The use of dye markers as a potential method of reducing pesticide use

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Summary

Colorants used as dye markers may allow better targeting of pesticide sprays and could offer the potential to reduce environmental pollution and overall levels of synthetic pesticide use. A series of experiments was set up to investigate the visibility of commercially available marker dyes, and also those derived from colorants developed for other industrial and scientific uses. Poor visibility and safety concerns with the other products led to effort being concentrated on the food dyes Acid Blue 9 and Acid Red 73. Both dyes gave good visibility on a variety of site types, being clearly visible when wet and dry, but fading within 21 days. However, experiments suggested that if relatively resistant weeds were treated, or low rates of some herbicides were used, dyes mixed with pesticides could reduce efficacy. The addition of 2 l ha^{-1} Acid Blue 9 (i.e. Dysol Turquoise at 2 per cent of final spray volume) as a marker dye may offer one immediate way of achieving better targeting, and hence some reduction in overall levels of pesticide use in UK forestry.

Introduction

In the UK, herbicides are the cheapest and hence the commonest method of achieving control of vegetation that competes for moisture and nutrients around young trees (I. Willoughby *et al.*, unpublished). However, there is increasing pressure from non-governmental organizations, and through voluntary certification schemes (UKWAS, 2000), to reduce reliance on pesticides. In addition, inappropriate use of herbicides can cause significant damage to crop trees or non-target vegetation and may pose a health risk to operators.

Research by the Forestry Commission in England into alternatives to herbicide use is focusing on stock type, the need for and intensity of weeding including critical period studies, alternative ground-cover crops, mycoherbicides, and the use of alternative silvicultural systems such as natural regeneration and direct seeding. However, progress in many of these areas can be relatively slow.

The addition of dye markers to herbicides

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could more rapidly help to reduce quantities applied by allowing greater precision and accuracy. A suitable dye marker allows spray patterns to be easily identified, giving early indications of drift to non-target areas. Colorants also help reveal any faults in nozzles, applicators or safety clothing, and so can help to reduce operator contamination. Potential disadvantages of dye markers are that they may alter pesticide efficacy, be costly, have negative impacts on the environment, be difficult to handle causing staining to skin and clothing, and, if persistent, may be visually intrusive to recreational users of the forest. Dye recognition may also be a problem for the 8 per cent of men and a much smaller percentage of women who have defective colour vision (Wright, 1969). The ideal characteristics of a colorant for use as a dye marker are for it to be of low cost, be safe to operators and the environment, have no effect on pesticide efficacy, be soluble in water, and to be visible during spraying and when dry on a variety of sites and vegetation types, but then fading after a few days.

Although some commercially formulated pesticide marker dyes are available on the market (see Table 1) and some are recommended on herbicide product labels (e.g. the herbicide Roundup Pro-Biactive; Monsanto, 1994), few are specifically designed for use with dilute herbicide sprays at medium volumes (200–700 l ha^{-1} diluent) in forestry conditions.

An alternative source of marker dyes is the many hundreds of colorants used world-wide in scientific and industrial applications such as textiles, cosmetics and food manufacture. Colorants are characterized by the selective absorption of certain wavelengths or colours in white light. The colour which is seen is due to the wavelengths that are not absorbed by the dye, i.e. reflected and scattered. Colorants may be divided into organic or inorganic (either naturally or synthetically produced) compounds, and are classed either as dyes or pigments. Dyes are completely or partially soluble in solvents. Pigments are insoluble and need to be attached to the substrate by addition of compounds such as polymer in paint (Zollinger, 1991); the need for additional compounds makes pigments less useful as herbicide markers than dyes.

Dyes are coloured, ionizing aromatic compounds that derive their colour from the relatively complex chromophore systems that they contain (Pagga and Brown, 1986). Chromophores are atomic configurations which can alter the energy within the dye, which results in the aromatic compound selectively absorbing visible radiation, thus producing colours (Llewellyn, 1998).

There are a number of different classes of dyes including azo dyes, di- and tri-phenylmethane dyes, fluorescent and methine dyes. Azo dyes are one of the most important groups, making up about half of the world dye production (Morrison and Bond, 1983; Zollinger, 1991). All azo dyes are synthetically manufactured from aromatic amines. These are organic compounds in which at least one of the hydrogen atoms of the ammonia molecule has been replaced by

Table 1: Some commercially available marker dyes

Commercially formulated pesticide dyes	Colour Index	Application	Manufacturer/ UK distributor
Blazon Liquid	Not disclosed	Liquid herbicides and fertilizers	Stewarts, Dalkeith, Midlothian, UK
Turfmark	Not disclosed	Liquid herbicides and fertilizers	Stewarts
Dyon	Not disclosed	Herbicide treatment for cut stumps	Rhone Poulenc, (Bayer), Hauxton, Cambs., UK – dye not available
Hortichem Scarlet Red	Acid Red 18 CI 16255	Weed wipers	Certis, Amesbury, Wilts., UK
Dysol Turquoise	Acid Blue 9 CI 42090	Stump treatment against *Heterobasidium annosum*	Albion Colours, Halifax, West Yorks., UK

another atom (Anon., 1994). This process is known as azo coupling, and is the most common method for producing azo dyes. Azo dyes include a wide range of colours (yellow, orange, red, blue and green) depending on their exact molecular structure, and they may either have acid or basic properties (Morrison and Bond, 1983). Many azo dyes have an acceptable intensity of colour, and these are water soluble and relatively inexpensive (Monsanto, 1993).

The molecular structure of tri-phenylmethane dyes is derived from $(C_6H_5)_3CH_3$, and a number of coal tar and synthetic dyes belong to this group (Pepling *et al.*, 1997). They have a greater colour intensity than azo dyes, but poor light stability which could be advantageous for short-term use, such as for markers in herbicides. They are relatively cheap, but are available in fewer colours than azo dyes (Monsanto, 1993). Fluorescent dyes give off their strong colour by absorbing near-ultra violet light and releasing energy by fluorescence in the visible light range (Zollinger, 1991). When wet, fluorescent dyes are much more visible than other dyes; however, they tend to be more expensive and less visible once dry (Monsanto, 1993). Another source of colorants may be the methine dyes that are used in photographic materials and pharmaceuticals.

Research into the potential applications of dyes in environmental settings is limited compared with industrial applications. Fluorescent dyes have been used for groundwater tracing (Field *et al.*, 1995), but perhaps of more direct relevance to herbicides are the studies carried out on the use of markers to ensure adequate coverage in the chemical and biocontrol stump treatments used to control *Heterobasidium annosum* (Fr.) Bref. Spencer (1998) reported on trials carried out on the effectiveness of Kenacid turquoise ANX50 (Acid Blue 9, CI 42090) added to urea for use as a temporary dye marker. [The name e.g. Acid Blue 9, and number e.g. CI 42090, given in parenthesis after the common name of the dye are the Colour Index (CI) name and number, which, where known, are used throughout this paper, as they provide an internationally recognized system for identifying commercially available dyes. The full Colour Index lists structural formulae, manufacturers, generic and commercial names (American Association of Textile Chemists and Colourists, 1999).] At all three concentrations tested (0.5, 1 and 2 cm^3 in 5 1 of urea solution), the dye gave an adequate visual indication for 1 week provided that there was little or no rain. Another example of marker dyes in stump treatment was reported by Web (1973), who stated that a marker dye (type unknown) was added to spore solutions of *Phlebiopsis gigantea* (Fr.) Jűlich (a biological control of the butt-rot fungi *Heterobasidium annosum*) in order to highlight stump coverage. Currently Acid Blue 9 (CI 42090) is used to colour spore solutions of *Phlebiopsis gigantea* (Fr.), and the identical Food Blue 2 (CI 42090) is used to highlight stump coverage (Katherine Thorpe, pers. comm.).

In comparison with stump treatment, there are fewer published results of trials comparing different pesticide marker dyes, and little reference to colorants for use in dilute herbicide sprays in UK forestry. One early example in the USA was the work carried out by White and Halan (1965). Under laboratory conditions they looked at a range of dyes (Fertilizer Blue (Colour Index name and number unknown), Pigment Red 3 (CI 12120), Pigment Red 74 (CI 11741), Pigment Blue 29 (CI 77007), Pigment Red 49 (CI 15630), and Pigment Red 101 (CI 77491)). Each was applied at four concentrations on to both green and brown oak foliage, and brown paper (to represent soil). The blue dyes were more visible than the red dyes. However, when the dyes were tested in the field by spraying with herbicide onto *Elytrigia repens* (L.) Nevski (couch or quack grass), the red dyes were more visible both when wet and after drying. The red dyes were persistent but the blue dyes less so, and did not last the winter; this might be an advantage in some circumstances.

Several marker dyes, including Basic Violet 10 (CI 45170) and Basic Violet 3 (CI 42555) have been used by the United States Drug Enforcement Administration (DEA) in its cannabis eradication programme. Marker dyes were incorporated into herbicides for selective and broadcast applications to verify that the intended target has been sprayed, and to stain the cannabis plants making them unmarketable. Pepling *et al.* (1997) compiled information on which commercially available dyes for use with herbicides might also be suitable for use in the cannabis eradication programme. They concluded that there was very little literature available on the use of marker dyes. Additionally, the assessment of potential risks associated with commercially formulated dyes was limited by the manufacturers' right not to disclose the components of the dyes.

The objective of the study reported here was to examine the effectiveness of diferent dyes as markers in pesticide applications in UK forestry. A series of experiments was set up over a 3-year period. The first series of experiments compared the visibility of a selection of dyes for use with herbicide treatment of different forest weeds. One experiment also investigated the potential of dyes as markers for an insecticide top-up spray with permethrin. This insecticide is used for the control of *Hylobius abietis* (L.), an insect which causes significant damage to the stems of transplants. The remaining experiments investigated the effect of dyes on the efficacy of three herbicides, and one insecticide commonly used in UK Forestry.

Materials and methods

Potential marker dyes

Table 2 gives details of the different dye types used in the experiments, the dye group to which they belong, their dye name or trade name, and their corresponding Colour Index name and number where known.

Visibility experiments

The general method used for the visibility experiments to examine the use of dye markers in herbicide treatments was the same on all experiments, although the dye type, dye concentration, site type and ground vegetation differed. All these experiments took place at Alice Holt Forest (Nat. Grid Ref. SU8042), and in each experiment a randomized design was used, with two replicates per treatment. The treatments were applied to 1.2 m wide $\times$ 10 m long swathes, using a knapsack sprayer. This simulated the method that might be used to spray dilute herbicide at medium volume in typical forestry situations. All dyes were mixed with water to the required concentration and sprayed at a volume rate of 200 l ha^{-1}. For example, a concentration of 0.05 per cent is equivalent to 0.1 l ha^{-1} of dye, at 200 l ha^{-1} volume rate. For all the experiments the concentration of dye used is expressed as a percentage of final spray volume.

Assessments were carried out by placing a 1-m-square quadrat every 2 m and the visibility of the dye assessed when wet (immediately after spraying) and dry, by the same three assessors standing directly above each plot. Dye visibility

Table 2: Dyes used in experiments

Dye group	Dye name (trade name)	Colour Index name (number)
Commercially formulated pesticide marker dyes		
Azo	(Hortichem Scarlet Red)	Acid Red 18 (CI 16255)
Tri-phenylmethane	50% Erioglaucine (Dysol Turquoise)*	Acid Blue 9 (CI 42090)
Not disclosed	(Dyon)	Not disclosed
Not disclosed	(Turfmark)	Not disclosed
Not disclosed	(Blazon Liquid)	Not disclosed
Cosmetic, food and textile dyes		
Fluorescent	Rhodamine B	Basic Violet 10 (CI 45170)
Tri-phenylmethane	Formyl Violet S4B	Acid Violet 17 (CI 42650)
Azo	Brilliant Crocein MOO	Acid Red 73 (CI 27290)
Methine	Astrazon Orange	Basic Orange 21 (CI 48035)
Tri-phenylmethane	Water green	Acid Green 50 (CI44090)
Tri-phenylmethane	Ethyl Violet	Basic Violet 4 (CI 42600)

* Acid Blue 9 applied as Dysol Turquoise = 50 per cent Acid Blue 9, i.e. 1 per cent Acid Blue 9 = 2 per cent Dysol Turquoise.

was then assessed at periodic intervals until dyes were invisible, using a subjective score ranging from 1 to 5, where 1 = not visible, and 5 = clearly visible.

In July 1997, the first experiment examined a range of colorants commercially marketed for use as marker dyes with pesticides. The treatments were: Acid Red 18 at 0.05, 0.1 and 0.2 per cent; 50 per cent Acid Blue 9 at 0.06, 0.12 and 0.24 per cent; Dyon at 0.5, 1 and 2 per cent; Turfmark at 0.25, 0.5 and 1 per cent; and Blazon Liquid at 0.25, 0.5 and 1 per cent. The lowest dye concentrations were the manufacturers' recommended rate, and the other concentrations were twice and four times the recommended rate (manufacturers' recommended rates were not necessarily for use as a dilute herbicide spray using 200 l ha^{-1} diluent). These treatments were applied to different site types dominated by *Pteridium aquilinum* (L.) Kuhn (bracken), *Deschampsia flexuosa* (L.) Trin. (wavy-hair grass), mown *Holcus lanatus* L. (Yorkshire fog) and *Digitalis purpurea* L. (foxglove).

The experiment was repeated in September of the same year using a range of cosmetic, food and textile dyes, not specifically formulated for use with pesticide sprays (Basic Violet 10, Acid Violet 17, Acid Red 73, Basic Orange 21, Basic Violet 4). All dyes were applied at a concentration of 1 per cent of final spray volume. The experimental area was a clearfelled site dominated by mature *Deschampsia flexuosa* (70 per cent), with 30 per cent *Senecio jacobea* L. (common ragwort) and dead vegetation.

In the third experiment carried out in October 1998, three cosmetic/food dyes were examined at a range of concentrations (Acid Red 73 at 2.5, 5 and 10 per cent; 50 per cent Acid Blue 9 at 0.5, 1, 2.5, 5 and 10 per cent; and Acid Green 50 at 0.5 per cent). Acid Blue 9 is a food dye, but is also sold pre-diluted as a marker dye for use with pesticides (see Table 1). The dyes were tested on a clearfelled area dominated by herbaceous vegetation comprising 50 per cent *Digitalis purpurea*, *Senecio vulgaris* L. (groundsel) and *Sonchus oleraceus* L. (smooth sowthistle), and 50 per cent bare ground, brash and dead vegetation.

A further visibility experiment took place at Headley Research Nursery (Nat. Grid Ref. SU808379) in September 1998, to assess the potential for using marker dyes in the application of the insecticide, permethrin, as a top-up spray treatment against *Hylobius abietis*. Three dyes (Acid Red 73, Acid Blue 9 and Acid Green 50) at two concentrations (0.5 and 1 per cent) were applied to 2-year-old *Picea abies* (L.) H. Karst. (Norway spruce) transplants as a 10 ml dose to the stem of each tree via a forestry spot gun. The experiment was set out as a fully randomized design with four trees per dye treatment. Once again, the visibility of the dye on the trees was assessed when wet and dry and, thereafter, at periodic intervals until dyes were invisible, using the same scoring system as before.

Efficacy experiments

Glasshouse trials

In 1998 and 1999, three experiments were set up at Long Ashton Research Station (Nat. Grid Ref. ST535699) to assess the effect of two food dyes (Acid Blue 9 and Acid Red 73) on the efficacy of three herbicides: glyphosate (as Roundup, 360 g l^{-1} glyphosate; Monsanto, Trumpington, Cambs., UK); propyzamide (as Kerb, 400 g l^{-1} propyzamide; SumiAgro, Epping, Essex, UK); and asulam (as Asulox, 400 g l^{-1} asulam; Aventis (Bayer), Hauxton, Cambs., UK). All experiments were on large established pot-grown plants. The efficacy of glyphosate and asulam was tested on pot-grown *Elytrigia repens* and *Rumex obtusifolius* L. (broadleaved dock) grown outdoors, using the dyes at a 10 per cent rate of final spray. The second experiment tested the effect of adding dyes at 1 per cent spray solution to three doses of propyzamide applied to outdoor-grown *Elytrigia repens* in winter. In the third experiment glyphosate +/– dyes at 1 per cent was sprayed on glasshouse-grown grown *Elytrigia repens*. In all experiments herbicides were applied using a laboratory track sprayer at 200 l ha^{-1}. The experimental design was fully randomized with six replicates per treatment. Shoot fresh weight was assessed after herbicide control treatments (no dye) had shown evidence of maximum kill of treated foliage and when re-growth from less effective treatments was occurring.

Field trials

A field trial was carried out at Failands, near Bristol (Nat. Grid Ref. ST518730), in September 1999. Acid Blue 9 and Acid Red 73 were mixed with different concentrations of glyphosate, and the effect on the herbicide's efficacy was tested against a range of field-grown herbaceous vegetation: *Ranunculus repens* L. (creeping buttercup), *Rumex obtusifolius* (broad-leaved dock), *Taraxacum officinale* Weber (common dandelion), *Trifolium repens* L. (white clover), *Urtica dioica* L. (common nettle) and *Elytrigia repens*. All these were established from seed in 1998 with the exception of *Elytrigia repens*, which was grown in large pots in the field. The dyes were added to the herbicides at a rate of 1 per cent of final spray volume, and applied to the vegetation at 200 l ha^{-1} using an Oxford precision sprayer. A randomized complete block design was used, with the dye treatment replicated three times in 5 m × 2.5 m plots. Percentage green cover was assessed 8 months after treatment.

The effect of the same two dyes on the efficacy of permethrin used as a top-up spray against *Hylobius abietis* was investigated in the final experiment carried out at Kilmichael Forest (Nat. Grid Ref. NR932916), in June 1999. The site had been clearfelled in the winter of 1998 and monitored in April and May of the following year to confirm that there was a high level of *Hylobius abietis* feeding activity. Transplants of *Picea sitchensis* (Bong.) Carr. (Sitka spruce) were then planted at the end of May 1998, and the treatments were applied at the beginning of June. The dyes were added at two concentrations (1 and 5 per cent) to a 0.4 per cent permethrin solution (as 2 per cent Permit, 200 g l^{-1} permethrin; Mitchell Cotts, Mirfield, West Yorks., UK) and applied as two 5 ml doses per tree (for 2500 stems ha^{-1} equivalent to 0.1 kg a.i. ha^{-1} permethrin), using a forestry spot gun. The experimental design consisted of four randomized blocks, each with six plots of 49 trees. The transplants were assessed for damage after sufficient *Hylobius abietis* feeding had occurred to discern differences between treatments. Plants were scored as follows: 1 = no damage, 2 = slight damage, 3 = serious damage (likely to lead to death of transplant), 4 = dead due to insect feeding, M = missing.

Statistical analysis

The data scores for the visibility experiments were measured on an integer scale making statistical analysis problematic. Although standard errors of differences of means (SED) were also generated for all the experiments using ANOVA, it was decided that plotting mean scores against days was a justified method of selecting dye combinations for further trials.

Data from the experiments to investigate the effect of dyes on the efficacy of herbicides was subjected to analysis of variance (ANOVA). Data from the permethrin efficacy experiment was subject to angular transformation of the variates prior to analysis (√arc-sine scale) to satisfy the underlying assumptions of ANOVA.

Results

Visibility experiments

The initial visibility experiment investigated a selection of five colorants that are commercially available for use as marker dyes in pesticides. The visibility of these dyes when sprayed onto bracken is shown in Figure 1. Differences were found between the different dyes at different concentrations, and between the wet and dry visibility of the dyes. All dyes were applied at three rates and when the dyes were applied at the lowest rates, which corresponded to the recommended rates suggested by the manufacturers for uses such as stump treatment, golf course herbicide applications and colour enhancement, they were either barely visible, or invisible. All the dyes' visibility increased when applied at two and four times the recommended rate, although none were highly visible when wet (immediately after spraying) with visibility scores ranging between 2.5 and 3.5. In all cases the dyes were invisible, or barely visible when dry. Visibility on other vegetation types was equally poor (data not presented).

The second experiment looked at the visibility of a range of five cosmetic/food and textile dyes applied at a 1 per cent concentration rate. Figure 2 shows that all the dyes had adequate visibility when wet (score ranging between 3.5 and 5), and were all still visible when dry (scores ranging between 3 and 4.5). Basic Violet 10 and Acid Red 73 were highly visible after 7 days (scores 5 and 4, respectively), and remained visible for the longest period of time (43 and 39 days, respectively). The remaining dyes had an average to low visibility after 7 days and all had completely faded after 11 or 21 days.

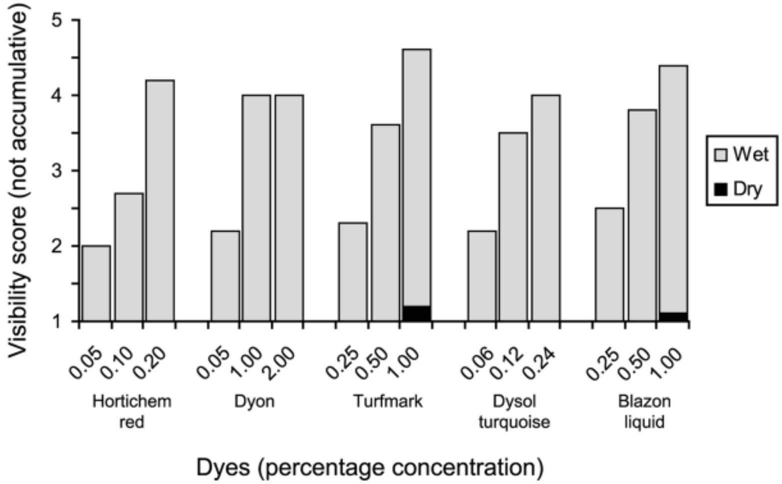

Figure 1. The visibility of commercially formulated dyes used as dilute sprays on bracken (SED = 0.085).

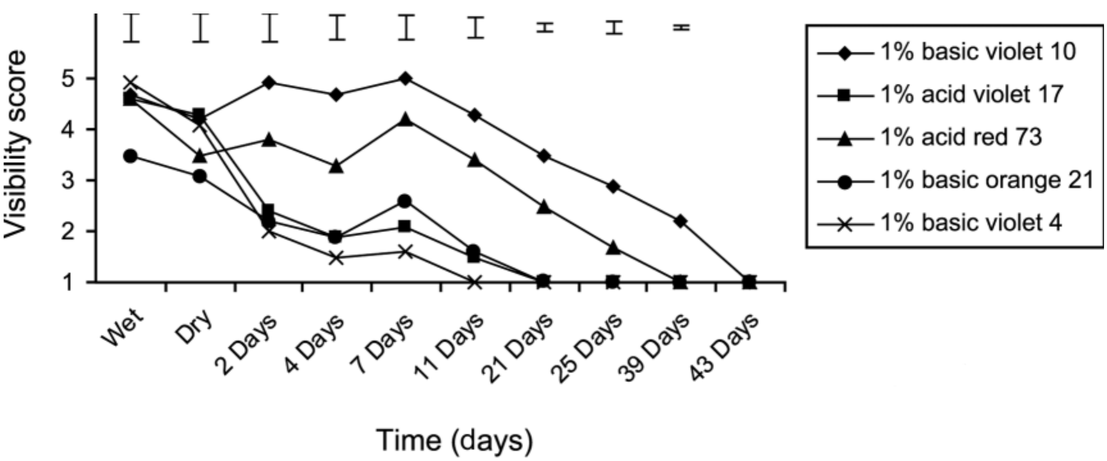

Figure 2. The visibility of textile and food dyes used as dilute sprays on mature grass. Error bars denote SED for all dyes within that time period. When not visible, SED < 0.1.

The third experiment compared three cosmetic/food dyes (Acid Blue 9, Acid Red 73 and Acid Green 50). Figure 3 shows the visibility of these dyes when sprayed on predominately herbaceous vegetation. From this it can be seen that seven of the dye treatments had good visibility when wet (score 5). However, 0.5 per cent Acid Green 50 and 0.5 per cent Acid Blue 9, had lower visibility scores of 3.5 and 4.5, respectively, and once dry, these two treatments were either of low visibility, or had faded completely. The remaining treatments remained visible (scores 3–5) with Acid Blue 9 being more visible than Acid Red 73, at all but the lowest concentration. All the treatments were invisible after 7 days and 5.1 mm of rainfall (a total of 5.7 mm rainfall since application). When the same treatments were applied to a site comprising bare ground, brash and dead vegetation the visibility recorded was higher, and lasted for a longer number of

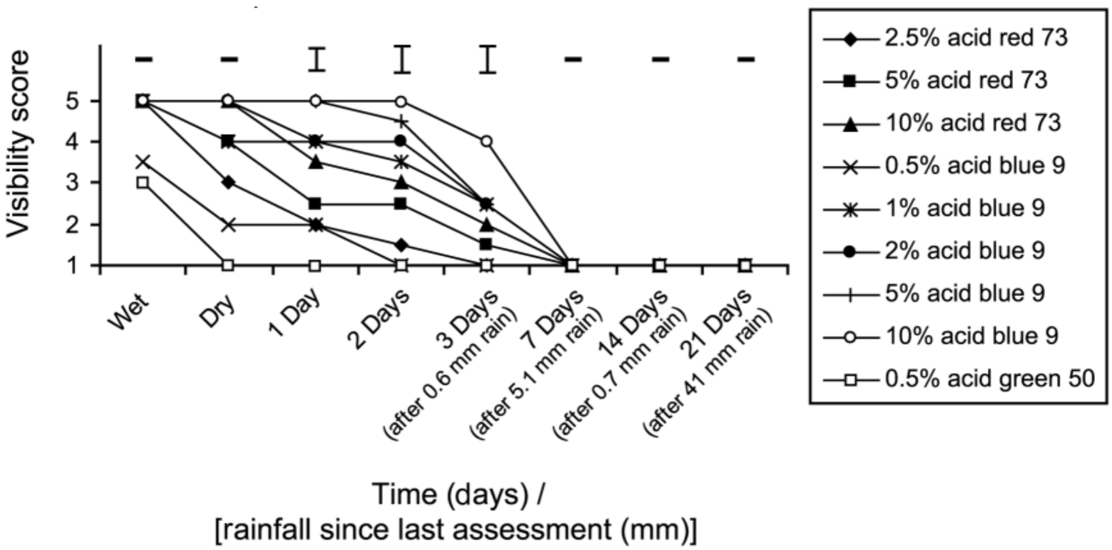

Figure 3. The visibility of food/cosmetic dyes used as dilute sprays on a restock site with predominately herbaceous vegetation. Error bars denote SED for all dyes within that time period. When not visible, SED < 0.1.

days. Here, six of the treatments were still highly visible after 2 days, and it took 21 days for all the treatments to become invisible or barely visible (scores 1–1.5) (data not presented).

The last of the visibility experiments compared the same three dyes in Experiment 3 for their use as marker dyes for top-up sprays of permethrin (see Figure 4). All the dyes, at both the concentrations were highly visible when wet, dry, and after 24 h. After this point the visibility of all the treatments dropped considerably, and by day 3 and 20.2 mm of rainfall the two concentrations of Acid Green 50, and the lowest Acid Blue 9 concentration had completely faded. The two Acid Red 73 dye treatments, and the 1 per cent Acid Blue 9 treatment were still visible after 7 days and 19.7 mm of rainfall (a total of 39.9 mm rainfall) but had completely faded after 14 days and 46.6 mm of rainfall (a total of 86.5 mm rainfall).

Efficacy experiments

Glasshouse trials

The initial efficacy experiments considered the effect of Acid Blue 9 and Acid Red 73 at different concentrations on the efficacy of glyphosate and propyzamide when applied to *Elytrigia repens*, and on the efficacy of asulam when applied to *Rumex obtusifolius*. The treatments were assessed in terms of foliage fresh weight, and a summary of the results can be found in Figures 5–8.

All rates of glyphosate significantly reduced *Elytrigia repens* weight ($P = 0.05$), although none gave total control. When 10 per cent Acid Blue 9 and 10 per cent Acid Red 73 were added there was a substantial reduction in efficacy at the highest rate of glyphosate (Figure 5). A second experiment was established using the dyes at a 1 per cent concentration, as this is the concentration which is more likely to be used in the field. Again, when used alone the glyphosate reduced the growth of *Elytrigia repens* at all the doses ($P \leq 0.05$). At the lower application rates of glyphosate (0.18 and 0.36 kg a.i. ha^{-1}) both the red and the blue dye noticeably reduced the efficacy of the glyphosate, as did the blue dye at the highest glyphosate application rate. However, at this higher herbicide application rate, the plant growth was still substantially reduced even when the dyes were present (Figure 6).

When the dyes were added to propyzamide (Figure 7) no consistent negative effect on efficacy

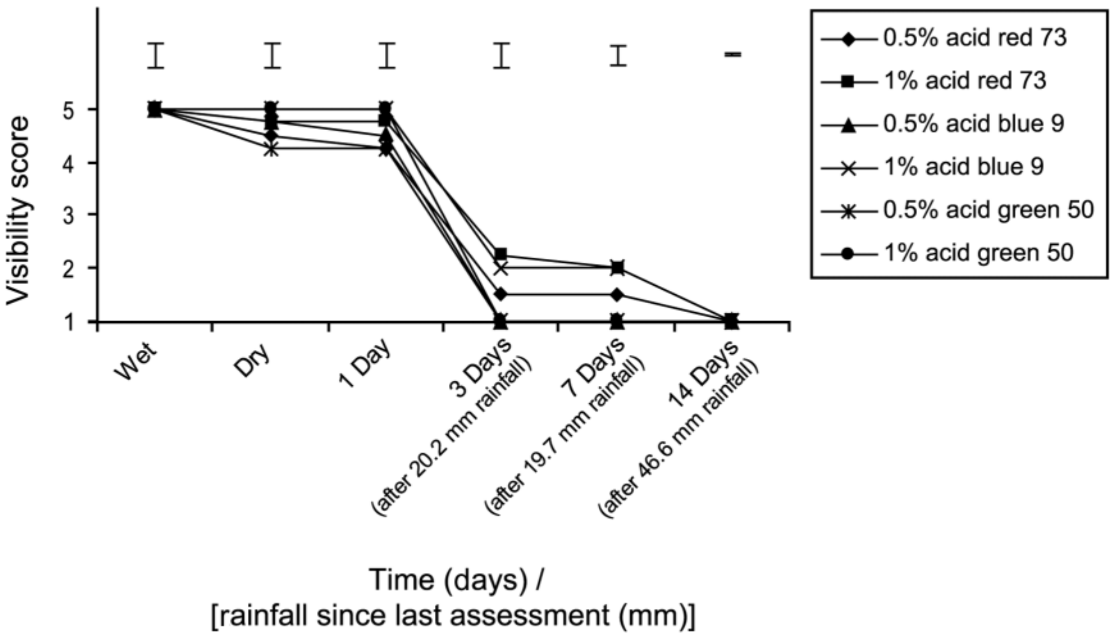

Figure 4. The visibility of food/cosmetic dyes used as top up spray on *Picea abies*. Error bars denote SED for all dyes within that time period. When not visible, SED < 0.1.

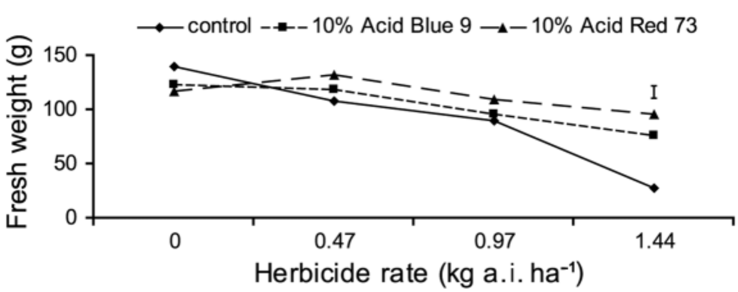

Figure 5. The effect of 10 per cent Acid Blue 9 and 10 per cent Acid Red 73 on the efficacy of glyphosate on *Elytrigia repens* (treatment date 16 July 1998; assessment date 21 October 1998). Error bar denotes SED.

was seen, even at the lowest herbicide application rate (0.75 kg a.i. ha^{-1}). When experiments were carried out using asulam +/– dyes, all the herbicide application rates reduced the growth of *Rumex obtusifolius* ($P = 0.05$). At the lowest herbicide rate both dyes reduced efficacy with the effect of the blue dye being greater; at the mid-rate of herbicide application (1.12 kg a.i. ha^{-1}), only the blue dye was found to reduce efficacy (Figure 8). At the highest rate of herbicide application neither dye substantially reduced the efficacy.

Field trials

The results of the field experiment designed to test the effect of the same dyes on efficiency of glyphosate against herbaceous weeds under field conditions are shown in Figure 9. Here glyphosate was used at the recommended doses

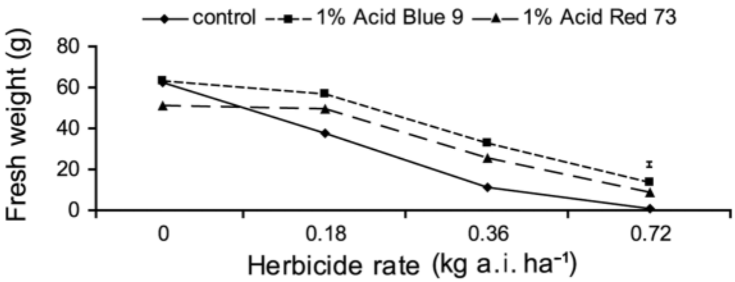

Figure 6. The effect of 1 per cent Acid Blue 9 and 1 per cent Acid Red 73 on the efficacy of glyphosate on *Elytrigia repens* (treatment date 20 January 1999; assessment date 21 December 1999). Error bar denotes SED.

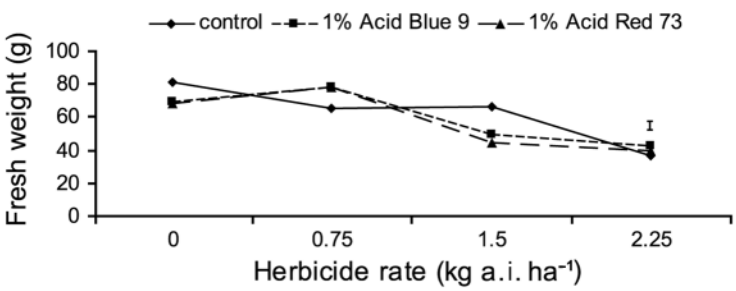

Figure 7. The effect of 1 per cent Acid Blue 9 and 1 per cent Acid Red 73 on the efficacy of propyzamide on *Elytrigia repens* (treatment date 21 December 1998; assessment date 16 June 1999). Error bar denotes SED.

of 1.8 kg a.i. ha^{-1}, and the effect on efficacy was assessed as a percentage of green cover remaining. All the glyphosate treatments caused severe damage to the target plants, and the dyes were not found to cause any significant differences in the incidence of death ($P = 0.05$).

The final experiment investigated the effect of these two dyes on the efficacy of permethrin when used as a top-up spray against *Hylobius abietis* (Figure 10). Significant differences were found between the control treatment (no permethrin) and the permethrin/dye treatments, with the control treatment having a significantly smaller percentage of undamaged transplants ($P < 0.01$), and a greater mean percentage of seriously damaged and dead plants ($P < 0.001$). However, none of the dye treatments was found to have a significant effect on the efficacy of permethrin, in any of the categories of damage.

Discussion

At the rates selected, visibility of the commercial dyes was poor, and most were invisible when dry. Many of these dyes were designed to be used as concentrated sprays, e.g. on stumps, where it remains economic to apply a higher concentration of dye on a relatively small part of the site. Several dyes were partly visible when wet if used at a rate that was higher than recommended. However, higher concentrations are likely to prove to be uneconomic in most cases (Acid Blue 9 being an exception), and hence this line of investigation was terminated.

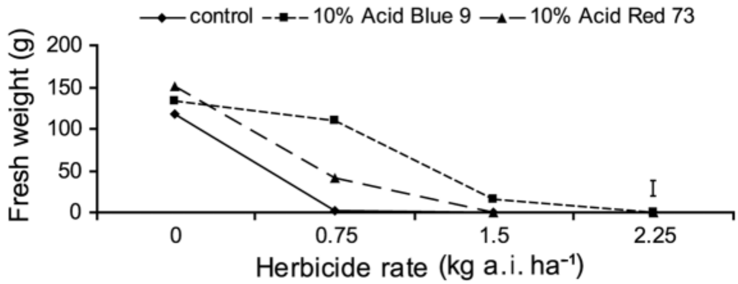

Figure 8. The effect of 10 per cent Acid Blue 9 and 10 per cent Acid Red 73 on the efficacy of Asulam on *Rumex obtusifolius* (treatment date 21 December 1998; assessment date 16 June 1999). Error bar denotes SED.

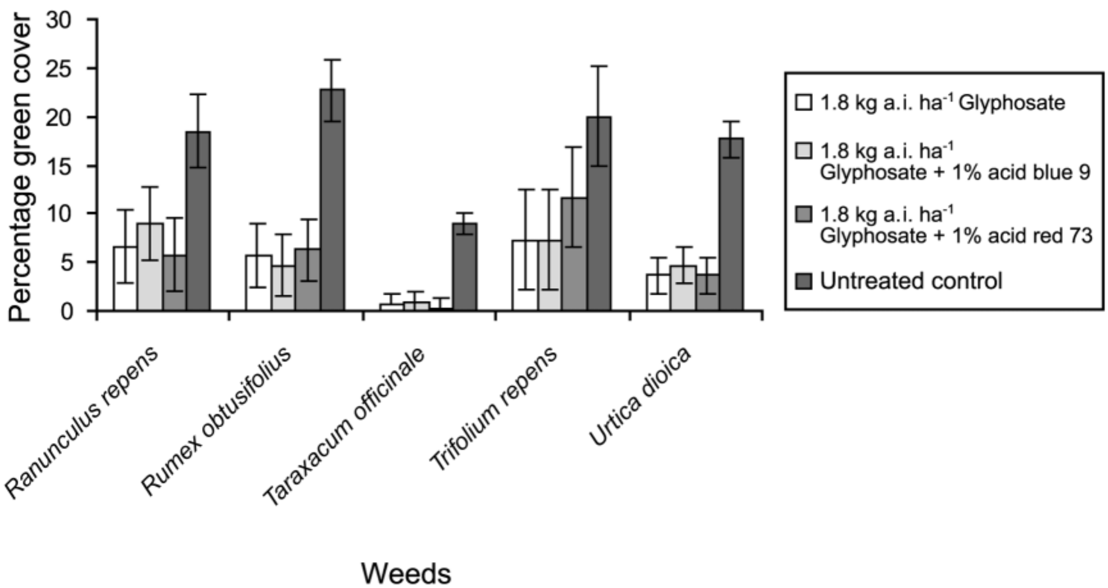

Figure 9. The effects of dyes on the efficacy of glyphosate on various mixed herbaceous weed species. Error bars denote SED.

Several textile dyes gave good visibility when wet or dry. Basic Violet 10 was the most visible and persistent, not fading completely until 43 days after application. However, there are a number of safety concerns relating to Basic Violet 10 (Field *et al*., 1995).

Subsequent experiments concentrated on cosmetic and food dyes, primarily from the azo and tri-phenylmethane dye groups, as these tend to be cheap and have acceptable colour intensity. In addition, although marker dyes are not covered by UK pesticide regulations (Pesticide Safety Directorate, personal communication), it seemed prudent to limit the choice of dyes to those approved for use in cosmetics and food to minimize the risk to operators or the environment from any spraying operation. Effort was therefore concentrated on Acid Blue 9, already in use with urea for stump treatment to protect against *Heterobasidium annosum* (Spencer,

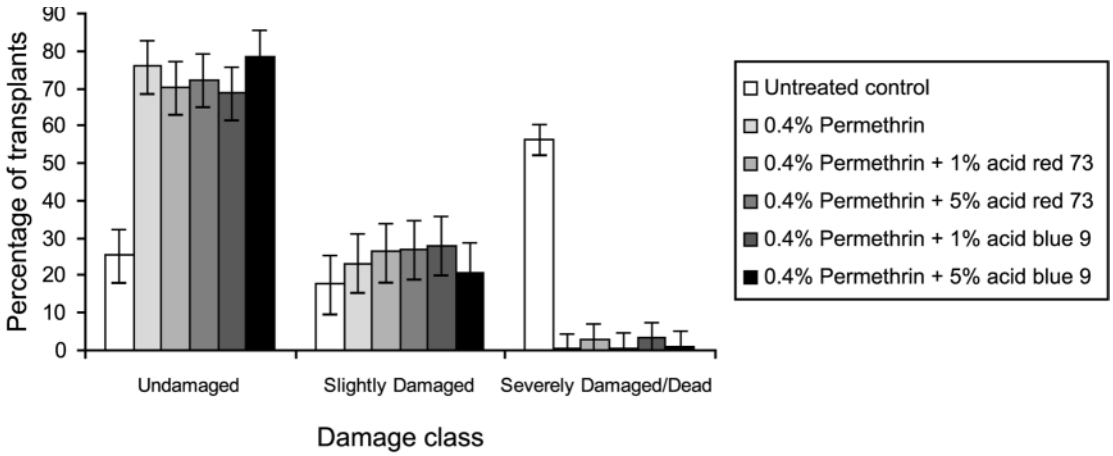

Figure 10. The effect of dyes on the efficacy of permethrin to prevent browsing by *Hylobius abietis* on *Picea abies* seedlings. Error bars denote SED.

1998) and Acid Red 73. The LD_{50} of Acid Red 73 could not be determined from the literature, but Acid Blue 9 has a low acute oral toxicity rate of LD_{50} >2000 mg kg^{-1} (Anon., 1999). The UK Pesticide Safety Directorate would usually class a pesticide product with this level of toxicity as not hazardous, and the World Health Organization assumes that an Oral LD_{50} of 2000 mg kg^{-1} for solids and 3000 mg kg^{-1} for liquids is so low that the hazard is negligible (Pesticide Safety Directorate, 2002; World Health Organization, 1997). A toxicity report (Anon., 1999) also found there to be no convincing evidence of reproduction toxicity, carcinogenicity or mutagenicity. A further food dye, Water Green 50, was also included in the third and fourth visibility experiments following promising results when used as a top-up spray with permethrin (Drake-Brockman, 1996).

On herbaceous vegetation, both Acid Blue 9 and Acid Red 73 were highly visible when wet, although Acid Blue 9 had greater and more persistent colouring at the higher concentrations. On bare ground and dead material the red dye was particularly visible. However, red dyes are less visible to people who have red/green colour-blindness. Both dyes reduced the efficacy of asulam and glyphosate, particularly at lower doses of herbicide. However when the dyes were added to propyzamide, or to glyphosate in the field trial, there was no significant reduction in efficacy. This suggests that if relatively resistant weeds are treated, spray distribution is poor, or low rates of glyphosate are used, dyes may significantly reduce the effect of glyphosate and possibly other foliar-acting herbicides. There was little evidence to suggest any consistent increase in efficacy from adding the dyes at the rates tested. Both dyes had acceptable visibility in the permethrin trials, having no perceptible effect on insecticide efficacy, suggesting either may have the potential for use as marker dyes for top-up sprays.

The small reductions in efficacy in asulam and glyphosate may have little practical impact as in field trials, at the higher rate usually used to control the weeds tested, efficacy did not appear to be affected. In addition, increasing the rates of these two chemicals is inexpensive, e.g. increasing the rate of glyphosate by 2 l ha^{-1} is unlikely to cost more than an additional £8 ha^{-1}. However in purely economic terms, the use of dye markers may not be justifiable if they are used simply to avoid the need for a repeat treatment of the target weed with a cheap herbicide.

The effect of the dyes on efficacy may mean that applying lower than recommended rates of foliar acting herbicides is not possible. However, the purpose of dyes is not to allow reduction in actual rates used, rather to allow better targeting of applications of pesticides at recommended rates. Thus dyes may help to avoid damage to non-target vegetation, reduce overdosing and remove the need for potentially damaging repeat applications. Dye markers may therefore allow a specific prescription for a particular chemical to be achieved more accurately in the field, thus helping to reduce the risk of inappropriate and potentially damaging misuse of pesticides.

Some herbicides are already distinctively coloured, and others have marker dyes recommended for particular operations on the product label. Where this is not the case, these experiments suggest that Acid Blue 9 or Acid Red 73 can be an effective marker dye for pesticides. Acid Blue 9 is available ready formulated in water as a liquid dye (Dysol Turquoise; Albion Colours), and hence is easier to handle than powder formulations of Acid Red 73. At a rate of 2 per cent of final spray volume, Dysol Turquoise costs around £68 ha^{-1} (for 200 l ha^{-1} diluent, an equivalent of 2 l ha^{-1} Acid Blue 9). This compares with a typical chemical cost ranging from approximately £20 ha^{-1} (for 5 l ha^{-1} glyphosate) to £85 ha^{-1} (for 3.75 l ha^{-1} propyzamide), with an additional cost of around £70 ha^{-1} for the manual application itself. For top-up sprays with permethrin against *Hylobius abietis*, only 25 l ha^{-1} diluent is used, resulting in a cost of around £17 ha^{-1} for Dysol Turquoise, compared with £60 ha^{-1} for the insecticide and application. Replacement insecticides for permethrin are likely to be considerably more expensive.

Although there appears to be little evidence of long-term studies, given its low toxicity, it seems unlikely that Acid Blue 9 will add to the environmental risk already present from applying any approved pesticide. However, in some instances more dye marker may be required than actual plant protection product, and land managers may need to take a judgement as to whether or not the benefits of the dye marker outweigh both its cost, and any risk they may perceive from adding a further synthetic chemical into the natural environment.

Visibility and persistence of dyes will depend on individual perception, spray quality, and on vegetation type and weather conditions. These experiments suggest that 2 l ha $^{-1}$ of Acid Blue 9 should be clearly visible when wet or dry on a variety of vegetation types, but subsequent visibility will vary. For this reason its use will be of most value to operators, not for follow-up supervisory purposes. The addition of Acid Blue 9 to pesticides appears to offer one immediate way of achieving better targeting and early identification of faulty equipment, and hence some pesticide reduction in UK forestry.

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