Weed control in mist-propagated Sitka spruce (*Picea sitchensis* (Bong.) Carr.) cuttings

by D.V Clay, F.L. Dixon and I. Willoughby

SUMMARY: When Sitka spruce is propagated in polytunnels, weed infestations can potentially interfere with the growth of cuttings and impact on general crop husbandry, as well as acting as a seed source to infest other parts of a nursery. Hand weeding can be carried out, but it is labour intensive. The use of herbicides might prove a cheaper option, but there are currently no products approved for Sitka spruce production in polytunnels in the UK. Hence in the work reported here, four weed species, hairy bitter-cress, annual meadow-grass, groundsel and common chickweed were grown in close proximity to Sitka spruce cuttings. The susceptibility of these species to the herbicides propyzamide, napropamide, simazine and propaquizafop (the latter on annual meadow-grass only) was assessed.

Propyzamide controlled chickweed when applied pre- or post-weed–emergence, and annual meadow-grass when applied pre- and post-weed-emergence. Propaquizafop controlled annual meadow-grass when applied post-weed-emergence and prevented seeding when sprayed post-flushing. Napropamide and simazine were not effective in preventing seeding of any weed species. Although the herbicides generally did not appear to have any adverse effect on the growth of Sitka spruce cuttings, further work is needed to confirm herbicide selectivity, particularly regarding adventitious root formation. Competition from weeds did not appear to affect the early shoot growth of cuttings. Whilst these results indicate that propaquizafop, and possibly propyzamide, may have the potential for safely controlling some annual weed species, in the short term good nursery hygiene, coupled with hand weeding where necessary, remains a more practical approach than using herbicides within polyhouses.

Introduction

Vegetative propagation of Sitka spruce (*Picea sitchensis* (Bong.) Carr.) has become popular since the advent of genetically improved material from tree breeding programmes, as it allows superior genetic material, for which seed is often very scarce, to be bulked up relatively cheaply for use in the forest (Rook, 1992; Mason and Jinks, 1994). A significant amount of this production is achieved by the rooting of small shoot cuttings in mist beds in polythene-covered tunnels (polytunnels). Approximately 10 million cuttings are produced annually in the British Isles using this technique.

Mist irrigation provides a film of water that cools leaves through evaporation. The lowered leaf temperature reduces the vapour pressure difference between the interior of the leaf and surrounding air, and so restricts the rate of water loss from the cuttings, helping to maintain them in a turgid condition (Mason and Jinks, 1994). Before cuttings of woody plants form adventitious roots, it is possible for some moisture to enter through leaves and via the rooting substrate into cut stems (Loach, 1988; LeBude et al., 2004). Optimum cutting water potential has been shown as important in maximising root initiation in woody cuttings, and this can be achieved by adjusting misting level and substrate moisture to provide a moderate level of water stress (LeBude et al., 2004 and 2005). Some photosynthesis is also thought to occur, although it is probably less important in unrooted cuttings than in rooted plants (Hartmann et al., 1990; LeBude et al., 2005).

In some propagation beds, infestations of annual weeds have been reported (J. Morgan, pers. comm.), which might potentially cause problems in several ways. The presence of weeds during rooting might shield cuttings from falling mist droplets, change the physical structure and water balance of the rooting medium, and encourage the development of other pests and diseases. Once adventitious root formation occurs, weed vegetation can compete for moisture, nutrients and light with young plants, hence reducing growth and survival as it would in the field or nursery bed (Macdonald, 1986; Davies, 1987; Williamson and Morgan, 1994). As well as any physiological impact, weed infestations can also cause cultural problems for the grower. If weeds are allowed to seed, they can spread, increase in density and infect other parts of the nursery. Weeds can also hinder other operations associated with cutting production, such as lining out of the rooted material (Macdonald, 1986; Williamson and Morgan, 1994). Problems arise from species with wind-blown seed such as groundsel (*Senecio vulgaris* L.) and American willow-herb (*Epilobium ciliatum* Rafin.) or those spread by nursery operations, such as annual meadow-grass (*Poa annua* L.). Much of the weed seed comes in on the tree cuttings themselves, but the peat-based rooting medium can also be a source of infestation (Macdonald, 1986).

If judged to be causing a problem by the grower, weeds can be removed by hand. However, this is both costly and may physically disturb tender young seedlings (Williamson and Morgan, 1994). A cheaper, less physically intrusive alternative might be to use herbicides, but none are currently approved by the UK Department of the Environment, Food and Rural Affairs for use in polytunnels producing spruce cuttings (Whitehead, 2005). Weed control in outdoor Sitka spruce seed beds in forestry nurseries is often achieved by the use of selective pre- and post-emergence herbicides (Williamson and Morgan, 1994), and some of the active ingredients used might be suitable for use in polytunnels. Although most residual herbicides are rendered inactive by the high organic matter content of peat soils, they may be more effective in the water-saturated mediums under mist propagation, where water is readily available and so adsorption of such herbicides by peat substrates may be reduced. Thus the residual herbicides napropamide, propyzamide and simazine may have potential for use in polyhouse cutting production. Apart from graminicides such as propaquizafop, there are few other candidate foliar-acting herbicides suitable for controlling problem weeds which are likely to be safe on the crop. Our experiment was therefore designed to study the effects of four potential herbicides (napropamide, propaquizafop, propyzamide and simazine) applied at three dates (pre-weed emergence, post-weed emergence and post-flushing of tree cuttings) on the growth and development of Sitka spruce cuttings, and on the control of four weed species hairy bitter-cress (*C. hirsuta* L.), annual meadow grass, groundsel and common chickweed.

Methods

Experimental design

Fifty six plastic seed trays (222 x 342mm) were filled with a mixture of medium grade Erin peat, Cambark and coarse perlite in a volume ratio of 1:1:1 and watered until moist. This gave four replicates each consisting of four herbicide treatments applied at three application dates plus two untreated controls (one with weeds and one without). The experiment was of a randomised block design with split plots, with the herbicides being the main plots and the weeds as sub plots. The trays were marked out in quarters and each section sown with one weed species on 24 March (Figure 1). Approximately 500 seeds of each of four species (0.059g hairy bitter-cress, 0.146g annual meadow-grass, 0.101g groundsel and 0.234g common chickweed) were sown in each tray by sprinkling on to the relevant section of the tray and very lightly incorporating into the surface, calculated to produce approximately 7000, 21,000, 6,000 and 9,000 seedlings m^{-2} respectively. Twelve of the trays (for the propaquizafop treatment) had only annual meadow-grass sown on the relevant section. Four trays were not sown with any weed seeds (forming the untreated, unweedy control plots). On the same day, thirty two unrooted Sitka spruce cuttings were then

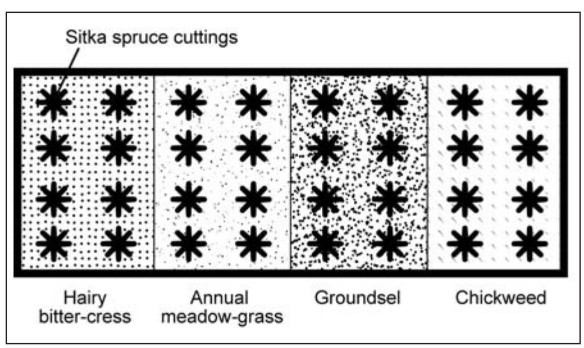

Figure 1. An example of a planted seed tray.

planted in each tray (forming 4 sub plots of 8 cuttings). The cuttings were 5 - 7cm long and unrooted.

Application of herbicides

The following herbicide treatments were applied to different, complete trays at three dates, using a laboratory track sprayer giving 252 litres ha^{-1} spray volume: napropamide (as Devrinol, 450g l^{-1} napropamide; United Phosphorus, Chadwick House, Birchwood Park, Warrington, Cheshire, UK) @ 1.0kg a.i. ha^{-1}; propyzamide (as Kerb Pro Flo, 400g l^{-1} propyzamide; SumiAgro, Merlin House, Falconry Court, Bakers Lane, Epping, Essex, UK) @ 1.5kg a.i. ha^{-1}; and simazine (as Unicrop Flowable Simazine, 500g l^{-1} simazine; Universal Crop Protection, Park House, Maidenhead Road, Cookham, Berkshire, UK) @ 1.0kg a.i. ha^{-1}. Propaquizafop (as Falcon, 100g l^{-1} propaquizafop; Makhteshim-Agan, Unit 16, Thatcham Business Village, Colthrop Way, Thatcham, Berkshire, UK) @ 0.15kg a.i. ha^{-1} was applied to the trays sown with annual meadow-grass only, because as a graminicide it was known to have very little activity on broad-leaved species.

The first treatment was sprayed on the day of planting (24 March) and all trays were placed into a mist propagation unit in a heated glasshouse. The second treatment was sprayed 17 days later after weeds had just emerged and formed very small seedlings (Figure 2). At this date spruce cuttings had not flushed, but buds were beginning to swell. The third application was made a further 30 days later on 9 May, when at least 90% of the spruce cuttings had flushed and had at least one new shoot up to 2cm long and roots 2 to 3cm long (Figure 3). By this third treatment stage the weed species, particularly annual meadow-grass and groundsel, were quite large and in some instances may have shielded the cuttings from spray applications (Figure 4). After each spray treatment, trays were left under cover for 24 hours to allow foliar herbicide opportunity to be adsorbed, before returning to the mist unit. Mist frequency was gradually reduced ten days after the final herbicide treatment, to wean the plants. The fungicide thiophanate-methyl (as Mildothane Liquid; Bayer CropSceince Ltd, Hauxton, Cambridge, UK) was applied at weekly intervals to protect against infection by grey mould (*Botrytis cinerea*).

Figure 2. Stage of growth at the second application date.

Figure 3. *Picea sitchensis* cuttings at the third application date.

Assessments and statistical analysis

Spruce and weed plants were assessed for health and vigour using a score of 0 - 7; where 0 = dead, 4 = 50% reduction in growth and 7 = as healthiest untreated. Health assessments were made two, four and eight weeks after each treatment stage. Control plots were common, but as each herbicide treatment date (pre-

Figure 4. Stage of growth at the third application date.

Figure 5. *Picea sitchensis* at the end of the experiment.

emergence, post-emergence, post flushing) was actually assessed at different calendar dates, the control plots were also re-assessed at this date, hence the three different means presented. Later in the experiment when weeds of all species were flowering on all untreated and many treated trays, a score of 5 or more indicated most plants in the treatment were flowering. At the end of the experiment in July, the number of spruce shoots per plant and length of new growth made by the main shoot were recorded (Figure 5). The percentage of spruce plants with roots greater than 5cm was also assessed. Data were subject to analysis of variance using Genstat (Genstat 5 Committee, 1993) and standard error of differences (S.E.D.s) generated. Using these values Fisher's test for least significant differences (Snedecor and Cochran, 1967) was then performed at the p=0.05 level, i.e. data points varying by more than the error bars presented have a 95% probability of being genuinely significantly different, rather than an apparent difference simply occurring by chance.

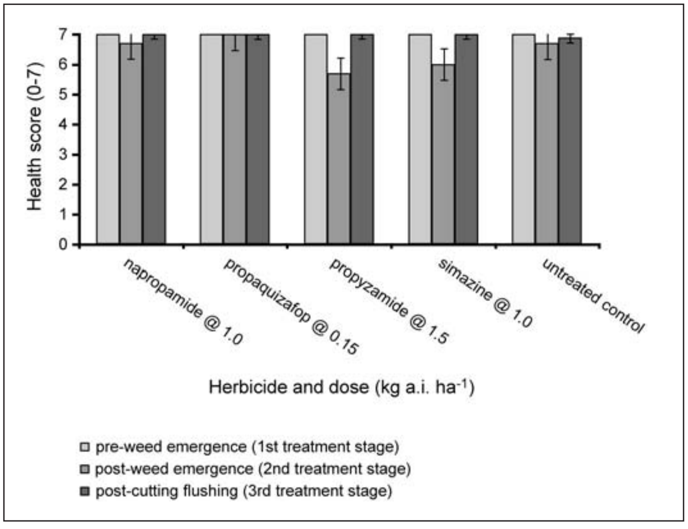

Figure 6. The effect of herbicides applied at three dates on the health of Sitka spruce cuttings – assessments made 2 weeks after each application date. Error bars represent S.E.D.s for comparison between herbicides, within each treatment date, residual df 16.

Results

The Sitka spruce cuttings remained healthy under the mist regime, flushing occurring on all trays from mid-April. Most species germinated and grew well, but with chickweed only five to ten plants emerged on untreated trays although these established well. Flowering occurred with chickweed and groundsel from the 20 May assessment, hairy bitter-cress from 5 June and annual meadow-grass later in June. Analysis of variance showed there to be overall significant differences between herbicide treatments at the p=0.05 level.

Sitka spruce

Data for the effect of herbicide on health of spruce cuttings two weeks after application are given in Figure 6. Data for the assessments at 4, 6 and 8 weeks after each application are not given as there were no significant differences in health at these dates.

Generally, the health of Sitka spruce appeared to be unaffected by any of the herbicide treatments and shoot growth appeared to be unaffected by weed species, therefore the data presented in Figures 6 and 7 are the means for all the weed species. The only exception to this was from the post-emergence treatment of propyzamide (Figure 6), where slight necrosis was noted on some plants two weeks after application but the necrosis was not evident in subsequent assessments.

Figure 7 shows the amount of new shoot growth of Sitka spruce on 10 July. In the case of shoot growth there was only one assessment date for all the treatments, hence the mean value for growth on all of the control plots is presented. The only statistically significant effect on shoot growth recorded was a reduction in the amount of new growth made by the cuttings sprayed with the pre-emergence application of napropamide. There was no difference in the amount of new growth made by the weed-free compared with the weedy control plants (data not shown). At the end of the experiment, all plots had a well developed root mat, and an assessment of the number of plants having roots greater than 5cm in length showed no consistent effect from any of the treatments, although there was considerable variation between plots (data not presented).

Weed species

Data for the health of the weed species four weeks after treatment are presented in Figure 8. Health scores for other assessment dates are not presented for reasons of brevity, and because in general the greatest effects on health were noted at 4 weeks after treatment. Hairy bitter-cress was not killed by any of the treatments. All applications significantly reduced plant growth, although this effect was transient and not evident at later application dates for the pre-emergence spray of simazine (data not presented). However, no treatment prevented the bitter-cress recovering sufficiently to flower and produce seed.

Annual meadow-grass was completely killed by the pre-emergence treatment of propyzamide. Napropamide applied pre-emergence also gave temporary reductions in growth. Propaquizafop and propyzamide applied post-emergence virtually killed all plants by eight weeks after treatment (data not shown). All other post-emergence (apart from simazine) and post-flushing treatments resulted in significant reductions in weed growth but did not prevent annual meadow grass forming seed.

Groundsel was not killed by any of the treatments. All applications of simazine significantly reduced growth, with the post-emergence and post-flushing treatments being the most effective. Post-emergence and post-flushing applications of napropamide also significantly reduced growth. Seed formation occurred with most treatments.

Chickweed growth was sparse throughout the experiment. Propyzamide applied both pre-emergence and post-emergence gave good control, killing most plants. Post-flushing applications of propyzamide and simazine both severely reduced growth. Pre-emergence treatments of napropamide also caused reduced growth. Seeding was only prevented by the pre- and post-emergence treatments of propyzamide.

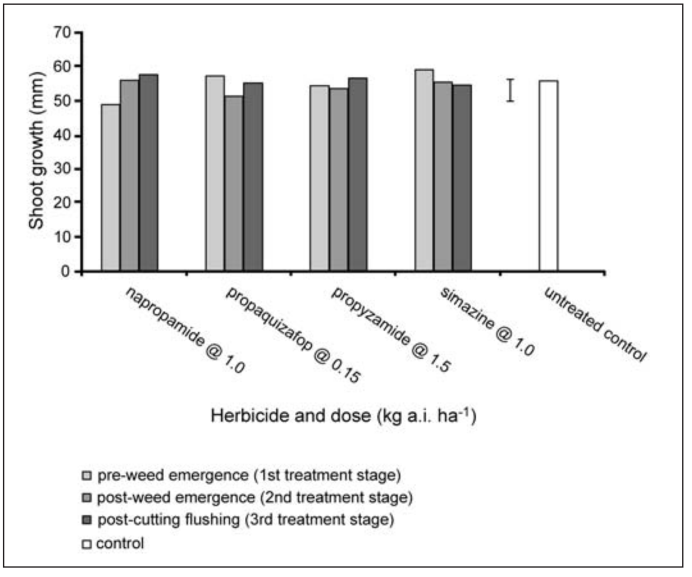

Figure 7. The effect of herbicides applied at three dates on the new shoot growth of Sitka spruce cuttings – assessments made 17 weeks after planting. Error bar represents S.E.D. for comparison between all treatments, residual df 40.

Discussion

Although some authors have reported that the stomata of cuttings stay closed until new roots are formed, more recent work suggests that stomata may remain open (Loach, 1988; LeBude et al., 2005). Unrooted cuttings may imbibe some water through leaves and also the stem base (Loach, 1988; LeBude et al., 2004). Before adventitious root formation, herbicides could therefore theoretically be taken up by cuttings, through open stomata, leaf cuticles and the cut stem via the rooting substrate. After rooting, herbicides could be taken up through roots and leaves.

Propyzamide and napropamide are both amide herbicides that are absorbed primarily though the roots and inhibit microtubule and cell division, in the latter case in the roots of newly germinating plants, leading to eventual death of all plant parts in susceptible species. Simazine is a triazine herbicide, mainly taken up by roots but also by foliage, that inhibits photosynthesis, leading to cell damage, bleaching of foliage and death of susceptible species. Propaquizafop is an aryloxyphenoxypropionate herbicide which is absorbed by both roots and foliage and inhibits lipid biosynthesis, hence disrupting cell membranes and leading to chlorosis and death but usually only in grass species (Tomlin, 1997; Reade and Cobb, 2002). Hence all of the herbicides tested in our work had a potential means of uptake by both rooted and unrooted cuttings, via foliage, roots, or the cut stem base. However, the only herbicide treatment to have any apparent long term adverse effect on the growth of Sitka spruce cuttings was the pre-emergence application of napropamide. This apparent tolerance may be due to either an inherent biochemical resistance of the spruce, or because insufficient absorption of the chemicals took place to cause damage. For simazine, propaquizafop and propyzamide, we would expect any damage symptoms to be visible on shoots in the form of chlorosis and necrosis, in addition to any impacts they

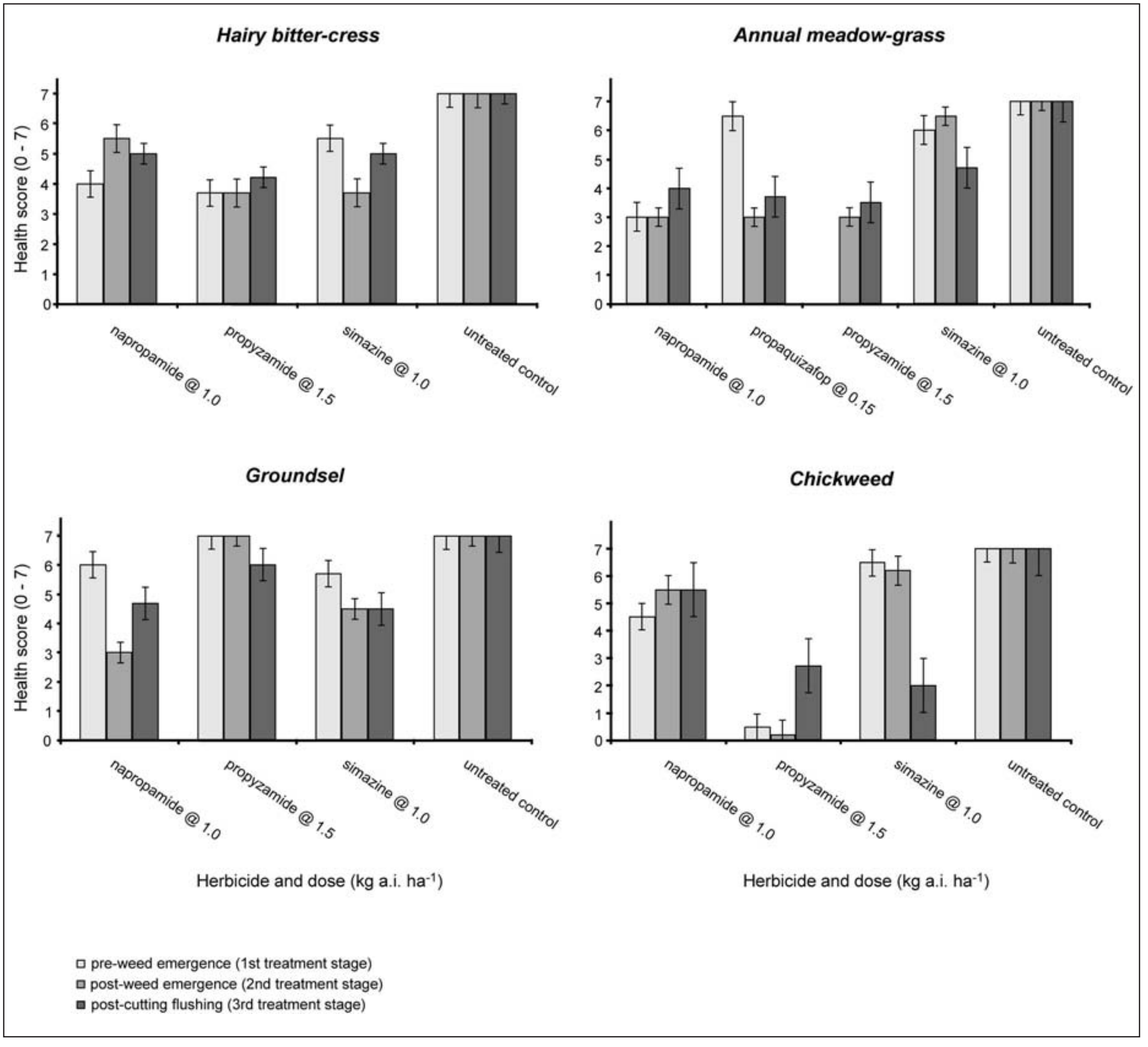

Figure 8. The effect of herbicides applied at three dates on the health of weed species – assessments made 4 weeks after each treatment date. Error bars represent S.E.D.s for comparison between herbicides, within each treatment date, residual df 9, except for annual meadow grass with a residual df of 12.

might have on adventitious root formation. Therefore based on our small scale experiment, several herbicides appear to have the potential to be safely used over cuttings of Sitka spruce propagated under mist systems, but more research is required to confirm adventitious root formation is not significantly affected.

Most of the weed species grew well in the wet conditions. No one herbicide treatment was effective in controlling all species, and very few treatments killed emerged plants. In most cases although growth was reduced, the plants grew on to produce seeds. Hairy bitter-cress and groundsel were not killed by any of the treatments. Pre-emergence application of propyzamide killed both the annual meadow-grass and chickweed, corresponding to known efficacy in nursery situations (Williamson and Morgan, 1994), and chickweed was also killed by the post-emergence application of propyzamide. Propaquizafop was only effective on annual meadow-grass when applied to newly-emerged seedlings, corresponding to the product label recommendation for propaquizafop in field crops which indicates control of this species is possible only up to the three leaves unfolded stage (Makteshim-Agan, 2002). The overall lack of efficacy of simazine may have been due to its adsorption on the organic substrate. Simazine is generally reliant on root uptake for efficacy, but contact damage on foliage is occasionally seen. In our experiment, this may account for the control of the common chickweed with the post-flush treatment. The poor control afforded by napropamide may be related to the dose used; in practice higher use rates up to 4kg a.i. ha^{-1} are usually needed for effective pre emergence weed control.

Although weeds could theoretically impact negatively on rooting development and growth of Sitka spruce cuttings, this was not observed with the annual species used in our experiment. Higher densities, or prolonged growth of the weeds such that they are permitted to overtop the rooted cuttings for longer, are likely to be more detrimental. However, it may be that the impact of weed infestations within polyhouses on the lifting and handling of rooted cuttings, and any risk these weeds pose for infesting other parts of the nursery, provide a more compelling justification for initiating control measures.

This research indicates that propaquizafop and propyzamide may be effective in killing or preventing seeding of established annual meadow-grass and common chickweed in polytunnels without damaging Sitka spruce cuttings, but further work is needed to confirm herbicide efficacy and selectivity, particularly regarding adventitious root formation. Currently, neither product is approved for use in Sitka spruce production in protected crops in the UK (Whitehead, 2005), although approval could be sought if there was sufficient demand from growers. However, good nursery hygiene including sterilisation or fumigation of the rooting substrate to prevent the spread of weed seed (Macdonald, 1988), reinforced by regular and early hand weeding of newly-emerged cotyledon stage weeds before they develop and interfere with cutting roots, may be the most economic approach to weed management in mist propagation systems in the short term.

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David Clay* and **Fiona Dixon*** have been researching weed control in forestry, amenity and non-crop situations.

Ian Willoughby** is a research programme manager for Forest Research, based at Alice Holt.

* Avon Vegetation Research Limited, PO Box 1033, Nailsea, Bristol BS48 4FH.

** Forest Research, Forestry Commission, Alice Holt Lodge, Farnham, Surrey GU10 4LH.