

**Project title:** Hardy nursery stock:  
Optimising rooting media for leafy cuttings

**Project number** HNS 98

**Project leader** Dr R.S. Harrison-Murray

**Report** Final report, June 2003

**Previous reports** Annual reports February 2001 and  
September 2001

**Key workers:** Mrs J.M. Taylor  
Mrs W.B. Oakley

**Location of project:** HRI-East Malling

**Project co-ordinator** John Hedger, New Place Nurseries Ltd.,  
London Road, Pulborough, W. Sussex, RH20  
1AT  
John Woodhead, Hilliers Nursery Ltd.,  
Ampfield House, Romsey, Hants, SO51 9PA

**Date project commenced:** 1 September, 1999

**Date completion due:** 31 August, 2002

**Key words:** hardy nursery stock, rooting, propagation,  
media,  
aeration, drainage, rotting, environment,  
water balance, peat, vermiculite, perlite,  
tension, mist, fog, cuttings, capillary  
drainage, capillary matting, necrosis,  
*Convolvulus, Garrya, Fremontodendron*

Whilst reports issued under the auspices of the HDC are prepared from the best available information, neither the authors nor the HDC can accept any responsibility for inaccuracy or liability for loss, damage or injury from the application of any concept or procedure discussed.

The contents of this publication are strictly private to HDC members. No part of this publication may be copied or reproduced in any form or by any means without prior written permission from the Horticultural Development Council.

# CONTENTS

<b>GROWER SUMMARY.....</b>	<b>1</b>
Headline .....	1
Background and objectives .....	1
Summary of the project and main conclusions.....	2
<i>Physical characteristics of media</i> .....	2
<i>Capillary drainage on the propagation bed</i> .....	3
<i>Rooting media trials</i> .....	3
<i>Principles underlying the response to rooting media</i> .....	4
Financial benefits .....	4
Action points for growers.....	5
<b>SCIENCE SECTION.....</b>	<b>8</b>
<b>Introduction.....</b>	<b>8</b>
<i>Purpose</i> .....	8
<i>A few basics</i> .....	8
<i>A few complications</i> .....	9
Work in Year 3.....	12
<i>The search for the correct measure of "medium wetness"</i> .....	12
<i>The nature of the adverse effect of 'overwet' media on cuttings</i> .....	13
<i>Alternatives to sand as a capillary drainage substrate</i> .....	14
<b>Materials and Methods.....</b>	<b>15</b>
Experiment 1. Influence of evaporative demand on the response to air/water status of the medium of <i>Garrya elliptica</i> and <i>Convolvulus cneorum</i> .....	15
<i>Media</i> .....	15
<i>Drainage</i> .....	15
<i>Water and air contents</i> .....	15
<i>Plant material</i> .....	16
<i>Propagation environment</i> .....	17
<i>Transpiration demand</i> .....	17
<i>Irrigation</i> .....	18
<i>Experimental design</i> .....	18
<i>Pre-rooting destructive measurements</i> .....	18
<i>Testing for the presence of ethanol in cuttings</i> .....	19
Experiment 2. The effect of rooting medium, drainage and transpiration demand on ethanol production in cuttings of <i>Convolvulus cneorum</i> .....	20
<i>Media</i> .....	20
<i>Drainage</i> .....	20
<i>Water and air contents</i> .....	20
<i>Plant material</i> .....	20
<i>Transpiration demand</i> .....	20
<i>Experimental design</i> .....	21
<i>Monitoring ethanol concentration and other physiological measurements</i> .....	21
Experiment 3. The effect of rooting medium, drainage and transpiration demand on ethanol production in cuttings of <i>Fremontodendron</i> 'California Glory'.....	22
<i>Media</i> .....	22
<i>Drainage</i> .....	22
<i>Water and air contents</i> .....	22
<i>Plant material</i> .....	23
<i>Transpiration demand</i> .....	23
<i>Experimental design</i> .....	23
<i>Monitoring ethanol concentration and other physiological parameters in the cuttings</i> .....	24
Experiment 4. Factors affecting contact between rooting media and drainage sand.....	24
<i>Medium</i> .....	24
<i>Drainage</i> .....	24
<i>Sample holders</i> .....	24
<i>Procedure</i> .....	25
<i>Treatments</i> .....	26
Experiment 5. On the problem of contact between rooting media and capillary matting .....	27

Medium .....	27
Drainage treatments .....	27
Procedure .....	28
<b>Results and Discussion.....</b>	<b>29</b>
Experiment 1. Influence of evaporative demand on the response of <i>Garrya elliptica</i> and <i>Convolvulus cneorum</i> to air/water status of the medium.....	29
(a) <i>Garrya elliptica</i> .....	29
Rooting and basal necrosis.....	29
Physical properties .....	30
Correlating cutting behaviour with physical properties .....	31
(b) <i>Convolvulus cneorum</i> .....	35
Rooting and basal necrosis.....	35
Stem density.....	36
Ethanol in cuttings and its interpretation .....	37
Correlating cutting behaviour with physical properties .....	37
Experiment 2. The effect of rooting medium, drainage and transpiration demand on ethanol production in cuttings of <i>Convolvulus cneorum</i> .....	43
Rooting and basal necrosis.....	44
Ethanol and acetaldehyde in basal stem segments.....	44
Experiment 3. The effect of rooting medium, drainage and transpiration demand on ethanol production in cuttings of <i>Fremontodendron</i> 'California Glory' .....	48
Rooting and basal necrosis.....	48
Ethanol and acetaldehyde.....	50
Experiment 4. Factors affecting contact between rooting media and drainage sand.....	53
Time course.....	53
Treatment effects on drainage.....	54
Experiment 5. On the problem of contact between rooting media and capillary matting .....	55
Increase in air content due to drainage.....	56
The 'problem' of contact.....	56
<b>Additional analysis of data from earlier years.....</b>	<b>57</b>
<b>Conclusions.....</b>	<b>58</b>
<b>GLOSSARY : TERMS, ABBREVIATIONS AND PRODUCTS USED .....</b>	<b>62</b>
<b>REFERENCES.....</b>	<b>64</b>

# GROWER SUMMARY

## Headline

Conditions in the medium can make the difference between 0% and 100% rooting of cuttings but this cannot be explained by differences in any single factor such as air content. Capillary drainage is widely beneficial and more hygienic alternatives to the traditional sandbed should make it more acceptable to growers. Localised oxygen starvation is common but is not responsible for all problems with rooting media.

## Background and expected deliverables

It has been estimated that about 25% of the 200 million HNS cuttings taken every year fail to root, though failure rates vary from nursery to nursery and from crop to crop. From experience, propagators know that the rooting medium, and the management of its water content, can have a profound influence on rooting failure rates. They modify their media in an attempt to reduce losses but useful basic principles and practical guidelines are lacking. This project addressed this need.

The nature of the adverse conditions in an 'overwet' medium has not been identified, despite a large volume of research comparing rooting in different media. It is often assumed to be poor aeration, leading to insufficient oxygen reaching the tissues in the cutting, but good evidence is lacking. Neither have experiments identified particular media that perform consistently better than others.

The expected deliverables from this project were:

- A technique for determining the water release characteristics of media adapted for the conditions under which rooting media are used, i.e. loosely packed media at very low suctions (0 to 10 cm of water).
- A simple index of drainage characteristics of rooting media, equivalent to AFP for growing media.
- Insights into the processes by which rooting media influence survival and rooting of cuttings

- Insights into the way that the aerial environment (evaporative demand and rate of water deposition from mist or fog) influences the optimum choice of rooting medium
- Guidelines for adjusting rooting mixes to achieve particular drainage characteristics
- Guidelines for correcting problems associated with rooting media and for selecting media and drainage arrangements which minimise the potential for problems

As well as the immediately applicable results, insights into role of the medium in rooting provide a foundation for further practical progress in this area.

## **Summary of the project and main conclusions**

### ***Physical characteristics of media***

A suitable method was developed for determination of the physical properties of rooting media based on a set of four 'tension tables' (or 'sand tanks') at tensions from 0 to 10 cm of water. A method was also devised to measure accurately the particle density of materials that float (e.g. perlite), so that total porosity and hence air content could be determined accurately. Water release curves (WRC) were generated for 24 media, key features of which are summarised in Figure 1. The main conclusions were:

- The volumetric air contents (VAC) at tensions of 2.5 and 10.0 cm of water provide a good index of drainage characteristics for rooting media (e.g. VAC<sub>2.5/10.0</sub> for fine peat is 3.6/16.6 and for 50:50 peat:perlite is 10.5/22.7).
- The two figures indicate the air contents that can be expected at the base of the cutting on a non-capillary surface (VAC<sub>2.5</sub>) and on a deep sandbed (VAC<sub>10.0</sub>).
- Addition of perlite or vermiculite to fine peat greatly increased the air content of the medium but only if the proportion of additive was 50% or more.

### ***Capillary drainage on the propagation bed***

In practice, rooting media may not behave exactly as predicted by their WRC, so experiments were conducted to compare actual and predicted drainage on sand and capillary matting in various configurations. The main conclusions were:

- Heavy wetting by mist or fog causes a small increase in water content compared to predicted values but not enough to invalidate the use of WRCs.
- In rooting trials, water contents were generally *lower* than predicted, suggesting that in practice media rarely become fully wetted. This is surprising and deserves further study as it may contribute to variability.
- It is not difficult to achieve adequate capillary contact between the rooting medium in a pot or tray and a capillary sandbed. Raking the sand before placing trays will help to ensure physical contact with every cell.
- The thickness of capillary matting is not enough to generate substantial suction but a 'tail' hanging below the main area of the mat generates additional suction. With a 3 cm tail, the air content of pure peat was increased by 8% (v/v).
- Capillary matting with a tail was less effective than the equivalent depth of fine sand. We have been unable to identify the reason for this.

### ***Rooting media trials***

Some of the media for which water release curves had been determined were selected for rooting trials with rot prone subjects (*Convolvulus cneorum*, *Fremontodendron* 'California Glory' and 'Pacific Sunset', and *Garrya elliptica* 'James Roof'). To look for interactions between rooting medium composition and other factors, these trials included contrasting aerial environments and drainage. The main conclusions were:

- Effects of the rooting media on rooting and rotting were very variable, indicating strong interactions with other factors and no medium was consistently better, either in terms of higher rooting percentage or reduced basal rotting. In separate experiments (see below) pure vermiculite was consistently better than peat or peat:vermiculite mixes in both respects.
- The most consistent response was an increase in rooting associated with capillary drainage, which increased the air content of all the media.
- Severe rotting was sometimes due to drought stress in an over-dry aerial environment.

### ***Principles underlying the response to rooting media***

To separate different components of the 'wetness' of a rooting medium (i.e. water content, water tension and air content) required media which had contrasting water release characteristics but were otherwise similar. Mixtures of peat and vermiculite were eventually selected because they are physically stable and of direct relevance to the industry. Physiological measurements were made on the basal 30 mm of the cutting stem to look for early changes that influence whether cuttings root or rot. For example, the concentration of ethanol in the stem tissue was measured as a test for oxygen starvation. The main conclusions were:

- The water tension and the air content of the medium had separate and independent effects on rooting.
- The effects on rooting and rotting of the proportion of peat in the medium could not be explained entirely in terms of physical properties. This suggests that there is an additional chemical, pH or micro-biological effect involved.
- Rooting increased and rotting declined as air content increased but the association was weak and did not fully account for the benefit of capillary drainage.
- There were strong interactions between factors, e.g., the benefit of capillary drainage on *Convolvulus* was much larger at low evaporative demand than at high demand. (Figure 2). Such interactions emphasise the need to trial media under a wide range of conditions.
- Measurements of ethanol in basal stem segments suggest that water seeping into the air-filled spaces between cells causes localised pockets of oxygen starvation.
- High ethanol in stem tissues was associated with poor rooting but the correlation was weak (e.g. Figure 3).
- Further work is required to find a reliable way of judging whether conditions are near optimal or not.

### **Financial benefits**

This project has highlighted the benefits of capillary drainage in the propagation environment and suggested ways in which it can be made compatible with high hygiene standards. Adopting capillary drainage will result in:

- Cost savings from reduced wastage.

- More consistent rooting, potentially leading to more uniform liners.
- More rapid bulking up of material that is in short supply due to changes in consumer demand.
- Reduced dependence on imported liners.

### **Action points for growers**

- **Capillary drainage** dramatically **increases the air content** of rooting media, especially in shallow trays. Combined with a direct benefit of the greater soil water tension, this will generally improve rooting and reduce rotting.
- A sandbed provides **the most reliable capillary drainage system** but capillary matting has a useful effect if a **hanging tail** can be formed to increase the water tension at the surface of the mat. 5 cm deep channels at ~50 cm intervals along a propagation bed would allow ‘tails’ to be formed.
- Alternatively, capillary matting could be used on top of a smooth and level sandbed as an easily replaced and hygienic surface.
- If adding a coarse material to peat to increase aeration, always add at least an equal volume of the coarse material (e.g. perlite). **Below 50%, the coarse particles have very little effect on air content.**
- Surprisingly good results were obtained with pure vermiculite (Pro Gro Medium). Where rotting is a persistent problem, vermiculite may be worth testing.
- **Rotting is not necessarily a sign that the medium is too wet.** It can also be a sign that the evaporative demand of the aerial environment is too high leading to desiccation stress.
- **The most reliable approach to managing the propagation environment** as a whole is to ensure that there is sufficient mist or fog to avoid drought stress and then use an open-textured medium combined with a layer of sand to ensure good capillary drainage. Additionally, raising humidity in a mist house will reduce the need for heavy misting (see reports for HNS 76).
- If doing your own trials of different media, bear in mind that the outcome is sensitive to many factors. Look for **consistency of performance** under different conditions (e.g. a wet vs. dry zone on the mist bed) and on different occasions.



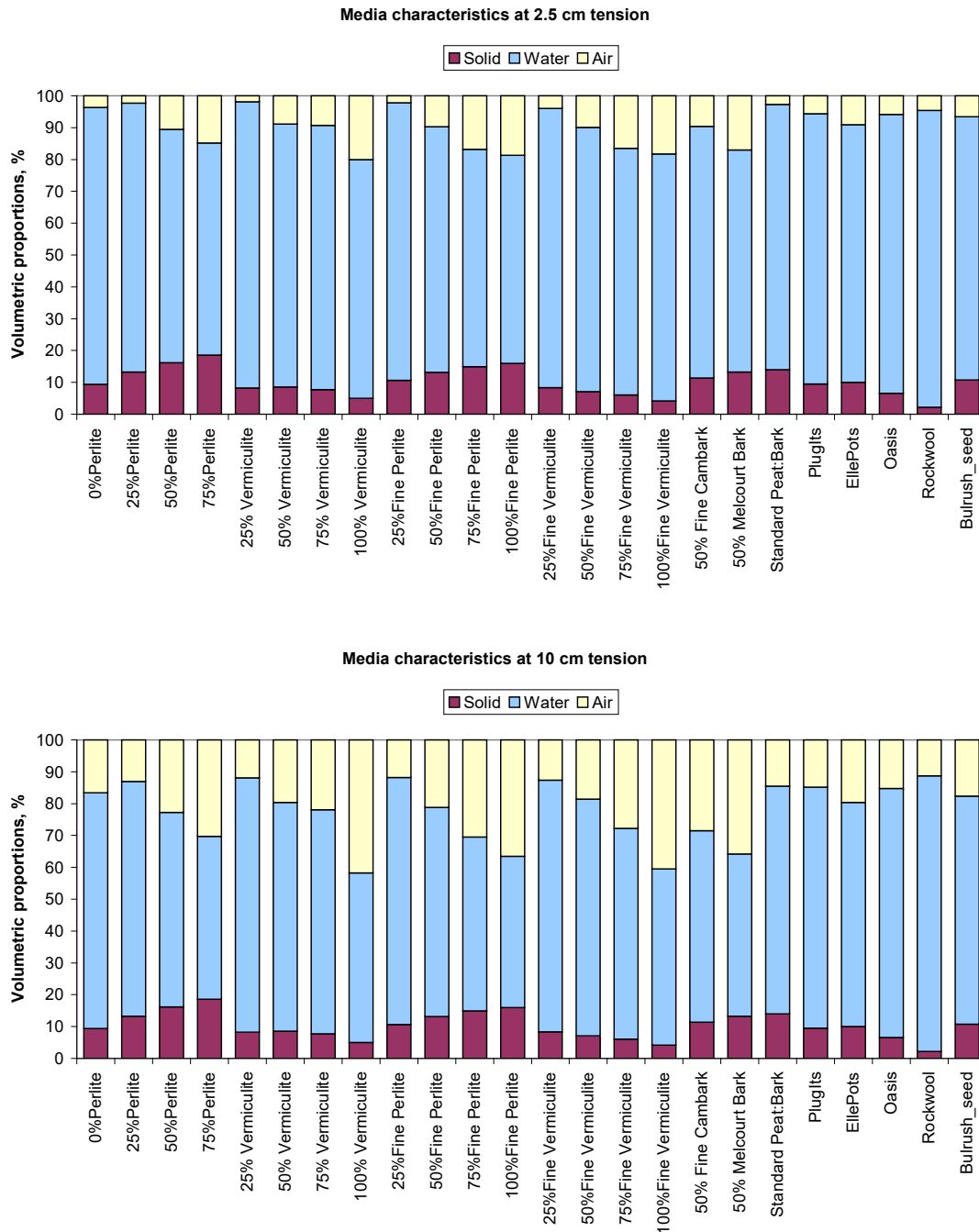


Figure 1. Summary of the key differences in water release characteristics among 24 rooting media. The bars show the volume occupied by solid particles, water and air as cumulative percentages of the total volume. The upper and lower graphs are for tensions of 2.5 cm and 10 cm respectively (measured at the top of a 2 cm deep sample and corresponding to a tray on a solid floor compared to 7.5 cm deep sandbed). 'Standard peat:bark' refers to a 50:50 mix of medium grade peat:fine grade Cambark

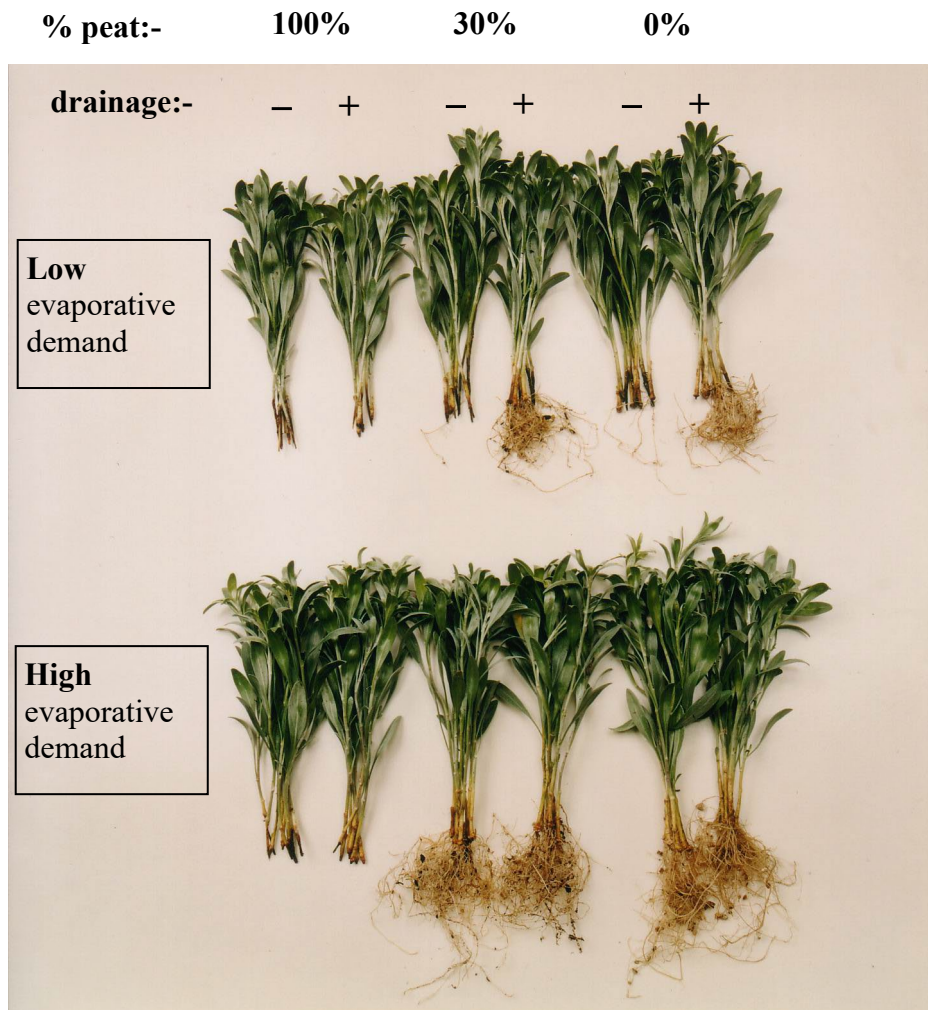


Figure 2. Cuttings of *Convolvulus cneorum* rooted in different ratios of peat and vermiculite,  $\pm$  capillary drainage, at two levels of evaporative demand.

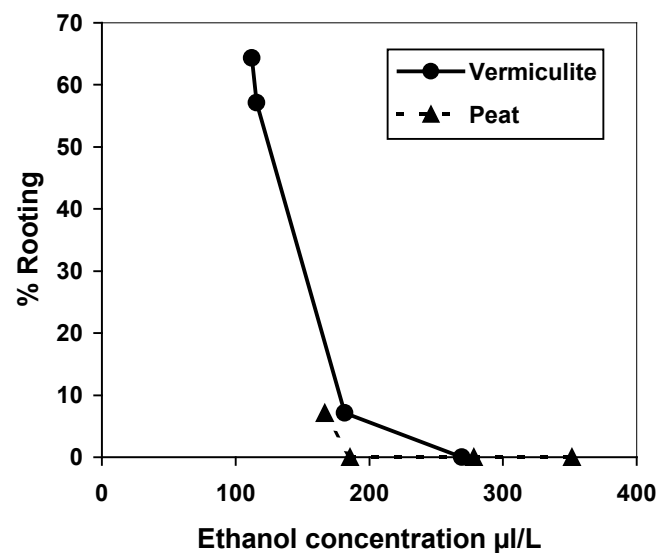


Figure 3. Rooting in relation to ethanol level in *Fremontodendron* 'California Glory'.

## SCIENCE SECTION

### Introduction

#### *Purpose*

The purpose of this project is to understand how the rooting medium contributes to successful rooting of cuttings so as to be able to recommend how growers should select and manage the medium for different types of cutting and different types of propagation environment.

The research was targeted closely on a number of current industry trends that are likely to render the drainage characteristics of the medium more critical:

- Many growers want to achieve more supportive aerial environments, which usually involves generous wetting of the foliage and hence increased danger of overwet media.
- There is a move away from sand under propagating trays, towards cleaner and more hygienic surfaces such as concrete which do not provide 'positive' (i.e. capillary) drainage.
- With increased use of shallow modular rooting trays, the depth of rooting medium below the base of the cuttings has decreased. This decreases the water tension and increases compaction of the medium at the base of the cutting, both of which tend to decrease aeration.

#### *A few basics*

The basic requirements of the rooting medium are:

- to hold the cutting upright
- to supply water
- to supply O<sub>2</sub> (oxygen) and remove CO<sub>2</sub> (carbon dioxide)

Therefore, mechanical strength, **water content**, and **air content** are likely to be the important properties. This helps us focus on the properties that determine air and water contents:

- The total volume of pores accessible to air and water (i.e. excluding closed air pockets that exist in materials such as pumice and perlite), known as the total porosity.
- The proportion of pores that are filled with water.
- This in turn depends on the size of the pores: the larger the pore the smaller the force required to pull the water out of it (as in a glass capillary tube).
- The forces tending to draw water out of the medium. In a rooting medium, gravity is the main source of such force, and its size depends on the height of water-filled pores which are acting like a hanging water column or siphon.
- If the propagation tray is on a non-capillary substrate, such as a solid floor or coarse grit, then the water columns ends at the base of the tray
- If the tray is in intimate contact with a capillary substrate such as fine sand then the hanging water columns can be much extended and drainage thereby improved.
- Therefore, the ratio air and water in the pores depends partly on the nature of the medium and partly on the drainage regime.

### *A few complications*

This simple model of the air/water relations of rooting media ignores a number of complicating factors. These are summarised diagrammatically in Figure 4

Firstly, water uptake by the cutting. This represents an additional force removing water from the system and, since it alters conditions in the immediate vicinity of the cut base of the cutting, it is potentially of great importance. The extent to which local depletion of water content occurs depends partly on the rate of transpiration and thus on the aerial environment to which the cutting is exposed. However, it will also depend on the rate at which water moves in from the surrounding medium to replace what has been taken up which depends on the hydraulic conductance of the medium. The hydraulic conductance increases rapidly with increasing water content so that significant localised drying around the base of the cutting will not occur if the medium is very wet to start with. Work with an artificial cutting suggested that local

drying in a pure peat medium does not start to become significant until the water content drops below about 70% (Thomas and Harrison-Murray, 1995). Therefore, it is unlikely to help maintain aeration of a medium that is too wet. Instead, it may add to the water stress suffered by cuttings exposed to high evaporative demand (i.e. sub-optimal aerial environment)

The second major complicating factor is the intermittent addition of water to the system in the form of mist or fog. This raises the water content above that predicted from laboratory determinations of drainage characteristics (i.e. the water release curve) by an amount that is hard to predict.

The most important area of uncertainty is how wet the medium can be before it starts to suppress rooting. Indeed, it is not known whether this depends on the volume of pores that remain filled with air, or the water content, or the tension with which the water is held. For a given medium, these three parameters are uniquely related and it is necessary to compare markedly different media to attempt to study their separate effects. One of the aims of the project was to attempt to achieve this separation though, from the outset, it was recognised that this would not be straightforward and success could not be guaranteed.

Despite such complexities, nurserymen have no doubt that the rooting medium, and the management of its water content, can have a profound influence on rooting failure rates. As a result they modify their media in an attempt to reduce losses. However, most recognise that there is little rationale behind these adjustments because useful basic principles and practical guidelines are lacking. This project addresses these needs.

To be in a position to offer robust guidance to practical nurserymen it was essential that we obtain a clearer understanding of the many complicating factors that are summarised in Figure 4. This requires an emphasis on the interactions between different parts of the system, which is central to this project.

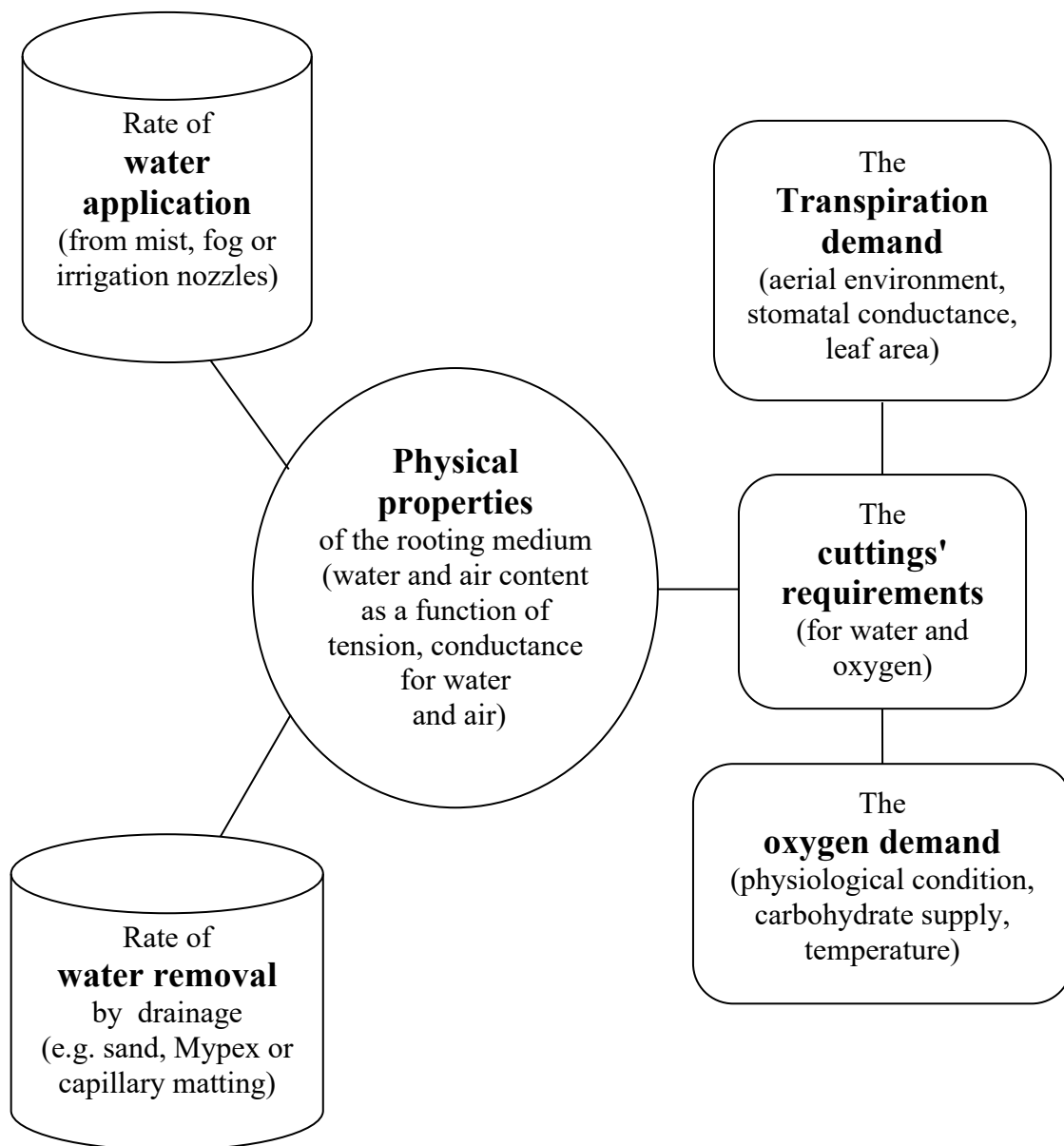


Figure 4. Model of the ways in which the influence of the physical properties of the rooting medium is predicted to interact with other elements of the propagation environment and by physiological differences between cuttings (between cultivars, stockplants, time of year, etc.)

### **Work in Year 3**

#### ***The search for the correct measure of "medium wetness"***

The primary objective in Year 3 was to determine which aspect of the wetness of a rooting medium has the greatest influence on the rooting process. In purely physical terms, the alternatives are basically the water content itself, the air content, or the tension with which water is held. When the water content of a particular medium changes then all three of these factors change and it is impossible to identify which aspect(s) of wetness actually influence cuttings. Identifying their separate effects requires experiments using a selection of media with contrasting water release curves, combined with drainage arrangements to create at least two levels of water tension. It had been hoped that different grades of sand and gravel would provide the required range of water release characteristics whilst being chemically identical. However, over the very narrow range of tensions that are of practical relevance in propagation, suitable sands could not be identified. Instead, we opted to work with mixtures of peat and vermiculite. These have the required drainage properties but are less physically stable and are not chemically identical. It was decided that this disadvantage was outweighed by their much greater commercial relevance as media for practical propagation.

Vermiculite was chosen in preference to the more widely used perlite for technical reasons. The cells within perlite granules are neither freely accessible to water nor completely closed so that water continues to be absorbed into particles over many months. This makes it difficult to monitor the air content of media containing perlite. The water release curves of peat:perlite mixtures are very similar to those of peat:vermiculite (Harrison-Murray et al., 2001), so that conclusions from experiments with peat:vermiculite mixes are likely to be applicable to peat:perlite mixtures.

Much of the variability in results of experiments with rooting media probably arises because of interactions with the aerial environment, particularly the extent to which evaporative demand is suppressed and with it transpiration from the cuttings. It was therefore essential to include variation in evaporative demand in experiments aimed at understanding the way in which the rooting medium influences the survival and rooting of cuttings. In Year 2, the variation in evaporative demand was created by

variation in radiation and leaf wetting in a special controlled environment chamber (the G-CPE: for details see Harrison-Murray, 2001). In Year 3, to accommodate a greater number of media, some of the experiments were transferred to a large fog house under natural illumination. In this facility (the 'Agritech' ventilated fog house) the decline in wetting with distance away from the fogger (a fan-assisted spinning nozzle design) was exploited to impose variation in evaporative demand on the cuttings.

### ***The nature of the adverse effect of 'overwet' media on cuttings***

If we understood what happens to the tissues of cuttings in situations where rooting is suppressed by an unsuitable medium, particularly an overwet medium, then it would make it much easier to design the ideal medium and drainage system. With this aim in mind, various hypotheses have been tested by making additional measurements on cuttings during the early stages of rooting, long before any roots emerge.

The primary hypothesis was that, in one way or another, an 'overwet' medium leads to some degree of oxygen starvation in the tissues which can either lead to death of cells (eventually visible as rotting) or reduce the amount of chemical energy available for physiological processes including root initiation. If this is the case then it is likely that the cells affected will switch over to using anaerobic respiration as a defence mechanism for surviving under low oxygen supply (hypoxia). Anaerobic respiration differs from normal aerobic respiration in being much less efficient and in producing ethanol as a waste product. Ethanol is not normally present in tissues which are well supplied with oxygen. Therefore, if ethanol is found, it represents strong evidence that somewhere in the tissues there are cells which are suffering hypoxia. Testing for the presence of ethanol is not difficult so that ethanol is a convenient 'marker' of tissues which have been starved of oxygen.

The secondary hypothesis was that hypoxia only occurs when the proportion of air filled pores in the medium falls below some critical threshold. The rate of oxygen diffusion through air is about 1000 times greater than the rate of diffusion through water so that the oxygen supply to the cutting depends on the air filled pores. The 'rule of thumb' for field soils is that an air content of 10% is generally enough to ensure sufficient oxygen reaches roots.



Therefore, if hypoxia (and therefore ethanol) occurs in a medium with an air content above about 10%, it would imply that there is another major barrier to oxygen diffusion apart from the medium itself, which must be located either around or within the cutting. A barrier of this sort could develop if, for example, water moved into the spaces between cells which are normally filled with air. Any such displacement of air by water would increase the density of the cutting which should be detectable.

Measurements of the density of the stem near the base of the cutting were therefore made at the start and during the rooting period.

#### *Alternatives to sand as a capillary drainage substrate*

Work in the Year 2 showed that capillary matting was much less effective than sand as a means of increasing water tension and air content in the rooting media in contact with it. What was surprising was that this was true whether or not there was a tail of mat hanging below the surface of the bed to increase the tension applied. Further experiments in Year 3 examined whether poor contact between mat and rooting medium was the factor limiting the benefit achieved from matting.

## Materials and Methods

### **Experiment 1. Influence of evaporative demand on the response to air/water status of the medium of *Garrya elliptica* and *Convolvulus cneorum***

#### ***Media***

These were mixtures of peat (Shamrock sphagnum peat, medium grade) and vermiculite (Pro Gro medium grade), as follows:

1. 0:100 Peat:Vermiculite
2. 30:70 Peat:Vermiculite
3. 50:50 Peat:Vermiculite
4. 70:30 Peat:Vermiculite
5. 100:0 Peat:Vermiculite

In addition, our standard rooting medium was included to allow a cross-referencing to previous work:

6. 50:50 Peat:Bark (Shamrock peat, medium grade, and Cambark, fine grade)

No fertiliser or any other amendment was added to any of the media.

#### ***Drainage***

There were two drainage treatments:

- + 10 cm deep bed of fine sand over a polythene membrane
- To interrupt the capillary link between the medium and the sand layer, a double layer of coarse nylon mesh (Netlon) was placed beneath the trays to create a capillary break from the sand bed beneath

#### ***Water and air contents***

Whenever cuttings were removed for destructive measurements, the water content of the medium in the pots from which they were removed was determined. The pots were 9 cm square pots with a depth of 7.97cm and a volume of 280 cm<sup>3</sup>. The volume of medium was estimated by measuring gap between the surface of the medium and the rim of the pot. The medium was then oven dried at 80 °C for 48 h to determine

the water content by weight loss. The following calculations were then applied to estimate the air and water contents on a volume percentage basis.

Where,

V = volume of medium, cm<sup>3</sup>

d = depth of medium, cm

Gd = dry weight of medium, g

Gw = wet weight of medium, g

Dp = particle density (from previous measurements, 1.22 g cm<sup>-3</sup> for pure peat and 2.4 g cm<sup>-3</sup> for pure vermiculite)

Db = dry bulk density, g cm<sup>-3</sup>

TP = total porosity, % (v/v)

θv = water content, % (v/v)

Av = air content, % (v/v)

then,

$$V = 280 \times d / 7.97$$

$$Db = Gd / V$$

$$TP = (1 - (Db \times Dp)) \times 100$$

$$\theta v = (Gw - Gd) / V \times 100$$

$$Av = TP - \theta v$$

### ***Plant material***

*Garrya elliptica* 'James Roof'. Apical cuttings were collected on 20 August, 2001, from well established field hedges and cut to about 15 cm long, the basal cut being about 2 mm below a node.

*Convolvulus cneorum*. Apical cuttings were collected on 13 September, 2001, from well established stock plants growing under glass in 3 L pots. Cuttings were prepared to about 12 cm long, the basal cut being about 2 mm below a node

All cuttings were treated with 1.25 g L<sup>-1</sup> of IBA in a 50:50 (v/v) mixture of acetone and purified water. Cuttings from two groups of stockplants were mixed within each plot.

### ***Propagation environment***

This was a 'ventilated wet fog' system. A combination of humidification and wetting was provided by a modified version of the Agritech spinning nozzle fogger. This type of fogger has a high enough output (about 150 L h<sup>-1</sup>) to humidify incoming air during forced ventilation before it reaches the cuttings. In this way it was possible to maintain a very high humidity (virtually 100% rh) around the cuttings without excessive heat build up (< 35 °C). The fogger was primarily controlled by an evaposensors so that evaporative demand on cuttings remained relatively stable under varying weather conditions. However, the quantity of water deposited on the leaves of cuttings decreased with increasing distance from the fogger creating a reproducible gradient of evaporative demand from one end of the house to the other.

The house was a 7 x 19.5 m polytunnel, well sealed to retain humidity, with reflective shade (Ludvig Svenson OLS 60) fixed over the outside of the tunnel, in contact with the polythene. Combined with the absorption of light by the polythene and reflection by condensation and fog, this resulted in about 20% of outside light reaching the cuttings. There was an extract fan in one end of the house which drew in air through a shuttered air intake at the opposite end. The fogger was located next to the air intake to humidify incoming air.

### ***Transpiration demand***

To impose two levels of transpiration demand, trays of cuttings were placed in different locations in the ventilated fog house. For the 'high' transpiration demand treatment, a location was selected where some wilting of cuttings in the early stages of the propagation could be expected. For the 'low' transpiration demand treatment, cuttings were placed close enough to the fogger to make any signs of desiccation stress most unlikely.

The choice of location (Table 1) was guided by previous experience of the range of conditions which selected species can tolerate.

Table 1. Details of locations, within the ventilated fog tunnel, used to create contrasting levels of transpiration demand.

Plant subject	Transpiration demand	Distance from fogger, m	Water deposition $\mu\text{m} / \text{h}$
<i>Garrya elliptica</i> 'James Roof'	Low	3	500
	High	9	76
<i>Convolvulus cneorum</i>	Low	7.5	105
	High	12	25

### ***Irrigation***

To ensure that the differences in water deposition did not have a substantial influence on the water content of the media, all trays were irrigated generously once per day by hand (1 L per tray, equivalent to 6 mm of irrigation).

### ***Experimental design***

A randomised block split-plot design was used. Drainage treatments were applied to main plots of 30 cuttings and the media treatments were applied to sub-plots of 5 cuttings. The media were packed into 7 cm square pots (0.25 L) arranged in plastic carriers holding 30 pots (Empot carriers), which thus represented one main plot. The six media were allocated at random to rows of 5 pots within each carrier (the sub-plots). There were four blocks, giving a total of 20 cuttings per treatment combination.

In summary, it was a multifactorial design as follows:

6 media x 2 drainage regimes x 4 blocks x 5 cuttings

### ***Pre-rooting destructive measurements***

After 4 days (or 5 days in the case of *Convolvulus*) one cutting per block was removed for visual assessment of early signs of necrosis and destructive measurements.

Measurements included the fresh and dry weight of the basal 3 cm segment of stem and the entire cutting, the volume and mean diameter of the basal segment. The volume was measured using a method based on Avogadro's Principle. A spring-

loaded pair of forceps, held at a reproducible height above a bench by a stand, was used to grip the stem segment while it was submerged in a beaker of water. The beaker was on an electronic balance which had been previously zeroed with the empty forceps in place. The balance registered a force equal to the upthrust on the cutting segment, which is equal to the weight of water displaced. Assuming the density of water to be  $1 \text{ g cm}^{-3}$ , the registered weight in grams was numerically equal to the volume of the cutting segment in  $\text{cm}^3$ .

These data were used to calculate the density of the basal segment from the formula

$$\text{Density} = \text{weight} \div \text{volume}$$

The fresh weight density was of interest because it would be expected to increase if some of the intercellular air spaces in the cutting were becoming filled with water.

### ***Testing for the presence of ethanol in cuttings***

The day after the main experiment was set up, additional cuttings were planted to provide additional material for destructive harvests so that cuttings could be tested for the presence of ethanol. Only three media were represented in this part of the experiment:

- 0:100 Peat:Vermiculite
- 50:50 Peat:Vermiculite
- 100:0 Peat:Vermiculite

The methodology of ethanol analysis is described under Experiment 2 (below)

## **Experiment 2. The effect of rooting medium, drainage and transpiration demand on ethanol production in cuttings of *Convolvulus cneorum***

### ***Media***

This experiment was confined to the two extremes of the range used in Experiment 1:

100% Peat (Shamrock sphagnum peat, medium grade)

100% Vermiculite (Pro Gro medium grade)

No fertiliser or any other amendment was added.

### ***Drainage***

There were two drainage treatments:

- + 10 cm deep bed of fine sand over a polythene membrane
- To interrupt the capillary link between the medium and the sand layer, a double layer of coarse nylon mesh (Netlon) was placed beneath the trays to create a capillary break from the sand bed beneath

### ***Water and air contents***

These were measured as for Experiment 1

### ***Plant material***

*Convolvulus cneorum*. Apical cuttings were collected on 18 October, 2001, as described above (Experiment 1)

### ***Transpiration demand***

Cuttings were placed in the G-CPE facility, which has been described in detail previously (Harrison-Murray *et al.*, 1998). Briefly, it is a controlled environment facility designed specifically for studying the response of cuttings to environmental conditions during rooting. Fog injected at one end of a polythene chamber creates a gradient of wetting along its length (10 m) while, high pressure sodium lights are arranged to create a gradient of light across the width (2 m) of the chamber.

For this experiment, all cuttings were within the three highest light zones (L1 to L3, PPFD = 180 - 230  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) so that variation in transpiration demand was dominated by location on the wetting gradient. The locations selected were wetting

zones W1 (1 to 2 m from the fog nozzles) and W6 (8.5 to 9.5 m from fog nozzles). These zones correspond to approximate mean water deposition rates of  $380 \mu\text{m h}^{-1}$  and  $3 \mu\text{m h}^{-1}$  respectively.

### ***Experimental design***

The factorial structure of the experiment was:

2 media x 2 drainage regimes x 2 transpiration demands = 8 treatment combinations

Drainage was applied as a main plot treatment with media subplots randomised within the main plots. A subplot consisted of 3 rows of 5 cuttings in 9 cm pots in an Empot carrier. Transpiration demand depended on location within the G-CPE so that factor could not be replicated. Within each transpiration demand treatment the media and drainage treatments were replicated twice.

### ***Monitoring ethanol concentration and other physiological measurements***

At intervals over the course of propagation, 2 cuttings were sampled at random from each subplot (i.e. 4 per treatment combination) to determine the concentration of ethanol and other volatile substances in the tissues at the base of the cutting.

Adhering medium was wiped off and a 30 mm long segment was excised from the base. The basal segment was placed in a  $4 \text{ cm}^3$  vial (Chromacol) with a septum lid (i.e. a screw top with a PTFE coated silicone rubber septum set into it) loosely closed. When a set was complete, the vials were transferred to a controlled environment room at  $20^\circ\text{C}$  and the lids were closed tightly. In the same room were vials containing known concentrations of ethanol in water, which acted as calibration standards. There were also standards for acetaldehyde, another volatile metabolite that can be produced by the enzymic oxidation of ethanol.

Ethanol and acetaldehyde were quantified on a gas chromatograph (Pye Unicam PU4500, fitted with a four foot long, 0.25 inch. OD glass column packed with 10% OV3 on Chromasorb W, AW, 80 mesh). The carrier gas was water saturated nitrogen. The injector and column were held at  $50^\circ\text{C}$  and detector was at  $100^\circ\text{C}$ . The detector was a flame ionisation detector with a hydrogen flame. Under these



conditions ethanol had a retention time of 1.2 min. and acetaldehyde had a retention time of 0.9 min.

After at least 30 min. equilibration at 20 °C, a 1 ml sample of the air in each vial was drawn into a glass syringe and injected into the gas chromatograph. By comparison with the standard solutions, peak heights measured from a chart recorder were used to estimate the ethanol concentration in the basal segments of cuttings. This method depends on the assumption that the partitioning of ethanol between water and air phases will be the same for ethanol dissolved in the water of the stem segment as it is for ethanol dissolved in pure water in the standard solutions.

When all vials had been sampled, the volume and other destructive measurements described earlier (see Experiment 1) were carried out.

### **Experiment 3. The effect of rooting medium, drainage and transpiration demand on ethanol production in cuttings of *Fremontodendron* 'California Glory'**

#### ***Media***

Like experiment 2, the media were the two extremes of the range used in Experiment 1:

100% Peat (Shamrock sphagnum peat, medium grade)

100% Vermiculite (Pro Gro medium grade)

No fertiliser or any other amendment was added.

#### ***Drainage***

There were two drainage treatments:

- + 10 cm deep bed of fine sand over a polythene membrane
- To interrupt the capillary link between the medium and the sand layer, a double layer of coarse nylon mesh (Netlon) was placed beneath the trays to create a capillary break from the sand bed beneath

#### ***Water and air contents***

These were measured as for Experiment 1

### ***Plant material***

*Fremontodendron* 'California Glory' . Single node cuttings collected on 6 December, 2001, from stock plants growing in a twin-span polythene house. The shoot apex and first expanded internode were discarded and cuttings were prepared from the next three internodes, designated as N1, N2, and N3 (moving towards the base of the shoot). Average length was 7-8 cm and each cutting had one expanded leaf.

### ***Transpiration demand***

Transpiration demand was varied by using contrasting locations in the G-CPE facility, as for Experiment 2. For this experiment, all cuttings were within the three highest light zones (L1 to L3, PPFD = 180 - 230  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) so that variation in transpiration demand was dominated by location on the wetting gradient. The locations selected were wetting zones W1 (1 to 2 m from the fog nozzles) and W5 (7 to 8 m from fog nozzles). These zones correspond to approximate mean water deposition rates of 380  $\mu\text{m h}^{-1}$  and 4  $\mu\text{m h}^{-1}$  respectively. However, it is in the nature of the G-CPE that there is a continuous gradient of water deposition along the chamber and therefore within the designated wetting zones. To assess whether this might have influenced the results in an important way, detailed measurements of wetting were made around each tray of cuttings.

### ***Experimental design***

The factorial structure of the experiment was:

2 media x 2 drainage regimes x 2 transpiration demands = 8 treatment combinations

Drainage was applied as a main plot treatment with media subplots randomised within the main plots. A subplot consisted of 3 rows of 5 cuttings in 9 cm pots in an Empot carrier. Transpiration demand depended on location within the G-CPE so that factor could not be replicated. Within each transpiration demand treatment the media and drainage treatments were replicated twice. Each row of 5 cuttings was a consistent mixture of cuttings from different nodes (1 x N1, 2 x N2, and 2 x N3).

### ***Monitoring ethanol concentration and other physiological parameters in the cuttings***

Sampling and analysis were as described for Experiment 2. Additionally, a sample of 10 cuttings was taken from among the prepared cuttings before they were inserted into the medium (i.e. a 'day 0' sample).

### **Experiment 4. Factors affecting contact between rooting media and drainage sand**

Rooting media exposed to repeated wetting from above, in the form of mist or fog, do not reach the static equilibrium water content predicted from their water release curve. Instead, a dynamic equilibrium is established in which water is draining out of the medium at the same rate that it is being deposited from above. This experiment investigated whether the care taken to ensure good contact between the rooting media and the sandbed on which it is placed could influence the equilibrium water and air contents. This is relevant both to the methodology for quantifying the drainage properties of rooting media in the laboratory and to improving the drainage of media in practical propagation on the nursery.

#### ***Medium***

100% Peat (Shamrock sphagnum peat, medium grade)

#### ***Drainage***

10 cm of fine sand

#### ***Sample holders***

For each treatment half the samples (i.e. 3) were in sample holders made by cutting down a 7 cm square plastic pot, as used in Years 1 and 2. The other half were in new samples holders designed and constructed for the purpose.

The new sample holder, which is illustrated in Figure 5, was made from a 4 cm length of Perspex tube, internal diameter 4.3 cm. The base was a disc of 1 mm thick stainless steel in which there were 4 holes with a diameter of 5 mm. This provided a much more rigid sample holder than the original. The main benefit of this additional rigidity was that the volume of the sample could be defined precisely, using a plunger

to compact the sample to a depth of 20 mm +/- 0.1 mm (Figure 6). Also, a line was marked on the outside of the Perspex tube to indicate the correct height of the top of the sample. This allowed visual checks to be made for any swelling or slumping of the sample over the course of the experiment.



Figure 5. Rooting media samples on a sandbed for a drainage experiment. The sample at the front left is in one of the new sample holders made from Perspex tube, the others are the original sample holders made from the base of 7 cm square plastic pots.



Figure 6. Close up of the new sample holder, with the plunger and lid assembly, used to compress the samples to a precise depth, still in place.

### ***Procedure***

- i. Stage 1 - sample preparation.

Based on the previously determined water content of the stock of medium, the weight of medium required to achieve a dry bulk density of 1.1 g/cm<sup>3</sup> in each type of sample holder was determined. Samples of medium were weighed, packed into sample holders and compacted to a 20 mm deep layer. With the original sample holders this depth was approximate but the actual depth was determined as the

average of three measurements with an electronic depth gauge at the end of the experiment.

ii. Stage 2 - wetting up the samples

The samples were placed in empty seed trays which were then positioned at 0.5 to 1.0 m from the fog nozzles in the G-CPE (wetting zone W0) at low light level (zone L8 to L10). Here water was deposited onto the samples at about 0.8 mm/h, sufficient to wet them quickly but not enough to cause any slumping or other structural changes.

iii. Stage 3 - adjust to reduced wetting

The trays of samples were moved to wetting zone W1, 1.0 to 2.0 m from the fog nozzles, where water deposition averaged about 0.4 mm/h. The samples were kept in the trays for 24 h to measure the effect of the 50% reduction in wetting before moving on to the drainage stage

iv. Stage 4 - add capillary drainage

Samples were transferred to the sandbed, the method of transfer varying between treatments (see below).

v. Stage 5 - adjust to zero wetting

The fog system was turned off so that water deposition ceased.

All samples were removed briefly from the G-CPE at intervals of 1 to 3 days and weighed to monitor changes in water content. A petri dish was used to collect any water draining out during this process so that the weight would accurately reflect the water content prior to removing the sample from its standing surface.

### ***Treatments***

Control	Smooth sand surface (i.e. not raked); sample holder pressed in firmly without sliding or other special procedure (i.e. the same as used in all previous work)
No pressure	No force was applied to press the samples into the sand but otherwise as Control
Slide	Samples slid from side to side as well as applying downward pressure, but otherwise as Control

Rake	Sand surface loosened with a rake before sample put in place but otherwise as Control
Disturbed	Placed on the sand exactly as the Control but removed for additional re-weighing 4 times in the first 24 h of the drainage stages (stages 4 and 5).
Non-cleaned	Unlike all other treatments, the base of the sample holder was not brushed clean of sand before weighing, potentially leading to overestimation of the water content of the medium.

### **Experiment 5. On the problem of contact between rooting media and capillary matting**

An experiment in Year 2 indicated that the drainage benefit of capillary matting was much less than that of a sand bed. This was true even when the capillary matting was allowed to hang over the edge of the supporting surface to create a 3 cm 'tail'. Such a tail should act like a hanging water column in a siphon tube to generate the same suction as a 3 cm depth of sand.

The objectives of the present experiment were (i) to confirm the earlier result and (ii) to test whether poor contact between the capillary matting and the rooting medium was the cause of the poor performance of capillary matting.

#### ***Medium***

100% Peat (Shamrock sphagnum peat, medium grade) packed into the original type of sample holders

#### ***Drainage treatments***

**Sand** 3 cm layer of well compacted fine sand in a seed tray. This was on a double layer of course nylon mesh (Netlon) to ensure that there was no capillary link with the main sandbed on which the tray was placed.

- Capillary mat            Grey Fibertex capillary matting. A single sheet, 350 x 480 mm, was placed on a smooth flat sheet of rigid plastic, 310 x 450 mm, supported on two inverted seed trays. The excess matting hung over the edge of the sheet forming a 3 cm 'tail' .
- Capillary mat + sand    As above, except that fine sand was sprinkled over the surface of the matting and rubbed into the fibres. Excess sand was scrapped off so that the matting was still visible through the sand.

### ***Procedure***

- i.        Stage 1 - sample preparation.  
          Samples prepared in original samples holders, filled to a depth of 20 mm
- ii.       Stage 2 - wetting up the samples  
          The samples were placed overnight in empty seed trays which were then positioned at 0.5 to 1.0 m from the fog nozzles in the G-CPE (wetting zone W0) at low light level (zone L8 to L10).
- iii.      Stage 3 - capillary drainage + reduced wetting  
          Samples were transferred to one of the three drainage regimes and wetting was reduced to a minimal level (0.001 mm/h) in wetting zone W6, light zone L8 to L10. The samples were put in place by pressing firmly downwards without sliding.
- iv.      Stage 4 - adjust to zero wetting  
          The fog system was turned off so that water deposition ceased completely.

The samples were removed for weighing at intervals to monitor the drainage process. After weighing they were replaced with firm downward pressure

## Results and Discussion

### Experiment 1. Influence of evaporative demand on the response of *Garrya elliptica* and *Convolvulus cneorum* to air/water status of the medium

The objective of this experiment was to accumulate evidence from which to identify the crucial component(s) of the physical environment of the rooting medium that determine the fate of the cutting and the way that it is influenced by the aerial environment. The presence or absence of contact between the medium and a sandbed created two levels of soil water tension (SWT) and varying the ratio of peat and vermiculite in the medium altered the air content independently of SWT. The experiment was conducted in our ventilated fog house where distance from the fogger was used to create two levels of evaporative demand.

The experiment included two species with very different environmental requirements: (a) soft cuttings of *Garrya elliptica* that require heavy wetting to limit evaporative demand sufficiently to prevent wilting, and can then achieve close to 100% rooting; (b) *Convolvulus cneorum* that tolerates relatively high evaporative demand and which fails to root if wetting is heavy, probably because it suppresses transpiration excessively. Both subjects are prone to basal necrosis but roots are often seen emerging above a rotted base (i.e. with a 'dead peg'), suggesting that the link between rooting and the absence of rotting is not very close.

#### (a) *Garrya elliptica*

##### *Rooting and basal necrosis*

Rooting was on average greater at low evaporative demand but the effect was small compared to the benefit of a high proportion of vermiculite in the medium (Figure 7). Capillary drainage was beneficial if evaporative demand was low but had an adverse effect for cuttings in a low peat mix at high evaporative demand, presumably by restricting water supply too much. This interaction between evaporative demand and capillary drainage was significant ( $P=0.05$ ).



Visible basal necrosis was evident in all cuttings in the pure peat medium, irrespective of other conditions, but became progressively less frequent as the proportion of vermiculite increased (Figure 8). The marked dip in the curves in Figure 8 is due to a substantial additional benefit of bark in the mixture, relative to vermiculite. Since there was no clear difference in air or water content between the bark and vermiculite mixes (see Figure 11 and Figure 12), it is likely to be attributable to a chemical or microbiological factor in the bark that suppressed the activity of the saprophytic micro-organisms that contribute to basal necrosis.

Absence of capillary drainage more consistently reduced root number (Figure 9) than it did rooting percentage (Figure 7). Similarly, there was a larger and more consistent reduction in the extent of basal necrosis with reducing peat content and capillary drainage (Figure 10) than there was on the percentage of cuttings affected (Figure 8).

### ***Physical properties***

As expected, air content of the medium increased as the proportion of peat in the mix was reduced, and was increased substantially by capillary drainage, on average by 19.3% (Figure 11), matched by a corresponding decrease in water content (Figure 12). The highest air content observed *without capillary drainage* was about 33%, in pure vermiculite, and this was approximately the same as in 100% peat *with drainage*. Comparison of these treatments should indicate the effect of SWT, independent of air content. Reference to Figure 7 shows that 100% of cuttings rooted in pure vermiculite without drainage, compared to 39% and 64% in pure peat with drainage. This suggests that the direct effect of SWT is to reduce rooting substantially but this conclusion is at odds with the small and inconsistent effect of capillary drainage on rooting percentage in any one medium. A more realistic interpretation of the results as a whole is that the peat content of the mix influenced rooting in a way that cannot be attributed to either air or water content. On that assumption, the direct effect of the higher SWT generated by a 10 cm deep sandbed was a small increase in rooting.

The absolute values of air content were substantially higher than those predicted from water release curves (WRC). The present data were obtained by recovering the medium from a sample of pots when cuttings were removed to record rooting at the

end of the experiment. This suggests a number of possible causes of the discrepancy compared to the earlier laboratory based data, the more likely of which are:

1. It was impossible to ensure that there was no additional drainage or evaporation before the medium was recovered and weighed prior to oven drying.
2. Compared to the 2 cm layer of medium used for the WRC, the present samples included the full depth of a 7 cm pot and therefore a wider range of SWT.
3. Possible differences between the peat used for the WRC and that used in the present experiment.
4. Differences in packing of the media, though this is unlikely since the bulk densities were similar.
5. The media in this experiment may never have wetted up sufficiently to reach the drainage state represented by the WRC. However, this seems unlikely because all media were watered generously once per day to prevent any difference in the media associated with the level of evaporative demand.

#### ***Correlating cutting behaviour with physical properties***

In view of the uncertainty about which data would more closely match the conditions actually experienced by the base of the cutting, both sets were used to study correlations between air content and rooting or basal necrosis. From the graphs in Figure 13, it is clear that neither of the data sets provide a good prediction of either rooting or basal necrosis but measured values are more useful than those predicted from the WRC. In general, there was an increase in rooting with increasing air content but increased aeration created by capillary drainage did not consistently improve rooting to a high level. Similarly, necrosis declined as aeration increased and capillary drainage did not consistently prevent a high frequency of necrosis. Within either drainage treatment, there was quite a close relationship between necrosis and water content (Figure 13 F).

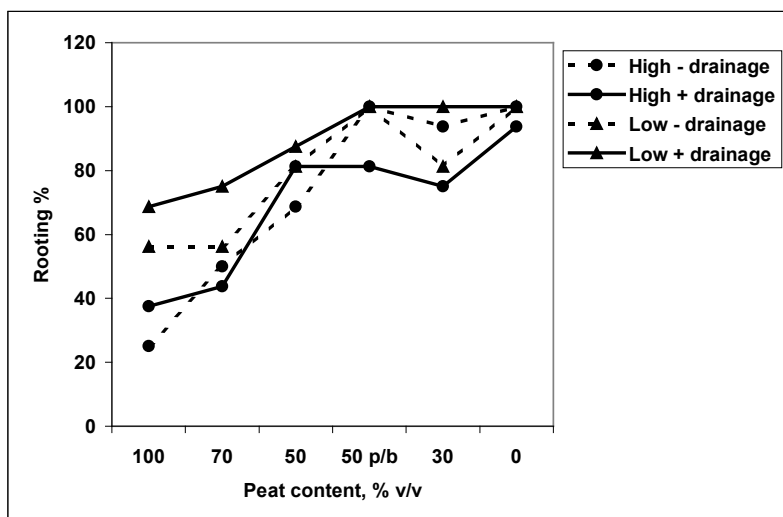


Figure 7. Rooting responses of *Garrya elliptica* 'James Roof' to rooting medium composition, as affected by evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Media were peat:vermiculite mixes except for '50 p/b' which was peat:bark. Significant effects: evaporative demand ( $P=0.002$ ), medium ( $P<0.001$ ), evaporative demand x drainage ( $P=0.05$ ).

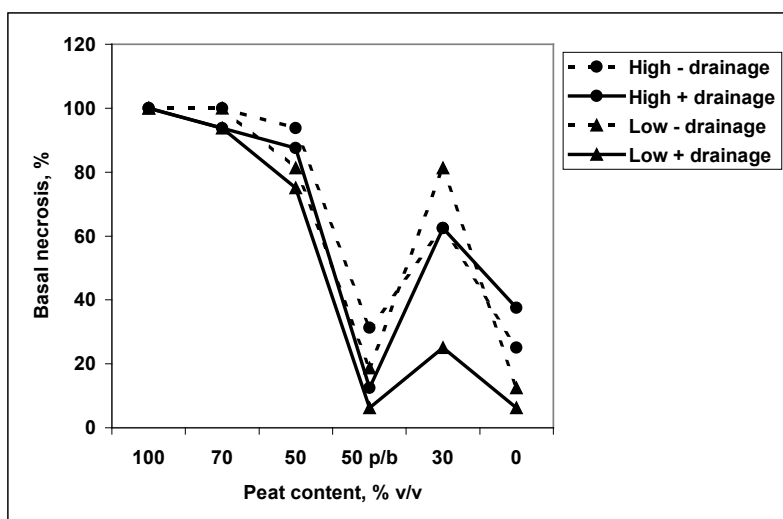


Figure 8. Effect of rooting medium composition on the frequency of basal necrosis (i.e. death leading to rotting) in cuttings of *Garrya elliptica* 'James Roof', as influenced by evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Media were peat:vermiculite mixes except for '50 p/b' which was peat:bark. Significant effects: medium ( $P<0.001$ ), drainage ( $P=0.01$ ).

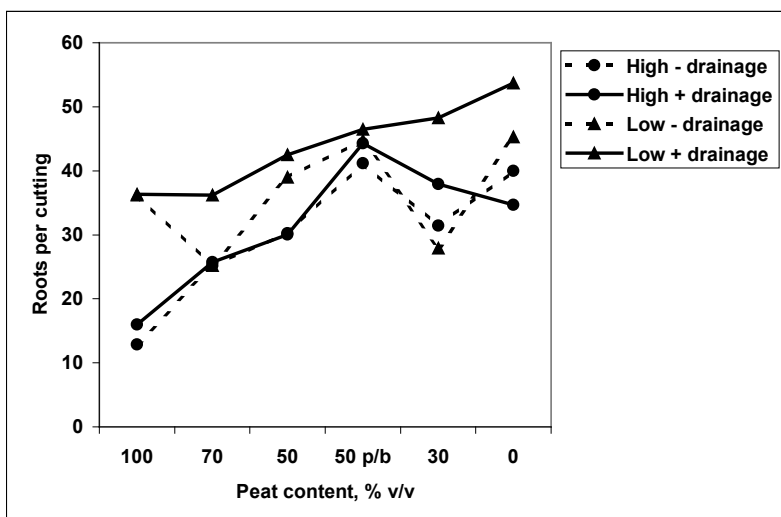


Figure 9. Effect of rooting medium composition on the number of roots per cutting in *Garrya elliptica* 'James Roof', as affected by evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Media were peat:vermiculite mixes except for '50 p/b' which was peat:bark. Significant effects: medium ( $P<0.001$ ), evaporative demand ( $P<0.001$ ), drainage ( $P=0.035$ ), drainage x evaporative demand ( $P=0.005$ ).

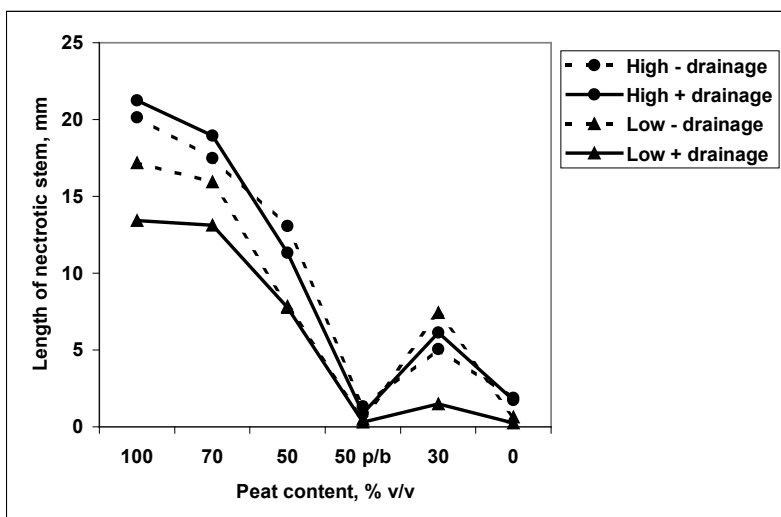


Figure 10. Effect of rooting medium composition on the extent of basal necrosis (i.e. rotting) in cuttings of *Garrya elliptica* 'James Roof', as affected by evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Media were peat:vermiculite mixes except for '50 p/b' which was peat:bark. Significant effects: medium ( $P<0.001$ ), evaporative demand ( $P<0.001$ ).

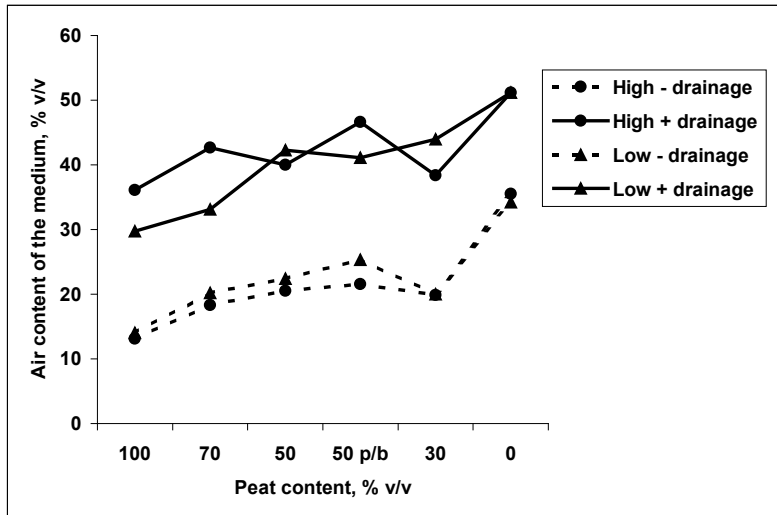


Figure 11. Air content of the rooting media at the end of Experiment 1. Plotted values are means of 3 pots (9 cm square pots) per treatment combination. Significant effects: medium ( $P < 0.001$ ), drainage ( $P < 0.001$ ).

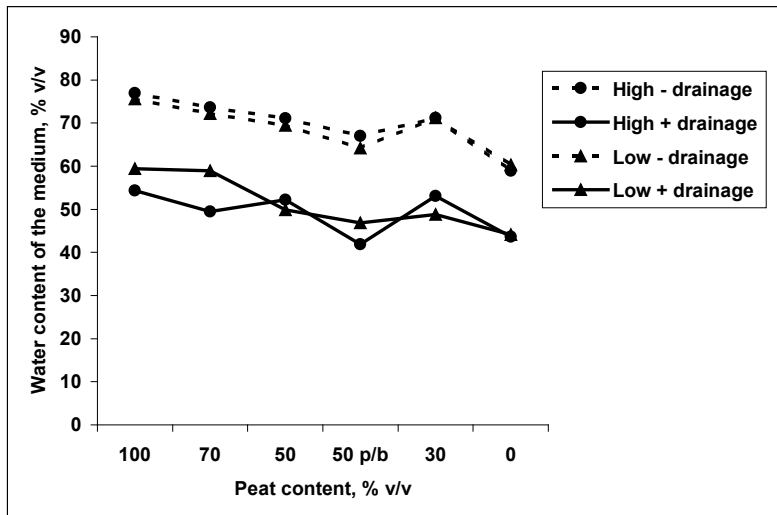


Figure 12. Water content of the rooting media at the end of Experiment 1. Plotted values are means of 3 pots (9 cm square pots) per treatment combination. Significant effects: medium ( $P < 0.001$ ), drainage ( $P < 0.001$ ).

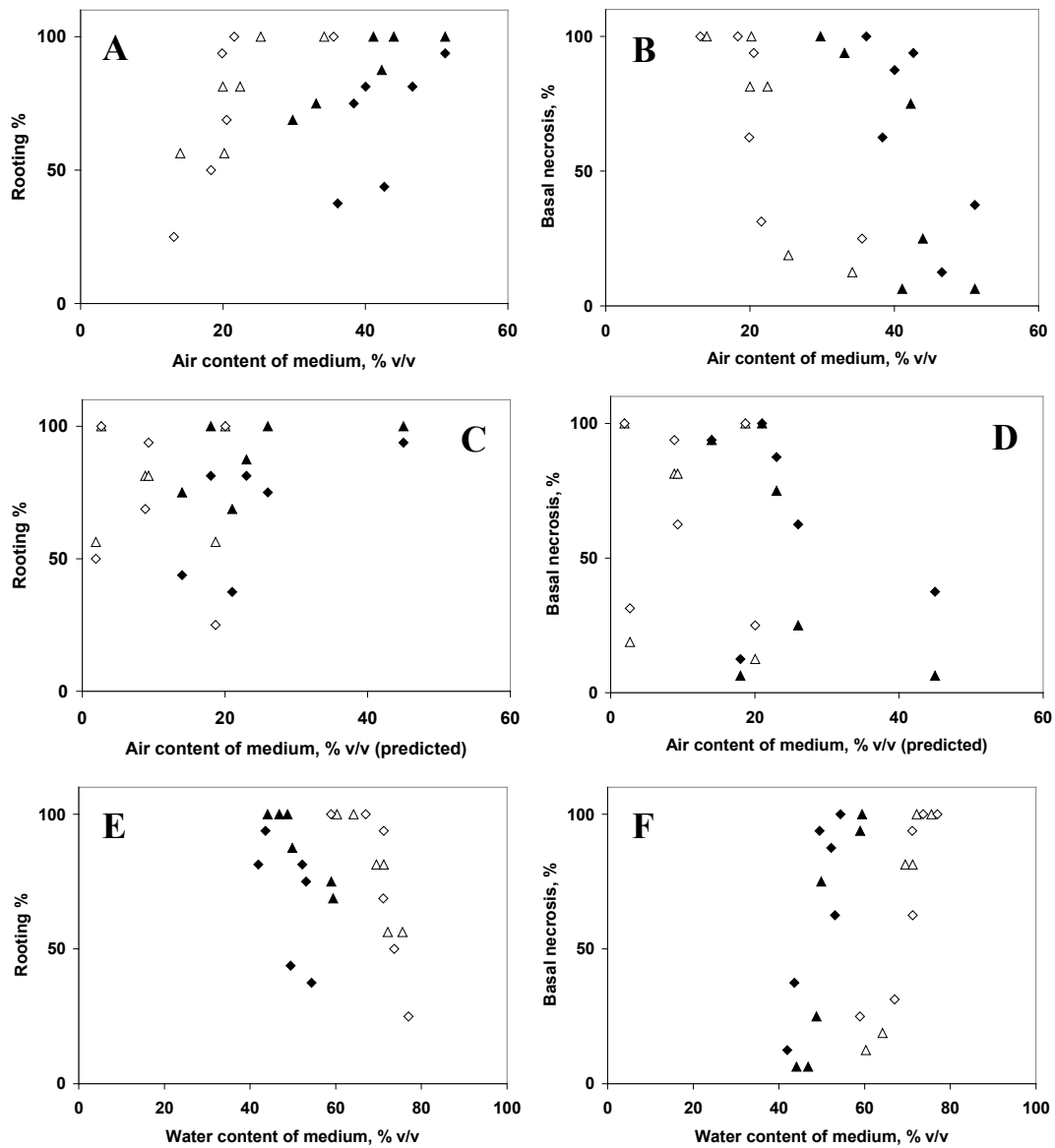


Figure 13. Rooting and basal necrosis (i.e. rotting) in *Garrya elliptica* 'James Roof', plotted against the air content (A and B) and water content (E and F) of the rooting medium. In C and D, the air content values are those predicted from water release curves for the same media determined in the laboratory. All other air and water contents were those determined at the end of the experiment. Key to symbols: Solid / open = + / - capillary drainage; triangle / lozenge = high / low evaporative demand. Plotted points represent means for different ratios of peat and vermiculite (n=16).

**(b) *Convolvulus cneorum***

***Rooting and basal necrosis***

As in *Garrya*, rooting of *Convolvulus* was greatest in pure vermiculite and least in pure peat but in other respects the responses were very different. Capillary drainage had a major effect that was mainly evident under low evaporative demand, where it

increased rooting percentage by up to 61%, and on average from 13% to 54% (Figure 14). By comparison, the average rooting percentage at high evaporative demand was 71.3% even without capillary drainage, rising to 80.6% with drainage. However, neither evaporative demand nor capillary drainage enabled any cuttings to root in pure peat.

These results demonstrate how highly interactive responses to rooting media can be. The interaction between drainage and evaporative demand is particularly clear in the photograph of representative cuttings from the experiment (Figure 16).

The frequency of basal necrosis declined gradually with decreasing peat content (Figure 15). The only exception to this trend was that, under high evaporative demand and with capillary drainage, replacement of 30% of the peat with vermiculite was enough to keep the incidence of basal necrosis to below 10% of cuttings, with little further improvement from reducing peat content any further. In contrast to the results for *Garrya*, the incidence of basal necrosis in the peat:bark mix was broadly in line with that in the peat:vermiculite mixes, though the extent of the necrosis was somewhat lower (Figure 17). Although basal necrosis occurred in 100% of cuttings in pure peat, irrespective of other treatment factors, the length of stem affected was reduced significantly by high evaporative demand and capillary drainage (Figure 17).

### ***Stem density***

The basal cut surface of a stem cutting exposes not only the cells of the internal tissues but the intercellular spaces between them. These are normally filled with air and provide a means of rapid movement of oxygen and carbon dioxide within the tissues. If water from the rooting medium were to infiltrate these air spaces then gas exchange within the tissues would be restricted and cells could run short of oxygen. In an attempt to determine whether this was happening and might be linked to necrosis or failure to root, the density of the basal 30 mm of stem was determined at 5 days after sticking. If air in intercellular spaces is replaced by water, the density of the tissue is increased.

The results shown in Figure 18 show a surprisingly high stem density was observed in cuttings from 100% peat, at high evaporative demand, plus drainage. However,

overall, there was no significant effect and it must be assumed that the high value was an error.

### ***Ethanol in cuttings and its interpretation***

A sub-experiment provided material for destructive harvesting to determine the ethanol content of the basal stem tissue. Ethanol, the alcohol produced yeast in brewing, is also produced by most higher plant cells when the supply of oxygen is insufficient to maintain normal aerobic respiration. Ethanol, therefore, has potential as a marker of the problem of poor oxygen supply. The results in Figure 22 show that ethanol was present in all treatments so that some restriction of oxygen supply was widespread. The ethanol concentration was highest in the cuttings in 100% peat and least in cuttings in 0% peat (i.e. pure vermiculite). However, the data was very variable and the difference between the media was only significant at high evaporative demand. There was quite a strong correlation between ethanol concentration and stem density (Figure 23), which suggests that the barrier to oxygen supply was probably largely water infiltrating into intercellular air spaces near the basal cut. It is tempting, therefore, to conclude that the reduction of ethanol level by high evaporative demand, evident in Figure 22, is attributable to removal of this excess water by transpiration. However, transpiration will also remove ethanol from the basal stem, transporting it to the leaves where it can evaporate or be metabolised. Therefore, while ethanol is a reliable marker of poor oxygen supply, small differences in ethanol concentration between treatments should be interpreted cautiously.

### ***Correlating cutting behaviour with physical properties***

None of the graphs in Figure 21 shows a close correlation between cutting behaviour and the air or water content of the rooting medium. However, there is a clear positive relationship between rooting and air content in graph A and a somewhat looser negative relationship between necrosis and air content. As with *Garrya*, the measured air content data were more useful than the values predicted from WRC.



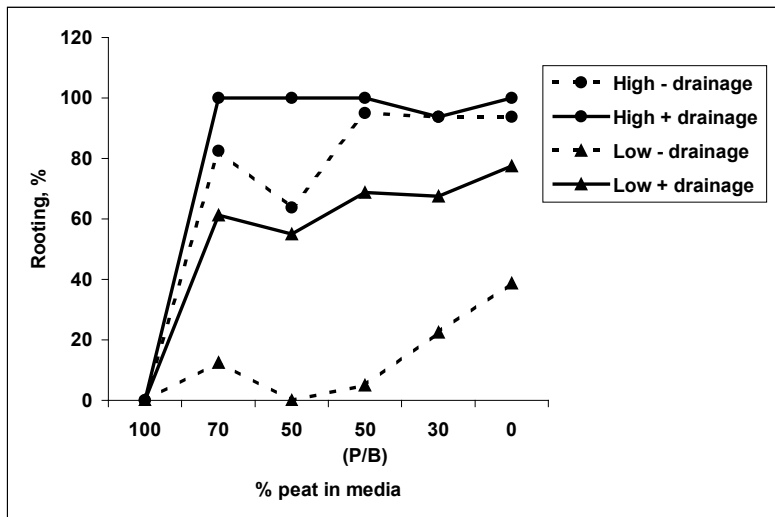


Figure 14. Rooting responses of *Convolvulus cneorum* to rooting medium composition, as affected by evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Media were peat:vermiculite mixes except for '50 (P/B)' which was peat:bark. Significant effects: medium ( $P=0.001$ ), drainage ( $P<0.001$ ), evaporative demand ( $P<0.001$ ).

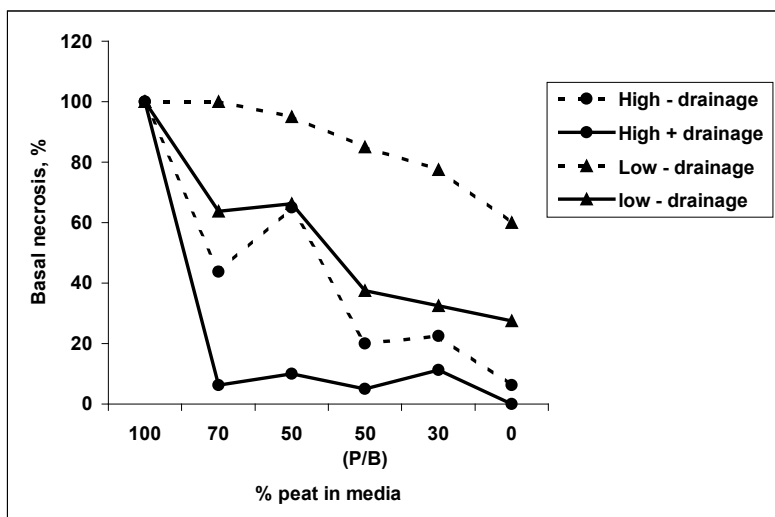


Figure 15. Effect of rooting medium composition on frequency of basal necrosis (i.e. rotting) in cuttings of *Convolvulus cneorum*, as affected by evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Media were peat:vermiculite mixes except for '50 (P/B)' which was peat:bark. Significant effects: medium ( $P<0.001$ ), drainage ( $P<0.001$ ), evaporative demand ( $P<0.001$ ).

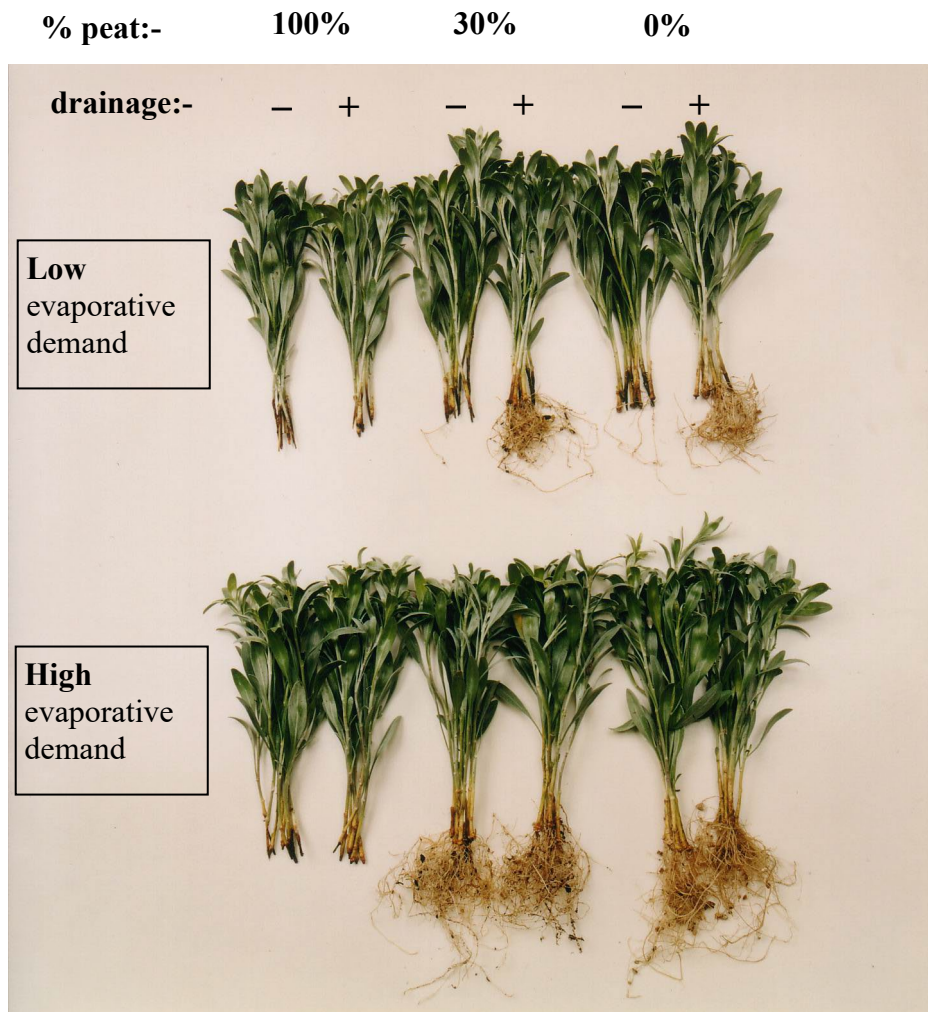


Figure 16. Cuttings of *Convolvulus cneorum* rooted in different ratios of peat and vermiculite,  $\pm$  contact with a sandbed to provide capillary drainage, at two levels of evaporative demand in a fog house. The close up shows extremes of the range of results: pure vermiculite with drainage (right) vs. pure peat without drainage (left).

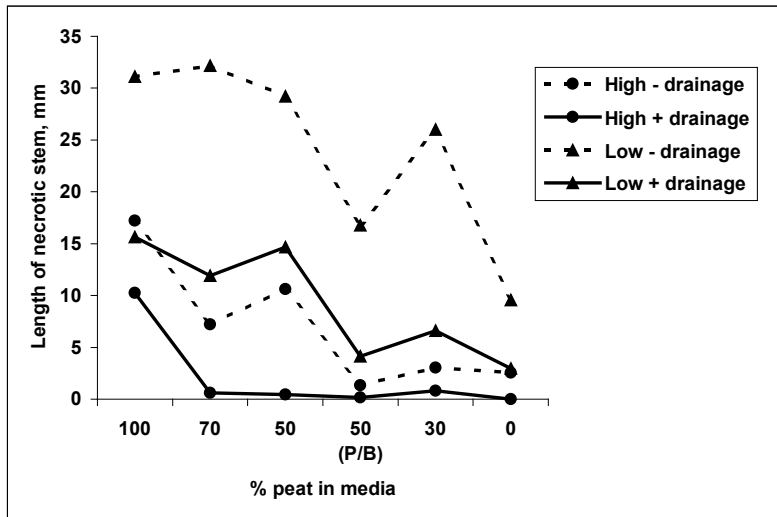


Figure 17. Effect of rooting medium on the extent of necrosis at the base of stem in *Convolvulus cneorum* cuttings, as affected by evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Media were peat:vermiculite mixes except for '50 (P/B)' which was peat:bark. Significant effects: medium ( $P<0.001$ ), drainage ( $P<0.001$ ), evaporative demand ( $P<0.001$ ), drainage x evaporative demand ( $P<0.001$ ), medium x evaporative demand ( $P=0.018$ ).

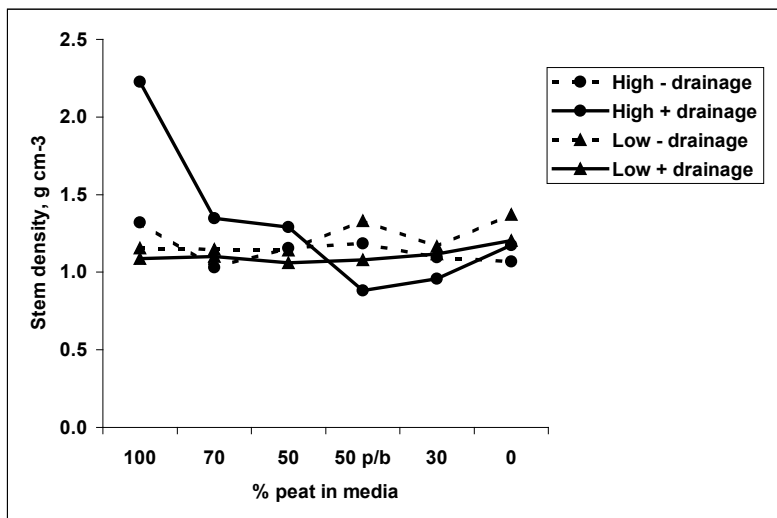


Figure 18. Fresh weight density of the basal 30 mm of the stem in cuttings of *Convolvulus cneorum*, on day 5 of Experiment 2. There were no significant effects.

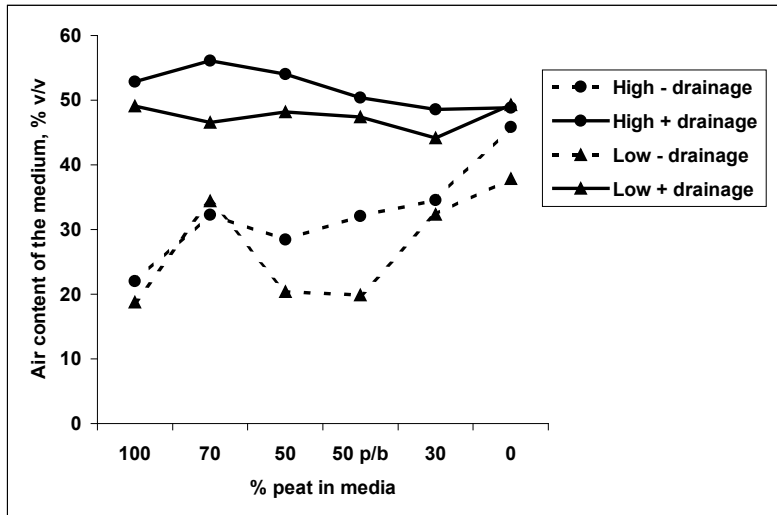


Figure 19. Air contents of the rooting media at the end of Experiment 1b. Plotted values are means of 3 pots (9 cm square pots) per treatment combination. Significant effects: medium (i.e. % peat,  $P=0.002$ ), drainage ( $P<0.001$ ), evaporative demand (i.e. high vs. low,  $P<0.001$ ), medium x drainage.

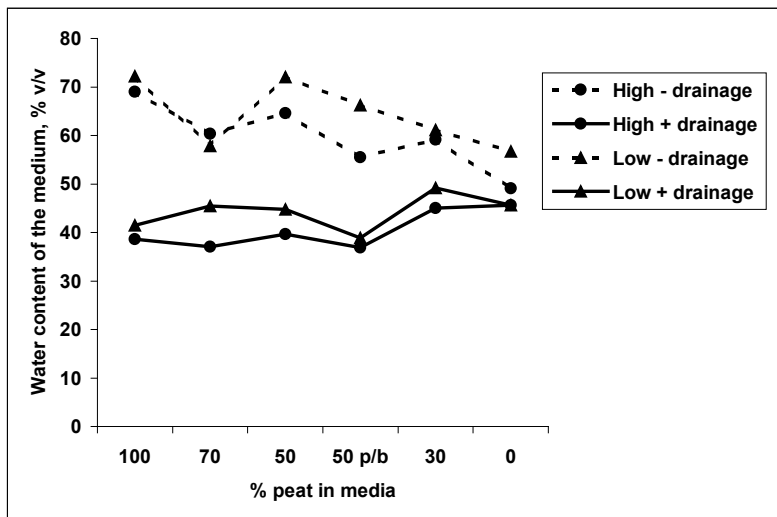


Figure 20. Water contents of the rooting media at the end of Experiment 2. Plotted values are means of 3 pots (9 cm square pots) per treatment combination. Significant effects: medium (i.e. % peat,  $P=0.012$ ), drainage ( $P<0.001$ ), evaporative demand (i.e. high vs. low,  $P=0.002$ ), medium x drainage ( $P<0.001$ ).

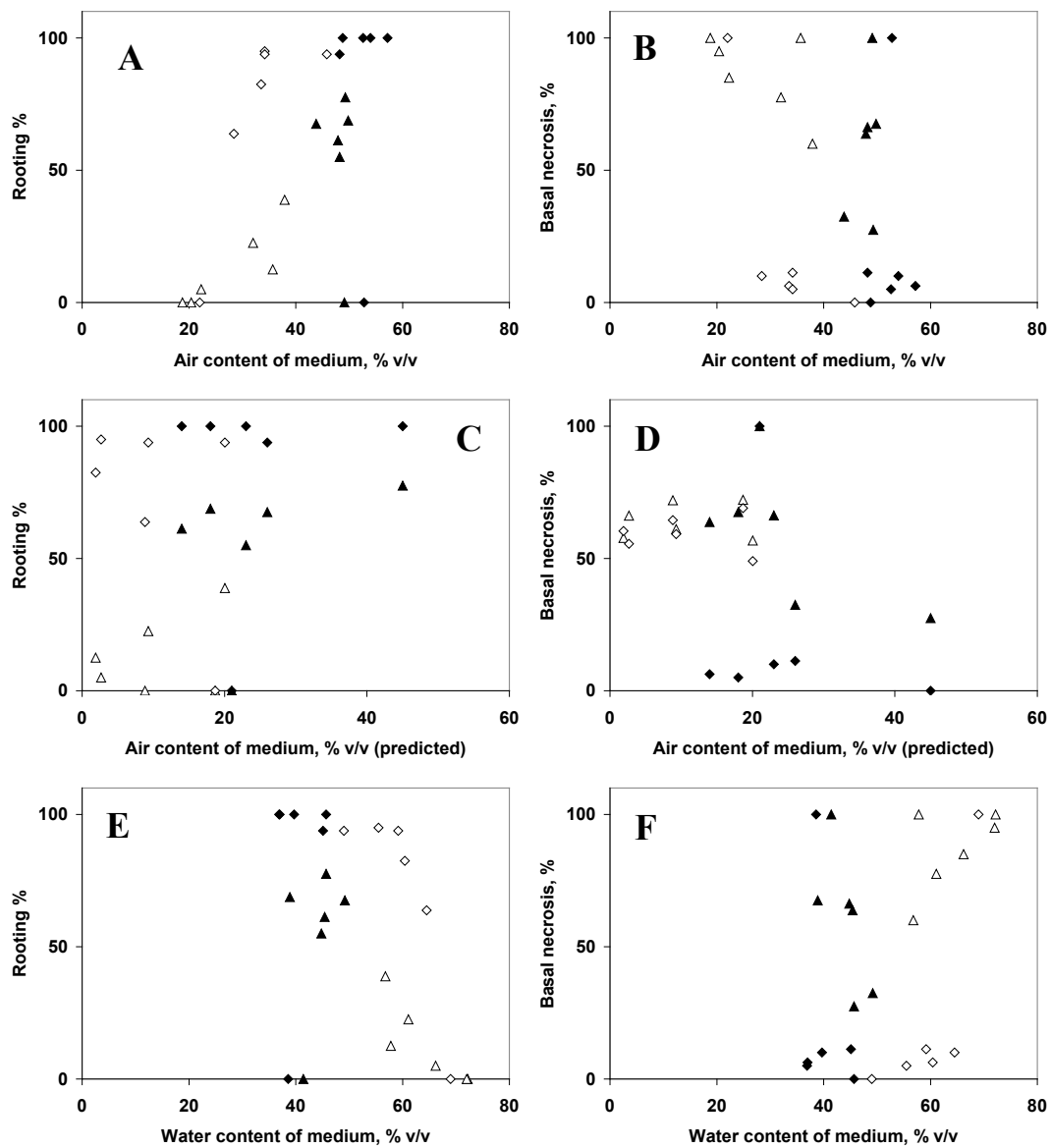


Figure 21. Rooting and basal necrosis (i.e. rotting) in *Convolvulus neorum*, plotted against the air content (A and B) and water content (E and F) of the rooting medium. In C and D, the air content values are those predicted from water release curves for the same media determined in the laboratory. All other air and water contents were those determined at the end of the experiment. Key to symbols: Solid / open = + / - capillary drainage; triangle / lozenge = high / low evaporative demand. Plotted points represent means for different ratios of peat and vermiculite (n=18).

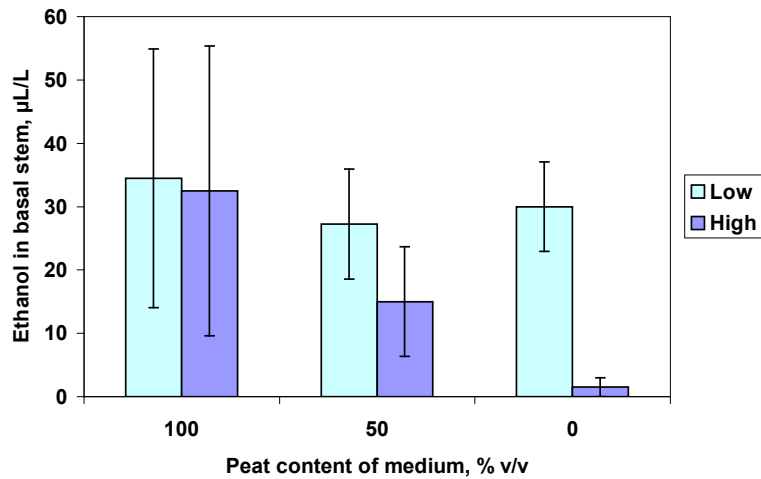


Figure 22. Concentration of ethanol in the basal 30 mm of the stems of *Convulvulus cneorum* cuttings, 24 days after sticking, and the influence of medium composition (% peat in a peat:vermiculite mix) and evaporative demand (High or Low). Plotted values are means  $\pm$  SE (n=4, combining  $\pm$  drainage).

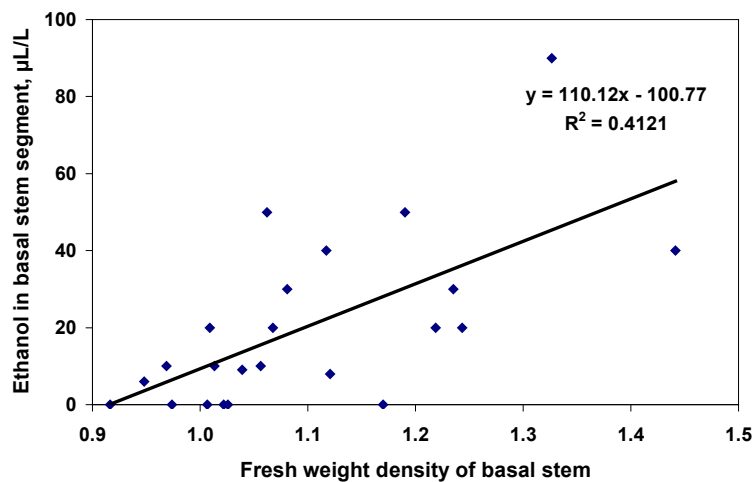


Figure 23. Relationship between the concentration of ethanol and the fresh weight density of the basal 30 mm of the stems of *Convulvulus cneorum* cuttings, 24 days after sticking.

## Experiment 2. The effect of rooting medium, drainage and transpiration demand on ethanol production in cuttings of *Convulvulus cneorum*

Following the ethanol results obtained in Experiment 1, this experiment looked more thoroughly at the accumulation of ethanol in *Convulvulus* cuttings, using only the extremes of the range of rooting media used in the earlier experiment and the more controlled conditions of the G-CPE.

### Rooting and basal necrosis

Responses shown in Figure 24 and Figure 25 were broadly similar to those seen in Experiment 1, allowing for the larger contrast between low and high evaporative demand that was possible using the G-CPE

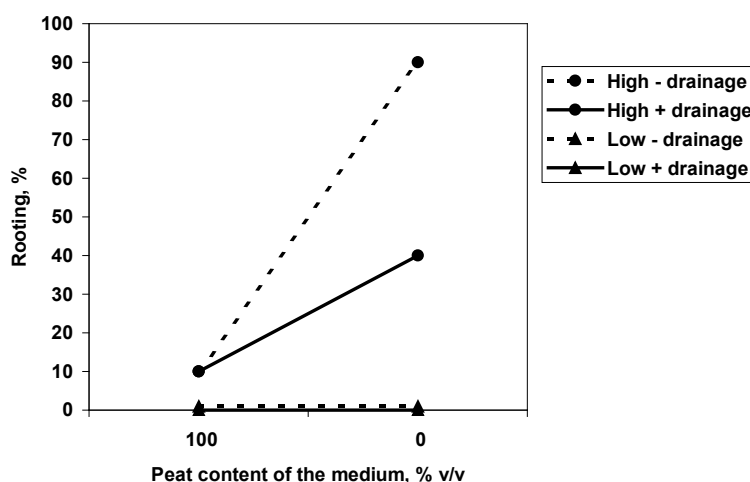


Figure 24. Rooting responses of *Convolvulus cneorum* to rooting medium composition (100% peat or 100% vermiculite), as affected by evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage) (n=10).

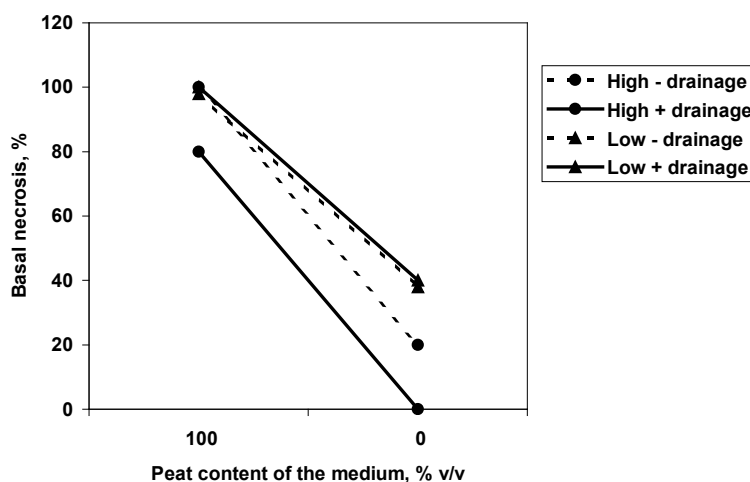


Figure 25. Frequency of basal necrosis (i.e. rotting) in *Convolvulus cneorum* cuttings, as affected by rooting medium composition (100% peat or 100% vermiculite), evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage) (n=10).

### Ethanol and acetaldehyde in basal stem segments

The data in Figure 26 show that there was a marked variation in ethanol concentration with time that was consistent in all treatments. There was a rapid rise in ethanol over the first few days, reaching a maximum on day 4 with mean values up to 86  $\mu\text{L/L}$ .

Thereafter, the concentration fell quite rapidly so that mean values were all below 20  $\mu\text{L/L}$  by day 20.

Treatment effects on ethanol concentration are more clearly seen in Figure 27, in which ethanol concentration during the early peak have been plotted in the same format as the rooting data in Figure 24 and the necrosis data in Figure 25. As in Experiment 1, high evaporative demand was the most effective way of keeping ethanol concentration low, but only when combined with capillary drainage. In contrast, capillary drainage was not necessary to achieve high rooting percentage (Figure 24) and ethanol concentration did not help to explain the much greater rooting observed in vermiculite than peat, so that the correlation between rooting and ethanol was weak (Figure 28).

As in Experiment 1, high ethanol concentration in individual cuttings tended to be associated with high stem density (Figure 29). The relationship did not account for the changes in ethanol with time, suggesting that the peak in ethanol early in the propagation process was due to higher respiration rate at this stage, associated with the induction of the wound healing process (callus formation, etc.).

In the presence of sufficient oxygen, ethanol is metabolised to acetaldehyde. This is likely to happen when ethanol in the transpiration stream reaches well aerated tissues above the surface of the compost. It might also happen in the basal stem segment if oxygen starvation is very localised. We therefore measured the concentration of acetaldehyde in the basal stem segments, in parallel with the ethanol measurements. Acetaldehyde was present at about 5% of the concentration of ethanol and there was a close correlation between its concentration and that of ethanol (Figure 30).

The close correlation of acetaldehyde and ethanol concentrations suggests that acetaldehyde production was limited by the supply of ethanol. This interpretation is consistent with existence of localised zones of oxygen starvation creating a supply of ethanol which is then metabolised to acetaldehyde at a more or less constant rate as the ethanol moves to better aerated zones. The ethanol concentration in the putative oxygen starved zones would have been many times higher than the average value



derived from the ethanol assay. That scenario would make it more likely that ethanol reached high enough concentrations to cause cell death and thus to start the process of necrosis. Acetaldehyde is more toxic than ethanol but, partly because it is more volatile, less likely to accumulate to locally high concentration.

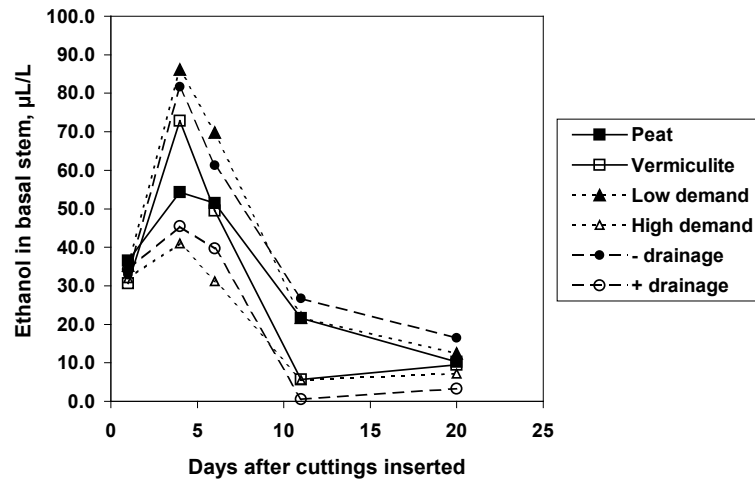


Figure 26. Changes in ethanol concentration in the basal 30 mm of *Convolvulus cneorum* cuttings over time in the rooting medium and the effects of medium (100% peat or 100% vermiculite), evaporative demand (High or Low), and capillary drainage. Plotted values are means of 8 samples and represent main effects averaged across other factors.

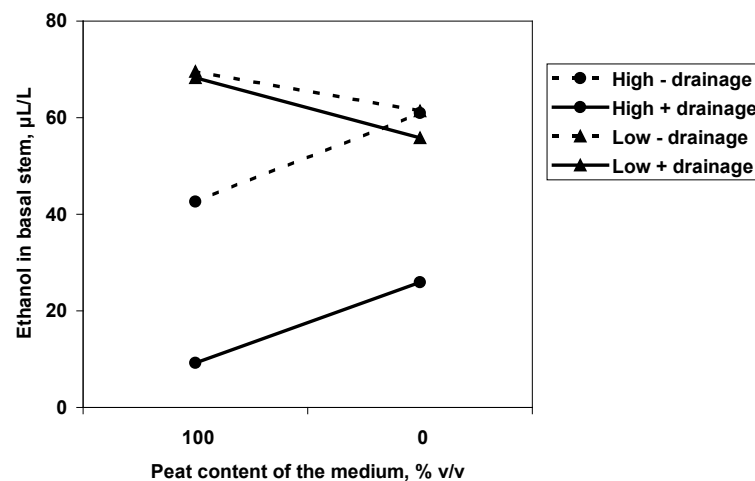


Figure 27. Ethanol concentration in the basal 30 mm of *Convolvulus cneorum* cuttings, as affected by medium composition (100% peat or 100% vermiculite), evaporative demand (High or Low), and contact with a sandbed to provide capillary drainage (+ drainage). Plotted values are means of 4 samples (days 4 and 6 combined)

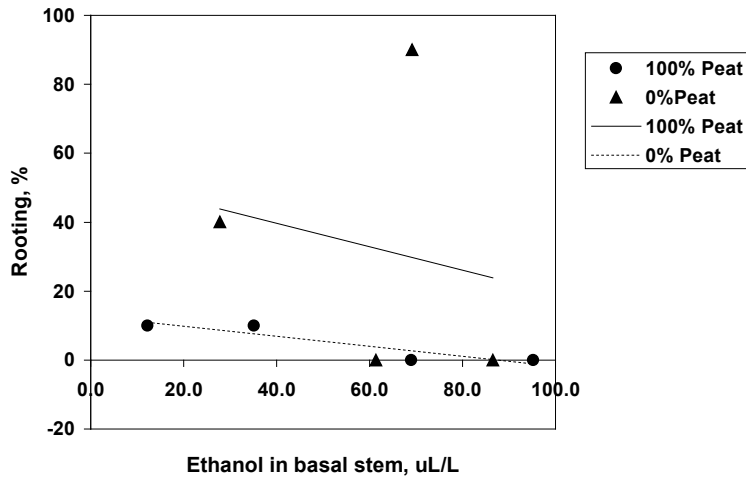


Figure 28. Relationship between rooting of *Convolvulus cneorum* cuttings and the ethanol concentration in the basal 30 mm (mean of days 4 and 6), as affected by medium composition (0% peat =100% vermiculite). Plotted values are based on 8 cuttings for ethanol and 10 cuttings for rooting. Lines were fitted by linear regression.

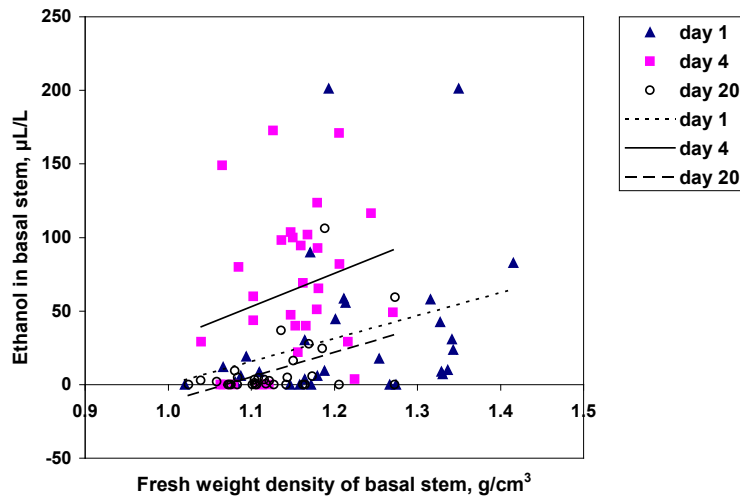


Figure 29. Relationship between the concentration of ethanol and the fresh weight density of the basal 30 mm of the stems of *Convolvulus cneorum* cuttings, at 1, 4 and 20 days after sticking. Lines were fitted by linear regression. Plotted values are from individual cuttings.

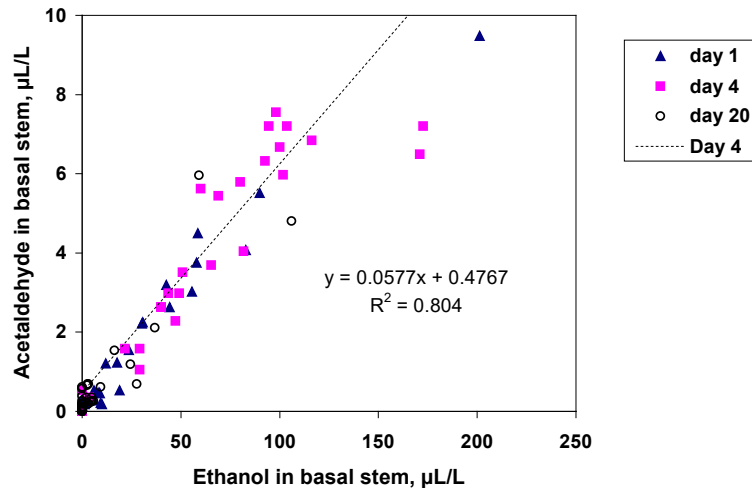


Figure 30. Relationship between the concentrations of acetaldehyde and ethanol in the basal 30 mm of the stems of *Convolvulus cneorum* cuttings, at 1, 4 and 20 days after sticking. The line was fitted to the day 4 data by linear regression. Plotted values are from individual cuttings.

### Experiment 3. The effect of rooting medium, drainage and transpiration demand on ethanol production in cuttings of *Fremontodendron* 'California Glory'

This experiment extended the study of the role of oxygen starvation in responses to rooting media to another species which is prone to basal necrosis and has previously been shown to benefit from capillary drainage of the rooting medium.

#### *Rooting and basal necrosis*

Rooting was poor in all treatment combinations except vermiculite combined with high evaporative demand (Figure 31). In contrast to earlier experience, capillary drainage had no effect. That experience was based mainly on experiments using peat:bark mixes in less extreme aerial environments (Harrison-Murray et al, 1998). This illustrates again the strong interactions that can occur in responses to the rooting environment.

Basal necrosis affected a high proportion of cuttings but the incidence was significantly greater in peat than in vermiculite (Figure 32). Furthermore, the length of stem affected was generally quite short in vermiculite but was on average nearly 20

mm in peat (Figure 33). There was a tendency for the length of stem affected to be reduced by capillary drainage and high evaporative demand but these effects were not significant.

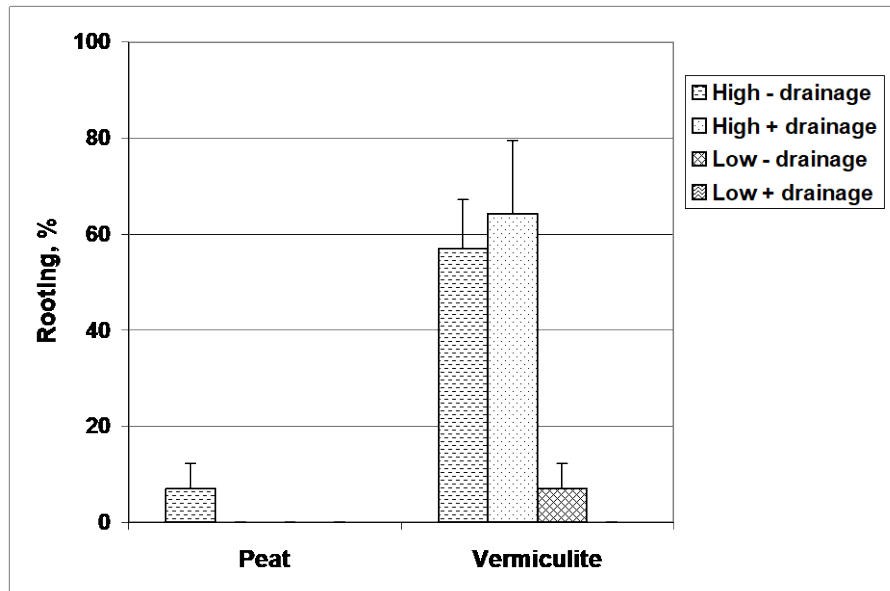


Figure 31. Rooting of *Fremontodendron* 'California Glory' as affected by rooting medium, evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Significant effects: medium ( $P=0.001$ ), evaporative demand ( $P<0.001$ ).

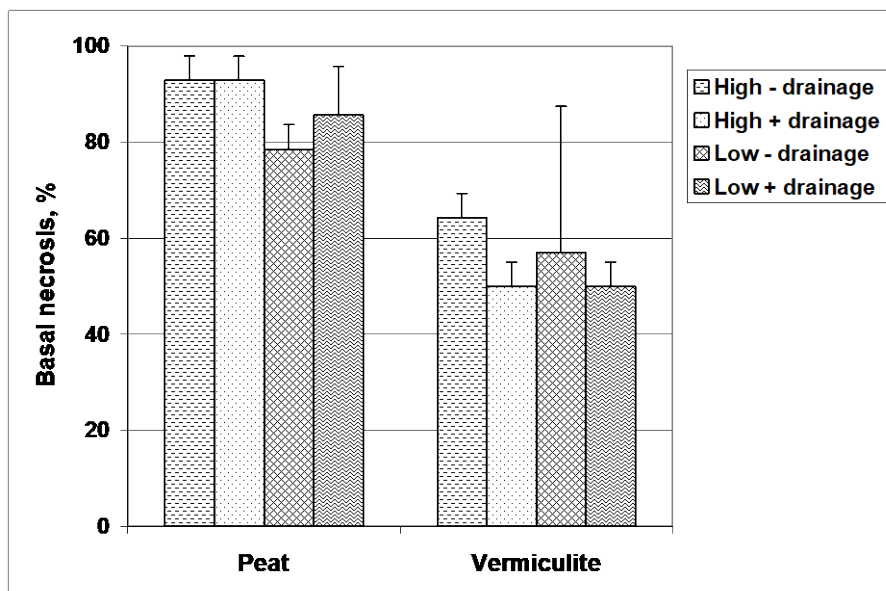


Figure 32. Percentage of *Fremontodendron* 'California Glory' cuttings with basal necrosis (i.e. rotting) as affected by rooting medium, evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Significant effects: medium ( $P=0.001$ ).

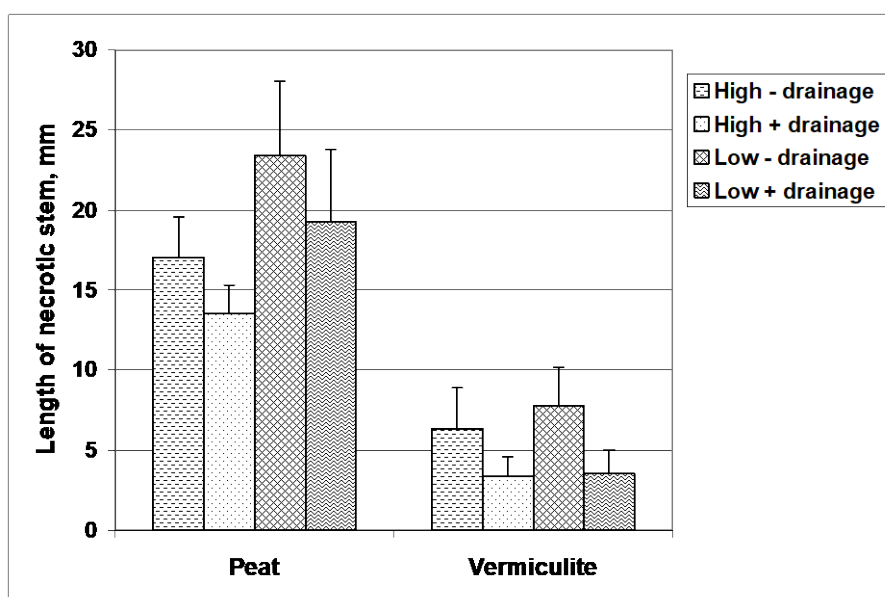


Figure 33. The extent of basal necrosis (i.e. rotting) in cuttings of *Fremontodendron* 'California Glory' as affected by rooting medium, evaporative demand (High or Low) and contact with a sandbed to provide capillary drainage (+ drainage). Significant effects: medium ( $P=0.001$ ).

### *Ethanol and acetaldehyde*

Ethanol reached concentrations up to about 500  $\mu\text{L/L}$ , more than five times higher than in *Convolvulus*. Also, the concentration continued to increase for a longer period so that it was not convincingly past its peak by day 14, when the last sample for analysis was taken (Figure 34). On average, the concentration was higher in peat than in vermiculite and under low evaporative demand than under high evaporative demand but drainage had no consistent effect (Figure 35). These effects roughly paralleled the effects on rooting so that a weak association between rooting percentage and ethanol concentration is evident in Figure 36.

As in all other experiments, ethanol concentration tended to increase with stem density but again the correlation was weak (Figure 37). This is not surprising if, as suggested above, ethanol production is confined to localised oxygen-starved zones created by an accumulation of water in a cluster of interconnected intercellular spaces. The same amount of water, if distributed more evenly, or in a previously empty xylem vessel, would increase stem density by the same amount but would be unlikely to cause oxygen starvation.

These results for *Fremontodendron* add weight to the hypothesis that local accumulation of water in the basal stem tissues, leading to localised oxygen starvation, may have an important role in the way that water availability in the rooting medium influences the physiology of cuttings.

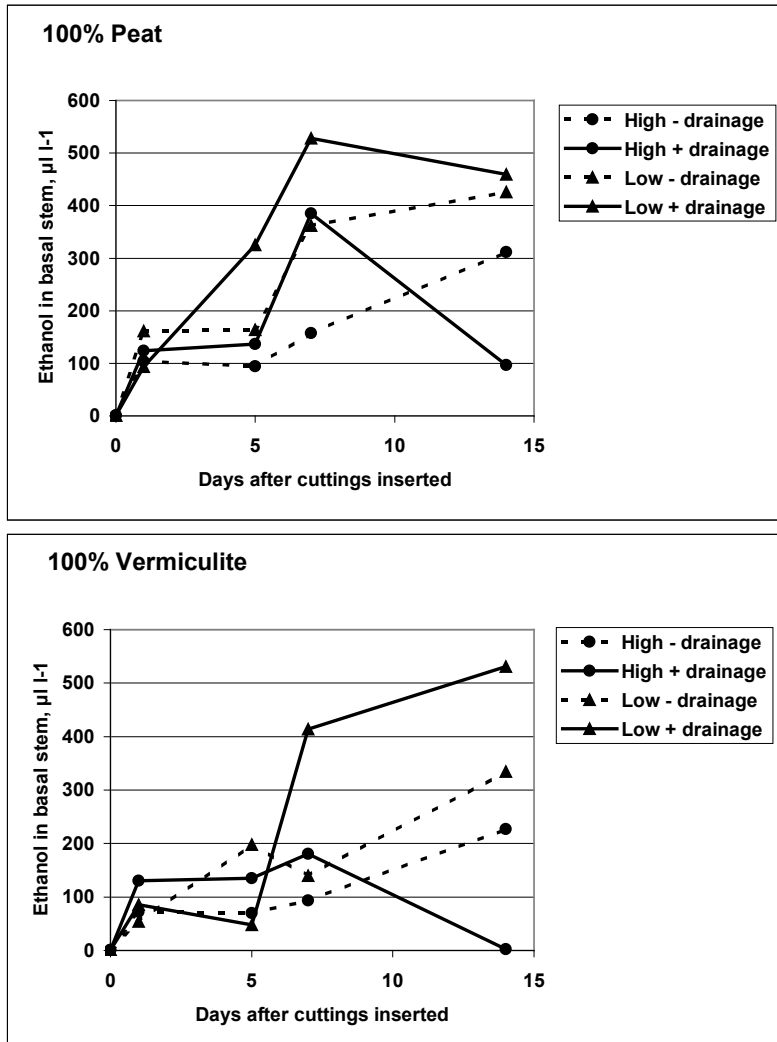


Figure 34. Changes in ethanol levels in the basal 30 mm of stem in cuttings of *Fremontodendron* 'California Glory' over the first 14 days after insertion. Plotted values are means of two samples.

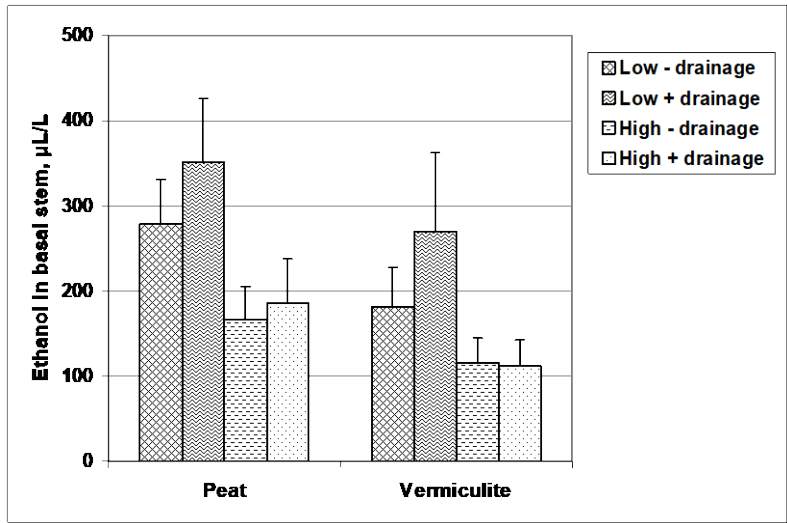


Figure 35. Ethanol levels in the basal 30 mm of stem in cuttings of *Fremontodendron* 'California Glory' averaged over the first 14 days after insertion. Plotted values are means and standard errors (n=8).

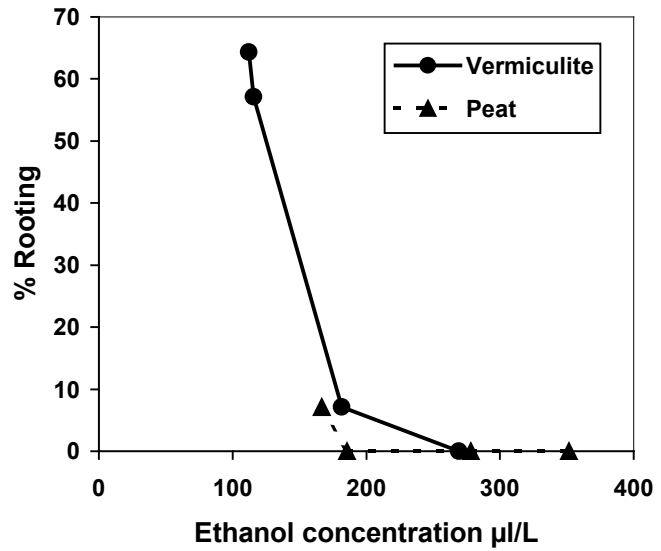


Figure 36. The relationship between rooting and ethanol levels in *Fremontodendron* 'California Glory'. Ethanol concentration is the average detected in the basal 30 mm of stem of cuttings sampled at 1, 5, 7 and 14 days.

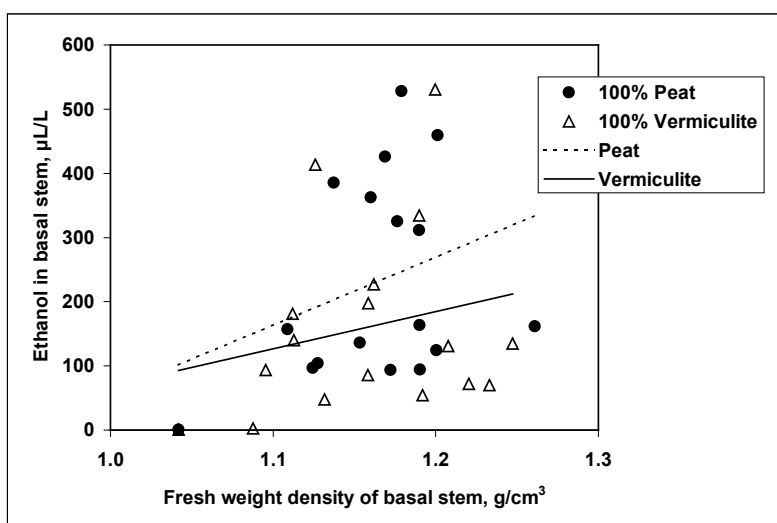


Figure 37. The relationship between ethanol concentration and density of the basal 30 mm of stem in *Fremontodendron* 'California Glory'. Plotted values are for individual cuttings, sampled at 1, 5, 7 and 14 days.

#### Experiment 4. Factors affecting contact between rooting media and drainage sand

Work in Years 1 and 2 had demonstrated the benefits of placing propagation trays on a sandbed to provide capillary drainage, thereby draining additional water from the medium and increasing air content. For this benefit to apply equally to every cell in a modular tray, it is essential that the medium in each cell makes contact with the sand. As long as the sand surface is loosened before the trays are stood down, some contact with every cell will generally be achieved. However, is 'some contact' enough? This experiment examined whether poor contact is likely to limit the practical benefit of placing propagation trays on a sandbed.

##### *Time course*

Figure 38 plots the time course of changes in weight of peat samples as they went through the various stages described in detail under Materials and Methods. Despite water deposition at almost 1 mm/h, samples were still increasing in weight slowly after 168 hours (7 days). This probably reflects the slow process of water displacing air from the microscopically small pores within the peat fibres. Such pores are not relevant to the supply of water or oxygen to the cutting but create difficulties in precisely quantifying the volume of the solid phase in the medium.



When moved to the sand , most drainage occurred in the first hour (not shown) and drainage was almost complete after 24 h. When water application ceased, a further small decrease in weight occurred as the water content reached static equilibrium with the suction generated by the sandbed.

### ***Treatment effects on drainage***

Differences due to treatment were too small to see clearly in a time course graph so they are shown separately as a bar chart in Figure 39. Neither raking, nor sliding improved drainage compared to control but, if the sample was placed on a smooth sand surface without applying any pressure, then the benefit of the sandbed was almost halved. However, on a repeat run of the experiment, even the 'No pressure' treatment drained as well as the control, indicating that it is actually quite difficult to *avoid* adequate contact with a sandbed.

One set of samples was removed from the sandbed for additional weighings at intervals over the first 24 hours (labelled 'Disturbed' in Figure 39). This had no detectable effect on the drainage process, which indicates that capillary contact is readily re-established, at least under the moist conditions of a propagation environment. The small decrease in drainage in the 'Non-cleaned' treatment indicates the scale of error that is introduced if sand is not brushed from the base of the sample holder before weighing. Although small compared to the volume of drainage measured in this experiment, in some cases it would be essential to avoid this error.

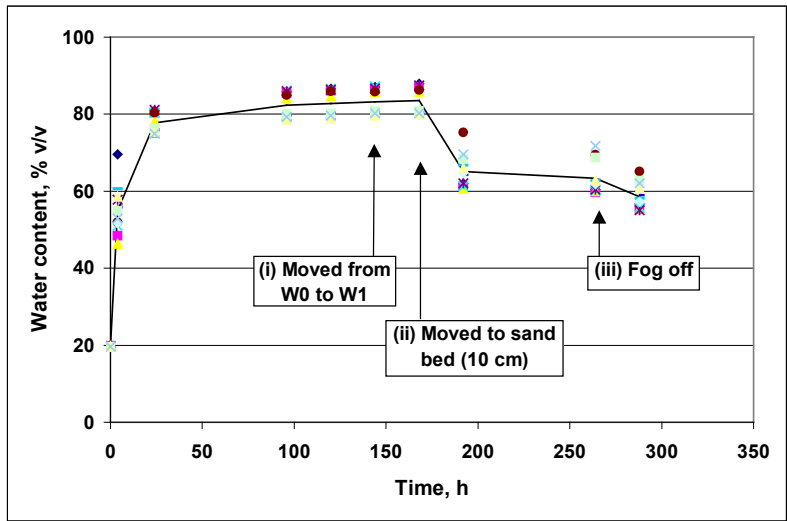


Figure 38. Changes in water content of peat during wetting up and following transfer to a 10 cm deep sandbed to provide capillary drainage. The arrows indicate the timing of three changes: (i) reduction in wetting from ca. 0.8 to 0.4 mm/h, (ii) transfer to sand and (iii) cessation of wetting. Plotted points are means of 3 samples and the plotted line is the overall mean.

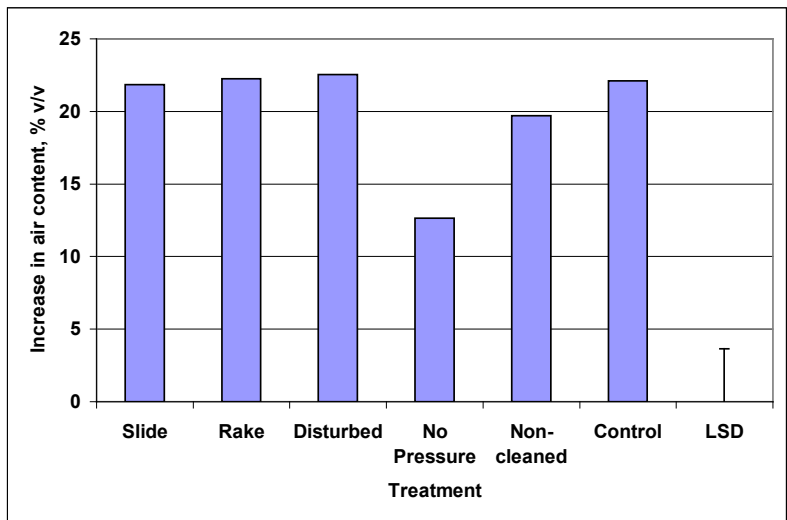


Figure 39. The increase in air content due to capillary drainage when samples of pure peat were transferred to a sandbed [i.e. stage (ii) in Figure 38]. The treatments differed in the steps taken to ensure good contact between the peat and the sand (see text for details).

### Experiment 5. On the problem of contact between rooting media and capillary matting

The objective of this experiment was to confirm and seek to explain the poor performance of capillary matting as a drainage medium that was observed in an earlier experiment (Year 2, Experiment 1, see Harrison-Murray, 2001). As normally used,

laid flat on the floor or bench, capillary matting does not have sufficient thickness to induce significant additional drainage compared with a solid or non-capillary surface. However, when a 'tail' is formed, hanging over the edge of a bench or dropping into a drainage channel, the water hanging in this 'tail' creates a suction that should be transmitted to the water in the horizontal part of the mat. The results of the earlier experiment showed some increase in drainage due to a 3 cm 'tail' but it was much smaller than the effect of a 3 cm deep sand bed.

### ***Increase in air content due to drainage***

The present experiment again showed that capillary matting with a 'tail' had a substantial drainage effect but that it was much less than achieved by an equivalent depth of sand. Figure 40 shows the decrease in air content as samples were wetted up overnight and the increase as water in the larger pores drained out under the suction generated by capillary drainage. Drainage had virtually ceased after 24 hours. On capillary matting with a 3 cm drainage 'tail', drainage increased the average in air content by 8.3% (v/v). This is enough to be of considerable practical value for media which are too water retentive. However, in samples placed on the 3 cm deep sandbed, air content increased by 23.3% (v/v), almost 3 times as much as on the capillary matting.

### ***The 'problem' of contact***

Incorporating a thin layer of sand into the surface of the matting, with the aim of improving contact between the media and the matting, had no significant effect. This suggests that the relatively poor performance of capillary matting is not due to poor contact with the rooting medium and must lie instead in its ability to create or transmit suction from the hanging 'tail'. The capillary rise observed with the matting used in this experiment (Fibertex) was about 10 cm, well above the height of the 'tail', so the matting should have remained close to saturation and it is hard to see what could prevent the transfer of suction. Further work is required to identify the nature of this problem and ways to overcome it in order to allow the maximum benefits of capillary drainage to be achieved without the hygiene and other practical difficulties associated with sand.

There was no significant effect of pressing the samples into the sand ('+ firming' in Figure 40). This result confirms that little care is required to ensure adequate contact with a sandbed to achieve the full benefit of capillary drainage.

When the fog was turned off there was a further increase in air content of the media on both sand and capillary matting. The change was smaller than seen in Experiment 4 because the rate of water deposition during stage 2 (drainage phase) was much lower.

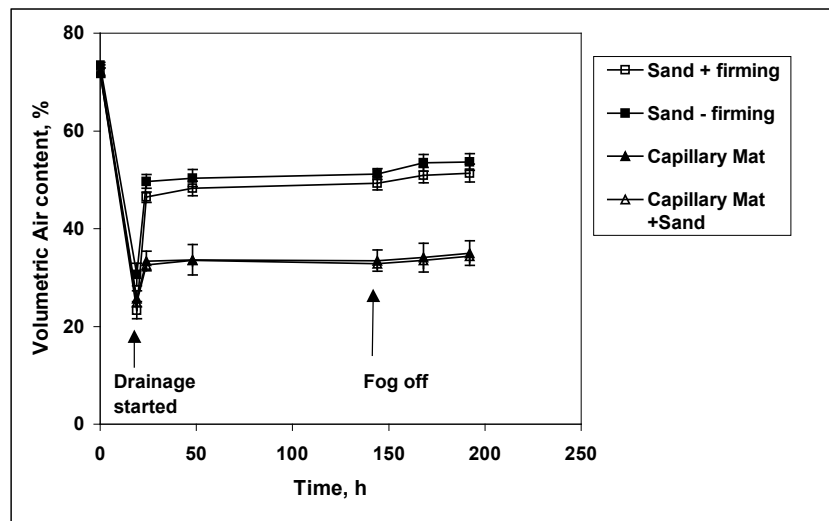


Figure 40. Comparison of the effectiveness of capillary matting and sand. Peat samples were wetted up for 19 h before transfer to either a 3 cm deep sand layer or capillary matting with a 3 cm 'tail'. On the sand, samples were either pressed firmly into the sand or placed without firming. On the capillary matting, samples were either placed directly on the matting or a little sand was sprinkled on the mat to help establish contact. Wetting was approximately 0.35 mm/h during wetting up and thereafter about 0.001mm/h, until the fog was switched off.

### Additional analysis of data from earlier years

All previous rooting trial data was re-examined for evidence of correlations between rooting or basal necrosis and physical conditions in the rooting medium (air content, etc.). The same approach was used as in Experiment 1 above, except that only predicted air content data was available. Rooting media trials with *Convolvulus* and *Fremontodendron* in Year 2 demonstrated the clearest correlations (Figure 41 and Figure 42)

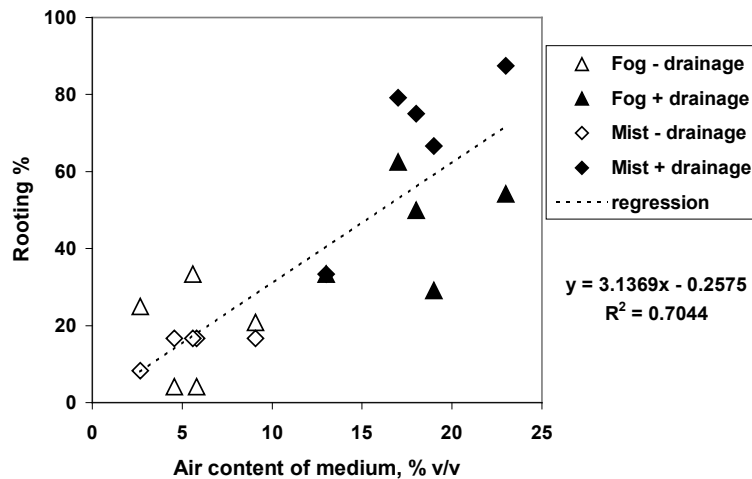


Figure 41. Rooting percentage of *Fremontodenron* 'California Glory' plotted against air content of the medium (predicted from water release curve). Plotted points represent means for different media (Elle pots, Plug Its, Oasis, Rockwool and 50:50 peat:bark) (n=24).

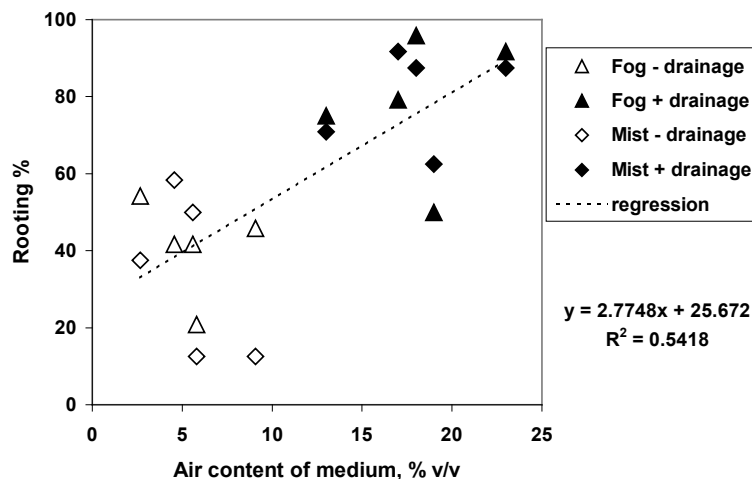


Figure 42. Rooting percentage of *Convolvulus cneorum* plotted against air content of the medium (predicted from water release curve). Plotted points represent means for different media (Elle pots, Plug Its, Oasis, Rockwool and 50:50 peat:bark) (n=24).

## Conclusions

The main conclusions from the final year of this project are as follows:

- Capillary drainage from a 10 cm deep bed of fine sand consistently increased the air content of rooting media, by about 20% v/v. As a proportion of the air content without capillary drainage, the effect was largest in media with a high proportion of peat.

- Capillary drainage sometimes greatly reduced necrosis and increased rooting (e.g. *Convolvulus cneorum*, especially when evaporative demand was low) and sometimes had little or no effect (e.g. *Garrya elliptica*).
- It is not difficult to achieve satisfactory capillary contact between the rooting medium in a pot or tray and a capillary sandbed. If there is physical contact that will generally be enough to obtain the benefit of capillary drainage. Raking the sand before placing trays helps to ensure that physical contact with every cell. Under the moist conditions of a propagation unit, contact is readily re-established if trays are lifted and replaced on the sand.
- The thickness of capillary matting is not enough to generate substantial suction in the way that a layer of sand does but this can be compensated for by creating a ‘tail’ hanging below the main area of the mat. With a 3 cm tail, air content of pure peat was increased by 8% (v/v). 5 cm deep channels at ~50 cm intervals along a propagation bed would allow ‘tails’ to be formed.
- Capillary matting with a tail was less effective than the equivalent depth of fine sand. We previously suggested that this was probably attributable to poor contact between the medium and the matting (Harrison-Murray, 2001) but the evidence we obtained this year did not support this hypothesis.
- In experiments with mixtures of peat and vermiculite, rooting increased and necrosis decreased as the proportion of peat was reduced. Although this corresponded with increasing air content in the mix, other evidence suggests that the effect of peat cannot be attributed to its effect on air content alone. Capillary drainage increased the air content of pure peat to equal that of vermiculite without drainage yet it increased rooting much less, e.g. in *Garrya*, Figure 7.
- Bark had a specific effect in reducing necrosis in *Garrya elliptica* when compared with vermiculite. This effect could not be related to its effect on air content, which was very similar to that of vermiculite, and is, therefore, probably a chemical or microbiological one. Bark had a much smaller effect on necrosis of *Convolvulus cneorum* cuttings.
- Rooting % tended to increase and the percentage of cuttings with basal necrosis tended to decrease as air content increased but the association was loose and did not fully account for the benefit of capillary drainage.

- A number of results demonstrated the strong interactions that frequently occur between factors influencing the rooting process, e.g., the benefit of capillary drainage on *Convolvulus* was much larger at low evaporative demand than at high demand. (Figure 14 and Figure 16).
- The use of ethanol as a biochemical indicator of oxygen starvation was very informative. It proved that oxygen supply to the base of cuttings is often restricted. Since ethanol was detected in cuttings from a well-aerated medium like vermiculite, the restriction of oxygen supply must be in or around the cutting itself.
- Detection of acetaldehyde in cutting bases, at a concentration correlating closely with that of ethanol, suggests that oxygen starvation is a very localised phenomenon. The weak but consistent correlation of ethanol concentration with the fresh weight density of the tissue at the base of the cutting strongly suggests that movement of water into the normally air-filled intercellular spaces is what causes localised oxygen starvation. This leads to a hypothesis that can be stated simply as: local water-logging of tissues leads to local oxygen starvation.
- This hypothesis is supported by the association between ethanol accumulation and environmental conditions likely to favour excess availability of water around the base of the cutting (i.e. a medium with high water content, lack of capillary drainage, low evaporative demand, e.g. Figure 26).
- The highest ethanol concentrations were usually observed in treatments which resulted in the highest proportion of necrotic cuttings and the lowest proportion of rooted cuttings. However, overall, the association between rooting and ethanol was rather weak (e.g. Figure 36)
- The concentrations of ethanol detected were too low (< 1%) to be toxic but probably masked much higher localised concentrations in waterlogged patches of tissue.
- Further work is required to test the localised tissue waterlogging hypothesis and to devise rapid non-destructive and sensitive sampling procedures for tissues very close to the cut base (e.g. rapid absorption of volatiles from the cutting base into a suitable absorbent cartridge). A non-destructive sampling procedure would allow the association between ethanol and rooting to be studied at the individual cutting level.

In summary, the results suggest that the rooting medium influences the health and rooting ability of tissues at the base of the cutting in complex ways that interact strongly with the evaporative demand of the aerial environment. While air content, water content, and SWT are all influence cutting behaviour, and restricted oxygen supply to respiring cells can reduce the likelihood of rooting, chemical and microbiological factors are also involved. It is, therefore, not yet possible to make generalisations about the role of the rooting medium that carry the same weight as those that have proved so useful in optimising the above ground part of the cutting's environment. Future research will need to encompass the putative chemical and/or microbiological differences between peat, bark and vermiculite as well as seeking to clarify the role of oxygen starvation and other aspects of the response to the physical environment. There is also a need to develop practical tools for monitoring parameters such as medium water content for use on the nursery and to use them to identify ways to maximise uniformity and consistency of rooting.

## **TECHNOLOGY TRANSFER**

Harrison-Murray, R.S., (1999). Propagation Environments - existing problems and future possibilities. Presented at the HDC Propagation Workshops, HRI East Malling, 22 & 23 September, 1999.

Harrison-Murray, R.S., (2002). Getting the environment right for rooting: fog, mist and media. Presented at the HDC Propagation Seminars, Bradbourne House, East Malling, 18 & 19 September, 2002

Harrison-Murray, R.S. (2003). The right media for rooting. *HDC News*, 89, 26-28.

## **ACKNOWLEDGEMENTS**

The skilful technical assistance of Linda Knight in the first two years of this project is gratefully acknowledged. We are also indebted to John Davies for construction of the special sample holders and repairs to the tension tables, to John Stow for advice on the ethanol analysis and to various colleagues, particularly Neil Hipps and Ross Cameron, for valuable discussions about the research.



## **GLOSSARY : terms, abbreviations and products used**

**Agritech fogger** - a machine in which large quantities of water (up to 135 L h<sup>-1</sup>) are atomised by nozzles mounted on the ends of a pair of rotating arms, and which incorporates a powerful fan to distribute the resulting fog. It produces a mixture of droplet sizes, ranging from mist-sized drops to fine fog droplets. It is no longer manufactured but alternatives are available. It is used in a polytunnel with an extract fan, that is referred to as the 'Agritech' ventilated fog house.

**apical cutting** - one which includes the shoot tip

**bulk density** - the density of the medium as a whole, including the pore space, i.e. the mass of a sample of the medium divided by its volume.

**CPE** - a 'Controlled Propagation Environment'.

**'environmental fingerprint'** - term coined for a 3D graph summarising the response of rooting to environment, in a particular species, cultivar, or type of cutting, based on results from the G-CPE (e.g. Figure 4 or 5).

**evaporative demand** - an imprecise term referring to the power of an environment to evaporate water. It differs from humidity in that it takes account of the many other factors which influence evaporation, such as irradiance. For a more precise definition it is necessary to specify a particular evaporative surface e.g. a leaf - see also potential transpiration.

**evapo-sensor** - an instrument invented at HRI - East Malling which provides an electrical signal approximately proportional to potential transpiration

**G-CPE** - the 'Gradient - Controlled Propagation Environment' in which gradients of two environment factors (currently light and wetting) interact to create a matrix of different environmental conditions.

**g L<sup>-1</sup>** - grams per litre.

**IBA** - indolyl butyric acid. This is a synthetic auxin used to stimulate rooting in cuttings and is the active ingredient in Seradix rooting powders.

**necrosis** - tissue death. Used in this report to refer to darkening of tissues that indicates that the cells have probably died so that the tissues will eventually break down.

**( $P < 0.05$ ,  $P < 0.01$ , or  $P < 0.001$ )** - a statement of the statistical probability (P) that the observed differences could have been due to chance. The smaller the value of P, the more certain we can be that the result is 'real'. A value of 0.05 is conventionally taken as the threshold for accepting the result, i.e. that an effect is 'statistically significant'.

**particle density** - the density of the solid material making up the 'particles' of the solid in the rooting medium.

**SWT** - soil water tension. See 'tension'.

**tension** (or soil water tension) - a measure of the capillary forces holding water in the soil (or rooting medium), and therefore the force required to remove water from it. It is synonymous with 'soil moisture suction' and numerically equal to the soil matric potential (though of opposite sign). In the present context it is convenient to express it in centimetres of water, i.e. the height of a column of water which would create the same tension.

**WRC** - water release curve. A graph which shows how the water content of a soil or artificial substrate decreases as soil water tension increases. It is usually obtained in the laboratory by applying progressively larger forces to drain water out of the samples. For the tensions up to about 0.1 atmospheres (i.e. 1 m of water), this is usually achieved by increasing the height of a hanging water column. This is the method used in this project, which was described in detail in the first annual report.

## REFERENCES

Anon. (1998). Methods for optimising the hydraulic properties of growing media for HNS. Final report on MAFF project HH1208SHN.

Harrison-Murray, R.S. (2001). Hardy nursery stock: optimising rooting media for leafy cuttings. Horticultural Development Council, 2nd Annual Report for project HNS 98.

Harrison-Murray, R.S., Howard, B.H. and Knight, L.J. (1998). Strategies for liner production designed to achieve high quality container plants. Horticultural Development Council, report for project HNS 55.

Thomas, A. and Harrison-Murray, R. S. (1995). What makes peat so good for growing. *Grower*, 124(21): 21-22.