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PRE-AND POST-ROOTING STORAGE OF SOFTWOOD AND HARDWOOD CUTTINGS

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HRI - EAST MALLING

CONTENTS

RELEVANCE TO NURSERYMEN AND PRACTICAL APPLICATIONS

Application

The purpose of this project was two-fold, 1. to investigate reasons why rooted cuttings failed during the first winter after propagation and 2. to determine if the use of cold storage could benefit nurserymen in their management of both softwood and hardwood cutting production.

1. Two prime reasons responsible for overwintering loss of cuttings were identified, namely their excessive exposure to environmental stress and the propagation of sub-standard material resulting in reduced cutting viability. Therefore, limiting production to only the very best quality cuttings and reducing their exposure to potentially damaging stresses, e.g. frost, excessive heat, overwatering, can ensure maximum cutting survival.

2. The research also demonstrated that the use of cold storage increases management flexibility by allowing both softwood and hardwood cuttings to be held for a number of weeks prior to propagation or to planting out after propagation, which can be useful for collecting together unrooted cuttings, for spreading workloads during busy periods or holding rooted cuttings during adverse weather conditions.

Summary

The broad aims of this project were to investigate problems associated with overwintering modular-grown, rooted cuttings and separately, to determine the feasibility of cold storage as an aid to the management of cutting production. There were three specific objectives; 1. to understand why many newly-rooted cuttings, often of highly-valued species, failed to survive their first winter after propagation, 2. to investigate if cold storing softwood cuttings could allow greater flexibility in the management of cuttings during the preparation and propagation stages and 3. to determine if cold storing hardwood cuttings was a feasible management tool to allow delayed field planting and thus avoid the need to plant out during periods of inclement weather.

Overwintering of cuttings

Many nurseries appear to successfully propagate a wide range of species and cultivars during the summer months, only to see numbers decline rapidly over the winter period. The levels of overwintering failure however, varies considerably between years, plant species and between different nurseries, making crop scheduling particularly difficult. On many nurseries reasons for failure are not obvious and are likely to relate to a number of different factors. The main conclusion from this research however, is that overwintering cutting losses can be avoided.

The extent to which young, newly-rooted cuttings were exposed to environmental stresses during the winter strongly influenced their viability. The most damaging stress was

exposure to frost, especially in situations where roots of cuttings were frozen for a number of hours. Temperatures of -3°C in the compost almost invariably resulted in plant death, regardless of the species under test. It is important for nurserymen to provide adequate frost protection to cuttings throughout the winter and avoid prolonged exposure to sub-zero temperatures.

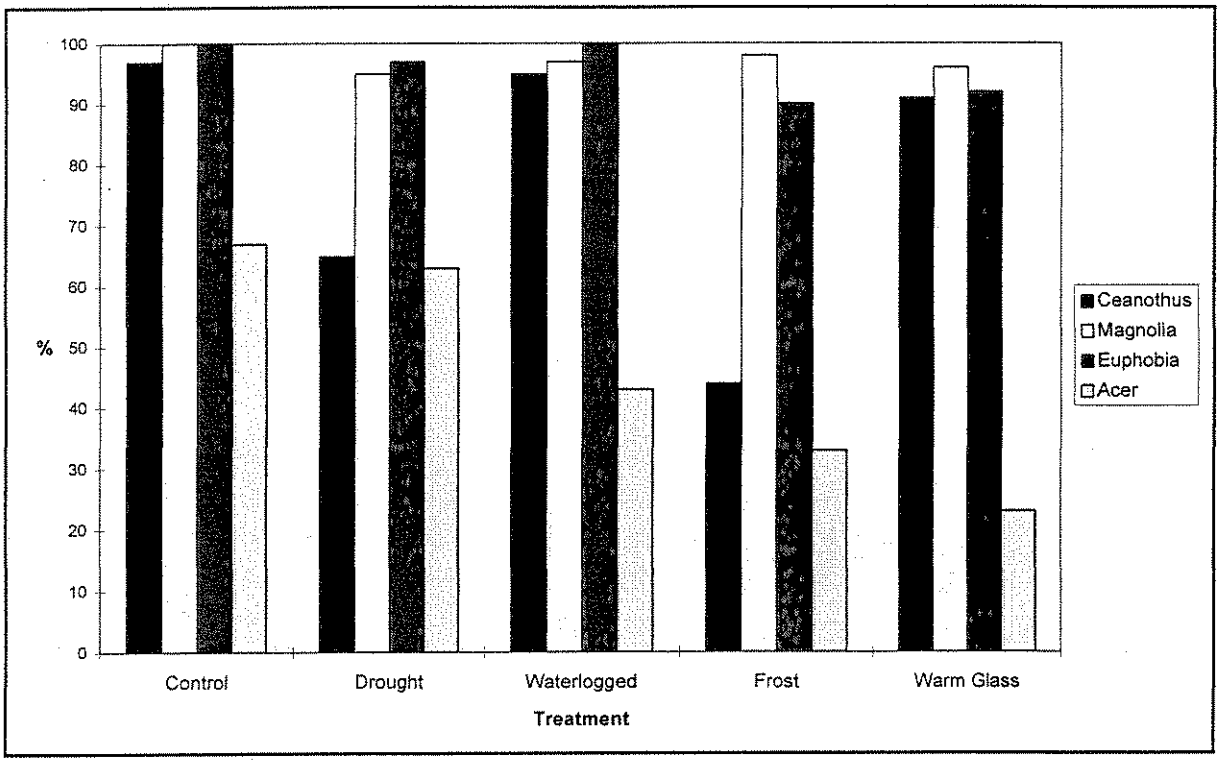
The extent of freezing damage either to leaves, stems or roots related to species, depth of temperature and exposure period. The evergreen *Ceanothus* 'Autumnal Blue' was particularly susceptible to foliar die-back, even at air temperatures as high as -3°C, although regrowth in the spring could be vigorous. Short, sharp frosts, where root systems remained unfrozen, could also prove fatal by direct injury to stems and buds. In the relatively robust *Magnolia* x *soulangiana*, air temperature of -7°C resulted in significant losses even though the exposure period was relatively short. Most experiments involving artificial freezing cycles used fully-dormant plants, but in situations where soft, non-acclimated growth may be present, e.g. in growth flushes after late propagation or early shoot growth in spring, cuttings would be even more susceptible to frost damage than in these experiments.

Generally, prolonging the freezing period resulted in increased damage for most species. The effects of this, however, could be off-set by potting-on modular cuttings into pots, where larger compost volumes conferred greater insulation to the roots during longer freezing cycles. However, if nurserymen wish to follow such procedures care should be taken because earlier potting-on may have other implications for crop management. In the final year of the project (1994-1995) cuttings of *Acer palmatum* 'Aureum' failed to establish after early potting-on, even without being exposed to freezing conditions. This was probably due to roots being exposed to anaerobic conditions within the greater volume of growing media, and irrigation regimes may need to be altered to reflect changes in plant requirements.

Although frost damage was found to be the most damaging stress, other forms of overwintering stress could also significantly affect cutting survival. The particular critical stress for each species however, tended to vary. *Ceanothus* was particularly sensitive to desiccation, whereas *Acer palmatum* 'Aureum' was more prone to injury relating to high overwintering temperatures (15 to 20°C), when maintained under heated glass, (Figure 1). As such, the management of cuttings in commercial situations must reflect the individual requirements of the species, e.g. evergreen species may need more frequent watering than deciduous subjects and should be placed under a separate irrigation system if necessary. A combination of different stresses is likely to prove to be more lethal than any individual form of stress, and nurserymen should try to manage plants appropriately in order to minimise the effects of any environmental stress.

Other factors may also influence viability and these can act in isolation, or interact with environmental stress to reduce overwintering success. Cuttings which are poorly rooted prior to winter will fail, although the stems may appear viable and healthy for many weeks after weaning. In this research up to one-third of overwinter losses in some trays could be attributable to limited rooting or the lack of an established root system. Even so, losses can still occur when all cuttings have rooted. This is primarily due to selection of inferior quality cuttings in the first instance, or trying to propagate material at inappropriate times, or under less than ideal conditions. Cuttings which were excised from older, less vigorous stockplants or propagated late in the season proved to be less successful at overwintering compared to

Figure 1. **The effects of various types of artificially imposed stress on percentage survival of rooted cuttings during winter 1993-1994**



Key: Control = side-ventilated polytunnel, with basal heat at 2°C; Drought = intermittant watering; Waterlogged = stood in shallow resevoirs of water; Frost = exposure to air temperatures of -4°C; Warm Glass = overwintered at 15 to 20°C.

cuttings derived from younger stockplants, or propagated in mid-summer when light levels were optimal, (Figure. 2).

The optimum temperature for cuttings during winter is between approximately 2 and 8°C and the use of a controlled environment, such as a cold store could avoid excessive cold or warmth. Indeed, a number of experiments demonstrated that high levels of survival could be obtained by cold storing rooted cuttings, although problems were encountered with the evergreen *Ceanothus*, primarily due to lack of light during storage. Cuttings would also need to be regularly monitored for fungal infection and drying out of compost, and more information is needed on their handling, especially storing bare-root. Where cold storage is a preferred method, cuttings may also need to be re-acclimatised before growing-on.

Alternatively, the standard of overwinter protection in polyhouses must be raised. In these experiments, high success rates were associated with the control treatment. This comprised a side-ventilated polytunnel with 'roll-down' sides, with trays placed on free-draining sandbeds and root systems protected from frost by basal heating cables which maintained beds at temperatures greater than 2°C.

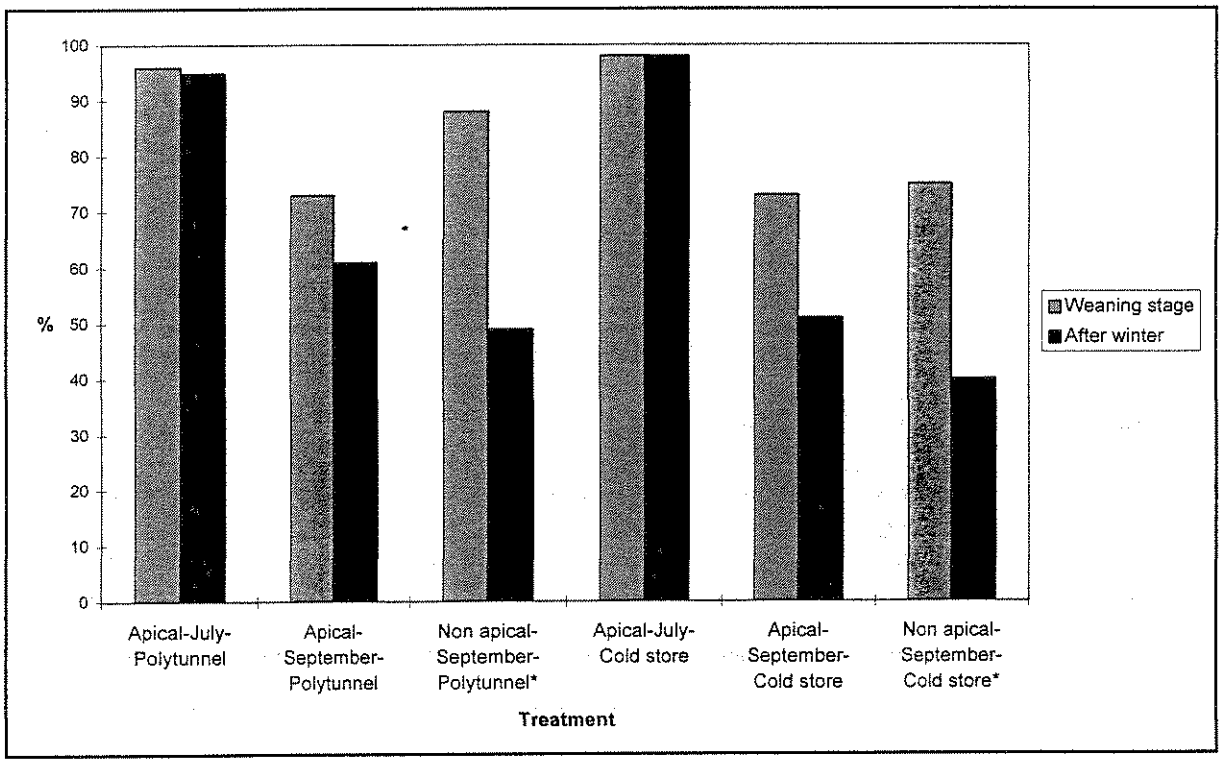
Softwood cutting storage

The use of short-term cold storage enables nurserymen to have more control over the propagation of cuttings. It allows the process of cutting collection to be separated from cutting preparation and planting, thus relieving the workload at any particular time and allowing for propagation to be managed over a number of days, if so required. Similarly, cutting transport, either in the pre-rooted or post-rooted form could be facilitated more readily. Although ideally the storage period should be kept to a minimum, this research demonstrated that many of the more robust, easier-to-propagate species such as *Forsythia* x *intermedia* 'Lynwood', *Weigela florida* 'Variegata' and *Buddleia davidii* 'Empire Blue' could be stored for a period of up to 4 weeks before being stuck and still retain high rooting potential.

In the majority of experiments successful storage was accomplished by soaking leafy cuttings prior to storage, allowing excess water to drain-off and simply placing them in thick-gauge polythene bags and sealing. Bags were then put into bin-liners for additional protection and cold stored at 2 to 3°C. The actual store used was jacketed with a high relative humidity, but this is not considered relevant for cuttings enclosed in double polythene bags. Generally, prior fungicidal treatment was not necessary.

In a number of species, the most notable being *Garrya elliptica* 'James Roof', a better rooting response was obtained after cold storage compared to propagating cuttings straight from the field. On the other hand, cold storage cannot be used to retain high rooting potential in species which have a limited optimum rooting period, i.e. *Syringa vulgaris* 'Madame Lemoine'; here survival and rooting was poor after prolonged cold storage.

Figure 2. *Magnolia* - **Percent cutting survival after weaning and overwintering during 1994-1995. Influence of cutting type, propagation date and overwintering environment**



Key: * rooted under artificial light.

Hardwood cuttings

Cold storage of hardwood cuttings gives nurserymen greater flexibility as to when to plant out cuttings and they can be successfully stored to avoid periods of wet or cold weather. The research also demonstrated that there were physiological aspects to be considered in relation to appropriate planting time and the extent to which cold storage was beneficial in different species. The amount of natural or artificial chilling that cuttings were exposed to could have a strong affect on their ability to establish subsequently. Elevated temperatures in the rooting bin could cause excessive bud and shoot development, especially after the bud chilling requirement had been completed and so this young growth could be susceptible to adverse conditions after planting out. In this respect, *Prunus* 'Colt' and *Tilia platyphyllos* clone 229 preferred propagation and planting in either autumn or mid-winter. Later propagation in the spring often correlated with advanced bud development which was prone to injury on planting out and therefore reduced the establishment rate. Pre-rooting cold storage in the spring compounded this effect even further.

In contrast, in *Cornus alba* 'Sibirica' highest success rates were associated with spring propagation and with treatments (such as prolonged cold storage), that maximised the amount of bud chilling prior to placement in the rooting bin. Treatments involving pre-rooting cold storage significantly increased survival rates over all three years of the project.

Action points for growers

Overwintering rooted cuttings

1. Minimise the effects of stress, especially exposure to frost. Provide frost protection measures wherever possible and ensure that roots are not frozen for prolonged periods. Potting into larger pots or providing basal heat will help protect vulnerable root systems.

2. Manage different species or plant types to reflect their sensitivity to various stresses. Avoid regimes which cause cycling between different stresses, e.g. high and low temperature, or under and over watering, as combinations of stress may be most detrimental.

3. Avoid late propagation when natural light levels are decreasing, because these less vigorous cuttings will suffer most from overwintering losses.

4. Select cuttings from only the most vigorous and healthy stockplants. Only the best quality cuttings will have enough reserves for both rooting and overwintering.

Cold storage of cuttings

1. Make sure softwood cuttings are turgid and not stressed prior to storage. Reduce moisture losses during storage by carefully sealing cuttings in polythene bags.

2. Avoid storing more sensitive, e.g. larger-leaved cutting types, for prolonged periods.

3. With respect to hardwood cuttings, avoid techniques or propagation periods (e.g. late spring), which encourage excessive shoot and root development before planting out.

EXPERIMENTAL SECTION

SECTION 1. THE EFFECTS OF OVERWINTERING CONDITIONS ON CUTTING SURVIVAL AND QUALITY

Introduction

Previous work in this project demonstrated that loss of cuttings overwinter was primarily due to environmental stress, although in some cases significant losses could also be attributable to poor rooting and lack of root development prior to winter. The ability to tolerate overwintering stress, however, could also be affected by factors not directly related to the overwintering environment and it is possible that 'pre-wintering' factors such as the origin of the cuttings, their size, propagation date, type of rooting and weaning environment may affect viability. Indeed, some of these factors may account for the large variations in survival rates that occur between different years, and different nurseries within the one year. A primary objective in the final year's research therefore, was to investigate the influence of propagation date, (and to a lesser extent cutting source) on the ability of cuttings to survive overwintering stress. An experiment was set up whereby cutting production was staggered during the summer period and cuttings from different propagation dates placed in either stressful or controlled overwintering environments. The type of stress imposed varied for each species and choice was dictated by the most damaging treatments (with the exception of frost) from the previous years' results, i.e. *Ceanothus* was exposed to drought, *Euphorbia* to high aerial humidity and *Acer* to relatively high overwintering temperatures in a glasshouse. *Magnolia* had generally proven resistant to most stresses imposed on it in the previous two years, so it was decided to use an extended period of cold storage, with cuttings being stored until late into the spring.

Of the range of stress treatments imposed on cuttings in the second year, exposure to frost was found to be the most damaging in the majority of species tested. Therefore, the objective of a second separate experiment was to investigate in more detail the range of temperatures and exposure periods which caused damage to each species, and to determine if the use of modular trays pre-disposed cuttings to greater freezing stress than the use of pots during the winter.

Because cold storage had appeared promising in year 1 it was decided, after consultation with nurserymen, to investigate if storage was a feasible mechanism for 'by-passing' many of the overwintering stresses which influenced cutting survival within polytunnels or glasshouses. Additionally, was it possible to save space within the cold store by removing plants from their trays and storing them in a more compact manner? This would also allow for rapid potting-on in the spring. To this effect, a third experiment was set-up to explore the potential of storing un-plugged cuttings and planting out directly, late in the spring after the threat of frost injury.

A. The effects of propagation time on subsequent cutting survival

Materials and methods

As in the previous year's work, the species selected for study were; *Ceanothus* 'Autumnal Blue', *Magnolia soulangiana*, *Euphorbia griffithii* 'Fireglow' and *Acer palmatum* 'Aureum'. All cuttings were prepared in a standardised way, with cuttings being dipped for 5 seconds in a 1,250 mg l^{-1} indole-3-butyric acid (IBA) solution (50 % acetone: 50 % water), left to dry and then stuck in compost (50% peat:50% fine Cambark) using rigid modular cellular trays (P.G. Horticulture Ltd.). Cell volume reflected cutting thickness, with *Magnolia* and *Euphorbia* rooted in 80 cm^3 cells and *Ceanothus* and *Acer* rooted in 55 cm^3 cells.

Ceanothus

Cuttings of between 60-80 mm in length were harvested from field-grown stockplants on three separate occasions, namely 14 July, 6 September and 3 October 1994, known as propagation times 1, 2 and 3, respectively. Cuttings from the first two harvests were rooted under natural light in a fog tunnel, whereas those harvested at the last date of 3 October 1994 were rooted in a fog compartment, provided with artificial light (400 W SON-T, sodium lamps) because natural irradiance levels had declined by this time. Plants were removed from the fog and weaned after approximately eight weeks. They were then placed on sandbeds in a side-ventilated polytunnel with 'frost-free' basal heat.

On 16 December 1994 trays representing each propagation date were divided into two batches and designated as either control or drought stress treatments. Both treatments were maintained on sandbeds within the polytunnel environment but trays in the drought treatment were watered intermittently when the growing medium had shrunk from the sides of most modules.

Magnolia

Apical cuttings were initially collected on 14 July 1994 from eight-year-old stockplants, grown in raised beds and protected from excessive wind exposure by a permeable windbreak. Unfortunately, regrowth was insufficient to allow further cutting collection from these stockplants during the summer and later propagation utilised material from younger stockplants grown at a different location. Growing conditions however, were similar between the two sites, with the latter stockplants also being grown in a protected raised bed. Cuttings from the second stockplant source were collected on two dates; originally apical cuttings were removed on 6 September 1994 with a subsequent harvest using sub-apical cuttings on 23 September 1994. Cuttings from this last collection (23 September) were rooted under artificial light, in contrast to the earlier collections where rooting was implemented under natural light in a fog tunnel. Cuttings collected on 14 July, 6 September and 23 September 1994 were labelled propagation times 1, 2 and 3, respectively.

Cuttings were rooted and weaned over an eight week period and maintained, as for

Ceanothus, in a polytunnel until 16 December 1994 before being allocated to two treatments. The control treatment consisted of retaining plants within the polytunnel, but the stress treatment was implemented by placing half the trays from each propagation date into cold storage from 16 December 1994 to 21 April 1995 at 2 +/- 1°C and 87% r.h. After storage, trays were placed back in the polytunnel prior to final assessments and planting out.

Euphorbia

Euphorbia cuttings were collected on two dates, 2 August (time 1) and 8 September 1994 (time 2) using material obtained from stockplants maintained in a protected raised bed. Rooting was carried out under fog in natural light and plants weaned after six weeks before being placed in the polytunnel to grow-on. Trays from each propagation date were divided on 16 December 1994 and half the cuttings placed under low polythene tents within the polytunnel, to create a high humidity environment. The sand bed was watered frequently to maintain humidity, and mean r.h. values were 66% compared to 48% around control plants in the open polytunnel. Humidity treatments were terminated on 21 April 1995, when the low polythene tent was removed.

Acer

Cuttings were collected on 29 June 1994 from two sources, namely containerised stockplants maintained in either a ventilated glasshouse or a polytunnel. Only active apical cuttings of approximately 100 mm length were collected. It was intended to take a second batch of cuttings from these stockplants, but as with *Magnolia*, lack of stockplant regrowth limited the number of appropriately sized cuttings that could be harvested on 16 August 1994. Therefore, to supplement these treatments cuttings were harvested from either field-grown stockplants or containerised plants held outside but provided with protection by windbreaks. As before, only vigorously growing cuttings of standard length were selected and there was no apparent visual difference between cuttings from different sources.

All cuttings were rooted in the fog tunnel using natural light and plants were weaned approximately eight weeks after propagation. Plants were allowed to establish in their modular cells before being divided into control and stress treatments on 16 December 1994. As with other species the control was overwintered in the polytunnel, but the stress treatment consisted of moving trays into a heated glasshouse and maintaining temperatures between 15 and 20°C until 21 April 1995, after which trays were moved back to the polytunnel.

Recording

Plants were assessed for rooting and tissue viability throughout the winter and spring with a final assessment being made between 24 and 28 April 1995. The data were analysed to determine initial rooting percentage, percentage survival after stress treatments of those cuttings that had originally rooted, and percentage of class 1 established cuttings (liners). Class 1 liners were those designated as having no signs of shoot, foliage or root die-back.

Additionally, a sample of cuttings from each treatment was planted out in a raised bed (comprising equal parts by volume of peat, bark, coarse grit and sand with lime at 3 kg m^3 and Ficote 180, 16:10:10 at 1 kg m^3 of compost) on 17 May 1995, to assess how propagation date and overwintering environment affected subsequent establishment. For the evergreen example, *Ceanothus*, chlorophyll fluorescence spectrometery was used on 3 February and 28 March 1995 to determine any effects due to drought stress. This technique measures the efficiency of photosynthetic electron transport, and the ratio of variable to maximum fluorescence (Fv/Fm) is sensitive to certain forms of environmental stress. Reduction in Fv/Fm values can indicate the extent of stress exposure before visual symptoms become apparent. A number of cuttings were also assessed for changes in the ratio of dry to fresh weight in December, prior to stress treatment, February during exposure to stress and in April, 4 days after removal from stress treatments. For safety reasons *Euphorbia* was not included because drying tissues of this species may have released volatile, irritable compounds.

Statistical analyses were based on least significant difference (LSD) at the 5% level. This indicates the size of difference between individual treatment means required to give a 95% probability that the effects are not due to chance.

Results

Rooting

Rooting rates varied with species, but with the exception of *Magnolia*, propagation time did not significantly affect the ability to root (Table 1). In *Magnolia*, later propagation in September (times 2 and 3) reduced the number of cuttings rooting compared to July propagation, although the effect appeared to be partially ameliorated by rooting under artificial light. Interestingly in *Acer*, later propagation, although not statistically significant resulted in marginally higher levels of rooting.

Survival and Quality

Survival rates and the number of class 1 liners were, as in the previous year, based only on the number of cuttings which successfully rooted, and nurserymen should remember that losses may be even greater when based on number of cuttings propagated.

In the majority of species, the establishment rates after planting out 'mirrored' results for overwintering survival and data are not shown. In *Magnolia*, however, plant losses continued after planting out and these data are included.

Ceanothus

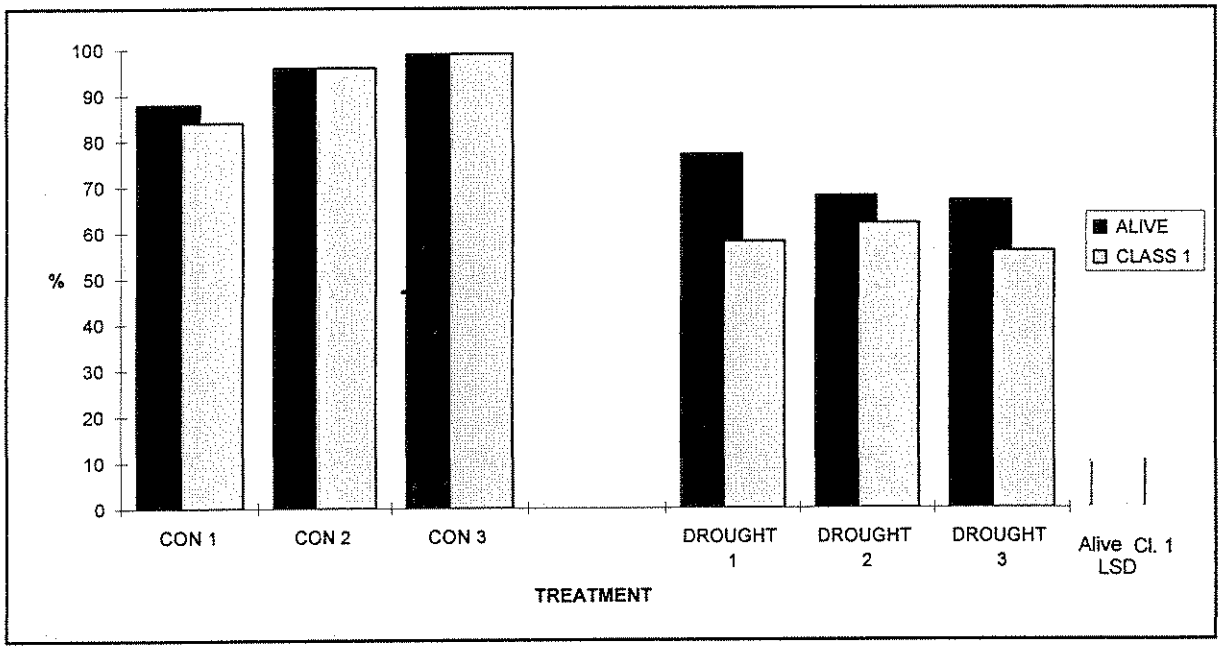
Exposing cuttings to drought stress during the winter reduced viability and the proportion of class 1 cuttings (Figure 3), and resulted in correspondingly lower chlorophyll fluorescence values Fv/Fm when assessed in March (Figure 4). Survival rates in some of the stress treatments were as low as 67%. There was no consistent effect due to propagation date

Table 1. Mean rooting percentages

Species	Propagation Time			
	1	2	3	LSD
Ceanothus	79.0	81.4	75.6	6.9
Magnolia	96.9.	71.2	80.8	7.1
Euphorbia	99.6	99.0	/	1.7
Acer	82.5	88.6	/	6.6

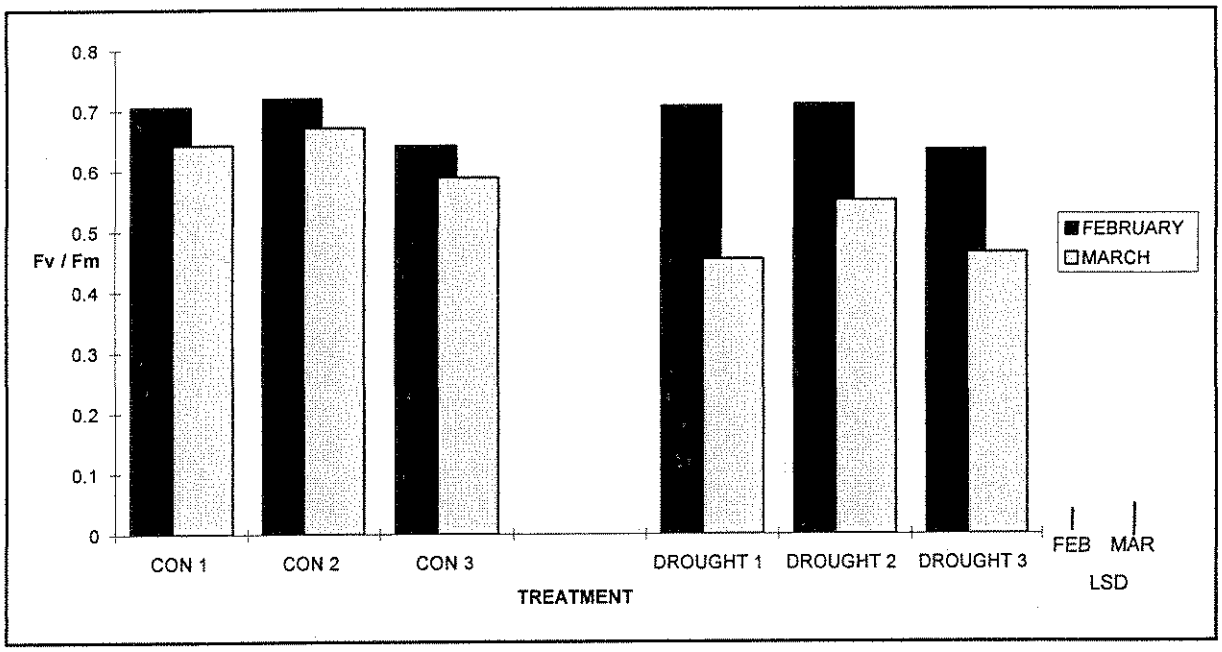
Key: Propagation times: *Ceanothus* 1 = 14/7/94, 2 = 6/9/94, 3 = 3/10/94.
Magnolia 1 = 14/7/94, 2 = 6/9/94, 3 = 23/9/94.
Euphorbia 1 = 2/8/94, 2 = 8/9/94.
Acer 1 = 29/6/94, 2 = 16/8/94.

Figure 3. *Ceanothus* **- Percentage survival and class 1 liners**



Key: Propagation times: 1 = 14/7/94, 2 = 6/9/94, 3 = 3/10/94.

Figure 4. ***Ceanothus*** **- Chlorophyll fluorescence values Fv/Fm recorded in February and March 1995**



Key: Propagation times: 1 = 14/7/94, 2 = 6/9/94, 3 = 3/10/94.

however, with early propagation of cuttings corresponding to lowest survival rates in the control treatments, but highest rates of survival when cuttings were exposed to drought stress.

There was a slight decrease in Fv/Fm values between February and March, suggesting viability decreases slowly with time throughout the winter, or that loss of viability may be accelerated as temperatures increase in spring, when light levels remain low and limit carbohydrate production from photosynthesis. Changes in dry to fresh matter ratios were rarely significant for either shoots or roots (Table 2), although there was a slight trend upwards in dry matter content in shoots between December and February in the stress treatment, possibly relating to less moisture in the tissues during exposure to drought. Any trend for decreasing ratios between February and April probably related as much to increased water uptake as buds began to swell, as to reduction in resources.

Table 2. *Ceanothus* - Ratio of dry to fresh weight in shoot and root tissues

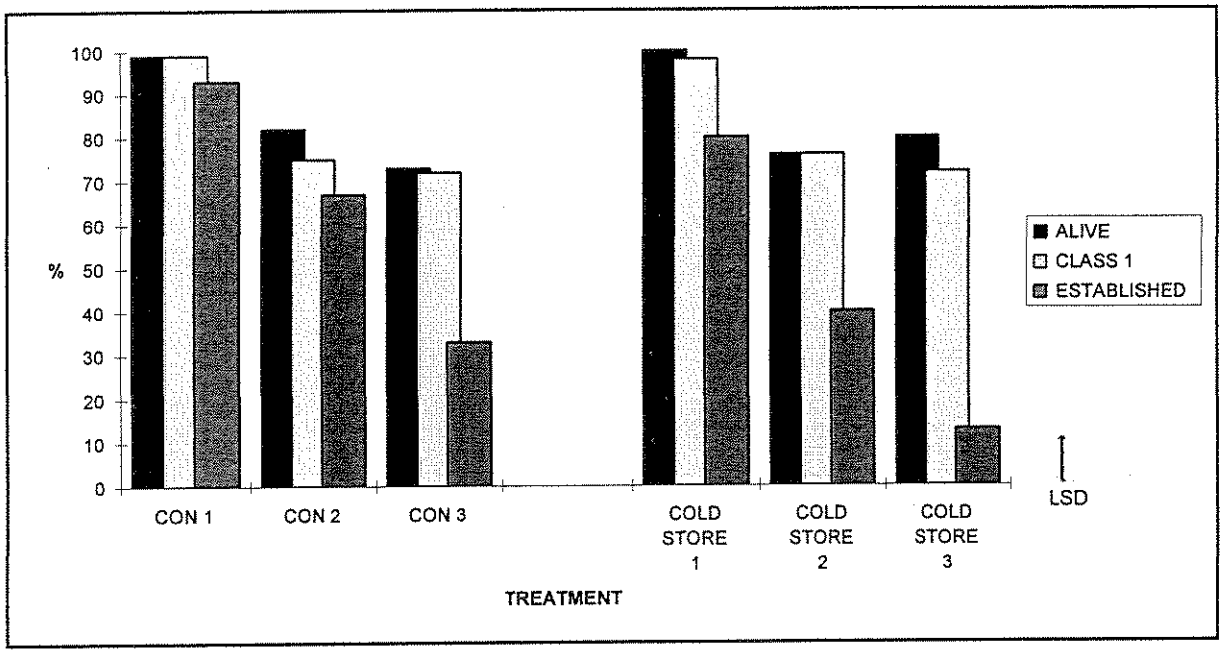
	Control			Drought stress			LSD
	1	2	3	1	2	3	n = 12
			Shoot				
December	0.37	0.36	0.40*	0.36	0.36	0.39*	
Ferbuary	0.36	0.38	0.40*	0.39	0.40	0.53*	
April	0.34	0.34	0.39	0.37	0.37	0.37	0.03
			Root				
December	0.23	0.18	0.18*	0.20	0.17	0.17*	
February	0.20	0.17	0.18*	0.21	0.17	0.17*	
April	0.21	0.24	0.22	0.24	0.22	0.20	0.04

Key: Propagation times: 1 = 14/7/94, 2 = 6/9/94, 3 = 3/10/94.
* reduced replication n = 7.

Magnolia

Propagation time had a greater effect than cold storage in determining overwintering survival of *Magnolia*, with cuttings propagated in July (time 1) having significantly greater survival rates than those propagated in September (times 2 and 3), regardless of storage (Figure 5). Subsequent establishment after planting out was also particularly poor for the non-apical cuttings from propagation time 3 (both cold stored and control), and for the cold stored

Figure 5. ***Magnolia*** **- Percentage survival, class 1 liners and establishment of rooted cuttings**



Key: Propagation times: 1 = 14/7/94, 2 = 6/9/94, 3 = 23/9/94.

cuttings from propagation time 2. As with *Ceanothus*, trends in dry to fresh weight ratios were difficult to determine as bud swelling had occurred by April and there was possibly greater volumes of water being absorbed at this time (Table 3). Nevertheless, in cold stored cuttings where bud expansion was retarded there was no apparent trend in weight ratios during the winter.

Table 3. *Magnolia* - Ratio of dry to fresh weight in shoot and root tissues

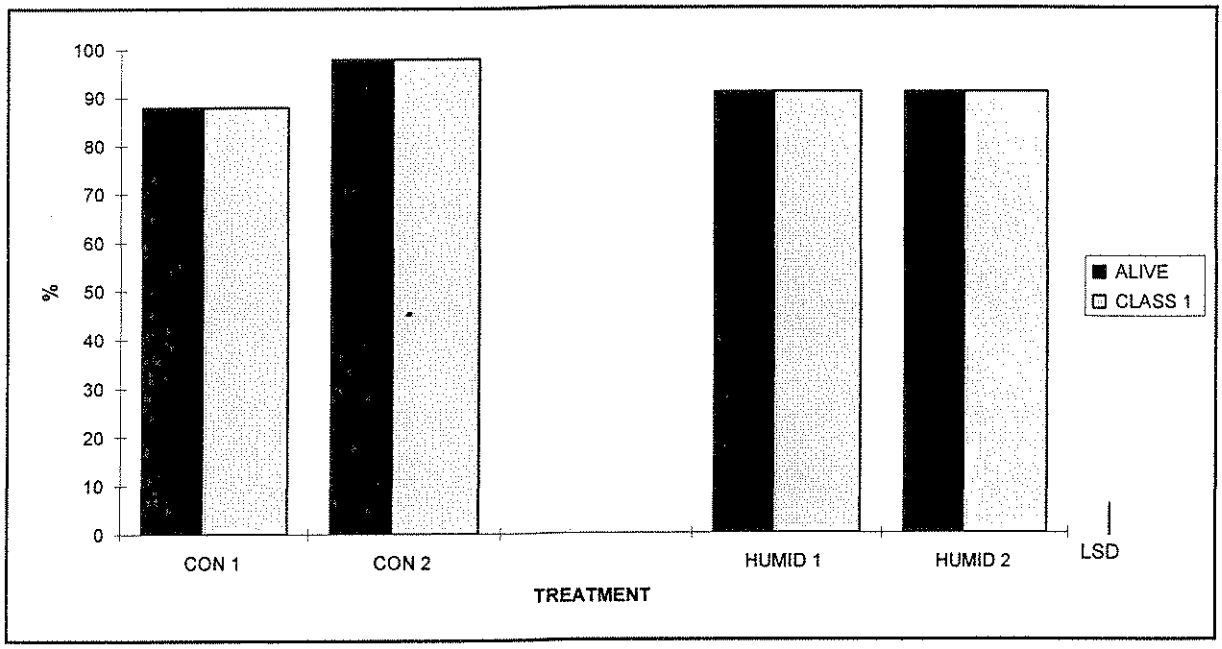
	Control			Cold storage stress			LSD
	1	2	3	1	2	3	n =12
			Shoot				
December	0.32	0.23*	0.29*	0.31	/	0.29*	
February	0.29	0.33*	0.31*	0.30	/	0.32*	
April	0.23	0.20	0.27	0.29	/	0.31	0.03
			Root				
December	0.17	0.13*	0.16*	0.17	/	0.16*	
February	0.16	0.15*	0.15*	0.16	/	0.16*	
April	0.13	0.12	0.16	0.17	/	0.16	0.02

Key: Propagation times: 1 = 14/7/94, 2 = 6/9/94, 3 = 23/9/94.
* reduced replication n = 5.

Euphorbia

The high humidity environment had no apparent effect on *Euphorbia* plant survival or quality (Figure 6). This was in contrast to the previous year's results where humidity treatments often resulted in loss of quality. However, in the original experiments (1993/94) the humidity effect was interacting with either an under-watering or over-watering of the growing media, suggesting a combination of stresses may be more detrimental than a single stress. Lowest success (88% survival) was associated with the control treatment of plants derived from the first propagation date, and this was significantly lower than control values for plants from the later propagation time, implying that factors associated with propagation may be influencing the ability of *Euphorbia* to overwinter successfully.

Figure 6. ***Euphorbia*** **- Percentage survival and class 1 liners**



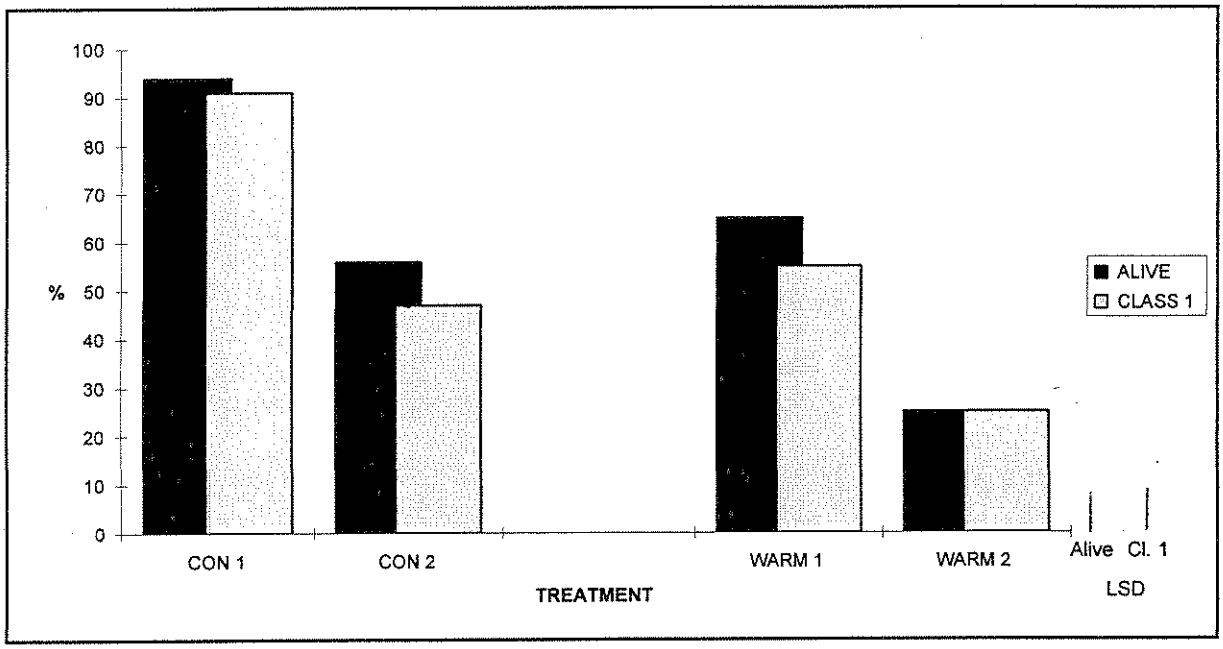
Key: Propagation times: 1 = 2/8/94, 2 = 8/9/94.

Acer

Cutting survival and quality were strongly affected both by propagation date and whether stress was imposed by placing trays in the warm glasshouse to overwinter (Figure 7). Later propagation and the exposure to stress resulted in greatest losses. Results also indicated that the source of cuttings, i.e. from which group of stockplants they were derived, significantly influenced the chances of the cutting surviving, (Table 4). It would appear that late propagation *per se* was not necessarily detrimental as cuttings obtained on 16 August 1994 (time 2) from containerised stockplants grown outside had 83 and 70% survival for control and stress treatments, respectively. This was in stark comparison to cuttings propagated at the same time from field-grown plants, where survival was 46% for controls and only 3% for stressed plants. It was interesting to note that in some of these treatments survival was not related to rooting ability, as cuttings from field-grown stockplants had rooted in excess of 90%.

Ratio of dry to fresh weights in shoot tissues generally show a downward trend from December to April, although there was no significant difference in percentage dry matter between stressed and control plants at the last sampling date, (Table 5). Losses of reserves within tissues therefore may only partially explain the reduction of viability in *Acer* during winter.

Figure 7. *Acer* **- Percentage survival and class 1 liners**



Key: Propagation times: 1 = 29/6/94, 2 = 16/8/94.

Table 4. *Acer* - Effects of cutting source on percentage rooting, survival and quality of rooted cuttings

Cutting source	Rooting	Alive	Class 1
Con 1			
Glasshouse	78	92	86
Polyhouse	92	97	97
Con 2			
Field	96	46	37
Outside protected	74	83	78
Warm 1			
Glasshouse	73	64	44
Polyhouse	86	66	66
Warm 2			
Glasshouse	74	24	24
Field	93	3	3
Outside protected	86	70	70

Key: Propagation times: 1 = 29/6/94, 2 = 16/8/94.

Table 5. *Acer* - Ratio of dry to fresh weight in shoot and root tissues

	Control		Warm stress		LSD
	1	2	1	2	n = 12
		Shoot			
December	0.43	0.36	0.45	0.46	
February	0.35	0.37	0.32	0.43	
April	0.31	0.31	0.31	0.29	0.04
		Root			
December	0.22	0.24	0.29	0.25	
February	0.25	0.24	0.25	0.22	
April	0.25	0.27	0.28	0.23	0.03

Key: Propagation times: 1 = 29/6/94, 2 = 16/8/94.

B. The effects of container size on cutting survival after exposure to freezing stress

Materials and methods

Cuttings were propagated in an identical manner to the first propagation treatment for each species in Part a), i.e. *Ceanothus* and *Magnolia* harvested on 14 July, *Euphorbia* on 2 August and *Acer* on 29 June 1994. On 5 October 1994 a number of trays for each species were selected and cut into individual modular cells, care being taken not to damage the rooted cuttings inside. These cuttings were graded and divided into three groups, where one group was potted-on into 11 cm pots, one group potted-on into 7 cm pots and the last group retained in their original modules. The potted-on plants were placed in a growing medium consisting of 50:50 peat:fine bark with 1 g l^{-1} Ficote 140, 16:10:10 controlled release fertiliser added. A number of fertiliser granules were also incorporated into the surface of the medium in the modular cells to simulate similar nutritional conditions to the plants potted-on. All plants were then placed back onto 'frost-free' sandbeds within a polytunnel and watered frequently to avoid any drying-out, especially in the 'now-isolated' modules.

Plants were grown-on for approximately three months in the polytunnel to allow some establishment of roots within the larger containers, then between 20 January 1995 and 28 February 1995 plants were moved progressively to a programmable freezer and exposed to a range of low temperature treatments. Four temperature regimes (Table 6), relevant to the cold tolerance determined for each species in previous experiments, were implemented within a week of one another to minimise variation due to natural deacclimation within the plants.

Each treatment comprised six replicate plants in each container size, i.e. 11 cm, 7 cm containers and 80 cm^3 modular cells. Plants were randomly distributed in the low temperature incubator to limit possible bias due to localised temperature variations. Before being placed in the incubator, plants were watered thoroughly bringing growing media to container capacity. Surface water on tissues and containers was removed before treatments commenced. All containers were placed in plastic trays containing coarse sand and enclosed in a polythene-covered box to create conditions similar to a 'mini-polytunnel'. Temperature values were recorded for air temperature within the mini-polytunnel and medium temperature in all three container sizes using thermistor probes and data loggers. Temperature cycles were arranged in a step-wise manner, thereby minimising tissue damage resulting from rapid rates of temperature change, and assisting the even temperature distribution within the incubator. Thus rates of air cooling and warming at sub-zero temperatures did not exceed 2°C h^{-1}. After treatment the plants were grown at 15 to 25°C and assessed for re-growth and damage after 42 days.

Results

Air temperatures recorded within the polythene box during freezing cycles (Table 7) were generally higher than those for the freezer itself (in some instances by a margin of approximately 3°C), and the use of the 'mini-polytunnel' and sand bases appeared to give some protection to the plants. The extent to which this applied however, varied with depth of temperature and length of exposure. Increasing the time of freezing cycles at any particular

Table 6. Low temperature regimes for containerised plants of *Magnolia, Ceanothus, Acer* and *Euphorbia*

Treatment	Freezer programme	
	Temp. (°C)	Duration (h)
Magnolia		
I	-10.0	1
II	-4.0	4
III	-4.0	12
IV	-4.0	36
Ceanothus		
I	-2.5	4
II	-5.0	4
III	-2.5	12
IV	-5.0	12
Acer		
I	-2.5	4
II	-5.0	4
III	-2.5	12
IV	-5.0	12
Euphorbia		
I	-5.0	4
II	-10.0	4
III	-5.0	12
IV	-10.0	12

Table 7. Lowest recorded air and growing medium temperatures during freezing, and number of *Magnolia, Ceanothus, Acer* and *Euphorbia* plants surviving. Results based on a replicate of six plants

Treatment	Air	Container size					
		11 cm		7 cm		Module	
	Temp (°C)	Temp (°C)	Alive	Temp (°C)	Alive	Temp (°C)	Alive
Magnolia							
I	-7.2	-1.5	3	-5.8	0	-8.4	0
II	-2.8	-0.3	5*	-0.2	5	-2.7	4
III	-2.8	-0.3	4	-0.2	4	-3.1	3
IV	-5.8	-6.5	0	-6.5	0	-6.0	0
Ceanothus							
I	-1.9	-0.3	6	-0.1	6	-0.4	6
II	-3.4	-0.4	5	-0.3	6	-1.9	5
III	-2.0	-0.4	5	-0.5	6	-2.2	6
IV	-4.3	-0.9	6	-3.7	1	-4.1	0
Acer							
I	-1.9	-0.3	0	-0.2	1	-0.3	2
II	-2.9	-0.4	0	-0.2	0	-2.8	1
III	-2.1	-0.5	0	-0.8	1	-2.5	3
IV	-4.6	-0.9	0	-4.0	0	-5.1	0
Euphorbia							
I	-2.7	-0.4	5	-0.2	6	-0.8	5
II	-7.8	-1.1	4	-4.6	0	-8.2	0
III	-4.5	-2.3	6	-3.7	1	-4.8	0
IV	-10.3	-9.8	0	-10.1	0	-10.6	0

Key: * Replicate of five plants.

temperature often resulted in lower air, and thus lower growing media temperatures. In certain cases this had a significant effect on survival, for example in freezing cycles II to IV with Magnolia, increasing the duration of the cold period to 4, 12 and 36 hours at a programmed -4°C (recorded air temperatures of -2.8, -2.8 and -5.8°C respectively) resulted in overall survival percentages of 83 +/- 8, 61 +/- 10 and 0%. Increasing freezing from 12 to 36 hours lowered recorded air temperatures by approximately 3°C and medium temperatures by as much as 6.3°C in 7 cm containers, (Tables 6 and 7).

The period of freezing within the growing medium and thus the temperatures that roots were exposed to generally influenced overall plant survival. Container size strongly influenced the temperatures recorded in the root zone, with larger containers 'buffering' against the lower temperatures. For example in a short severe 'frost' such as cycle I in *Magnolia*, temperatures within modules (-8.4°C) reached similar levels to those recorded in the surrounding air (-7°C), but were marginally higher in 7 cm containers (-5.8°C), and considerably higher in 11 cm containers (-1.5°C). Similarly, in more prolonged but less severe freezing cycles, temperature remained higher in larger containers and resulted in subsequently greater survival, (e.g. *Ceanothus* cycle IV). When results were pooled from different freezing cycles for each species, overall survival was significantly affected by container size. In *Euphorbia*, survival rates were in the order of 62.5 +/- 6% in 11 cm, 29.2 +/- 5 in 7 cm and 20.8 +/- 5 in modular containers.

For all species with the exception of *Acer*, greatest damage (i.e. ≤ 50% plant survival) was correlated with treatments where growing medium temperatures dropped below -3°C, regardless of the period of the exposure. Where temperatures at the root zone remain above -3°C, survival was generally high, even after extended periods of sub-zero air temperatures, (e.g. 12 hours freezing in cycles III of *Ceanothus* and *Euphorbia*).

In treatments where air temperatures were particularly low, plant death appears to have resulted directly from damage to the shoots, (e.g. in a number of the *Magnolia* plants in 11 cm containers during treatment I), as relatively high sub-zero temperatures were recorded in the root zone, (i.e. -1.5°C). The evergreen *Ceanothus* was particularly sensitive to low air temperatures with early leaf-tip dieback being apparent and extensive defoliation occurring subsequently in some replicates. In plants exposed to freezing cycles I to III however, regrowth was rapid and percentage survival was high when growing on at 15 to 20°C.

The trend in survival for *Acer* was opposite that for the other three species, with greatest survival often being associated with the smallest container size regardless of cold treatment. The reasons for this are unclear, but are probably linked to factors not directly associated with the freezing cycles, e.g. extent of root anaerobism. Potting into larger volumes of media is likely to have restricted the oxygen supply to the relatively small root-ball, resulting in root anaerobism. This would be exacerbated by the frequent watering regime applied to all cuttings in order to ensure that modular plants did not dry out.

C. The use of cold storage to overwinter rooted cuttings which have been removed from modules

Materials and methods

Two trays of cuttings for each species were propagated by the standard method described in Part a), with *Ceanothus* being harvested on 6 September, *Magnolia* on 14 July, *Euphorbia* on 2 August and *Acer* on 16 August 1994. Rooted cuttings were maintained on sandbeds within the side-ventilated polytunnel until 16 December 1994, when every alternate plant in each tray was carefully removed and placed (whilst trying to maintain the root-ball) onto a 'bakers' plastic tray (74 x 45 x 16 cm), with louvred sides and base. Any cuttings which had not rooted or had a poor root system were discarded at this stage. Once full, each bakers tray was covered with a black polythene 'bin-liner' to avoid excessive desiccation, and stored one on top of another, inside a large mobile bulk-bin. A top was placed on the bin and the whole bin wheeled into a cold store set at 2 +/- 1°C and 87% r.h. Cold storage treatment lasted for 18 weeks, and the bin was periodically opened and cuttings inspected for disease. Generally, infection levels were low and cuttings were not sprayed with fungicide throughout the storage period.

On 24 April 1995 cuttings were removed from cold storage and along with polytunnel-maintained plants assessed for viability before being planted out into a protected raised bed (equal parts peat, bark, coarse grit and sand with lime at 3 kg m^{-3} and Ficote 180, 16:10:10 at 1 kg m^{-3}) to grow-on. A second assessment of establishment was made on 14 June 1995.

Results

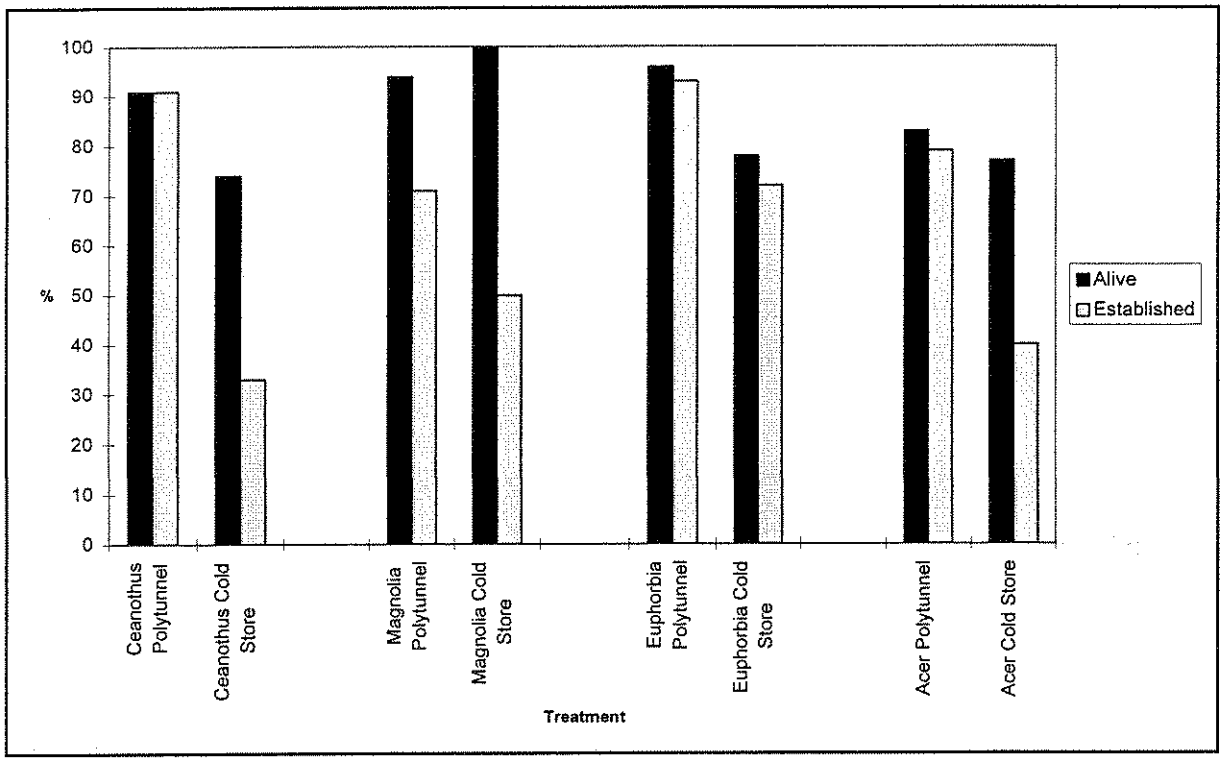
Removing plants from their modular cells and placing into cold storage from December to April resulted in a marginal decline in viability for most species compared to cuttings overwintered in the polytunnel, (the exception being cold-stored *Magnolia* cuttings, where all appeared viable). Nevertheless, nearly three-quarters of all cuttings in each species survived the prolonged cold storage, (Figure 8). The rate of establishment after planting out the cold stored plants was generally poor, however, with viability decreasing sharply for *Ceanothus*, *Magnolia* and *Acer*. In contrast, relatively high levels of control plants of *Ceanothus*, *Acer* and *Euphorbia* survived the planting out process.

The reasons for the decrease in establishment of cold stored plants is not clear, but may relate to; 1. the cold storage process stressing plants and reducing their reserves, 2. the removal of cuttings from their modules either damaging the roots directly, or causing root desiccation, or 3. the sudden movement from cold store to outside conditions *per se* resulting in stresses that damaged the cuttings.

Conclusions

The type and extent of overwintering stress, the date of propagation and in some cases the source of cutting material all influenced overwintering capability. In *Ceanothus*, exposing

Figure 8. **Cold storage of 'de-plugged' cuttings. Percentage survival and establishment**



plants to drought stress during winter was the most influential factor, whereas in contrast, propagation date and propagation environment had a significantly greater effect on survival in *Magnolia* than the imposition of artificial stress. Later propagation during the summer resulting in greater susceptibility to overwintering stress. In *Acer*, late propagation and stress due to overwintering environments combined to adversely affect survival rates, and cutting source had a strong influence on subsequent tissue viability. This was the case, even when cuttings appeared healthy, and where rooting percentages were in excess of 90%. Such information highlights the importance to nurserymen of ensuring that only the highest possible quality material is propagated, thus avoiding problems manifesting themselves at later stages of production. Removing cuttings from sub-standard stockplants or old stockplants with limited vigour should be avoided, because such cuttings may not have the resources to support both rooting and overwintering processes.

Although in *Acer* the trend in dry/fresh weight ratios declined with time, there was no apparent difference between cuttings in the control and stress treatments at the last sampling date. Therefore, the loss of carbohydrate and other resources may not be the only influencing factor in determining survival, and alterations in hormone levels or the production of particularly damaging compounds associated with stressful environments could also contribute to the loss of viability.

Where nurserymen are faced with the possibility of the cuttings being exposed to frost, e.g. unheated polytunnels and glasshouses, overwintering in modular trays may, unfortunately, induce greater susceptibility to frost damage compared to overwintering in pots, where there is a larger volume of growing media. This may be especially true during periods of prolonged cold where temperatures remain consistently below 0°C for a number of days. Roots are generally the most susceptible part of the cutting to freezing injury and the results presented here suggest that when temperatures in the growing media drop below approximately -3°C, plant death invariably occurs.

Although earlier potting-on into larger containers reduced the likelihood of frost injury, the technique was not beneficial to *Acer* cuttings, where a large proportion of the cuttings failed after potting-on, even without exposure to frost. This technique therefore, may be of use for more vigorous lines, but at this stage cannot be recommended for weak or slow growing species.

Removing cuttings from their modules and cold storing them closer together in trays, although giving quite encouraging results initially, resulted in poor subsequent establishment in the raised bed. The reasons for this, however, may not relate to the cold storage itself, but to the way the cuttings were handled, because cuttings from previous cold storage experiments gave better establishment rates. The cuttings in these original experiments were retained in the modular trays during storage and were placed back in a polytunnel for a number of weeks prior to planting out. Therefore, removing cuttings from the modules may predispose roots to more physical damage or desiccation stress. Likewise moving cuttings straight from cold storage (2°C) to the raised bed (where temperatures were as high as 22°C on occasions), could have imposed significant additional stress on tissues. It may be that cold stored cuttings need to be 'weaned' from low temperature and light environments, by one or more intermediate stages before being exposed to outdoor conditions. Another possible factor explaining loss of viability is that the storage period may be critical, and holding cuttings back for too long in

spring proves detrimental. Further research is required to illuminate some of the more specific problems relating to cold storage and to give clearer guidelines to the advantages/ disadvantages of overwintering storage within certain practical considerations such as not increasing total handling between propagation and potting-on.

Section 2. Cold storage of summer softwood cuttings

Introduction

It has been demonstrated in this project that short-term cold storage allows greater flexibility in the management of softwood cuttings during summer. An additional and indirect consequence of the research so far, however, has been the indication that cold storage may have a beneficial effect on rooting ability in some species. The objective of this third year's work was to study this aspect of storage in more detail. Three species were used: *Cornus alba* 'Sibirica' as a comparison to hardwood cutting production where rooting appears to be stimulated after cold storage; *Syringa vulgaris* 'Madame Lemoine' to determine if chilling can re-activate the rooting response that generally declines after mid-summer; and as a repeat experiment, *Garrya elliptica* 'James Roof', which responded well to chilling in the previous summer.

Materials and methods

Apical cuttings of *Cornus*, *Syringa* and *Garrya* were collected from stockplant hedges on 1 August 1995 and trimmed to size (150-200 mm long) before either being propagated (Control 0 days) or cold stored for 14 days at 2°C, (Cold store 14 days). On 14 August 1995 additional cuttings were taken from hedges (Control 14 days) and propagated along with the cuttings from cold storage. Cold storage treatments consisted of storing cuttings sealed inside heavy grade, clear polythene bags, which in turn were placed in black bin liners for extra protection. Propagation of cuttings was by the standard method, i.e. bases dipped in 1,250 mg l^{-1} IBA for 5 seconds, left to dry before being stuck (two cuttings per pot) in 9 cm pots containing 50% peat, 50% fine bark. Rooting was accomplished by placing pots in an Agritec fogging tunnel until 1 October 1995, at which time cuttings were weaned-off in an open mist bed by progressively reducing the misting frequency. Cuttings from Control 0 days were assessed for rooting and viability on 16 October 1995 and the other two treatments 14 days later on the 30 October 1995. The extent of rooting in each species dictated the assessment method, with the degree of 'rooting-through' the pots being measured in the rapidly rooting *Cornus*. Additionally, overall plant quality was assessed on a scale of 1 to 4, with values of 1 denoting highest quality. In contrast, the slower rooting *Syringa* and *Garrya* were knocked-out of pots before assessments were made. Cuttings were graded on a scale of 1 to 4 for both the extent of shoot die-back or root development. Lower values denoting better shoot quality and greater root development.

Results

The effects of the cold storage treatment varied with species. In *Cornus* cold storage resulted in lower rooting percentages and loss of quality compared to either control treatment, (Table 8). The opposite was true however in *Garrya*, where the storage treatment resulted in all cuttings rooting well with a significantly better mean rooting grade than control values (Table 9). Poorest result were associated with the later control treatment. Cold storage of

Table 8. *Cornus* - The effects of cold storage on rooting performance in softwood cuttings

Treatment	No. rooted (/40)	Extent of rooting (/8)	Mean grade*	Mean root length (cm)
Con 0 d.	40	7.45	1.15	9.82
Con 14 d.	40	7.65	1.08	7.93
Cold store 14 d.	35	5.25	1.90	4.19
LSD		0.99	0.27	1.18

Key: * Lower grading values denote better quality, i.e. 1 = Good; 4 = Poor.

Table 9. *Garrya* - The effects of cold storage on rooting performance in softwood cuttings

Treatment	No. rooted (/40)	Extent of rooting (/8)	Mean shoot grade*	Mean root grade*	Mean root length (cm)
Con 0 d.	36	0.50	1.20	1.78	7.47
Con 14 d.	26	0.60	1.58	2.38	4.78
Cold store 14 d.	40	1.45	1.20	1.20	7.15
LSD		0.70	0.25	0.44	1.64

Key: * Lower grading values denote better quality, i.e. 1 = Good; 4 = Poor.

Syringa resulted in a decline in rooting ability compared to cuttings excised from stockplants at the same time (Table 10). Performance however, was comparable or even marginally better than that of the second control treatment.

Table 10. *Syringa* - The effects of cold storage on rooting performance in softwood cuttings

Treatment	No. rooted (/40)	Extent of rooting (/8)	Mean shoot grade*	Mean root grade*	Mean root length (cm)
Con 0 d.	31	2.65	1.35	2.33	7.37
Con 14 d.	17	2.40	2.25	3.08	4.07
Cold store 14d.	21	2.08	2.15	2.65	5.90
LSD		1.89	0.46	0.57	2.81

Key: * Lower grading values denote better quality, i.e. 1 = Good; 4 = Poor.

Conclusions

Response in terms of enhanced rooting due to artificial chilling appears to relate strongly to the species in question. The results for *Garrya* were similar to the results in 1994, where cold storage increased rooting response and so chilling appears to be of some benefit in this species. Cold storage is not a feasible mechanism for retaining the rooting capacity found in *Syringa* during early summer, or re-stimulate tissues to root more readily later in the year.

Section 3. Cold storage of winter hardwood cuttings

Introduction

The main objective of this experiment was to determine if there were any beneficial effects induced by artificial chilling (removing cuttings and cold storing them) compared to natural chilling (by leaving the cuttings on the stockhedges) on rooting and establishment. In the previous year's research, cold storage of *Cornus alba* 'Sibirica' cuttings prior to rooting had resulted in higher rates of establishment than either control or post-rooting storage treatments. As such, the influence of artificial chilling by way of cold storage was investigated further in both the relatively difficult-to-propagate *Cornus* and *Tilia platyphyllos* clone 229 by implementing some of the treatments earlier in the winter, when natural chilling requirements may not have been satisfied. Such treatments would also yield more accurate information on the optimum time during the winter for propagation of *Tilia* and *Cornus*.

Materials and methods

The handling of cuttings was similar to experiments of previous years, although only pre-rooting storage was investigated in this year's work. Cuttings of *Tilia* and *Cornus* were excised from stockplants, trimmed to 600 mm basal cuttings and graded into four blocks of ten cuttings according to stem thickness. Prior to rooting, cuttings were dipped into 2,500 mg l^{-1} IBA in 50 % acetone solution for 5 seconds and left to dry. To initiate roots, cuttings were placed in a rooting bin for five weeks with bases being maintained at 18 to 20°C. After rooting, cuttings were planted out into a raised bed consisting of equal parts by volume of peat, bark, coarse grit and sand with Ficote 140, 14:8:8 controlled releaser fertiliser added at 1.5 kg m^{-3}. Cold stored cuttings were enclosed in polythene bags and stored at 3°C in a jacketed store at 84 % r.h.

Specific treatments were as follows:

A Cuttings harvested on 5 December 1994, cold stored for 5 weeks until 9 January 1995, placed in rooting bin for a further 5 weeks and planted out on 13 February 1995.

B Cuttings left on stockplants to experience natural chilling until harvested on 9 January 1995, placed in rooting bin for 5 weeks and planted out on 13 February 1995.

C Cuttings harvested on 5 December 1994, cold stored for 10 weeks until 13 February 1995, placed in rooting bin for further 5 weeks and planted out on 20 March 1995.

D Cuttings harvested on 9 January 1995, cold stored for 5 weeks until 13 February 1995, placed in rooting bin for further 5 weeks and planted out on 20 March 1995.

E Cuttings left on stockplants to experience natural chilling until harvested on 13 February 1995, placed in rooting bin for 5 weeks and planted out on 20 March 1995.

Results

There was a distinct difference in the ability of the two species to establish after planting out, with *Tilia* cuttings from all treatments establishing readily, compared to relatively poor survival in *Cornus*, (Tables 11 and 12). This was probably due to *Cornus* being more susceptible to damage from late frosts, which occurred shortly after the last planting date and again between 19 and 22 April 1995. Presumably *Cornus* cuttings had less frost tolerance primarily because a greater proportion of the buds had broken dormancy and were in active growth before the frosts, in contrast to *Tilia* cuttings, where relatively few of the buds were growing by this stage, (on average, less than one per cutting).

Fresh weight changes during cold storage were relatively small for both species with usually less than 0.5% weight loss occurring, even in cuttings stored for 10 weeks, (Table 13). This indicates that cold storage treatments were relatively successful in limiting water and carbohydrate loss within the tissues.

There were no significant differences between treatments for *Tilia* in terms of establishment, with 90% survival or over being recorded in all treatments. There appeared to be no direct correlation between establishment rates and previous extent of callus production or rooting, although results from treatment B suggest that early root development in the bin, may not be beneficial in promoting establishment in the field. Early harvesting and placement into cold storage, e.g. treatments A and C, marginally reduced the extent of bud activity after rooting, compared to other treatments. Cold storing cuttings resulted in slightly greater callus production, but less actual rooting after removal from the heated bin, when compared to leaving equivalent cuttings on the stockplants.

Dry to fresh weight ratios in *Tilia* decreased with later sampling of cuttings excised straight from the stockplants. This implies either loss of reserves throughout the winter due to cell respiration, or possibly progressive water movement into stems prior to budbreak in the spring, (Table 14). Placing cuttings into cold store generally resulted in lower weight ratios, i.e. from 0.452 to 0.447 in cuttings harvested on 5 December 1994 (for both 5 and 10 week storage) and from 0.445 to 0.434 in cuttings excised on 9 January 1995, but such changes were not statistically significant. Trends in the weight ratio before and after rooting in the bin were inconsistent and varied with respective treatments; cuttings from treatments A, B, and C decreased, whereas cuttings from D and E increased.

In *Cornus*, slower rooting in treatment C, (i.e. lower percentages of rooting on removal from the bin and shorter average root length) corresponded to the highest subsequent establishment rate of 40% and the later root development may have to some extent off-set the deleterious effects of frost. As with *Tilia*, cold storage appeared to marginally reduce the number of cuttings where rooting had occurred within the bin, although callus production was always high.

Removing *Cornus* cuttings directly from stockplants between 5 December 1994 and 13 February 1995 resulted in slight decreases in dry/fresh weight ratios, i.e. from 0.457 to 0.445, (Table 15). There was no consistent trend due to cold storage however, but weight ratios decreased significantly in cuttings from all treatments during the rooting process in the

Table 11. *Tilia* - The effects of propagation date and cold storage treatments on rooting and establishment of hardwood cuttings

Treatment	Callus %	Rooting %	Mean longest root (mm)	Bud growth /stem	Establish. % (June)
A	90	25	62	0.02	98
B	70	53	108	0.18	90
C	100	45	123	0.12	100
D	98	40	95	0.50	98
E	50	48	67	0.58	98
LSD	26	32	68	0.33	11.2

Key: Treatments:

A. Cuttings harvested on 5 December 1994, cold stored for 5 weeks until 9 January 1995, placed in rooting bin for further 5 weeks and planted out on 13 February 1995.

B. Cuttings left on stockplants to experience natural chilling until harvested on 9 January 1995, placed in rooting bin for 5 weeks and planted out on 13 February 1995.

C. Cuttings harvested on 5 December 1994, cold stored for 10 weeks until 13 February 1995, placed in rooting bin for further 5 weeks and planted out on 20 March 1995.

D. Cuttings harvested on 9 January 1995, cold stored for 5 weeks until 13 February 1995, placed in rooting bin for further 5 weeks and planted out on 20 March 1995.

E. Cuttings left on stockplants to experience natural chilling until harvested on 13 February 1995, placed in rooting bin for 5 weeks and planted out on 20 March 1995.

Table 12. *Cornus* - The effects of propagation date and cold storage treatments on rooting and establishment of hardwood cuttings

Treatment	Callus %	Rooting %	Mean longest root (mm)	Bud growth / stem	Establish. % (June)
A	100	65	115	3.83	0
B	100	80	118	3.65	2
C	98	58	58	4.70	40
D	100	65	102	4.62	25
E	88	78	95	4.97	2
LSD	7.3	24	33	0.74	26

Key: Treatments:

A. Cuttings harvested on 5 December 1994, cold stored for 5 weeks until 9 January 1995, placed in rooting bin for further 5 weeks and planted out on 13 February 1995.

B. Cuttings left on stockplants to experience natural chilling until harvested on 9 January 1995, placed in rooting bin for 5 weeks and planted out on 13 February 1995.

C. Cuttings harvested on 5 December 1994, cold stored for 10 weeks until 13 February 1995, placed in rooting bin for further 5 weeks and planted out on 20 March 1995.

D. Cuttings harvested on 9 January 1995, cold stored for 5 weeks until 13 February 1995, placed in rooting bin for further 5 weeks and planted out on 20 March 1995.

E. Cuttings left on stockplants to experience natural chilling until harvested on 13 February 1995, placed in rooting bin for 5 weeks and planted out on 20 March 1995.

Table 13. Percentage fresh weight change in *Tilia* and *Cornus* cuttings during storage treatments

	Cold store 5 weeks 5 Dec-9 Jan	Cold store 10 weeks 5 Dec-13 Feb	Cold store 5 weeks 9 Jan-13 Feb	LSD
Tilia	-0.525	-0.527	-0.320	0.278
Cornus	-0.360	-0.571	-0.101	0.183

Table 14. *Tilia* - The effects of previous treatment and date of analysis on ratio of dry to fresh weight

	Date			
Previous treatment	5 Dec.	9 Jan.	13 Feb.	20 Mar.
None	0.452	0.445	0.430	/
Rooting bin	/	/	0.443	0.442
Cold store 5 wks	/	0.447	0.434	/
Cold store 5 wks + rooting bin	/	/	0.442	0.463
Cold store 10 wks	/	/	0.447	/
Cold store 10 wks + rooting bin	/	/	/	0.430
LSD				0.015

Table 15. *Cornus* - The effects of previous treatment and date of analysis on ratio of dry to fresh weight

Previous treatment	Date			
	5 Dec.	9 Jan.	13 Feb.	20 Mar.
None	0.457	0.454	0.445	/
Rooting bin	/	/	0.410	0.393
Cold store 5 wks	/	0.449	0.457	/
Cold store 5 wks + rooting bin	/	/	0.393	0.411
Cold store 10 wks	/	/	0.456	/
Cold store 10 wks + rooting bin	/	/	/	0.402
LSD				0.010

bin. This was presumably due to the large extent of shoot growth that occurred in the dark.

Conclusions

In *Cornus*, late spring frost damage appears to have interfered with treatment responses. Interestingly, the most successful treatment was the 10 week artificial cold storage, which was planted out at the later date of 20 March 1995. This treatment gave similar results to spring cold stored treatments in the previous year, but significantly greater establishment than cuttings planted out at the same time, but not previously cold stored. This was true despite both cold stored and control cuttings having high levels of bud activity and it may be that there is a residual cold storage effect even after placement in the rooting bin. It is possible that cold storage has either delayed the deacclimation (dehardening) process or retarded root emergence sufficiently to resist the late frost damage.

All treatments with *Tilia* resulted in relatively high rates of establishment, possibly due to the limited bud activity at planting out, and it would appear that this species is best propagated either in autumn or mid-winter before the buds have been sufficiently chilled. This avoids any early growth which may be prone to subsequent cold injury.

Acknowledgements

I should like to thank Mrs Anne King and Mr Roy Taylor for their technical assistance and help in processing the data.

Contract between HRI (hereinafter called the "Contractor") and the Horticultural Development Council (hereinafter called the "Council") for a research/development project.

PROPOSAL

1. TITLE OF PROJECT **Contract No: HNS/44**

PRE- AND POST-ROOTING STORAGE OF SOFTWOOD AND HARDWOOD CUTTINGS

2. BACKGROUND AND COMMERCIAL OBJECTIVE

Propagation annually of many millions of shrubs and some tree cuttings creates peak demands for labour, propagation and growing-on facilities. There is little management flexibility because the time of propagation is determined by the availability and condition of cuttings, and the need to schedule production towards specific market opportunities. When under pressure nurserymen risk compromising their future success by collecting over-mature cuttings or reducing inputs during cutting preparation, thus increasing the risk of variable rooting and failure.

Not all cuttings progress rapidly from propagation to container, and some subjects are overwintered in flats before being potted-off, with no guarantee that apparently rooted cuttings ~~will~~ survive and grow. Many high value subjects are affected, and this problem is of major concern to many nurserymen.

The purpose of this project is to investigate the opportunities for storing cuttings both before and after rooting for relatively short periods, in order to spread peak workloads to the benefit of both the cutting material and management of staff and facilities, and to store successfully overwinter difficult deciduous and evergreen species that die-off in their cutting trays. Storage of micropropagules to accumulate production ready for preferred spring establishment may be considered also, in conjunction with HNS 32.

Because storage is aimed essentially at retarding the deterioration of cuttings but cannot prevent it, it follows that the effectiveness of propagation treatments and environments in determining cutting survival and quality of rooting is inseparable from the storage phase in determining overall success. This project is opportune in view of the progress made in these related areas.

3. POTENTIAL FINANCIAL BENEFIT TO THE INDUSTRY

This is difficult to assess accurately because of the lack of essential statistics, and because propagation success ranges from very high to very low among the many thousands of subjects produced commercially, with a further opportunity to succeed with hitherto 'impossible' subjects as technology improves. However, given that the annual

output of 158 million container grown shrubs indicates the minimum number of cuttings propagated, and given that expected failure rates (estimated by some nurserymen to approach 40% overall) encourage taking even more cuttings to compensate for losses, the scale of the national problem is evident. Benefits from this work are likely to be measured by reduced management pressure as well as by space saving and increased success rates.

4. SCIENTIFIC/TECHNICAL TARGET OF THE WORK

The aim is to investigate the response of softwood and hardwood cuttings to pre- and post-rooting cool or cold storage in terms of survival, rooting and growth as appropriate, and to identify and correct the causes of losses in newly rooted cuttings during their first winter.

The outcome will be determined by the interaction of the following factors, which will be included in factorial experiments where possible:-

(a) The ease of rooting of the subject and the extent of cutting deterioration during collection, handling and preparation.

(b) Storage conditions, including humidity, temperature, treatment against pathogens and the presence or absence of light.

(c) Of particular importance will be environmental conditions used for rooting, which for pre-rooting storage can be seen as providing post-storage resuscitation, and for post-rooting storage creating a pre-storage reserve of establishment potential.

(d) Whether or not cuttings are able to increase reserves after rooting and before storage, and whether they are able to develop important seasonal conditions such as winter hardiness, will be relevant, especially in the case of overwinter losses.

5. CLOSELY RELATED WORK - COMPLETED OR IN PROGRESS

The opportunity and justification for this work are increased by progress in understanding and improving propagation environments (HO/9) and rooting potential and weaning (HNS 27), linked to strategic MAFF funding in L102A of £110K p.a. The general literature on cutting storage deals mainly with herbaceous species and the work is largely of an *ad hoc* nature. The diversity of shrub and tree species, and their propagation, justify an HNS initiative in this area.

The already excellent facilities at the Propagation Science laboratory, East Malling, are being further improved and extended as part of the HRI restructuring programme, and a

programmable cold cabinet is available in addition to direct cooled and jacketed cold stores. A specialist post-harvest section is available to advise on novel approaches of controlled atmosphere or hyperbaric storage if this is justified later.

6. DESCRIPTION OF THE WORK

It is not possible to justify on scientific or cost grounds a comprehensive investigation of the many interactive conditions described in paragraph 4. The work will start by screening on a relatively small scale those treatment combinations of most scientific and commercial relevance, leading to the more comprehensive assessment and development of promising procedures in the later stages of the project.

Milestones

Year 1. (the first year programme will depend on start date) - Softwood cuttings. Identify subjects of differing rooting ability and set up and monitor propagation environments at different stress levels, e.g. wet fog, mist, polythene.
Root cuttings for post-rooting storage including bare-root for potting-off in the short-term, and for overwintering in flats undisturbed.
Carry out pre-rooting storage treatments with subsidiary experiments on storage conditions, e.g. time of day for cutting collection, progressive v immediate low temperature and comparison of temperature levels.
Effect of pre-treatment with fungicides.
Effect of photoperiodic light, and daylight v artificial light.
(Similar factors will be taken into account for post-rooting storage of both bare-root cuttings and cuttings overwintered in flats as appropriate).
Follow through post-storage rooting or post-rooting storage as appropriate and make appropriate measurements to describe conditions after storage (e.g. fresh weight and dry weight changes); pot up samples.
Hardwood cuttings: Attempt pre- and post-rooting storage of an autumn-propagated subject, e.g. *Prunus* Colt, under 'bare-root' jacketed cold store conditions compared to bagged direct cold store conditions.
Measure fresh and dry weight changes.
Propagate in hardwood cutting bins under conditions providing relatively rapid and relatively slow drainage to assess the key environmental interaction.
Repeat for spring subject, e.g. *Tilia* spp.

Year 2. Softwood cuttings. Continue with the variety x environment interaction, scaling-up experiments to allow statistical analysis of data, using the best combinations of other factors. Assess growth of plants from year 1.
Continue to investigate storage conditions and introduce

effects of condition of cuttings entering storage, and of auxin treatment.
Identify the key physiological problems preventing successful storage.
With advice from nurserymen, consider the relevance of holding-back direct stuck cuttings for better targeting of markets.
Hardwood cuttings. Continue with autumn and winter schedules, adjusting storage and propagation conditions to improve performance. Assess earlier results.

Year 3. Experiments will be largely determined by progress but the aim will be to present an understanding of the opportunities for, and constraints on, storage and overwintering of softwood and hardwood cuttings, with practical recommendations.
If justified, alternative methods of storage will be attempted; for example, appropriate controlled atmosphere conditions can be assessed, but the main emphasis of this project is to work in conditions that are readily accessible to nurserymen now.

7. COMMENCEMENT DATE AND DURATION

October 1992 for 3¼ years to provide 3 full seasons for softwood cuttings and 3 seasons for hardwood cuttings.

8. STAFF RESPONSIBILITIES

Within the general supervision of Dr.B.H.Howard and Dr.R.S.Harrison-Murray, HRI East Malling, subject to restructuring current staffing commitments in association with funding from other projects.

9. LOCATION

HRI-East Malling.