Project title	Optimising defoliation in young trees
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Project leaders:	Neal Ward, University of Reading Dr Ross Cameron, University of Reading
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Key staff:	Neal Ward Dr Ross Cameron Dr Gillian Rose
Location of project:	University of Reading
Project coordinator:	Jamie Dewhurst, J&A Growers Nick Dunn, Frank P Matthews
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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

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Headline

Understanding, managing and enhancing defoliation in young trees

Background

Nurserymen are concerned that natural leaf abscission on field-grown trees is occurring later each year, due to milder autumns. A consequence of this is that tree lifting can be delayed with nurseries failing to meet early demand from the landscape sector, or that some nurseries are being forced into lifting trees to meet orders whilst the foliage is still attached. Chemical defoliants are available to nurserymen, but these need to be applied with care to promote a strong enough abscission response, yet avoid damaging the crop.

This project aims to optimise the use of existing chemical products, and to explore cultural and alternative techniques that either enhance the effectiveness of these, or provide an alternative mechanism for defoliation. Work is focused on trying to defoliate field-produced stock through a number of field trials at both commercial holdings and the University of Reading. Experiments in controlled conditions help determine the relationship between potential defoliants and the physiological stage of the crop at the time of application.

Summary

- 'Leaf Fall' a commercial product containing Copper-EDTA) remains the most effective chemical for defoliating young trees

- Effectiveness of 'Leaf Fall' remains variable, however, de[ending on crop type and time of application (e.g. 60-80% defoliation in *Crataegus* compared to only 15-33% in *Malus 'Bramley'*

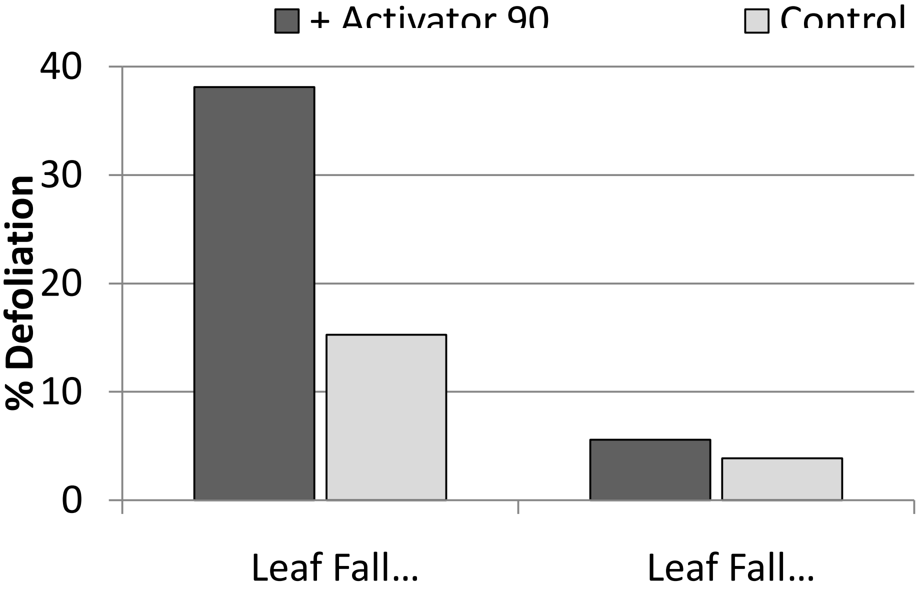
- Alternatives (including Iron-EDTA) did not perform as well as 'Leaf Fall' or provide any economic advantage.

- The addition of a wetting agent improved the action of 'Leaf Fall' when applied at the recommended rate of 20 ml^{-1} , but not when the rate was dropped to 5 ml $^{-1}$. (Figure A).

- Mild drought stress may enhance natural leaf abscission in warmer autumns.

- A secondary effect of defoliating with ‘Leaf Fall’ may be stronger re-growth in spring.
- Late season applications of growth retardants did not significantly improve the effect of ‘Leaf Fall’ and reduced spring re-growth.
- Simulating frost conditions aided defoliation, but only when shoot activity / auxin transport was disrupted in the young tree.
- Chilling temperatures (+20°C

Figure A. Defoliation recorded in *Malus* ‘Bramley’ at site B on 31st October 2008, after applying 2 concentrations of ‘Leaf Fall’ +/- Activator 90 two weeks previously.



Financial benefits

- Feasible, alternative defoliants to ‘Leaf Fall’ evaluated in the project to date, appear to be no more cost effective than ‘Leaf Fall’.
- Savings can be optimised by adding wetters / considering concentrations of ‘Leaf Fall’ required.
- Techniques that disrupt apical activity late in the growing season (undercutting, moderate drought imposition, lower fertilizer concentrations) may help reduce the requirements for chemical defoliants. The challenge remains in meeting the height and quality specifications for the crop.

Action points for growers

- Currently, ‘Leaf Fall’ remains the most effective way of defoliating young trees. Nurserymen, should consider how its use can be optimised to maximise effect and minimise costs
- Wetting agents may improve the action of ‘Leaf Fall’ by up to 100% when both products are used in late autumn at the recommended rate
- A half rate of ‘Leaf Fall’ with wetter may be worth trying (at least for those cultivars that appear more responsive to defoliants anyway)
- Applying mild drought stress during late summer / autumn may encourage defoliation. Field irrigation schedules should be reduced when warm ‘Indian Summer’ conditions are experienced and the crop has attained its specified height.
- Nurseries with containerised stock grown under protection, and which require early defoliation to aid subsequent cold storage or transportation, may wish to consider reduced irrigation / controlled drought as a tool.
- Triazole-based growth regulators have no universal effect on autumn defoliation, but can inhibit re-growth in the following spring.

Science Section

Introduction

Society still tends to discuss climate change in the context of what will happen in future, but there is growing evidence that alterations in the natural world due to temperature shifts are already being observed. Although there are strong seasonal effects from one year to the next, tree nurserymen have noticed over the last decade that autumns periods have been progressively warmer, with fewer and later instances of frost being experienced (Semenov, 2007). Although a longer growing season brings some advantages, one key disadvantage highlighted by nurserymen is that leaf abscission is later. This has resulted in tree crops being lifted and cold stored before the foliage has fallen off completely, as nurserymen attempt to meet market demand from the landscape sector. Nurserymen, however, are running the risk of lowering crop quality by storing material with leaves still present; as the presence of leaves can promote tissue desiccation, cause localised heating, reduce air movement around the crop and encourage pathogens whilst in storage. Lifting the trees early, before dormancy is complete may also have implications for growth in the spring, through reducing carbohydrate and amino acid levels in the overwintering buds. Manually removing leaves is labour intensive and not cost-effective for many tree crops, and so a number of growers are attempting to induce earlier and most consistent defoliation through chemical means (defoliants).

For nursery tree crops defoliants need to be able to encourage natural leaf drop rather than kill leaves outright (otherwise they remain attached to the branches as moribund tissue – ‘stuck leaves’). Parts of the industry have come to rely on the product ‘Leaf Fall’ (a copper EDTA based compound) to enhance leaf abscission, others have suggested that this material, despite its potential advantages, may be too expensive to apply and thereby further reduce the profit margin. Thus, this project aims to evaluate the potential for chemical or non-chemical means to aid leaf abscission in young tree crops in a reliant and cost-effective manner. There are 4 specific objectives:

1. Determine the optimum use of ‘Leaf Fall’ (timing – ideally in relation to physiological crop stage, concentration, and any additional factors that promote its effectiveness).
2. Evaluate if there are other (potentially cheaper) chemical compounds that encourage leaf abscission, but do not kill leaves *per se*, and if these have any market potential.
3. Investigate if any non-chemical, practical management techniques could be used to help leaf abscission or improve the effectiveness of chemical defoliants.

4. Within the context of a changing climate, provide nurserymen with some guidance of how seasonal affects may influence their ability to lift the crop at the appropriate time.

Leaf abscission

The biochemistry of the leaf abscission process is only partially understood. The two most important hormones identified in the control of autumnal leaf senescence and abscission are auxin (primarily indole-3-acetic acid) and ethylene (C_2H_4). The highest concentration of the former is found in young leaves, whilst synthesis of the latter is enhanced by wounding. The concentrations of these two substances in the cells around the pre-formed abscission zone (AZ) at the base of the leaf petiole control the process of abscission. In essence, a reduced concentration of auxin in the presence of ethylene in these tissues will drive leaf abscission, whilst the higher concentrations of auxin will prevent or retard the abscission process.

Copper ions have an important relationship with both auxin and ethylene, which appears to explain the effectiveness of 'Leaf Fall' in enhancing natural defoliation. The action of copper ions has been identified as threefold.

1. The phytotoxicity of high concentrations of copper causes damage to cell membranes and subsequent ethylene evolution (Ben-Yehoshua and Biggs, 1970; Fernandes and Henriques, 1991; Luna et al., 1994; Chen and Kao, 1999; Chatterjee et al., 2006; Zhang et al., 2008)
2. Copper ions appear to inactivate auxin (Ben-Yehoshua and Biggs, 1970)
3. The ability of specialist proteins within cells to detect ethylene is reliant upon the presence of copper ions (Rodríguez et al., 1999; Binder et al., 2007)

The first year of the project focussed on 'Leaf Fall' and potential chemical alternatives. Results showed, however, that 'Leaf Fall' applied in late September, either with or without Cuprokylt (copper oxychloride) and urea was the most effective product tested at encouraging leaf abscission. Unlike other copper-based compounds, the mode of action with 'Leaf Fall' appears to relate to the copper ion being readily available in solution (i.e. it can interact directly with the plant cells). This may not be true, for example with copper-based fungicides. Early applications of 'Leaf Fall' (August) unfortunately did not promote leaf abscission or earlier lifting (suggesting there is a distinct interaction between the chemical and the physiological status of the leaf).

A second experiment tried to determine how important the copper ion was in the leaf defoliation process. For example, could a similarly structured compound – chelated (sequestered) iron (FeEDTA) be just as effective? Data suggested though, that FeEDTA was a far less effective defoliant over a constrained time period than that of ‘Leaf Fall’ (at least at comparable concentrations), although levels of leaf tissue damage were similar.

A third strategy was to investigate the relationship between the activity of the apical bud and the potential for the tree to abscise its leaves. Essentially, it is the physiological state that helps promote leaf abscission dependant on the terminal bud stopping growth (and or entering dormancy). Triazole based fungicides were evaluated in this respect in year 1, and direct effects on leaf abscission (through tissue damage) were observed in addition to the expected reduced apical growth. Some literature has also investigated the potential of these compounds to induce early entry into dormancy (MacDonald, 1995). Initial trails with alder (*Alnus glutinosa)* under controlled conditions were encouraging; three weekly applications of the triazole, Folicur (tebuconazole 250 mg/l) to at a rate of 1 ml l^{-1} improved the subsequent effectiveness of ‘Leaf Fall’. Indeed, four applications of Folicur without any ‘Leaf Fall’ gave rise to similar defoliation rates. One ‘side-effect’ of the triazole, however, was a reduction in plant vigour. Whether Folicur was as effective in the field and across other species at a commercially-relevant frequency and concentration however, remained to be determined.

The plan for year 2 was worked up in conjunction with the project co-ordinators and attempted to address some key questions:

1. Can growth regulators be used in practice to reduce late season crop vigour, induce earlier bud dormancy and hence aid leaf abscission (either with / without a defoliant)?
2. Can ‘Leaf Fall’ be made more effective through the use of a spray adjuvant (wetting agent)?
3. Does temperature and or soil moisture availability in the autumn influence potential for leaf abscission?
4. If anecdotal evidence suggests frost is the most effective natural defoliant available to nurserymen, is the action physical (ice formation) or physiological (chilling). Are there alternative techniques that could replicate this mode of action?
5. Allied to 1 and 4 above, is frost only effective once the plant has formed a resting apical bud (and the transport of auxin from the apical bud to the leaves terminated (Addicott, 1982))? If so, perhaps alternative techniques may require a dormant apical bud or reduced auxin signal before they can be effective?

The research was carried out via a number of field trials using two commercial nurseries (Site A – seedling material used for hedging and Site B – two-year old trees derived from budded stock) as well as a field site at the University of Reading (Shinfield). In addition to field trials, pot–based experiments were used to test the efficacy of novel chemicals or plant responses in a more controlled manner, e.g. under specific temperature regimes. Specific details of plant species used and methodology are provided under each experimental heading (see below).

Statistical Analyses and Presentation of Results

In order to ascertain where treatment differences lay, normally distributed data were analysed using a standard parametric ANOVA in Genstat 10, using contrast matrices to give accurate probabilities (P=). Where data transformation was necessary before analysis, the untransformed data is displayed, although treatment differences and probabilities quoted apply to the transformed data. Probabilities were assessed at the 95% confidence interval, i.e. when $P< 0.05$ treatment differences were deemed significant.

Where problems arising for non-homogeneous variances could not be resolved with standard transformations, the Kruskal-Wallis non-parametric ANOVA test was used. Binomial defoliation data (leaves fallen/total nodes) was analysed using a generalised linear model for binomial distribution with a logit link function. Count data was analysed using chi-squared (X^2) distribution tests. Data are presented in figures with standard error (SE) or least significant difference (LSD) bars, when appropriate.

Exp. 1. Possible alternatives to CuEDTA

As a preliminary investigation to work in year 2, possible alternatives to ‘Leaf Fall’ (CuEDTA) were evaluated in a small scale glasshouse experiment using *Alnus*. Literature suggested that cell wall damage (lipid peroxidation) and resultant ethylene evolution were consequences of high foliar doses of copper (Bousquet and Thimann, 1984; Chen and Kao, 1999). The aim here was to evaluate other non-copper compounds that promoted similar tissue damage, and compare their performance with ‘Leaf Fall’ prior to their possible inclusion in a full field-scale experiment.

Materials and methods

Alnus glutinosa were raised from seed in an unheated glasshouse and potted into 9cm pots during spring 2008. The pots remained in the glasshouse throughout and were treated with the following chemicals on 6th August using hand sprayers:.

A. Jet-5 glasshouse sanitizer (peracetic acid) (50 ml l^{-1})

B. Librel hydroponic / foliar fertiliser (iron-EDTA complex, 13.2% Fe) (20 g l^{-1})

C. Urea (300 g l^{-1})

D. ‘Leaf Fall’ (copper-EDTA complex, 9.1% Cu) (5 ml l^{-1})

E. ‘Leaf Fall’ (copper-EDTA complex, 9.1% Cu) (20 ml l^{-1})

After three weeks, defoliation was recorded as (leaves abscised / total leaves at the beginning of the experiment) x 100 to give a % defoliation value for each treatment.

Results

As in the previous year, 'Leaf Fall' induced abscission to a greater extent than other treatments (Fig. 1). Despite urea showing the lowest defoliation, it resulted in the most leaf damage – essentially the leaves were killed and then remained stuck on the branches. Previous work with urea had showed little response at all, but this data suggested there was a physiological reaction and some form of intermediate concentration may prove useful (hence it was included in the following experiment). Jet-5 also demonstrated some promise, but again the concentration used was high, and unlikely to meet commercial criteria on cost or environmental legislation. One notable side effect of both 'Leaf Fall' treatments, not observed in any other plants was the appearance of new shoots at the base of the stem (Fig. 2). The shoot apex in these treatments was not visibly damaged by the copper applications.

Figure 1. *Alnus glutinosa* (University of Reading). Defoliation (%) recorded 22 days after application of potential alternatives to the recommended rate of 'Leaf Fall'. Mean values shown, n=10.

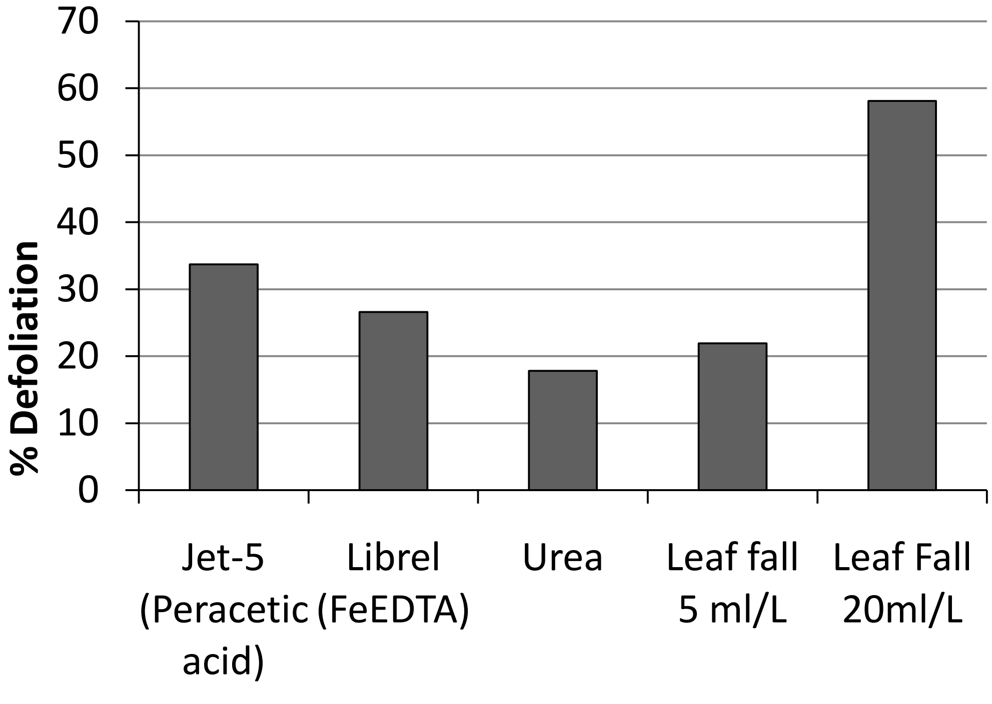


Figure 2. *Alnus glutinosa* (University of Reading glasshouse) basal-shoot emergence after treatment with 'Leaf Fall' 20ml l^{-1}.

Exp. 2. Reducing plant vigour to improve the effectiveness of defoliants

Continuing with the hypothesis that reduced vigour in juvenile plants was paramount in maximising the effects of defoliants, this experiment sought to investigate the effects of plant growth regulators (PGRs) on subsequent application of defoliants. Consultation was undertaken with the nurserymen who had been involved in the previous autumn's evaluations, and treatment schedules for large-scale experiments were devised to examine ways to reduce plant vigour chemically prior to the application of defoliants. The results from a small experiment conducted in early 2008, using the triazole fungicide Folicur (tebuconazole) as a pre-treatment before 'Leaf Fall' application, suggested that growth retardation (via inhibiting gibberellin synthesis) may be useful in improving the action of CuEDTA. An unrelated trial of a second triazole fungicide, Nativo (tebuconazole + trifloxistrobin) on *C. monogyna* at site A was also reported by one of the project collaborators to have a side-effect of reducing plant vigour.

The primary objective of this experiment therefore was to ascertain whether a late season application of a triazole compound would slow growth in juvenile plants and encourage earlier terminal bud formation. In addition to the two fungicides Folicur and Nativo, Cultar, another triazole compound was also included in order to evaluate whether the effect of this compound was greater, as it is specifically marketed as a plant growth regulator (PGR) (Table 1).

The action of ‘Leaf Fall’ (copper-EDTA) and other defoliants is often quoted as acting through the evolution of the plant hormone ethylene, released after the copper ion (or other antagonist) has caused stress in the leaf tissue (Luna et al., 1994). A secondary objective therefore was to assess whether the defoliant action of ‘Leaf Fall’ related solely as a result of enhanced ethylene production from leaf tissue damage. Two additional potential defoliants that were also associated with leaf injury were thus examined in comparison; urea and Cerone. Urea was selected as Exp. 1 suggested that an intermediate concentration of this chemical was worth further evaluation. Cerone was included however, as it is an ethylene-evolving PGR (Table 1) and may be able to induce abscission through ethylene release.

Table 1. Technical details for compounds used in Exp. 1 (2008 field evaluations).

Product	Type / use	Active Ingredient(s)/ concentration	Manufacturer
Urea	Defoliant	Laboratory grade urea $(NH_2)_2CO$	Fisher Scientific
Leaf Fall	Defoliant	$CuNH_4$.EDTA	Protex Chemicals
Cerone	Growth regulator Cereals, fruit crops	2-chloroethylphosponic acid (480 g l^{-1})	Bayer CropScience
Cultar	Growth regulator	paclobutrazol (250 g l^{-1})	Syngenta Crop Protection UK
Folicur	Fungicide Cereals, vegetables	tebuconazole (250 g kg^{-1})	Bayer CropScience
Nativo 75WG	Fungicide Cereals, vegetables	tebuconazole (500 g kg^{-1}) trifloxistrobin (250 g kg^{-1})	Bayer CropScience

Materials and methods

A factorial experiment was set up with a range of growth retardants (or water) being applied to crops, followed by a range of potential defoliants (i.e. 16 treatment combinations, Table 2). Treatments were applied to seed-grown *Crataegus monogyna* and *Alnus glutinosa* at Site A and to Malus ‘Bramley’ at Site B. At the University of Reading (Shinfield site) 1 year old *Crataegus monogyna* received the treatments. Applications of these treatments were made in mid-September (retardants) and at the beginning of October (defoliants) at all three sites. Sprays were applied to manufacturers' instructions using a Cooper Pegler CP 15 knapsack sprayer fitted with a fine nozzle suitable for fungicide application. At site A, plants were grown in 5-row seed beds. Each experimental treatment block was allocated as a 3 m section of the bed. The bed was further divided into 3 equal sections to allow 3 randomised blocks, each comprising the 16 treatments. One metre buffer zones were marked between blocks to minimise the effects of spray drift. Within these blocks, a central group of 10 plants were selected and labelled to provide detailed recordings.

At site B and at the University of Reading, plants were located in rows with approximately 0.5 m spacing. Again the experimental population was divided into 3 blocks across the fields to allow a blocked experimental design (10 replicates per treatment per block).

Table 2. Treatment schedule for experiment 1 (All sites).

Retardant	Concentration (g l^{-1} / ml l^{-1})		Defoliant	Concentration (g l^{-1} / ml l^{-1})
Cultar	8.9	**X**	Leaf Fall	20
Folicur	3.3		Urea	90
Nativo	0.8		Cerone	2.5
Water	n/a		Water	n/a

Prior to the application of growth retardants, the activity of the apical shoot was recorded at University of Reading and Site A. At the former this took the form of a score from 1 – 10. At Site A, there were low levels of activity in both *C. monogyna* and *A. glutinosa* and thus a binary scoring system was adopted, with plants being awarded a score of 1 if there was some apical shoot activity and 0 if there was not. *M.* 'Bramley' displayed consistent growth patterns as in the previous year, with vigorous activity being noted in nearly every plant. Therefore plant heights were recorded at Site B to the nearest 0.5 cm at the time of application of the first and second treatments. Formation of a resting bud was recorded separately for these plants.

Defoliation was recorded as a percentage score on *C. monogyna* at University of Reading. At site A, both species were awarded a score of 1-5 according to the following criteria:

1 - <20% defoliated

2 - 20-39% defoliated

3 - 40-59% defoliated

4 - 60-79% defoliated

5 - >80% defoliated

Malus 'Bramley' were awarded a percentage defoliation score for each stem zone, as in experiments carried out in 2007.

Results

Cessation of growth

No significant growth retardation was observed following the application of potential retardants to *C. monogyna* (both sites) or *A. glutinosa* (data not shown), but the rate of stem elongation in *M.* 'Bramley' was significantly reduced in all three retardants compared to the control (water; Table 3). At the second recording point, however only 3 *M.* 'Bramley plants had formed a terminal bud (one in the control and two in the retardant treatments).

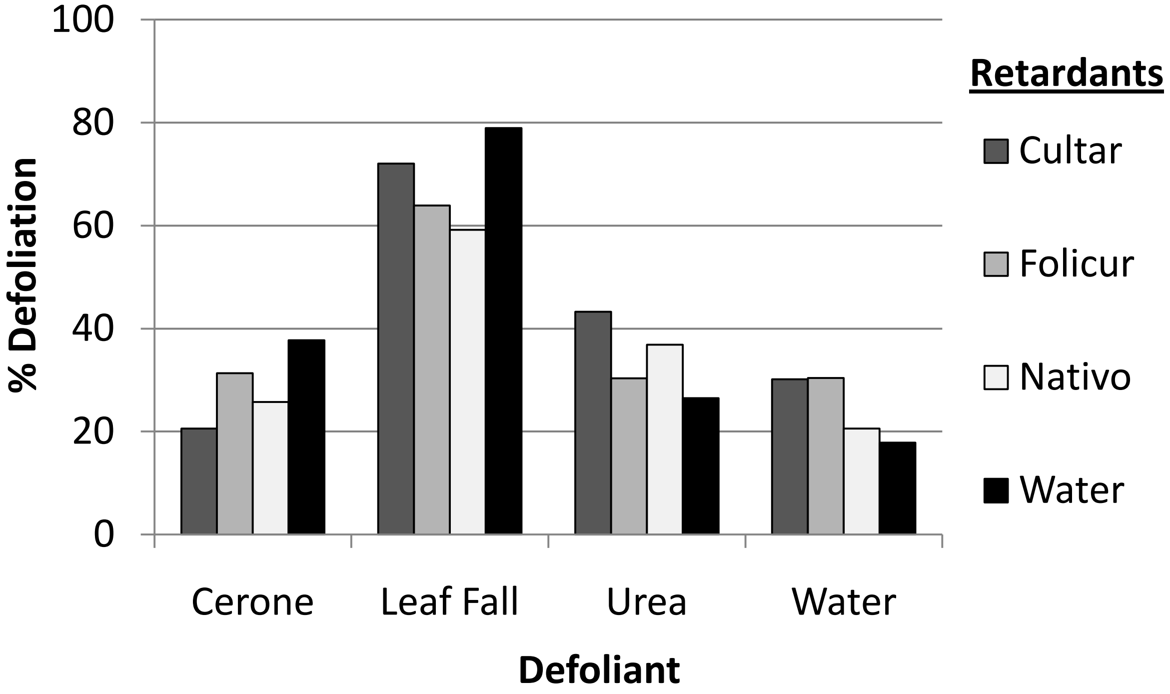
Table 3. *Malus* 'Bramley' (Site B). Extension growth (cm) of apical stem recorded between 16th September and 9th October

Retardant	**Growth (cm)**
Cultar	3.45
Folicur	2.88
Nativo	3.07
Water	4.23
LSD (P=0.05)	**0.76**

Defoliation

Once again, 'Leaf Fall' proved significantly more effective than any of the other treatments at defoliating the species under test. There was no obvious effect of any pre-treatment at the University of Reading (Fig. 1). In fact the *C. monogyna* plants that had received only water as the pre-treatment displayed the highest levels of defoliation at University of Reading in mid November (79%).

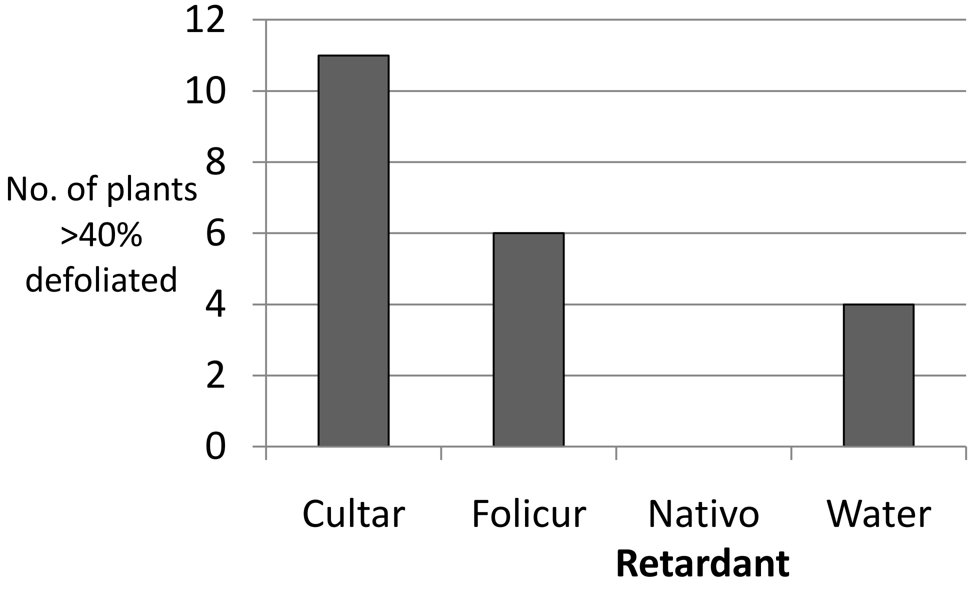
Figure 1. ***Crataegus monogyna*** **(University of Reading). Defoliation (%) recorded on 12th November 2008 (n=30).**



At site A, *Alnus glutinosa* responded more quickly to ‘Leaf Fall’, with over 80% of the plants scoring 3 or above on the 23rd October compared to 30% or less in all other treatments (data not shown). However by 14th November, almost all plants were >80% defoliated. There was no apparent effect of pre-treatment in this species.

Crataegus monogyna was less responsive at site A. The action of ‘Leaf Fall’ did not appear to be enhanced by pre-treatment with any of the triazole fungicides; however Cultar appeared to mildly enhance the action of ‘Leaf Fall’ (Fig. 2.). The enhancement was not apparent for any other defoliants.

Figure 2. ***Crataegus monogyna*** **(Site A) Count of plants >40% defoliated 14th November 2007, ‘Leaf Fall’ treatments only (n=30).**



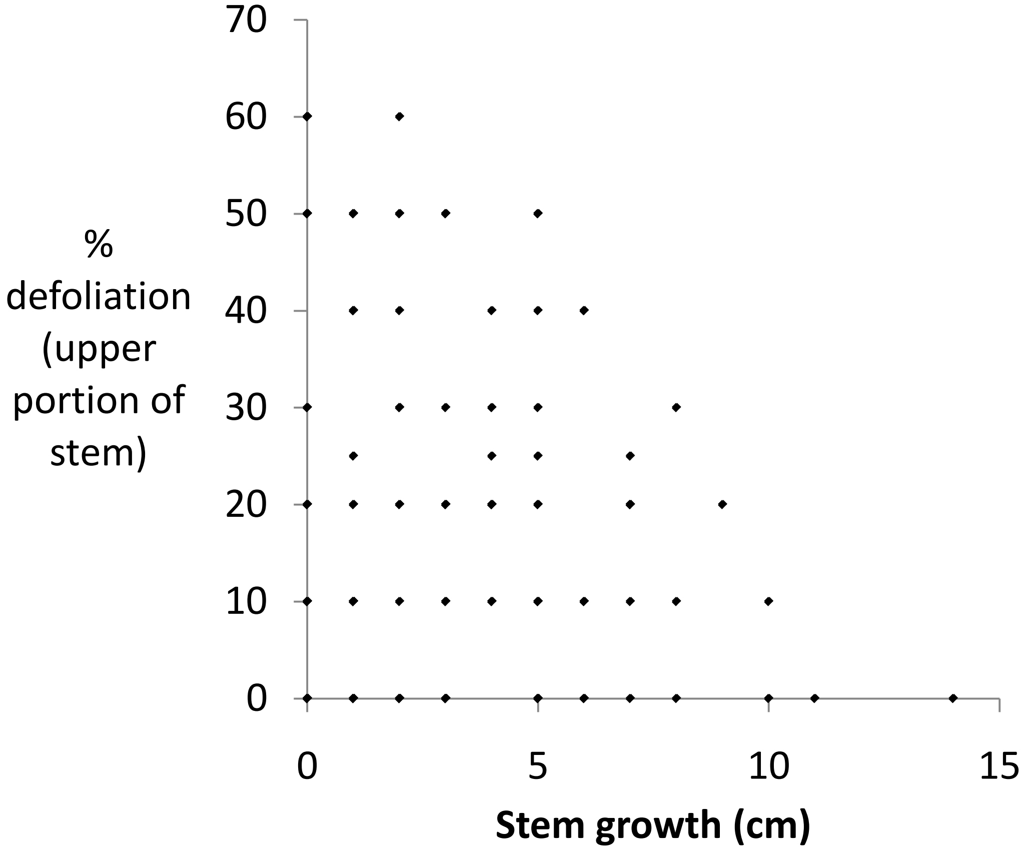
At site B, *Malus* 'Bramley' showed no significant response to any of the defoliants other than 'Leaf Fall', although pre-treatment with Nativo did appear to cause a more amplified response to the defoliant than the other pre-treatments (Table 4). Analysis of whole-tree defoliation for 'Leaf Fall' treatments using a Kruskal-Wallis non-parametric ANOVA revealed significant differences between pre-treatments of Folicur and Nativo (H=14.29, P<0.001) and between Folicur and the water control (H=7.01, P=0.008).

The upper portion of the stem was again least responsive to 'Leaf Fall' as had been observed in 2007. The negative effect of strong growth on defoliation is amplified in this top portion, and although there was no significant correlation, the upper portion of the stem in this species was most difficult to defoliate when the plants were growing most strongly (Fig. 3).

Table 4. *Malus* 'Bramley' (Site B). Defoliation after treatment with 'Leaf Fall' and a variety of triazole-based growth regulators recorded on 31st October 2008. *Only minimal defoliation was achieved with other defoliants in this test and the data are omitted for clarity.*

Pre-treatment	Defoliation Upper %	Defoliation Middle %	Defoliation Lower %	Defoliation Whole %	Stem growth 16th September – 9th October (cm)
Cultar	25	41	29	32	2.2 ± 0.38
Folicur	22	39	31	31	3.4 ± 0.49
Nativo	32	73	59	55	2.1 ± 0.43
Water	7	25	18	17	4.9 ± 0.67
Overall mean for 'Leaf Fall'	**21**	**44**	**34**	**33**	**3.1**

Figure 3. ***Malus*** **'Bramley' (site B) % defoliation of upper stem plotted against stem growth (between retardant and defoliant treatment dates) for 'Leaf Fall' treatments only.**



Exp. 2b. Re-growth of *Crataegus monogyna* following application of growth retardants, defoliation and winter cold-storage

Although none of the defoliants used on *Crataegus* at site A proved ideal (Fig. 2), the most successful, 'Leaf Fall' (where even here, only 18% of plants were > 40% defoliated on 14th November 2008) was selected to investigate the relationship between growth retardants and shoot vigour in the following spring.

Materials and methods

Plants from the 'Leaf Fall' treatments in experiment 1 (Table 5) were lifted and bundled by treatment into plastic sacks to reduce desiccation during storage. The plants were cold-stored at 1°C at University of Reading from 21st November 2008 until 27th February 2009. Upon removal from storage, the plants were potted in 11cm pots of peat-based media with no additional fertilizer and placed in the experimental grounds.

Following 50 days growth, new apical shoots were removed at the point of the origin with a razor blade and the tissue dried and weighed. In order to account for varying plant size, the dry weight of the new growth was divided by the length of the stem to give values of in mg cm^{-1}.

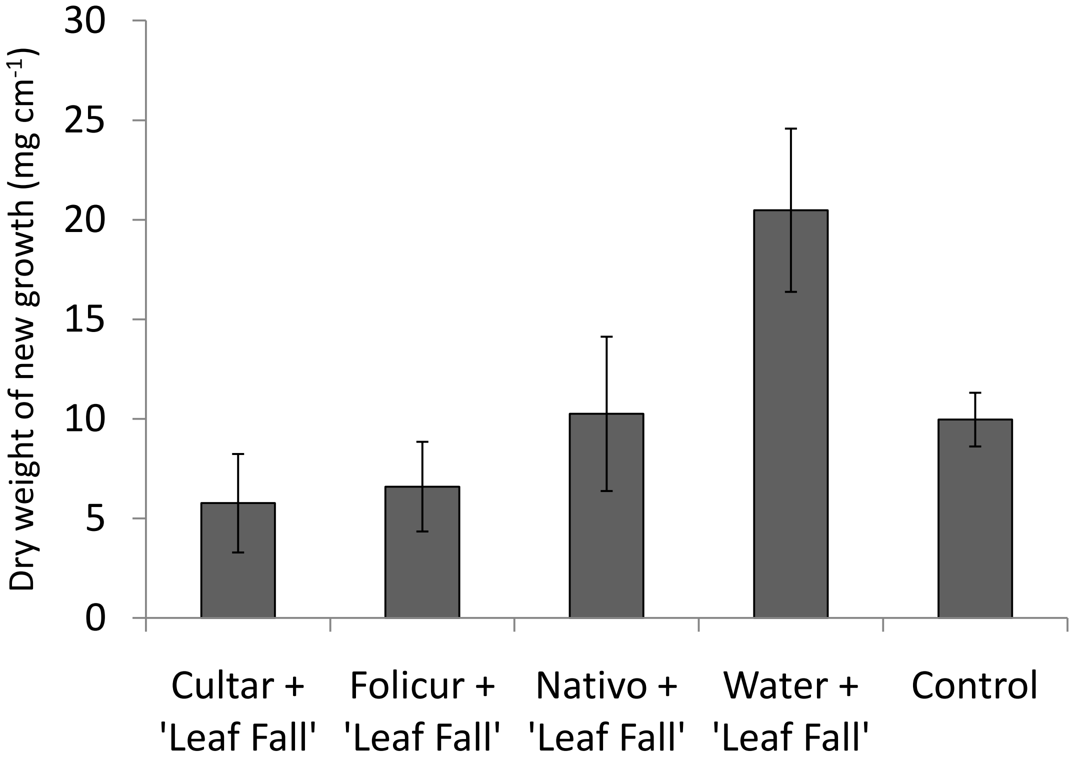
Table 5. Treatments selected for examination of re-growth.

Pre-treatment	Defoliant
Water	Water
Water	'Leaf Fall'
Cultar	'Leaf Fall'
Nativo	'Leaf Fall'
Folicur	'Leaf Fall'

Results

Spring growth was reduced in plants treated with the triazole compounds prior to 'Leaf Fall' application the previous autumn, compared to plants just treated with 'Leaf Fall' alone (Fig. 4). When the data was transformed by square root, differences were shown to be statistically significant (transformed data not shown). Similarly, treating plants with just 'Leaf Fall' implied better retention of shoot reserves in the autumn compared to no treatment, as spring growth was also significantly stronger than the controls (Fig 4) This replicates results from 2007.

Figure 4. *Crataegus monogyna* (Site A). Dry weight of new shoot growth per cm of stem, recorded in spring after 3 months of cold storage (1°C) following lifting from in November 2008. Error bars represent S.E. (n=6)



Exp. 3. Improving the action of 'Leaf Fall' using a spray adjuvant

Nurserymen are keen to optimise the performance of 'Leaf Fall', but minimise costs of application. Results from 2007 field evaluations were encouraging in that they suggested that a half-strength application of 'Leaf Fall' produced comparable results to the full dose. Anecdotal evidence from nurserymen, however, indicates that responses to 'Leaf Fall' can be inconsistent between years, and so it was decided to investigate mechanisms to improve the uptake and retention of the chemical. Therefore, an experiment in 2008 at site B using *M*. 'Bramley' aimed to examine the effects of adding a wetting agent (Activator 90, containing alcohol ethoxylates and fatty acids) to determine if this could enhance defoliation through better retention / penetration of the 'Leaf Fall'. In addition, this provided an opportunity to test if a $^1/_4$ concentration (5 ml l^{-1}) of 'Leaf Fall' could also maintain defoliation capacity compared to the recommended rate of 20 ml l^{-1}.

Materials and methods

Trees were sprayed to the point of run-off with the following treatments on the 9th October using a Cooper Pegler CP15 knapsack sprayer:

- 'Leaf Fall' 20 ml l^{-1} + Activator 90 10 ml l^{-1}
- 'Leaf Fall' 20 ml l^{-1}
- 'Leaf Fall' 5 ml l^{-1} + Activator 90 10 ml l^{-1}
- 'Leaf Fall' 5 ml l^{-1}
- Water + Activator 90 10 ml l^{-1}
- Water

Results

Applying 'Leaf Fall' at the recommended rate of 20 ml l^{-1} proved more effective than the 5 ml l^{-1} rate, 15% compared to 4% whole tree defoliation (Table 6). However, the use of a wetting agent significantly increased mean defoliation (Table 6). The Kruskal-Wallis non-parametric ANOVA used in these results indicates significant differences in whole-tree defoliation using 'Leaf Fall' 20 ml l^{-1} +/- Activator 90 at the $P<0.001$ level (n= 22 and 24 respectively, H=21.2, d.f. = 1, values in Table 6 in bold). No other significant whole tree effects were detected with this test.

Table 6. *Malus* 'Bamley' (Site B). Defoliation (%) recorded on 31st October 2008 after spraying with full (20 ml l^{-1}) and quarter (5 ml l^{-1}) concentrations of 'Leaf Fall' +/- Activator 90 wetting agent (0.1%) on 9th October 2008.

		Mean defoliation (%)	
		+ Activator 90	**Control**
'Leaf Fall' 20 ml l^{-1}	Upper	19.8	7.3
	Middle	39.4	18.3
	Lower	55.2	20.2
	Whole tree	**38.9**	**15.3**
'Leaf Fall' 5 ml l^{-1}	Upper	1.7	0.8
	Middle	6.1	7.3
	Lower	8.9	3.5
	Whole tree	5.6	3.9
Control	Upper	0	0
	Middle	0	0
	Lower	0	0
	Whole tree	0	0

Exp. 4. Assessing the effects of mild drought on natural leaf abscission

Developing practical, non-chemical defoliation techniques remains challenging at a field scale, due largely to costs or the infra-structure required. Whilst temperature and photoperiod are outside the control of most nurserymen, the manipulation of water availability may provide more opportunities, albeit many techniques may still be difficult / expensive to implement in practice. Nevertheless, a better understanding of the relationship between water availability and leaf defoliation may prove useful at a number of levels: -

- It may allow nurserymen to relate / predict defoliation rates more effectively and to implement some management strategies, e.g. in a dry autumn, field irrigation could be reduced to help natural defoliation in the crop.

- It may illustrate that techniques that are feasible for some growers e.g. undercutting field crops in late summer, are effective by restricting / limiting water supply for short periods.
- For those nurserymen that grow crops under protection or even in container beds outside, they may be able to use controlled irrigation as a tool to help induce defoliation.

In an attempt to investigate the effects of water availability during autumn on leaf abscission of young trees, container-grown *Crataegus monogyna* were subjected to 'mild' or 'severe' drought stress in two temperature regimes (a polythene tunnel and an unheated glasshouse). The interaction of water deprivation and temperature were assessed through measurement of leaf abscission, stomatal conductance and plant growth.

Materials and methods

Plants were grown from pre-statified seed (Region 402 [UK] supplied by ForestArt, Hadnall, Shrewsbury, Shropshire, UK). In March 2008 seeds were sown in peat-based media in trays, and seedlings transplanted into 9 cm pots in early June, using a 1:1 mix of John Innes no. 2 soil-based compost and peat-based potting media with Osmocote 3-4 month continuous release fertilizer added at the recommended rate. Prior to the experiment, in early September, the plants were transferred to 11 cm pots of John Innes no.2 compost.

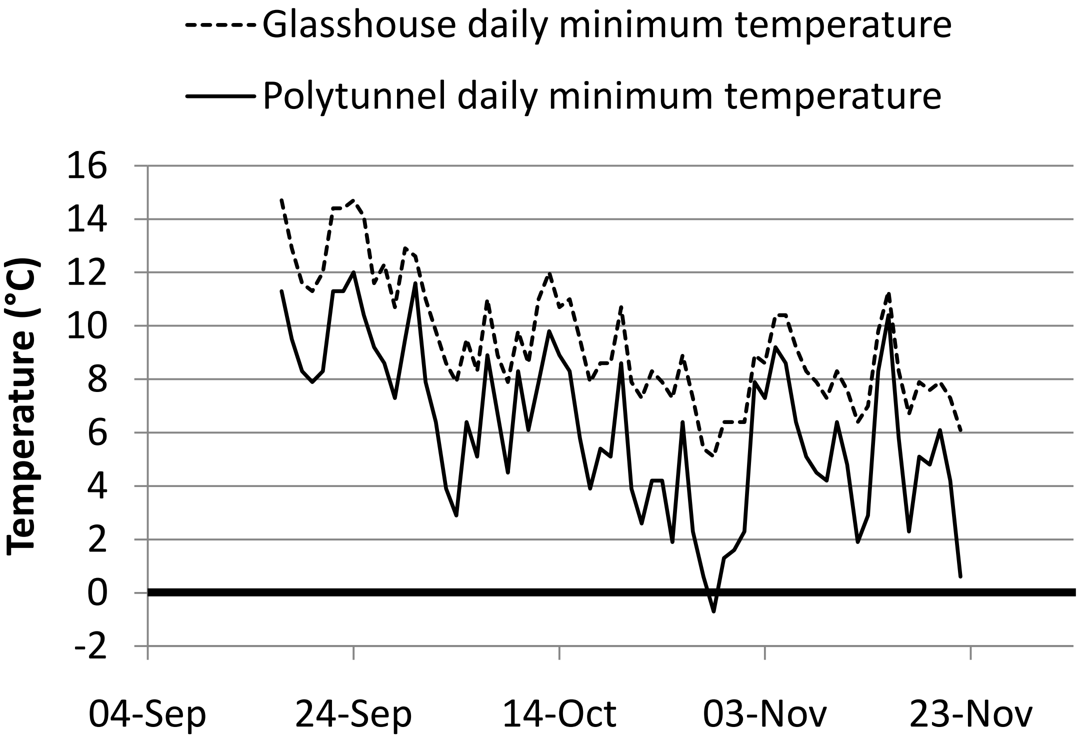
Mean water use was calculated for each temperature treatment between 23rd and 25th September 2008 using subsets of plants in each. Plants were brought to container capacity, weighed and then re-weighed two days later to determine water loss through evapo-transpiration. Thereafter, 100% 50% or 25 % of the calculated water loss in each temperature regime was applied to give differential irrigation regimes (60 ml, 30 ml and 15 ml respectively in the glasshouse and 30 ml, 15 ml and 7.5 ml in the polytunnel). Water was applied every two days from 26th September (1g = 1ml).

The water content of the potting media was measured at approximately weekly intervals using a Delta-T theta probe. Two measurements per pot were taken to allow for variation within the media. Stomatal conductance was recorded weekly using a Delta-T AP4 porometer. Measurements were taken on two leaves on each of 4 randomly selected plants per treatment, giving a total of 8 replicates per treatment at each recording point. Temperature was recorded in each structure using TinyTag temperature loggers recording temperatures hourly. The total number of nodes on 4 randomly selected plants per treatment was recorded prior at the start of the experiment. These plants were monitored weekly and the number of missing leaves recorded in order to calculate defoliation as a proportion.

Results

Daily minimum temperatures remained above freezing in the glasshouse throughout the experiment, but frost was recorded in the polytunnel on 2 Nov. (Fig. 5).

Figure 5. Minimum daily temperatures recorded using TinyTag temperature sensors in an unheated glasshouse and open-ended polythene tunnel at the University of Reading over the course of experiment 4, autumn 2008.



Glasshouse plants

Moisture content in the media was reduced in the 50% and 25% irrigation treatments (Fig. 6) and generally resulted in lower stomatal conductance values in these treatments (although daily fluctuations could be high due to prevalent weather conditions affecting light levels, humidity etc.) (Fig. 7). Defoliation rates were also quite variable between reps within the one treatment, and although rates were higher in the 50% treatment compared to the 100% differences were not statistically significant (Fig. 8). By 19th Nov, however, plants in the more severe 25% treatment were significantly more defoliated than the 100% control.

Polytunnel plants

Moisture content in the growing media dropped relatively uniformly within the two drought treatments, and the water content was significantly higher in the 100% irrigation treatment for the majority of the experiment (Fig. 9). In contrast to plants in the glasshouse, stomatal conductance values decreased after early October and remained low in all treatments thereafter, suggesting limited or no photosynthesis was taking place (Fig.10). Stomatal conductance values did not correlate to media moisture contents in the different treatments, indicating that some other factor; perhaps temperature or aerial humidity, was affecting stomatal aperture directly. Defoliation rates were generally low (10-20%) until frost was experienced in early November, at which point defoliation increased (Fig 11). Interestingly, the data suggests that defoliation was most rapid in the 100% control treatment, but differences between treatments were not significant following the frost (H=2.159, n=4, P>0.1).

Figure 6. *Crataegus monogyna* (Reading - Glasshouse). The volumetric media moisture content (m^3 m^{-3}) of plants exposed to differential irrigation treatment (100, 50 and 25%) during autumn 2008.

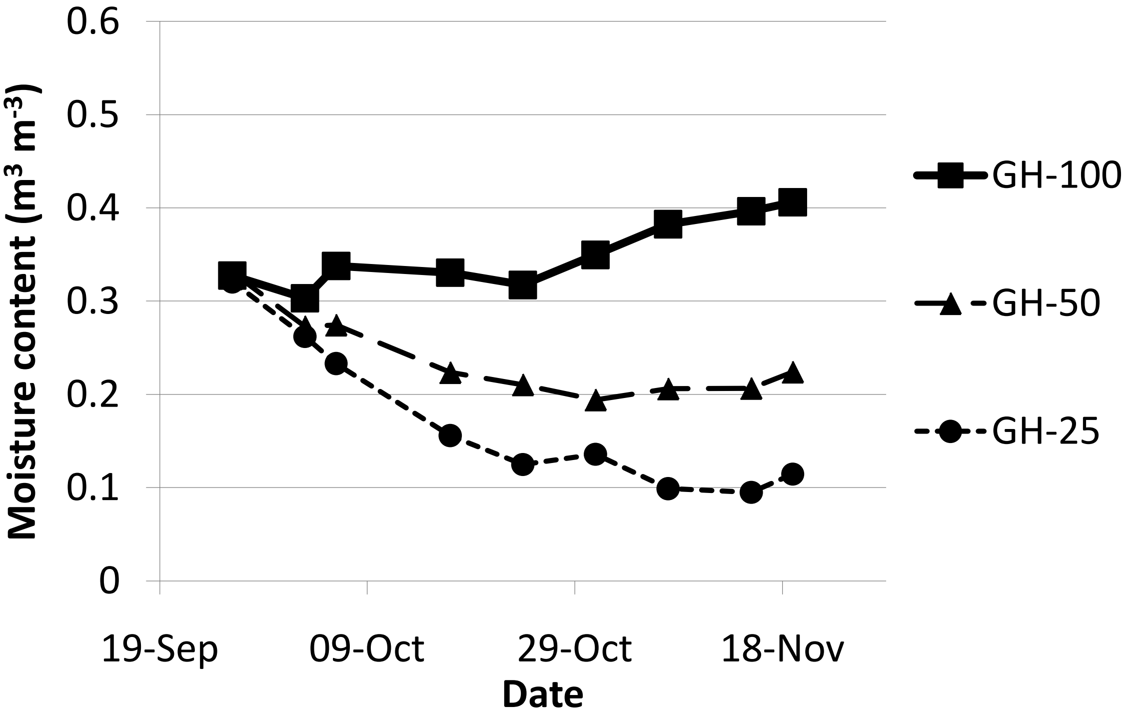


Figure 7. ***Crataegus monogyna*** **(Reading - Glasshouse). Stomatal conductance (mmol m^{2} s^{-1}) of plants exposed to differential irrigation treatment (100, 50 and 25%) during autumn 2008.**

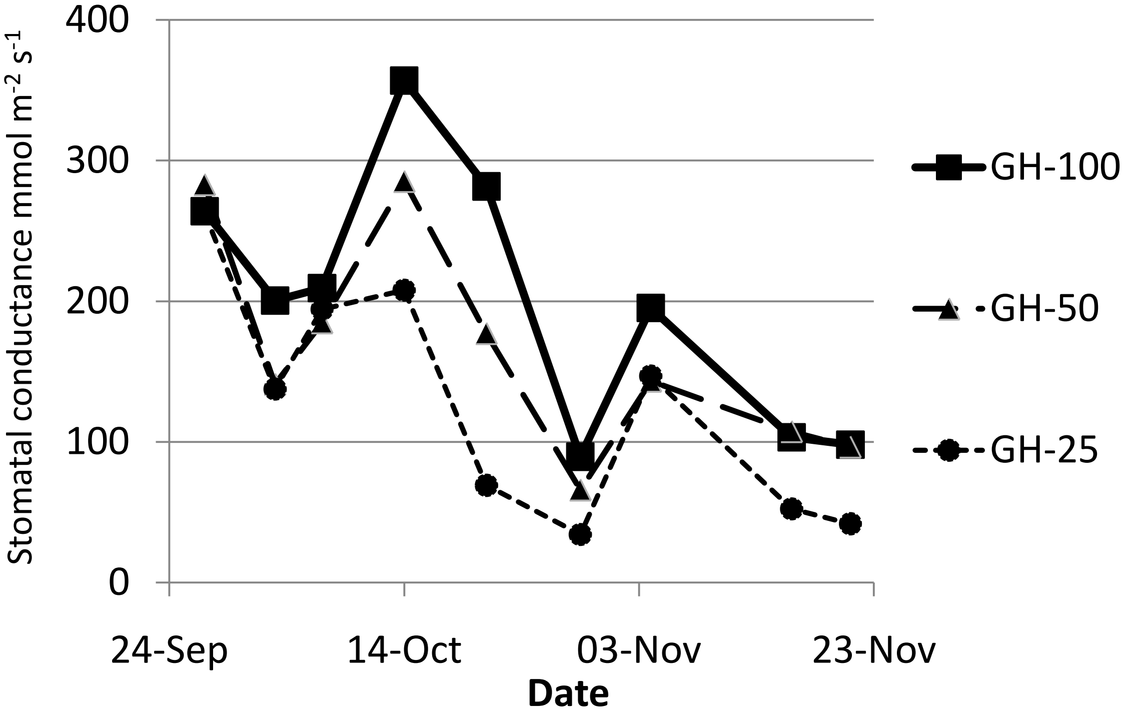


Figure 8. ***Crataegus monogyna*** **(Reading - Glasshouse). Leaf defoliation (%) of plants exposed to differential irrigation treatment (100, 50 and 25%) during autumn 2008.**

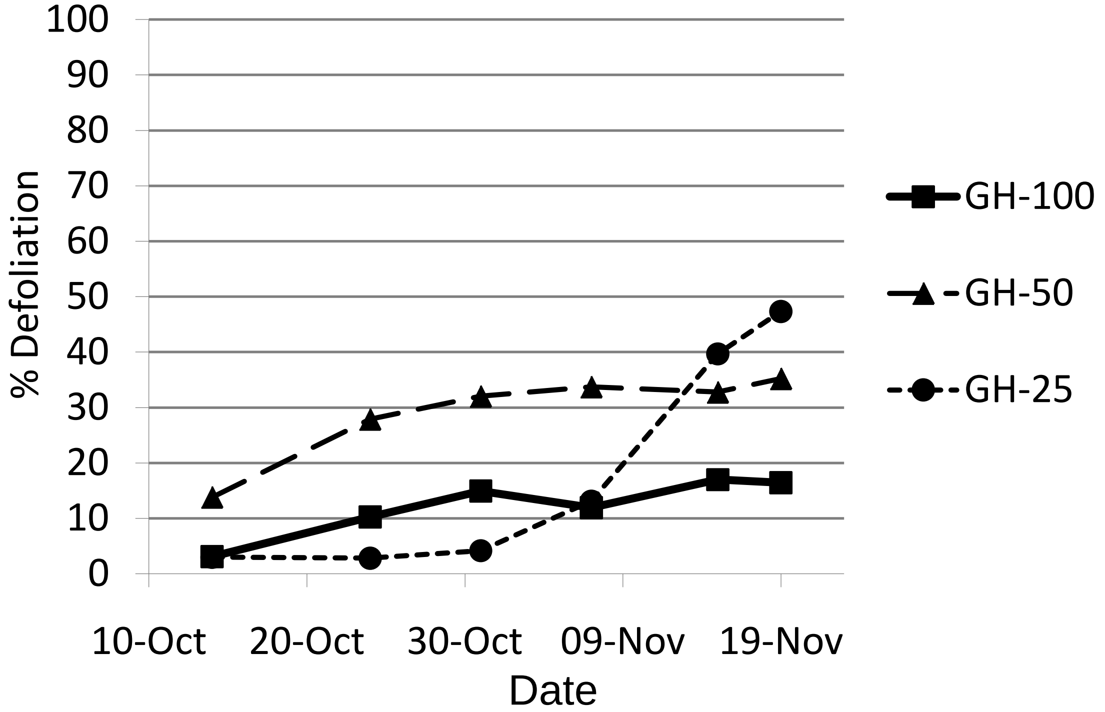


Figure 9. *Crataegus monogyna* (Reading - Polytunnel). The volumetric media moisture content (m^3 m^{-3}) of plants exposed to differential irrigation treatment (100, 50 and 25%) during autumn 2008.

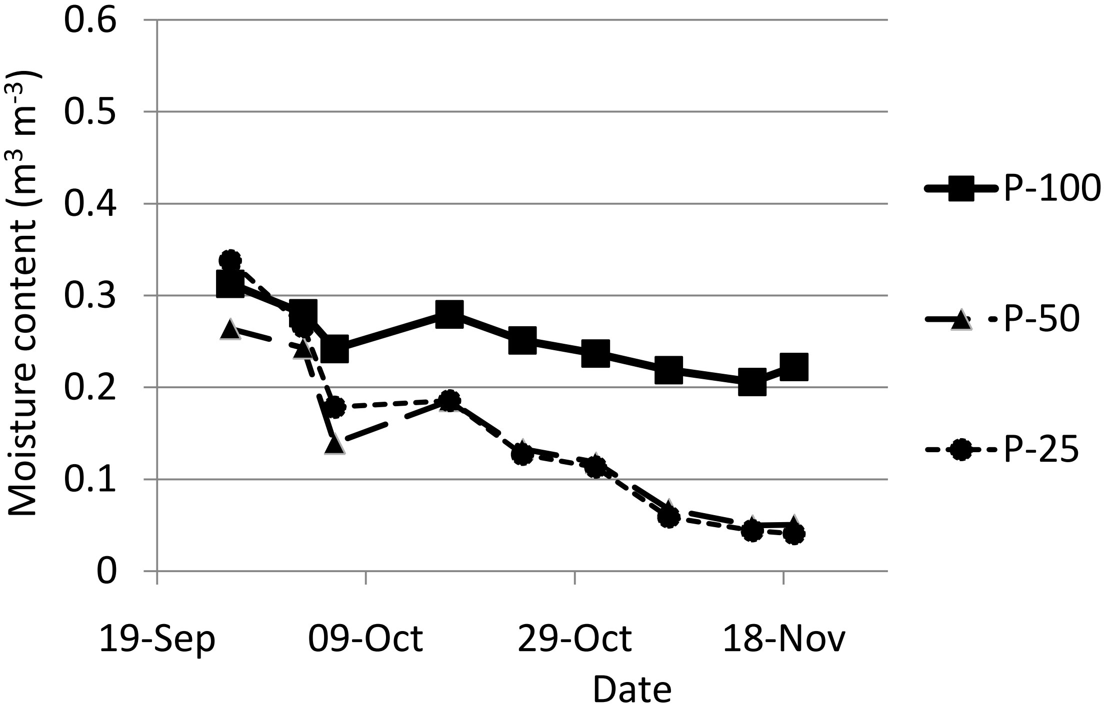


Figure 10. *Crataegus monogyna* (Reading - Polytunnel). Stomatal conductance (mmol m^2 s^{-1}) of plants exposed to differential irrigation treatment (100, 50 and 25%) during autumn 2008.

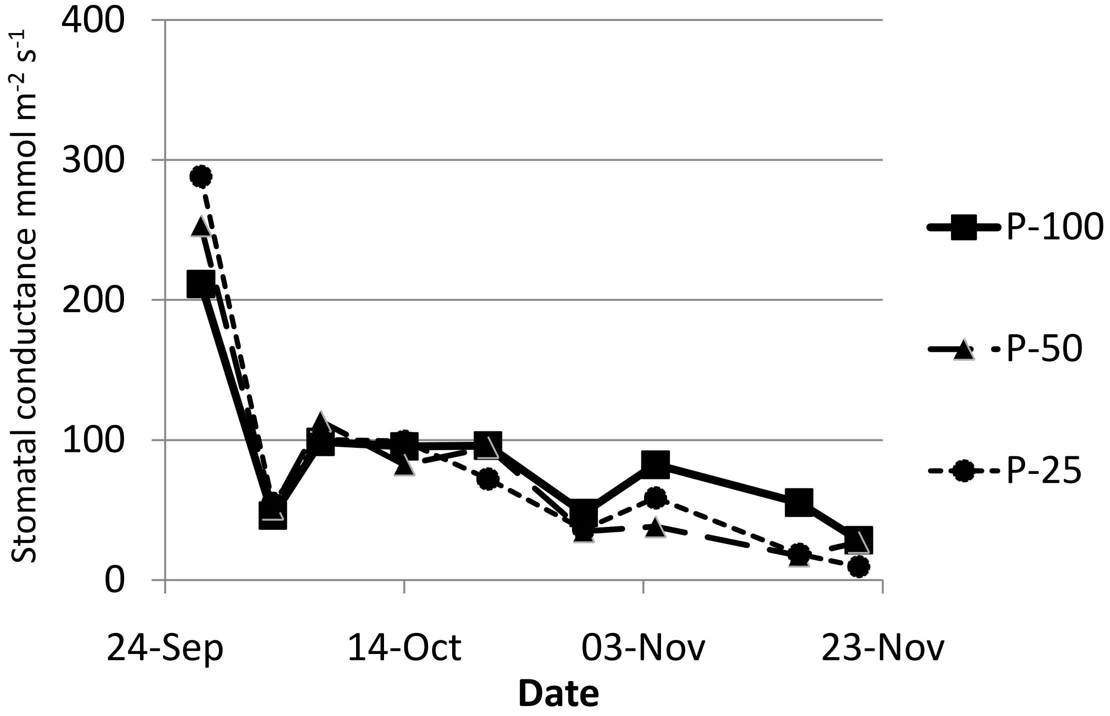
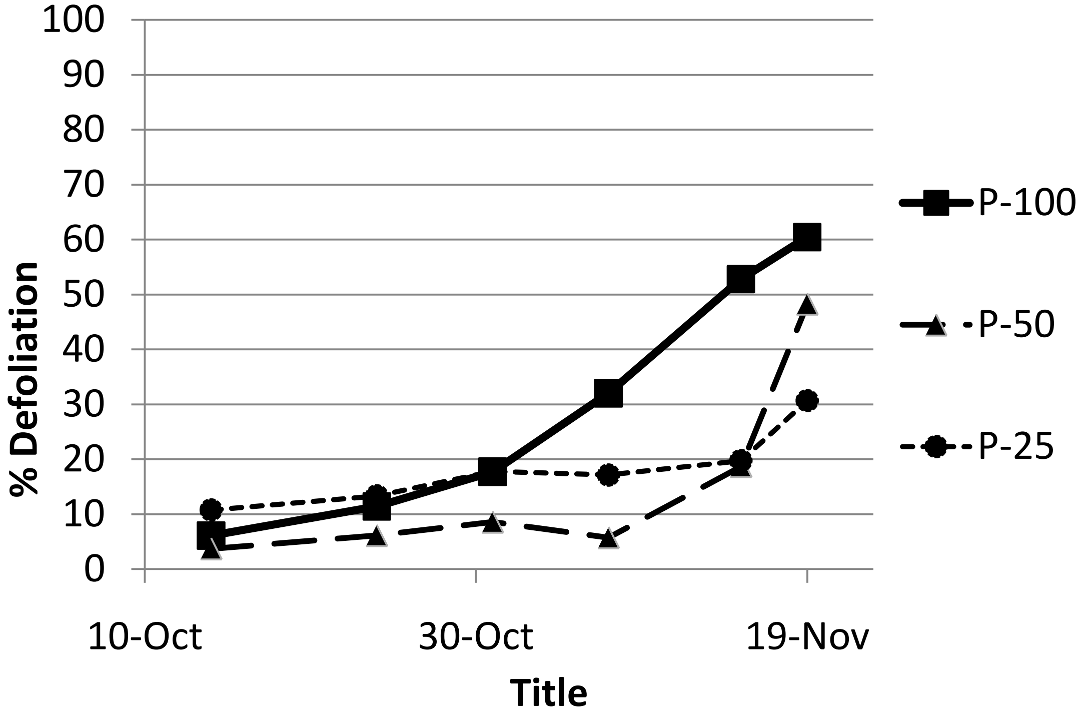


Figure 11. ***Crataegus monogyna*** **(Reading - Polytunnel). Leaf defoliation (%) of plants exposed to differential irrigation treatment (100, 50 and 25%) during autumn 2008.**



Experiments conducted under controlled environment conditions

Exp. 5. Temperature and the disruption to polar auxin (IAA) transport from the shoot tip on leaf senescence and abscission in *Crataegus monogyna*

Progressively lower night temperatures are widely accepted as a trigger for woody species to enter endodormancy (Arora et al., 2003). Results from field trials suggested that lower plant vigour and reduced rates of apical meristem (AM) activity gave rise to more effective chemical defoliation in autumn. If as a result of climate change, autumn temperatures remain higher, potentially lengthening the growing season, nurserymen who wish to artificially defoliate their crop may need to improve the efficacy of defoliants by providing additional dormancy-inducing stimuli, either chemically or culturally.

In an attempt to build on the theories explored in Exp. 1, controlled environment facilities at the University of Reading were exploited to investigate in more detail the relationship between growth activity, temperature and leaf abscission. An experiment was set up to explore two avenues for inducing earlier plant dormancy and leaf senescence. One was to inhibit auxin movement within the plant and the second was to use mechanical stress in an attempt to induce early dormant bud formation.

Auxin is produced in young actively-dividing tissues such as leaf tips and apical meristems. Within an individual leaf, a decline in auxin movement through the petiole from leaf to stem is thought to initiate senescence and sensitivity to ethylene, and encourage the formation of an abscission zone (Taiz and Zeiger, 1998). It may also be the case that auxin efflux from the leaf is influenced by other auxin pathways too, including movement of auxin down the main stem from the apical meristem. Termination of this polar auxin transport (PAT) from the apex, may be one of the precursors for dormancy induction and one of the first signals to induce defoliation. Plant responses to mechanically induced stress are well documented too and the effects are widely known as thigmomorphogenesis (Jaffe, 1973). The effects of physical disturbance of woody, deciduous species can be premature terminal bud formation and leaf senescence.

The objectives of the study were therefore to quantify the effects of mechanically induced stress (MIS) and the polar auxin transport (PAT) antagonist 2,3,5-triiodobenzoic acid (TIBA) on plant growth and development in three temperature regimes and to ascertain whether inhibition of auxin movement down the stem, or a thigmomorphogenetic response in low temperatures can trigger whole-plant dormancy.

Materials and Methods

Crataegus monogyna were grown from seed sown in spring 2008 in modular trays. Once large enough to handle, they were transplanted into 9cm pots containing a 50:50 mix of potting compost and John Innes no. 2 soil based media and grown under heated glass period prior to the experiment. The plants were potted on once more into 14 cm pots with 100% John Innes no.2 media and placed into Fisons 600 series growth cabinets. Initially the temperature remained at 15°C whilst the plants became acclimatised and, in order to reduce the effects of sudden exposure to cold, the night temperatures were reduced by one degree per day until the desired temperature is reached. The plants were then held at this temperature for the remainder of the experiment (3 weeks). Lighting was provided for 12 hours of each 24 hour period by tungsten bulbs and fluorescent tubes, supplying approximately 140µmol m^{-2} s^{-1}. Irrigation was effected manually with pots standing in saucers.

Treatment schedule

Temperature regime

A. CONTROL 15°C day / 15°C night

B. NIGHT CHILL 15°C day / falling night temp ending at 2°C

C. NIGHT FROST 15°C day / falling night temp ending at -2°C

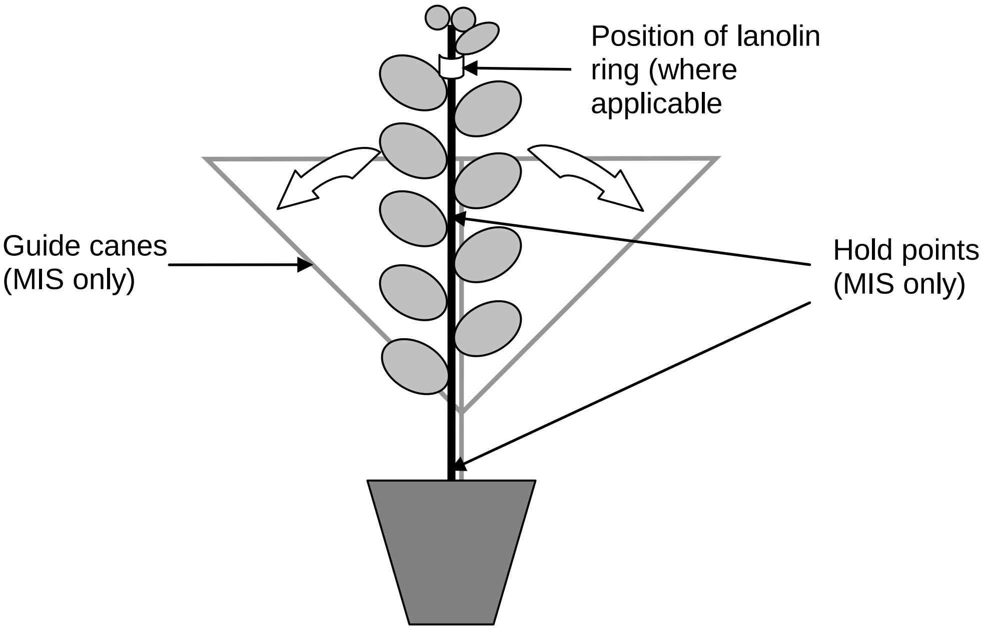
Treatment

1. Vigorous stem shaking once every 24h (MIS)
2. TIBA 10mM applied 1.5 cm below apical meristems in lanolin
3. TIBA 20mM applied 1.5 cm below apical meristems in lanolin
4. Acetone and lanolin applied under apical meristems
5. Control.

Plants receiving MIS were gripped between thumb and forefinger with the left hand at the base of the stem and at a point approximately halfway along the stem. Vigorous oscillations were made to 45° each side of the vertical axis for 30 seconds at a rate of approximately 2 per second. A triangular frame was constructed to ensure all plants received the same degree of bending (Fig. 12)

Treatments 2 and 3 were implemented by applying a small ring of TIBA + lanolin mixture to the stem using a 1 ml syringe. The required mass of TIBA was dissolved in 1 ml of acetone before being mixed with 9 ml of liquid acetone. This mixture was then drawn into the syringe and allowed to harden before application to the plants. Between 0.03 ml and 0.04 ml of the mixture was required to form a ring around each stem (Fig. 12).

Figure 12. Diagrammatic representation of the application of the MIS treatment. Also shows the position of the Lanolin (+/- TIBA) rings approximately 1.5cm below the shoot apex.



Owing to the possible defoliating effects of the contact itself in the MIS treatment, the bottom 5 cm of each stem was excluded from all data recording in all treatments.

Experimental layout

In order to reduce the effects on other plants of the daily removal of those receiving the MIS treatment, these were placed close to the access door of the cabinet (Fig. 13). Whilst this does not represent a fully randomised design, the layout of the lighting and the continuous circulation of air were, for the sake of practicality, deemed to provide a satisfactorily homogenous environment. The initial proposal was to administer the MIS treatment whilst the plants were in the cabinet; however, it became clear that it was necessary to remove these plants to apply the treatment. With hindsight, it is clear that an additional ‘treatment’ of removal from the cabinets was applied to these plants and future work of this nature would require removal of all plants from the cabinet daily to maintain statistical robustness.

Figure 13. Distribution of treatments within each temperature regime. The same design was used for each temporal block.

Cabinet A (15°C /15°C)					
5	5	4	4	2	3
3	4	2	2	3	3
5	2	2	4	5	4
3	1	1	5	1	5
1	3	1	2	1	4

Door

Cabinet B (15°C /2°C)					
4	3	5	3	5	4
5	2	4	3	4	2
3	5	5	2	2	2
4	1	4	1	3	5
1	3	1	2	1	1

Door

Cabinet C (15°C /-2°C)					
3	2	3	5	5	3
2	2	5	5	3	4
5	4	3	4	3	4
4	1	2	1	4	2
1	1	1	5	1	2

Door

Plant growth (height) was measured using a standard metal tape to the nearest 0.5 cm at the start of the experiment. Plants were measured one week later and then at weeks 3 and 5.

Chlorophyll content was measured in relative units at the same time intervals using a Hansatech CL-01 chlorophyll content meter for one leaf in the upper ½ of the stem and one in the lower ½, over 3 plants in each treatment. In addition the following parameters were measured at the end of week 5:

Defoliation – 20 plants were selected at random and the mean number of nodes calculated. This result was then used as the common denominator to calculate defoliation as a proportion (%) of leaves missing on each plant.

Leaf colour score – all plants were scored 0, 1, 2 or 3 depending on the following criteria:

0 – all leaves green

1 – Yellowing stipules / mild yellowing of whole leaves in lower 1/3 of stem

2 – Some reddening discernable in upper leaves or yellowing of the majority of leaves.

3 – Bright red / orange leaves on the whole plant

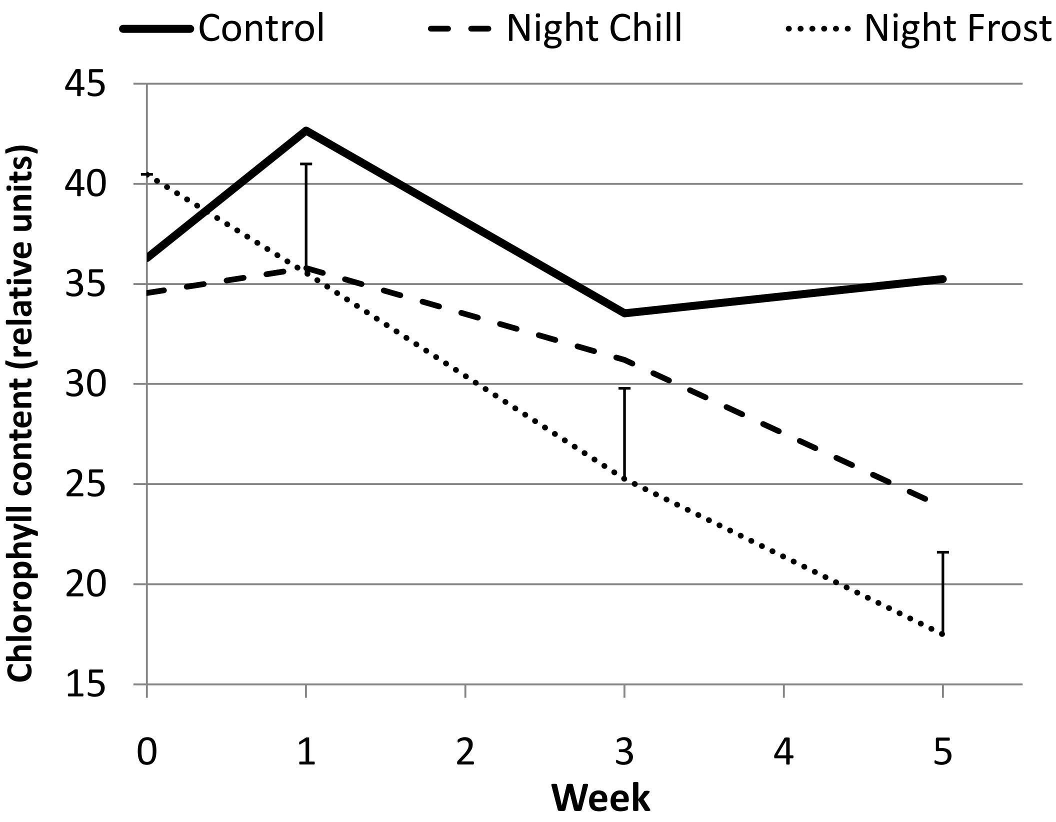
Leaf detachment force – Recorded in two randomly selected plants per treatment using a fruit penetrometer. Six measurements were made for each plant for the top 3 and bottom 3 nodes on each stem. The penetrometer was used to push down vertically at the junction between the petiole and the stem. In cases where leaves were missing, or became detached during handling prior to measurement, a force of 0 g was recorded.

Results

Owing to the potential effects of removal from the cabinets, MIS data have been excluded from some analysis.

Neither temperature, nor treatment had a significant effect on growth (P=0.54 and P=0.21 respectively). Visual leaf colour differences were obvious in some plants in the frost treatment after one week of falling night temperatures. This was confirmed after analysis of leaf chlorophyll content values (Fig. 14). Chlorophyll content of both the night chill and night frost treatments continued to decline over the course of the five-week experiment, whilst that of the control plants were not significantly different at the beginning and end of the experiment.

Figure 14. *Crataegus monogyna* (Reading – Controlled Environment). Mean chlorophyll content (relative units) for plants maintained at 15°C (Control), or where temperature progressively lowered to 2°C (Chill) or -2°C (Frost) night temperatures. Bars represent least significant difference at the P=0.05 level (n=12).



After 5 weeks under controlled conditions, plants subjected to night temperatures of -2°C (Night Frost) had lost approximately twice as many leaves as those in the control environment (data pooled across all treatments; Figs 15 and 16). Plants treated with TIBA 20mM in the night frost regime had lost more leaves than those in either the control (P<0.001) or the night chill (P=0.055) treatments (using standard ANOVA after square-root transformation). Defoliation of plants treated with TIBA 20mM in the night chill was also significantly higher than those at the control temperature (P=0.007). Analysis of the defoliation data recorded only in the night frost regime highlighted significant differences across all 5 treatments (P<0.001). However, there was not a significant difference between plants treated with 10 mM TIBA and 20 mM TIBA (P=0.164).

The frost temperature regime also gave rise to the highest levels of leaf colouration, with the TIBA 20 mM treatment having the highest mean score (2.5). The leaf detachment force was significantly lower than all other treatments in the night frost + TIBA 20 mM treatment (direct comparison with the same chemical treatment under night chilling revealed significant differences at the P=0.008 level, Fig. 17).

In an attempt to ascertain whether leaf colouration was an indicator of abscission zone formation, two plants from the frost + TIBA 20 mM were selected, one with very little leaf reddening and one whose leaves were all red/orange. Twenty-five leaves were tested with a penetrometer and the values analysed using ANOVA. The mean leaf detachment force of the predominantly red-leaved plant was significantly lower than that of the green leaved plant ($P<0.05$).

Mechanically induced stress (MIS) also showed some positive responses in terms of leaf defoliation (Fig 13). The relatively high value for the control in the MIS, however, would tend to suggest that at least some of the defoliation may be due to moving the plants in and out of the cabinets (but of course this may be a form of thigmomorphogenesis in itself!).

Figure 15. ***Crataegus monogyna*** **(Reading – Controlled Environment). Defoliation (%) for plants maintained at 15°C (Control), or where temperature progressively lowered to 2°C (Chill) or -2°C (Frost) night temperatures. (n=12).**

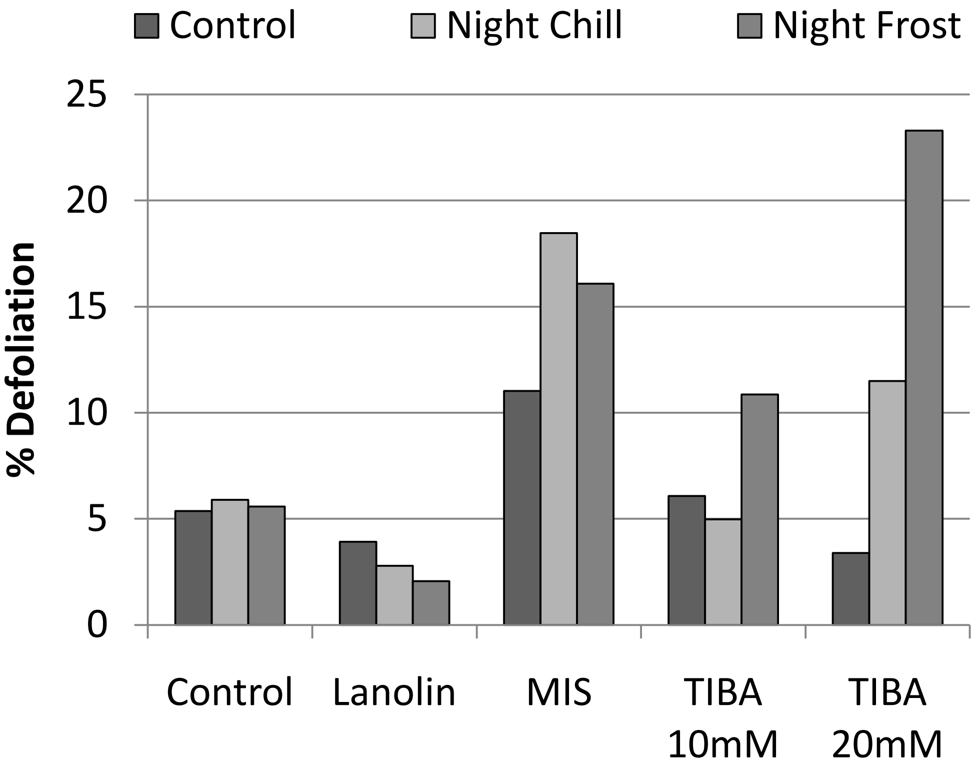


Figure 16. *Crataegus monogyna* (Reading – Controlled Environment). Examples of defoliation achieved in the three temperature environments after applying TIBA (20mM) rings to the stem below the shoot apex (visible on far right plant).

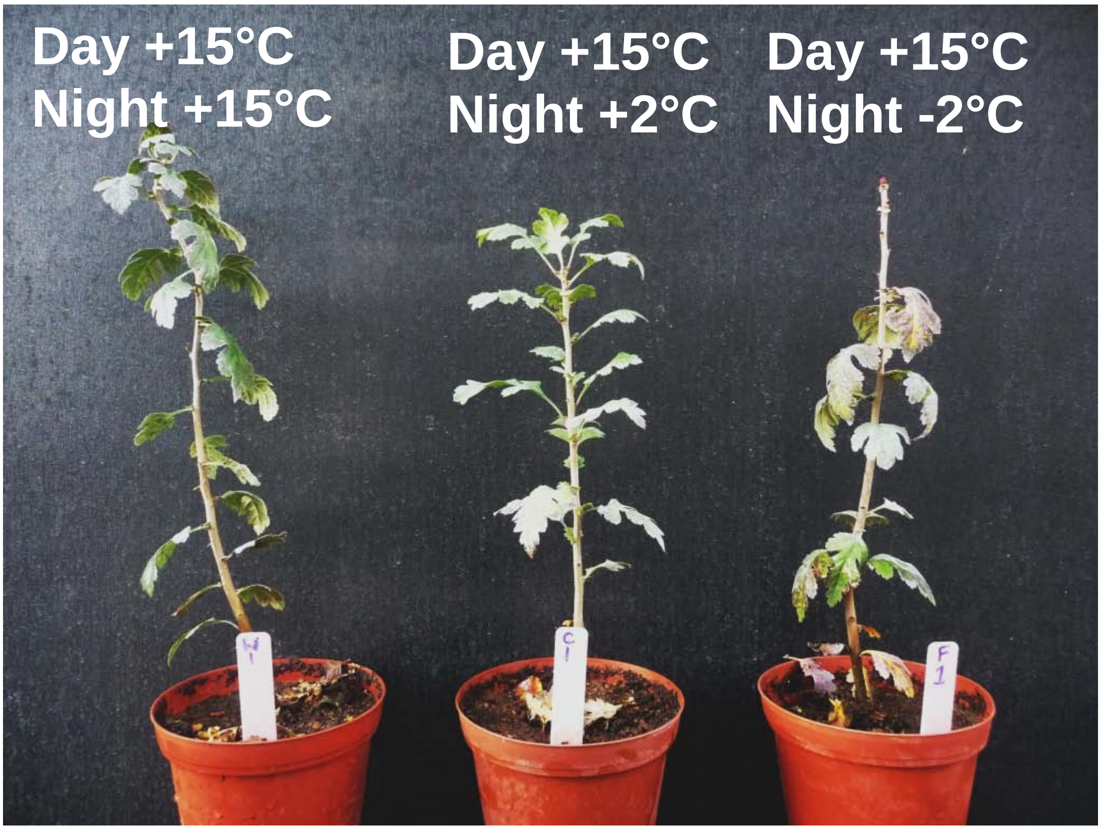
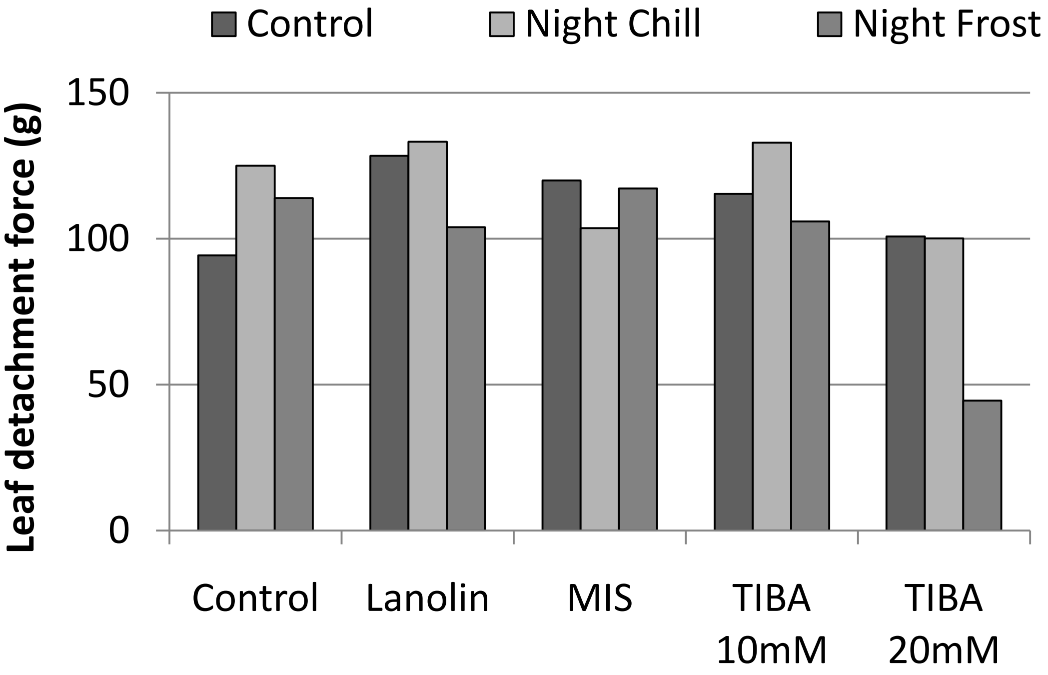


Figure 17. *Crataegus monogyna* (Reading – Controlled Environment). Mean leaf detachment force (g) for all treatments (n=24)



Exp. 6. Investigating the relationship between copper dose on subsequent ethylene evolution in *Crataegus monogyna* explants (data collection and analysis ongoing)

Data from the literature suggests that the major mode of action of Cu^{2+} ions in leaf abscission is likely to be promotion of the synthesis of ethylene gas through cellular damage (Bousquet and Thimann, 1984). In order to ascertain the relationship between the concentration of the copper solution applied to the leaf and the subsequent ethylene evolution and leaf abscission, single-leaf explants of *Crataegus monogyna* were dipped in solutions of $CuNa_2$.EDTA of varying concentrations and the production of ethylene gas was monitored. Secondary measurements of leaf chlorophyll content and leaf detachment force were also recorded.

Materials and methods

Prior to the experiment, a half strength Murashige and Skoog (M&S) tissue culture media was prepared using 2.2 g M&S basal salts (Duchefa Biochemie, Haarlem, NL) per litre of water. Bacto Agar (BD Biosciences, Erembodegem, Belgium) was dissolved at a rate of 7 g per litre of nutrient solution before dispensing into 350 ml capacity honey jars. The plastic lids of the jars had been modified to incorporate a gas tight septum to facilitate the removal of gas samples via a syringe.

Treatment solutions were prepared using crystalline copper disodium EDTA (Sigma Aldrich, Dorset, UK) and distilled water:

Treatments

A. 60mM (2.4 g/100 ml) solution of copper disodium EDTA ($CuNa_2$.EDTA)

B. 30mM (1.2 g/100 ml) solution of copper disodium EDTA ($CuNa_2$.EDTA)

C. 15mM (0.6 g/100 ml) solution of copper disodium EDTA ($CuNa_2$.EDTA)

D. 7.5mM (0.3 g/100 ml) solution of copper disodium EDTA ($CuNa_2$.EDTA)

E. Distilled water (Control)

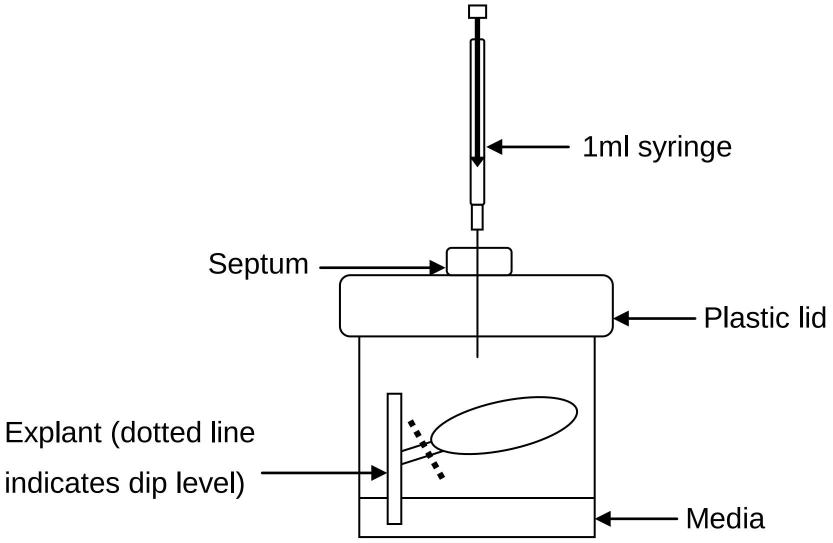
Background ethylene control:

F. empty jar + agar (no leaf)

Explants were removed from *Crataegus monogyna* plants comprising of a single leaf opened between 1 and 2 months prior to the start of the experiment, attached to approximately 4 cm of stem. Before applying treatments, the chlorophyll content of each leaf was measured using a Hansatech CL-01 chlorophyll content meter. After dipping the leaf into the treatment solutions to the level of the petiole for 5 seconds, the explants were shaken to remove excess solution and left for 1 minute on paper towel. Holding with forceps, the stem was re-cut under water to reduce the chance of air embolism within the xylem tissue. The stem was quickly inserted into the solid media at the bottom of the jar and the modified lids were attached and sealed with adhesive tape to prevent the loss of ethylene gas.

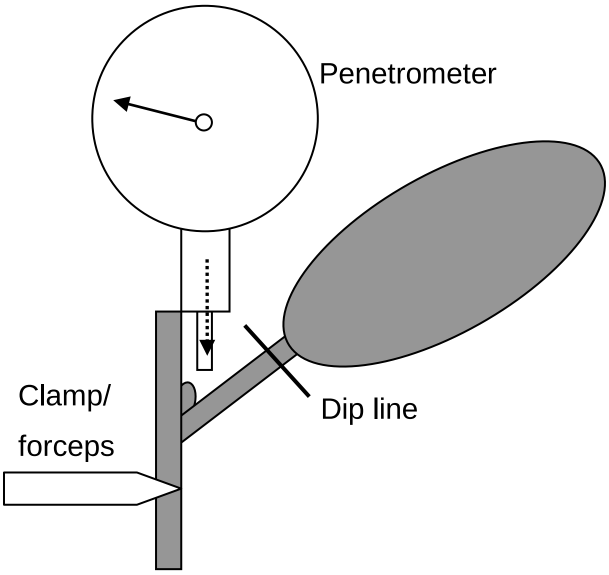
After 1 hour, the headspace of the jar was sampled for ethylene content. This was achieved by inserting a 1 ml syringe into the jar through the septum (Fig. 17), drawing a sample of head space gas to fill the barrel and flushing 5 times to mix gases (Fang et al., 2008). Subsequently 0.1 ml of the headspace gas was drawn off and injected into an Agilent Technologies (Palo Alto, CA) 5890 series 2 gas chromatograph with a flame ionisation detector, fitted with a 30m J&W Scientific (Folsom, CA) GS-Q porous layer open tubular column x 0.53mm internal diameter (oven temperature 60°C isothermal; injector temperature 60°C, detector temperature 200°C; helium carrier at 12.5 ml min^{-1}, split ratio 4:1). The jars were placed in a tissue culture growth room at 24°C (±1°C) and monitored daily.

Figure 17. Schematic diagram of the explant / media technique employed by this experiment showing the withdrawal of a headspace sample for analysis by gas chromatography.



At the end of the experiment explants were removed, weighed and the chlorophyll content measured. The explants were held by the stem vertically using forceps and a fruit penetrometer was used to measure leaf detachment force (Fig. 18). The detached leaves were rinsed with distilled water and allowed to dry on paper towel before being oven dried for 48 hours. This material is currently awaiting analysis for copper content by mass spectrometry.

Figure 18. Measurement of the leaf detachment force at the end of the experiment using a fruit penetrometer.



Results

Ethylene evolution increased in all treatments during the first 72 hours (Fig 19.). By the end of the experiment, however, the concentration of headspace ethylene had started to fall in all but the 7.5 mM treatment, probably due to the combined effect of an imperfect seal on the jar lid and a reduced rate of ethylene synthesis. Owing to the variability of the data, it was only possible to detect differences of statistical significance between the control and the 7.5 mM treatment at +144 hours using a Kruskal-Wallis one-way non-parametric ANOVA (H = 4.81, df = 1, X^2 p = 0.028).

The mean chlorophyll content increased in all treatments over the duration of the experiment (Table 7). This was unexpected as the copper solutions had caused visible damage to the leaf tissue by the time the leaves were removed. Furthermore, the only two occurrences of complete leaf abscission were in the control treatment, whilst the highest concentration treatment of $CuNa_2$.EDTA gave rise to the highest mean leaf detachment force.

Figure 19. Headspace ethylene detected above single leaf explants of *C. monogyna* after treatment with varying concentration solutions of $CuNa_2$.EDTA (n=5).

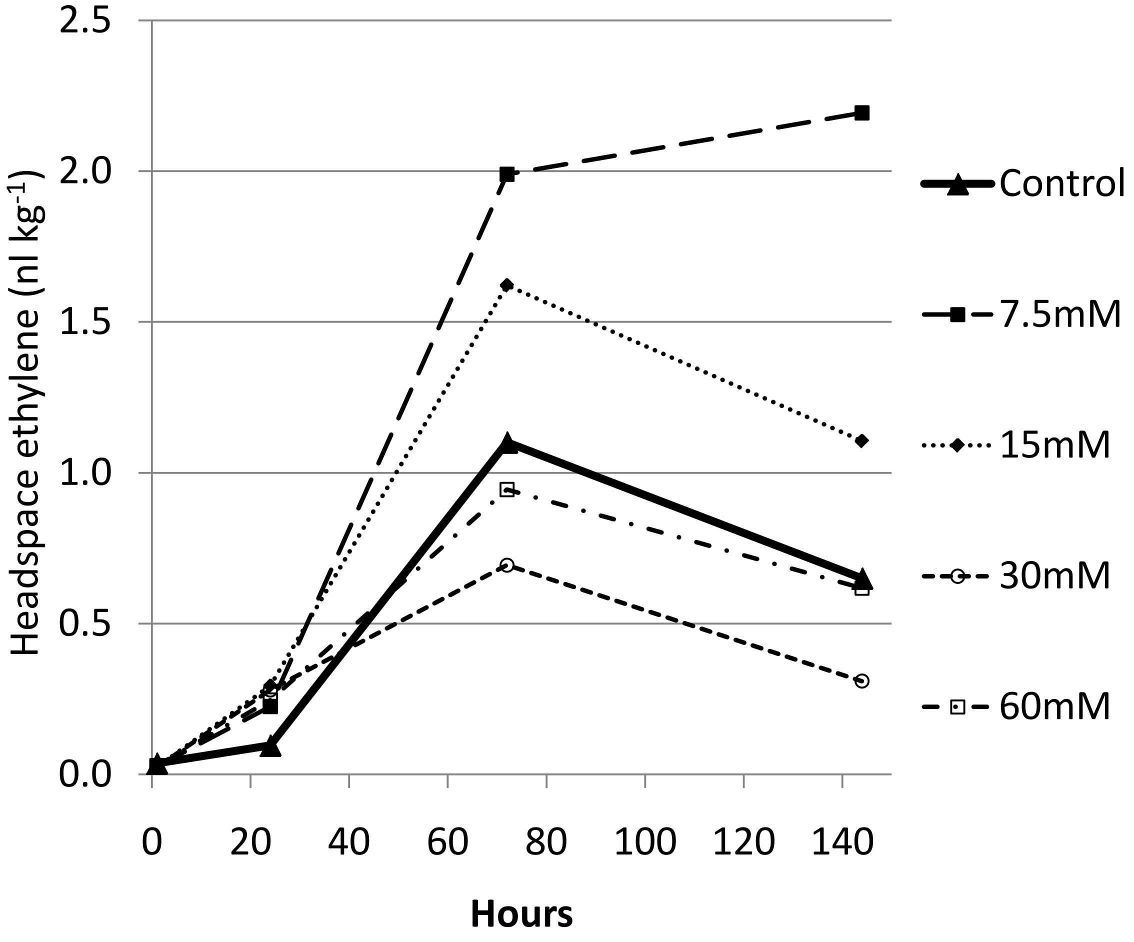


Table 7. Increase in chlorophyll content and leaf detachment force recorded in explants of *C. monogyna* at the end of the experiment after treatment with varying concentrations of $CuNa_2$.EDTA for 6 days (n=5, means shown ± s.e.).

$CuNa_2$.EDTA concentration (mM)	Increase in chlorophyll content (relative units)	Mean leaf detachment force (g)
0	6.5 ± 1.9	175 ± 74
7.5	8.5 ± 1.6	175 ± 10
15	7.2 ± 3.6	246 ± 53
30	3.9 ± 2.1	217 ± 53
60	6.1 ± 1.3	287 ± 49

Discussion

Improving the effectiveness and reliability of ‘Leaf Fall’

Data from year 2 of the project indicates that ‘Leaf Fall’ is the most appropriate chemical means of defoliating young trees; in some situations ‘Leaf Fall’ helping promote 60-80% defoliation. Effectiveness of the chemical, however, can vary with species, weather conditions and stage of plant development. Potential cost of this chemical is also a concern for some nurserymen. Field trials carried out in 2007 suggested that a half strength application of ‘Leaf Fall’ could be as effective on *Malus* as the recommended rate. By adding a wetting agent to the spray mix, in an attempt to improve foliar penetration, it was hoped that similar results could be achieved if the rate of application was reduced still further. The inclusion of a wetting agent did improve the action of ‘Leaf Fall’ when applied at the recommended rate of 20 ml l^{-1} but the inclusion of this adjuvant did not result in successful defoliation of *M.* ‘Bramley when the application rate of ‘Leaf Fall’ was reduced further to 5 ml l^{-1}.

Leaves located in the upper stem were least affected by defoliant treatments in *M.* ‘Bramley’. It is possible that due to high auxin:ethylene ratios in these leaves there is reduced capacity to form abscission zones (Taiz and Zeiger, 1998). Ethylene is usually released from injured tissues (e.g. following cellular damage from high doses of copper) but very little tissue damage was actually observed in the uppermost leaves, even though they are generally perceived as being less robust and more prone to damage than older leaves. A lack of injury in the younger leaves suggests that retention or penetration of the defoliants was poor. This may relate to the angle the leaves are held in relation to the stem i.e. the more acute the angle; the less spray will be retained. In *M.* ‘Bramley’, the pubescence of young leaves may also have reduced the effectiveness of defoliants, whereas in other species, the integrity of cuticular wax may have been greater in young leaves, giving rise to the same phenomenon. The fact that a wetting agent in Exp. 3 improved the effectiveness of ‘Leaf Fall’ (across all leaf types) implies that penetration of copper ions may be one of the reasons behind inconsistent results with ‘Leaf Fall’, and why young leaves particularly, are resilient. Interestingly, in relation to this, work by Arman and Wain (1960) suggested the absorption capacity of copper (II) ions by leaves increases from spring to summer and then declines as leaves move toward senescence and abscission.

The action of copper

Copper ions, like those of other heavy metals cause lipid peroxidation, damaging cell membranes (Chen and Kao, 1999; Luna *et al.*, 2001). ‘Leaf Fall’ is effective as the Cu^{2+} ions are in solution whereas other, insoluble copper compounds are reliant upon biological exudates or physical weathering to dissolve them slowly, which makes them useful as fungicides (Hassall, 1990; Cremlyn, 1991). However, whilst the high level of solubility is beneficial insofar as a larger amount of absorption may occur over a given amount of time, this property does render 'Leaf Fall' more susceptible to dissolution by rain and dew once it is on the leaf. To be effective, any defoliants must achieve maximum leaf penetration as rapidly as possible and this occurs via cracks in cuticular wax or via the stomata, which are mainly located on the abaxial surface of the leaf (Schönherr, 2001).

Applications of potential alternative defoliants such as FeEDTA and urea did not achieve comparable rates of defoliation when compared to 'Leaf Fall', even though there was often significant damage to the leaves of plants receiving these treatments. One notable effect of a high concentration application of urea was a complete absence of an abscission zone, although leaves were significantly damaged after several days. This suggests that copper-EDTA does not encourage abscission solely by tissue damage and resultant ethylene evolution. Whether this additional action is caused by copper (II) ions, or by the EDTA molecules with which they are complexed warrants further investigation. Copper has also been shown to inactivate the auxin IAA, which may enhance the effects of any ethylene produced (Ben-Yehoshua and Biggs, 1970).

Copper – ethylene relations in excised stem tissues

Whilst the experiment to investigate the relationship between the amounts of copper applied to a leaf and the resultant ethylene synthesis has not yielded any significant results from the preliminary analysis of the data, it has proved the robustness of the method for examining single leaves in isolation. Several more experiments using this basic protocol are planned, although the deletion of nutrients from the growth medium may be beneficial in order to accelerate the action of any defoliant treatments (the chlorophyll content increase may have been due to the provision of macro- and micro-nutrients in the media). Although some results were not initially expected, this in itself, may provide further evidence that juvenility is the overriding factor determining the response of a leaf to copper-based defoliants. The high levels of force required to remove leaves from explants treated with high doses of copper may also reinforce the concept that applying copper above the optimum defoliation concentration may be so physiologically damaging that the biochemical processes required to hasten abscission cannot proceed. Conversely, the experiment may suggest that CuEDTA applied solely to the leaf blade is not sufficient to trigger ethylene-mediated abscission, and that the primary defoliant effect of CuEDTA may, in fact be the direct action of copper ions and/or EDTA on cells of the apical zone. When the data set is completed with the analysis of each leaf for copper content, more meaningful conclusions may be drawn.

Pre-treatment with Growth Retardants

As outlined above, results from field trials in both 2007 and 2008 demonstrate that CuEDTA in the form of 'Leaf Fall' can be a highly effective defoliant. It would appear though that the desired effects of pre-treatment with triazole compounds, however, to slow growth and encourage earlier entry into dormancy were inconsistent across species. The poor weather conditions during summer 2008 may have masked the effects of the growth regulators as most plants, even those in control treatments were showing little sign of shoot activity prior to applying the defoliants. Regrowth of *C. Monogyna* treated with ‘Leaf Fall’ was significantly greater in the spring following defoliation in those plants that had not received a growth regulator as a pre-treatment. Hence, any retardation of bud-burst in the following year would seem to make such treatments practically unsuitable, even if a repetition of the field trial was to yield more positive results.

Temperature and implications for hormonal regulation of leaf abscission.

Exp. 5 clearly demonstrated that leaf abscission could be enhanced when young plants were exposed to a combination of frost (-2^{o}C) and a disruption to their auxin transport (through the use of auxin blocker TIBA). Providing cool, but non freezing conditions (+2^{o}C) were not as effective, similarly, exposure to frost (-2^{o}C), but without TIBA being used, did not enhance defoliation to the same extent. This is an important result, in that it implies that reduction of auxin transport is a required pre-condition in woody plants before any subsequent stress will be successful in inducing leaf abscission. The finding is in direct contradiction to work on cotton (*Gossypium hirsutum*) which suggests that defoliants will only work when the auxin signal from the shoot tip is maintained (Morris, 1993). Also important, the data confirms nurserymen’s experiences in that frost is the best ‘defoliant’ and even cool, but non-freezing temperatures were not as effective. It is unlikely that at -2^{o}C that ice crystals penetrated the xylem vessels of the stem or the leaves, but ice formation on the outside of the leaf, may have caused cellular desiccation and / or induced biochemical changes within the tissues (Cameron and Dixon, 2000).

Drought Stress

Controlling environmental factors that influence defoliation remains challenging for nurserymen, and likely mechanisms could be very expensive or difficult to implement in a practical manner. Of the possible applications though, water is probably the most widely and easily controlled. Climate change may also give rise to drier summers in most of the UK, meaning that by withholding irrigation, some nurserymen may be able to expose plants to a controlled stress. Experimentally, the potential effects of mild drought in two temperature regimes were examined. Whilst, due to the small scale of the project, some data analysis proved non-significant, there appeared to be an important difference between the treatments. Even though the water content and stomatal conductance in the colder environment were lower than in the warmer glasshouse, the stress imposed by withholding water had no effect. The opposite was true in the warmer environment, suggesting that whilst the most important cue for leaf abscission is cold (or even freezing) temperatures, the imposition of mild drought stress during warm periods in early-mid autumn may aid subsequent defoliation.

Conclusions and future work

Copper complexed with EDTA proved to be the most important aid to defoliation examined in field trials. Whilst this compound is widely used, the full effects on abscission physiology are not completely understood. Whilst copper is widely used as a pre-harvest defoliant in horticulture, work on the action of copper compounds with respect to this process has been reasonably rare in the last fifty years. Current industry practice based on the work carried out by a number of researchers allows nurserymen to achieve acceptable levels of defoliation, however more unpredictable weather patterns may mean plants continue to grow strongly later in the calendar year. This is especially true of juvenile material. Data collected during this project suggest that reducing plant vigour (or interrupting auxin supply) at the end of the season by applying mild stress may be key in amplifying the effects of natural stimuli such as reduced photoperiod and frost and in guaranteeing the future efficiency of copper defoliation treatments. Work with the plant growth regulators though suggests that slowed extension growth alone may not necessarily hasten the formation of a completely dormant apical bud, which has stopped exporting auxin.

Planned Experiments 2009–10

Applying stress, whether chemically or physically warrants further investigation as a method of curtailing the growth of juvenile woody plants and potentially increasing the effectiveness of any subsequent defoliant treatments. Furthermore, since ‘Leaf Fall’ appears to be the most effective abscission promoter used during the first two years of the project, it would be useful to understand its mode of action further in order to refine its use or suggest alternatives. Experiments in the final growing season will therefore focus on the following:

a) Comparing the effects of water availability and mild doses of copper on plant growth, leaf senescence and abscission (Semi controlled conditions, University of Reading)

b) The use of mild copper doses as auxin antagonists to inhibit late-season growth and potentially hasten the formation of an apical bud (Field trial, commercial site)

c) Understanding how the constituent parts of ‘Leaf Fall’ act on the abscission process, and how the product may be made more effective in various environmental conditions (e.g. the effects of rain and temperature)(Field trial, commercial site)

d) In order to gain further data on the importance of auxin flow from the shoot tip, and the potential of copper to antagonise this signal, single leaf explant studies will allow the fine control and detailed observation of the abscission process in a controlled environment.

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Appendix 1. Project Milestones with Comments (from 2008 report)

1. M.Phil / Ph.D. student in place (Jul 07)

Completed

2. Determine suitable concentrations of alternative chemical sprays (Aug 07)

Completed – *Covered in first year report*

3. Assess leaf abscission rates after treatments at Reading and on commercial nurseries (Dec 07)

Completed – *Covered in first year report*

4. Carry out extensive lit review on mechanical / microbial / light influences on leaf abscission (Jan 08)

Completed – *Covered in first year report*

5. Identify 3-4 potential (non-chemical) alternative approaches to leaf abscission that may have application for industrial use (Mar 08)

Completed – *Covered in first year report*

6. Identify most promising rate and timing of chemical application (June 08)

Completed – *Covered in first year report*

7. Correlate treatment responses with potential physiological keys (June 08)

Completed – *Covered in first year report*

8. Provide annual report (Jul 08)

Completed

9. Set up small scale experiments to test the most promising practical cultural factor identified from the literature (e.g. thigmomorphogenesis [brushing] and to provide a greater understanding of environmental factors that influence leaf abscission on juvenile trees (e.g. temperature, water availability, photoperiod). (Dec 08)

Completed – Drought and thigmomorphogenesis experiments conducted 4th quarter 2008.

10. Evaluate a small number of growth regulator chemicals to induce earlier and more consistent cessation of apical bud growth. Investigate their interaction with defoliant chemicals as a mechanism for more effective leaf abscission (June 09)

Covered in this report

11. Identify the potential of the cultural technique used in the small scale controlled experiments to aid leaf abscission (June 09)

Covered in this report

12. Determine the extent to which key environmental factors affect leaf abscission in juvenile trees and provide growers with information in context to aid management in the field (e.g. how temperature or rainfall factors may influence timing of defoliants) (June 09)

Covered in this report

13. Provide annual report 2 (Jul 09)

14. Determine if the non-chemical alternative approach has application and if so, incorporate into field tests in comparison with one or more chemical approaches. If not, further refine chemical approaches alone (Dec 09)

15. Provide Open Day for nurserymen to review results (Jun/Jul 10)

16. Reassess most promising chemical and cultural techniques (Jul 10)

17. Provide final report (Jul 10)