ANNUAL REPORT

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HDC Project HNS 138
Annual Report 2006

Manipulation of the root environment in HNS production

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Commercial - In Confidence

Grower Summary

HNS 138

MANIPLUATION OF THE ROOT ENVIRONMENT IN HNS PRODUCTION

Annual Report 2006

Project title:	Manipulation of the root environment in HNS production.
HDC project number:	HNS 138
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Signed on behalf of:	**Warwick HRI**
Signature:	…………………………………………………………………
Name:	Professor Brian Thomas Deputy Director
Date:	11 May 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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Manipulation of the root environment in HNS production

Headline

pH alone does not appear to have a dramatic effect on rooting of cuttings in a sand based system.

Background and expected deliverables

The estimated 25% failure rate for the 200 million cuttings that are rooted every year is the main issue being addressed in year 1 of the project. The success rate in rooting cuttings has a subsequent effect on plant uniformity.

Previous work has examined how rooting is influenced by environmental factors such as relative humidity (RH), temperature and light. The effect of different substrates on the balance of air and water in the growing medium has also been investigated. The effects of the chemical environment (specifically pH) has been investigated for the rooting of cuttings in tissue culture but no reports of work carried out in more conventional substrate based systems were not found. Anecdotal evidence from a commercial heather grower however suggests that manipulation of pH in conventional growing systems may encourage rooting of heathers and may merit investigation in other hardy nursery stock (HNS) species.

The main objective of year one was therefore to develop a system to examine the response of rooting to pH and then use this system to test some representative HNS species.

Summary of the project and main conclusions

Initial work was undertaken to develop an ebb and flood system. This involved a series of tasks:

1. Testing suitable rooting systems
2. Examining the stability of pH adjustments with time
3. Checking that the pH in the root zone would be comparable with that of the irrigation supply (a low concentration nutrient solution)
4. Testing representative cuttings for ability to root in the system
5. Devising a suitable scoring system

A number of methods were developed from these studies as follows:

- The use of sand filled modules or troughs in which to root cuttings (figure 1).

- Pre-conditioning the sand to minimise its effects on the pH of the nutrient solution. This involved mixing the washed sand with nutrient solution at the correct pH with regular agitation over a 48 hour period prior to filling modules.
- Adjusting nutrient solution pH with sulphuric acid (for pH 4-7) or sodium hydroxide (pH 8).
- A qualitative scoring system was developed for rooting as follows:

  score 0 = dead cutting

  score 1 = viable cutting with no signs of rooting

  score 2 = thickening of stem at cut surface or lenticels or development of a distinct callus

  score 3 = root initials, one or several up to 5mm in length

  score 4 = root development i.e. extension growth of the developing root system, roots 5-10mm but not branching

  score 5 = good root development with branching, root system to support cutting growth after potting on.

Figure 1 Use of sand filled modules / troughs to root heather cuttings (struck 4th May 2006)

Seven species were screened in experiments in the summer of 2006. Cuttings were struck and assessed for their response to pH. The following is a brief summary of results by species assessed:

Ceanothus

Both the root scoring system and dry weight indicated that best rooting was achieved at a level of pH 7.

Rosemary

It was considered that pH levels of 7 and 8 were least favourable for rooting. Lower pH treatments (pH 4) gave better results with an average root score of 4.8. They also provided the least variability in root dry weight.

Fuchsia

Lower pH levels appeared preferable for fuchsia rooting in this experiment, with pH 5 producing the highest overall scores and dry weight. Rooting was however rapid and successful across the full range of pH levels assessed.

Erica tetralix

The best root score and fresh weight were achieved at pH 5, with pH 6 and above giving slightly poorer rooting. It was surprising that responses to higher pH levels were not greater than observed given the preference of Ericaceous plants for lower pH.

Erica carnea, Erica vegans and *Calluna vulgaris*

Problems experienced with rooting in this experiment were thought to be related to high temperatures. Cuttings began to die back after the high temperatures experienced and before any significant progress had been made towards rooting. These species will be examined again in further experiments starting in the autumn/winter 2006/07 using species normally rooted at this time of year, when the environment will be less stressful.

Repeat experiments are underway to verify results in year 1, but at present there is little evidence to suggest that pH alone had a dramatic affect on rooting in a sand based system. Rooting in growing media however is also affected by the interactions that occur between pH and nutrient availability and so pH may have a bigger part to play in these more complex systems.

Financial benefits

At this stage of the project, insufficient results have been obtained to ascertain any financial benefits.

Action points for growers

At this early stage in the project, no action points can be drawn up for growers.

SCIENCE SECTION

2.1. 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 current state of 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 such sector which would provide a good starting point to investigate the benefits of this technology.

The project overall was designed to examine responses to pH initially (as covered in this report) with later work focussing on either pH or nutrition depending on initial progress.

OBJECTIVES (Year 1)

1. Undertake pilot studies to modify an ebb and flood system to ensure treatments can be applied accurately and that the system is capable of producing rooted cuttings of acceptable quality.

2. Use the system developed to carry out screening experiments which test the relationship between pH and rooting performance of cuttings.

2.2. MATERIALS AND METHODS

2.2.1. Pilot studies

Initial work focussed on adapting an existing ebb and flood system for use in the assessment of pH and pilot experiments were designed to determine suitable methods and ensure cuttings could be successfully rooted. The system consisted of twenty independent trough, pump and reservoir systems which were recirculating with gravity drainage of the 'nutrient solution' draining back to a reservoir (figure 5). Key initial questions included determining a suitable growing system for HNS type material and establishing a suitable method for changing and maintaining different pH levels without the complication of differences in nutrient availability that results when the pH of a fertilised growing medium such as a peat based compost is altered.

Figure 5. Photograph of pilot studies work with different media types and illustration of system layout.

Development of a system to support cuttings

Suitable methods of supporting unrooted cuttings within the system were investigated. The preferred option for this work was to root the cuttings in the absence of a growing medium to eliminate any factors that may interact with the pH of the 'nutrient solution'. Modular trays were tested for supporting cuttings (each tray had an array of seven by six cells). This included covering the tray with light-proof polythene with a small hole inserted over each cell for placement of a cutting or inverting the tray and using the drain hole at the base of each cell to support the cutting (with polythene covering again to light-proof the root environment). Previous experience with hydroponic systems for protected chrysanthemums (PC 24), suggests that an inert substrate such as sand improves plant production possibly due to the higher buffering potential compared with substrate free systems where roots will experience periods of no moisture when the nutrient solution is not circulating. Sand filled modular trays were therefore used along side systems designed to root cuttings without a growing substrate. The sand used in initial experiments had the following particle size distribution:- 4.2% >6mm, 13.2% 2-4mm, 12.2% 2-4mm, 15.2% 0.6-2mm, 43.8% 0.2-0.6mm and 11.5% <0.6mm. The sand was later modified following review with the project steering group by sieving out particles above 6mm.

Development of the irrigation/treatment system

The frequency of the flood cycle in the ebb and flood system was tested for its suitability by trial and error using cuttings of *Erica carnea* and *Calluna vulgaris* grown in the different types of modules described above. The aim of the tests was to identify a flood cycle long enough to cover the base of cuttings unsupported by a substrate or to make the surface of the sand substrate glisten (i.e. indicating a flood depth just below the sand surface). The rest period between flood cycles needed to be sufficiently short to prevent drying out of the cuttings unsupported by substrate whilst sufficiently long to aerate the sand.

Tests with a limited number of pH treatments (pH 4, 6 and 8) were also carried out to evaluate stability of pH over time and devise suitable maintenance protocols. This included a comparison of acid type (sulphuric acid compared with nitric acid) for its effects on stability of achieved pH over time and any detrimental effect on the cutting material. A low concentration nutrient solution was used as the background irrigation solution with nitrate-N set to 10 mg/l, potassium at 23 mg/l and phosphate at 1.3 mg/l. This solution was then adjusted to the required pH by addition of acid or sodium hydroxide. The pH of the nutrient solution before adjustment was around pH 7.8.

Small scale tests were also carried out to assess if the use of sand in the rooting modules would interact with the nutrient solution applied affecting achieved pH at the root zone. This was tested by mixing pH adjusted nutrient solution with sand in a beaker and measuring the pH of the supernatant after agitation and subsequent settling. The low pH of the sand reduced the pH of the nutrient solution initially by 0.5 to 0.8 units. Repeated cycles of replacing the supernatant with fresh pH adjusted solution (as would happen in an ebb and flood system) gradually reduced the impact that the sand had on pH of the solution which recovered to its original level after 6 to 10 cycles of ebb and flood. Sand to be used as a substrate in modules was therefore 'pre-conditioned' before the start of a new experiment. This involved mixing the sand with the relevant nutrient solution and regularly agitating over a period of 48 hours before draining the sand and using it to fill modules. Subsequent tests were carried out on the sand medium 'in situ' using pH indicator papers that could be dipped into a well within a sand filled module to test root zone pH. The colour change was in good agreement with that observed by dipping indicator paper directly into the nutrient solution.

Since cuttings were to be rooted in open troughs regularly flooded with the pH adjusted nutrient solution, mist propagation was not suitable for maintaining high RH in the aerial environment due to the dilution effect it would have on each solution. Rooting was therefore carried out under polythene. A frame to support a polythene canopy was constructed over the benches used for the ebb and flood systems. The polythene at the sides and end of the structure were clipped to the sides of the bench to raise the humidity above the system. Fleece was draped over the polythene to reduce radiation on sunny days. The glasshouse compartment was set to 7°C heating with venting and set to 18˚C and forced ventilation (via fans) at 20°C. Shade screens at the top and south end of the compartment were also set to close at light intensities higher than 500W/m².

The above factors were tested in small scale experiments using cuttings of *Erica carnea*, *Calluna vulgaris*, Rosemary, Fuchsia (cvs Beacon Rosa and Beacon) and Ceanothus which were struck between 30th January and 8th February 2006.

2.2.2. Main experiments

Rosemary and Ceanothus cuttings were rooted in sand filled modules in initial experiments. Problems with sand siphoning out of the drainage holes of these modules made handling difficult and so for fuchsia and heather experiments, the whole trough was filled with sand and the cuttings struck directly into this. The sand to fill modules and toughs was the pre-conditioned sand prepared as described in 2.2.1 above. The low concentration nutrient solution was adjusted to pH 4, 5, 6, 7 or 8 using sulphuric acid or sodium hydroxide as required. Each replicate system had its own reservoir for the pH adjusted nutrient solution which was topped up with fresh nutrient solution at monthly intervals. Each reservoir was tested for pH three times a week and adjusted back to target level when it deviated by 0.2 units or more. 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. The ebb and flood system was set to give a 2 minute flood period per 12 hours.

Seven species were tested for pH response as summarised in table 1. Initial experiments consisted of four replicates of each pH level. This was subsequently reviewed and reduced to two replicates to allow for testing of a wider range of species.

Table 1. Species tested for pH response in year one.

Species	Date struck	Module size (cm^3)	No. reps
Rosemary	30/03/06	20	4
Ceanothus	30/03/06	20	4
Erica carnea cv	04/05/06	Rooted in trough	2
Erica tetralix cv	04/05/06	Rooted in trough	2
Erica vagans cv	04/05/06	Rooted in trough	2
Calluna vulgaris cv	04/05/06	Rooted in trough	2
Fuchsia	12/05/06	Rooted in trough	2

Unprepared cuttings were obtained from plant suppliers (except for Fuchsia which had already been cut to length). In all unprepared material it was necessary to use a mixture of tip and stem cuttings which were distributed evenly through the treatments to avoid interactions with cutting type. Material was graded to give material as uniform as possible at the start of the experiment. Woody cuttings were struck directly into the sand whilst holes were dibbed prior to striking soft cuttings.

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 slower to root than expected 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.

Interim rooting inspections consisted of allocating a root score on a scale 1 to 5 where:

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 growth of the developing root system, roots 5-10mm but not branching.
5 Good root development with branching, root system to support cutting growth after potting on.

Examples of these scores are given in Appendix 1.

Final rooting assessments were carried out once a whole row of plants from one of the troughs assessed for interim inspections had reached a score of 5. Cuttings were assigned a score for rooting as described above and root weight was measured. For Ceanothus, Rosemary and Fuchsia, root weight was taken as all material below the surface of the sand. For the heathers however, the original, woody cutting stem formed a significant proportion of the material below the surface of the sand. Hence root weight was determined by slicing off roots from the original stem material and weighing these.

2.3. RESULTS

2.3.1 Pilot studies

Sulphuric acid and nitric acid produced comparable results when used to adjust reservoir pH to desired treatment level (figure 7) and treatments remained distinct from each other throughout the pilot test period. Sulphuric acid treated reservoirs were slightly more stable over time and also avoided the problem of different N levels in each pH treatment which would result by using nitric acid. Sulphuric acid was used to lower tank pH in the main experiments.

Figure 7. The influence of acid type on maintenance of treatment pH (as measured in the reservoir) over time, where a = sulphuric acid and b = nitric acid.

Cuttings rooted in the pilot experiments were assessed at intervals with number rooted counted at each inspection (figures 8 and 9). In these initial experiments rooting was assessed as any signs of root development beyond callus stage. Samples were photographed and used to help develop the score system used in later experiments. More cuttings rooted in the sand filled modules than in modules with no substrate. Rooting of *Erica carnea* was affected by the module system to a greater extent than *Calluna vulgaris*, but overall the evidence suggested that sand filled modules were the more reliable system in which to carry out future experiments. These pilot studies also compared cutting response to acid type and there was no evidence to suggest that pH adjustment with sulphuric acid rather than nitric acid would be detrimental to rooting.

Small scale tests evaluated other media to support cuttings of Fuchsia and Rosemary, perlite, vermiculite and mineral wool all gave good results in terms of cuttings rooting successfully (appendix 2) whilst rooting was slower and/or poorer on the system with no substrate. Sand was however considered to be the most suitable substrate because of the ease with which roots could be inspected whilst minimising damage.

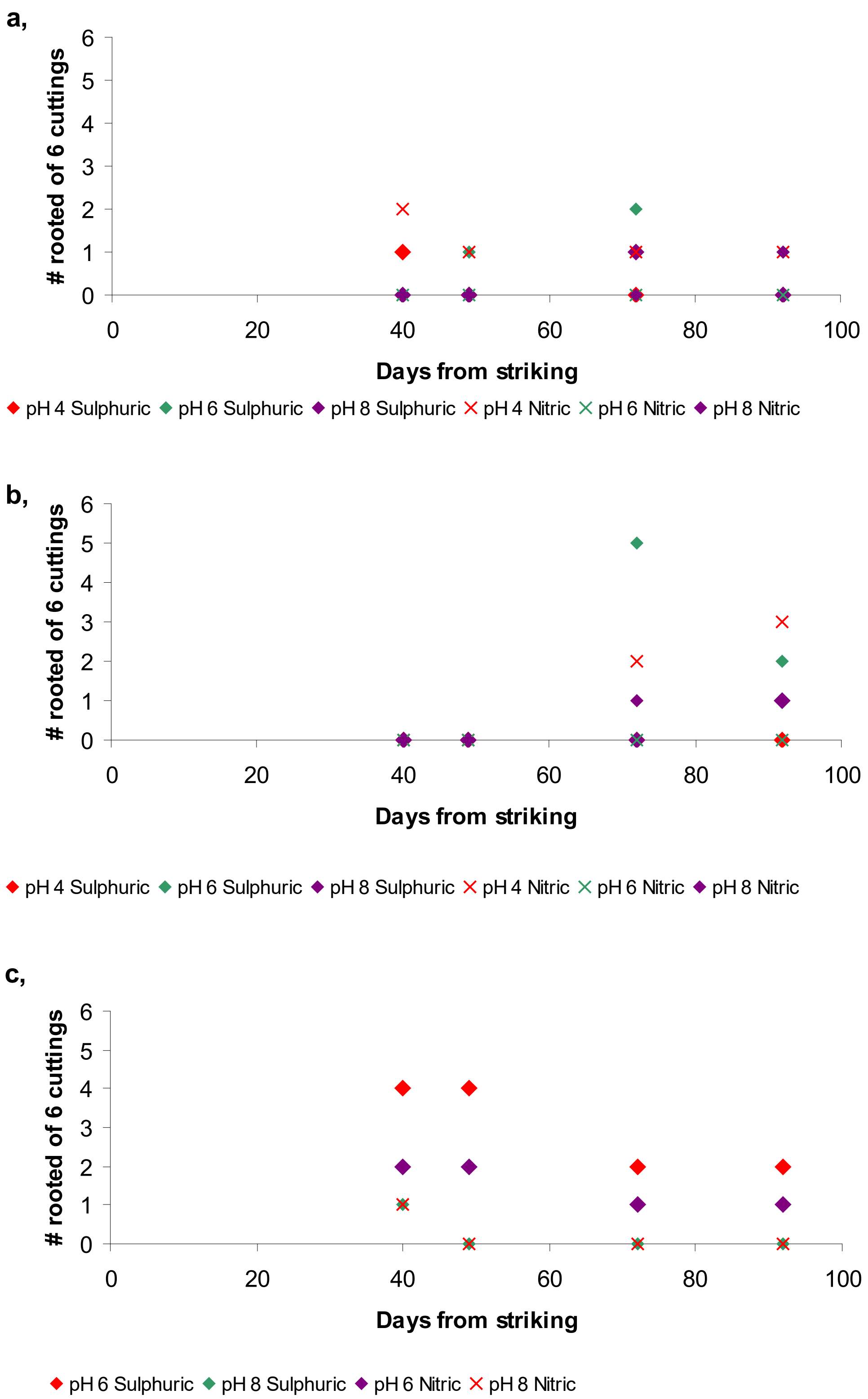


Figure 8. The influence of growing system and acid type on rooting of Calluna vulgaris, where a = cuttings raised without substrate in upright module trays, b = cuttings raised without substrate in inverted module trays and c = cuttings raised in sand substrate in module trays.

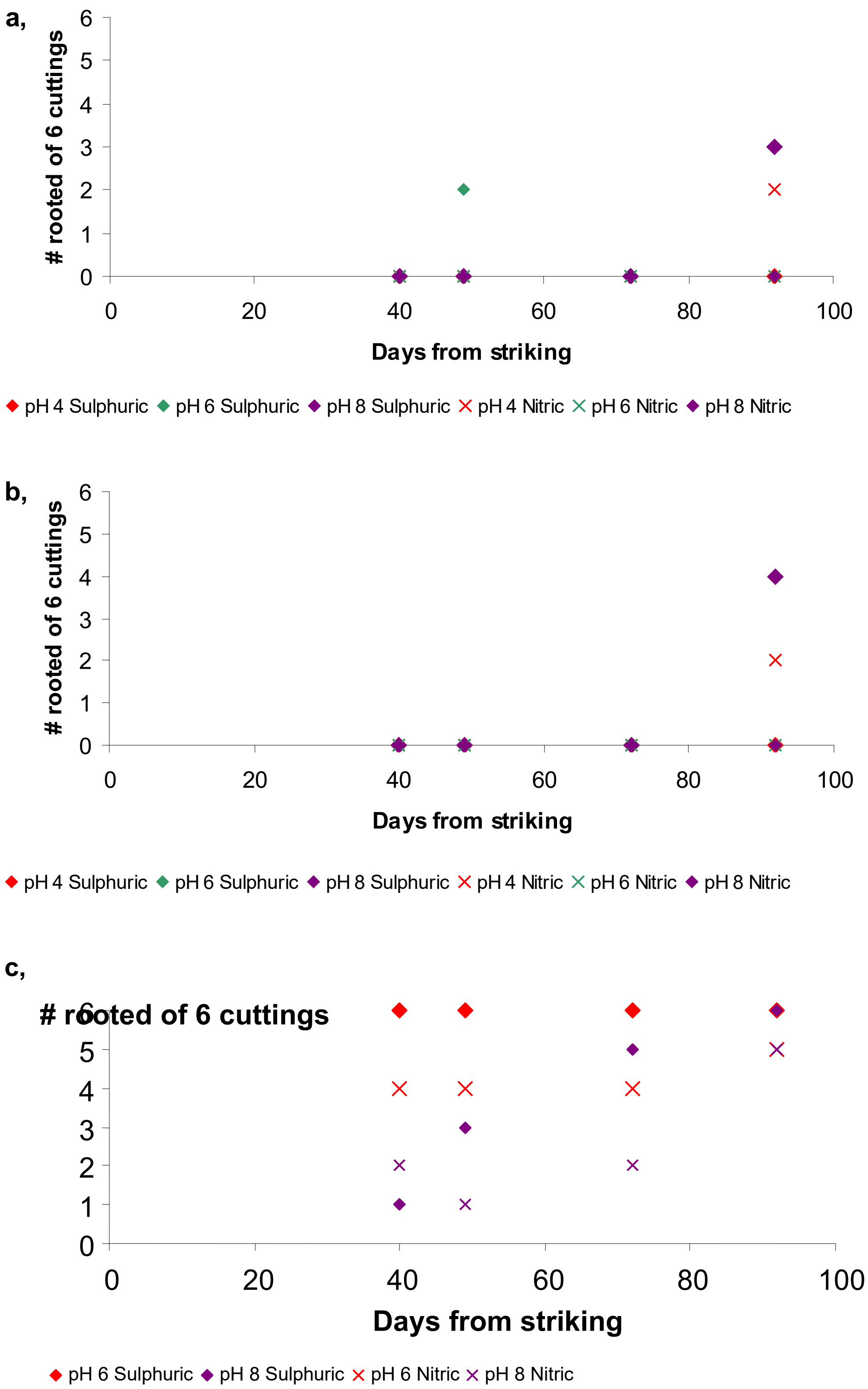


Figure 9. The influence of growing system and acid type on rooting of Erica carnea, where a = cuttings raised without substrate in upright module trays, b = cuttings raised without substrate in inverted module trays and c = cuttings raised in sand substrate in module trays.

2.3.2 Main experiments

Seven species were assessed for response to pH treatments ranging from pH 4 to pH 8. 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%)

<u>Ceanothus experiment (4 reps)</u>

Ceanothus struck on 30 March 2006 took around 77 days to root in the experimental system. Analysis was carried out on each weekly set of assessments separately with no differences found between pH treatments in any of the weeks that assessment was carried out. (figure 10). Mean root score in the last half of the experiment had wider variation than at the start of the experiment.

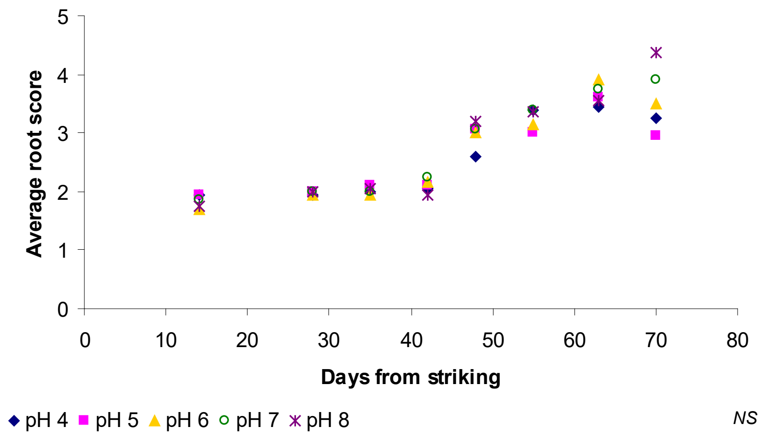


Figure 10. The influence of pH on root scores assessed weekly over time for Ceanothus.

Final root scores were significantly (P<0.001) higher at higher pH (figure 11). It is assumed that the reason for differences in significance of response to pH between scores taken as final inspections (figure 11) compared with weekly scores (figure 10) is at least in part due to sample size. Five plants were recorded per replicate for the interim inspection set of plants and ten plants were recorded per replicate as a final sample, this increase in replication may have improved the sensitivity of the analysis. The interim sample plants were also taken from a larger set so a different set of 5 were assessed each week to minimise disturbance of individual cuttings. This method may be expected to introduce greater variation than if the same set were re-examined every week (but should also make the assessments more robust).

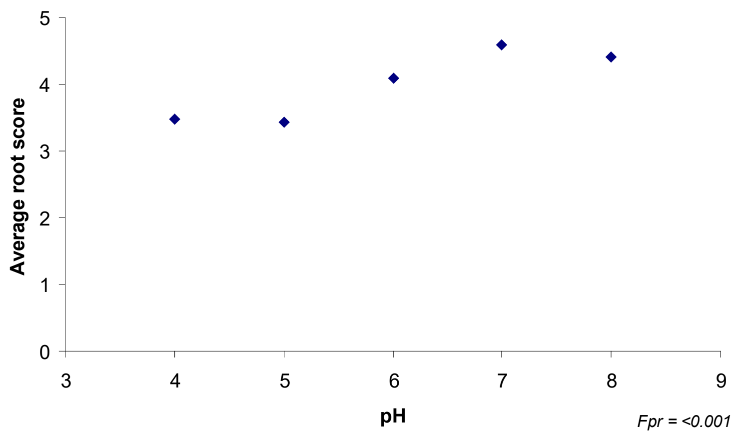


Figure 11. The influence of pH on final root score for Ceanothus (l.s.d.(5%) = 0.40).

The effect of removing cuttings for interim inspection was assessed by comparing scores for cuttings that had been removed for interim inspection with those that had been left in situ throughout the experiment (removed and not removed). There were no consistent trends to suggest that removal for inspection and subsequent replacement had reduced final root score (figure 12). It therefore appears that the strategy for interim inspections and the methods used to remove and replace cuttings did not inhibit rooting.

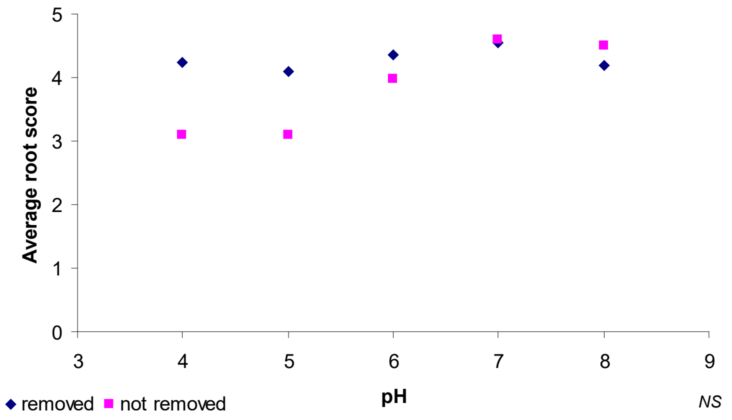


Figure 12. The influence of cutting removal and replacement for inspection and pH on final root score for Ceanothus.

Root dry weight was variable from rep to rep which may in part reflect the variability in initial material since root weight was taken as all material below the surface of the module. Despite this variability there was an increase in root dry weight for cuttings rooted at pH 7 and 8 compared with lower pH (figure 13).

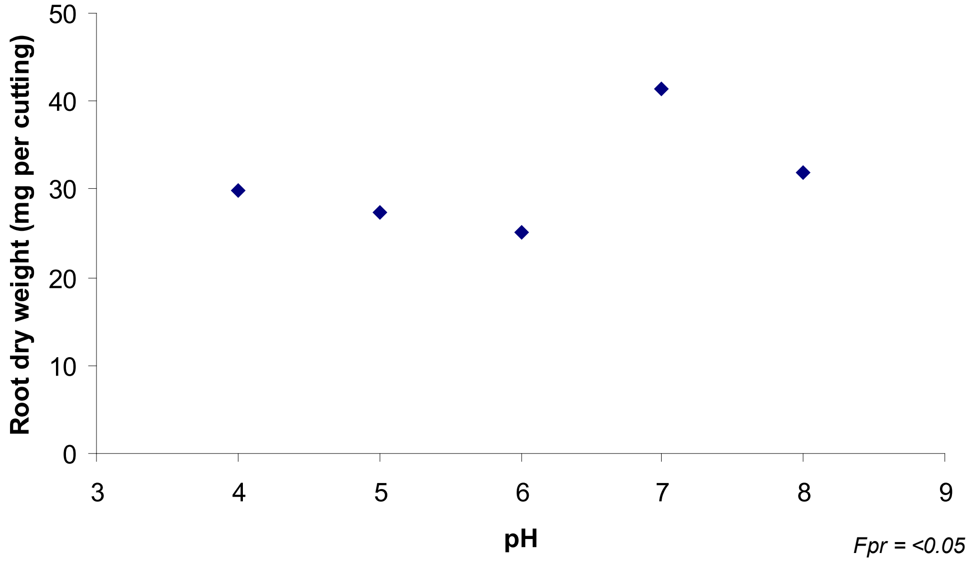


Figure 13. The influence of pH on root dry weight of Ceanothus (5% l.s.d.= 10.5).

<u>Summary for Ceanothus</u>

Rooting assessed by both root score and dry weight was best overall at pH 7 with pHs 6 and 8 coming close to pH 7 in terms of root score. Lower pH levels had lower root scores. Difficulties with variability in material used for dry weight need to be considered in interpreting the root weight data.

Rosemary experiment (4 reps)

Rosemary struck on 30 March 2006 rooted within 26 days from striking with no significant differences between pH treatments found for interim assessments of root score (figure 14).

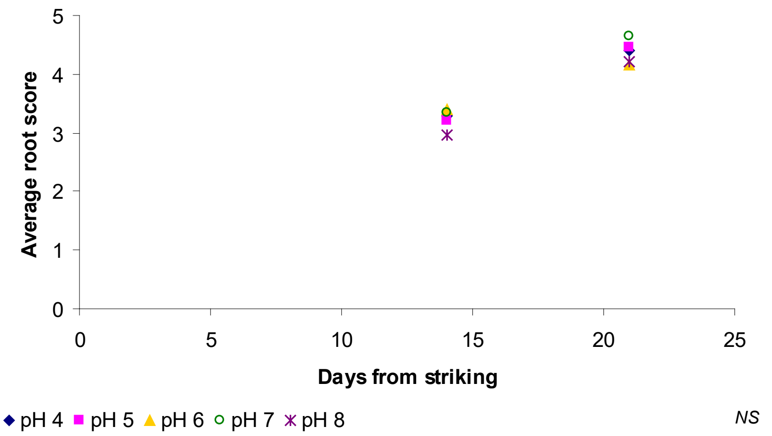


Figure 14. The influence of pH on average interim root score for Rosemary.

At final harvest, a slight decrease in root score was associated with the pH 7 treatment (figure 15), although a significant interaction with replicate suggests that this reduction was due to a low average score for two of the four replicates at pH7 (figure 16).

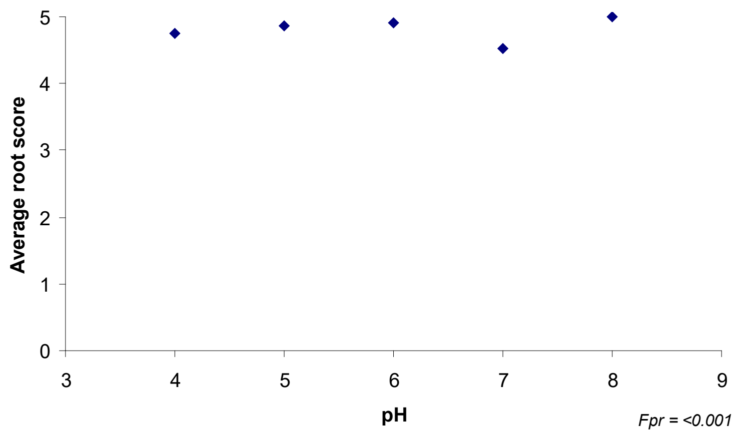


Figure 15. The influence of pH on average final root score for Rosemary (5% l.s.d.= 0.20).

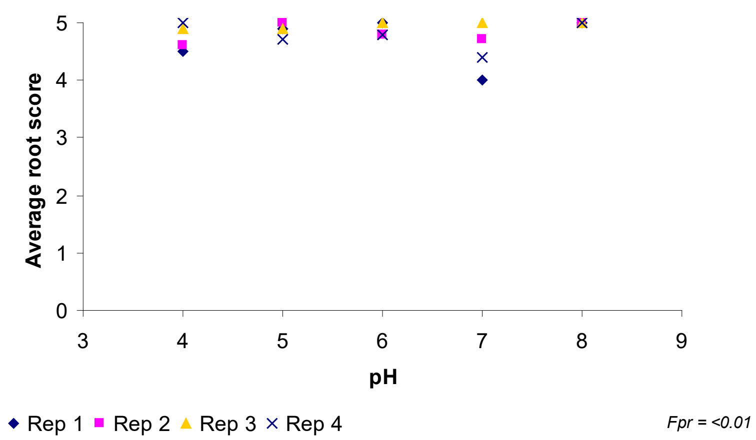


Figure 16. The interaction between replicate and pH on average final root score for Rosemary (5% l.s.d.= 0.40).

The reduction in root score at pH 7 is also reflected in a slight decrease in root dry weight at final harvest for both pH 7 and pH 8 (figure 17).

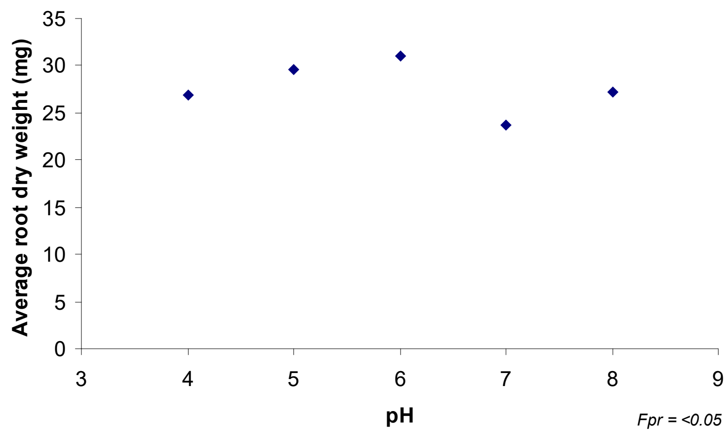


Figure 17. The influence of pH on average final root dry weight of Rosemary (5% l.s.d.= 4.6).

<u>Summary for Rosemary</u>

Overall, whilst some significant differences suggest that pHs 7 and 8 were least favourable for rooting, the differences between treatments were small considering the wide range of pH levels assessed. The best of the lower pH treatments was pH 4 which gave an average root score of 4.8.

Fuchsia (Beacon Rosa) – 2 reps

Fuchsia struck on 12 May 2006, rooted rapidly in all systems, achieving the maximum score 19 days from striking. Differences between pH treatments were not significant for the interim scores assessed prior to the final sample (figure 18).

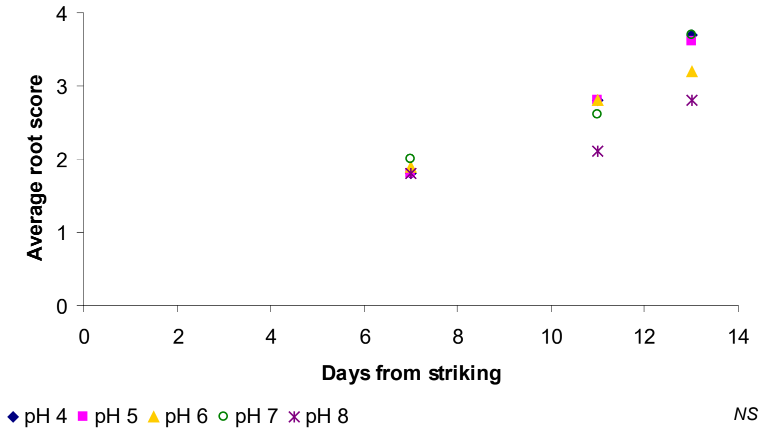


Figure 18. The influence of pH on change in average root score over time for Fuchsia.

Significant differences were found between pH treatments for plants assessed as final samples (figure 19). Root scores decrease slightly at pH 7 and 8 compared with pHs 4-6. However the differences between treatments were quite small and all treatments achieved a score of 4 to 5 indicating good levels of root establishment in all cases

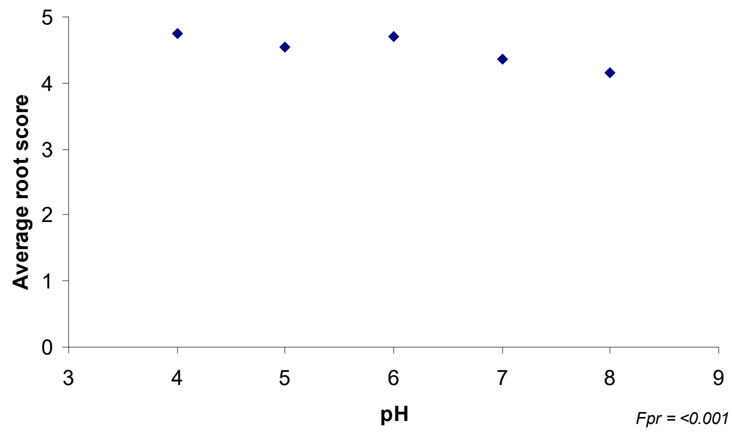


Figure 19. The influence of pH on average final root score for Fuchsia (5% l.s.d. = 0.30)

Cutting removal for interim inspection had no significant influence over the final root score recorded for each treatment (figure 20).

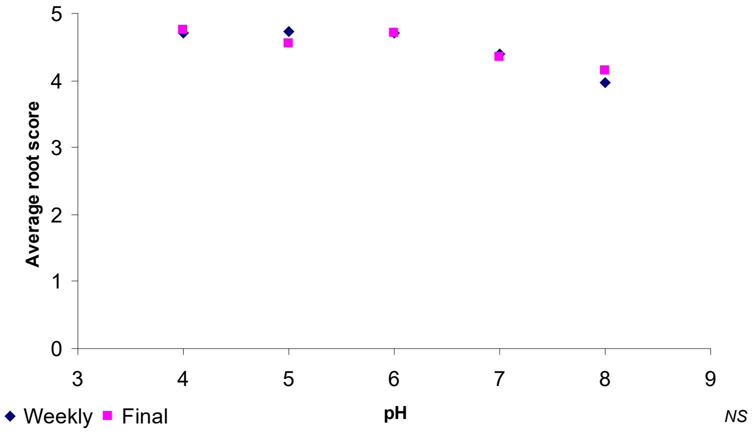


Figure 20. The influence cutting removal and replacement for inspection and pH on average final root score for Fuchsia.

Root dry weight followed a similar trend to root score, with pH 5 giving the highest dry weight and root weight decreasing with increase in pH above pH 5 (figure 21).

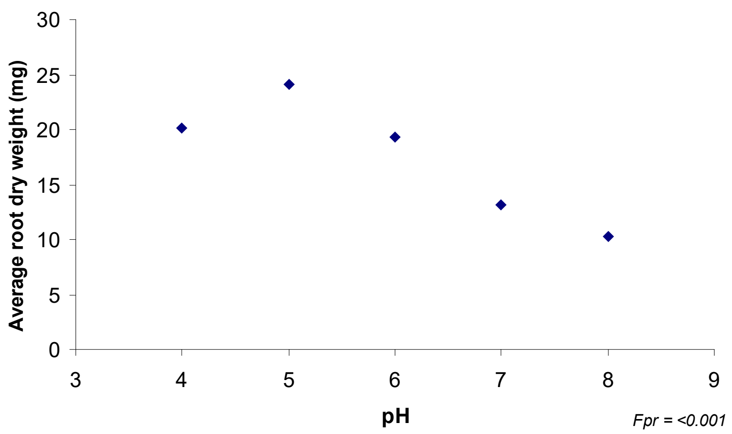


Figure 21. The influence of pH on average root dry weight final harvest for Fuchsia (5% l.s.d.= 5.13).

<u>Summary for Fuchsia</u>

Lower pHs appeared preferable for fuchsia rooting in this experiment with pH 5 producing the highest overall scores and dry weight. Rooting was however rapid and successful across the range of pHs assessed.

<u>*Erica tetralix* experiment (2 reps):</u>

Erica tetralix struck on 4 May 2006 rooted within 42 days of striking. No significant differences were found in root score between treatments assessed at weekly intervals prior to final samples (figure 22).

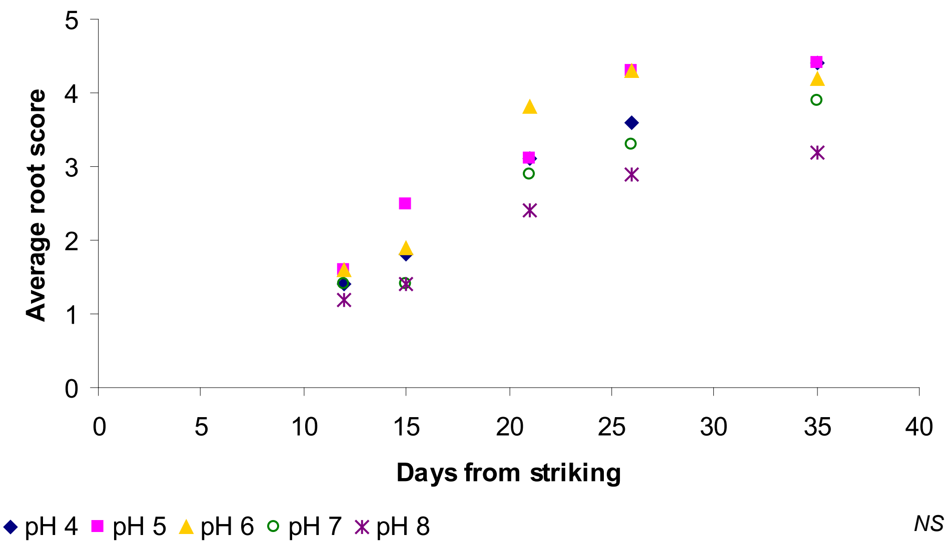


Figure 22. The influence of pH on change in average root score over time for Erica tetralix.

At final root assessments, significant differences were found, with root score decreasing at higher pH (figure 23), and no significant difference between pH 4 and 5. The differences between results found during weekly interim sampling and final samples (figures 22 and 23) may be related to sample size as discussed previously for Ceanothus. It is apparent in figure 22, that despite there being no significant difference in the weekly assessment data, the trend from the second assessment onwards was of higher scores associated with lower pH's.

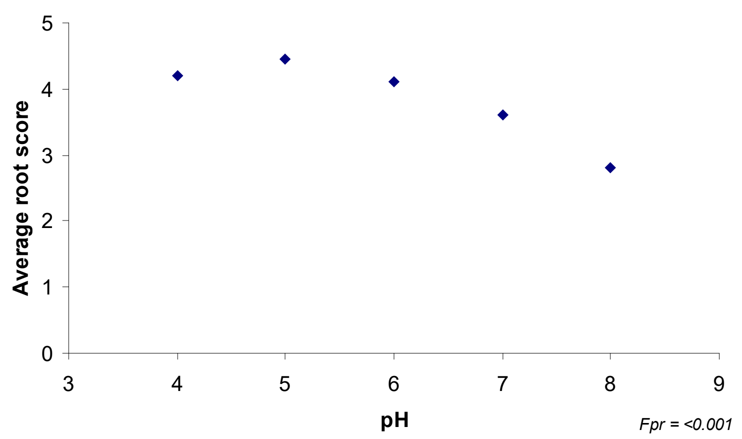


Figure 23. The influence of pH on average final root score for Erica tetralix (5% l.s.d. = 0.37)
Unlike Ceanothus and Rosemary, variability in root score was not affected by pH treatment with similar differences between the two replicate sets of data from *Erica tetralix*.

No significant differences were found between interim weekly samples (i.e. from plants removed and replaced in the sand) and final samples (i.e. plants not removed from the growing medium prior to final assessment) suggesting that disturbing the cuttings for interim inspections did not influence root score allocated (figure 24).

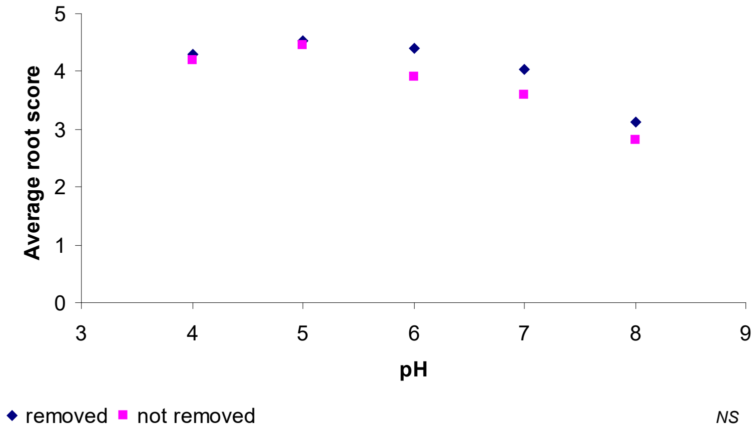


Figure 24. The influence cutting removal and replacement for inspection and pH on average root score at final harvest for Erica tetralix.

Root weight was difficult to assess accurately. On average the new root growth generated since striking the cuttings constituted between 3 and 17% of the dry weight of the material below the surface of the sand. The remaining material originated as the stem from the cutting which varied in diameter and hence weight. To eliminate this variation from the final assessments, the new root material was detached from the main stem using a scalpel blade prior to weighing. The new root material was however very fine and difficult to handle accurately, introducing error into the assessments. Despite these difficulties the overall trend in fresh weight of new root growth showed a similar trend to root scores, with weight decreasing at higher pH (figure 25). There was particular variability between the 2 replicates at pH 5 which can not be attributed to just one or two erroneous data points. The reason for this variability is unclear.

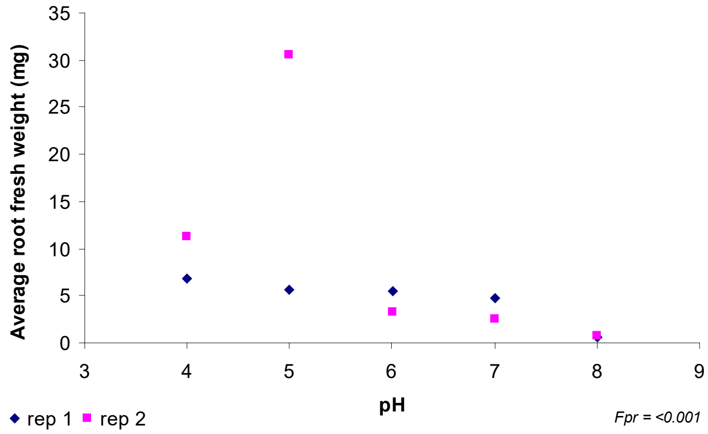


Figure 25. The influence of pH on average final root fresh weight for Erica tetralix (5% l.s.d. = 7.19)

<u>Summary for *Erica tetralix*</u>

Overall differences between treatments were small but pH 5 appears to have given the best root score and fresh weight with pH 6 and above giving slightly poorer rooting. It is surprising that responses to higher pH are not greater than observed given the preference of Ericaceous plants for lower pH.

<u>Erica carnea (2 reps)</u>

Erica carnea struck on 4 May 2006 failed to root before 10 August (98 days from striking) when there were too few viable cuttings remaining for assessment. The higher scores allocated to cuttings in the earlier stages of assessment relate to the cutting remaining apparently viable (score 1), with these scores degrading to 0 (dead) with time (figure 26). The death of cuttings over time is illustrated in figure 27.

The reasons for this failure have been explored with the project steering group and problems with high temperature and low air filled porosity (AFP) were considered to be the primary factors responsible for the poor rooting in this summer experiment (which contrasts with the successful rooting achieved with the winter experiment as part of the initial pilot studies). Data collected to investigate this problem are presented in Appendix 3.

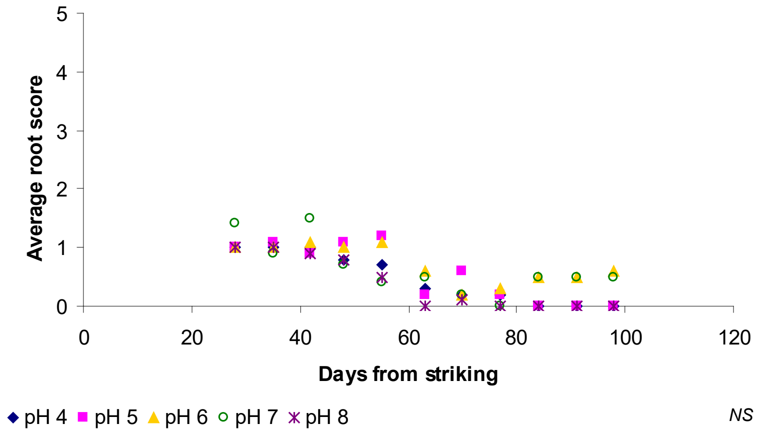


Figure 26. The influence of pH on change in average root score over time for Erica carnea.

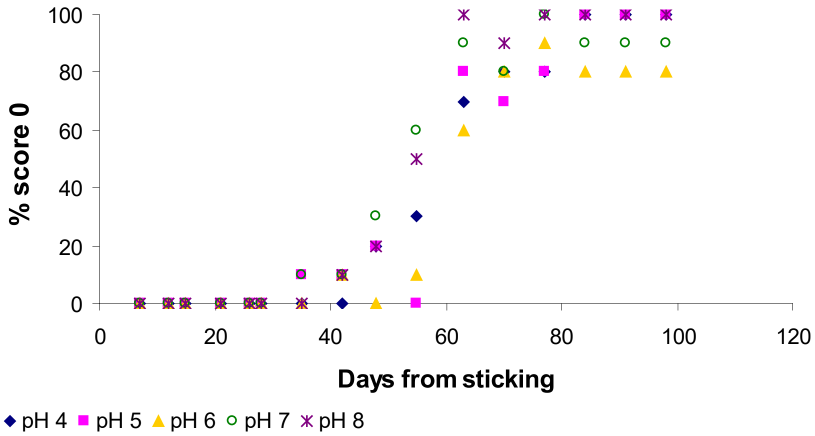


Figure 27. The influence of pH and time from striking on cutting death for Erica carnea.

Erica vagans (2 reps)

Erica vagans was also difficult to root in the experiment struck on 4 May 2006 which was finished 98 days from striking due to the high incidence of cutting death (figure 28). Average scores were higher than for *E.carnea* outlined previously, indicating some initial root development in the experiment. However average root score subsequently declined as cuttings died back which is indicated by proportion of cuttings at score 0 (i.e. dead or dying back – figure 29). Incidence of dead cuttings increased from around 70 days from striking onwards.

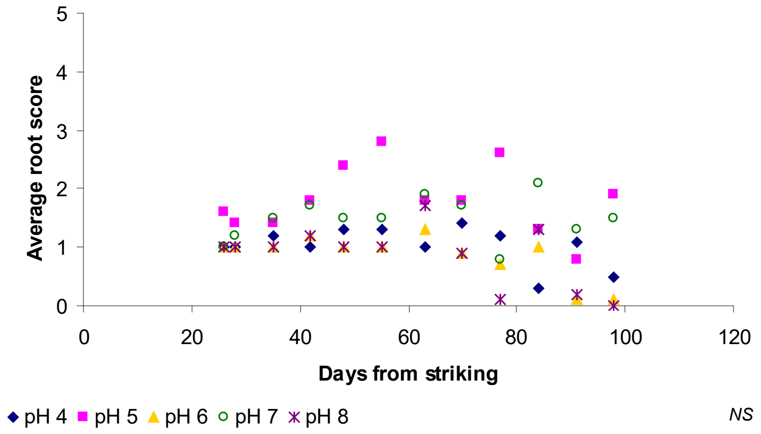


Figure 28. The influence of pH on change in average root score over time for Erica vagans.

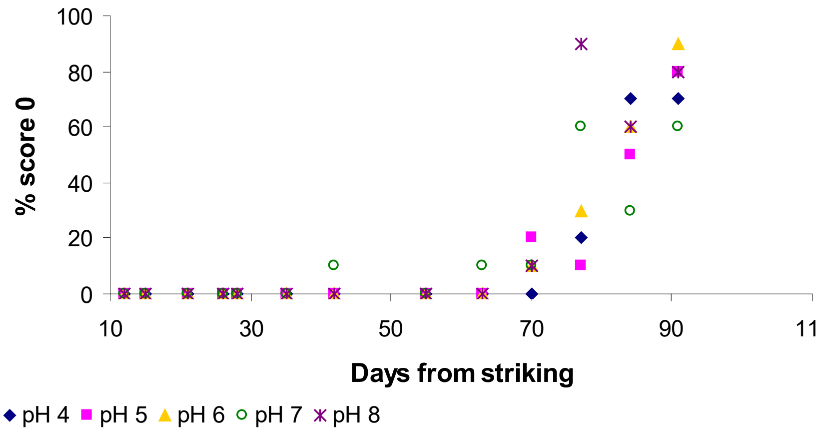


Figure 29. The influence of pH and time from striking on cutting death for Erica vagans.

Callluna vulgaris (2 reps)

Calluna vulgaris also had difficulties rooting in the summer 2006 experiment with root score reaching a maximum of 2 which indicates the appearance of callus type material (figure 30). Root scores did not then progress to 5 (indicating successful rooting) but declined as cuttings died back which is illustrated by the change in % of 0 scores (figure 31). Cuttings that have died off are scored 0 and an increase in the proportion of plants with this score was noted from 70 days from striking onwards.

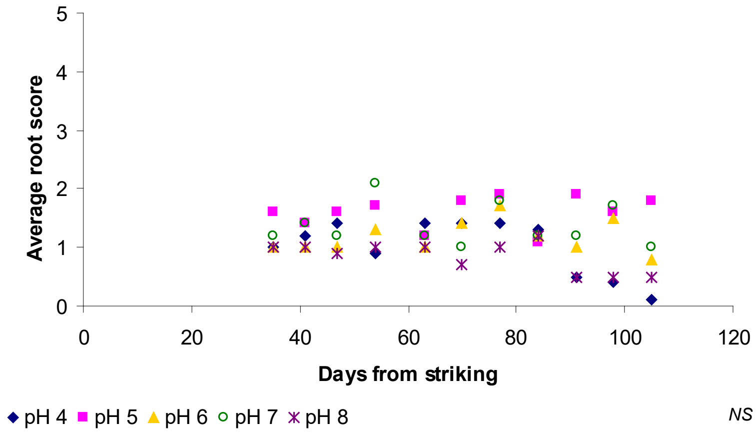


Figure 30. The influence of pH on change in average root score over time for Calluna vulgaris.

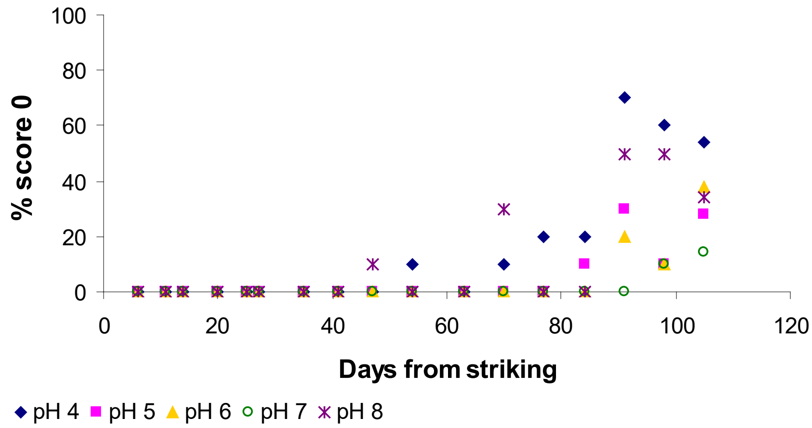


Figure 31. The influence of pH and time from striking on cutting death for Calluna vulgaris.

Summary for *Erica carnea*, *Erica vagans* and *Calluna vulgaris.*

The problems with rooting experienced with these three species make it impossible to interpret the limited data with any confidence. These species will be examined again in further experiments starting in the Autumn/winter when the environment will be less stressful using species normally rooted at this time of year.

2.4 DISCUSSION

Of particular interest in the experiments covered in Year 1 is the wide range of pH levels in which the species tested have developed root systems. Some species appear to have responded to pH; for example Ceanothus rooting was better at pH 7 and 8 than pH 4 and 5 and was also more variable at low pH than higher pH. Fuchsia cv Beacon Rosa and Erica tetralix both rooted best at around pH5. Whilst statistically significant, these differences appear unlikely to have a large impact on commercial rooting systems. Since the current work was designed to form an initial screening system, replication was limited and would need further verification.

In these studies, interim root scores were designed primarily to keep track of rooting progress and identify a suitable time to evaluate final scores on cuttings not previously disturbed by the interim assessments be measured as a qualitative score as well as by root weight. Whilst quantitative data is preferable to qualitative assessments, practical difficulties introduced variability in the assessment of root weight. Root weight in the first experiments to reach final assessment stage (i.e. Ceanothus, Fuchsia and Rosemary) was taken as all material below the surface of the sand substrate. This material therefore included original stem material from the cutting as well as new root growth. In woody cuttings in particular, the variability from cutting to cutting in weight of original stem material may have masked rooting effects with the new fleshy root material forming a small proportion of the total root weight. For the heather assessments therefore new root growth was separated from the original stem material before weighing but the small size of roots made this process difficult to carry out with any confidence in accuracy. A further problem resulting from this variability may be the impact on rooting itself if the original stem material is variable. Future experiments will therefore focus on qualitative scores rather than root weight and with increased emphasis on uniformity of initial material (although cuttings from commercial sources will continue to be sourced to ensure commercially relevant material is being screened).

There is evidence from plants grown in tissue culture that pH influences rooting, and it is commercial practice to adjust substrate pH according to species being rooted; however pH interacts with other factors such as nutrient availability. As the current experiments were designed to minimise interactions with nutrient availability it is possible that it is not pH (i.e. concentration of hydrogen ions) that is directly influencing rooting. Given the problems experienced with 3 of the species tested however further experiments are required to verify the results emerging from these initial experiments. Through discussion with the project co-ordinators, modifications to be made to further experiments include reducing moisture levels in the sand modules, use of commercially prepared cuttings that are as uniform as possible and striking cuttings under less stressful environmental conditions.

2.5 CONCLUSIONS

Species with some response to pH of low nutrient level irrigation supply in a sand based substrate included Ceanothus, Fuchsia and *Erica tetralix*, with average amount of root developed and variability in rooting affected.

The response of all species was generally small in comparison with the wide pH range selected for these initial experiments, especially in the case of the ericaceous subjects.

Rosemary rooting was largely unaffected by pH in these experiments.

Other species, namely *Erica carnea, Calluna vulgaris* and *Erica vagans*, failed to root in these experiments and died back at around 70 days from striking. Adverse temperatures may have been responsible for the die back but the reasons for the slow initial root development remain unclear, especially for the first two species that did establish roots in pilot experiments.

2.6 TECHNOLOGY TRANSFER

Members of the HTA technical committee were shown experiments as part of their first technical committee meeting at Wellesbourne in 2006. The two grower co-ordinators have been also been updated on progress via written summaries and review meetings with information passed on to members of the HNS panel at formal meetings.

2.7 REFERENCES

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

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

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

Rahman SM, Hossain AKM, Islam R, Joarder OI. 1992. Effects of media composition and culture conditions on in vitro rooting of rose. Scientia Horticulturae 52: 163-169.

2.8 ACKNOWLEDGEMENTS

The project co-ordinators Neal Wright and David Edge have provided valuable support and feedback in both developing the trial and running the main experiment.

Appendix 1. Photographic key to the root scoring system

Score 0 The cutting is dead. Most likely as a result of die-back from the cut surface

Score 1 The cutting shows no signs of die-back at the cut surface, is healthy but not producing a callus or any visible signs of rooting

Erica tetralix

Score 2 The cutting has produced callus or positive indications that rooting has started eg swelling, lesions

Ceanothus

Score 3 The cutting has produced one or more identifiable roots. A score of 3 has a range from a single identifiable root to several roots indicating the establishment of a root system. This can be a long stage if root growth is slow.

Erica tetralix

Calluna vulgaris

Ceanothus

Score 4 The cutting has a developing root system made up of several roots which may show some branching, but is not of sufficient development to be self supporting. If the root system is developing rapidly this is a short stage.

Erica tetralix

Calluna vulgaris

Ceanothus

Score 5 The root system is well branched and developed enough to make the cutting self sufficient.

Erica tetralix



Appendix 2. Data from pilot experiments assessing rooting of Rosemary and Fuchsia in different media.

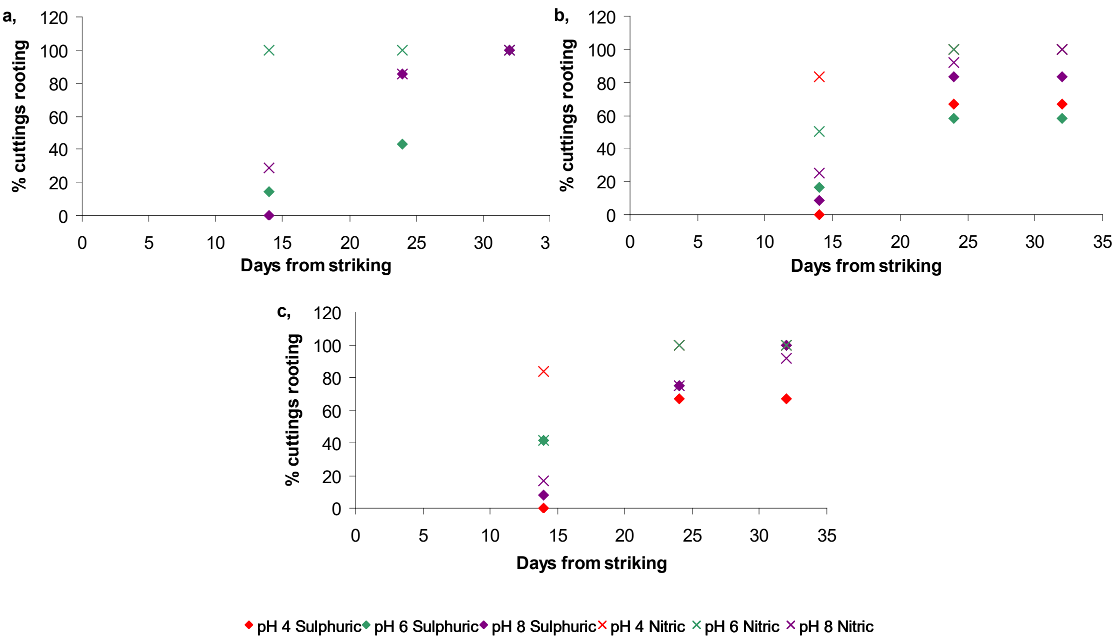


Figure 35. Rooting of Rosemary cuttings in:- a = sand; b = vermiculite, c = perlite.

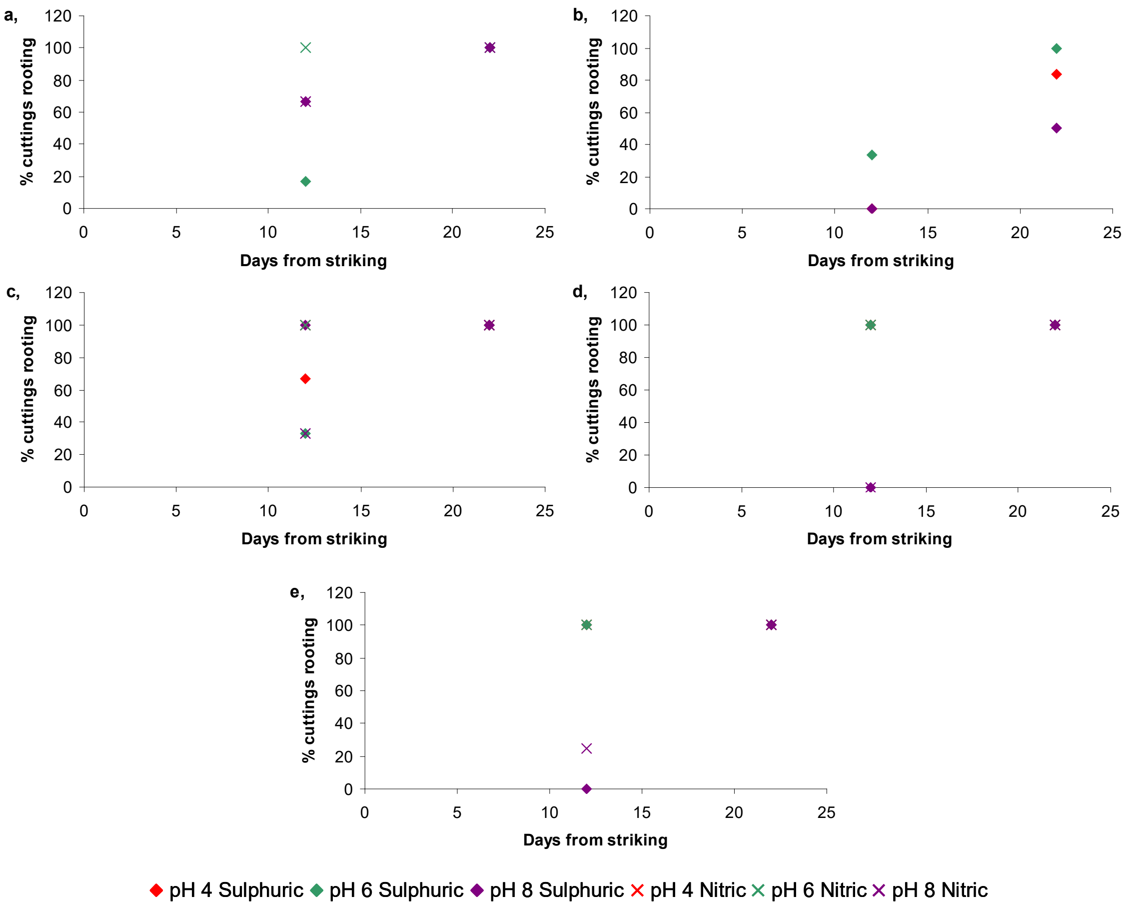
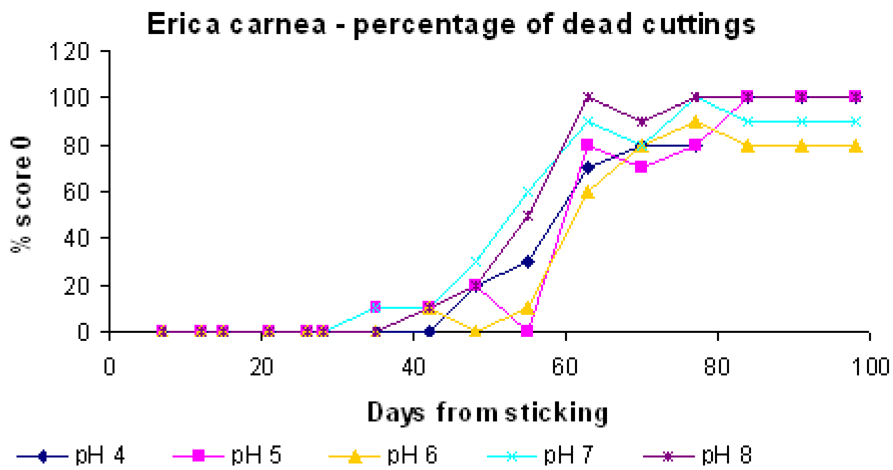


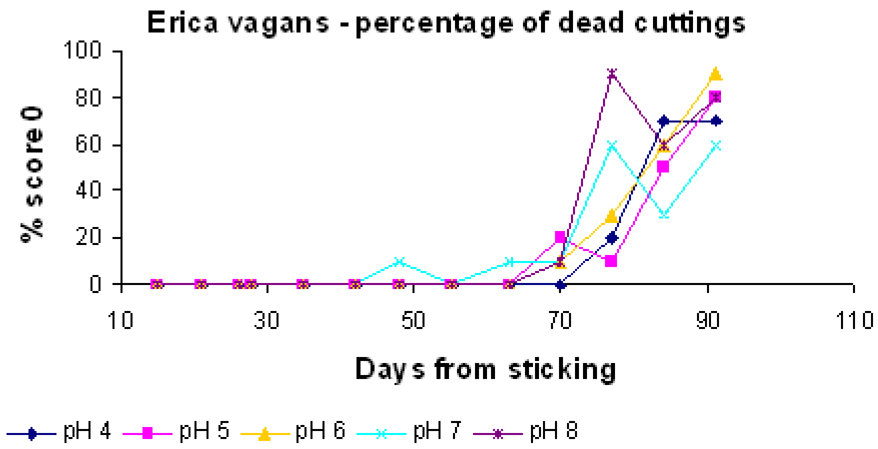
Figure 36. Rooting of Fuchsia cv Beacon Rosa cuttings in:- a, sand; b, no substrate; c, mineral wool; d, vermiculite; and e, perlite.

Appendix 3: Factors contributing to cutting death in summer 2006 trials:

The die back of cuttings of *Erica* carnea, *Erica* vagans and *Calluna* vulgaris prevented assessments of response to pH for these species in the summer 2006 experiments. Reasons for the die back were explored since successful rooting had been observed in pilot study work.

Cuttings were expected to root within 6 weeks (42 days from sticking) and die back appeared from 50 to 60 days from sticking for *Erica.carnea* and from 70 days from sticking for *Erica vagans* and *Calluna vulgaris* (figure 37).





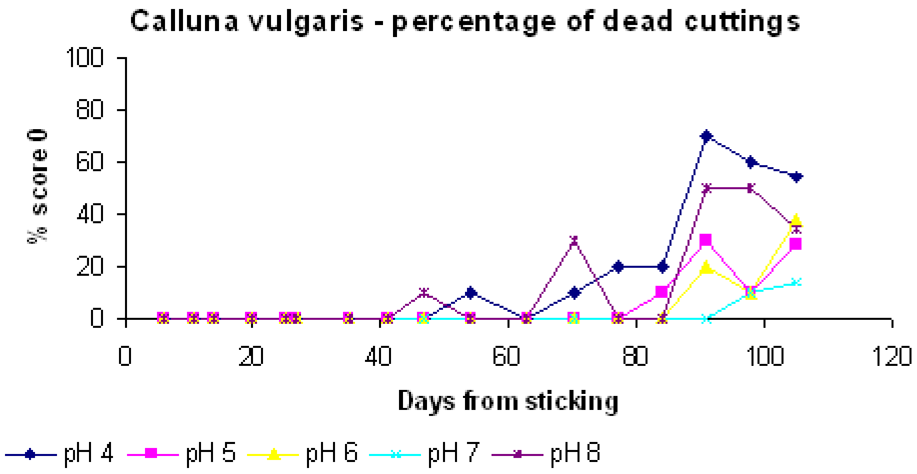


Figure 37. Rate of die back of cuttings over time as indicated by percent at score 0 (indicating a dead cutting).

The death of cuttings was attributed to temperature with high temperatures in July coinciding with the increase in % of cuttings with score 0 (i.e. dead). However the cuttings should have rooted earlier than this and hence the reason for slow rooting needs to be examined. Since the die back was observed soon after a period of high temperatures environmental data was examined alongside the time course of cutting deaths.

A brief period of high temperature was experienced in May soon after sticking (0-10 days from sticking). Tent temperature was on average 8.6°C above external temperature in early May compared with 4.4 to 4.8°C after 13th May when the forced air cooling was switched on (figure 38).

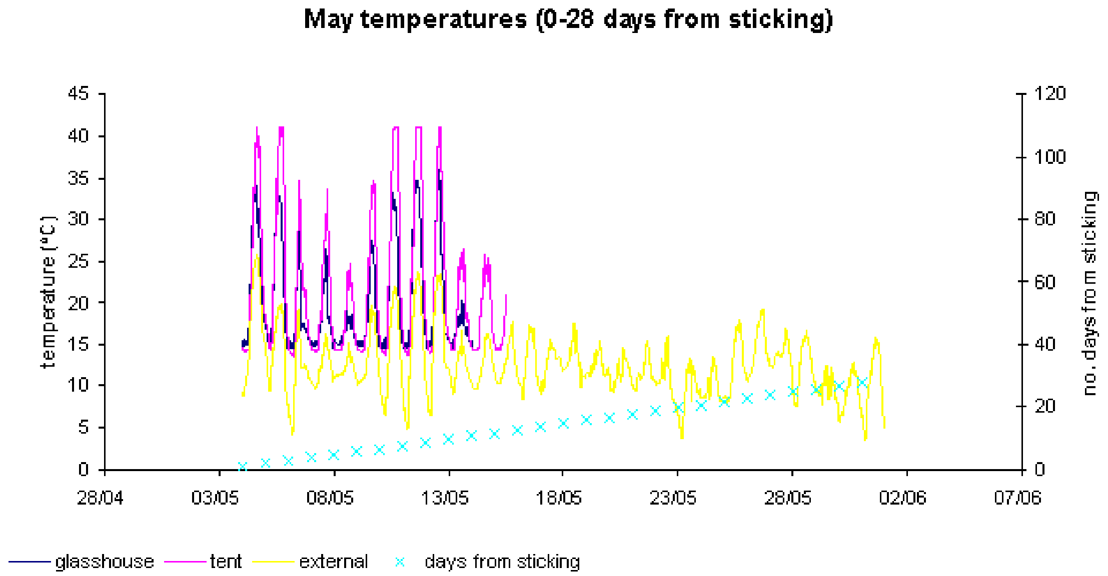


Figure 38. Temperatures in the glasshouse, under the polythene tent and outside in relation to time from striking heather cuttings during May 2006.

High external and glasshouse temperatures (28-35°C) were also observed at the beginning of June (29-39 days from sticking) when tent temperatures may be expected to be between 30 and 37°C (i.e. 2°C above glasshouse temperature); which may have resulted in some stress (figure 39).

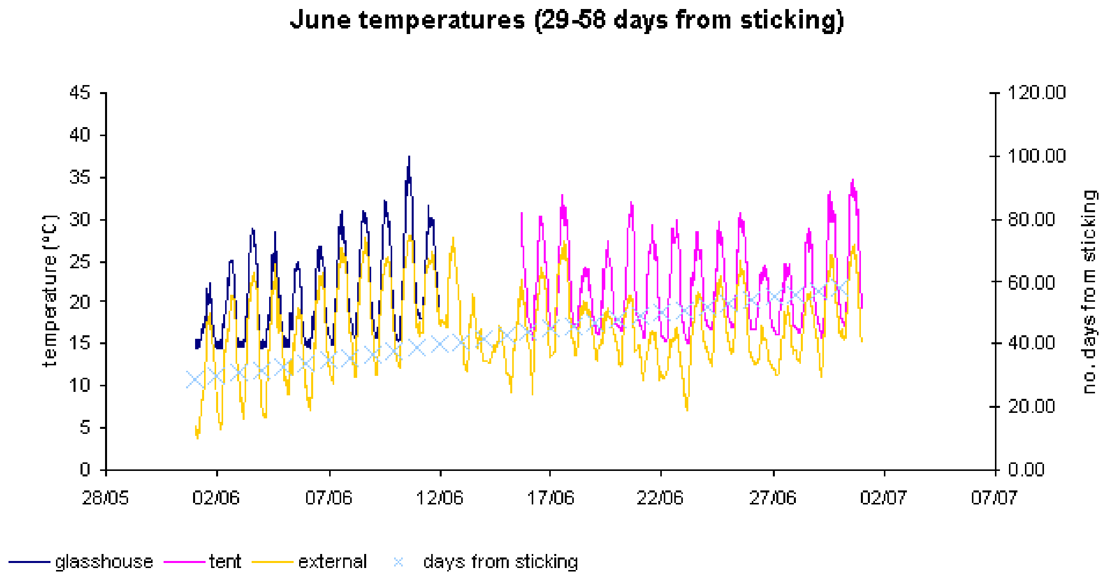


Figure 39. Temperatures in the glasshouse, under the polythene tent and outside in relation to time from striking heather cuttings during June 2006.

Extremes of temperatures were observed in July (59-63 days from sticking at 37 - 40°C) which coincides with the increase in dead cuttings of *Erica vagans* and *Calluna vulgaris* but not of *Erica carnea* (figure 40).

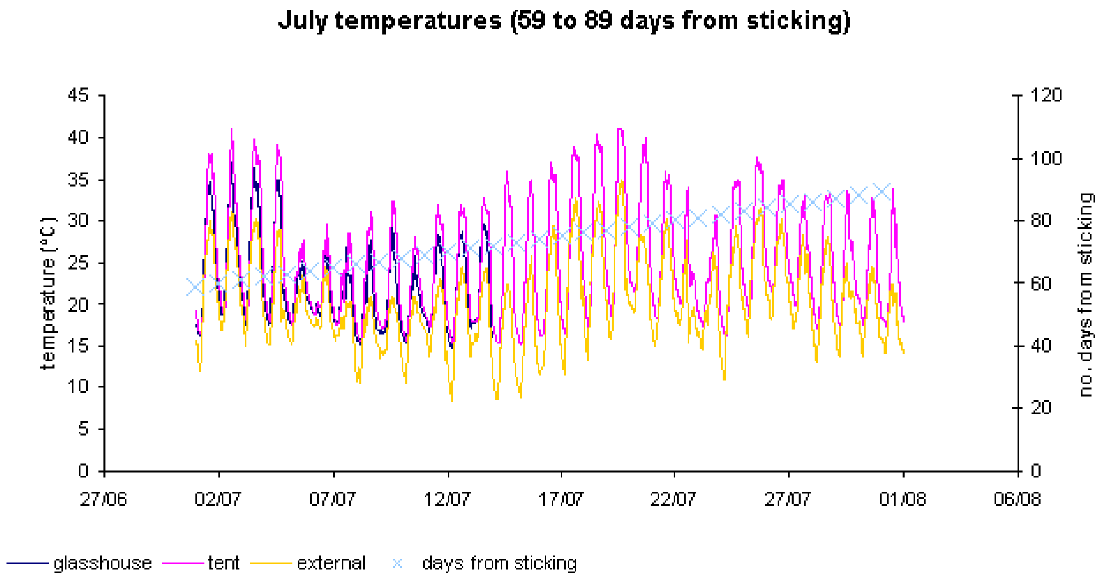


Figure 40. Temperatures in the glasshouse, under the polythene tent and outside in relation to time from striking heather cuttings during July 2006.

In pilot studies, Erica carnea had at least 50% rooting in most treatments 3 weeks after sticking (although this took 9 weeks for the pH 8 treatment using nitric acid). Calluna vulgaris was less successful in sand. Over 50% cuttings had rooted after 3 weeks but this number declined with time.

Hence factors other than treatments imposed may be limiting rooting. Observation by grower coordinators suggested that the sand may not have had sufficient air filled porosity. An estimate of AFP following a method similar to Bragg and Chambers (1988) suggests the sand used had 5% AFP (it has been sieved at the start of the experiment due to concerns over the size of the coarse material included). Future (year 2) experiments will therefore use a fresh supply of horticultural sand.

Bragg, N.C. and Chambers, B.J. (1988). Interpretation and advisory applications of compost air-filled porosity (AFP) measurements. *Acta Horticulturae*, 221 pp35-44.