

Project title Optimising defoliation in young trees

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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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AUTHENTICATION

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GROWER SUMMARY

Headline

- Natural leaf abscission is promoted by consistent (day and night) low (chilling) temperature; brief warm periods during the autumn delay the response.
- Once young trees meet the specification for height, it is important to encourage dormant bud formation; continued shoot extension results in leaf abscission being delayed.
- The project confirmed that Copper EDTA, a foliar feed fertiliser (known by some growers internationally as the product 'Leaf Fall') is effective as a defoliant. However, Copper EDTA is not registered for use within plant protection products in the UK and therefore cannot be used as a defoliant.

Background and expected deliverables

There is concern in the industry 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.

This project aimed to investigate what factors promoted or delayed natural leaf fall and how, through a better understanding of these, growers could better predict when leaves would be shed and the crop lifted. Through a review of literature, a number of cultural approaches were identified and the most promising were examined within the project. A second objective was to determine whether there were any management tools, chemical or cultural, that growers could exploit to aid defoliation and help crop scheduling. Investigating non-chemical approaches was desirable due to pressure to reduce the reliance on agro-chemicals, and the costs incurred to register new products. Nevertheless, both chemical and non-chemical approaches were explored to compare effectiveness.

Work 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 were also utilized to help determine the relationship between potential defoliants and the physiological stage of the crop at the time of application. One of the key triggers for leaf abscission in nature is thought to be exposure to frost and this project aimed to verify the effect of low temperature and whether other abiotic stress factors, such as controlled drought or waterlogging, could substitute for this stimulus.

Summary of the project and main conclusions

Important note – None of the fertilisers, fungicides, adjuvants or disinfectants mentioned are currently approved as growth regulators or defoliant (see Table 1). Only products officially approved for use as plant protection products should be applied to control pest, disease and weed problems and provide growth control. You should consult your BASIS-registered agronomist for advice on appropriate products to use on your crops.

Physiology of the young tree

A key objective of the project was to try to optimise leaf defoliation treatments through a better understanding of when the trees would be responsive. Young nursery trees tend to be juvenile in character, and this combined with cultivation techniques that maximise growth (to meet the market specification), promote continued shoot activity late into the growing season. Data indicated that defoliation techniques were often only effective after the shoot extension had slowed, or that a resting bud was forming. The relationship was not absolute – leaves adjacent to dormant buds did not always abscise, but in general acquiring dormant shoots aided defoliation (Figure 1). Figure 1 (*Crataegus*) also alludes to the fact that in most species tested, it is the younger leaves in the upper part of the stem that are least likely to defoliate when the plant is still active. This implies that a second factor, probably the plant growth regulator auxin, is determining leaf abscission potential. When auxin transport is disrupted within the plant (e.g. by stopping transport from the apical meristem or across the leaf petiole using the auxin blocker TIBA), then leaves become better disposed to abscission (e.g. after the application of a defoliating chemical such as Copper EDTA ('Leaf Fall') – Figure 2; or on subsequent exposure to chilling temperatures).

In practical terms, growers need to monitor for the development of a resting apical bud and the end of leaf expansion before they can be assured that any subsequent defoliation treatment will be effective.

Environmental stress

Anecdotal evidence suggests that exposure to frost is the primary inducer of leaf abscission (and the current problem is exacerbated by the lack of autumn frosts). In an attempt to understand the action of frost and whether other environmental stress factors could be substituted for it, experiments investigated how stress affected defoliation. Exposing trees to simulated chilling and frost conditions indicated that sub-zero temperatures (-2°C) were much more effective than merely chilling (+2°C).

Figure 1. *Crataegus monogyna*. The relationship between shoot activity and percentage defoliation in upper and lower sections of stem.
(Plants with dormant apical buds are more likely to shed their leaves. When buds are still active, young leaves in the upper sections are the most difficult to remove).

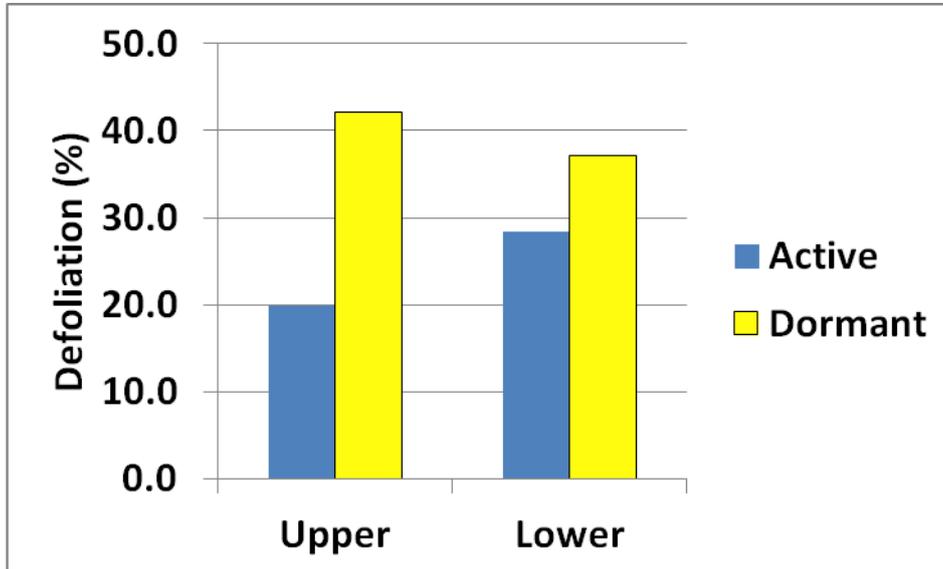
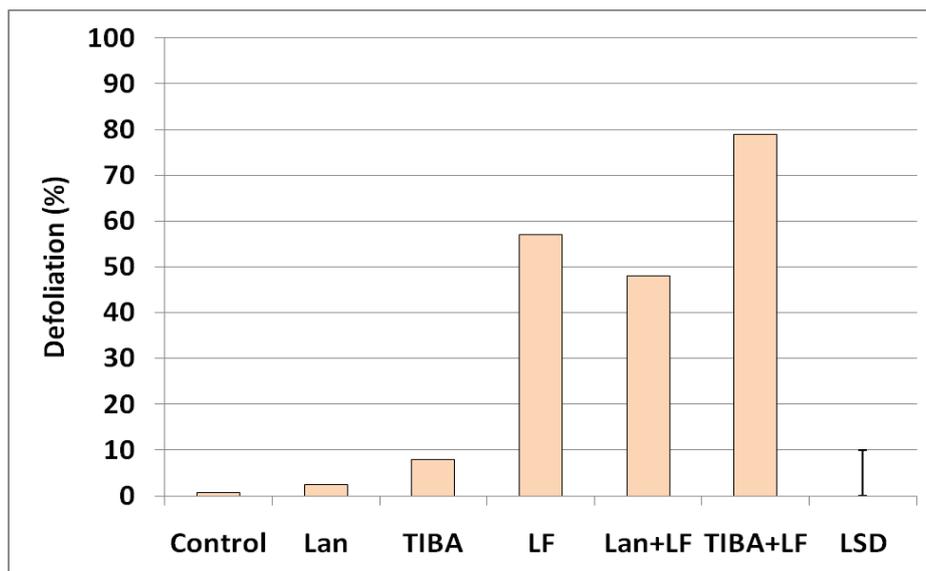


Figure 2. *Crataegus monogyna*. Defoliation percentage on 3 November 2009, after treatments with the 'non active' Lanolin or auxin-blocker TIBA in August and subsequently sprayed with water or Copper EDTA ('Leaf Fall -LF') on 13 October.



Maximum defoliation was observed when sub-zero conditions were combined with the application of the auxin blocker. To maximise responses, low temperature exposure during the day appeared important, i.e. it was not sufficient just to expose trees to low temperatures at night. The formation of abscission zones was enhanced when plants were kept at 10°C day and night, and then sprayed with a chemical defoliant (in this case Copper EDTA 'Leaf Fall'), rather than to allow the day temperature to rise to 20°C.

Both controlled 'moderate' drought stress and waterlogging, imposed on containerised plants for 6 weeks in autumn, encouraged leaf abscission, with waterlogging being particularly effective in the trial. Admittedly, the use of such techniques is unlikely to be feasible in large scale field production (although the results do provide some indication of responses in either a very wet or dry summer / autumn), but for those businesses who can manipulate irrigation such additional factors may help substitute for the lack of frost.

Chemical approaches

The use of Copper EDTA

Copper EDTA, available within UK as a foliar fertiliser (Protex Chemicals Ltd) and known by some growers internationally as the product 'Leaf Fall' (not available within the UK), was the most effective chemical trialled which induced defoliation. For some species, such as *Crataegus monogyna* and *Malus* 'Profusion Improved', defoliation rates were as high as 60-97%. Results, however, were not consistent across different species, locations or seasons; in the case of *Malus* 'Bramley' for example, defoliation varied between 17 and 57%. None of the potential alternative chemicals tested proved to be as effective as Copper EDTA. Combinations of chemicals or mechanisms to manipulate the crop growth, however, may go some way to improving the efficacy of Copper EDTA products.

With the difficulty to defoliate *M.* 'Bramley' adding the wetting agent Activator 90 to the spray mix with Copper EDTA improved defoliation rates from 15 to 40%; suggesting that part of the difficulty with using Copper EDTA is ensuring penetration to the leaf. Indeed, the youngest leaves were the most difficult to remove in this cultivar and this may be due to juvenility, but also the fact that such leaves / petioles were heavily pubescent (hairy) (Figure 3) which retards the penetration of defoliants. Unlike other copper compounds used regularly in crop protection the copper in Copper EDTA contains copper in solution, which defoliates the young trees more effectively than insoluble copper compounds found in some fungicides. The disadvantage of this however is that the compound may be more prone to being washed off by rainfall.

In an attempt to determine the effects of lower concentrations of Copper EDTA (e.g. to facilitate repeat applications or save costs) field trials were carried out in 2007 on *Crataegus* using a reduced strength application (10 ml/l rather than 20 ml/l). This proved equally effective as the full concentration on this species, however, further reductions to a 5 ml/l application in 2008, were ineffective on *M. 'Bramley'* even with the addition of a wetter. The effectiveness of chemical defoliants such as Copper EDTA was optimized when plants were in a more 'favourable' physiological state prior to application. This could include pre-treatment with other chemicals, a reduction in shoot activity or disruption to endogenous auxin (plant hormone) supply, or exposure to moderate levels of environmental stress (chilling, drought, waterlogging etc.).

Figure 3. *Malus 'Bramley'*. Young leaves and stems showing their pubescent nature which retards the penetration of defoliants such as Copper EDTA.

(Note also the damage to leaf tips where the defoliant has run-off the leaf surface and accumulated. These young shoots and leaves are likely to be the source of endogenous auxin that is inhibiting the formation of abscission zones).



Alternative chemical defoliants

Of the potential alternative chemicals to Copper EDTA which induced defoliation (see Table 1), none performed as well and inconsistencies were apparent based on concentration applied and crop growth stage. Nevertheless, urea at 90g /l, the plant

growth regulator Cerone (2-chloroethylphosphonic acid), and the triazole fungicide Folicur (tebuconazole) all showed some promise, as did Jet 5 (peracetic acid) at 50ml/l. The wetting agent Silwet L-77 also showed some potential as a defoliant. In contrast, other copper compounds such as copper sulphate (Cuproxtat FL) and copper oxychloride (Cuprokylt FL) showed only very limited defoliation potential. Chelated Iron (iron EDTA e.g. micronutrient fertilizer Librel Fe-LO) also appeared a far less effective defoliant than Copper EDTA, its copper equivalent.

Financial benefits

The work highlights that there are alternatives to natural frost in encouraging leaf abscission in young trees. Chemicals, particularly Copper EDTA ('Leaf Fall') have potential to induce defoliation, but costs for registration as a pesticide are generally prohibitive (currently, Copper EDTA is used as a fertiliser to avoid copper deficiency, but is not registered as a plant protection product).

Reduced sales potential due to poor leaf abscission has been estimated at £510,000 per annum (assuming a 1% direct loss within tree, hedging and rose crops, but not including any secondary costs such as extra labour for manual stripping of leaves, storage or transport problems etc.).

As Copper EDTA can only currently be used as a fertiliser to avoid copper deficiency other techniques that disrupt apical activity in the latter part of the growing season should be considered (undercutting, moderate drought imposition, lower fertiliser concentrations), leaving the crop in a more favourable physiological state. Associated costs will vary with area and value of the crop, but the key challenge will remain that of ensuring the crop meets specifications for height and quality at the end of the season.

Action points for growers

- Once the crop specification is met, look for opportunities to reduce growth vigour, e.g. via lower fertilisation or irrigation rates or checking growth again via undercutting. This will aid natural defoliation.
- Be aware that a range of environmental factors, not just frost can influence both late season growth and the likelihood of leaf defoliation. Look to exploit both periods of particularly dry or wet weather from late August onwards to schedule earlier lifting. Moderate levels of stress in the crop during late summer / autumn will encourage earlier abscission.

Table 1. Approval status (during the period of the project) and technical data for compounds referenced within the Grower Summary

Product	Active ingredient	Use	Crop use	Approval status on ornamentals
'Leaf Fall'	Copper EDTA Ethylene-diaminetetra-acetic acid copper (II) diammonium salt (Cu(NH ₄) ₂ .EDTA)	Fertiliser	Various	Fertiliser only, not approved as a plant protection product
Librel FE-LO	Iron.EDTA 13.2%	Fertiliser	Various	Fertiliser only, not approved as a plant protection product
Urea	Urea	Fertiliser	Various	Fertiliser only, not approved as a plant protection product
Cerone	2-chloroethyl-phosphonic acid (480g/l)	Growth regulator	Cereals, ornamentals, tree fruit and tomatoes	SOLA approval for growth control
TIBA	2,3,5-Triiodobenzoic acid	Growth regulator	None	Not approved as a plant protection product
Copper sulphate (available commercially as Cuproxat FL)	Copper sulphate	Fungicide	Potatoes	Not approved
Cuprokyt FL	Copper oxychloride (270g/l)	Fungicide	Top fruit, tomatoes and a range of other horticultural crops	Permissible under LTAEU for disease control
Folicur	Tebuconazole (250g/l)	Fungicide	Cereals, rape and a range of horticultural crops	Permissible under LTAEU for disease control
Nativo 75WG	Tebuconazole (500g/kg), trifloxistrobim (250g/kg)	Fungicide	Brassica crops, carrot and leek	Permissible under LTAEU for disease control
Activator 90	Alcohol ethoxylates (750 g/kg), natural fatty acids(150 g/kg)	Adjuvant	Cereals, fruit, vegetables	Permissible as a wetting agent
Silwet L-77	Polyalkylene-oxide modified heptamethyl-trisiloxane (80-85%)	Adjuvant	Cereals, fruit, vegetables	Permissible as a wetting agent
Jet-5	Peracetic acid (5%), hydrogen peroxide (20%), acetic acid (10%)	Disinfectant	Hard surfaces	Permissible for use as a disinfectant on hard surfaces only

SOLA – Specific Off-Label Approval.

LTAEU –Long Term Arrangements for Extension of Use.

SCIENCE SECTION

INTRODUCTION

Important note

None of the fertilisers, fungicides, adjuvants or disinfectants mentioned are currently approved as growth regulators or defoliants (see Table 2). Only products officially approved for use as plant protection products should be applied to control pest, disease and weed problems and provide growth control.

You should consult your BASIS-registered agronomist for advice on appropriate products to use on your crops

There are many theoretical discussions about climate change, but the horticultural industry is very much in the 'vanguard' in experiencing the consequences in practical terms. If seasonal changes are occurring, then this impacts directly on growers who need to sow crops, apply appropriate management regimes and market their produce all in accordance with the predominate weather patterns. Growers have always had to account for variability in the weather, but stronger climatic shifts will bring a new range of problems and opportunities. Some of these may already be evident. Tree nurserymen have noticed over the last decade that autumns have been progressively warmer, with fewer and later incidences 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 increasingly wish to induce earlier and more consistent defoliation through alternative means..

For nursery tree crops chemical defoliation could be an option. However, they 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 consider that copper-EDTA complexes, available as foliar fertilisers, to have potential to enhance leaf abscission but 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 5 specific objectives:

1. Determine the optimum use of Copper EDTA ‘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.
5. Through a review of the literature (see Appendix 1) and additional small scale experiments, provide industry with a more complete understanding of the factors that can influence leaf abscission, and which may be exploited in future.

Leaf abscission

The biochemistry of the leaf abscission process is only partially understood (See Review of Literature – Appendix 1). The two most important hormones identified in the control of autumnal leaf senescence and abscission are auxin (primarily indole-3-acetic acid) and ethylene (C₂H₄). 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 Copper EDTA ‘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 project aimed to assess whether Copper EDTA has potential for use as a commercially-viable chemical defoliant. Copper EDTA is available within UK as a foliar fertiliser (Protex Chemicals Ltd) and known by some growers globally as the product 'Leaf Fall' (not available within the UK). This product is essentially a Copper EDTA salt. It is known that the effectiveness of Copper EDTA can be variable across species and seasons, and the mode of action in relation to forming a leaf abscission zone is not clearly understood. Therefore, it was important to determine if there were either other copper-based products, or indeed other EDTA compounds, that were equally effective at promoting leaf abscission. Similarly, we wished to evaluate other potential chemical alternatives, - particularly ones that were more likely to achieve registration as a plant growth regulator, were less expensive or were more benign in environmental terms (e.g. not leaving a residual metal ion after degradation).

There was also a desire within the industry to determine if non-chemical means could be explored to enhance abscission, or at least understand more fully how crop development, husbandry and environmental factors interact to influence abscission. Many of the current problems being experienced with late natural defoliation have been linked to the reduced incidence of frost during the autumn period, but it is not clear just how low temperatures need to be before leaf abscission is encouraged. Similarly, if frost occurrence is going to be less frequent in future, how do other abiotic stresses that crops may experience in the late summer / autumn, e.g. drying or waterlogged soils affect the potential for leaf abscission?

In commercial practice nurserymen need to strike a fine balance. They are required to optimize growth during summer to ensure the crop attains specifications for size (and with some fruit varieties – shape, e.g. early branching, 'feather production'), and yet also require the crop to cease growing quickly once the specification is met, and then promote dormant bud formation. Maximising growth, e.g. via high nitrogen fertilizers, however, is likely to prolong shoot growth, delay resting bud formation, and potentially impact on degree and timing of leaf defoliation. Therefore we aimed to investigate more fully the relationship between meristem activity, bud dormancy and leaf abscission, and to seek practical solutions that may promote dormant bud formation and defoliation at a convenient time.

MATERIALS AND METHODS

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 chemicals or plant responses in a more controlled manner, e.g. under specific temperature regimes. Also a range of laboratory techniques were developed to assess the mode of action of some of the defoliant used in the project.

Six tree species were identified after consultation with nurserymen as being representative of the type of material in which defoliation has become difficult in recent years. *Crataegus monogyna* (hawthorn), *Alnus glutinosa* (alder) and *Quercus robur* (oak) were produced from seed as hedging material. *Malus domestica* ‘Bramley’ (culinary apple), *M. x moerlandsii* ‘Profusion Improved’ (ornamental crab apple) and *Pyrus communis* ‘Conference’ (pear) were field-grown grafted examples grown for wholesale or garden centres.

Field experiments

University of Reading, (UoR)

Maiden whips of *Crataegus monogyna* were planted at the University field unit, in rows (2 x 0.5 m spacing) during winter / spring of each year. Plants were watered at planting and in most experiments irrigation provided during the growing season via drip irrigation. Rows were divided into blocks and treatments, with each experimental unit being represented by at least 6 individual plants. The nutritional status of the soil did not necessitate the application of any fertilisers. Fields were kept weed free through manual cultivation or alleyways of polypropylene fibre (Mypex) were used to suppress weeds between the experimental rows.

Site A

Both *Crataegus monogyna* and *Quercus robur* were utilised with seed field-sown in spring of each year. Plants were grown at high density (c. 125 plants / m²), in 5-row beds. Experimental treatment blocks were allocated as 3 m sections of the beds. One metre buffer zones were marked between blocks to minimise effects due spray drift. Experimental treatments were imposed over 3-4 experimental blocks within the fields to reduce effects due to any localized conditions.

Site B

Each year one-year-old chip-budded specimens of *Malus domestica* ‘Bramley’, *Pyrus communis* ‘Conference’ and *Malus x moerlandsii* ‘Profusion Improved’ were used and

sections selected from rows of commercially produced trees. As before, sites were divided into blocks and treatments represented within each block, with usually a minimum of 10 reps per treatment / block. Soils were base-dressed with 55kg N: 22kg P: 94kg K ha⁻¹ with a later top-dressing of 105kg N: 12kg P: 4kg K ha⁻¹ each year.

Control of Pests and Pathogens

Both commercial growers used a programme of pesticides throughout the season in line with their normal annual regime and as such no significant outbreaks of pests or pathogens were noted at either site. Plants at UoR were sprayed periodically with 'Nimrod T' (bupirimate, triforine) and 'Systhane' (myclobutanil) to control powdery mildew (*Podosphaera clandestina*) and with 'Chess' (pymetrozine) to control aphids.

Glasshouse and polytunnel experiments

One-year-old *Crataegus monogyna* were grown under protection (usually in 2, 3 or 5 l pots) within a range of glasshouse environments and side-ventilated polytunnels (depending on requirements of individual experiments) at the University of Reading. Plants were grown in 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 4 g l⁻¹ and irrigated by drip lines.

Laboratory experiments

An *in vitro* experimental system was developed to understand more effectively how chemical manipulations of the leaf influenced the development of an abscission zone. Explants were removed from *Crataegus monogyna* plants comprising of a single leaf attached to approximately 4 cm of stem. These were inserted into 'honey' jars containing half strength Murashige and Skoog (M&S) tissue culture media, prepared from 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 l⁻¹ of nutrient solution before dispensing into 350 ml capacity jars. Either normal jars lids were used, or in some cases where the objective was to determine if ethylene gas was involved in the leaf abscission process, lids were modified to incorporate a gas tight septum which facilitated the removal of gas samples via a syringe. In the *in vitro* system chemicals could be applied to the entire leaf or sections of it. To mimic spray application in the field whole leaves would be dipped in solutions of defoliant and other chemicals. Alternatively chemicals could be applied discretely, via pipettes, or incorporating the active chemical in a lanolin paste (to improve retention and uptake of the chemical).

Assessments

Assessments focused on recording key parameters associated with tree development and abscission, but methodologies varied depending on the scale of the experiment. For example, in field experiments a sub-sample of shoots or trees may be selected for evaluation, whereas in glasshouse experiments the total number of leaves per tree would be recorded.

Key measurements were:

Defoliation

Percentage defoliation was calculated as (number of leaves abscised / total leaves on tree x100). Often the shoot was divided into three sections upper, middle and lower and the percentage defoliation recorded in each.

Leaf Injury

A scoring system was used to determine direct effects of chemicals on leaf status:

0	No damage
1	≤ 50% necrotic tissue
2	51 – 100% necrotic tissue
3	Abscised

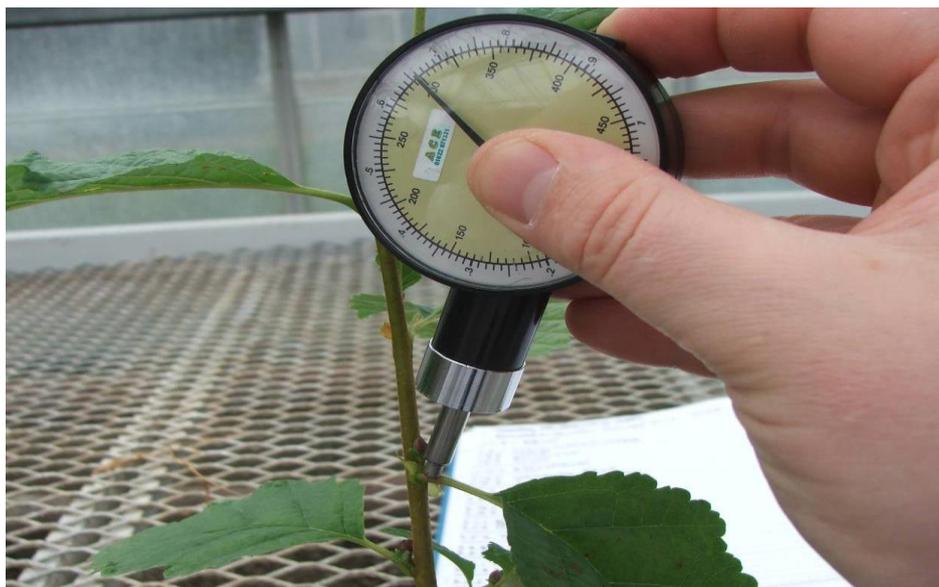
Detachment Force

Leaf abscission rates could be influenced by direct weather events e.g. wind speed and rain, as well as the physiological state of the abscission zone. A methodology was therefore developed to test the likelihood that a leaf was ready to abscise, by using a fruit penetrometer. The amount of force required to separate a leaf from the stem was recorded using the penetrometer to press down on the leaf petiole at the point of attachment (Figure 5). If the leaves were absent, a force of 0 was recorded.

Shoot activity

In an attempt to correlate the effectiveness of defoliant with the overall vigour of the plant at the time of treatment, a score was given based on the visible activity of the apical meristem. In controlled environment / glasshouse experiments plants were scored for vigour of apical growth between 0 (least) and 10 (greatest), or shoot extension rates recorded. In field experiments, bud activity was categorized as 0 = dormant, 1 = recent, unopened leaves beginning to senesce or change colour and 2 = vigorous production of new leaves still apparent.

Figure 5. Use of a penetrometer to record detachment force.



Bud burst and re-growth

The percentage of dormant buds that flushed the following spring and their growth rates were recorded in some experiments to determine if defoliation treatments had any effect on the longer-term viability of the trees.

Ethylene gas

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 were dipped in solutions of $\text{CuNa}_2\text{.EDTA}$, (the laboratory equivalent of the Copper EDTA product know as 'Leaf Fall') placed into agar gel within jars and the production of ethylene monitored over time. Ethylene was abstracted from the jars by inserting a 1 ml syringe into the jar through the lid septum, drawing a sample of head space gas to fill the barrel and flushing 5 times to mix gases. 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 0.53 mm 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 ($\pm 1^\circ\text{C}$) and monitored daily.

Chlorophyll content

Reductions in chlorophyll are associated with leaf senescence and stress responses, e.g. exposure to copper or ethylene. Where appropriate chlorophyll content was monitored using a Hansatech CL-01 chlorophyll content meter.

Dry weight, Nitrogen and Copper analyses

Tissue samples were dried at 70°C for 5 days. Dried plants were divided into root and shoot sections and ground separately in a large Retsch Müller hammer mill with a 2 mm sieve plate. Samples were then ground again in a smaller hammer mill using a 1 mm sieve plate. Plants selected from each treatment were bulked together in groups of three, giving three replicates composed of three plants each. Total nitrogen content as a percentage of sample weight was obtained using an automated micro-Dumas method (Ma and Rittner, 1979) using a Europa Roba Prep elemental analyser. In some experiments dried samples were also used to determine copper content via mass-spectrometry.

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. Two-way analyses of the same data to take into account the effects of treatment timing were carried out using the Scheirer-Ray-Hare extension of the Kruskal-Wallis test (Scheirer *et al.*, 1976) (using the 'R' software environment). This allowed comparisons between treatment effects (H test values 'H' - where higher values indicate greater significance between treatment effects and Probability 'P values'), but could not account for variance around specific mean values (i.e. figures do not have error bars). 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.

CHEMICAL DEFOLIANTS AND MODE OF ACTION

Experiments were set up within the project to identify potential chemical defoliants (technical data and current approval status for all products trialled in this project are detailed in Table 2). The experiments also investigated how results could be influenced by species, growth phase and seasonal factors.

Table 2. Approval status and technical data for compounds referenced within the project.

Product	Active ingredient	Use	Crop Use	Approval status on Ornamentals	Experiment
'Leaf Fall'	Copper EDTA Ethylene-diaminetetra-acetic acid copper (II) diammonium salt (Cu(NH ₄) ₂ .EDTA)	Fertiliser	Various	Fertiliser only, not approved as a plant protection product	1,2,3,4,5,6,7,11,12,13,15
Sigma-Alrich Copper EDTA (laboratory grade)	Ethylene-diaminetetra-acetic acid copper (II) disodium salt (CuNa ₂ .EDTA)	Fertiliser	n/a	Not approved	7,8
Jet-5	Peracetic acid (5%), hydrogen peroxide (20%), acetic acid (10%)	Disinfectant	Hard surfaces	Permissible for use as a disinfectant on hard surfaces only	2
Librel FE-LO	Iron.EDTA 13.2%	Fertiliser	Various	Fertiliser only, not approved as a plant protection product	2
Urea	Urea	Fertiliser	Various	Fertiliser only, not approved as a plant protection product	1,2
Copper sulphate (Sigma Alrich laboratory grade used but available commercially as Cuproxat FL)	Copper sulphate	Fungicide	Potatoes	Not approved	6,7
Cuprokyt FL	Copper oxychloride (270g/l)	Fungicide	Top fruit, tomatoes and a range of other horticultural crops	Permissible under LTAEU for disease control	1,9

Table 2. Approval status and technical data for compounds referenced within the project.

Product	Active ingredient	Use	Crop Use	Approval status on Ornamentals	Experiment
Folicur	Tebuconazole (250g/l)	Fungicide	Cereals, rape and a range of horticultural crops	Permissible under LTAEU for disease control	3
Nativo 75WG	Tebuconazole (500g/kg), trifloxistrobim (250g/kg)	Fungicide	Brassica crops, carrot and leek	Permissible under LTAEU for disease control	3
Cerone	2-chloroethyl-phosphonic acid (480g/l)	Growth regulator	Cereals, ornamentals, tree fruit and tomatoes	SOLA approval for growth control	3
Cultar	Paclobutrazol (250g/L)	Growth regulator	Ornamentals (outdoors)	Permissible under LTAEU	3
TIBA	2,3,5-Triiodobenzoic acid	Growth regulator	None	Not approved as a plant protection product	10,12
Activator 90	Alcohol ethoxylates (750 g/kg), natural fatty acids(150 g/kg)	Adjuvant	Cereals, fruit, vegetables	Permissible as a wetting agent	4,5
Silwet L-77	Polyalkylene-oxide modified heptamethyl-trisiloxane (80-85%)	Adjuvant	Cereals, fruit, vegetables	Permissible as a wetting agent	6
EDTA	Ethylene-diaminetetra-acetic acid disodium salt	n/a	n/a	Not approved	6,7

Experiment 1 (2007). Optimising the use of chemical products (timing and concentrations)

Three chemical treatments, Copper EDTA ('Leaf Fall'), 'Cuprokylt FL' (copper oxychloride) and Urea, were applied in a range of combinations (see Table 5 in Results).

Copper EDTA, which is available within UK as a foliar fertiliser (Protex Chemicals Ltd) and is known by some growers internationally as the product 'Leaf Fall' (not available within the UK), has been used to defoliate deciduous nursery crops outside of the UK in the past. The active ingredient $\text{Cu.NH}_4\text{EDTA}$ (abbreviated to Copper EDTA throughout) is a source of Cu^{2+} in solution (9% Cu), allowing effective leaf penetration. Although deemed to be a relatively effective defoliant it is apparent that the problem of retarded leaf senescence and abscission has become more acute since the initial trials with Copper EDTA were undertaken (Knight, 1983) and that some refinement may be required in light of climatic changes.

'Cuprokylt FL' (Universal Crop Protection Ltd - UNICROP) is another copper containing compound (copper oxychloride), widely used in a variety of agricultural and horticultural contexts as a protectant fungicide. This compound is routinely used as an end of season preventative treatment against apple and pear canker (*Nectrina gallegina*) and nurserymen have observed that it can provide initial mild stress to the leaf which in turn may aid in the formation of abscission zones, or help pre-dispose the leaf to subsequent defoliants. It is supplied as a suspension concentrate containing 270 g l^{-1} copper as copper oxychloride ($\text{Cu}_2\text{Cl}(\text{OH})_3$).

Urea (Laboratory grade urea, $(\text{NH}_2)_2\text{CO}$ - Fisher Scientific) application prior to rapid artificial defoliation has been cited as a way of mitigating the reduced nitrogen recovery in comparison to that recycled during natural leaf senescence and abscission (Guak *et al.*, 2001).

Tree species used were *Crataegus monogyna* (both 18 month old specimens at Reading and 6 month old trees at Site A), *Quercus robur*, *Malus domestica* 'Bramley', *M. x moerlandsii* 'Profusion Improved' and *Pyrus communis* 'Conference'. Sprays were applied using a Cooper Pegler CP 15 knapsack sprayer fitted with a fine nozzle suitable for fungicide application. Trees were sprayed to provide as much leaf coverage as possible with minimal run-off. Applications were made in dry weather conditions and low wind speeds. Block and rep numbers varied with site, but in each case there was a minimum of 10 plants per block and 3 blocks per experiment.

Experiment 2 (2008). Possible alternatives to Copper EDTA

Possible further alternatives to Copper EDTA 'Leaf Fall' 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 non-copper compounds that promoted similar tissue damage, and compare their performance with Copper EDTA 'Leaf Fall' prior to their possible inclusion in a full field-scale experiment. Particularly of interest is the iron-EDTA complex foliar fertiliser product 'Librel', as this will help to determine how important the metal ion component is within Copper EDTA 'Leaf Fall'.

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.

Experiment 3 (2008). Reducing plant vigour to improve the effectiveness of defoliants

Data from Experiment 1 suggested that the cessation of shoot extension and the formation of a dormant apical bud may be linked with increased potential for leaf abscission. Continuing with the hypothesis that reduced vigour in juvenile plants may be paramount in maximising the effects of defoliant, this experiment sought to investigate the effects of other plant growth regulators (PGRs) on subsequent application of defoliant such as Copper EDTA. The results from a small experiment conducted in early 2008, using the triazole fungicide 'Folicur' (tebuconazole) as a pre-treatment before Copper EDTA 'Leaf Fall' application, suggested that growth retardation (via inhibiting gibberellin synthesis) may be useful in improving the action of Copper EDTA. 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 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 2).

The action of Copper EDTA ‘Leaf Fall’ and other defoliant 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 Copper EDTA ‘Leaf Fall’ related solely as a result of enhanced ethylene production from leaf tissue damage. Two additional potential defoliant that were also associated with leaf injury were thus examined in comparison; urea and ‘Cerone’. Urea was selected as Experiment 2 suggested that an intermediate concentration of this chemical was worth further evaluation. ‘Cerone’ was included however, as it is an ethylene-evolving PGR and may be able to induce abscission through ethylene release.

A factorial experiment was set up with a range of growth retardants (or water) being applied to crops, followed by a range of potential defoliant (i.e. 16 treatment combinations, Table 3). Treatments were applied to *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 (defoliant). Sprays were applied to manufacturers’ instructions using a Cooper Pegler CP 15 knapsack sprayer fitted with a fine nozzle suitable for fungicide application. As before, a minimum of 3 blocks were used in each site with treatments being represented by >10 plants in each treatment within a block.

Table 3. 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		‘Leaf Fall’	20
Folicur	3.3		Urea	90
Nativo	0.8	X	Cerone	2.5
Water	n/a		Water	n/a

Plants from the Copper EDTA ‘Leaf Fall’ treatments were assessed for re-growth potential the following spring (Table 4). Field-grown trees were lifted, bundled into plastic sacks and

cold-stored at 1°C at University of Reading from 21st November 2008 until 27th February 2009. Upon removal they 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 4. Treatments selected for examination of re-growth.

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

Experiment 4 (2008). Improving the action of Copper EDTA using a spray adjuvant

This experiment aimed to optimise the performance of Copper EDTA 'Leaf Fall', but minimise potential costs of application. Results from Experiment 1 field evaluations were encouraging in that they suggested that a half-strength application of Copper EDTA 'Leaf Fall' produced comparable results to the full dose; although a quarter strength application appeared less effective in Experiment 2. Anecdotal evidence indicates that responses to Copper EDTA 'Leaf Fall' can be inconsistent between years, and it was decided to investigate mechanisms that may improve uptake and retention of the chemical. Therefore, this experiment using *M. 'Bramley'* (Site B) aimed to examine the effects of adding a wetting agent ('Activator 90', [900 g/l alkyphenyl hydroxypolyoxyethylene and natural fatty acids], De Sangosse Ltd, Swaffham, UK) to determine if it improved efficacy of Copper EDTA 'Leaf Fall'. In addition, this provided an opportunity to re-test the quarter strength application. Trees were sprayed to the point of run-off with the following treatments on the 9th October 2008 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

Experiment 5 (2009). Can 'Activator 90' improve the performance of Copper EDTA on other cultivars?

The aim of this experiment was to determine if any perceived advantage of using Copper EDTA 'Leaf Fall' in combination with the wetting agent 'Activator 90' to aid leaf abscission was evident in species other than *M. 'Bramley'*. Field-grown trees of *M. 'Bramley'*, *M. 'Profusion Improved'* and *P. 'Conference'* were sprayed with water, (Control), Copper EDTA 'Leaf Fall' (20 ml l⁻¹) or Copper EDTA 'Leaf Fall' (20 ml l⁻¹) plus 'Activator 90' (10 ml l⁻¹) on 14 Oct. Trees were divided into 3 experimental blocks, with 10 trees of each species being treated with each chemical within a block. Plants were tested for defoliation potential using the penetrometer on 21 October.

Experiment 6 (2009). To determine if the leaf wetter 'Silwet' has defoliation properties

Although the wetter 'Activator 90' showed no advantage in inducing abscission on its own (it only enhanced the effect of Copper EDTA 'Leaf Fall'), there are anecdotal reports of another wetter 'Silwet' (Silwet L-77, [polyalkyleneoxide], De Sangosse Ltd, Swaffham, UK) having abscission-inducing properties. This compound is a non-ionic organosilicone surfactant and has been considered to have cellulase inducing properties (Reese and Maguire, 1969; Castanon and Wilke, 2004), i.e. could help in the breakdown of cells in the leaf abscission zone. A small experiment was set up to determine if 'Silwet' had defoliant properties and could be used as an alternative product to Copper EDTA 'Leaf Fall'.

Crataegus monogyna were grown in a heated glasshouse (min temp 14°C) and treated with 'Silwet', Copper EDTA 'Leaf Fall' and a laboratory formulation of Copper EDTA (Cu.Na₂EDTA). 'Silwet' was applied at a dilution of 0.1% in distilled water; the two copper compounds were applied at 20 ml l⁻¹ and 60 mM respectively. All plants were sprayed to run-off on 28th January 2010, following approximately six months active growth. All shoot apical meristems were inactive during the experiment.

Experiment 7 (2009). How important are the copper and EDTA components of the Copper EDTA molecule?

Results from previous experiments suggest that Copper EDTA 'Leaf Fall' has been the most consistent product in terms of eliciting an abscission response, albeit overall effectiveness can depend on species, timing etc. Evidence from the literature suggests that copper is a key component in aiding abscission (possibly by stimulating a wound response and releasing ethylene, e.g. Luna *et al.*, 1994, or even directly acting on auxin movement, e.g. Ben-Yehoshua and Biggs, 1970). This was reinforced when we used the equivalent FeEDTA salt but this proved less effective compared to CuEDTA. Some of the previous experiments, however, also trialled compounds (e.g. 'Cuprolyt') that contained copper, but these were relatively ineffective compared to Copper EDTA 'Leaf Fall'. Therefore, Experiment 7 was devised to try and determine the relative merits of both the copper and EDTA component parts of the Copper EDTA 'Leaf Fall' product, by applying the component parts separately to leaf tissue.

The *in vitro* system was used to evaluate which chemical compounds were influencing the formation of an abscission zone. Leaves and stem sections of *Crataegus* were placed into agar within jars and drops of chemical applied via lanolin (0.03 ml) to either the basal part of the leaf blade (just above the abscission zone - BLADE) or on the petiole itself on the abscission zone (AZ). Chemicals applied were 30 mM CuNa₂EDTA, 30 mM CuSO₄, 30 mM EDTA, distilled water or lanolin used without additional chemicals (Control). (All chemicals supplied by Sigma-Aldrich Co. Ltd. Dorsert, UK). Jars were maintained at 23°C from 21 September to 4 October 2009, before leaves were determined for detachment force. Each treatment was represented by 10 leaves, randomly distributed within a growth cabinet.

ETHYLENE

Experiment 8 (2009). Investigating the relationship between copper dose on subsequent ethylene evolution in *Crataegus monogyna* explants

Ethylene has been closely associated with the formation of abscission zones (See Lit Review) and it has been considered that copper (Cu²⁺) may act by injuring leaf cells and inducing ethylene formation (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 CuNa₂.EDTA of varying concentrations and the

production of ethylene gas was monitored *in vitro*. Leaf chlorophyll content and leaf detachment force were also recorded. Leaves were exposed to the following treatments:

- A. 60 mM (2.4 g/100 ml) solution of copper disodium EDTA (CuNa₂.EDTA)
- B. 30 mM (1.2 g/100 ml) solution of copper disodium EDTA (CuNa₂.EDTA)
- C. 15 mM (0.6 g/100 ml) solution of copper disodium EDTA (CuNa₂.EDTA)
- D. 7.5 mM (0.3 g/100 ml) solution of copper disodium EDTA (CuNa₂.EDTA)
- E. Distilled water (Control)

Background ethylene control:

- F. empty jar + agar (no leaf)

At the end of the experiment explants were removed, weighed, chlorophyll content measured and detachment force measured.

GROWTH PHASE AND DORMANCY INDUCTION

Experiment 9 (2009). Dormancy induction with 'Cuprokyt' (copper oxychloride) [Cu₂Cl(OH)₃] and effect on defoliation

Results from Experiment 3 had suggested that slowing growth in the autumn may enhance subsequent defoliation in *M. 'Bramley'*, but there was actually little evidence of dormant bud formation occurring after treatments with growth retardants. This experiment was designed to explore the relationship with shoot bud activity and leaf abscission. It returned to the use of 'Cuprokyt' (Copper oxychloride) which had been used in Experiment 1 as a pre-treatment prior to the use of Copper EDTA 'Leaf Fall', the primary defoliant. Discussions with growers regarding this product at the highest permissible doses suggested it may encourage the formation of woody stem tissue. Scientific literature also alludes to a. the ability of copper ions to inactivate auxin (Ben-Yehoshua and Biggs, 1970) and b. the role of reduced auxin in wood formation (Nilsson *et al.*, 2008). The following experiment sought to investigate whether 'Cuprokyt' could be used to induce early dormancy, thus enhance natural leaf abscission. Trees at Site A were utilized to compare shoot activity and defoliation potential by cutting back (to 5 cm from base) some of the trees on 10 July 2009 to induce new growth from the base and essentially delaying the onset of natural dormancy (Re-Grown). Comparisons were made with non-cut trees (Control) and to half of each population 'Cuprokyt' was applied at 5 ml l⁻¹ concentration on 9th September, 18th September and 24th September 2009. Trees were assessed for growth, apical meristem activity and defoliation on 4th November.

There were 3 experiment blocks of *Crataegus monogyna*, with 10 trees of each treatment combination in each block.

DORMANCY STATUS AND LOW TEMPERATURE EXPOSURE

Progressively lower night temperatures are widely accepted as a trigger for woody species to enter endodormancy (Arora *et al.*, 2003). Results from Experiments 1 and 9 suggested that lower plant vigour and reduced rates of apical meristem (AM) activity may aid defoliation in autumn.

Experiment 10 (2008). Temperature and the disruption to polar auxin (IAA) transport from the shoot tip on leaf senescence and abscission in *Crataegus monogyna*

In an attempt to build on the theories explored in Experiments 1 and 9, 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 if inhibiting polar auxin transport (PAT) from the apex, using the antagonist 2,3,5-triiodobenzoic acid (TIBA, Sigma-Aldrich Co. Ltd. Dorset, UK) would induce earlier dormancy and leaf senescence.

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. The aim of this study was therefore to quantify how temperature and disruption to polar auxin transport influenced defoliation.

Crataegus monogyna were grown in 2 litre pots under heated glass (15-25°C) prior to being 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 was 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.

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. TIBA 10 mM applied 1.5 cm below apical meristems in lanolin
2. TIBA 20 mM applied 1.5 cm below apical meristems in lanolin
3. Lanolin applied under apical meristems
4. Control.

Experiment 11 (2009). How does temperature during the day influence defoliation?

Low night temperatures appeared beneficial in encouraging defoliation, with sub-zero (freezing) temperatures being particularly useful compared to those above 0°C (chilling). This experiment wished to explore further how increasing the amount of chilling, by keeping day temperature low might also affect abscission. A range of temperature regimes were set up using the controlled cabinets to represent weather scenarios that might be encountered during late summer / autumn. These were:

- | | |
|--------------------------------|-----------------------|
| A. DAY CHILL / NIGHT CHILL | 10°C day / 10°C night |
| B. DAY WARM / NIGHT CHILL | 20°C day / 10°C night |
| C. DAY VERY WARM / NIGHT CHILL | 28°C day / 10°C night |

Crataegus monogyna in 2 litre pots and previously grown in a glasshouse at 15-25°C were placed into Fisons 600 cabinets as before on 9th October 2009. Copper EDTA 'Leaf Fall' (20 ml l⁻¹) was sprayed onto the relevant trees three days later to run-off as in previous experiments and the plants returned to the cabinets. Copper EDTA 'Leaf Fall' was applied to a second group of plants again on 3rd November. After returning these plants to the cabinets, trees were maintained in their respective temperature regimes until 2nd December and monitored for ease of leaf detachment.

A 3 x 3 factorial design was utilised, with treatments randomised within the growth cabinets. Initially 10 replicates were selected for each treatment, however only 7 replicates in each of the 'November Copper EDTA 'Leaf Fall' and 'Control' plants were used in each cabinet for analysis due to mildew infection on some plants.

Experiment 12 (2009). Controlling shoot activity in the field via TIBA to induce earlier dormancy and defoliation

The auxin blocker, TIBA had proven useful in controlled environment experiments in inducing earlier dormancy and aiding defoliation processes. This experiment was designed to determine if such results could be replicated at the field scale using Site A.

Field-grown seedling *C. monogyna* were treated with Lanolin paste (Sigma Aldrich, Dorest, UK) or TIBA (incorporated in Lanolin, 20 mM) on 9 September 2009, with the paste being attached as a ring around the stem 20-30 mm below the apical bud. Some trees were left untreated (Controls). Half the trees in each treatment were sprayed with 'Leaf Fall' at 20 ml l⁻¹ on 13th October. Plants were monitored for dormant bud formation and defoliation from August onwards and assessed on 3 November 2009. Each treatment combination was represented by 30 plants divided into 3 positional blocks.

EFFECTS OF WATER STRESS AND WATERLOGGING

Developing practical, non-chemical defoliation techniques remains challenging at a field scale, due largely to costs or the infrastructure 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.

Experiment 13 (2008). Assessing the effects of controlled drought on leaf abscission

To investigate the effects of water availability during autumn on leaf abscission of young trees, container-grown (2 l) *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.

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 (1 g = 1 ml). In this 2 x 3 experimental design, 2 randomised complete blocks were used in each location (glasshouse or polytunnel) with a total of 8 replicates per treatment.

Experiment 14 (2009). Assessing the effects of controlled drought on leaf abscission II

Data from the polytunnel component of Experiment 13 was quite variable (see Results section), so a similar experiment was conducted in 2009. Young *C. monogyna* plants in 2 l pots that had been growing for approximately 5 months, and had recently finished a period of strong 'flushing' extension growth were selected for this experiment. On 2nd November the plants were labelled by treatment and arranged in one randomised block in a heated glasshouse (min. temperature 14°C). Trees were brought to container capacity then three irrigation treatments imposed on a 20:5:1 ratio, i.e. Well-watered Controls = 100 ml; Mild Drought = 25 ml and Severe Drought = 5 ml of water per pot every 3 days. Moisture content in the pots was monitored via a soil moisture probe and the actual volumes could be altered depending on the prevalent weather conditions to avoid treatments getting too wet or dry, e.g. under cool conditions with little evapo-transpiration taking place 20, 5 and 1 ml could be substituted for the three differential treatments, respectively. The control plants were free draining, but saucers were placed under the drought treatments to ensure that any run-through irrigation was absorbed back into the base of the media. Each treatment was represented by 7 replicate plants randomly distributed within the experimental design.

Experiment 15 (2009). Plant responses to drought and waterlogging

On 15 June 2009, seedling plants were graded and 80 of similar size and habit were selected. Plants were transferred to 10 litre pots containing John Innes no. 2 compost. Plants were moved to their final positions in an open-sided polytunnel at this time and placed on inverted saucers to prevent rooting into the polytunnel floor. Each 10 l pot was irrigated using push-in drippers placed at the base of the plant stem. Drippers were connected to 2 l h⁻¹, pressure compensated drippers (Netafim, USA) via 'spaghetti' irrigation tube. Differing irrigation regimes were carried out using two main 16 mm LDPE pipes, connected to two outlets of a manifold. Outlet valve solenoids were controlled using a Galcon 4 irrigation timer.

On 15th September, stress treatments commenced. Control plants and those to receive chemical stress received four 1 minute irrigation applications at 4-hourly intervals between 0800 and 2000 (approximately 132 ml in total). Plants subjected to the mild drought treatment received water for 1 minute at 0800 only (approximately 33 ml). Those plants subjected to waterlogging were immersed in 10 l plastic buckets filled with water. The water level was maintained above the surface of the potting medium for the duration of the treatment period (6 weeks - 15th Sep to 27th Oct.) with manual watering.

After 1 week, media moisture content measurements suggested the irrigation schedule was too generous and thus applications were reduced to Monday, Wednesday, Friday and Sunday only. This change was made to both the both drought and non-drought treatments. Drought treatments were imposed for 6 weeks. Mild chemical stress consisted of two, weekly applications of 15 mM Cu.EDTA (applied as Copper EDTA 'Leaf Fall' diluted to a concentration of 5 ml l⁻¹). Plants were sprayed to run-off on 21st September and 29th September 2009 [LF-Sep]. The defoliant treatment consisted of one 60 mM application of Cu.EDTA (as 'Leaf Fall' 20 ml l⁻¹). Plants were sprayed to run-off as above on 22nd October [LF-Oct].

Treatments were based on a 4 x 2 factorial design with two randomised complete blocks with ten replicates of each stress / defoliant treatment combination in each:

<u>Stress</u>		<u>Defoliant</u>
A. Drought		
B. Waterlogged		1. 60 mM Leaf Fall [LF-Oct]
C. 2 x 15 mM Leaf Fall spray [LF-Sep]	X	2. Control (water)
D. Control		

RESULTS

CHEMICAL DEFOLIANTS AND MODE OF ACTION

Experiment 1 (2007). Optimising the use of chemical products (timing and concentrations)

Results showed that most defoliation was associated with the application of Copper EDTA 'Leaf Fall' in September. The exception was with *Quercus robur*, which showed very little defoliation throughout (data not shown). In some situations the effect of Copper EDTA 'Leaf Fall' was enhanced by the application of Urea or 'Cuprolyt', but these applied in isolation were not particularly effective compounds. Table 5 provides a summary of species responses and full details of the results are provided in Report 1. Data from *Crataegus* suggested that the half concentration (10 ml l⁻¹) was as or possibly more effective than the full concentration (20 ml l⁻¹) and this aspect was investigated in subsequent experiments. There was also a correlation between apical meristem activity and the ability of the trees to abscise their leaves, i.e. leaf abscission was enhanced when the trees had stopped growth and the apical bud had become dormant. Note also the variation in defoliation for *Crataegus* between Reading and Site A, highlighting how factors such as age and geographical location (climate) can influence the response.

Conclusions

- **Copper EDTA 'Leaf Fall' was the most effective compound tested.**
- **A reduction in the rate of Copper EDTA 'Leaf Fall' from 20 to 10 ml l⁻¹ was equally effective in promoting defoliation in some cultivars.**
- **Shoot stem activity may provide guidance to the extent to which trees are able to abscise their leaves.**

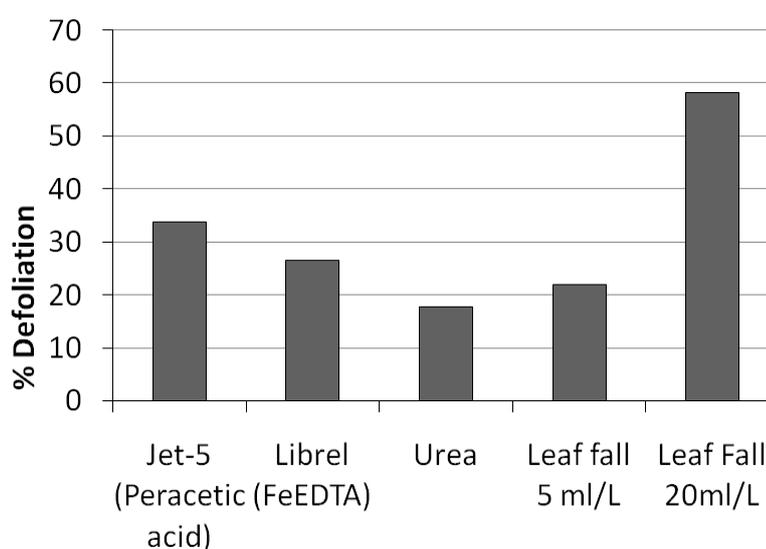
Table 5. Defoliation (%) as affected by chemical defoliant Copper EDTA ‘Leaf Fall’, ‘Cuprokylt’ and Urea applied at different combinations and times to *Crataegus monogyna* (at Reading and Site A), *Malus* ‘Bramley’, *Malus* ‘Profusion Improved’ and *Pyrus* ‘Conference’. Defoliation rates > 40% highlighted.

Treatment	<i>C. monogyna</i> Reading	<i>C. monogyna</i> A	<i>M.</i> ‘Bramley’	<i>M.</i> ‘Profusion Improved	<i>P.</i> ‘Conference’
Control	14	7	1	26	4
Water	13	10			
Leaf Fall 20 ml l ⁻¹ August	40	14			
Leaf Fall 20 ml l ⁻¹ September	39	46	57	97	37
Leaf Fall 20 ml l ⁻¹ October	39	41			
Leaf Fall 10 ml l ⁻¹ August	47	17			
Leaf Fall 10 ml l ⁻¹ September	54	62			
Leaf Fall 10 ml l ⁻¹ October	21	38			
Cuprokylt + Leaf Fall 20 ml l ⁻¹ August	42	16			
Cuprokylt + Leaf Fall 20 ml l ⁻¹ September	48	63	50	97	33
Cuprokylt + Leaf Fall 20 ml l ⁻¹ October	49	30			
Cuprokylt September	4	13	2	37	5
Urea + Leaf Fall 20 ml l ⁻¹ August	43	28			
Urea + Leaf Fall 20 ml l ⁻¹ September	47	57			
Urea + Leaf Fall 20 ml l ⁻¹ October	49	24			
Urea September	8	10			
Cuprokylt + Urea + Leaf Fall 20 ml l ⁻¹ August	52	29			
Cuprokylt + Urea + Leaf Fall 20 ml l ⁻¹ September	58	45	70	96	41
Cuprokylt + Urea + Leaf Fall 20 ml l ⁻¹ October	54	38			
Cuprokylt + Urea September	4	9	0	40	3

Experiment 2 (2008). Possible alternatives to Copper EDTA

Copper EDTA 'Leaf Fall' again induced abscission to a greater extent than other treatments (Figure 6). Transformed values showed the Copper EDTA 'Leaf Fall' to have significantly higher abscission rates than other treatments (data not shown). 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 is a physiological reaction and some form of intermediate concentration may prove useful. 'Jet-5' also demonstrated some promise, but again the concentration used was high, and unlikely to meet commercial criteria on cost or environmental legislation.

Figure 6. *Alnus glutinosa* (University of Reading). Defoliation (%) recorded 22 days after application of a lower rate of Copper EDTA 'Leaf Fall'. Mean values shown, n=10.



Experiment 3 (2008). Reducing plant vigour to improve the effectiveness of defoliants

The influence of growth retardants was only evident with *M.* 'Bramley' where the rate of stem elongation in *M.* 'Bramley' was significantly reduced in all three retardants compared to the control (water; Table 6). 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). The growth reduction in *M.* 'Bramley' aided defoliation after the application of Copper EDTA 'Leaf Fall', with 'Nativo' being the most effective combination (Table 6). Analysis of whole-tree

defoliation for Copper EDTA '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 ($H=7.01$, $P=0.008$). Breakdown of leaf abscission based on location, showed that the youngest leaves in the uppermost sections were usually the most difficult to remove in *M. Bramley* (Table 6) and indeed in most other species. The exception was *Pyrus* 'Conference', however, where it was often the older more mature leaves, lower on the stem that were most resilient (data not shown).

Copper EDTA 'Leaf Fall' was again the best defoliant for other species tested, but no significant growth retardation (and hence additional benefit) was observed following the application of potential retardants to *C. monogyna* (both sites) or *A. glutinosa* (data not shown).

Table 6. *Malus* 'Bramley' (Site B). Defoliation after treatment with Copper EDTA 'Leaf Fall' and 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				Stem growth
	Upper %	Middle %	Lower %	Whole %	16 th September – 9 th October (cm)
Cultar	25	41	29	32	2.2
Folicur	22	39	31	31	3.4
Nativo	32	73	59	55	2.1
Water	7	25	18	17	4.9
Overall mean for 'Leaf Fall'	21	44	34	33	3.1

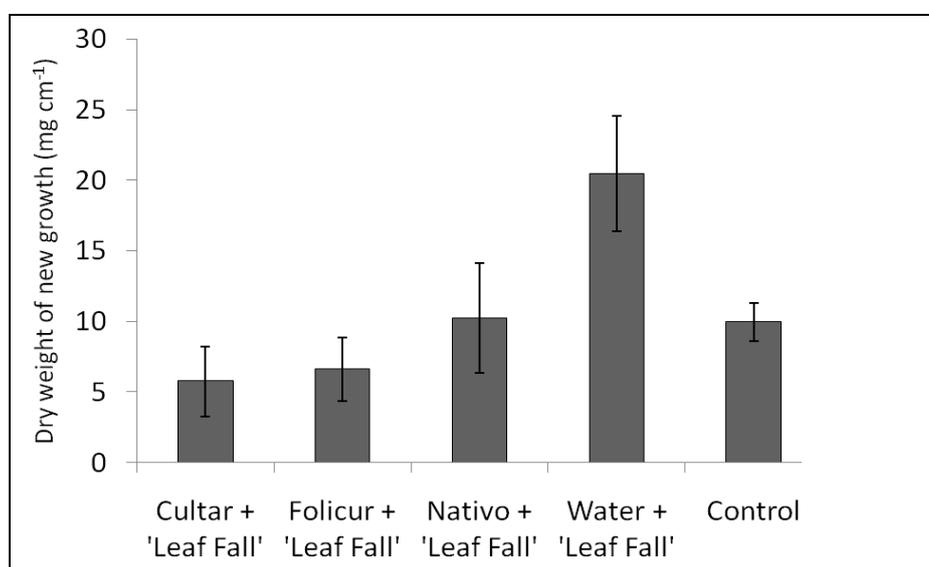
Spring Re-growth: Spring re-growth was reduced in plants treated with the triazole compounds prior to Copper EDTA 'Leaf Fall' application the previous autumn, compared to plants just treated with Copper EDTA 'Leaf Fall' alone (Figure 7). When the data was transformed, differences were shown to be statistically significant (transformed data not

shown). Similarly, treating plants with just Copper EDTA '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 (Figure 7).

Conclusions

- Growth retardants had no effect on *C. monogyna* or *A. glutinosa*, but did enhance leaf removal in *M. 'Bramley'* when plants were subsequently sprayed with Copper EDTA 'Leaf Fall'.
- Growth retardants slowed growth of *M. 'Bramley'*, but did not necessarily induce early terminal bud dormancy.
- Growth retardants appeared to affect dry matter accumulation in the spring, and potentially new season growth could be negatively affected, by autumnal applications.

Figure 7. *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)



Experiment 4 (2008). Improving the action of Copper EDTA using a spray adjuvant

Applying Copper EDTA '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 7). The

use of the wetting agent significantly increased mean defoliation. The Kruskal-Wallis non-parametric ANOVA used in these results indicates significant differences in whole-tree defoliation using Copper EDTA '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).

Conclusions

- A ¼ strength application of Copper EDTA 'Leaf Fall' on the 'difficult to defoliate' M. 'Bramley' had little impact on defoliation.
- 'Activator 90' appears to provide an added benefit when used in conjunction with Copper EDTA 'Leaf Fall' in aiding leaf abscission on M. 'Bramley' – a 'difficult to defoliate' cultivar.

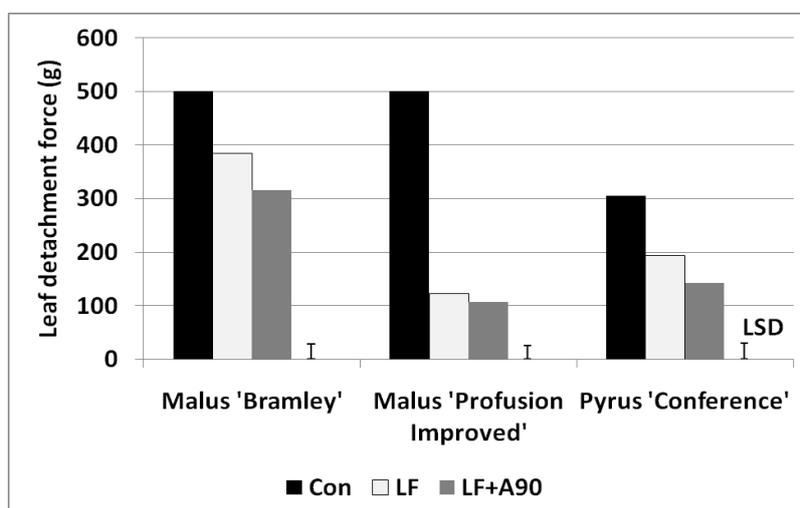
Table 7. *Malus* 'Bramley' (Site B). Defoliation (%) recorded on 31st October 2008 after spraying with full (20 ml l⁻¹) and quarter (5 ml l⁻¹) concentrations of Copper EDTA 'Leaf Fall' +/- 'Activator 90' wetting agent (0.1%) on 9th October 2008.

		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

Experiment 5 (2009). Can 'Activator 90' improve the performance of Copper EDTA on other cultivars?

Malus 'Profusion Improved' readily responded to Copper EDTA 'Leaf Fall' with leaves showing little resistance to the abscission pressure, and there was no added advantage to adding 'Activator 90'. In contrast, the 'Activator 90' provided additional benefits to leaf abscission in both *M.* 'Bramley' (as before) and *Pyrus* 'Conference', with significantly less pressure required to encourage leaf drop than applying Copper EDTA 'Leaf Fall' alone (Figure 8).

Figure 8. Leaf detachment force required to remove leaves on 21 Oct from *M.* 'Bramley', *M.* 'Profusion Improved' and *P.* 'Conference' (Site B) after applications of Copper EDTA 'Leaf Fall' and Copper EDTA 'Leaf Fall' + 'Activator 90' on 14 Oct.



Conclusions

- Adding the wetter 'Activator 90' improves the performance of Copper EDTA 'Leaf Fall' in two out of three cultivars tested – *M.* 'Bramley' and *P.* 'Conference'.

Experiment 6 (2009). To determine if the leaf wetter 'Silwet' has defoliation properties

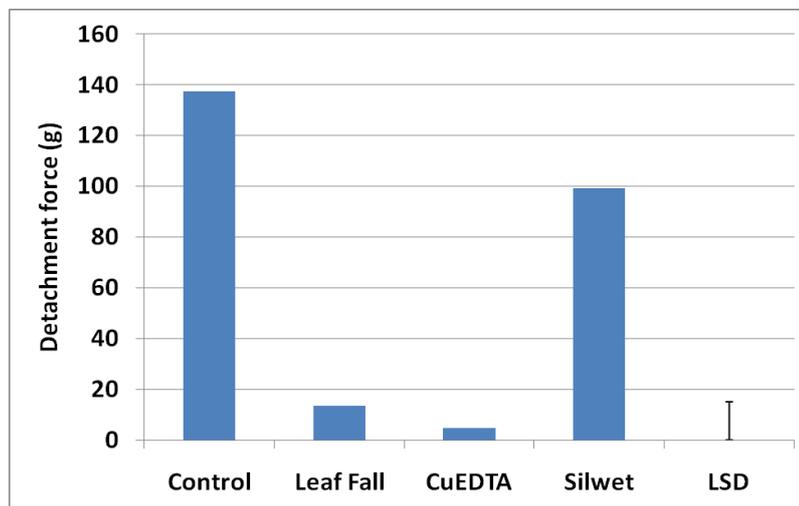
Experiments using a penetrometer suggested that 'Silwet' did significantly improve defoliation over controls, but was not as effective as both the Copper EDTA 'Leaf Fall' and

the laboratory synthesised Copper EDTA (Figure 9). Despite there being a reduction in detachment force with 'Silwet', no natural (i.e. non-forced) abscission was observed in this treatment, unlike the Copper EDTA 'Leaf Fall' and laboratory synthesised Copper EDTA (data not shown). Also 'Silwet' was only used in this experiment at the recommended rate as a spray additive (0.1%) and higher rates of this chemical alone may be worth investigating in future research. In addition, as 0.1% 'Silwet' significantly decreased abscission zone break-strength, the manual handling that occurs in commercial operations during harvest may be enough to dislodge many of the leaves (not tested).

Conclusions

- 'Silwet' demonstrated some defoliant potential, but was less effective than Copper EDTA 'Leaf Fall' in actual defoliation.
- Future research may wish to evaluate this product more thoroughly, especially in a commercial setting (e.g. by varying rates of application).

Figure 9. Leaf detachment force required to remove leaves on 12th February from *C. monogyna* after applications of 'Silwet', Copper EDTA 'Leaf Fall' and the 'laboratory Copper EDTA' on 28th January 2010.



Experiment 7 (2009). How important are the copper and EDTA components of the Copper EDTA molecule?

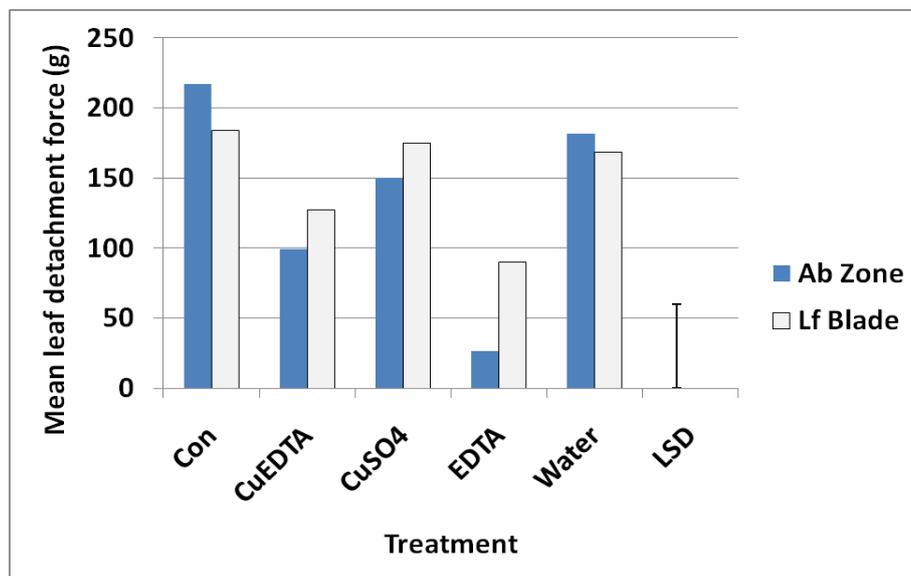
Defoliation was most readily induced in the EDTA treatment, especially when directly applied to the abscission zone (Figure 10). Although not significantly better than the CuEDTA

treatment, the EDTA did appear more effective than CuSO_4 and the control treatments. Although, to some extent responses do depend on relatively concentrations of the compounds applied, (e.g. we may have used a sub-optimal concentration of CuSO_4) there is evidence that EDTA is having a significant factor on the effectiveness of Copper EDTA 'Leaf Fall', i.e. copper may not be the sole 'active ingredient' in eliciting abscission of the leaf.

Conclusions

- **EDTA may be a key component in the effectiveness of Copper EDTA 'Leaf Fall' as it itself appears to have a role in the formation of an abscission zone.**

Figure 10. Leaf detachment force required to remove leaves of *Crataegus* held within honey jars and exposed to Copper EDTA, CuSO_4 , EDTA or water applied to the leaf blade (BLADE) or petiole (AZ) of individual leaves. Control plants were not treated with any chemicals.



ETHYLENE

Experiment 8 (2009). Investigating the relationship between copper dose on subsequent ethylene evolution in *Crataegus monogyna* explants

Spraying increasing concentrations of Copper EDTA onto leaves resulted in corresponding increases in leaf tissue copper (Figure 11). Ethylene evolution increased in all treatments

during the first 72 hours (Figure 12). Values peaked with the 7.5 mM treatment, but values were not significantly greater than other concentrations owing to the variability of the data.

The mean chlorophyll content increased in all treatments over the duration of the experiment (Table 8). 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 Copper EDTA gave rise to the highest mean leaf detachment force.

Figure 11. *C. monogyna* leaf tissue copper concentration following application of three concentrations of Copper EDTA

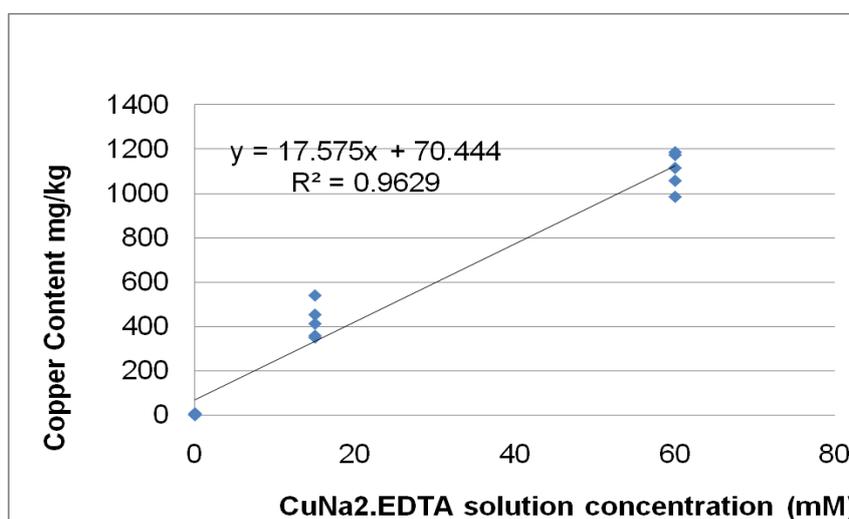


Figure 12. Headspace ethylene at 72 hours detected above single leaf explants of *C. monogyna* after treatment with varying concentration solutions of Copper EDTA (n=5).

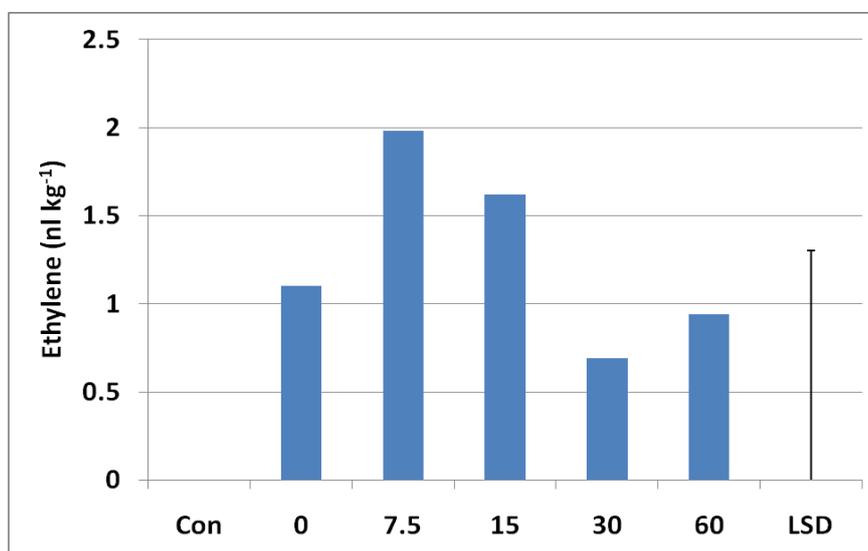


Table 8. Increase in chlorophyll content and leaf detachment force recorded in explants of *C. monogyna* at the end of the experiment after treatment with varying concentrations of Copper EDTA for 6 days (n=5, means shown \pm s.e.).

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

Conclusions

- Results were variable regarding direct correlations between ethylene release and ease of abscission.
- Nil and Low (7.5 mM) applications of copper were associated with greater abscission potential, but ethylene was only enhanced with the low treatment, and even then not significantly more so than other concentrations.

GROWTH PHASE AND DORMANCY INDUCTION

Experiment 9 (2009). Dormancy induction with ‘Cuprokyt’ (copper oxychloride [Cu₂Cl(OH)₃]) and effect on defoliation

Growth and shoot activity was extended into the season, by cutting trees back in July (Figures 13 and 14). In those trees cut and which re-growth was vigorous, ‘Cuprokyt’ reduced growth rate (Figure 13). Dormant bud formation, however, was marginally non-significant between the Re-Grown and Re-Grown + ‘Cuprokyt’ (Figure 14). At the time of harvest though, ‘Cuprokyt’ had no effect on defoliation of the Re-Grown trees and only a marginal effect on Controls (16% - Figure 15).

Conclusions

- ‘Cuprokylt’ (copper oxychloride) reduced growth rate on re-grown shoots, and had lower bud activity (not significant) to non-treated plants.
- ‘Cuprokylt’ (copper oxychloride) did not significantly enhance defoliation.

Figure 13. Mean apical shoot growth in *Crataegus monogyna* as affected by cutting back and re-growth (Re-Grown) and ‘Cuprokylt’ - $\text{Cu}_2\text{Cl}(\text{OH})_3$ application.

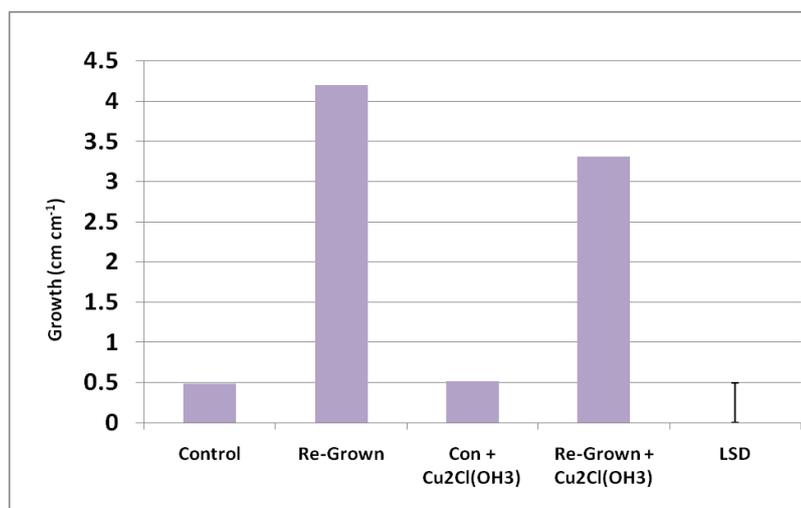


Figure 14. Shoot activity (% of trees with active apical bud) in *Crataegus monogyna* as affected by cutting back and re-growth (Re-Grown) and ‘Cuprokylt’ - $\text{Cu}_2\text{Cl}(\text{OH})_3$ application.

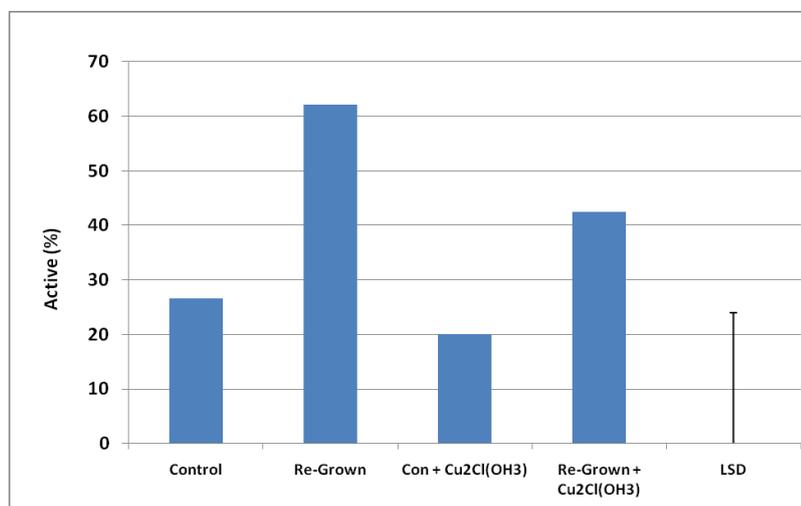
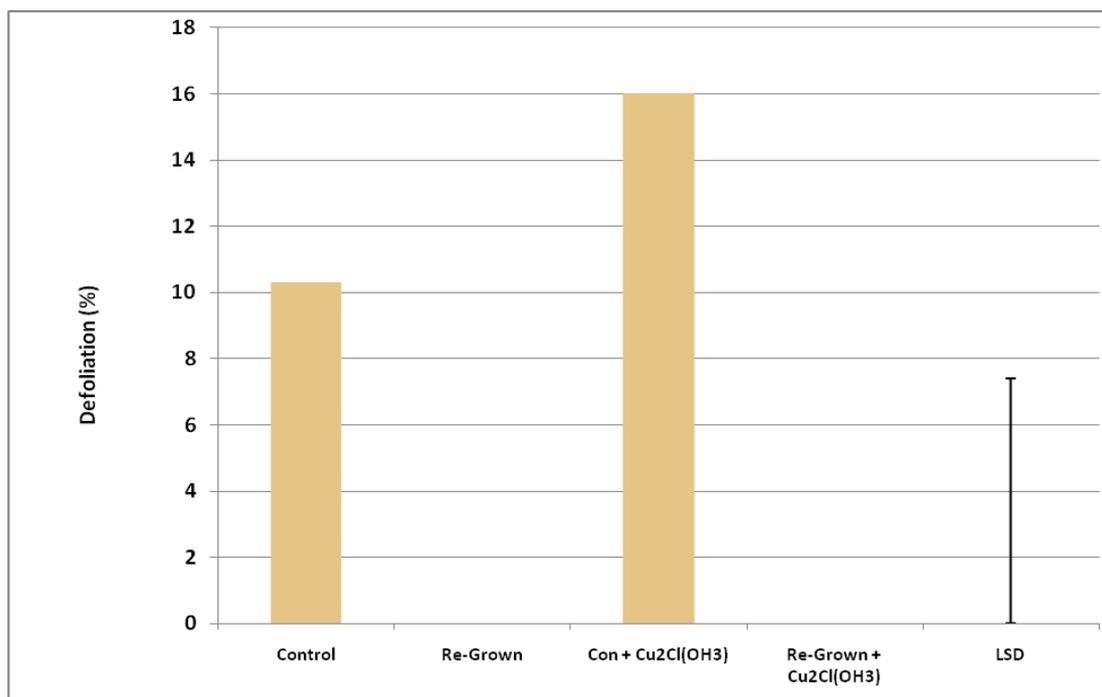


Figure 15. Defoliation (% of leaves) in *Crataegus monogyna* as affected by cutting back and re-growth (Re-Grown) and 'Cuprokylt' - $\text{Cu}_2\text{Cl}(\text{OH})_3$ application.



DORMANCY STATUS AND LOW TEMPERATURE EXPOSURE

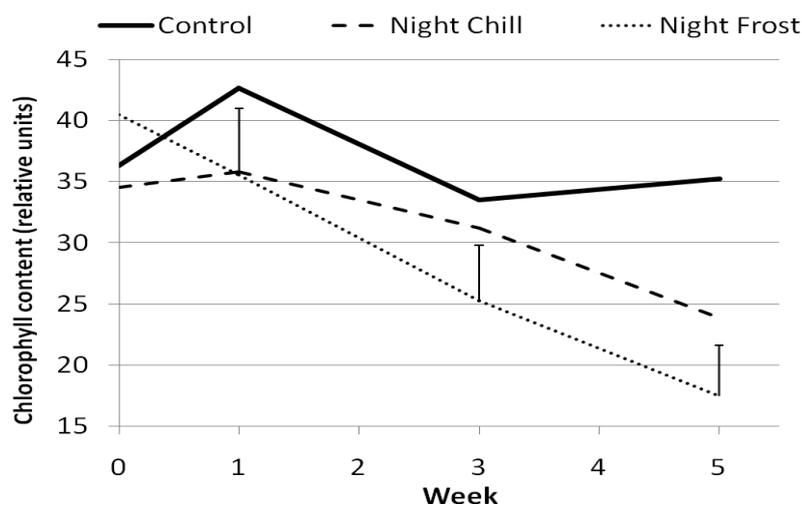
Experiment 10 (2008). Temperature and the disruption to polar auxin (IAA) transport from the shoot tip on leaf senescence and abscission in *Crataegus monogyna*

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 (Figure 16). 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.

After 5 weeks under controlled conditions, plants subjected to night temperatures of -2°C (Night Frost) and TIBA tended to abscise more leaves than other treatments (Table 9 and Figure 17). Plants treated with TIBA 20 mM in the night frost regime had lost significantly 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 20 mM 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 ($P<0.001$).

Figure 16. *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 ($n=12$).



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 (e.g. 42 g force compared to >95 g in the control treatments including the night frost without TIBA Table 9). (Direct comparison with the same chemical treatment under night chilling revealed significant differences at the $P=0.008$ level).

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 ($P<0.05$).

Conclusions

- **TIBA in combination with night frost enhances defoliation.**

- Reducing the flow of auxin from the shoot apex, combined with a stress factors such as freezing night temperatures appears to encourage leaf colouring and abscission potential.

Figure 17. *Crataegus monogyna* (Reading – Controlled Environment). Examples of defoliation achieved in the three temperature environments after applying TIBA (20 mM) rings to the stem below the shoot apex.

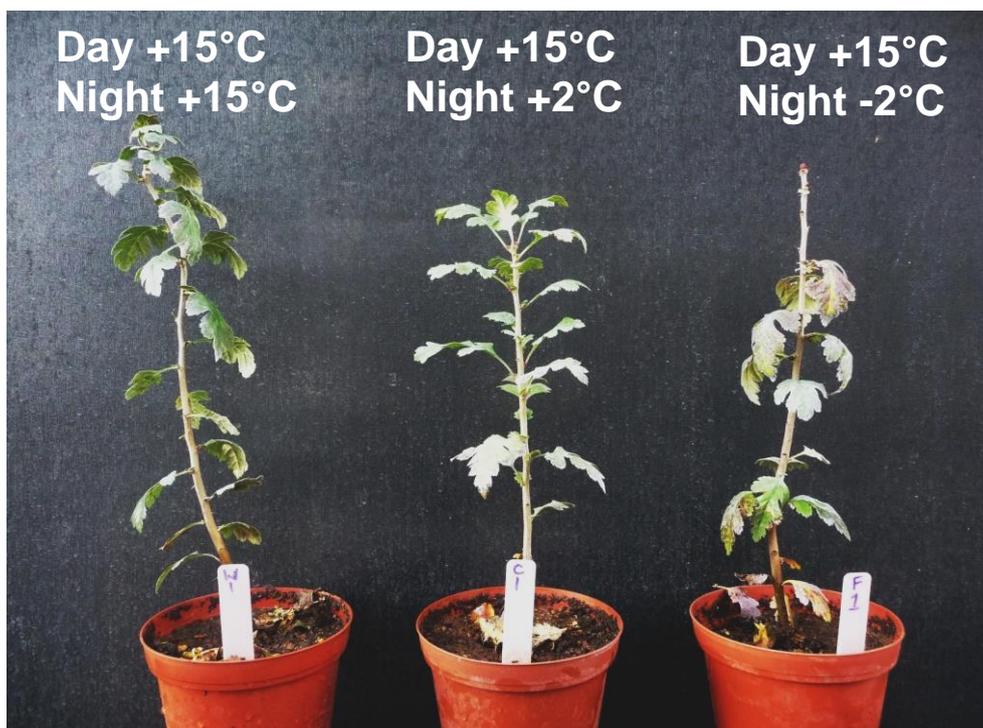


Table 9. *Crataegus monogyna* (Reading – Controlled Environment). Defoliation (%) and Leaf Detachment Force (g) for plants maintained at 15°C (Control), or where temperature progressively lowered to 2°C (Chill) or -2°C (Frost) night temperatures. (n=12).

	Control	Night Chill	Night Frost
	Defoliation (%)		
Con	5	6	5
Lanolin	4	3	2
TIBA (10 mM)	6	5	11
TIBA (20 mM)	3	12	24
	Detachment Force (g)		
Con	95	125	115
Lanolin	130	132	105
TIBA (10 mM)	117	138	107
TIBA (20 mM)	100	100	42

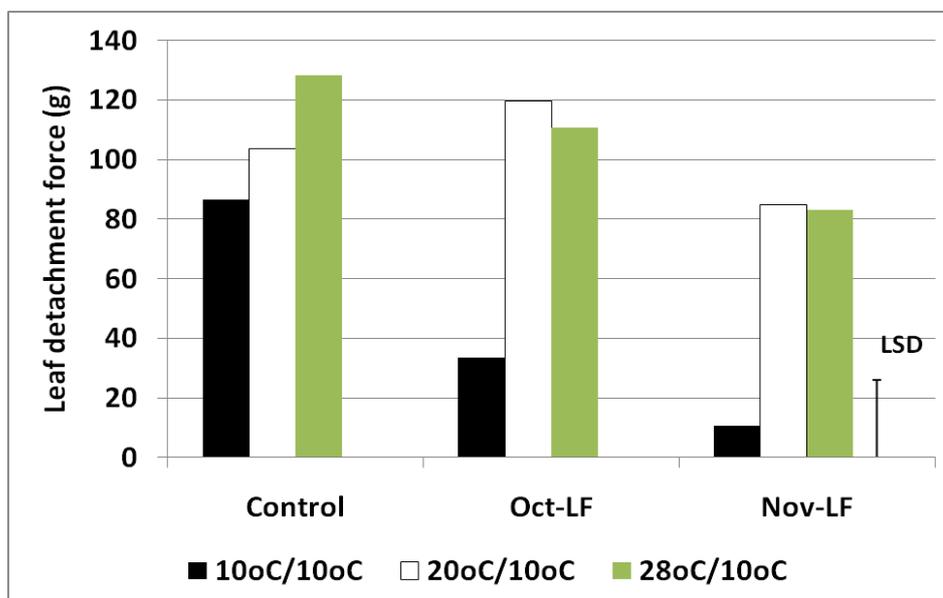
Experiment 11 (2009). How does temperature during the day influence defoliation?

Continual low temperatures (10°C/10°C) significantly reduced the leaf detachment force, particularly so when used in combination with Copper EDTA 'Leaf Fall' (Figure 18). Leaves abscising readily in the continual chill treatment after both October and November applications of Copper EDTA 'Leaf Fall'. Later Copper EDTA 'Leaf Fall' applications had a beneficial effect even in the 28°C/10°C regime, but the effects were less marked than those of the continual chill treatment.

Conclusions

- Increasing the degree of chilling the trees experience enhances the effectiveness of Copper EDTA 'Leaf Fall' in promoting defoliation
- In some scenarios chilling can aid defoliation without the absolute requirement for *sub-zero* temperatures.

Figure 18. *Crataegus monogyna* (Reading – Controlled Environment). Mean leaf detachment force (g) for all treatments (n=24)



Experiment 12 (2009). Controlling shoot activity in the field via TIBA to induce earlier dormancy and defoliation

Results showed that TIBA induced early bud dormancy in the young field grown specimens of *Crataegus* (Figure 19). At the time of final recording there was a suggestion that both TIBA and Copper EDTA 'Leaf Fall' had reduced shoot activity, independently (Figure 20), but that greatest dormancy was induced by the combination of the two – 'TIBA+LF' (Figure 20). In addition, this treatment significantly improved leaf abscission (Figure 21), with almost 80% of leaves abscising by this time.

Conclusions

- Application of TIBA to trees in the field proved useful in inducing early bud dormancy and promoting leaf abscission through the subsequent application of Copper EDTA 'Leaf Fall'.
- The disruption to polar auxin transport in a field setting is encouraging in that it implies that some form of anti-auxin or even anti-gibberellin, growth regulating chemicals may have application in the future to improve the effectiveness of Copper EDTA 'Leaf Fall'.
- Data again suggests that auxin is a key signal in maintaining shoot growth and resisting leaf removal.
- There are implications that non-chemical factors that encourage early resting bud formation and growth cessation may help in leaf removal.

Figure 19. *Crataegus monogyna* (Site A). Bud dormancy (%) as affected by TIBA or lanolin and measured on 3 Nov 2009.

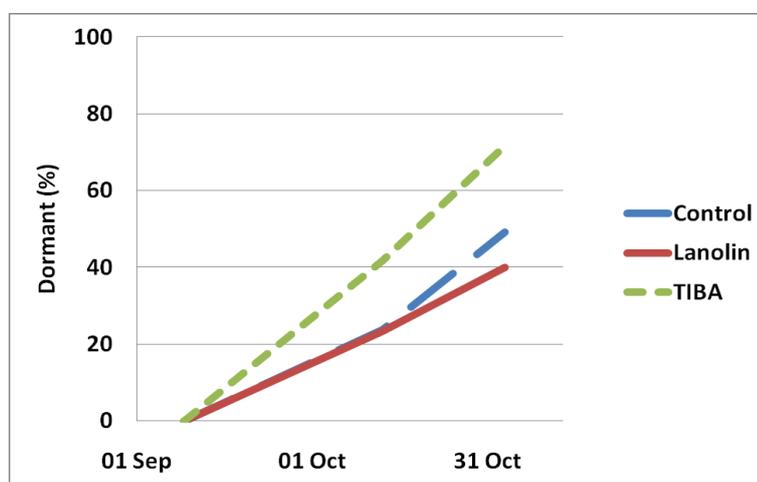


Figure 20. *Crataegus monogyna* (Site A). Shoots (%) still considered to be actively growing on 3 November 2009, after treatments with Lanolin or TIBA in August and subsequently sprayed with water or Copper EDTA 'Leaf Fall' (LF) on 13th October

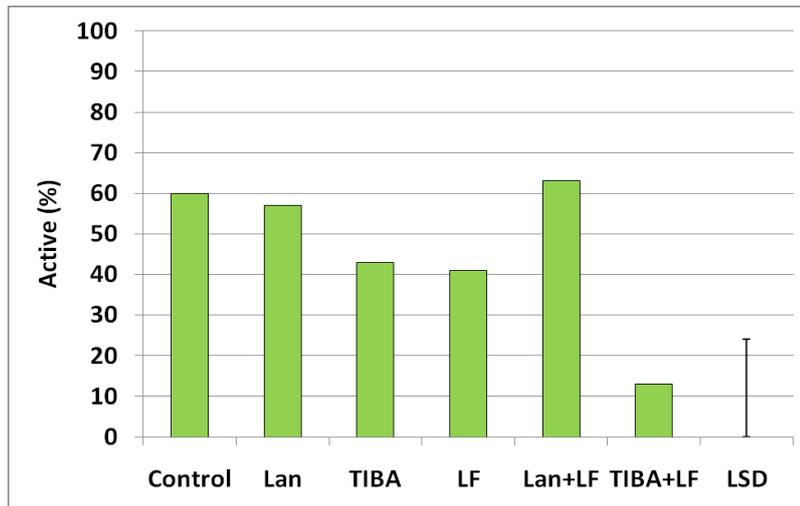
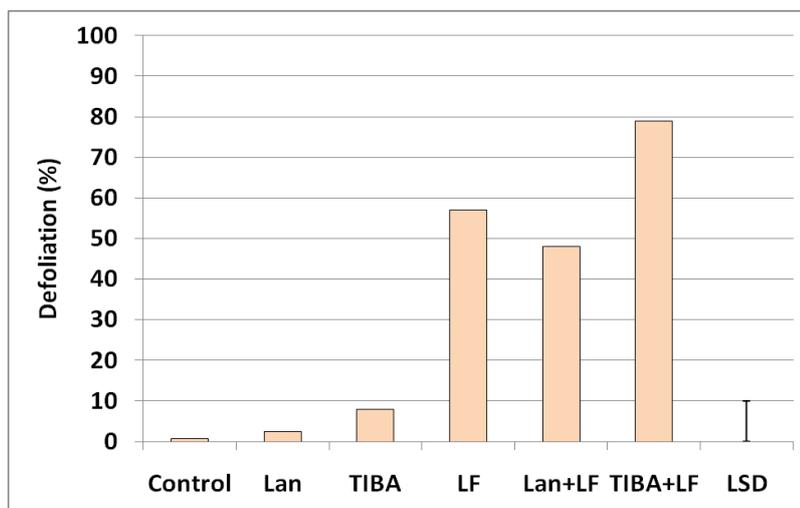


Figure 21. *Crataegus monogyna* (Site A). Defoliation (%) on 3 November 2009, after treatments with Lanolin or TIBA in August and subsequently sprayed with water or Copper EDTA 'Leaf Fall' (LF) on 13th October



EFFECTS OF WATER STRESS AND WATERLOGGING

Experiment 13 (2008). Assessing the effects of controlled drought on leaf abscission

Defoliation rates were quite variable between replications within the one treatment, possibly reflecting the variability in actual water content in individual pots. This was particularly so for plants in the polytunnel, where condensation forming on the roof of the tunnel resulted in water dripping off the polythene onto the plants (data from stomatal conductance studies reinforced the notion that there was a high degree of variability irrespective of treatment – data not shown). Defoliation rates were generally low in the polytunnel (10-20%) until frost (-0.5°C) was experienced in early November, at which point defoliation increased (Table 10). Interestingly, the data suggests that defoliation was most rapid in the 100% control treatment, but differences between treatments were not significant following the frost ($H=2.159$, $n=4$, $P>0.1$). Conditions were more controllable in the Glasshouse and here there was an increase in defoliation associated with the drier regime. By mid November Glasshouse plants in the more severe 25% treatment were significantly more defoliated than the 100% control, but not the 50% treatment.

Table 10. *Crataegus monogyna* (Reading). Leaf defoliation (%) of plants grown in a Glasshouse or Polytunnel and exposed to differential irrigation treatment (100, 50 and 25%) during autumn 2008.

	Glasshouse	Polytunnel*
100 %	18	60
50%	35	49
25%	49	30
	Sig v con	Not sig

*Plants in Polytunnel experience night frost to -0.5°C

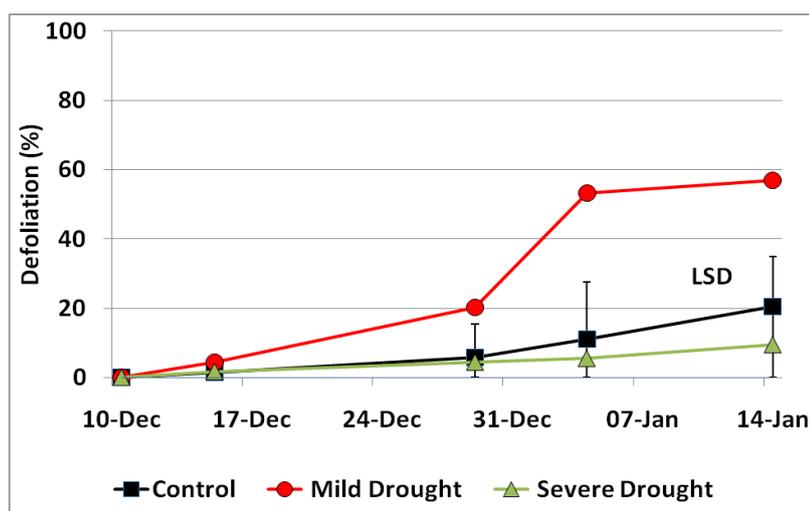
Conclusion

- **Some suggestion that under warmer autumn temperatures, that controlled drought stress may help defoliate plants.**

Experiment 14 (2009). Assessing the effects of controlled drought on leaf abscission II

This experiment re-assessed the influence of drought after some of the variable results encountered in Experiment 13. Conditions in the relatively warm polytunnel were not conducive to leaf abscission, and leaves were retained on the young trees well into December 2009. After that point however, the Mild Drought treatment increased defoliation and by 4 January 2010 leaf abscission was significantly greater than the control plants (Figure 22). In contrast to the Mild Drought, the Severe Drought brought on stress too rapidly, with leaves tending to desiccate and shrivel on the tree rather than fall (although the trees themselves remained alive).

Figure 22 *Crataegus monogyna* (Reading). Defoliation (%) over time during autumn 2009 with Mild or Severe Drought imposed.



Conclusions

- Controlled drought stress has the potential to aid defoliation, but care is required to ensure a consistent moderate degree of stress to optimise abscission.

Experiment 15 (2009). Plant responses to drought and waterlogging

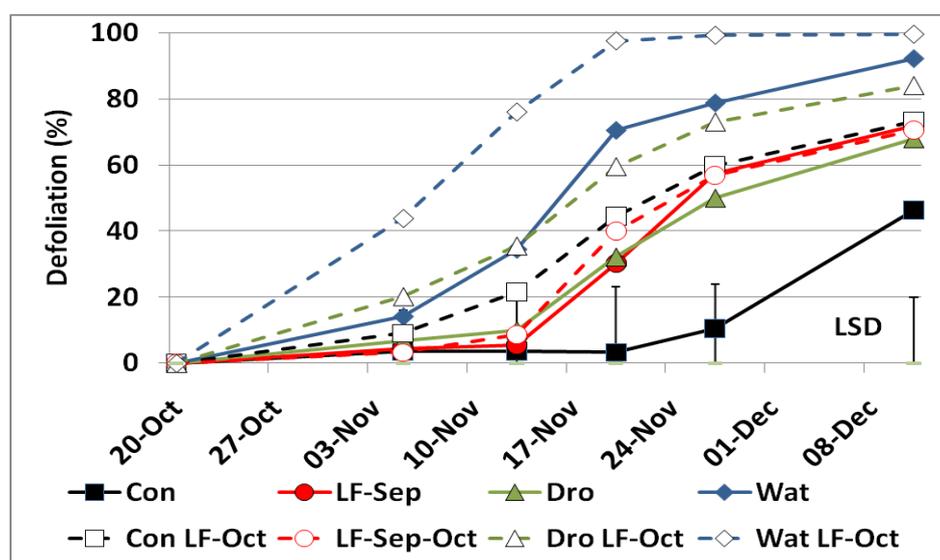
Typical soil moisture values for waterlogged pots were $0.68 \text{ m}^3 \text{ m}^{-3}$, whereas droughted plants averaged $0.29 \text{ m}^3 \text{ m}^{-3}$ and Controls and Copper EDTA 'Leaf Fall' were approximately $0.39 \text{ m}^3 \text{ m}^{-3}$. Waterlogging trees increased defoliation rates compared to Controls (Figure 23) and the effect was enhanced with the application of Copper EDTA 'Leaf Fall' in October

(100% defoliation being recorded on 20th Nov.). Either drought stressing trees or applying Copper EDTA 'Leaf Fall' in September also increased abscission rates compared to Controls. Again applying Copper EDTA 'Leaf Fall' in October to the drought stressed specimens provided additional advantage, but there was little difference in response to a double dose of Copper EDTA 'Leaf Fall' (Sep+Oct) compared to a single application (Sep) (Figure 23). No fatalities were recorded in any treatments, and indeed there was no suggestion of a growth penalty the following spring after drought or waterlogging.

Conclusions

- Stress treatments such as waterlogging and drought, when applied at moderate levels appeared to provide some benefits in encouraging defoliation.
- Waterlogging was more effective at inducing leaf abscission than a double dose of Copper EDTA 'Leaf Fall', suggesting that if stress treatments could be induced in a controlled and cost effective manner in a commercial context, they may be an opportunity to reduce the need for chemical applications.

Figure 23 (2009). *Crataegus monogyna* (Reading). Defoliation (%) over time after treatments with Copper EDTA 'Leaf Fall' (LF) Drought (Dro) or Waterlogging (Wat). Drought and waterlogging was applied from 15th September, and Copper EDTA 'Leaf Fall' applied on 21st and 29th September at quarter-strength as a stress (LF-Sept) and/or at full strength on 22nd October as a defoliant (LF-Oct).



CONCLUSIONS

Current industry practice, based on the work carried out by a number of researchers in the last 20 years, allows nurserymen to achieve acceptable levels of defoliation. Climate change is beginning to reduce the effects of these regimes as plants now continue to grow strongly later in the calendar year. Nurserymen were keen to evaluate if the use of potential chemical defoliant, such as Copper EDTA 'Leaf Fall', could counteract some of the problems of recent seasons. This involved an evaluation of Copper EDTA 'Leaf Fall' taking account growth phase and crop species / variety, and potential alternative chemicals.

Results derived early on in the project demonstrated that Copper EDTA 'Leaf Fall' appeared to be the most effective potential defoliant. Results did vary with species / cultivar with *Malus* 'Bramley' proving to be the most difficult to defoliate (17 to 57 %) although even here, better retention of the Copper EDTA 'Leaf Fall' through the application of an additional wetter 'Activator 90' induced positive responses (e.g. increasing % leaf abscission from 15 to 40% in Bramley when the wetter was applied). Relatively good defoliation results were also achieved with this 'difficult' cultivar when Copper EDTA 'Leaf Fall' was combined with urea and 'Cuprokylt' (70% - Experiment 1); or the growth rate was slowed prior to application, using a previous application of the growth regulator 'Nativo' (55% - Experiment 3). The disadvantage of these growth regulators however, appeared to be reduced bud vigour the following spring. The fact that *M.* 'Bramley' has a fairly pubescent (hairy) leaf, may suggest that part of the problem with this cultivar is the inability of the active elements to penetrate the leaf. The addition of wetters provide an opportunity when dealing with cultivars of this type. This is backed by the fact that an additional wetter – 'Silwet' (Experiment 6) also appeared to have some benefit in aiding abscission zone formation in one of the later experiments carried out.

Much of the literature would suggest that it is the copper ions that are conferring the advantage with a product such as Copper EDTA 'Leaf Fall', but there is some evidence to indicate that the EDTA component could be critical too. We observed conflicting results in this matter as iron chelate, (FeEDTA) appeared to be less effective than CuEDTA ('Leaf Fall'), but EDTA applied alone showed very favourable results in Experiment 7. Taking the evidence overall we would conclude that Copper EDTA 'Leaf Fall' provides an advantage over many other compounds due to the presence of the copper ion, the solubility of the ion within the EDTA chelate and that the EDTA itself may also possess defoliant characteristics.

Unfortunately, over the three years of the project we found no 'off-the-shelf' (i.e. registered) chemicals that acted as a practical defoliant. Out of all the chemicals trialled, Copper EDTA 'Leaf Fall' was by far the most effective,

The research data however, alluded to the factors that control the physiology of leaf abscission itself. Again much of the literature would suggest that the formation of an abscission zone is determined by the actions of endogenous auxin and ethylene. The results here support the influence of auxin in retaining the leaves. Where the disruption of auxin movement was induced, trees were more susceptible to defoliation. The use of the auxin blocker, TIBA, proved useful in controlled experiments (and more surprisingly in the field) in altering physiology in such a way that subsequent factors (chilling, frost, Copper EDTA 'Leaf Fall') proved to be significantly more effective in removing leaves. The disruption to polar auxin transport in a field setting is particularly encouraging in that it implies that some form of anti-auxin (or even anti-gibberellin), growth regulating chemicals may have application in the future to aid natural defoliation. In contrast to the fairly clear story associated with auxin, the role of ethylene was more difficult to determine. In a controlled *in vitro* experiment (Experiment 8) defoliation potential was enhanced after no or low copper (7 mM) applications, but associated ethylene levels were variable within treatments, and although highest recorded values were linked with the 7.5 mM copper treatment, these were not significantly different from other treatments. These results suggest however, (and this is consistent with other studies), that an excess application of a toxic chemical (in this case copper) reduces abscission potential (i.e. overly damaged leaves do not readily form an abscission layer).

A key element of the project was to seek non-chemical means to aid defoliation. Within the constraints of maximising crop growth for much of the season, and within the limits of open ground production, where narrow profit margins provide little scope for new capital infrastructures (e.g. leaf blowing machinery), the opportunities for environmental manipulations are restricted. Nevertheless, results clearly demonstrated that environmental factors and moderate degrees of abiotic stress in particular, can induce leaf abscission. Abscission was accentuated under either low temperature, controlled 'moderate' drought or waterlogging regimes. As such, there may be feasible manipulation techniques for growers who use protected structures or grow in containers etc., yet field scale manipulations still remain elusive or are unlikely to be cost effective. With a strong desire to reduce reliance on chemicals, however, future research and development activities may wish to explore this avenue more fully.

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APPENDIX 1

Literature Review

Possessing a feasible and cost-effective management tool to aid leaf abscission in the autumn would be a great bonus for nursery managers. Globally, chemical defoliants have been widely used in tree and other crops (Stahler, 1953; Jones *et al.*, 1973; Forbes and Pratley, 1983; Metzger and Keng, 1984), but effectiveness can vary between species, chemicals used, concentrations applied and effects due to different growing seasons (Knight, 1979; Guak and Fuchigami, 2001; Bi *et al.*, 2005).

The area of artificial defoliation has received intensive research globally for many years, and encompasses the development of chemicals such as Agent Orange, used for forest defoliation in the Vietnam war, through to compounds that act more benignly through the release of ethylene (e.g. Ethephon). The vast majority of research has focussed on chemical defoliants (Jones *et al.*, 1973; Dong *et al.*, 2002; Bi *et al.*, 2005), with only limited application of mechanical (Anon, 2006) or heat related (Funk *et al.*, 2006) techniques. Much of the more recent research involving defoliants has been associated with cotton (*Gossypium* spp.), where desiccating and removing the foliage aids the harvesting of the cotton bolls (Sanders, 2005). With young tree crops, the objective is to induce leaf abscission, not by rapidly killing the leaf, but in a non-lethal manner that encourages the formation of an abscission zone.

Decreasing photoperiod and decreasing temperature are universally accepted as triggers for temperate zone, deciduous trees to begin entry into a period of quiescence (Arora *et al.*, 2003). Most authors cite a combination of both of these abiotic factors as the initiators of the entry into endodormancy, and it is generally accepted that this holds true for most species. The relationship between photoperiod and temperature in the induction of endodormancy, leaf senescence and abscission and cold hardening/acclimation is highly variable across genera and even species. In *Vitis labruscana*, for example, Fennell and Hoover (1991) were able to induce dormancy using only reduced photoperiod. Conversely, for other species, dormancy may be induced by reduced temperatures independently of photoperiod (Arora *et al.*, 2003).

Photoperiod

Phytochromes are the active compounds which allow plants to sense day length and have been widely researched since their existence was first suggested in the first half of the 20th century (Borthwick and Hendricks, 1960). The perception of reducing day length through these substances induces a switch from the production of genes coding for enzymes

required for photosynthesis and active growth to those coding for enzymes that facilitate the breakdown of leaf tissue constituents (Taylor and Whitelaw, 2001). The ratio of different phytochromes resulting from reduced photoperiods appears to cause increased levels of endogenous ethylene (Goeschl *et al.*, 1967). This then initiates the production of carbohydrases, lipases and proteases important in leaf abscission (Abeles and Leather, 1971; Thompson *et al.*, 2000).

Temperature and carbon dioxide

The ability of a plant to sense reducing temperatures is key to winter survival but does not appear to come about through any particular sensory substance. Drawing on both plant and animal physiology, Sung *et al.* (2003) suggest a number of mechanisms for sensing temperature change; these include altered gene expression through changes in the fluidity of cell wall membranes and a possible mechanism of calcium ion influx to the cell homologous with recently discovered mammalian mechanisms. Again work on *Arabidopsis thaliana* has identified the genes expressed during exposure to cold (Medina *et al.*, 1999). Increased atmospheric CO₂ which has been linked to increased photosynthetic potential in plants has also been linked recently with interfering with leaf senescence (Taylor *et al.*, 2008).

Leaf senescence and abscission

Whether induced by the gradual reduction in photoperiod and average temperatures, a period of stress, or by natural senescence, foliar abscission is the result of complex and coordinated changes in cell structure, metabolism and gene expression brought about by the sensing and transduction of signals from within the plant and from its surroundings (Taylor and Whitelaw, 2001). Artificial control of abiotic factors often results in accelerated or delayed foliar abscission (Olmsted, 1951; Addicott and Lynch, 1955; Arora *et al.*, 2003).

Natural leaf abscission is largely regulated by the plant hormone auxin (Abeles and Rubinstein, 1964; Ayala and Silvertooth, 2006). A young, growing leaf is a source of auxin and the hormone is transported from the leaf across the leaf petiole into the stem. Once a leaf stops growing and senescence begins, auxin transport decreases and compounds within the leaves (chlorophyll, RNA, carbohydrates, proteins and inorganic ions) are broken down and translocated away from the leaf to the stem. These are then stored in the stem and used to facilitate new growth in the following spring. To allow for the movement of these compounds the water conducting tissues in the leaf and petiole need to remain alive. The reduction in auxin movement, however, also stimulates the promotion of ethylene within the leaf petiole, and this in turn helps activate enzymes (pectinase, cellulase, IAA-oxidase) that

begin the break down of the cell walls in the abscission zone (Gomez-Cadenas *et al.*, 1996). Once a sufficient number of cells have been weakened (i.e. an abscission zone has formed), movement by wind or physical abrasion is enough to snap the petiole and cause the leaf to drop.

The most effective artificial defoliant are those that help activate the natural processes of nutrient translocation and abscission zone formation. Use of an inappropriate chemical (or too strong a concentration) or excessive stress, however, result in direct damage to the leaf (usually via desiccation) and provides no opportunity for either movement of solutes or the formation of the abscission zone (Del Arco *et al.*, 1991). The consequences of which are that the young buds are 'starved' of reserves for proper growth in the spring, and dead leaves remain attached to the branches ('stuck' leaves) and become a source for pathogen infection.

The majority of techniques used to induce artificial defoliation have relied on chemical means. As outlined above, however, for these to be effective they need to be compatible with the natural processes involved in leaf abscission. For this reason, timing and concentration of chemical sprays is often critical to optimise the response. A large range of chemical compounds have been used in different crop types to promote defoliation within research trials. Copper EDTA has shown potential in previous trials for defoliation of nursery stock (Knight, 1983), requiring a wetter in some species. At the correct concentrations, it promotes adequate leaf abscission, and is most effective when applied 2-3 weeks before the period considered for natural leaf abscission. Manually stripping the leaves after treatment may still be required. A further benefit of Copper EDTA may be the prophylactic fungicidal properties, reducing the possibility of pathogens entering leaf scars. Applied too early, or at too high a concentration, though, it can scorch the leaves rather than encourage drop.

Applying copper oxychloride (e.g. 'Cuprolyt FL') may also aid the defoliation process as well as provide a general protectant against a number of bacterial and fungal pathogens.

Copper ions have an important relationship with both auxin and ethylene, which appears to explain the effectiveness of Copper EDTA '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).

Other compounds that have been used as defoliants include:

- Copper products in combination with other compounds e.g. Cu + urea
- Potassium iodide
- Bromodine
- Phosphate containing compounds
- Sodium chlorate
- Surfactants and mineral oils
- Growth retardants e.g. succinic acid-2,2 dimethylhydrazide
- DEF (S,S,S-tributylphosphorotrithioate)
- Abscisic acid (ABA)
- Aminolevulinic acid
- Ozone
- Ethephon (ethylene induction)
- Thidiazuron

Promoting abscission through mild plant stress

As early as the 19th century, research into the effects of stress showed that shorter growing seasons and early entry into dormancy could be achieved by applying moderate amounts of stress to the actively growing plant (Müller Thurgau 1885 in Arora *et al.*, 2003). These early studies also indicated that a reduced number of bud chilling hours were also required as a result of a growing season where stress was applied. In contrast, studies by Chandler and Tufts (1934) demonstrated that by extending the period of shoot growth in peach (*Prunus persica*), bud burst was delayed in the following growing season due to an increased chilling requirement. Again, this has ramifications for the UK nursery sector in that plants exposed to longer growing seasons as a result of climate change may be slow to establish the following year due to a delayed resumption of growth.

Moderate stress (enough to activate stress responses but not to irreparably damage the plant) and the activity of certain pest and pathogen species (Mao *et al.*, 1989; Michaeli *et al.*, 2001) may reduce the synthesis of hormones associated with active growth and lead to dormancy and leaf abscission responses. High levels of stress may induce rapid leaf senescence resulting in an absent or poorly formed abscission zone, meaning leaves are not easily detached.

Water

When available water is scarce, leaf abscission is also a method of leaf area adjustment which regulates the plant's fitness for the water status of its environment (Salisbury and Ross, 1992). Water stress, either through drought or flooding results in increased production of abscisic acid (ABA) and subsequent reduction of stomatal aperture (Wadman-van

Schravendijk and van Andel, 1985). However, leaf abscission under water stress occurs mainly as a result of greater amounts of ethylene being synthesised (El and Hall, 1974; Gomez-Cadenas *et al.*, 1996). ABA has also been implicated in short-day-induced entry into bud dormancy (Guak and Fuchigami, 2001). Exogenous ABA has also proved successful as a defoliant, appearing to fulfil the requirements of reducing plant vigour and promoting abscission (Addicott, 1982; Larsen and Higgins, 1997) required by nurserymen. Guak and Fuchigami (2001) also reported an increased mobilisation of nitrogen from the leaves into woody tissues when using ABA as a defoliant. Following this study, the proposal to undertake further studies, using cheaper ABA analogues, was made by the authors.

Pests and pathogens

Leaf abscission promoted by attacks from fungi and pests can largely be attributed to the release of ethylene from damaged tissues (Ketring and Melouk, 1982). ABA, though, is also produced by many fungi, and it is not clear whether pathogen-derived ABA has any significant effect on leaf abscission. The use of pathogens as a tool to defoliate leaves late in the season is theoretically possible, but one that may alarm commercial growers. High populations of fungal spores and bacterial cells could readily overwinter, and re-infect the crop in the spring, potentially causing significant damage to the young leaves and developing shoots. The use of more 'benign' microbial organisms (such as phylloplane yeasts species e.g. *Sporobolomyces*, *Cryptococcus*, *Rhodotorula*) that encourage abscission zones to form, may also be feasible, but problems maintaining the appropriate conditions for these micro-organisms to thrive, e.g. leaf surfaces may need to be kept continuously wet, may be prohibitive to their application.

Mechanically induced stress

Neel and Harris (1971) found that moderate shaking of *Liquidambar* trunks for 30 seconds daily reduced height growth to only 20 to 30 percent of that of trees not shaken. In the same trial 75% of the shaken trees set terminal buds within 3 weeks of the start of the treatment whilst no unshaken plants formed terminal buds. The authors therefore suggested that this represented an endogenous mechanism for regulating tree growth in windy situations. This phenomenon was later termed thigmomorphogenesis by Jaffe (1973) after experiments in which internode regions were manually rubbed to temporarily retard stem elongation. Biddington (1986) also cites the retardant effects of stem bending in some plants within the context of 'mechanically induced stress' (MIS). The phenomenon is now regarded as a stress-induced strategy to prevent mechanical damage in some species (Salisbury and

Ross, 1992) and the role it plays in the encouragement of terminal bud formation may have some use in field situations.

Light level and quality

Reductions in the amount and spectrum of light reaching the photosynthetic tissues within a leaf have been shown to affect its longevity. Guamet *et al* (1989) demonstrated that the ratio of red : far-red (R:FR) light reaching a single leaf had a direct influence on senescence using spectral filters, indicating the role of phytochromes in the process. This study concluded that a ratio below 0.45 could significantly increase rates of chlorophyll and leaf protein degradation in soybean (*Glycine max*). Such studies on individual leaves, are not, however indicative of whole plant responses to shade in a field situation.

When the whole plant is in conditions of high irradiance, young leaves act as strong sinks, drawing the nitrogen required for their construction from both the soil and older leaves. The older leaves therefore senesce more quickly (Nambiar and Fife, 1987). The situation is amplified if irradiance is high but nitrogen availability is low as the source status of mature leaves will increase in relation to the roots (Hikosaka, 2005).

In a crop canopy, shaded mature leaves senesce more rapidly if young leaves still receive high irradiance (Evans, 1989; Hikosaka, 2005).

When the whole plant is shaded, young leaves are less photosynthetically active meaning slower replacement of resources used to construct them (Williams *et al.*, 1989). This results in a longer life span. Furthermore, mobile nutrients are translocated less rapidly from mature leaves and there is also an increase in their longevity.