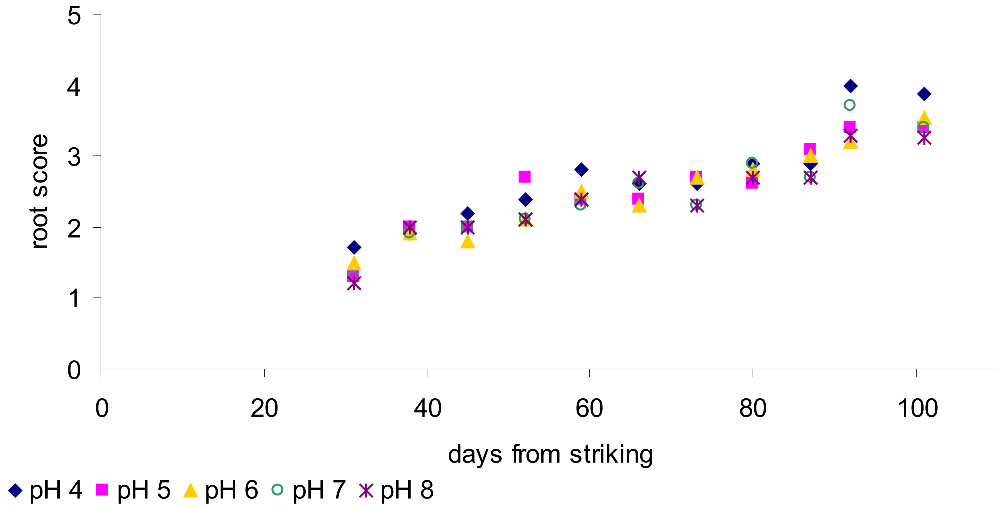


Figure 16. *The influence of pH on rooting score over time for Pieris* Purity.

Average root score at final assessment varied between 3.1 (root initials starting to develop) to 3.7 (root initial extending but not much branching) across treatments with no significant differences relating to pH (figure 17).

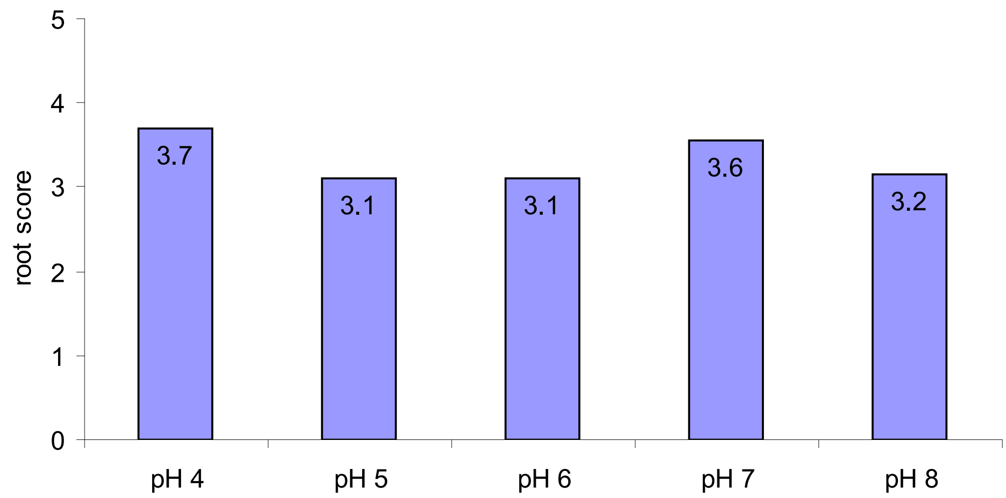


Figure 17. *The influence of pH on final rooting score for Pieris Purity (101 days from striking).*

<u>*Fuchsia* Dollar Princess</u>

Fuchsia rooted fastest out of the species tested with initial development noted by 10 days from striking and with cuttings rooted by 24 days from striking (figure 18). The was no significant influence of pH on interim rooting scores.

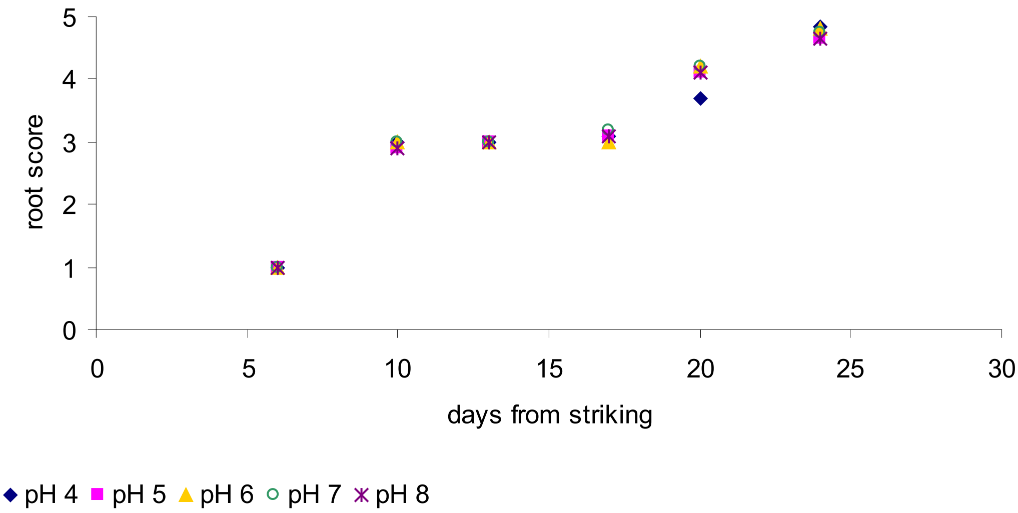


Figure 18. *The influence of pH on rooting score over time for Fuchsia Dollar Princess.*

Cuttings from all treatments were well rooted (score 4.7 to 4.9) when final assessments were carried out 24 days from striking with no significant differences between pH treatments (figure 19).

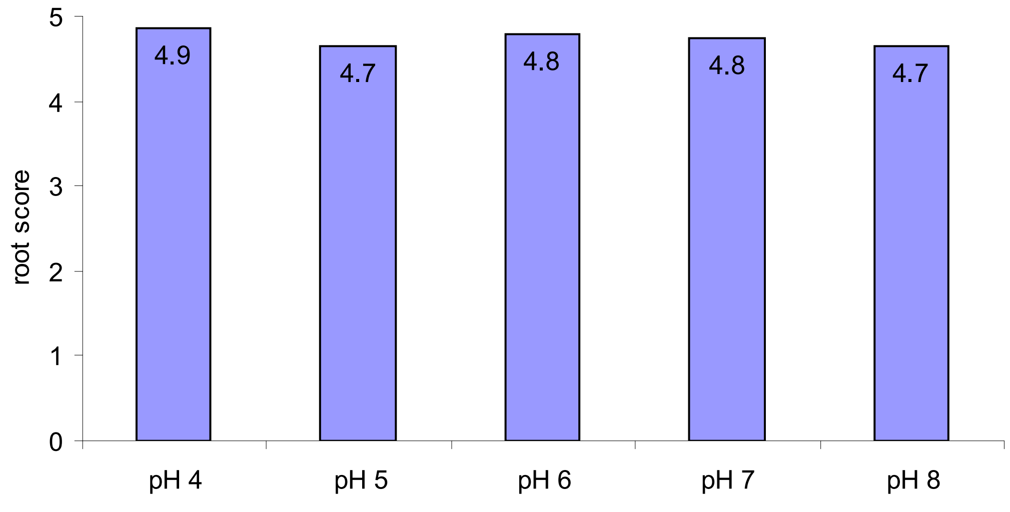


Figure 19. *The influence of pH on final rooting score for Fuchsia Dollar Princess (24 days from striking).*

Data from the current, year 2 experiments for *Fuchsia* Dollar Princess are in agreement with year 1 data where *Fuchsia* Beacon Rosa was the fastest to root out of the species tested;

however rooting in year 2 (with striking in January) was slower than in year 1 (struck in May). There were also significantly lower final rooting scores associated with the higher pH treatments in year 1 but no significant differences found in year 2.

DISCUSSION

The high rate of cutting death experienced with heathers in the year 1 experiments was not repeated in year 2 experiments and so it is assumed that the system / environmental conditions benefited from the changes in system outlined previously. However there remain concern over the speed of rooting in general. For example it was expected from commercial experience that heathers would root in around 6 weeks (42 days) when in fact the shortest rooting period was 78 days for *Erica* carnea Pink Spangles and *Erica vagans* Cornish Cream and this increased to 148 days for *Erica tetralix* Riko.

There was consistently no significant response to pH in the tests carried out in year 2 which is unexpected given the wide range of pH treatments assessed (i.e. pH 4 to pH 8) and reasons for this lack of response have been sought through literature searching and discussion with others.

It is clear from the literature that rooting should be considered in at least two separate phases i.e. initiation and subsequent development (Anderson, 1986; Davies, 1988; Snyder, 1974; Tukey, 1979). Furthermore the expected responses from commercial growers may be based on the more established response of potted up plants in different pH environments which is related to the growth of whole plants rather than the very early stages of root initiation as has been examined in this project so far.

It is well established that root initiation is the result of hormonal triggers (IAA). Chemical factors in the root environment are also reported to have an influence over root development (Anderson, 1998; Snyder, 1974; Tukey, 1979). Indeed a direct link between IAA movement and pH within the cutting has been suggested (Liu et al; 1993) and also that IAA movement across cell membranes may be assisted in low pH environments where the undissociated form of the molecule predominates. Work *in* vitro with apple micro cuttings suggests that the pH effect on IAA movement is due to uptake rather than metabolism for the initiation of adventitious roots (Harbage et al; 1998). Other chemical factors reported to influence initiation include B (McGuire, 1980; Davies, 1988) and Zn, (Davies 1988), but there are conflicting reports as to whether these factors stimulate or inhibit rooting which may in part be related to concentration. Ca, N, P, K are also thought to be important for adventitious rooting (Anderson 1986), although probably only in 'low' quantities. Turning to pH specifically, there are reports of work on how pH influences rooting both *in vitro* (Leifert, 1992; Rahman et al; 1992; Williams et al; 1985) and *in vivo* (Hitchcock, 1928; Economou, 1968; Paul & Leiser, 1968; Kelly, 1976; Willumsen, 1986; Andrews & Hammer, 2006). There are however difficulties in interpreting the work because of the methods used to adjust pH. Early work by Hitchcock (1928) for example looked at different proportions of sand and peat in rooting media to give pH treatments from 4.5 to 7.0. 96 varieties from 43 genera were characterised into pH response groups according to these experiments, and majority (61%) of species tested showed no preference to pH for rooting. The limitation here of course is that the physical properties of the substrates varied as well as the pH level. There are other examples of experiments where no pH preference was found, e.g. Paul and Leiser (1968) altered pH in sphagnum peat by different amounts of Ca saturation which altered both Ca concentration and pH. Species varied in responses but the ericaceous Rhododendron rooted well over the whole range of Ca concentrations/pH treatments; despite the assumption that the *Ericaceae* are calciphobes.

An interesting study with sunflower seedlings demonstrated that low pH increased the number of root initials developed, but that the response to pH diminished with time and in fact pH triggered these differences within 5 hours of exposing the newly cut surface of the seedling to pH treatments (Lui et al; 1993). If response to pH in woody HNS species was similarly short lived there would be little practical application for pH treatments at the propagation stage, although they may be potential for treatments applied as cuttings are removed from stock plants.

The experiments reported here were designed to take a step back from conventional peat based substrates because changes in pH in these media also alter the availability of nutrients, in particular phosphorus, iron manganese and boron (Bunt, 1988). Hence a more inert medium was used. A similar approach to this was adopted by Holt et al; (1999) who looked at rooting of rhododendron in perlite using a sub irrigation system. The work was limited to two pH levels (pH 4.5 and pH 7.5) which were achieved by either using untreated perlite (pH 7.5) and irrigating with water at pH 7.5 or acid washed perlite (pH 4.5) irrigated with water adjusted to pH 4.5 with sulphuric acid. This experiment is very close to the current project in design and suggested that the Rhododendron preferred the lower pH treatment. This conflicts with the results of Paul and Leiser (1968) above where

Rhododendron rooting was not influenced by pH. It is not clear which species of Rhododendron was used in each case, but it does underline the importance of experimental design to the results achieved.

Although considerable care was taken to ensure pH in the rhizosphere matched the pH treatments desired, it is clear from the literature that mineral exchange at the root surface can change rhizosphere pH by up to 1 unit (Kinraide, 1997; Blossfeld, 2007). The cation exchange capacity in sand may be inadequate for buffering these localised differences whereas a peat based medium adjusted to the desired pH with lime may have greater buffering capacity and hence ability to influence rhizosphere pH. Given the range of pH treatments used in previous experiments, localised pH changes of up to 1 unit would still have given a wide range of pH treatments in which to initiate responses. Lui et al; (1993) used pH adjusted water as well as a range of pH buffers in their studies with sunflower, and whilst they suggest that buffers are more suitable due to the lack of drift in pH over time, they presented results illustrating similar responses to pH from both pH adjusted water and buffer based treatments.

Another issue to address is the apparent plateau reached in root development for a number of the species tested in year 2. The slowness in rooting experienced in year 2 may have been a result of this apparent stalling in root development once callus or early root initials had developed. Although propagation is associated with low nutrient requirement it is suggested by Anderson (1986) that there is a real nutrient requirement during propagation. One of the reasons for this very slow root development may therefore have been lack of nutrient. Over time, the ebb and flood system would replenish nutrient supply in the sand substrate but the low levels of nutrient added may have made this replenishment too slow for the demands of the cuttings. Air/water balance is of course also important in the rooting medium and perlite, for example, is commonly added to peat propagation mixes for the aeration rooting media. The sand substrate was replaced for the year 2 experiments given that air/water balance was a concern in the year 1 experiments and sharp sand sold for use in propagation was used in the year 2 experiments. AFP measured by the rapid method was around 3.5% in year and 5.0% in year 2 which falls short of the desired AFP of 15-20% for rooting media. It should be noted however that the AFP method is difficult to manage accurately with sand due to the very high initial rate of drainage which makes capture of all the drained water difficult when containers are transferred from the water bath to the funnel for collection of drainage water.

The original plan for the third year of work under this project was to follow through the most promising pH treatments in conventional media and to consider nutritional factors in the rooting process. There is of course no clear direction at present from year 1 and 2 experiments to direct year 3 experiments. It is proposed therefore that a multifactorial experiment is carried out at the start of year 3. The aim of this work will be to address why rooting was apparently slow in previous experiments whilst moving the project forward in terms of understanding how pH influences adventitious rooting. The experiment will examine how the air/water balance of the rooting medium might influence rooting by comparing sand, perlite and a 50/50 peat/perlite mix. This would give inert media with contrasting aeration properties as well as a media that growers are familiar with. These media would be tested with a low, medium and high pH (i.e. pH 4, pH 6 and pH 8) which will cover the original range tested and help to test if pH has more of an effect under different physical conditions in the rooting medium. Feed will be introduced as a factor with the 3 pH levels combined with either the low feed from year 2 or a higher feed equivalent to a standard PG mix at the rate recommended for propagation. Rooting stage will also be considered by either striking cuttings directly into treatments or by pre-rooting cuttings to the early root initials stage in a conventional peat/perlite rooting medium before transplanting into the treatments already described. Irrigation will be applied from the substrate surface via trickle tape rather than as an ebb and flood system which will run to waste, this will increase the frequency of replenishing the pH adjusted solutions to minimise pH drift in solution and will also ensure that the root zone if regularly percolated with the desired treatment pH. Finally, as pH influences nutrient availability, some observation treatments on cuttings in peat/perlite mixes with micronutrient feeds will be carried out. Species tested will be confined to two species of heather given the high number of treatments. More focussed treatments will then be examined in follow up experiments in the spring of year 3.

CONCLUSIONS

Root initiation and early development of commercially prepared cuttings of 8 different species did not respond to differences in pH treatments ranging from pH 4 up to pH 8 when rooted in a sand based closed system.

The lack of response to such a wide pH range appears surprising although there are other examples of little response to similar pH ranges in the literature, with workers giving conflicting views on the pH response of the same species (although not necessarily the same cultivar).

Components of the experimental design that may have resulted in the slow rooting experienced with some of the species tested have been reviewed and year 3 experiments designed to take these factors into account.

REFERENCES

Anderson, A.S. (1986). Environmental influences on adventitious rooting in cuttings of non-woody species. In: New root formation in plants and cuttings. (Developments in plant and soil sciences; 20), pp 223-253.

Anon. 1999. Strategies for liner production designed to achieve high quality container plants. HDC Final Report HNS 55.

Blossfeld, S. and Gansert, D. (2007). A novel non-invasive optical method for quantitative visualization of pH dynamics in the rhizosphere of plants. Plant, Cell and Environment 30: 176-186.

Brauer, D. & Staley, T. (2005). Early developmental responses if white clover roothair lengths to calcium, protons and aluminium in solution and soil cultures. Crop Science, 45 (4): 1216-1222.

Bunt, A.C. 1988. Media and mixes for container grown plants. Unwin Hyman Ltd., London.

Davies, F.T. (1988). Influence of nutrition and carbohydrates on rooting of cuttings. The international plant propagators society combined proceedings, 38: 432-437.

Economou, A.S. and Read, P.E. (1986). Influence of pH and medium composition on rooting of hardy deciduous azalea microcuttings. Journal of the American Society for Horticultural Science 111(2): 181-184.

Harbage, J.F., Stimart, D.P. and Auer, C. (1998). pH affects 1H-indole – 3- butyric acid uptake but not metabolism during initiation phase of adventitious root induction in apple microcuttings. Journal of the American Society for Horticultural Science 123(1): 6-10.

Harrison-Murray R. 2003. Hardy Nursery Stock: Optimising rooting media for leafy cuttings. HDC Final Report HNS 98.

Hitchcock, A.E. (1928). Effect of peat moss and sand on rooting response of cuttings. The Botanical Gazette LXXXVI (2): 121-151.

Holt, T.A., Maynard, B.K. and Johnson, W.A. (1999). Low pH enhances rooting of stem cuttings of Rhododendron in subirrigation. J. Environ. Hort. 16(1): 4-7.

Kelly, J.C. (1976). Propagating media: Effect of pH on rooting. In: An Foras: Horticulture research report. Published by An Foras Talúntais, 19 Sandymount Avenue, Dublin 4. P15.

Kinraide, T.B. (1994). Use of a Gouy-Chapman-Stern model for membrane-surface electrical potential to interpret some features of mineral rhizotoxicity
Plant physiology 106 (4): 1583-1592.

Leifert, C., Pryce, S., Lumsden, P.J. & Waites, W.M. (1992). Effect of medium acidity on growth and rooting of different plant species growing in vitro. Plant Cell, Tissue and Organ Culture 30: 170-179.

Liu, J.H., Mukherjee, I. and Reid, D. (1993). Stimulation of adventitious rooting in sunflower (Helianthus annus)by low pH: possible role of auxin. Canadium Jouranl of Botany 71: 1645-1650.

McGuire, J.J (1980). Root initiation: a survey of current literature. The international plant propagators society combined proceedings, 30: 282-287.

Paul, J.L and Leiser, A.T (1968). Influence of calcium saturation of sphagnum peat on the rooting of some woody plants. The international plant propagators society combined proceedings, 18: 170-180.

Pierick RLM, Steegmans HHM. 1975. Analysis of Adventitious Root Formation in Isolated Stem Explants of Rhododendron. Scientia Horticulturae 3: 1-20.

Rahman, S.M., Hossain, M., Rafiul Islam, A.K.M. and Joarder, O.I. (1992). Effects of media composition and culture conditions on in vitro rooting of rose. Scientia Horticulturae 52 : 163-169.

Snyder, W.E (1974). Physiology of rooting. The international plant propagators society combined proceedings, 24: 384-387.

Tukey, J.R (1979). Back to the basics of rooting. The international plant propagators society combined proceedings, 29: 422-427.

Williams, R.R., Taji, A.M. and Bolton, J.A. (1985). Specificity and interaction among auxins. light, and pH in rooting of Australian woody species in vitro. Hortscience 20(6): 1052-1053.

Willumsen, J. (1986). Liming of peat for pot plant growing. Acta Horticulturae 178: 205-212.

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Appendix 1. Photographic illustration of rooting scores

Score system descriptions:

Score	Description
0	Dead and/or decaying .
1	Sound cut surface, no decay.
2	Thickening of stem at cut surface or lenticels or development of a distinct callus.
3	Root initials, one or several up to 5mm in length.
4	Root development, extension, roots 5-10mm but not branching.
5	Good root development with branching, sufficient to support growth after potting on.

Rooting scores for *Camellia japonica*.

Ro

otin

g scores for *Daphne odora*.

Rooting scores for Heathers (*Erica carnea* ‘Pink Spangles x 10 magnification used in this example).