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The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

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

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Grower Summary

Headline

- Results indicate the continued use of 'Leaf Fall' to be the most effective way of defoliating young trees.

Background and expected deliverables

Nurserymen are concerned that natural leaf abscission on field-grown trees is occurring later each year, due to milder autumns. As such, the period in which field-grown trees can be lifted is becoming restricted. Some nurseries are being forced into lifting trees to meet orders whilst the foliage is still attached. Chemical defoliants are available to nurserymen, but these need to be applied with care to promote a strong enough abscission response, yet avoid damaging the crop.

This project aims to optimise the use of existing chemical products, and to explore cultural and alternative techniques that either enhance the effectiveness of these, or provide an alternative mechanism for defoliation. The first year's work comprised a rigorous field trial over three sites to examine the effectiveness of current defoliants in isolation, combination and varied application timings.

Summary of the project and main conclusions

Year 1 – Optimising the use of existing chemical products

Studies into defoliation regimes on a variety of species commenced in August 2007 at the University of Reading (UoR) and two commercial nurseries. Site A was a producer of hedging plants, grown from seed, lifted, cold-stored and distributed within one season. Site B produced grafted fruit and ornamental trees to be sold either bare root or potted. Combination treatments of "Cuprokylt" (copper oxychloride) +/- urea +/- 'Leaf Fall' (copper in solution as a copper-EDTA complex), were made over three consecutive months (August – October). *Crataegus monogyna*, *Quercus robur*, *Pyrus communis* 'Conference', *Malus domestica* 'Bramley' and *Malus* x *moerlandsii* 'Profusion Improved' were selected for treatment.

The activity of the apical meristem was recorded at the time of chemical treatment in order to allow analysis of the relationship between plant vigour and the effectiveness of each treatment. Detailed observations of leaf abscission were made in order to ascertain how the position of each leaf on the stem may affect its sensitivity to defoliants. At UoR, this took the form of nodal maps, scoring each leaf on the leader for level of damage or abscission; levels of damage to the stem were also recorded in detail. Due to the density of planting and the size of the material at site A (*C. monogyna* and *Q. robur*) whole plant scores for percentage damage and defoliation were recorded. At site B, the percentage of leaves damaged and absent on the upper, middle and lower thirds of each plant were recorded.

Treatments that included 'Leaf Fall' were the most effective on all the species tested, but the total amount of leaf abscission varied greatly across species and at individual plant level (Table A). August applications of defoliant treatments were least effective as plants continued to produce new leaves at both the shoot apex and secondary growth points after spraying and initial treatment effects. At 50% of the recommended application rate (10 ml l^{-1}) 'Leaf Fall' gave rise to similar levels of defoliation to the full rate (20 ml l^{-1}) when applied to *C. monogyna* in September. Therefore, there may be an opportunity to reduce costs as long as plants have reached the required size by this time.

Table A. Summary of the most effective treatments across sites and species trialled in autumn 2007. Defoliation is expressed as a percentage of lost foliage at the time of lifting. NB some data sets have been omitted from the summary as it was not guaranteed that chemical penetration was effective in October, e.g. *Q. robur* at site A.

Site	Plant material	Most successful treatment		% Defoliation
University of Reading	*Crataegus monogyna*	‘‘Cuprokylt’’ + Urea + ‘Leaf Fall’	September	**59**
Site A	*Crataegus monogyna*	‘‘Cuprokylt’’ + ‘Leaf Fall’	September	**64**
Site A	*Quercus robur*	‘‘Cuprokylt’’ + ‘Leaf Fall’	August	**5**
Site B	*Pyrus* ‘Conference’	‘‘Cuprokylt’ + Urea + ‘Leaf Fall’	September	**44**
Site B	*Malus* ‘Bramley’	‘‘Cuprokylt’’ + Urea + ‘Leaf Fall’	September	**52**
Site B	*Malus* ‘Profusion Improved’	‘Leaf Fall 20ml l^{-1}’	September	**97**

Re-growth following chemical defoliation

A sample of *C. monogyna* plants recovered from site A was cold stored for several weeks at the University of Reading and then moved into a warm glasshouse during spring in order to examine the effects of defoliation on the shoot re-growth. Treatments selected for this exercise represented the highest levels of defoliation for both September and October regimes.

Untreated plants produced the lowest amount of new stem and foliage growth. Conversely, the plants that had highest levels of defoliation showed the most vigorous re-growth. This may have resulted from reduced moisture loss in the defoliated plants during storage, compared to controls. Alternatively, the non-treated controls may have entered dormancy later, and the subsequent artificial chilling was not sufficient to break dormancy fully. Analysis of the total nitrogen content of plants in the selected treatments did not reveal a relationship between nitrogen content and the amount of new growth produced. Interestingly, plants treated with urea were no more abundant in nitrogen than those receiving no urea.

In Summary

- 'Leaf Fall' applied in late September, either with or without "Cuprokylt" (copper oxychloride) and urea was most effective at encouraging leaf abscission
- 'Leaf Fall' contains copper in solution (CuEDTA), which defoliates young trees more effectively than insoluble copper compounds found in most fungicides
- The most effective defoliation treatments also caused most plant damage
- August treatment with the same defoliants did not induce enough defoliation to facilitate early lifting

Supplementary experiments

Chelated Iron

Research conducted in the 1980s suggested that chelated iron, FeEDTA has similar abscission-promoting properties to 'Leaf Fall'. A small trial was imposed that examined the performance of 'Leaf Fall' alongside a 20g l^{-1} solution of 'Librel' hydroponic nutrient (13.2% FeEDTA) on *Salix* sp. The iron compound was a far less effective defoliant over a constrained time period, although levels of leaf tissue damage were similar. This suggests that higher concentrations of FeEDTA may give rise to more commercially significant results.

'Folicur'

Following meetings with other nurserymen, a trial was undertaken at the University of Reading to asses the effects of the triazole fungicide 'Folicur' (tebuconazole) when used in conjunction with 'Leaf Fall'. A small experiment conducted using *Alnus glutinosa* showed that three, weekly applications of this compound at a rate of 1 ml l^{-1} improved the subsequent effectiveness of 'Leaf Fall'. Additionally, omitting 'Leaf Fall' altogether and making four applications of 'Folicur' gave rise to similar defoliation rates. It was apparent, however, that one 'side-effect' of this fungicide is a reduction in plant vigour (triazoles also have growth regulator properties).

Areas for further Research

Following the initial trials conducted in 2007, it is hoped that further experiments in the following areas may yield data that contribute to a fuller comprehension of artificially induced abscission:

- Developing a greater understanding of the biochemical action of 'Leaf Fall' and using these data to evaluate possible alternatives. These may be less environmentally persistent or less costly.
- Evaluating the role of late season applications of growth retardants. Vigorous plant growth late into the year has been cited as a major constraint on defoliation regimes and as such, plant growth regulators may represent a way of mitigating the effects of warmer autumns.
- Investigate the potential of brushing treatments (thigmomorphogenesis) as a practical non-chemical approach to inducing earlier bud dormancy and encouraging leaf abscission.
- Investigations under controlled conditions into the effects of various climate variables on the relationships between, and possible delays in plant dormancy, leaf senescence and leaf abscission.

Financial benefits

- End-of-season application of ''Cuprokylt'' may not be required if applying 'Leaf Fall', saving at least £60/ha (assuming 2 applications of ''Cuprokylt'')
- Reduced rates of 'Leaf Fall' application on some species could potentially reduce costs by 50%.

Action points for growers

- Initial results indicate the continued use of 'Leaf Fall' to be the most effective way of defoliating young trees.
- 'Folicur' (tebuconazole) applied to HONS in higher concentrations or at reduced intervals under a SOLA may represent a less environmentally-persistent defoliant than copper.

Science Section

Introduction

Deciduous tree crops are grown by the UK nursery sector for both hedging and as specimen trees. Hedging trees are field-grown, with seed sown in winter / spring and trees lifted the following autumn. These bare-root plants are bundled and stored in refrigerated cold-rooms prior to retail to landscapers, who will plant during the dormant season. Specimen trees tend to be of higher value, and often consist of a varietal scion budded onto a rootstock during summer of year 1. The rootstock is 'headed back' and the scion allowed to produce a saleable tree of the desired size and vigour by the early autumn of year 2. Specimen trees are also lifted as bare-root plants, either to be sold as such or, more commonly, to be potted before distribution to garden centres and nurseries.

Traditionally, nurserymen would lift trees once the crop had been exposed to early frost and leaves were beginning to abscise naturally. In more recent times, prolonged growing seasons, and the reduced incidence of early frost (Semenov, 2007) mean that trees are entering dormancy later and retaining their leaves for longer. Indeed they may still be growing actively at the time they should be harvested (Guak and Fuchigami, 2001). On commercial nurseries high fertilisation and irrigation regimes, combined with the juvenility of the plants, may exacerbate the problem. To this end nurserymen are finding that there is an increasing need to artificially induce dormancy in order to facilitate harvesting at the end of the growing season.

There is a paradox in this situation in that nurserymen require trees to grow strongly throughout the summer so as they meet the specifications of the market (set height, girth etc.), but also require growth to cease very rapidly in the autumn; thereby stimulating effective defoliation and facilitating lifting of the crop. Problems perceived by UK growers which can arise as a result of lifting non-dormant plants still in leaf include:

- increased labour costs due to prolonged handling times per plant
- increased risk of plant desiccation due to evapotranspiration
- possible increase in pathogens carried on leaves
- possible injury to tender stem tissue due to leaves heating as they decompose
- increased storage costs due to bulkier material

Whilst there are a large number of chemical defoliants available, inappropriate choice of chemical, concentration or timing can actually kill a leaf through desiccation rather than encourage abscission, resulting in dead leaves remaining on the tree. Indeed, many defoliants only promote optimum leaf abscission when the tree is at an appropriate physiological stage, for example, once auxin is no longer transported from the leaf to the petiole (Addicott, 1982). As such, nurserymen are keen to understand better timing of chemical application in relation to crop stage. There is also a desire to move away from reliance on chemicals both from an environmental and cost point of view. This raises the question as to the extent to which other practical management factors might be employed to encourage leaf defoliation (e.g. reducing irrigation or altering fertiliser regimes towards the end of the growing season). This three-year project both aims to evaluate current chemical defoliation regimes and optimize their use, but also investigate alternative practices that may replace or reduce amounts of chemicals used in defoliation programmes. In this first year of the project the focus has been on evaluating feasible chemical approaches and investigating the effect of timing of application.

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. 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).

<u>Photoperiod</u>

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 20^{th} 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

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).

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 co-ordinated 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 defoliants 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 of crops 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. For nursery trees in the UK, 'Leaf-Fall' appears to be the most popular. This is a copper-EDTA complex, with a recommended application rate in water of 20 ml l^{-1}, sometimes applied with a wetter. At these concentrations, it promotes adequate leaf abscission, and is most effective when applied 2-3 weeks before the period considered for natural leaf abscission. Some growers manually strip the leaves after treatment, and report prophylactic properties against disease entering leaf scars. Applied too early, or at too high a concentration it can scorch the leaves rather than encourage drop.

A number of nurserymen will also use copper oxychloride (e.g. ‘‘CuprokyIt’’) two weeks prior to applying ‘Leaf-Fall’, as this may also aid the defoliation process as well as provide a general protectant against a number of bacterial and fungal pathogens.

Other compounds that have been used 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. succinicacid-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 *Sporoboblomyces, 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. Guimet 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.

Statistical analysis

Various statistical models were used to analyse data within the project, based on the specific parameters measured in individual experiments. Parametric and normally distributed data were analysed using a standard Analysis of Variance test (ANOVA) undertaken using GenStat. The results were expressed in terms of the least significant difference (LSD) at the 5% level. This indicates a 95% probability that the value reported is the true difference between population means.

Percentage (proportion) data were arcsine transformed prior to preliminary descriptive statistical analysis. This showed that transformed data did not fulfil the requirements for standard parametric tests and thus non-parametric analyses were used in these instances. Box plots for untransformed data are shown in Appendix 2.

One-way analyses of variance for non-parametric and non-normally distributed data were made using the Kruskal-Wallis test (GenStat). 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 subsequent calculations to ascertain where significant differences occurred were not possible (i.e. figures do not have error bars).

Where it is inappropriate to display LSD bars, standard error (s.e) bars have been used (where valid) to demonstrate variation around a mean value. Similarly, where error bars may misrepresent the data distribution, these are been excluded.

Correlations including proportion data were analysed by calculating a Spearman Rank Correlation Coefficient (r_s), again using the GenStat package.

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

Five 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 and *Quercus robur* are produced from seed as hedging material.

Malus domestica 'Bramley', *M.* x *moerlandsii* 'Profusion Improved' and *Pyrus communis* 'Conference' are field grown grafted examples grown for wholesale to garden centres.

'Leaf Fall' was identified from both previous research, and from consultation with nurserymen to be the most effective, defoliation available. However, it was also apparent that the problem of retarded leaf senescence and abscission has become more acute since the initial trials were undertaken (Knight, 1983) and that some refinement of current practices is required.

Also containing copper, ''Cuprokylt FL' is widely used in a variety of agricultural and horticultural contexts. This compound is routinely used as an end of season preventative treatment against apple and pear canker (*Nectrina gallegina*) and nurserymen may also use it to provide initial mild stress to the leaf prior to the application of 'Leaf Fall'.

Urea 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).

Materials and methods

Treatments

Three chemical treatments, 'Leaf Fall' (CuEDTA), 'Cuprokylt' (copper oxychloride) and laboratory grade urea, were applied in the combinations shown (Table 1).

'Cuprokylt FL' – Universal Crop Protection Ltd (UNICROP)

'Cuprokylt' is a protectant fungicide for the control of a variety of pathogens, including apple and pear canker (*Nectrina galligena*). It is supplied as a suspension concentrate containing 270 g l^{-1} copper as copper oxychloride ($CuCl_2 \cdot 3Cu(OH)_2$).

'Leaf Fall' – Protex Chemicals

This product has been widely used to defoliate deciduous nursery crops for over two decades. The active ingredient CuEDTA is a source of Cu^{2+} in solution (9% Cu), allowing effective leaf penetration.

Urea

Laboratory grade urea, $(NH_2)2CO$, supplied by Fisher Scientific was utilised.

Treatment schedule

The programme of treatment applications ran over three consecutive months (Table 1). Where treatments consisted of two chemical applications, the first was applied in the middle of the month and the second at the end of the month. This programme was adhered to as closely as weather conditions permitted.

Although the first portion of the October treatments was applied to all relevant trees at site B, 'Leaf Fall' was applied to the entire crops by staff prior to the end-of-October experimental applications. Treatments 10 and 18 thus received two doses of 'Leaf Fall' and the final data could not be included in analysis.

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.

Table 1. Treatments applied and date of application. Starred [∗] treatments indicate applications made at site B. All treatments were applied at University of Reading and site A.

Treatment	Application 1	Rate ($ml\ l^{-1}$/$g\ l^{-1}$)	Application Date 1	Application 2	Rate ($ml\ l^{-1}$)	Application Date 2
1∗	No spray					
2	Water	n/a	Late Oct			
3	'Leaf Fall'	20	Late Aug			
4∗	'Leaf Fall'	20	Late Sep			
5∗	'Leaf Fall'	20	Late Oct			
6	'Leaf Fall'	10	Late Aug			
7	'Leaf Fall'	10	Late Sep			
8	'Leaf Fall'	10	Late Oct			
9	'Cuprokylt'	5	Mid Aug	'Leaf Fall'	20	Late Aug
10∗	'Cuprokylt'	5	Mid Sep	'Leaf Fall'	20	Late Sep
11∗	'Cuprokylt'	5	Mid Oct	'Leaf Fall'	20	Late Oct
12∗	'Cuprokylt'	5	Mid Sep			
13	Urea	30	Mid Aug	'Leaf Fall'	20	Late Aug
14	Urea	30	Mid Sep	'Leaf Fall'	20	Late Sep
15	Urea	30	Mid Oct	'Leaf Fall'	20	Late Oct
16	Urea	30	Mid Sep			
17	'Cuprokylt' + Urea	5 / 30	Mid Aug	'Leaf Fall'	20	Late Aug
18∗	'Cuprokylt' + Urea	5 / 30	Mid Sep	'Leaf Fall'	20	Late Sep
19∗	'Cuprokylt' + Urea	5 / 30	Mid Oct	'Leaf Fall'	20	Late Oct
20∗	'Cuprokylt' + Urea	5 / 30	Mid Sep			

Field Trial Sites

<u>University of Reading, Whiteknights site.</u>

Plant material:

Crataegus monogyna

During April 2007, 600 maiden whips were planted in the university's experimental grounds. Trees were arranged in a 60 x 10 block and planted through woven plastic landscaping fabric. T-tape irrigation pipe was installed under the landscaping fabric along each row prior to planting to be utilised if dry periods threatened to put the plants under drought stress. A 2 m gap was left between rows and 0.5 m between plants. After planting, the trees were watered in to aid establishment.

The plot was divided into 3 equally sized blocks and the 20 treatments were allocated randomly to blocks of 10 trees across all 3 blocks. The nutritional status of the soil did not necessitate the application of any fertilisers.

<u>Site A</u>

Plant material:

Crataegus monogyna

Quercus robur

Both species were field-grown from seed sown in spring 2007. Plants were grown at high density (c. 125 plants / m^2), in 5-row beds.

The experimental treatment blocks were allocated as 3 m sections of the beds. One metre buffer zones were marked between blocks to minimise the effects of spray drift. Beds of *C. monogyna* were long enough to accommodate all treatments repeated randomly over 3 blocks, however, block 3 of the *Q. robur* was carried over to an adjacent bed as these plants were being grown in a shorter field.

<u>Site B</u>

Plant material:

Malus domestica 'Bramley'

Pyrus communis 'Conference'

Malus x *moerlandsii* 'Profusion Improved'

Nine treatments (those with * - Table 1) were applied to all three species, also according to a randomised block design.

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 P leucotricha*) and with 'Chess' (pymetrozine) to control aphids.

Data recorded

<u>Shoot activity at time of treatment application</u>

In an attempt to correlate the effectiveness of defoliants 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 *C. monogyna* and *Q. robur* plants were scored for vigour of apical growth between 0 (least) and 10 (greatest) (See Appendix 1).

Growth in all three tree species at site B was very uniform and a broad scale was not appropriate. Scoring was based on criteria relating to individual species / cultivars:

- *Pyrus* 'Conference' trees were awarded a score of 0 if the apical meristem was dormant, 1 if the most recent, unopened leaves were beginning to senesce, and 2 if vigorous production of new leaves was apparent.

- *Malus* 'Bramley' received a score of 1 if 10 cm or less of new unlignified stem growth was apparent, and 2 if there was more than 10 cm.

- New leaves on *Malus* 'Profusion Improved' were red in colour. All trees were producing new leaves on the main leader only were given a score of 1, whilst trees that also had new, red leaves on lower branches scored 2.

Leaf damage

It was important to determine the extent to which chemicals injured leaves and either encouraged abscission, or alternatively, resulted in moribund dead leaves remaining on the stem. At all sites each plant was awarded a score between 1 and 100 to represent the total percentage of brown leaf tissue. The score applied to those leaves present and was not related to defoliation. Therefore plants whose leaves had been totally desiccated and had subsequently lost 90 % of them could receive damage and defoliation scores of 100 and 90 respectively.

Defoliation

The method of recording leaf loss was different at each site:

At UoR, fifteen plants were randomly selected from each treatment for detailed measurement of the condition of every leaf on the leader. Each leaf on the main stem was scored for damage / abscission at regular intervals from September to December according to its state:

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

Additionally, percentage leaf loss was recorded on two separate occasions near the end of the season by visual inspection of the entire population.

At site B defoliation was recorded as a percentage by visual inspection for upper, middle and lower thirds of the main stem at two week intervals after completion of each tranche of applications. Approximately 20 leaves were present in each third of the leader, and thus therefore 1 missing leaf equated to a 5% reduction in foliage.

Plants treated at site A were not divided for recording due to their smaller size. Approximately 35 – 40 leaves were counted per stem on a sample of the population and thus every 2 missing leaves was taken to represent 5% defoliation.

Results

University of Reading

Apical Meristem Activity

Apical meristem activity (AMA) scores were inversely correlated with time. The Spearman Rank Correlation Coefficient (r_s) of the relationship between AMA and Julian date was -0.62 (n=584, $p<0.001$).

Treatment Effects

Comparison of defoliation levels two weeks after each application showed no significant differences (H=6, p=0.19) for treatments that included 'Leaf Fall'. Timing of application, however, had a significant effect on the level of defoliation after two weeks (H=71, $p<0.001$). There was no significant interaction between the treatment and timing factors (H=14, p=0.09) for the data sets collected two weeks following application.

Analysis of data collected in mid-November though, showed no significant effect of application timing on end of season defoliation levels (H=2, p=0.29). At this recording point, however, significant differences in the amount of defoliation could be attributed to treatment (H=11, p=0.02) and to treatment + timing interactions (H=17, p=0.03).

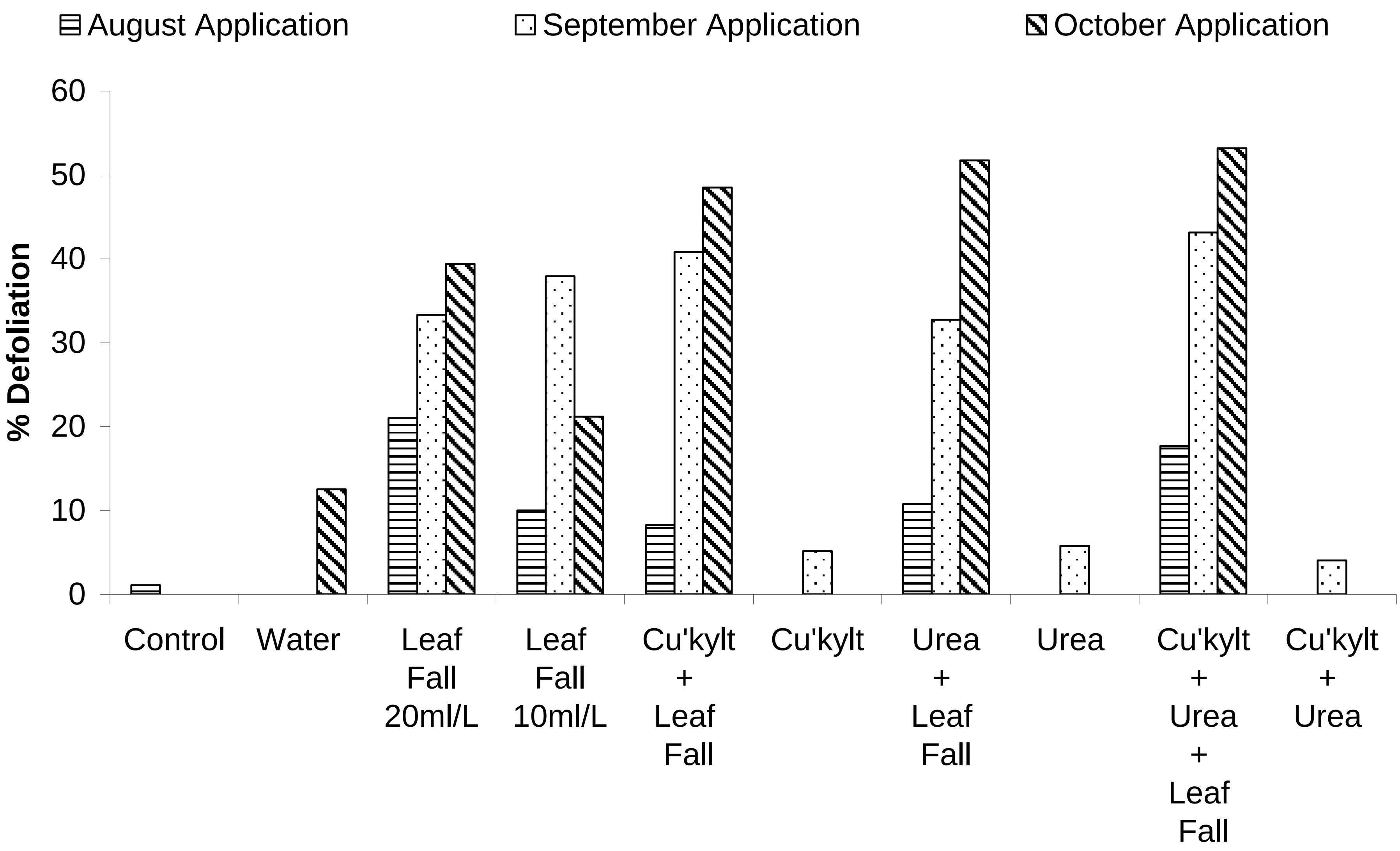
August Treatments

After the August treatments, (assessed mid-September), the greatest amount of defoliation was observed in the plants that had been sprayed with the recommended dose of Leaf Fall (Fig. 1). Prior treatment with 'Cuprokylt' in August did not significantly increase defoliation. By the assessment in mid-September (see 'August' columns Fig. 1), defoliation by the 50% (10ml l^{-1}) concentration of Leaf Fall was less than half that observed in plants treated with the full recommended concentration.

September Treatments

Two weeks after completion of the September treatments (mid-October) leaders of all plants that had received 'Leaf Fall' at the recommended rate in September had lost at least 30% of their foliage. The mean level of defoliation of plants receiving a half-strength application of 'Leaf Fall' at the same time was slightly higher (Fig.1). At this time, plants that had received 'Cuprokylt' and / or urea, but not 'Leaf Fall' suffered less defoliation than all of the treatments that included 'Leaf Fall'. The level of defoliation of control plants, those treated with urea only (September) and 'Cuprokylt' and urea (September) was comparable at this time (i.e. 1-10%). The plants sprayed with a 50% dose of 'Leaf Fall' in August continued to produce new leaves (not shown).

Figure 1. Defoliation （%） of *C. monogyna* recorded two weeks after treatments completed in late August, September and October respectively at the University of Reading.


August Application
September Application
October Application
% Defoliation
60
50
40
30
20
10
0
Control
Water
Leaf Fall 20ml/L
Leaf Fall 10ml/L
Cu'kylt + Leaf Fall
Cu'kylt
Urea + Leaf Fall
Urea
Cu'kylt + Urea + Leaf Fall
Cu'kylt + Urea

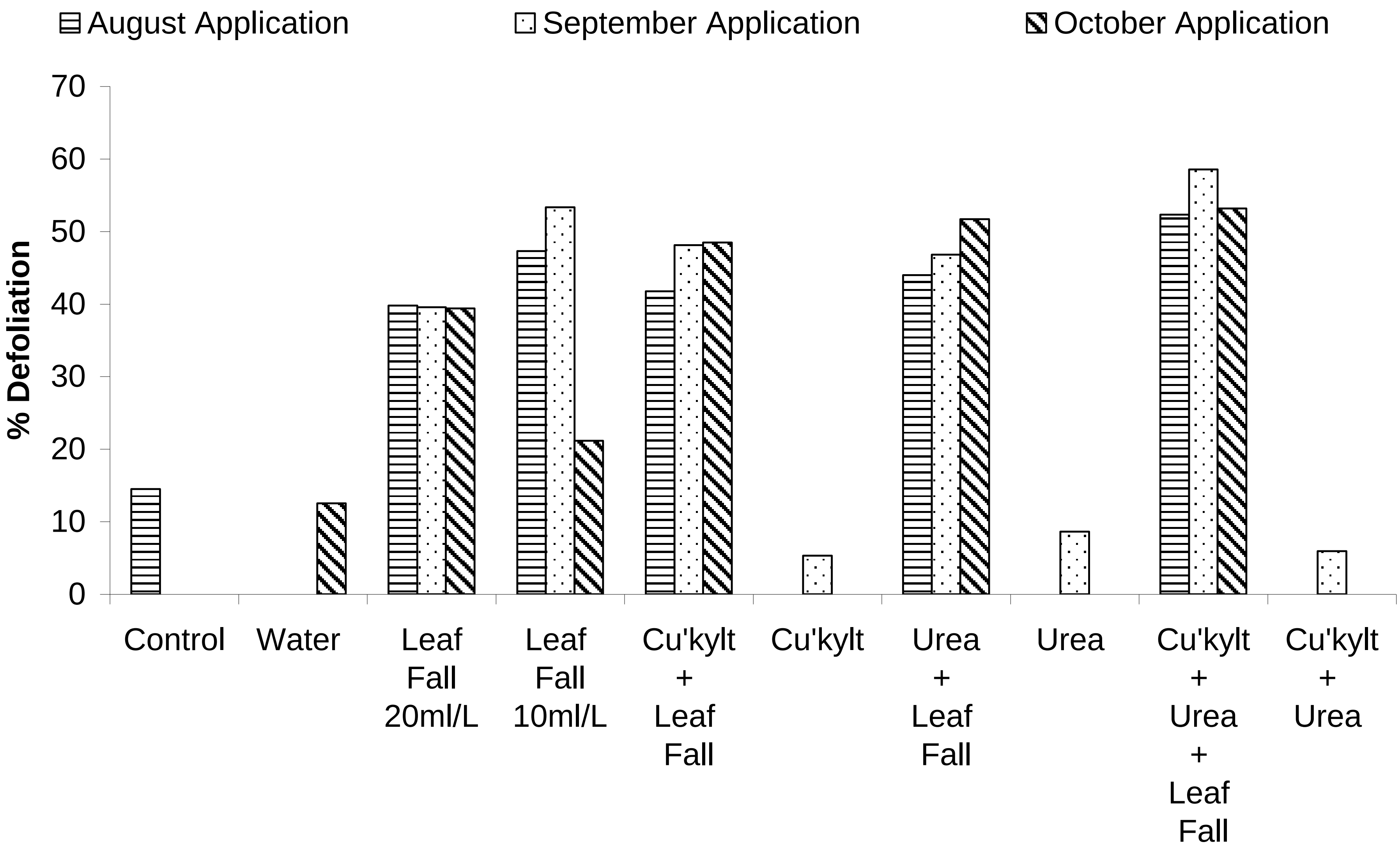
October Treatments

Defoliation with 'Cuprokylt' plus 'Leaf Fall' (both with and without urea) was marginally more effective two weeks after the October application (>50%) than it had been after the September application (>40%) (Fig.1). Urea + 'Leaf Fall' was also a fairly effective treatment when applied in October.

November Assessments

By mid-November all the treatments that had incorporated 'Leaf Fall' at its full rate (Fig 2.) showed consistent positive results (although maximum rates of defoliation were still only 60%). Interestingly, plants sprayed with a 50% dose of 'Leaf Fall' in both August and September were more defoliated than the full-strength dose applied at the same time. (but were less for the October application). 'Cuprokylt' and urea applied in the absence of 'Leaf Fall' resulted in similar levels of defoliation to the control and water treatments (Fig. 2).

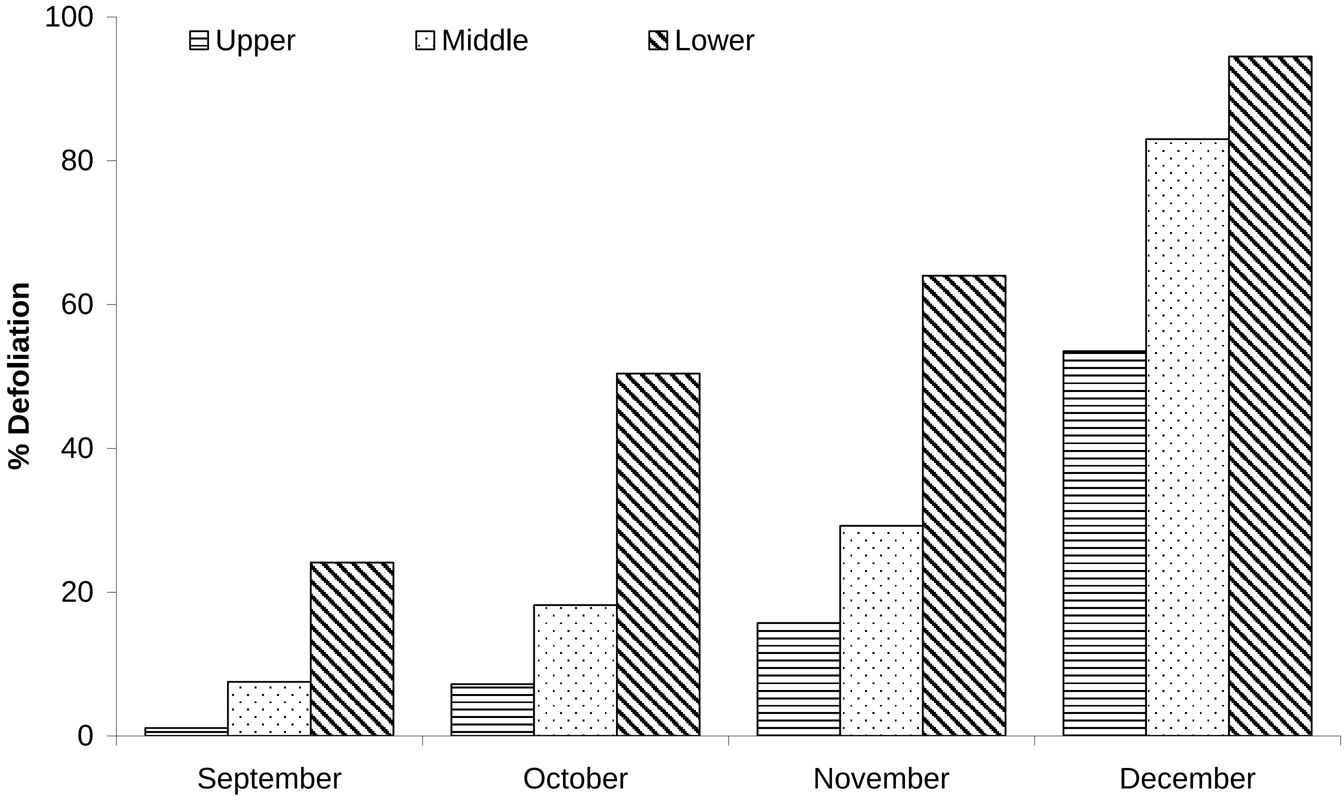
Figure 2. Defoliation (%) of *C. monogyna* recorded in mid-November at the University of Reading.



August Application
September Application
October Application
% Defoliation
70
60
50
40
30
20
10
0
Control
Water
Leaf Fall 20ml/L
Leaf Fall 10ml/L
Cu'kylt + Leaf Fall
Cu'kylt
Urea + Leaf Fall
Urea
Cu'kylt + Urea + Leaf Fall
Cu'kylt + Urea

When data from all the treatments was pooled, it was evident that the leaves in the basal portion of the stem were more inclined to drop than middle or upper sections (Fig. 3 Furthermore, leaves in the middle portion of the stem were also more likely to abscise compared to those at the top. Analysis demonstrated a significant difference in sensitivity to defoliants across the three stem zones ($P<0.001$ for all months).

Figure 3. Distribution of abscised leaves over *C. monogyna* leaders at the University of Reading. Pooled data for all treatments.



Phytotoxicity

Whilst the treatments that included 'Leaf Fall' were effective at causing rapid defoliation when applied in September, this timing also gave rise to the highest amount of apical meristem damage (Table 2.). Leader tip death was observed in around half of the plants in these treatments in comparison with 3% or less in the treatments that did not include 'Leaf Fall'. In general, less damage was induced by October applications of 'Leaf Fall', possibly due to the greater lignification in the stem tissues.

Table 2. Phytotoxicity of treatments measured as % of plants with dead apical meristems. Measured 27th November 2007, University of Reading.

Treatment	% of plants with apical meristem necrosis
Control	0
Water	0
'Leaf Fall' 20 ml l^{-1} August	23
'Leaf Fall' 20 ml l^{-1} September	40
'Leaf Fall' 20 ml l^{-1} October	7
'Leaf Fall' 10 ml l^{-1} August	37
'Leaf Fall' 10 ml l^{-1} September	51
'Leaf Fall' 10 ml l^{-1} October	4
'Cuprokylt' + 'Leaf Fall' August	20
'Cuprokylt' + 'Leaf Fall' September	44
'Cuprokylt' + 'Leaf Fall' October	10
'Cuprokylt' September	3
Urea + 'Leaf Fall' August	17
Urea + 'Leaf Fall' September	45
Urea + 'Leaf Fall' October	7
Urea September	0
'Cuprokylt' + Urea + 'Leaf Fall' August	25
'Cuprokylt' + Urea + 'Leaf Fall' September	42
'Cuprokylt' + Urea + 'Leaf Fall' October	24
'Cuprokylt' + 'Leaf Fall' September	0

SITE A

C. monogyna

Apical Meristem Activity

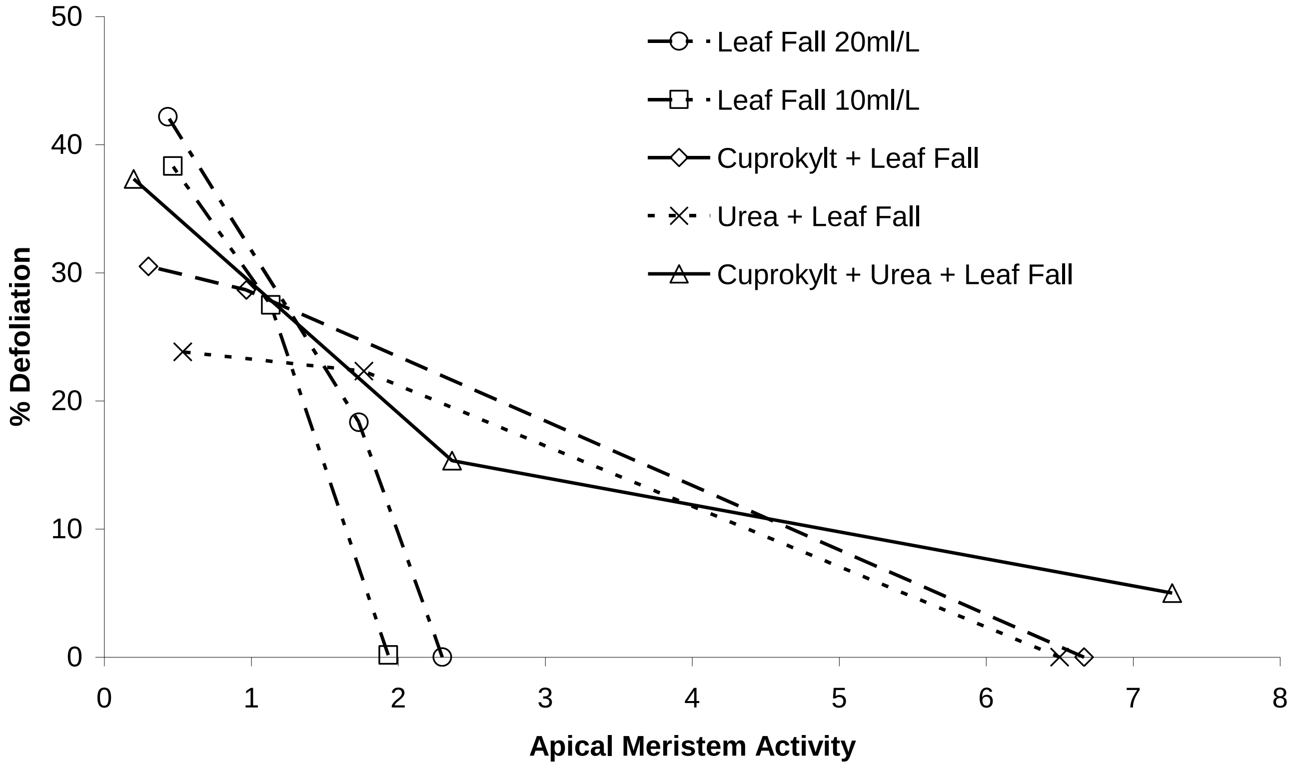
Analysis of apical meristem activity (AMA) over time using the Spearman Rank Correlation Coefficient indicated a strong inverse correlation between AMA and date (r_s = -0.74 adjusted for tied data sets, n=600, p<0.001). Greater defoliation was associated with treatments (and times) that reduced apical bud activity (Fig. 4). The discrepancy in apical bud activity between 'Leaf fall' (approx. 2 %) and other treatments (6-8%) (see points near x-axis in Fig. 4) relates to treatment and recording dates. Plants that had been treated with 'Leaf Fall' alone were recorded after the crop had been undercut, whereas the data for the other treatments represents a period before undercutting.

Treatment Effects

Comparison of mean defoliation levels two weeks after application for treatments that included 'Leaf Fall' only, showed no significant differences (H=3, p=1). Timing of application had a significant effect on the level of defoliation after two weeks (H=138, p<0.001). There was no significant interaction between the factors of treatment and time (H=13, p=0.12).

Analysis of data collected in mid-November showed a significant effect of application timing on end of season defoliation levels (H=83, p<0.001). At this recording point, no significant differences in the amount of defoliation could be attributed to treatments including 'Leaf Fall' (H=1, p=1), but could to treatment + timing interaction (H=23, p=0.003).

Figure 4. Mean apical meristem activity score (10=high, 0=low) vs. mean % defoliation 2 weeks after selected treatments incorporating 'Leaf Fall' applied Aug, Sep, Oct 2007 to *C. monogyna* at site A.



<u>August Treatments</u>

Approximately two weeks after completion of the August treatments (mid-September) some plants showed significant levels of leaf tissue damage, but no plants were more than 15% defoliated. Plants sprayed with 'Cuprokylt' + 'Leaf Fall' were defoliated most (Fig. 5).

<u>September Treatments</u>

Treatments involving 'Leaf Fall' were the most successful in inducing defoliation, when applied in September. 'Leaf Fall' sprayed at 50% of the recommended dose and 'Cuprokylt' + 'Leaf Fall' were the most successful treatments two weeks after completing the applications (Fig. 5).

October Treatments

Of the October treatments, the most successful was the recommended rate of 'Leaf Fall', resulting in more than 40% defoliation after two weeks (Fig. 5). This treatment was not, however, significantly more successful than the 50% dose of 'Leaf Fall', or the application of 'Cuprokylt' + urea + 'Leaf Fall' applied at the same time.

November Assessments

At the later recording period of mid-November (Fig. 6) control plants were still < 10% defoliated. Low defoliation rates were also recorded in those treatments not involving 'Leaf Fall'. At this period 'Leaf Fall' treatments applied in September were giving optimum defoliation, but later recordings (data not shown) indicated that applications in October were also equally (or in some instances more) effective.

Figure 5. Defoliation （％） recorded two weeks after application for all treatments applied to *C. monogyna* at site A.

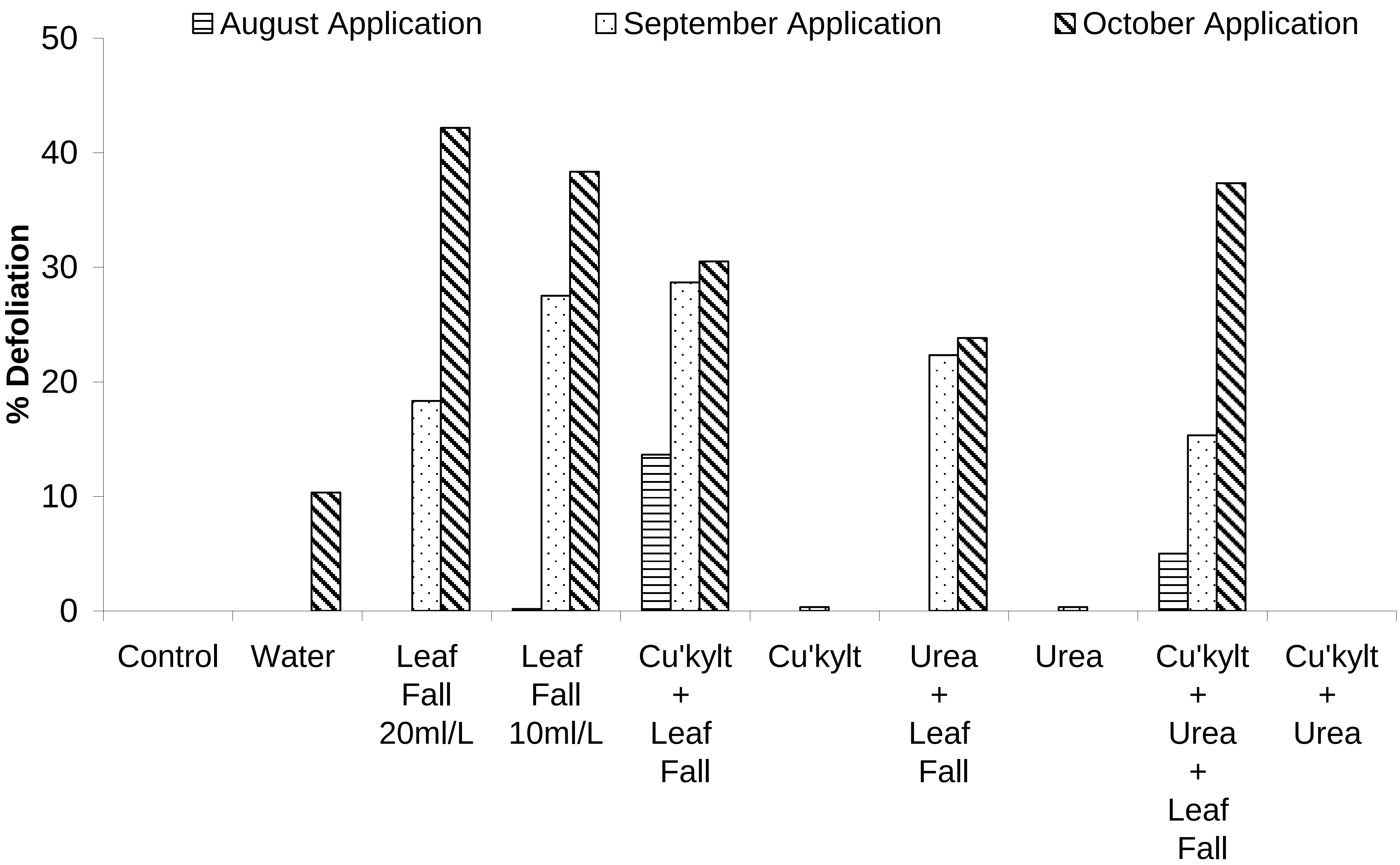
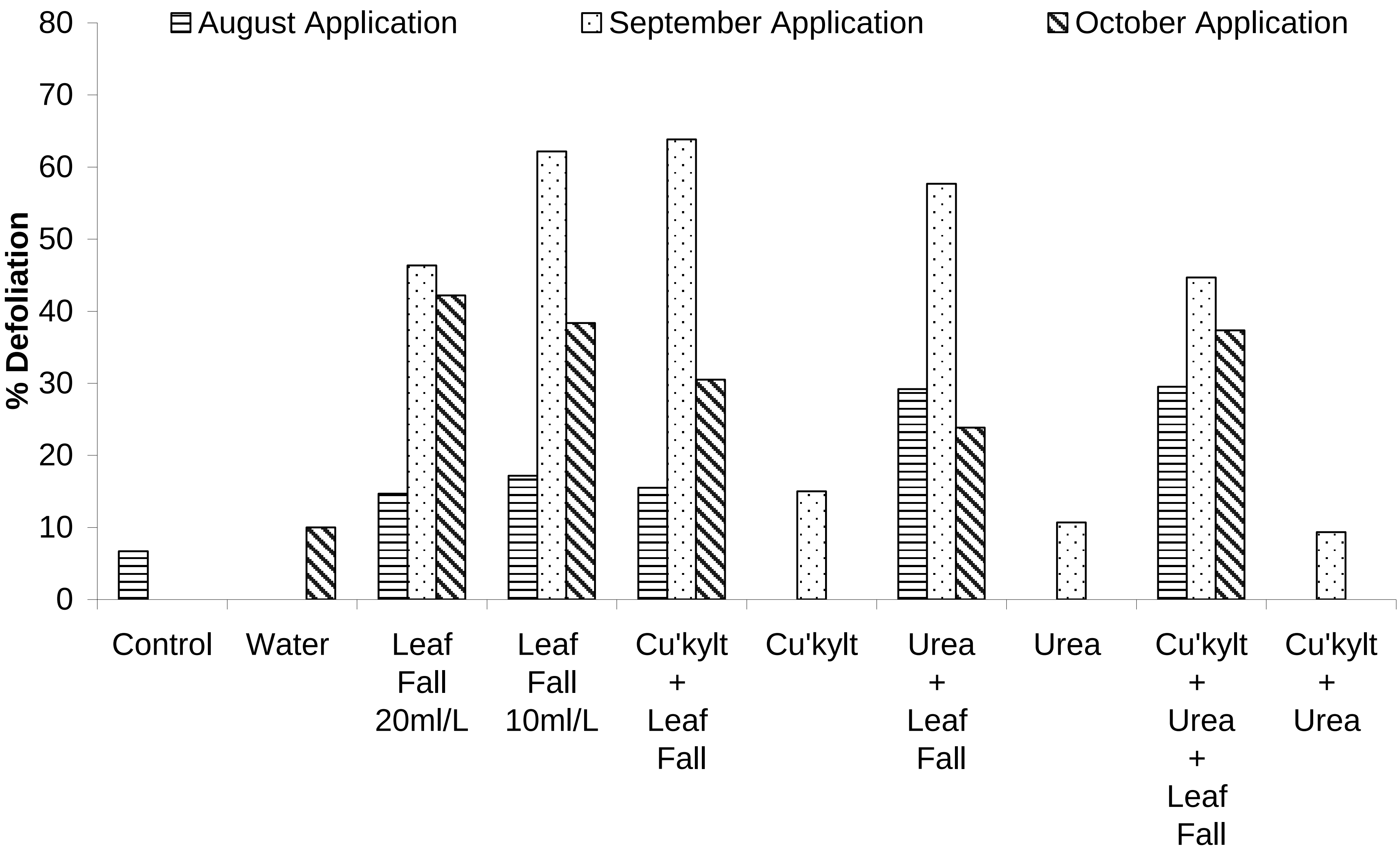


Figure 6. Defoliation (%) recorded on 14th November 2007 for all treatments applied to *C. monogyna* at site A.


% Defoliation
80
70
60
50
40
30
20
10
0
August Application
September Application
October Application
Control
Water
Leaf Fall 20ml/L
Leaf Fall 10ml/L
Cu'kylt + Leaf Fall
Cu'kylt
Urea + Leaf Fall
Urea
Cu'kylt + Urea + Leaf Fall
Cu'kylt + Urea

Q. robur

Due to poor growth rates, these plants were not lifted at the end of 2007 as originally intended and remained *in situ* to be lifted in autumn 2008. Considerable weed growth occurred in the experimental blocks by the time of the last chemical applications and thus spray penetration may have been compromised in the October treatments. There was greater leaf retention in this species compared to the *Crataegus*, with little evidence of leaf abscission until mid November (or evidence of leaf injury until mid-October; Table 3). Neither treatment, nor timing, caused significant differences in mean levels of defoliation ($H=0.1$, $P=1$ and $H=0.7$, $p=0.7$ respectively).

Table 3. Damage and defoliation of *Q.robur* at site A.

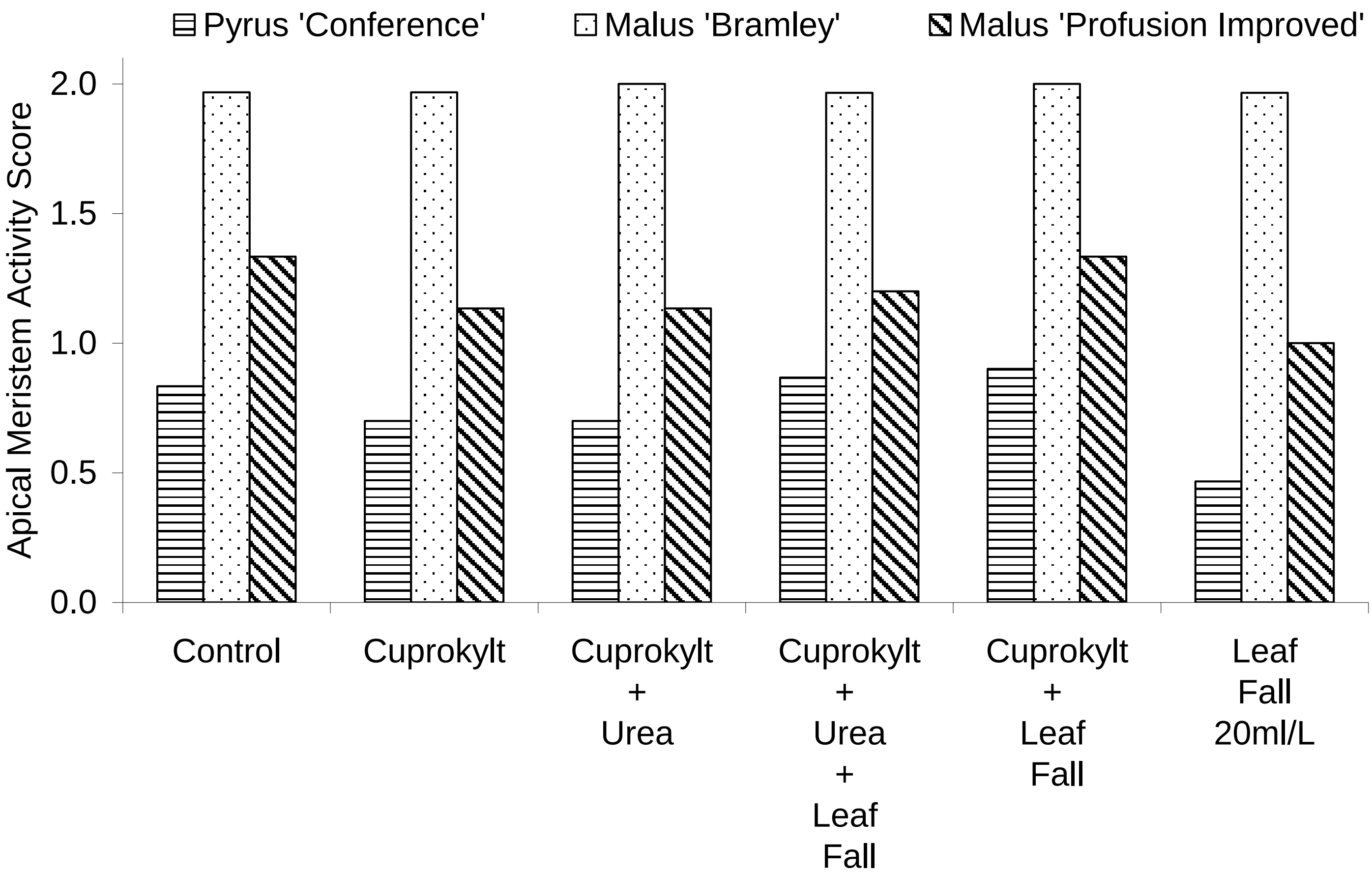
	Mean Apical Meristem Activity Score at Treatment	Mean % Damage			Mean % Defoliation
		17-Oct	30-Oct	13-Nov	13-Nov
Control	6.5	1.8	3.5	9.7	0.0
Water	1.0			13.2	0.3
'Leaf Fall' 20 ml l^{-1} August	2.3	4.3	6.7	10.5	0.0
'Leaf Fall' 20 ml l^{-1} September	2.4	8.7	18.0	29.2	1.3
'Leaf Fall' 20 ml l^{-1} October	0.3			17.5	0.0
'Leaf Fall' 10 ml l^{-1} August	1.9	4.3	8.3	14.8	0.0
'Leaf Fall' 10 ml l^{-1} September	2.3	12.7	22.2	30.0	3.7
'Leaf Fall' 10 ml l^{-1} October	1.1			23.2	0.0
'CuprokyIt' + 'Leaf Fall' August	5.0	5.5	14.3	18.0	5.0
'CuprokyIt' + 'Leaf Fall' September	2.1	4.7	7.3	14.0	0.0
'CuprokyIt' + 'Leaf Fall' October	1.7			18.8	0.0
'CuprokyIt' September	2.4	3.2	6.5	11.2	0.7
Urea + 'Leaf Fall' August	6.5	5.2	6.2	8.2	0.0
Urea + 'Leaf Fall' September	1.9	10.8	15.8	25.5	0.7
Urea + 'Leaf Fall' October	1.6			33.2	0.0
Urea September	2.4	4.3	9.8	16.8	0.0
'CuprokyIt' + Urea + 'Leaf Fall' August	6.6	8.7	12.7	17.3	0.0
'CuprokyIt' + Urea + 'Leaf Fall' September	3.1	8.3	14.5	28.0	1.7
'CuprokyIt' + Urea + 'Leaf Fall' October	1.3			20.0	0.3
'CuprokyIt' + 'Leaf Fall' September	2.6	7.0	9.7	17.3	1.0

Site B

Apical Meristem Activity

Pyrus 'Conference' showed noticeably reduced AMA in comparison with *Malus* 'Bramley' and *Malus* 'Profusion' (Fig. 7). Of those plants that received a score of 0, many carried senescent and abscising young leaves immediately below the shoot tip.

Figure 7. Mean apical meristem activity score recorded at the time of treatment application for *Pyrus* 'Conference', *Malus* 'Bramley' and *Malus* 'Profusion Improved' Site B.



Defoliation

By the end of October, approximately four weeks after completion of the September treatments, there was a significant difference in defoliation across treatments ($p < 0.001$)

Those plants that had been sprayed with 'Leaf Fall' showed far greater levels of defoliation than those that had not in all three species (Fig. 8). *Malus* 'Profusion Improved' was easiest to defoliate with the treatments employed and four weeks after completion of the applications, most trees sprayed with 'Leaf Fall' were almost totally leaf-free (Fig. 8). The untreated control plants of this species had lost approximately 25% of their leaves through natural stimuli alone.

In contrast to *Malus* 'Profusion Improved', *P.* 'Conference' displayed less than 50% defoliation across all six treatments. *Malus.* 'Bramley' was intermediate in its tendency to abscise leaves, with again 'Leaf Fall' being critical to obtaining a response (Fig. 8).

Trends were similar for October applications (data not shown), although in *Malus* 'Bramley' the amount of defoliation resulting after applying 'Cuprokylt', urea and 'Leaf Fall' was marginally lower.

In contrast to both *Malus* spp., where sensitivity to treatments increased down the leader, *Pyrus* 'Conference' was most sensitive to the defoliants in the upper third of the stem (Fig. 9, 10, 11) This species also displayed the lowest levels of AMA at the time of treatment application.

Figure 8. Defoliation (% of whole plants) of *Pyrus* 'Conference', *Malus* 'Bramley' and *Malus* 'Profusion Improved' Site B recorded 29th October 2007.

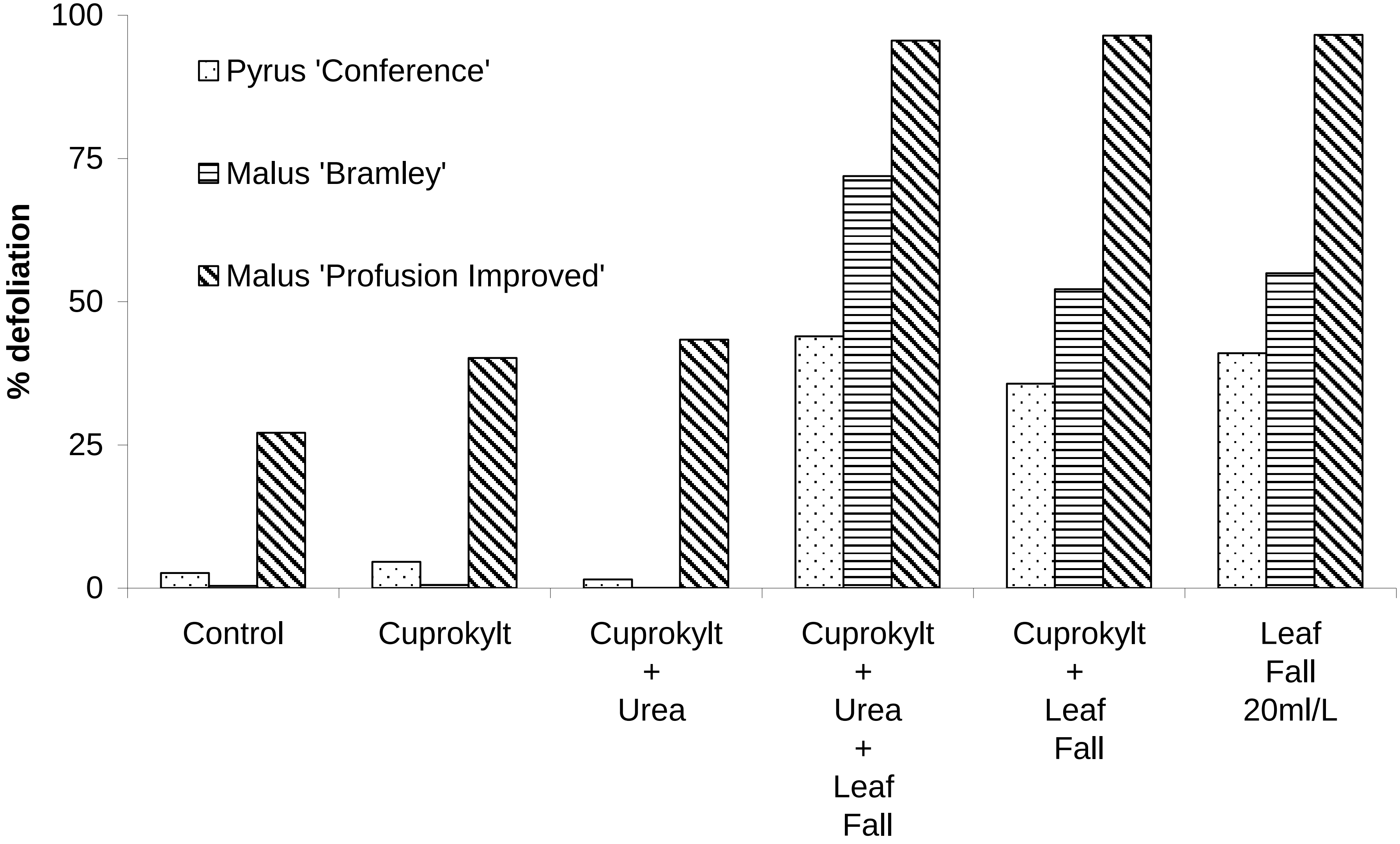


Figure 9. Defoliation (%) of *Pyrus* 'Conference' by stem zone recorded at site B 29th October 2007.

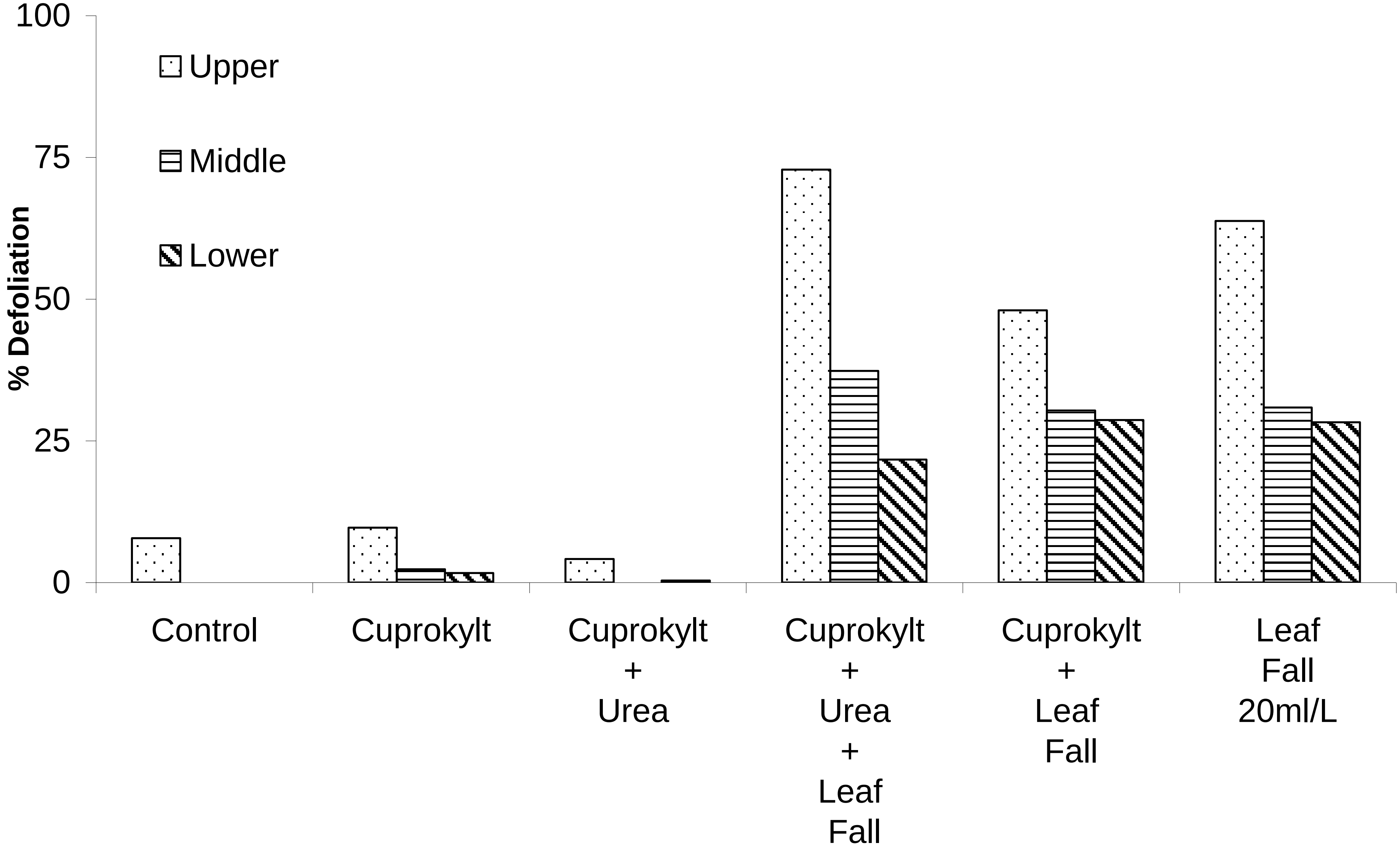

100
75
50
25
0
% Defoliation
Upper
Middle
Lower
Control
Cuprokylt
Cuprokylt + Urea
Cuprokylt + Urea + Leaf Fall
Cuprokylt + Leaf Fall
Leaf Fall 20ml/L

Figure 10. Defoliation (%) of *Malus* 'Bramley' by stem zone recorded at site B 29th October 2007.

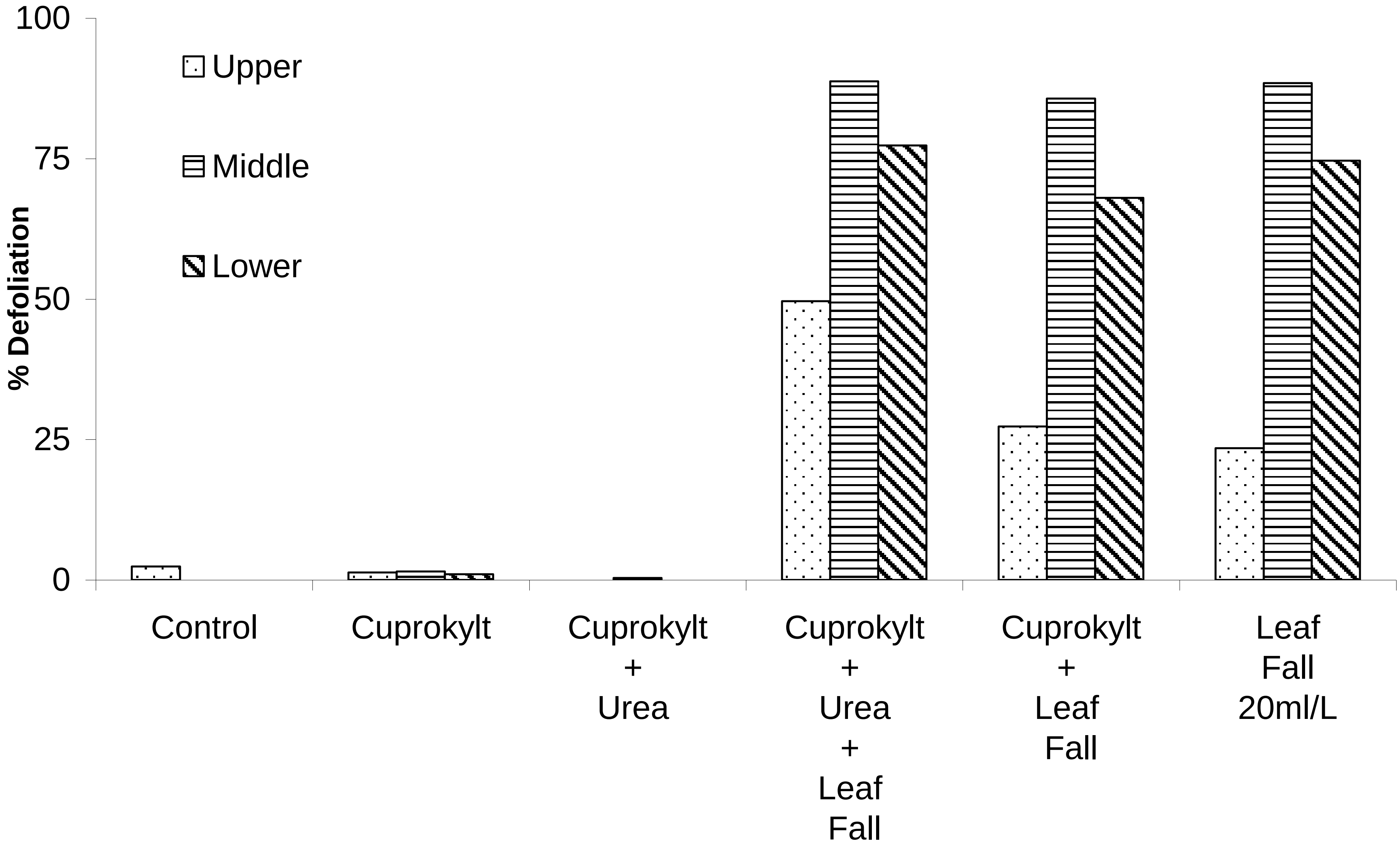
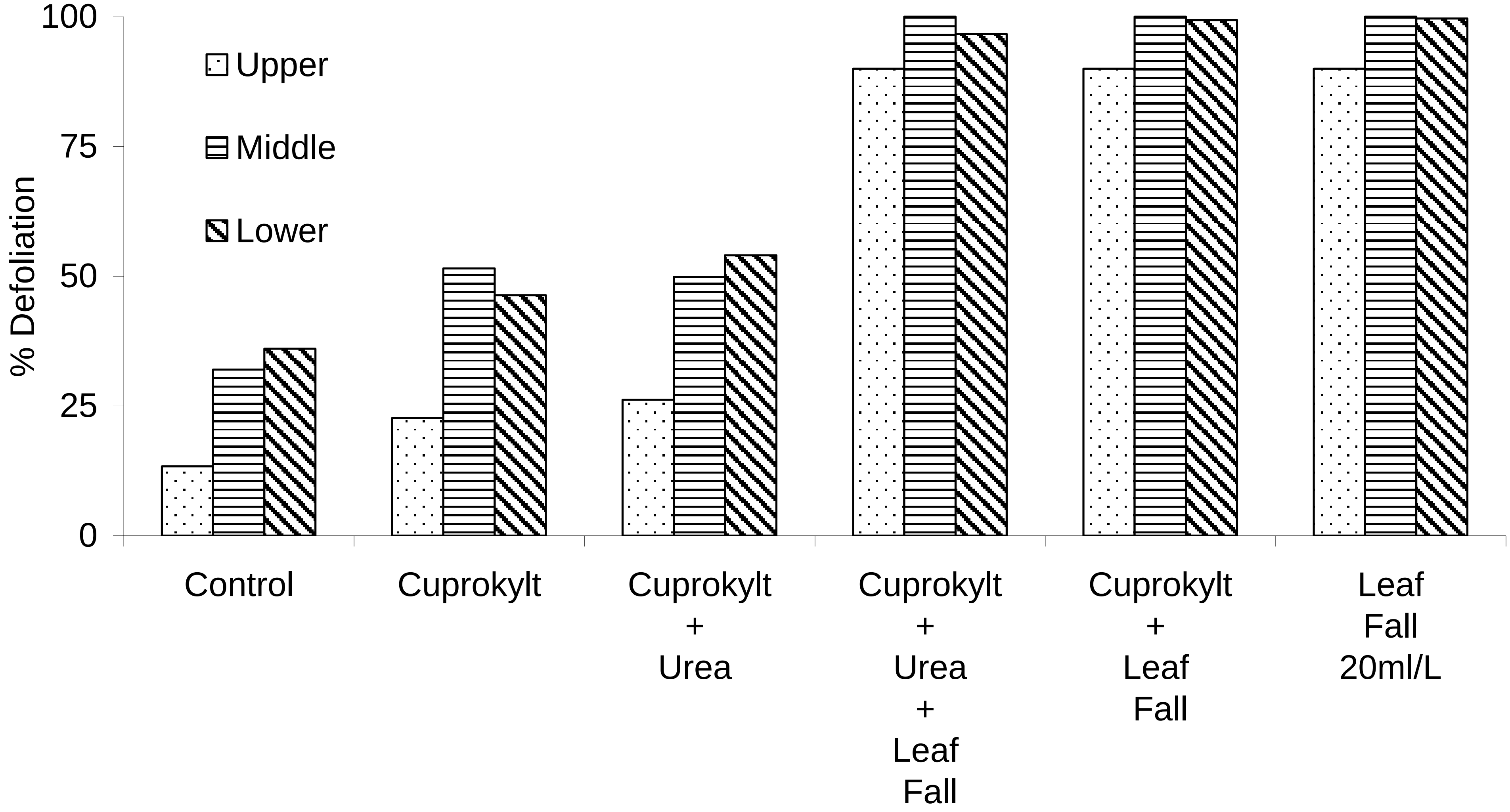

100
75
50
25
0
% Defoliation
Upper
Middle
Lower
Control
Cuprokylt
Cuprokylt + Urea
Cuprokylt + Urea + Leaf Fall
Cuprokylt + Leaf Fall
Leaf Fall 20ml/L

Figure 11. Defoliation (%) of *Malus* 'Profusion Improved' by stem zone recorded at site B 29th October 2007.



Experiment 2. Regrowth in *C. monogyna* during spring 2008

It was important to determine whether chemical treatments applied (and subsequent leaf abscission characteristics) influenced bud development in the spring following application. A separate experiment was employed to assess this and utilised plants retained from the earlier trial. These plants were then used to assess re-growth potential and tissue nitrogen content.

Materials and Methods

Thirty plants (10 from each block) from the following treatments were lifted on the 22nd November and reserved following the defoliation trials at site A:

Control

'Leaf Fall' 20ml l^{-1} (October)

'Leaf Fall' 10ml l^{-1} (October)

'Cuprokylt' + 'Leaf Fall' (September)

'Cuprokylt' + 'Leaf Fall' (October)

'Cuprokylt' + Urea + 'Leaf Fall' (September)

'Cuprokylt' + Urea + 'Leaf Fall' (October)

All plants were wrapped in 80 litre bags and cold-stored at 2°C for 29 days. No leaves were removed manually prior to storage as this treatment aimed to replicate storage conditions that commercially produced hedging plants experience prior to planting out.

Upon removal from storage the plants from each treatment were divided into two groups. 21 plants from each treatment were planted in 11 cm pots containing a mixture of 75% peat and 25% Perlite. These plants were grown in a heated glasshouse (temp range 13 – 34°C) with supplementary lighting to provide a 12-hour photoperiod. The remaining nine plants from each treatment 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. Following this operation, the samples were 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. The samples were stored in air-tight plastic bags at room temperature until further analysis.

Nitrogen analysis

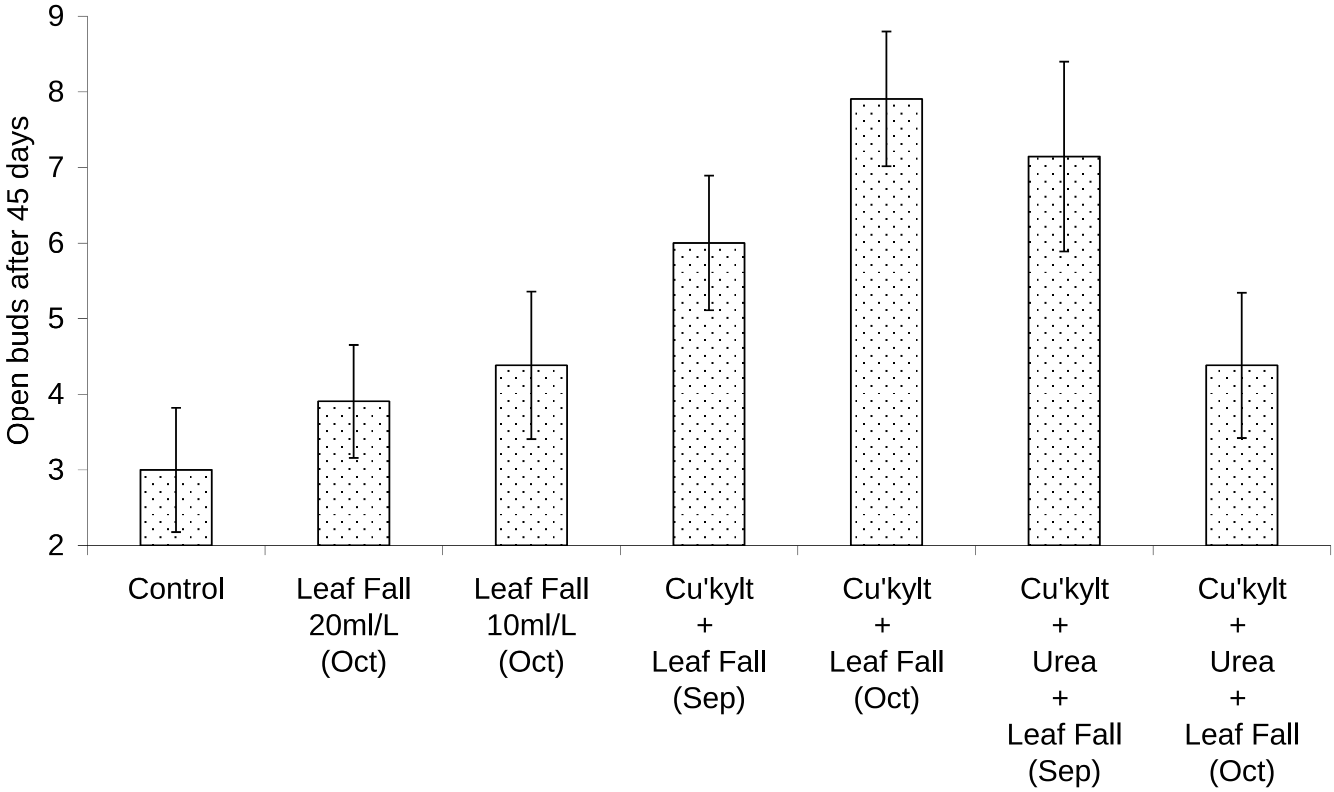
Total nitrogen content as a percentage of sample weight was obtained using an automated micro-Dumas method (Ma and Rittner, 1979) using a Europa Roba Prep elemental analyser.

Results

Bud break and regrowth

Seventeen days after being placed in the glasshouse bud burst was noted in all but the control and 'Leaf Fall' 20ml l^{-1} October treatments. By day 20 only the control plants remained dormant; no regrowth occurred on these until day 27, ten days after the quickest plants to break bud. From day 20 onwards, greatest growth activity was associated with the 'Cuprokylt' + 'Leaf Fall' (Oct) treatment (Fig. 12).

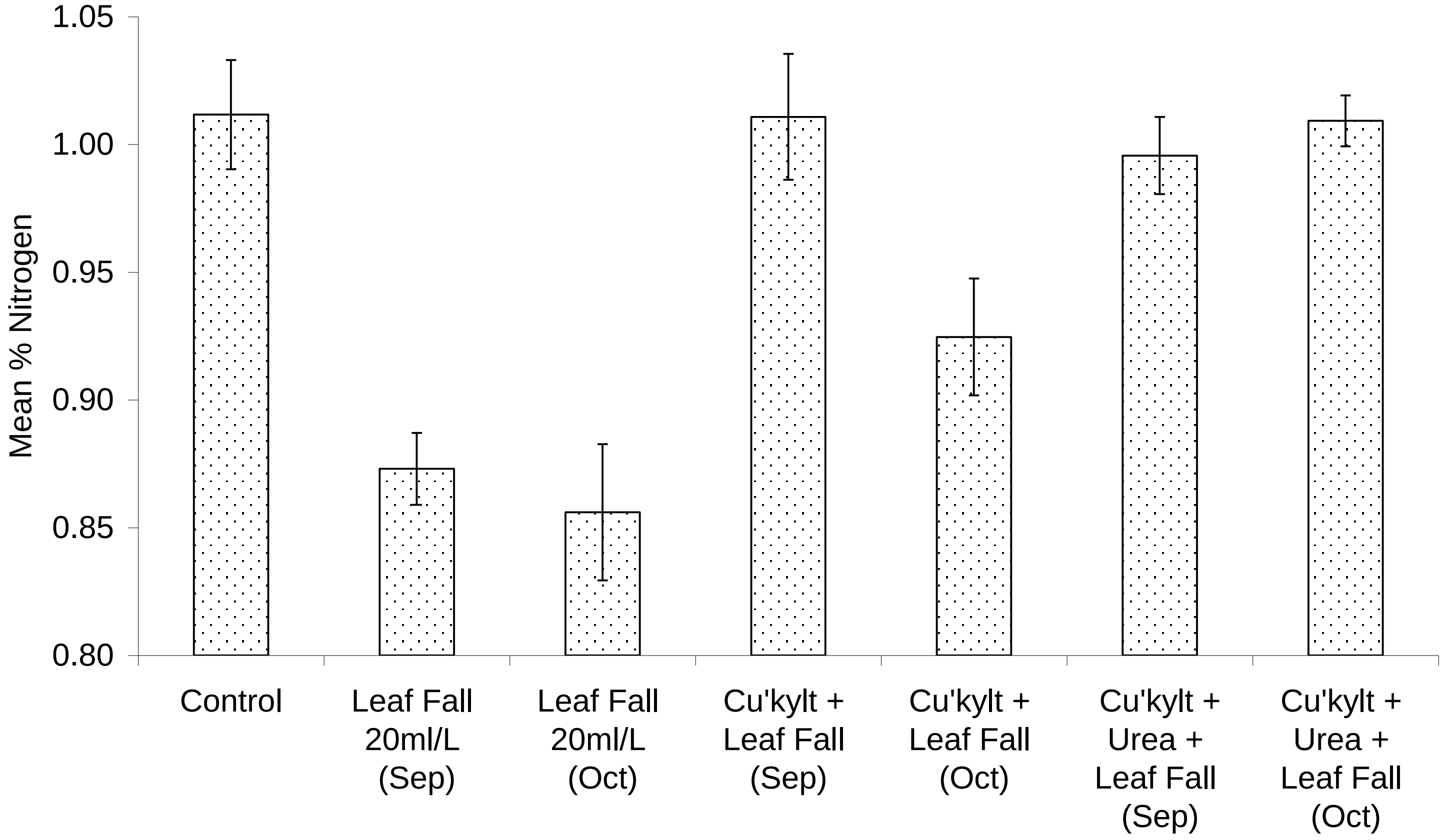
Figure 12. Mean number of active buds per plant after 45 days of regrowth for selected treatments made in autumn 2007 to *C. monogyna* at site A. (n=21, error bars represent s.e.)



Nitrogen Content

The total nitrogen content was higher in the control plants and those that received two-part treatments (Fig 13). Plants that had received urea in the treatment programme did not have the highest percentage of nitrogen by weight. Treatment with 'Cuprokylt' + 'Leaf Fall' in September resulted in plant tissue samples containing more nitrogen than the same treatment applied in October.

Figure 13. Total nitrogen content of plants from selected treatments. (Stem tissue only; n=3, error bars represent s.e.).



Dry weight accumulation

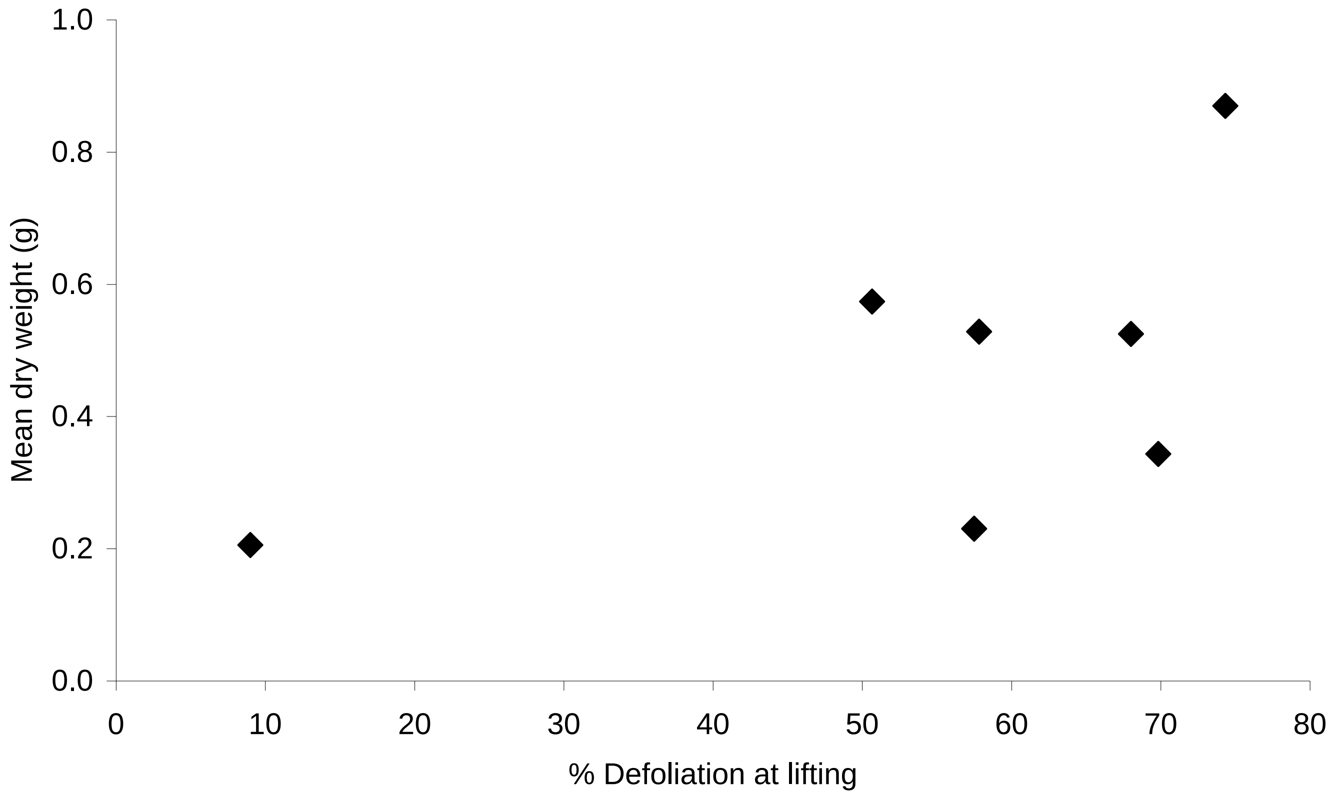
Control plants accumulated the lowest amount of dry weight over the period in the glasshouse (Table 4), which corresponded with the lowest number of active buds at the end of the trial (Fig. 12). Conversely plants treated with 'Cuprokylt' and 'Leaf Fall' in October produced both the highest mean number of buds and the most biomass per plant. Where growth was most vigorous, the activity tended to be confined to a small number of buds.

Table 4. Mean dry weight of new growth after 45 days of regrowth (n=7)

Treatment	Mean Dry Weight Accumulation (g)
Control	0.21
'Leaf Fall' 20ml l^{-1} (October)	0.53
'Leaf Fall' 10ml l^{-1} (October)	0.23
'Cuprokylt' + 'Leaf Fall' (September)	0.34
'Cuprokylt' + 'Leaf Fall' (October)	0.87
'Cuprokylt' + Urea + 'Leaf Fall' (September)	0.57
'Cuprokylt' + Urea + 'Leaf Fall' (October)	0.53
Least significant difference (P=0.05)	0.13

Of the seven treatments selected for the regrowth trial, the mean dry weight accumulation and the mean percentage defoliation (at the time of lifting on 22nd November) were both highest for plants treated with 'Cuprokylt' and 'Leaf Fall' in October and lowest in the control plants. The control plants, which had been defoliated least, also displayed the least accumulated dry weight after 45 days. For the remaining treatments, this small-scale experiment did not indicate a strong correlation between defoliation and post-storage plant vigour (Fig. 14).

Figure 14. Defoliation (%) for 22nd November (n=30) vs. mean dry weight increase in spring (n=21) for selected treatments.



Experiment 3. 'Folicur' fungicide pre-treatment to improve the efficiency of + 'Leaf Fall'

Observations by nurserymen suggest that the application of 'Folicur' fungicide (tebuconazole), a member of the triazole family, leads to a cessation of growth when used on *Crataegus monogyna* and various vegetable crops. Since the trials conducted in autumn 2007 showed that early applications of immobile ionic copper (Cu^{2+}) can result in the defoliation of the lower regions of a plant whilst the apical shoot continues to produce new healthy leaves, the possible combination of the two products may promote more effective defoliation of a field-grown deciduous tree. For this experiment juvenile Alder (*Alnus glutinosa*) were utilised. This species had been identified during initial consultations with nurserymen as one that has become increasingly prone to late season leaf retention. Furthermore, from a practical viewpoint, it represents a useful model species as it grows rapidly from seed and its large leaves facilitate data recording.

Materials and Methods

Table 5. Application schedule for the trial.

	Week			
Treatment	**1**	**2**	**3**	**4**
0,0,0,0	No spray	No spray	No spray	No spray
0,0,0,LF	No spray	No spray	No spray	'Leaf Fall' 20 ml l^{-1}
0,0,0,W	No spray	No spray	No spray	Water
Fo,Fo,Fo,LF	'Folicur' 10ml l^{-1}	'Folicur' 10ml l^{-1}	'Folicur' 10ml l^{-1}	'Leaf Fall' 20 ml l^{-}
Fo,Fo,Fo,Fo	'Folicur' 10ml l^{-1}	'Folicur' 10ml l^{-1}	'Folicur' 10ml l^{-1}	'Folicur' 10ml l^{-1}
W,W,W,W	Water	Water	Water	Water

The experiment consisted of six treatments, three involving 'Folicur' and 'Leaf Fall' and three controls applied to *A. glutinosa* over a period of four weeks (Table 5). Folicur (250 g l^{-1} tebuconazole) was applied at a rate of 10 ml l^{-1}. This corresponds to the manufacturers instructions to apply 1 litre of product in 100 litres of water. 'Leaf Fall' was applied at the recommended rate of 20 ml l^{-1} in line with the earlier stages of this project.

The plants were placed in an unheated glasshouse and given supplementary lighting from 1600 – 2000 to give a twelve hour photoperiod. Night temperatures did not fall below 10°C for the duration of the experiment. Pots were spaced so that plants were not touching, and held in place using plastic multiple pot holders.

Data recorded 21 days after completion of treatments

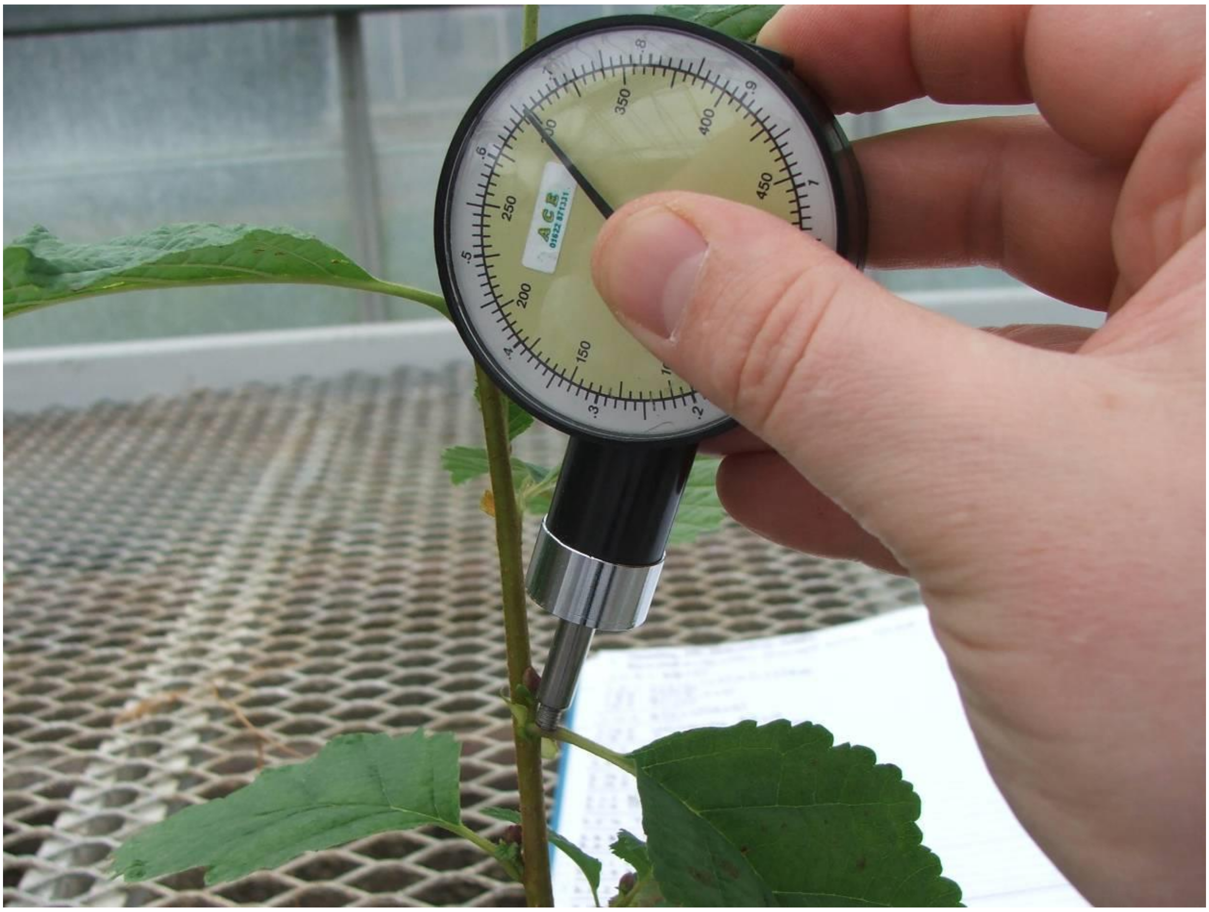
Leaf damage was recorded as a visual percentage score of damaged leaf tissue present as in previous experiments.

Leaf number per plant at application and at the end of the trial was recorded to ascertain the defoliant effects of each treatment.

Plant height (to the nearest 0.5 cm) and number of nodes was recorded at the beginning and end of the experiment. This data enabled the calculation of a growth rate for each treatment.

The amount of force required to separate the uppermost 8 leaves from the stems of 6 plants per treatment were recorded using a penetrometer to press down on the leaf petiole at the point of attachment (Fig. 15). If the leaves were absent, a force of 0 was recorded.

Figure 15. Use of a penetrometer to record detachment force of *A. glutinosa* leaves.



Results

Damage to leaves resulting from the 'Folicur' treatments was apparent 24 hours after the first application. Following applications of this treatment over the three subsequent weeks gave rise to increased damage and, on some plants, limited leaf abscission.

Four weekly applications of 'Folicur', gave rise to the highest levels of leaf loss at the end of the trial (Fig. 16). One application of 'Leaf Fall' made in week 4 resulted in less than 20% defoliation. The remaining leaves were also only mildly damaged, compared to those plants that were sprayed with both 'Folicur' and 'Leaf Fall'.

Figure 16. Mean % damage and defoliation in *Alnus glutinosa* 21 days after treatment with 'Folicur' and / or 'Leaf Fall' (n=24, error bars represent s.e.).

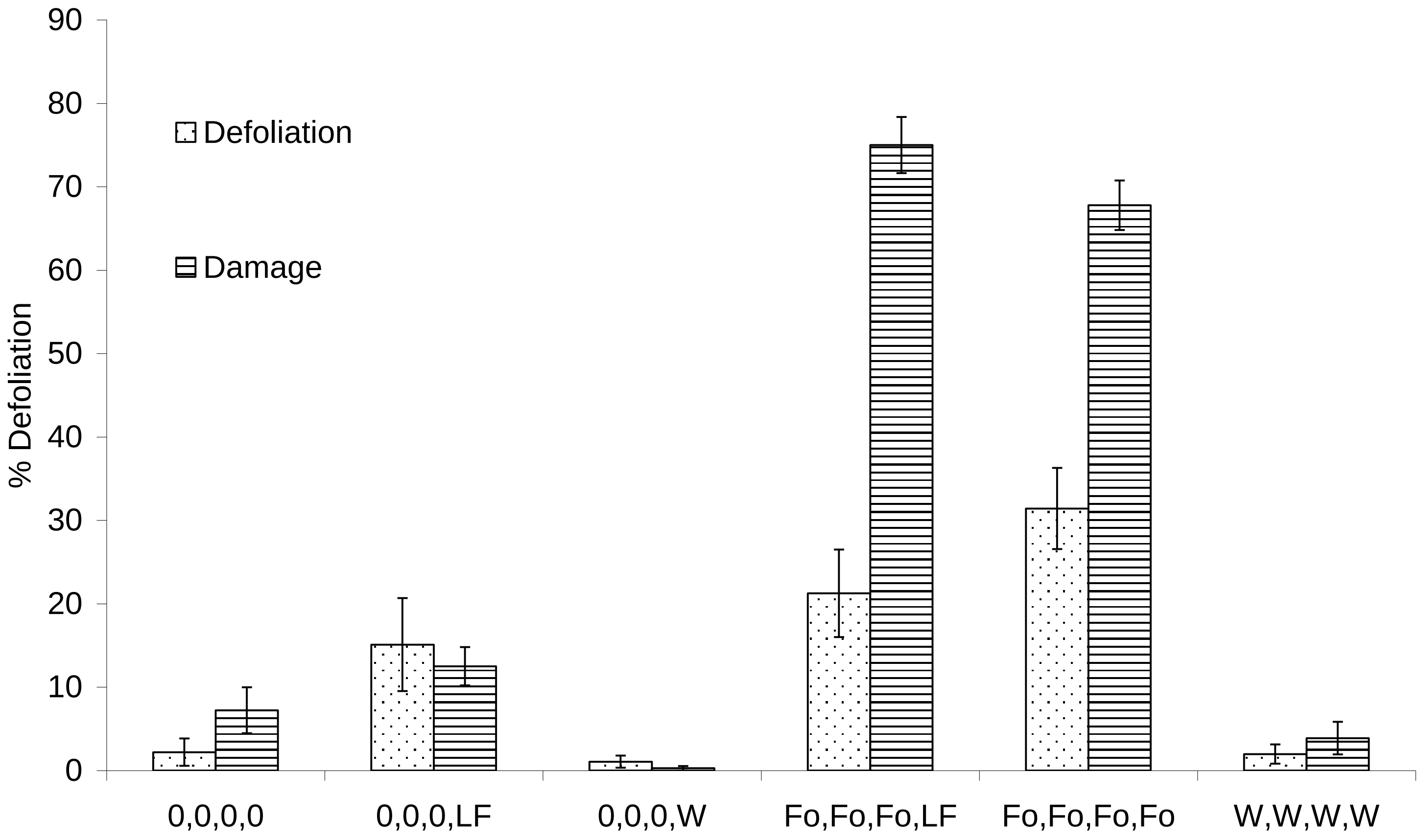
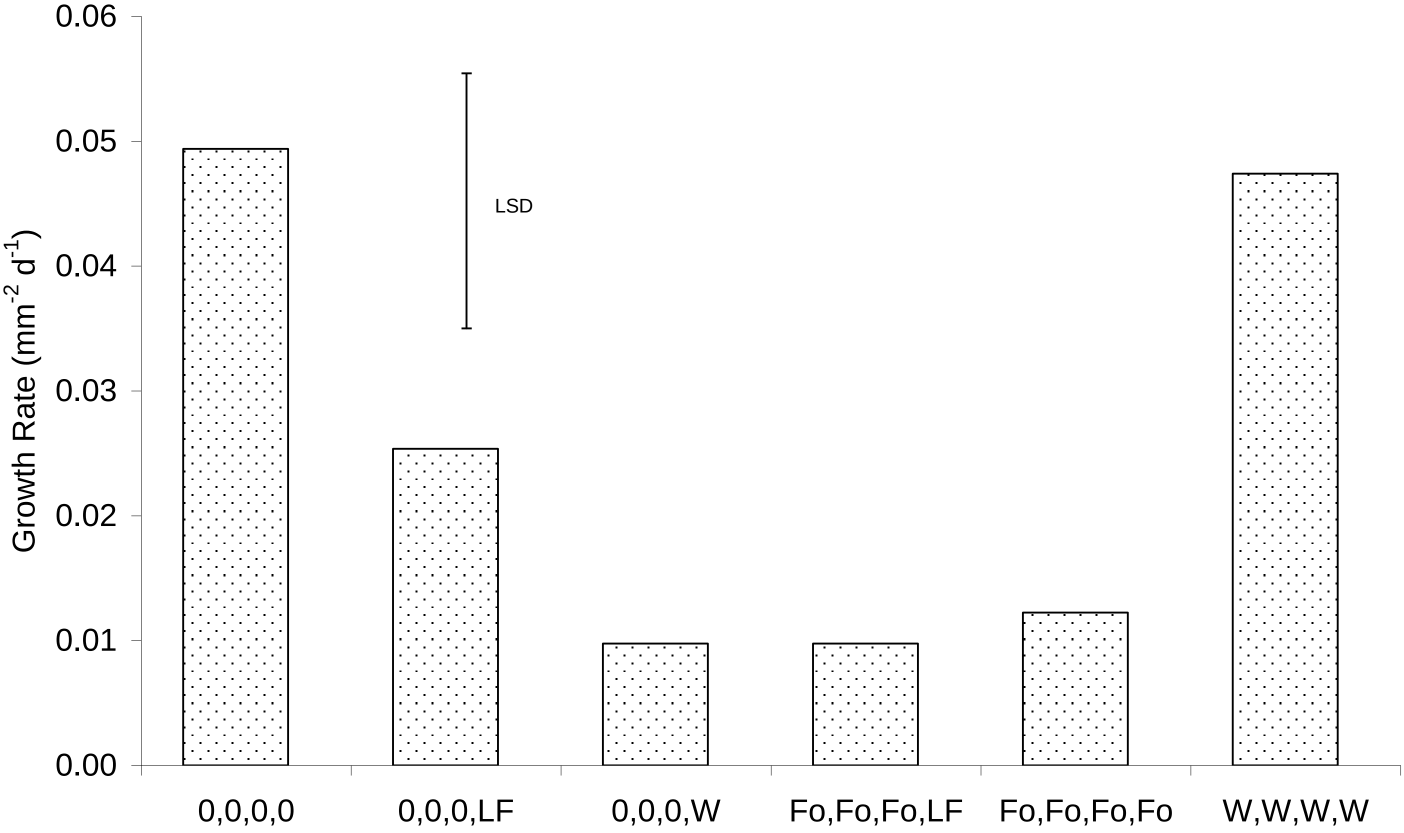


Figure 17. Mean growth rate of *Alnus glutinosa* measured as stem length increase between treatment and the end of the trial (p=0.05).



Growth rate was reduced by the application of 'Folicur' (Fig. 17), however the plants sprayed with water in week 4 also displayed little stem elongation during the trial. The plants sprayed 4 times with water and those receiving no spray showed significantly greater rates of stem elongation than the two 'Folicur' treatments.

Leaves were most easily removed after 4 treatments of 'Folicur' (Table 6). Those plants receiving 'Leaf Fall' in the final week instead of 'Folicur' were also relatively easy to detach (differences not significant from 'Folicur'). The application of 'Leaf Fall' alone caused leaves to require significantly more detachment force. The leaves of plants in this treatment were, however more easily detached than those from control plants.

Table 6. Mean force required to separate leaf petioles from stems at the point of abscission zone formation. This figure represents recorded forces on 8 leaves across 6 plants per treatment.

Treatment	Mean removal force (g) (n = 48)
Fo,Fo,Fo,Fo	156.6
Fo,Fo,Fo,LF	178.6
0,0,0,LF	332.8
0,0,0,W	442.9
W,W,W,W	464.5
0,0,0,0	477.4
Least significant difference (P=0.05)	68.4

Experiment 4. The effectiveness of FeEDTA as an alternative to CuEDTA ('Leaf Fall')

Knight (1983) found FeEDTA to cause limited defoliation on St. Julien A plum rootstock and *Q. rubra*, however, at the time the compound was not widely available commercially. This study used a commercially available nutrient containing FeEDTA designed for use in hydroponic systems to ascertain its effectiveness as an alternative to 'Leaf Fall'. *Salix* sp. was chosen for this experiment owing to its high vigour when struck from stem cuttings. It was envisaged that this attribute would amplify any difference between the two compounds.

Materials and Methods

The following treatments were applied in a warm glasshouse in long-day conditions to young *Salix* 'Tora' plants.

1. 'Librel' (13.2% w/w FeEDTA) 20 g l^{-1}
2. 'Leaf Fall' (9% Cu) 20 ml l^{-1}
3. Water
4. No Spray

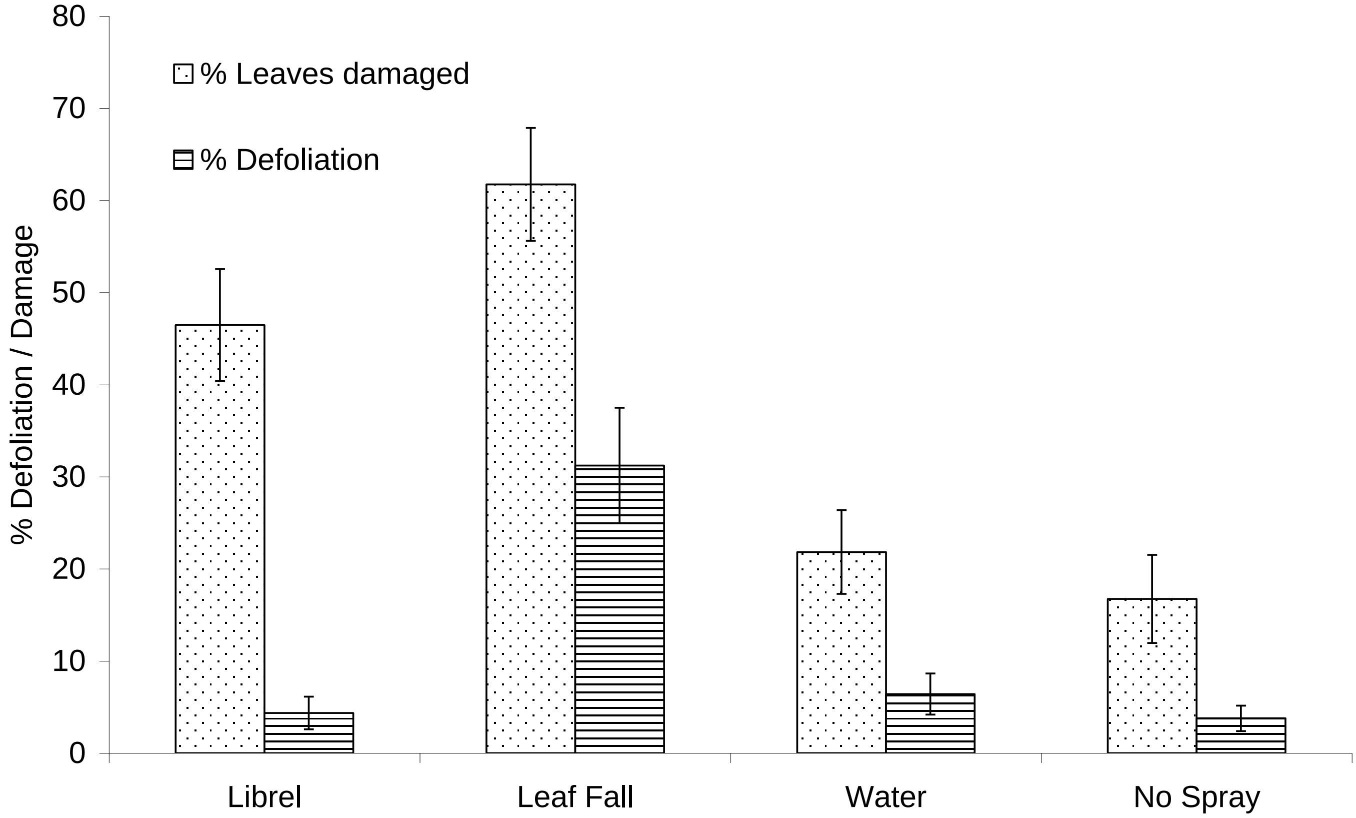
The plants had been grown from cuttings in warm glasshouse conditions under supplementary lighting for approximately three months prior to treatment. Fifteen plants were allocated to each treatment in a 3-block randomised design. Three weeks after treatment, the number of leaves with tissue damage and the number of absent leaves was recorded for each plant.

Results

21 days after treatment, over 60% of the leaves of the plants treated with 'Leaf Fall' were either damaged or had abscised (Fig 18). These plants had lost over 30% of their leaves compared with only 4% of the leaves of the plants sprayed with 'Librel'. Considerable leaf damage was also recorded on plants in the two control treatments;

however this appeared to be as a result of caterpillar damage and was reasonably constant across all plants in the trial.

Figure 18. Damage and defoliation (%) to *Salix* cv.'Tora' 21 days after treatment with FeEDTA or 'Leaf Fall'. (error bars represent s.e.).



Discussion

Of the defoliation regimes examined, only those that included 'Leaf Fall' gave rise to significant levels of leaf abscission. Even so the whole-plant response was limited on a number of species. The most effective treatments made to *C. monogyna* at UoR achieved 50 – 60% leaf loss. Results at site B were similar for *M.* 'Bramley' and *P.* 'Conference'. Each lost approximately 50% of their leaves by the end of October. *M.* 'Profusion Improved' showed the greatest response to treatment, with near complete leaf loss resulting from all treatments incorporating 'Leaf Fall'. By mid-November, 64% defoliation of *C. monogyna* was achieved by applying 'Cuprokylt' and Leaf Fall in September at site A. At the same site, *Q. robur* was least responsive to the defoliants; no treatment resulted in more than 5% of the leaves to be lost.

When 'Leaf Fall' was applied on its own to *C. monogyna*, the 50% strength treatment (10 ml l^{-1}) applied in September was most successful at both sites. In fact at site A, the amount of defoliation achieved by applying a half-strength dose in September was comparable to that achieved with the recommended concentration in October. This suggests that if plants have reached the required size by the end of September, this earlier treatment may be more economical. Measured at UoR, however, the September application of 50% strength 'Leaf Fall' gave rise to the most plants showing apical meristem necrosis, although this was not significantly greater than other treatments that resulted in similar levels of defoliation. So, even at this concentration there is danger of injury to tissues when the 'Leaf Fall' is applied early.

Copper ions, like those of other heavy metals cause lipid peroxidation, damaging cell membranes (Chen and Kao, 1999; Luna *et al.*, 2001). 'Leaf Fall' is effective as the Cu^{2+} ions are in solution whereas other, insoluble copper compounds are reliant upon biological exudates or physical weathering to dissolve them slowly, which makes them useful as fungicides (Hassall, 1990; Cremlyn, 1991). However, whilst the high level of solubility is beneficial insofar as a larger amount of damage may occur over a given amount of time, this property does render 'Leaf Fall' more susceptible to dissolution by rain and dew once it is on the leaf. To be effective, therefore, all defoliants must achieve maximum leaf penetration as rapidly as possible and this occurs via cracks in cuticular wax or via the stomata, which are mainly located on the abaxial surface of the leaf (Schönherr, 2001). Cuticle damage in *C. monogyna* at the University of Reading, caused by powdery mildew (*Podosphaera clandestina*) may thus have increased the effectiveness of the treatments allowing more to enter the leaf (Isaac, 1992). Reduced control of leaf pathogens by nurserymen towards the end of the growing season may therefore improve the efficacy of defoliants and reduce costs. This, of course, has implications for disease control in the plants in the following year.

The leaves of the upper portion of the main stems of *M.* 'Bramley', *M.* 'Profusion' and *C. monogyna* were all substantially less affected by defoliation treatments. The effect of ethylene, released from damaged leaf tissue is reduced in young leaves where its ratio to auxin content is lower (Taiz and Zeiger, 1998). However, it was also observed that these uppermost leaves actually showed very little tissue damage, even though such leaves are generally perceived as being less robust and more prone to damage than older ones. This may also be as a result of the angle at which they are held in relation to the stem i.e. the more acute the angle; the less spray will be retained. In *M.* 'Bramley', the pubescence of young leaves may have reduced the effectiveness of defoliants, whereas in other species, the integrity of cuticular wax may have been greater in young leaves, giving rise to the same phenomenon. Wetting agents and penetrants may therefore improve the effect of defoliants on this type of leaf and further research on this species may be warranted.

Undercutting *C. monogyna* during August at site A significantly reduced the activity of the apical meristem; even so, it was possible to detect moderate to strong correlations between shoot activity and date. The plants at UoR were one year older than those at site A, thus it may be fair to assume a slightly earlier cessation of growth associated with a reduced 'juvenility effect' in these plants. There was no such correlation in *Q. robur* at site A or all three species at site B. Whilst the negative relationship between date and apical meristem activity was stronger at UoR, the most effective treatments resulted in higher levels of defoliation at site A. Abscisic acid (ABA) associated with drought stress is recognised as a factor in ethylene production, and thus organ abscission, as well as hastening entry into dormancy. (El and Hall, 1974; Guak and Fuchigami, 2001) A reduction in the plants' capacities to take up water as a result of undercutting may therefore have played an important role in improving the action of the defoliants.

When plants were selected for regrowth assessment and tissue analysis, higher levels of total plant nitrogen were associated with two-part defoliation regimes. In such treatments, the most damaging compound, 'Leaf Fall' was applied second, potentially highlighting the benefits of a low level of initial stress to initiate the senescence of the leaf and prompt the breakdown and recovery of proteins and amino acids.

Although there was no significant linear correlation between the variables, the highest levels of defoliation gave rise to the greatest amount of new growth (dry weight) in the regrowth trial; the plants defoliated least, the control plants, produced the lowest amounts of new growth. The poor regrowth in control plants may be as a result of disrupting the dormancy induction process (buds less responsive to the subsequent 29 days chilling compared to those that had been chemically treated) or possibly greater desiccation in storage due to the high amount or retained foliage. Significantly, the largest amount of regrowth corresponded to only the fourth highest total nitrogen content, suggesting that other factors, such as carbohydrate content or increased desiccation may be more important than nitrogen recovery in determining the success of plant establishment.

Applications of FeEDTA in the form of 'Librel' did not achieve comparable rates of defoliation in *Salix* sp. when trialled alongside 'Leaf Fall'. There was significant damage to the leaves of plants receiving 'Librel', but within the duration of the experiment, defoliation was not significant. Further experiments may be warranted with this compound in order to ascertain its potential at greater concentration as a less environmentally damaging defoliant.

Folicur gave encouraging results at UoR. The active ingredient, tebuconazole, is a member of the triazole fungicide group, and is used to control numerous fungi in agricultural crops, especially wheat and Oil Seed Rape. The fungicidal action of triazole fungicides is attributable to their ability to reduce the biosynthesis of sterols that are essential in maintaining the stability of lipoprotein membranes (Hassall, 1990). Like heavy metal ions, then, tebuconazole appears to cause leaf damage by disrupting cell membranes. The ensuing synthesis of ethylene as a result of this damage then drives leaf abscission. Again, further trials, in a commercial context may yield useful data on the potential of this compound as an aid to defoliation.

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. Whether this is primarily due to higher autumn temperatures (Bisgrove and Hadley, 2002) or increasing concentrations of CO_2 (Taylor *et al.*, 2008), data collected during these experiments suggests that reducing plant vigour at the end of the season may be key in guaranteeing the future efficiency of chemical defoliation treatments. A portion of future work on this project must therefore be concerned with understanding how the relationships between plant dormancy, leaf senescence and abscission will be affected by future changes in climate. Development of techniques that mitigate the effects of climate change and induce earlier dormancy or at least slow growth will also form a part of this work. Stem manipulation (Jaffe, 1973) or the induction of water stress may represent two possible cost-effective cultural methods to achieving this. Alternatively further investigation of the use of growth retardants to induce dormancy (MacDonald, 1995) may have some merit where the cost of treatment in relation to the value of the plants being produced is not inhibitive.

Due to results from year 1 and further consultations with nurserymen, the milestones of the project have been revised. These are now outlined in Appendix 3.

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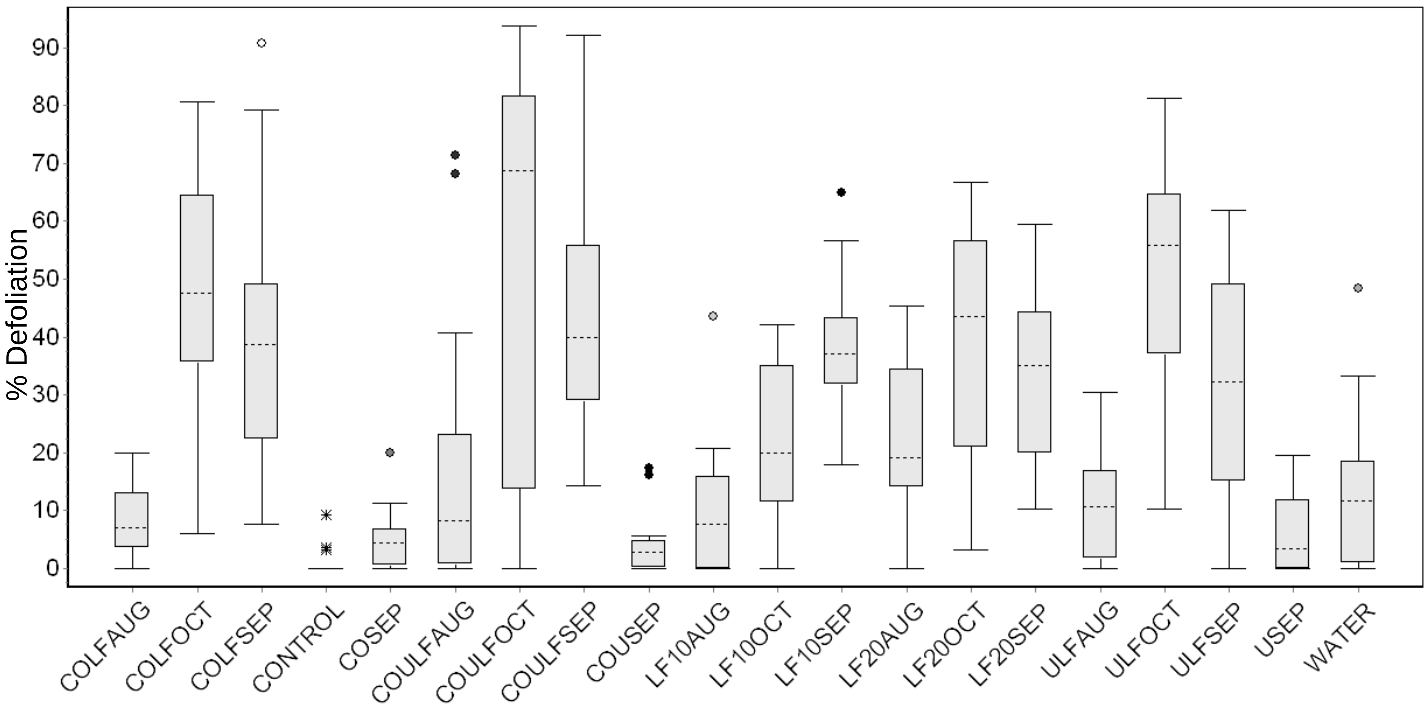
Appendix 1. Scoring Criteria for *C. monogyna* and *Q. robur*

Score	*C. monogyna* UoR	*C. monogyna* SITE A	*Q. robur* SITE A
0	Lignified stem, dark green leaves to top of stem No red-stemmed new growth		Terminal bud not enlarged
1	Enlarged terminal bud		
2	Tiny new red leaves discernable	1 or 2 new red leaves opening	Opening apical bud
3	Leaves larger Up to 3 cm stem growth	3 – 4 new leaves opeing	Individual leaves discernable
4	3-5 cm stem growth	As 4 + red, unlignified stem extension below bud	As 3 but some stem extension below bud
5	Small new leaves fully open	New leaves turning pale green Up to 2cm stem extension	New leaves opening
6	Large but pale green new leaves	2-3 cm new growth	Pronounced sinuate margins on new foliage
7	5-10 cm of new stem growth	3-4 cm new growth	Substantial stem extension below apical bud
8	10+ cm stem extension Stems are not robust	4-5 cm new growth	Axillary buds on stem opening
9	First leaves of new flush darkening	> 5 cm red extension growth	Secondary stems growing at lower nodes
10	>5 growth points Thick red-stemmed extension growth	As 9 + new leaves now turning darker green	Vigorous growth from 2 or more nodes

Appendix 2. Preliminary data analysis of whole plant defoliation data for all sites

Boxplot of *C. monogyna* defoliation two weeks after treatment at University of Reading.



Boxes represent the inter-quartile range (between Q1 and Q3)

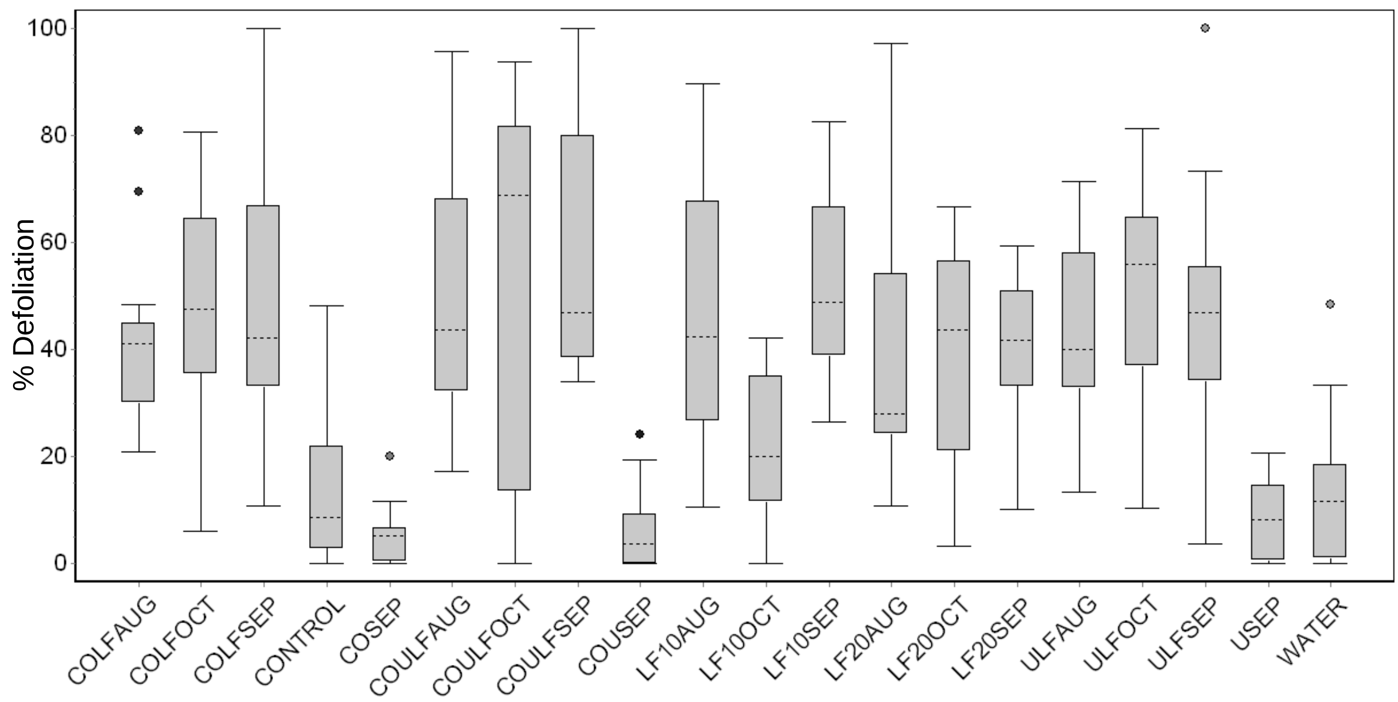
'Whiskers' represent the extremes of observed (non-outlier) values

KEY (all boxplots)

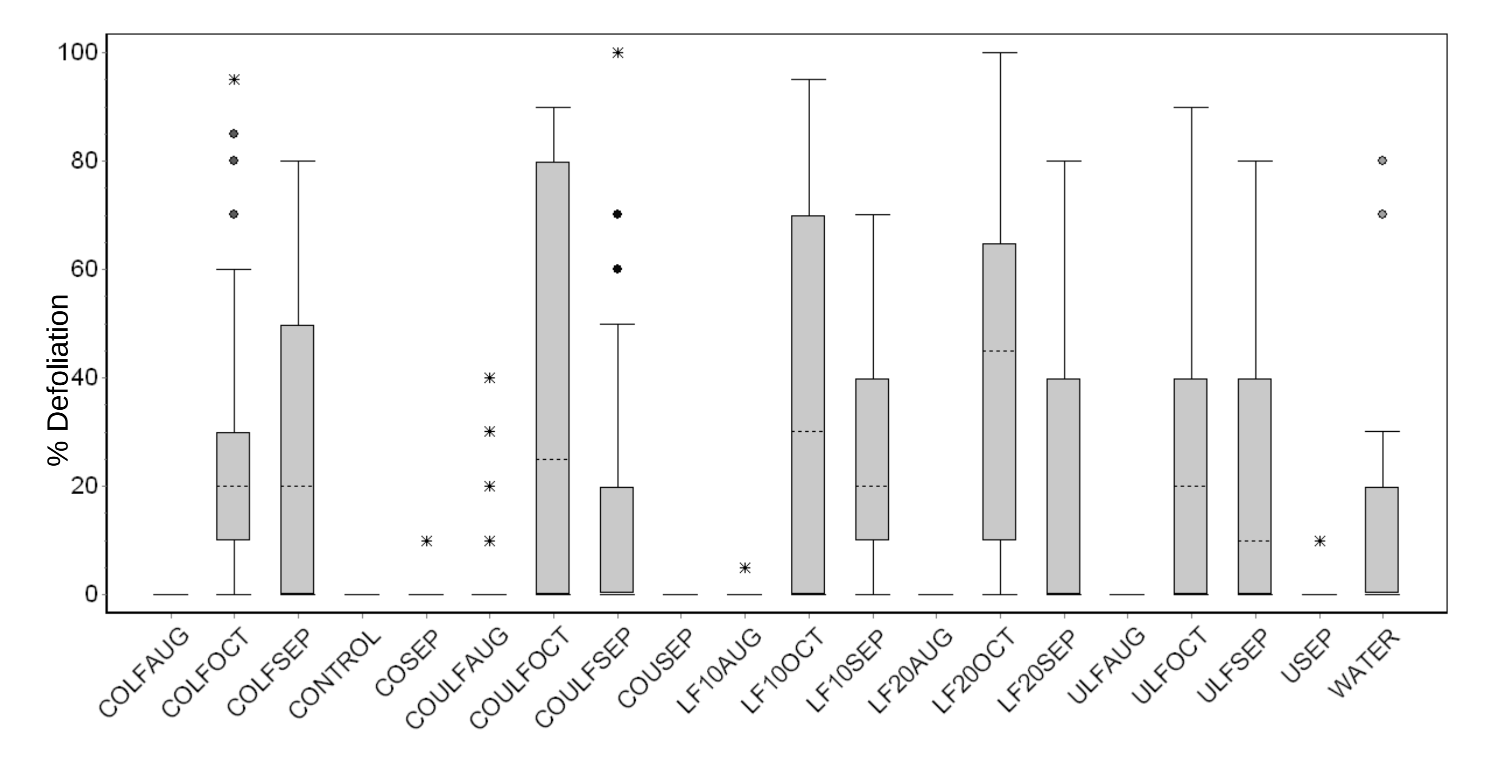
---	= median
X	= extreme outlier
O, ●	= mild outlier
CONTROL	Control
WATER	Water
LF20AUG	'Leaf Fall' 20 ml l^{-1} August
LF20SEP	'Leaf Fall' 20 ml l^{-1} September
LF20OCT	'Leaf Fall' 20 ml l^{-1} October
LF10AUG	'Leaf Fall' 10 ml l^{-1} August
LF10SEP	'Leaf Fall' 10 ml l^{-1} September
LF10OCT	'Leaf Fall' 10 ml l^{-1} October
COLFAUG	'Cuprokylt' + 'Leaf Fall' August
COLFSEP	'Cuprokylt' + 'Leaf Fall' September
COLFOCT	'Cuprokylt' + 'Leaf Fall' October
COSEP	'Cuprokylt' September
ULFAUG	Urea + 'Leaf Fall' August
ULFSEP	Urea + 'Leaf Fall' September
ULFOCT	Urea + 'Leaf Fall' October
USEP	Urea September
COULFAUG	'Cuprokylt' + Urea + 'Leaf Fall' August

COULFSEP 'Cuprokylt' + Urea + 'Leaf Fall' September
COUSEP 'Cuprokylt' 'Leaf Fall' September
COULFOCT 'Cuprokylt' + Urea + 'Leaf Fall' October

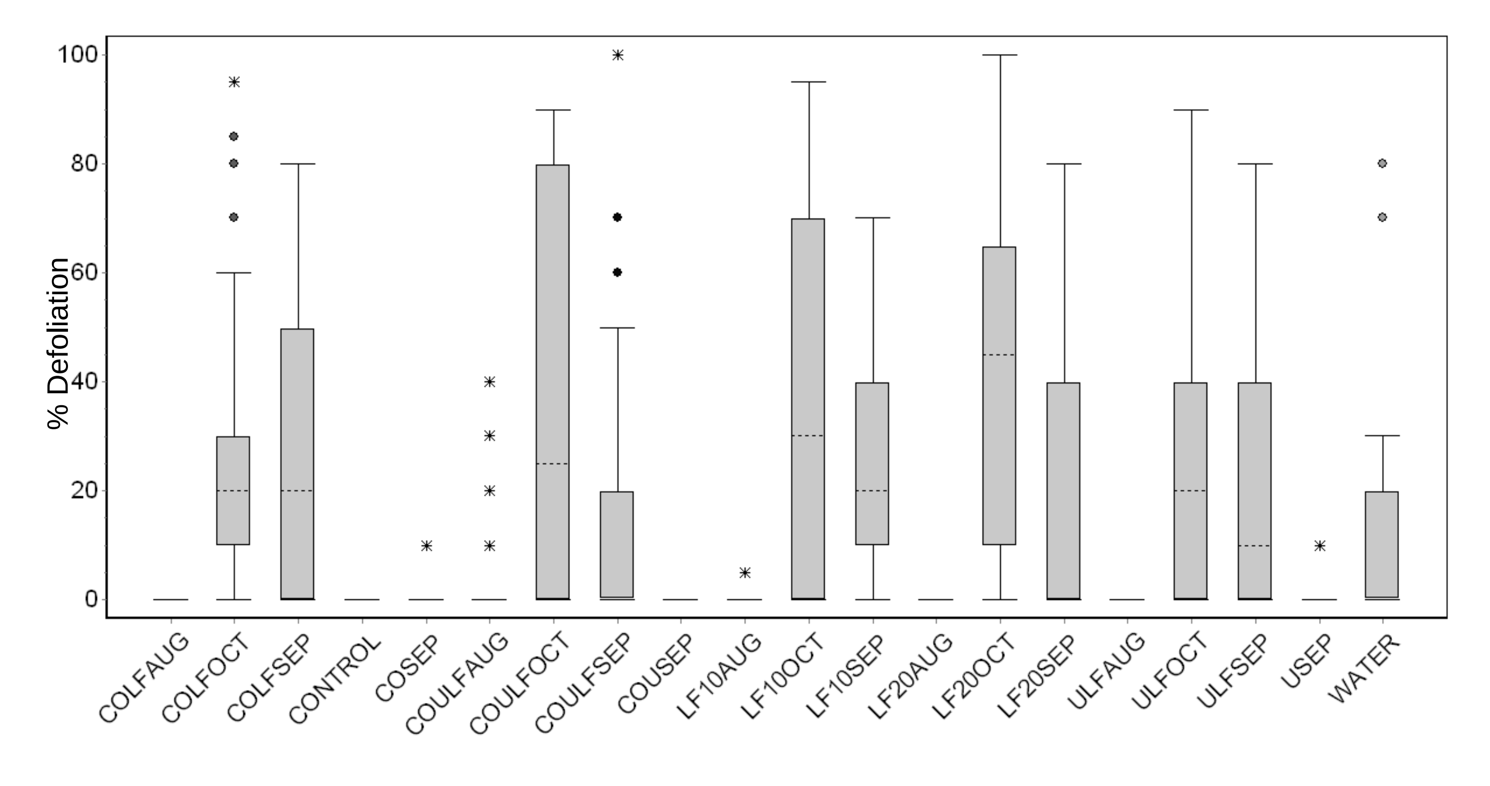
Boxplot of *C. monogyna* defoliation in mid-November at University of Reading.



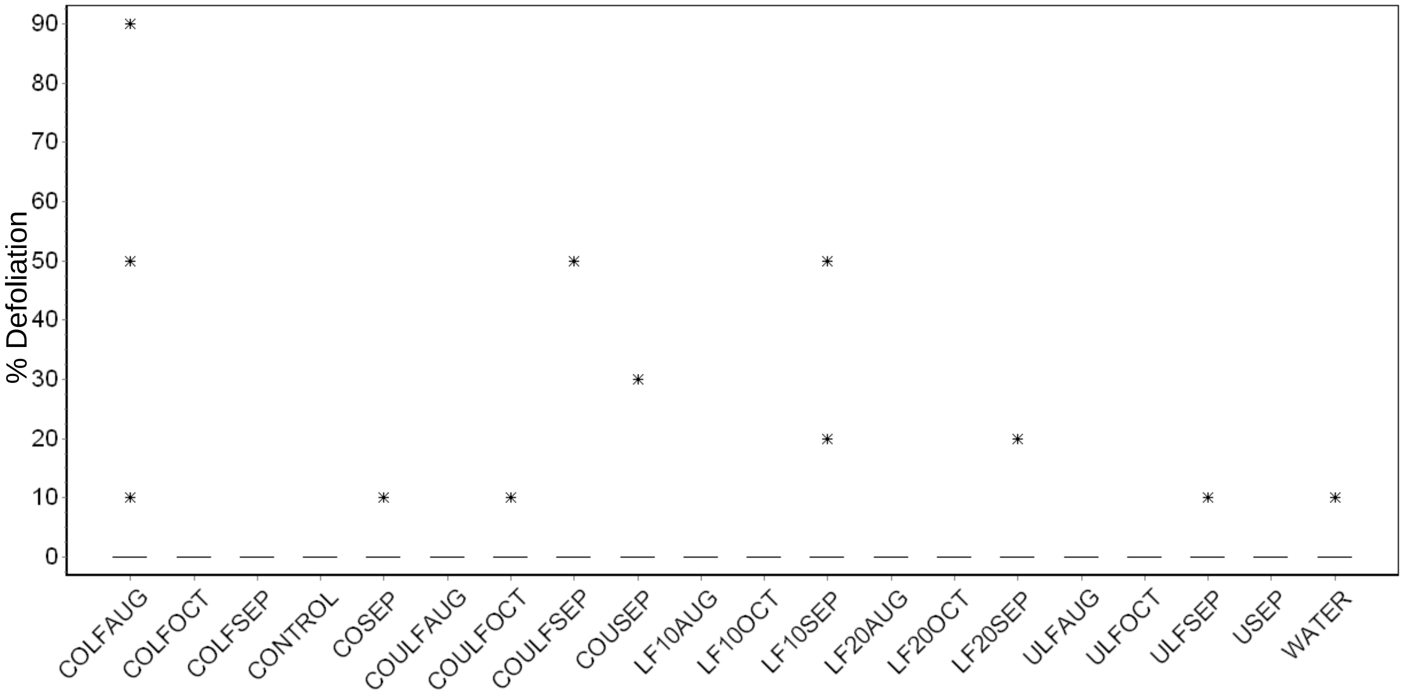
Boxplot of *C. monogyna* defoliation two weeks after treatment at site A.



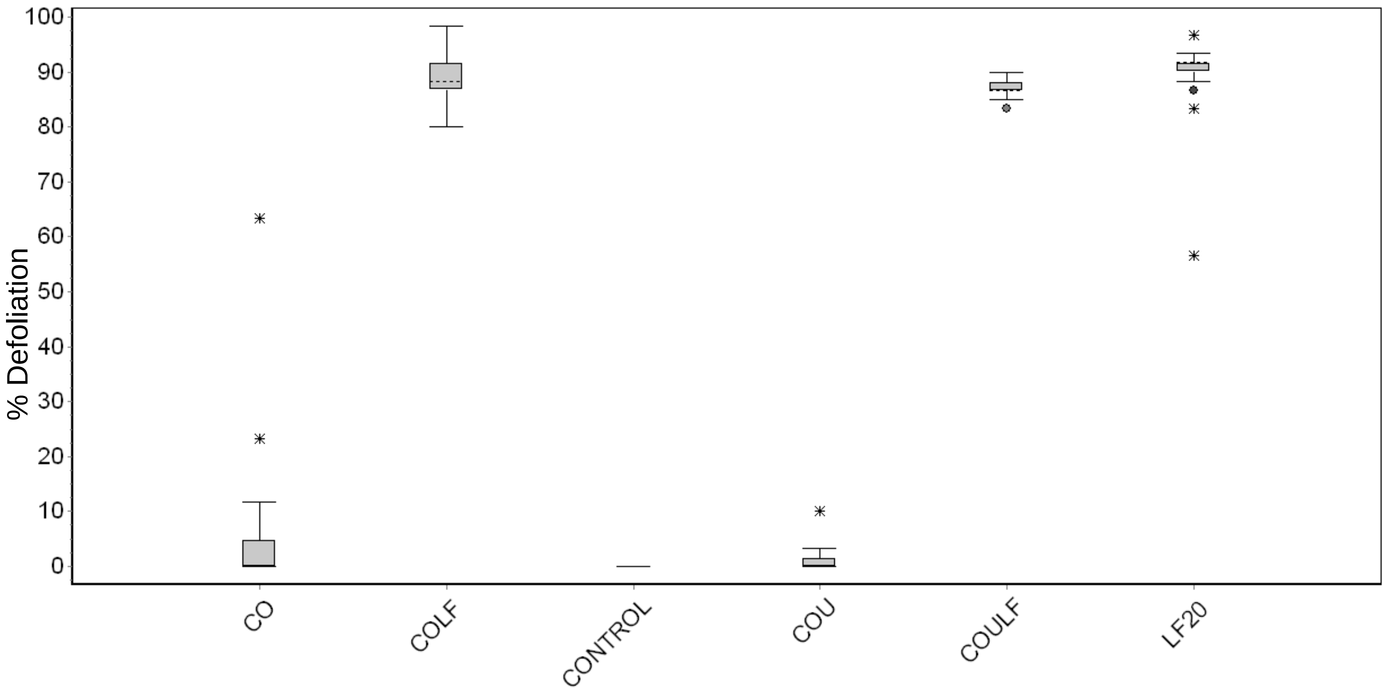
Boxplot of *C. monogyna* defoliation in mid-November at site A.



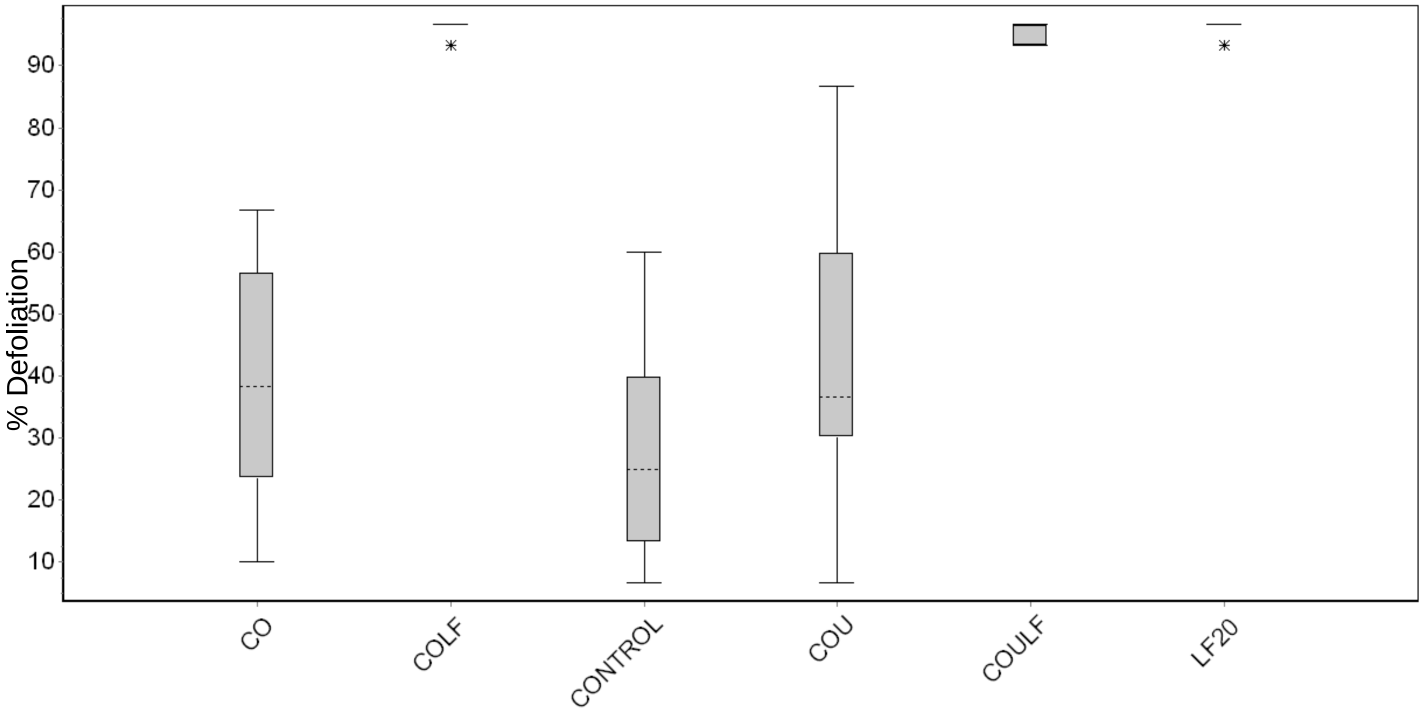
Boxplot of *Q. robur* defoliation in mid-November at site A.



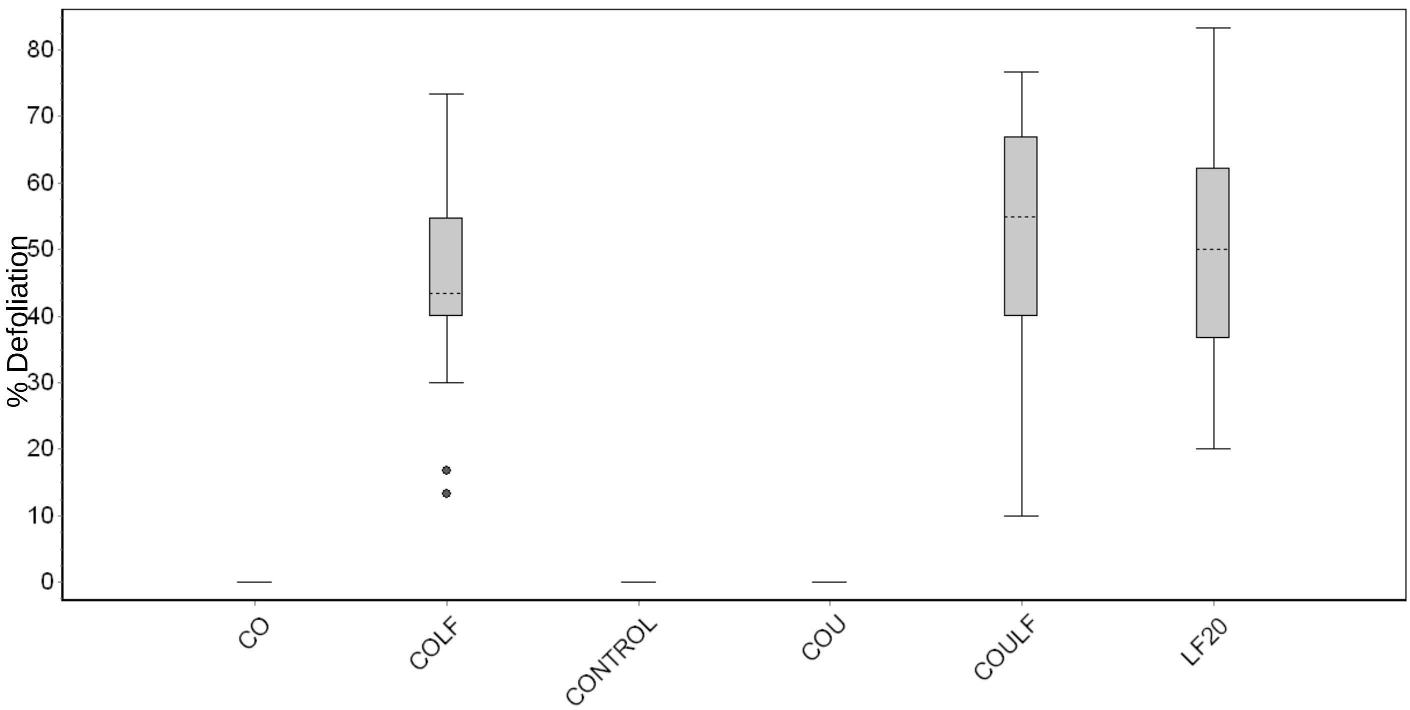
Boxplot of *M.* 'Profusion Improved' defoliation 2 weeks after treatment at site B



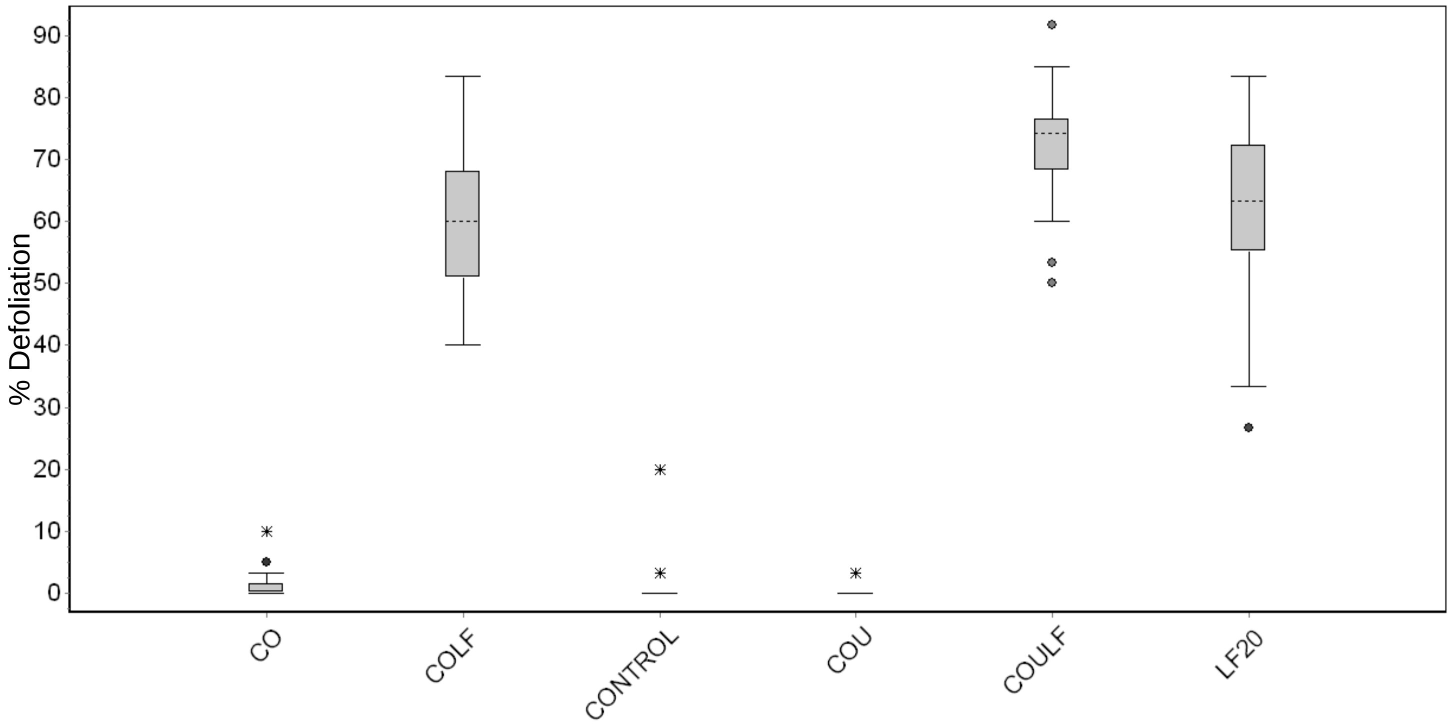
Boxplot of *M.* 'Profusion Improved' defoliation 29th October 2007 at site B



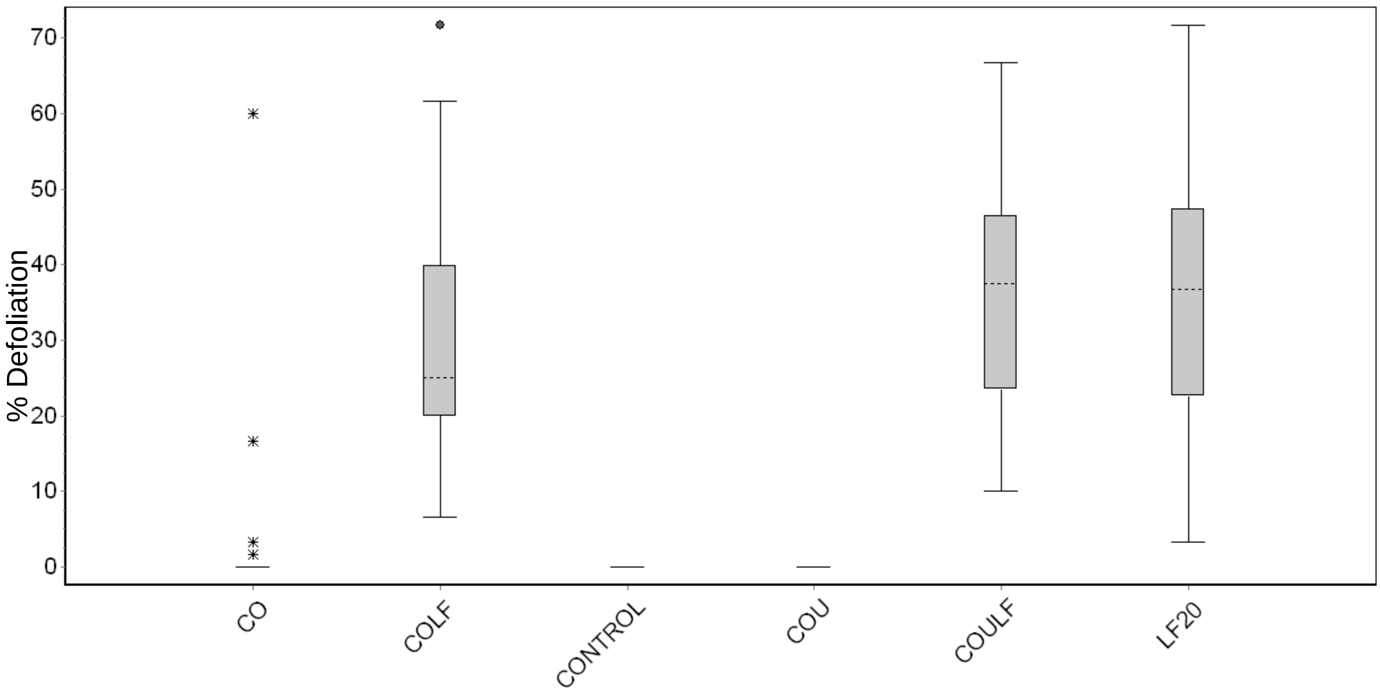
Boxplot of *M.* 'Bramley' defoliation 2 weeks after treatment at site B



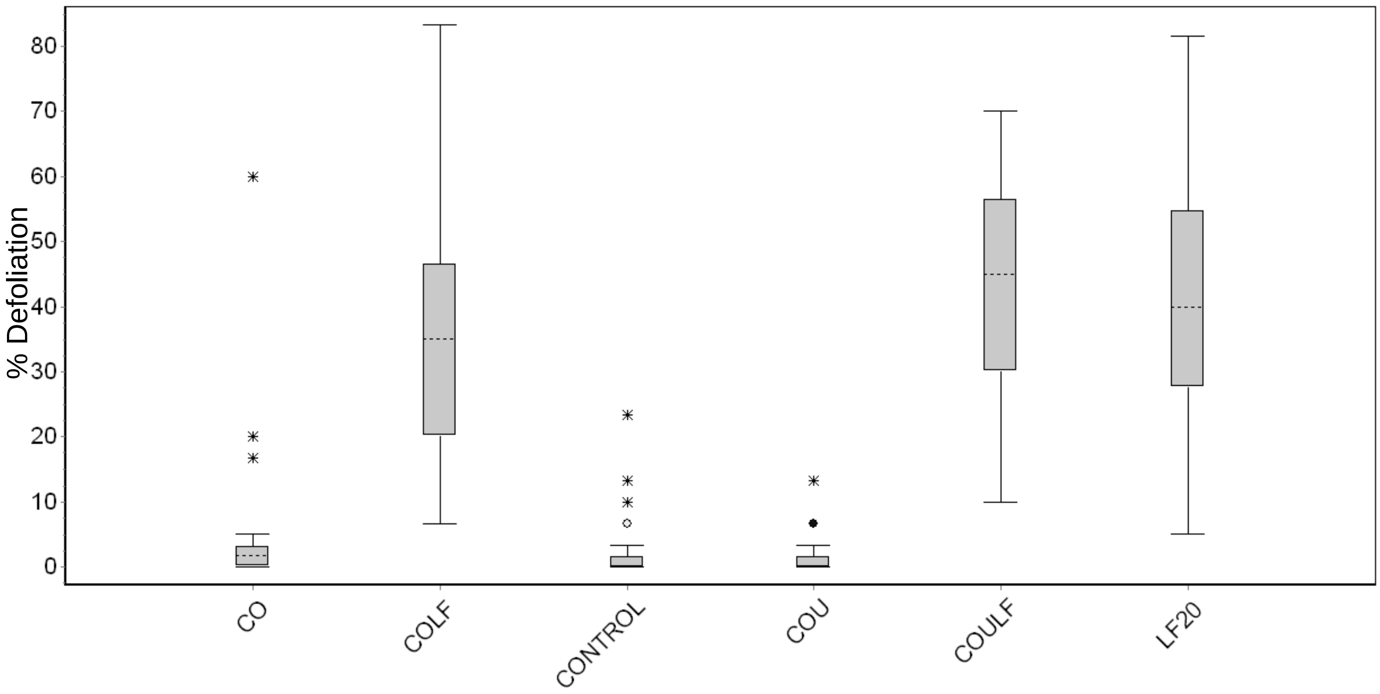
Boxplot of *M.* 'Bramley' defoliation 29th October 2007 at site B



Boxplot of *P.* 'Conference' defoliation 2 weeks after treatment at site B



Boxplot of *P.* 'Conference' defoliation 29th October 2007 at site B



Appendix 3. Revised milestones with comments

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

N. Ward appointed 25/06/07

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

This was largely obtained from the literature, using commercial recommended rates or in discussion with growers. Small scale tests were applied at Reading under glass on urea concentration to determine scorch , but time was limited to assess responses before full treatments were required for field evaluations (starting in August). It was agreed to follow the agreed plan of action for Exp 1, with follow up refinements on concentration (and possibly timing) as required.

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

See this report

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

Abbreviated form provided in this report. An on-going full literature review is available from N. Ward. It became evident from an early stage that many potential techniques (such as microbial, e.g. Phylloplane yeast species) were unlikely to have strong practical relevance to growers under UK climatic conditions. Similarly many mechanical means were deemed inappropriate (leaf scarring) or not cost effective (cost of machinery).

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

The key physiological stress that elicits leaf abscission is low temperature, although shortening photoperiods can help induce 'competence to abscise'. Other factors can mimic the cold stress response to a greater or lesser degree, but these need to *be 'subtle' enough not to kill the leaf outright, nor desiccate it excessively. There are number of factors that have the potential to slow active shoot growth and / or induce leaf abscission, namely; controlled drought, water-logging, high temperature, low temperature, altering photoperiod, photo-spectrum or light intensity, pathogen infection and physical rubbing or abrasion (thigmomorphogenesis). Although many of these merit scientific study the ability to implement them in commercial holdings often appears to be a limiting factor. After discussions with nurserymen, it was decided that the most feasible cultural approach was probably thigmomorphogenesis via brushing or some other physical means. This will be studied in year 2.*

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

    See this report

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

    See this report

8. Provide annual report (Jul 08)

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

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

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

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

13. Provide annual report 2 (Jul 09)

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

15. Provide Open Day for nurserymen to review results (Mar 10)

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

17. Provide final report (Jul 10)