# POSTER SESSION 8D

# DEVELOPMENTS IN HERBICIDE APPLICATION AND FORMULATION TECHNOLOGY

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Poster Papers: 8D-1 to 8D-7

# The characteristics of sprays produced by air induction nozzles

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## ABSTRACT

This paper reports on measurements of the characteristics of sprays produced by five commercially-available air induction nozzles in terms of the flow rate of air into each nozzle, droplet size distributions and droplet velocities and considers the implications for the quantity of included air in spray droplets and the potential risk of spray drift.

Results suggest, for a given nozzle size and pressure, sprays with a larger droplet size have a greater flow of air into the nozzle and a larger percentage of included air in droplets. The quantity of included air in spray droplets reduces as nozzle size increases. The risk of spray drift is strongly dependent on droplet size.

## INTRODUCTION

A range of designs of air induction nozzle are commercially available that use a Venturi to draw air into the nozzle before atomising the liquid. It has been shown that the different designs produce droplets with a wide range of characteristic droplet sizes (Piggott & Matthews, 1999), although the consequences of these differences in terms of spray performance has not yet been evaluated.

Previous work with a test nozzle (Butler Ellis *et al.*, In preparation A) evaluated air intake, droplet size distributions, droplet velocities and risk of spray drift. The quantity of air contained in droplets was estimated and these parameters were related to changes in nozzle design. Here, we use similar techniques to compare the characteristics of sprays produced by five commercially-available air induction nozzles, and together with measurements published elsewhere (Butler Ellis *et al.*, In preparation A & B,), consider the implications for spray drift.

## MATERIALS AND METHODS

Five nozzle designs were evaluated (Table 1). Measurements were made of rate of air flow into the nozzle, droplet size distributions and droplet velocities. All measurements were made with the "02" size (0.8 litres/min at 3.0 bar) and some with the "04" size (1.6 litres/min at 3.0 bar).

Nozzles were inserted into a brass case (Figure 1) which enabled the equipment for measuring air flow rates described by Butler Ellis *et al.* (In preparation A) to be attached to an inlet port. Air flow measurements were not made with nozzle 2 because its design was not compatible with the geometry of the case in Figure 1.

Table 1. Air Induction nozzles selected

| Nozzle number | Manufacturer | Nozzle description |
|---|---|---|
| 1 | Billericay Farm Services | Bubblejet |
| 2 | Lurmark Ltd | DriftBeta |
| 3 | Hardi International | Injet |
| 4 | Spraying Systems | Teejet AI |
| 5 | Sprays International | Pneujet |

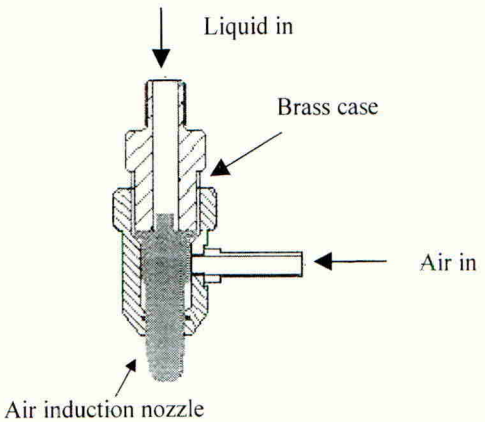


Figure 1. Arrangement for measurement of air flow into air induction nozzles

Droplet size distributions of the full spray from each of the nozzles at 2,3 and 4 bar were made with a particle/droplet image analysis (PDIA) system (Visisizer, Oxford Lasers Ltd) also described by Butler Ellis *et al.* (In preparation A). The data were analysed to determine volume median diameter (VMD) of the spray, although this is only a nominal value since liquid volumes cannot be measured directly when sprays contain air-included droplets.

Droplet velocities were measured vertically below the nozzle using PDIA. Measurements were made with the nozzle operating at 3.0 bar, spraying both water alone and 0.1 % surfactant (Agral). The mean droplet velocity for each droplet size was calculated. These velocities were used as input to a model of droplet trajectories in order to estimate droplet densities, as described in Butler Ellis *et al.* (In preparation A).

## RESULTS AND DISCUSSION

### Droplet size and air intake

As expected, VMD reduced as pressure increased, although unlike conventional nozzles, the VMD did not necessarily increase with nozzle size (Figure 2). This relationship only holds with conventional nozzles because the single orifice controls both droplet size and nozzle output. With an air induction nozzle, the first orifice controls flow rate and the final orifice controls spray droplet size and so droplet size is essentially independent of nozzle output.

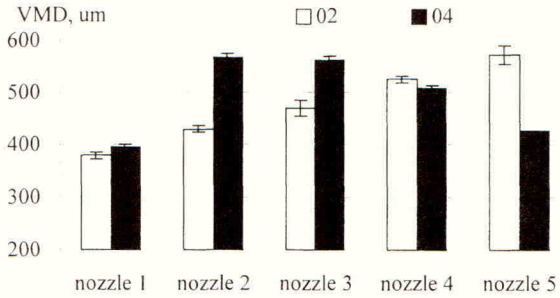


Figure 2. The effect of nozzle output on VMD

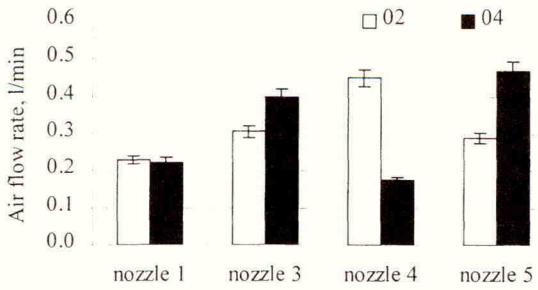


Figure 3. The effect of nozzle size on air intake

The flow rate of air into the nozzle increased with liquid pressure, as expected, but there was no consistent relationship between air intake and nozzle size (Figure 3). The proportion of air in the liquid/air mixture leaving the nozzle varied only slightly with pressure but is very dependent on nozzle size, with the 04 nozzle typically resulting in a lower proportion of air, sometimes considerably so (Figure 4). Previous work showed that the quantity of air in droplets was strongly influenced by the proportion of air exiting the nozzle (Butler Ellis *et al.*, In preparation A), suggesting that the 04 size nozzles produced droplets with less included air than the equivalent 02.

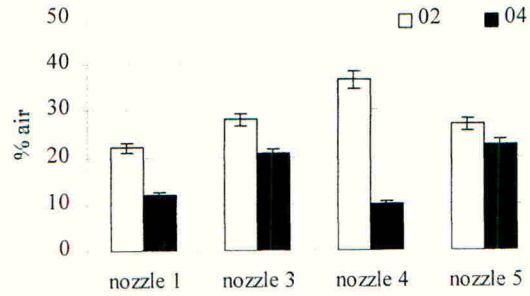


Figure 4. The effect of nozzle size on the proportion of air exiting four air induction nozzles

**Droplet velocities**

The relationship between droplet size and velocity for AI nozzle 5 is shown in Figure 5. Velocities of droplets are significantly lower with air induction nozzles than with a conventional flat fan. With the flat fan nozzle, velocities of droplets containing surfactant are the same as those consisting of water only, as would be expected with droplets of the same density. However, with the air induction nozzles, at 600 mm from the nozzle the velocities of droplets containing 0.1 % non-ionic surfactant are lower than droplets of water only, indicating the presence of air inclusions.

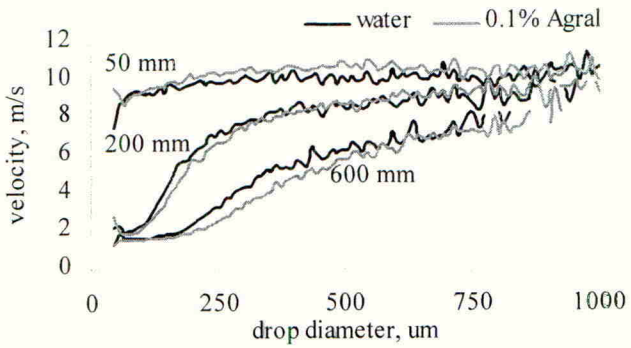


Figure 5. Variation of velocity with droplet size at three distances from AI nozzle 5

The change in velocity between 200 and 600 mm below the nozzle can be used to estimate the density of droplets, (Butler Ellis *et al.*, In preparation A). Table 2 shows the characteristics of sprays from the "02" size nozzles at 3.0 bar. For larger droplets with a greater percentage of included air, the estimated droplet density agreed well with the calculated density of the liquid/air mixture exiting from the nozzle. However, nozzle 1 had significantly less air in droplets than the air/liquid mix suggested.

Table 2. Characteristics of sprays from "02" nozzles, measured at 3.0 bar spraying 0.1 % surfactant

| Nozzle number | VMD, μm | Mean velocity of 300μm droplets 200 mm from nozzle, m/s | % air in air/liquid mix | Estimated % air in spray droplets |
|---|---|---|---|---|
| 1 | 379±7 | 10.3 | 22 | 10 |
| 2 | 430±7 | 7.4 | - | 10 |
| 3 | 469±15 | 8.5 | 28 | 25 |
| 4 | 525±6 | - | 27 | - |
| 5 | 572±18 | 7.5 | 36 | 35 |

**Spray drift**

Measurements of horizontal drift profiles in a wind tunnel were made previously with a test nozzle and showed that characteristic droplet size was the most important indicator of the risk of drift (Butler Ellis *et al.*, In preparation A). Measurements of wind tunnel drift profiles were also made with the nozzles of Table 1 and a range of liquids to determine how spray liquid might influence spray performance (Butler Ellis *et al.*, In preparation B). There is also a considerable unpublished body of data concerning drift and spray droplet size from a variety of sources. Some of these data were used to calculate a drift length scale (Walklate *et al*, 2000) and compared with VMD, as shown in Figure 6.

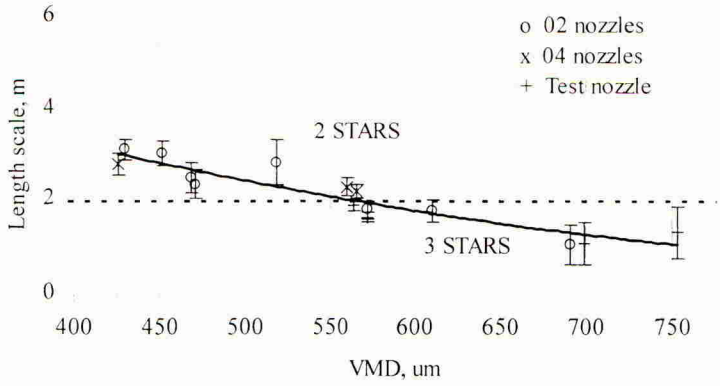


Figure 6. Relationship between VMD and drift length scale for a range of air induction nozzles at a range of pressures, compared to a standard flat fan "03" reference nozzle

Despite each nozzle producing sprays with very different droplet velocities, air velocities and droplet densities, the effect of droplet size appears to dominate the calculated drift length scale. The threshold for a three star rating by this calculation appears to be around 575 μm (as measured with PDIA using the settings described in Butler Ellis *et al.*, In preparation A).

## CONCLUSIONS

Air induction nozzles produce sprays with a range of droplet sizes, droplet velocities and quantities of included air. Increasing the nozzle operating pressure increased liquid flow rate and air flow rate, and reduced droplet size but had only a small effect on the proportion of air in the fluid exiting from the nozzle.

The relationship between droplet size and nozzle output for conventional nozzles does not necessarily apply to air induction nozzles. All of the four air induction nozzles tested showed a lower proportion of air with the 04 size than with the 02 size.

The major factor determining the risk of drift, as measured in a wind tunnel, was spray droplet size.

## ACKNOWLEDGEMENTS

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Walklate P J; Miller P C H; Gilbert, A J (2000). Drift classification of boom sprayers based on single nozzle measurements in a wind tunnel. *Aspects of Applied Biology*, **57**, Pesticide Application, 49 – 56

# An investigation into the deposition and efficacy of pesticide sprays from air induction nozzles

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## ABSTRACT

Experiments to evaluate the differences in deposit and biological control between air induction (AI) nozzles and conventional nozzles, with different designs of AI nozzle, different spray liquids, at two volume rates and on a range of target plants are reported. Statistical significance was rarely obtained, but flat fans nozzle resulted in the greatest deposit more frequently than did AI nozzles. At 100 litres/ha, the flat fan nozzle resulted in the best control more frequently than AI nozzles, and an AI nozzle resulted in the best control for all 200 litres/ha treatments.

## INTRODUCTION

The use of air induction (AI) nozzles in pesticide application is widespread. They reduce drift significantly (e.g. Lund, 2000) and are therefore valuable when conventional nozzle technology might lead to unacceptable risk of drift, e.g. low volumes. Droplets in sprays from AI nozzles are larger than from conventional nozzles (Piggott & Matthews, 1999) and there are concerns that their use may compromise the quantity of pesticide retained on the target plant and efficacy. It is recognised that herbicide activity can be reduced as droplet size increases when conventional nozzles are used (Knoche, 1994). The characteristics of AI nozzles vary between designs, with consequences for spray drift (Butler Ellis *et al.*, 2001) and potentially for efficacy. The quantity of spray liquid retained by the target plant is dependent on both the spray and the characteristics of the target itself. It has been suggested that small, upright plants are more difficult to deposit spray on than larger, horizontal targets with coarser sprays that have fewer droplets (Jensen, 1999), although there is little evidence yet to support this.

For a given forward speed, the deposit from conventional flat fan nozzles is generally expected to increase as volume rate, and therefore nozzle size, is reduced probably as a consequence of the reduction in spray droplet size. There is no similar relationship between droplet size and nozzle output for AI nozzles (Butler Ellis *et al.*, 2001) and therefore the effect of reducing volume rates is less certain.

The performance of a spray nozzle is dependent on spray liquid, but the effect on spray droplet size with AI nozzles can be opposite to that of conventional nozzles (Butler Ellis & Tuck, 2000), with water-soluble surfactants increasing droplet size and emulsifiable concentrate (EC) formulations decreasing droplet size compared to water alone. The addition of a surfactant is likely to increase the quantity of included air and may therefore affect retention more with an AI nozzle than a conventional flat fan. The consequences for spray deposit on target plants of changing from a conventional to an air induction nozzle may thus depend upon AI nozzle design, spray liquid, volume rate and the target plant.

This paper reports the results of a series of experiments to evaluate the differences in deposit between AI nozzles and conventional nozzles, and between different designs of AI nozzle with different spray liquids and on a range of target plants.

## MATERIALS AND METHODS

### Laboratory measurement of deposit on tray-grown crops

A conventional flat fan, (02F110YE, Lurmark Ltd) and two AI nozzles, (02 Bubblejet, BFS Ltd and 02 Pneujet, Sprays International) were used. The VMDs, measured with an imaging system (Oxford Lasers Visisizer) when spraying 0.1 % surfactant (Agral) at 3.0 bar were 194, 379 and 571 µm and were denoted FF, AIS and AIL respectively. Three liquids were selected to produce a wide range of droplet sizes with AI nozzles: a water-soluble surfactant, (Genapol LRO, Clariant Ltd, UK); a blank EC formulation, (Aventis CropScience UK Ltd) and water alone. 0.5 % Eurocert Green S E142 tracer dye was added to each spray liquid.

Three different outdoor-grown crops were used: a dense crop of oilseed rape (Apex), winter wheat (Claire) at GS 14 and spring wheat (Chablis) at GS 13. Three nozzles were arranged 0.5 m apart on a boom section, 0.5 m above the crop. Trays of plants were arranged as a four-by-three array. The boom, mounted on a transporter, had a forward speed of 2.2 m/s. Samples were taken of five plants from the middle of each of the two central trays, one directly below the middle nozzle and the other between two nozzles. These were bulked together and washed off in 50 ml distilled water. Two replicate measurements were made for each treatment. The data were analysed to determine the quantity of spray liquid per unit weight of plant material for each replicate.

### Field trials

A total of nine different application treatments (Table 1) were evaluated for deposit on the crop and for biological control. A surfactant (Genapol LRO) was added at 0.1% to one set of treatments. Dose rates of herbicide were lower than recommended rates in order to discriminate between treatments.

Table 1. Spray treatments and nozzle VMD, measured with PDIA, when spraying 0.1 % non-ionic surfactant at 3.0 bar

| Nozzle code | Nozzle | VMD μm | App. rate, l/ha | Added surfactant |
|---|---|---|---|---|
| FF | 04F110RE, Lurmark Ltd | 233 | 200 | No |
| AI1 | 04 Bubblejet, BFS Ltd | 389 | 200 | No |
| AI2 | DB04F120, Lurmark Ltd | 553 | 200 | No |
| FF | 02F110YE, Lurmark Ltd | 194 | 100 | No |
| AI1 | 02 Bubblejet, BFS Ltd | 379 | 100 | No |
| AI2 | DB02F120, Lurmark Ltd | 430 | 100 | No |
| FF | 02F110YE, Lurmark Ltd | 194 | 100 | Yes |
| AI1 | 02 Bubblejet, BFS Ltd | 379 | 100 | Yes |
| AI2 | DB02F120, Lurmark Ltd | 430 | 100 | Yes |

*Experiment 1*: A crop of winter wheat containing wild oats was treated at GS 33 and 38 with 62.5 ml/ha of clodinafop-propargyl (Topik) plus 1 litres/ha mineral oil. Tracer (Helios SC500) was added to each tank mix at 50 g/ha. Ten wild oat plants were sampled and cut into either three sections for GS33 or four sections for GS38, as described by Marshall *et al*, 2000. Control was assessed approximately 30 days after application.

*Experiment 2*: Amidosulfuron (Eagle, 75% w/w WDG) was applied at 20 g/ha. At site 1, cleavers at growth stages between 25 and 34 were treated in a crop of winter wheat at GS 31-32. At site 2, cleavers at GS 37 were treated in a crop of winter wheat at GS 32-33.

## RESULTS AND DISCUSSION

### Measurements of deposit

The effects of nozzle and liquid on the quantity of spray liquid deposited on trray-grown plants, per unit weight of plant material, are shown in Figure 1 for rape and spring wheat (winter wheat deposits are not shown). Each data point is the average of four (two replicates below the central nozzle, and two from between the nozzles). The mean quantities of plant material per sample were 45g for rape, 3.4 g for spring wheat (8.3 g for winter wheat). The total quantity of active ingredient deposited in field trials at the first and second timings is shown in Figure 2.

There was no statistical significance in deposit between most treatments, particularly with the tray-grown crops. It is only possible therefore to consider trends. On rape, the FF nozzle produced the highest deposit but there were no clear trends on wheat. The small spring wheat plants could be harder targets for the low droplet numbers produced by the large-droplet AI nozzle but this was not apparent, suggesting that if there is a target size below which AI nozzles become less efficient, it is smaller than those used here. The effect of spray liquid was similar for all three nozzles, with no increase in deposition with the surfactant and AI nozzles above that expected with conventional nozzles.

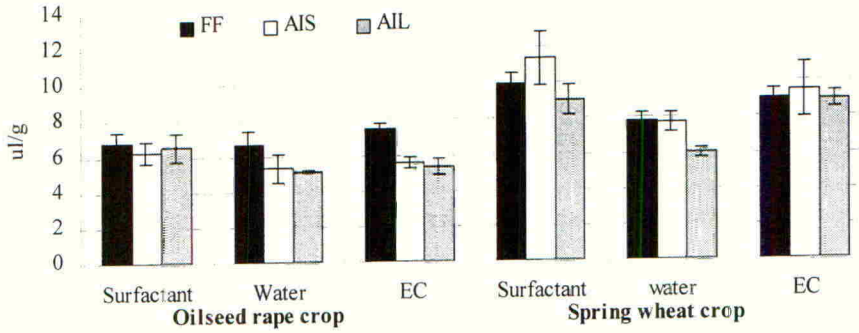


Figure 1. Mean deposit on oilseed rape and winter wheat with three nozzles and three liquids. Error bars denote standard error.

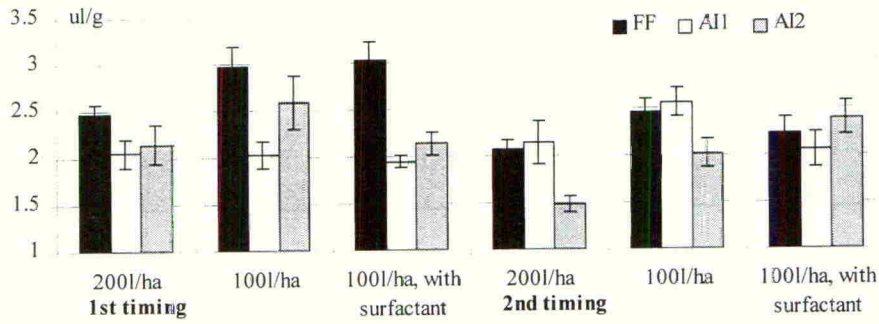


Figure 2. Deposit of active ingredient on wild oat leaves 1-3 at the 1st timing, and leaves 1-4 at the 2nd timing. Error bars denote standard error.

At 100 litres/ha in the field, the FF nozzle had the greater deposit for the first timing, though not the second. It was not possible to distinguish consistent differences in deposit between the two AI nozzles either in the lab or the field, despite different spray characteristics, Comparing 200 with 100 litres/ha also showed the expected increase in deposit with the FF nozzle due to finer droplets. The AI nozzles did not show this, despite AI2 also producing smaller droplets at the lower flow rate. At the later growth stage, either AI1 or AI2 produced the largest deposit, similar to the trends observed by Marshall *et al.* (2000) where an AI nozzle deposited more, particularly on lower leaves, than a flat fan nozzle. The addition of surfactant had a similar effect for FF and AI nozzles.

**Efficacy assessments**

Again, there was no statistically significant differences between nozzles, so only trends are considered. Estimates of control of *Avena fatua* are shown in Figure 3. The slightly better retention of the FF nozzle at the first timing was not reflected in biological control, whereas the slightly poorer retention of AI1 was.

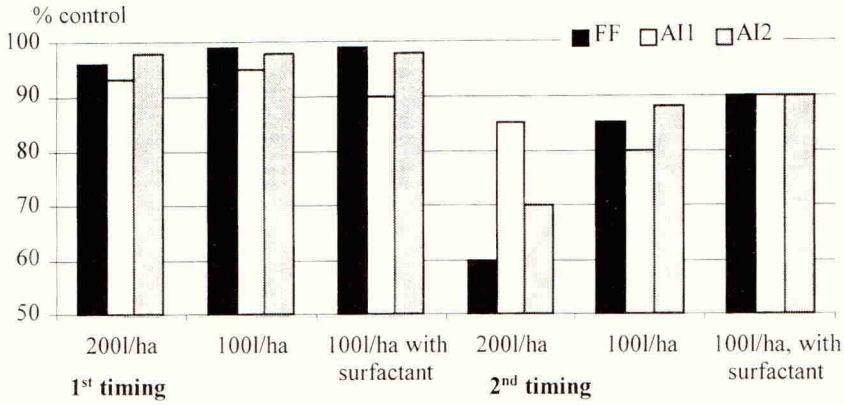


Figure 3. Control of *Avena Fatua*, 30 d after spraying (statistical analysis unavailable)

At the second timing, there was a large improvement in control with AI2 and FF nozzles between 200 and 100 litres/ha, both of which have reduced droplet size at the lower volume rate.. The control of *Gallium aparine* (Figure 4) at 100 litres/ha was slightly better with the FF nozzle than AI nozzles, with improved control between 200 litres/ha and 100 litres/ha for the FF nozzle. The addition of surfactant had a similar effect for both FF and AI nozzles.

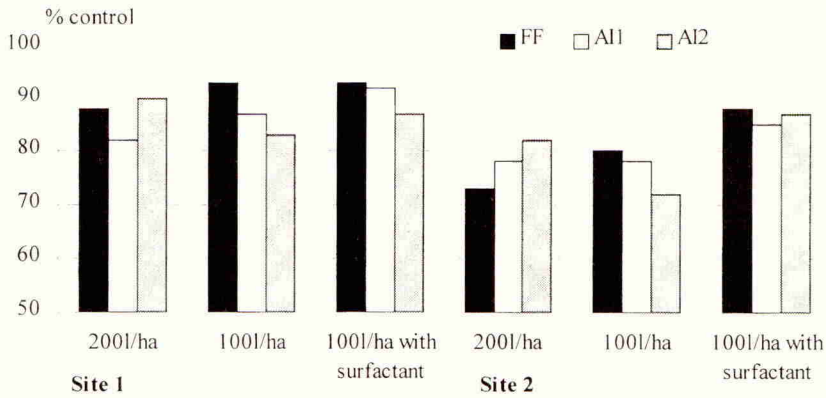


Figure 4. Control of *Galium aparine*. LSD = 8.6 % control at site 1 and 11.6 % control at site 2

Of the 12 treatments overall, the FF nozzle had better control with six, an AI nozzle with five and one showed no difference. AI nozzles performed better, relative to the FF nozzle, at 200 litres/ha than at 100 litres/ha, with the large-droplet AI2 performing better than the smaller droplet AI1. It appeared that improvements in efficacy with reducing volume occurred only with flat fan nozzles, i.e. the performance of AI nozzles was independent of nozzle output. For retention, where there were 15 comparisons, the FF nozzle produced the greatest deposit in 10 cases, and an AI nozzle in only five. There was a poor correlation

between deposit and control, suggesting that the distribution of the deposit may be of more significance than the quantity.

## CONCLUSIONS

The quantity of pesticide spray deposited on a range of crops from air induction and flat fan nozzle spraying different formulation types was measured. The variation between replicates was such that statistical significance was rarely obtained. On balance, the flat fan nozzle resulted in the greatest deposit more frequently than did the AI nozzles. There was more pesticide deposited at 100 litres/ha than at 200 litres/ha with flat fan nozzles because of the smaller droplet size, but this relationship was not apparent with AI nozzles, even with an AI nozzle that had a smaller droplet size at the lower application rate.

When determining efficacy, the flat fan had the best control more frequently than the AI nozzles at 100 litres/ha. An AI nozzle had the best control for all 200 litres/ha treatments. The trends in control between nozzles were not well correlated with trends in deposit, suggesting that distribution of deposit over the plant is potentially more important than its quantity.

## ACKNOWLEGEMENTS

This work was funded by MAFF, Lurmark Ltd, Syngenta Crop Protection UK Ltd and Aventis CropScience UK Ltd through the SAPPIO LINK programme.

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Piggott S; Matthews G A (1999). Air Induction nozzles: a solution to spray drift? International Pest Control, January/February 1999: 24-28

# Classification and imaging of agricultural sprays using a particle/droplet image analyser

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## ABSTRACT

The principles and uses of a new laser-based high-speed imaging system that utilises an infra-red laser and a high-speed digital camera are described. Drop sizing and statistical corrections are achieved through software. Measurements of droplet size were made using reference nozzles from the BCPC and the International classification schemes. Suitable grids were obtained that should enable ranges of nozzles to be classified. The use of the system to produce sequences of high-speed images was explored. Side views of break-up from standard and air-induction flat-fan nozzles showed that air-induction nozzles produce thick spray plumes and periodic flow instabilities may occur.

## INTRODUCTION

Hydraulic nozzles produce sprays with a range of droplet sizes and velocities (Lefebvre, 1989). These variations can affect the efficient application of agricultural sprays. Parkin (1993) reviewed methods of measuring droplet size with agricultural sprays. The most common laser based methods were; laser diffraction (e.g. Malvern); droplet imaging (e.g. PMS), where size and velocity is determined through a small sample volume; and phase Doppler analysis (e.g. Aerometrics PDA or Dantec PDPA) where, droplet velocity and size were determined by phase difference in scattered light. These techniques were incorporated within the BCPC nozzle classification system (Doble *et al.*, 1983), which places nozzles in categories based on their spray quality (i.e. droplet size). Droplet size distributions of test nozzles are compared against standard nozzles, allowing the relative quality of the test spray to be determined. The scheme was later modified and extended (Southcombe *et al.*, 1997).

However, the increasing use of twin-fluid and air-induction nozzles has recently created difficulties. It appears that the classification of these designs could be hindered by the suitability of some of the available measurement systems. Sprays produced by the interaction of air and liquid are known to produce droplets with air inclusions (Rutherford *et al.*, 1989). It has been recognised that these inclusions could cause difficulties with techniques that rely on diffraction and refraction (Tuck *et al.*, 1997). This could limit the measurement systems that can reliably be used for these nozzle designs to those based upon imaging. There has also been increased interest in imaging as a diagnostic tool in atomisation research caused by the need to explore the effect of adjuvants on spray behaviour and investigate the mechanisms involved in atomisation by air-induction nozzles.

The Oxford Lasers PDIA (Particle/Droplet Image Analysis) is an imaged-based system with a large sampling volume that is capable of determining droplet size and velocity. It is capable

of capturing video images of high-speed processes and, using its VisiSizer software, is able to measure droplet size and velocity. In this paper we describe the use of a PDIA system for characterising and visualising the atomisation process that occurs in agricultural sprays.

## METHOD AND MATERIALS

### PDIA technique

Image acquisition was by a high-speed CCD camera with illumination provided by a diode laser and a diffuser (Figure 1). The 8-bit camera operated at 30 Hz for drop sizing and 250 Hz for high-speed imaging. The image resolution was 512 x 480 pixels.

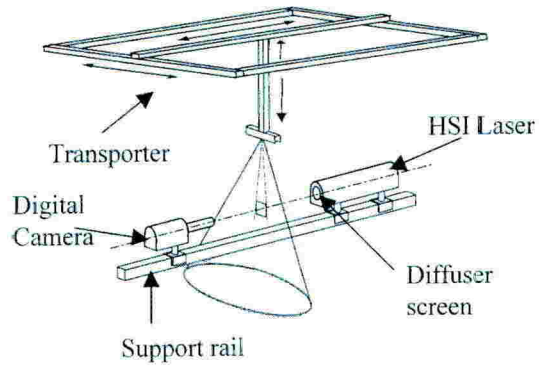


Figure 1. Layout of main components of the PDIA

The technique uses backlit imaging (Figure 1) to produce shadow images of the droplets (Figure 2). In this typical image (Figure 2) the sized droplets are numbered and the X indicates a focus rejection. (For purposes of illustration rejection has been set at a more stringent level than normal.) Images of droplets touching the image border have been automatically rejected along with those that occupy fewer than 10 pixels.

The laser freezes the motion of the droplet (maximum allowed movement is 10 % of the particle diameter, during the laser pulse) which appears dark on a light background. A threshold grey-level is set on the images, and droplet areas are measured by software. In order that droplets are accurately sized, the intensity gradient at the edge of the droplet is measured to determine the degree of focus of the image. This enables large droplets that are in focus and small droplets that are out of focus to be distinguished and accurately sized (Figure 2). It can also allow for statistical corrections relative to different particle sizes having different depths of focus. The semi-empirical system proposed by Yule *et al.* (1978), in which the gradient of intensity at the edges of the droplets on the image is measured, is used. The software has the ability to measure non-spherical particles or droplets, and shape parameters derived from the images can be used to distinguish non-spherical or overlapping droplets.

To date the smallest particles that have been sized by PDIA systems are 1 μm diameter. This is the practical limit imposed by diffraction. Larger particles may be sized by a suitable choice of imaging optics. The dynamic range of sizing depends on the number of pixels in the image. The smallest particle must be imaged to approximately 5 pixels width, while the

largest should be no more than 20 % of the total image size. For a 500 pixel sensor, this corresponds to dynamic range of 20, comparable with PDA/PDPA (Tuck *et al.* 1997).

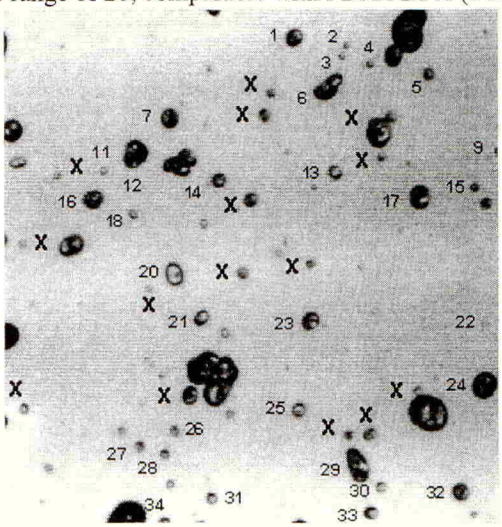


Figure 2. Processed images from an agricultural spray

All droplet sizing techniques suffer to some extent when the droplets are non-spherical. However, PDIA has the advantage of providing clear images of the droplets being sized; for example where air inclusions are clearly visible in the droplet (Figure 3).

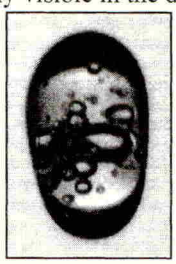

Figure 3. In-flight image of a *ca* 1 mm diameter water droplet with air inclusions

**Droplet sizing and imaging**

To obtain a representative sample of the spray below the nozzle, test nozzles were mounted on a 1100 mm square PC-controlled X-Y transporter (Figure 1) operating with an accuracy of 0.1 mm at a speed of 50 mm/s. A short axis sample was used (Tuck *et al.*, 1997). When imaging, the nozzle was fixed and optics with lower magnification and an additional diffusion screen were used.

**Nozzles and Spray Liquids**

To assess the capability of the PDIA to classify agricultural sprays, sets of reference nozzles from the original BCPC (Doble *et al.*, 1983) and modified International (Southcombe *et al.*, 1997) schemes were tested and classification grids established. These tests used a 0.1 % solution of non-ionic surfactant (Agral; 900 g/l alkyl phenol ethylene oxide).

An investigation also compared the atomisation of conventional and air-induction 110-03 flat-fan nozzles using the imaging capabilities of the PDIA. Unlike most previous studies (e.g. Butler Ellis *et al.*, 1997) side views of the liquid sheet were obtained. The spray liquid was a 0.5 % v/v solution of a cationic surfactant (Ethokem; 870 g/l polyoxyethylene tallow amine).

## RESULTS

### Droplet Sizing

PDIA measurements of the nozzles that define the classes in the original BCPC and modified International schemes are shown in Tables 1 & 2.

Table 1. Original BCPC classification grid using the PDIA

| Nozzle (Lurmark) | Pressure (bar) | VMD (µm) | Boundary VMD (µm) | Category Width (µm) | Description |
|---|---|---|---|---|---|
| 01-F110 | 4.5 | 159 | 160 | | Very Fine / Fine |
| | | | | Fine 56 | |
| 02-F110 | 3.5 | 187 | 216 | | Fine / Medium |
| | | | | Medium 57 | |
| 04-F110 | 2.5 | 245 | 273 | | Medium / Course |
| | | | | Coarse 56 | |
| 08-F110 | 2 | 301 | 329 | | Coarse / Very Coarse |

Table 2. International classification grid using the PDIA

| Nozzle | Pressure (bar) | Boundary VMD (µm) | Category Width (µm) | Description |
|---|---|---|---|---|
| Delevan 11001 | 4.5 | 154 | | Very Fine / Fine |
| | | | Fine 54 | |
| Lurmark 31-03-F110 | 3.0 | 208 | | Fine / Medium |
| | | | Medium 39[1]/59[2] | |
| Lechler LU12006 | 2.0 | 247[1]/267[2] | | Medium / Course |
| | | | Coarse 126[1]/106[2] | |
| TeeJet 8008 | 2.5 | 373 | | Coarse / Very Coarse |

[1] Scan limited by transporter width. [2] Sum of two half-scans.

The original BCPC scheme required interpolation for the category boundaries. The modified International scheme uses sets of stainless steel references nozzles to define boundaries.

### Imaging

In side view the characteristics of the spray plume from the air-induction nozzle are markedly different from those of the standard nozzle (Figure 4). A periodic instability appears to be generated within the nozzle that causes fluctuations in the plume.

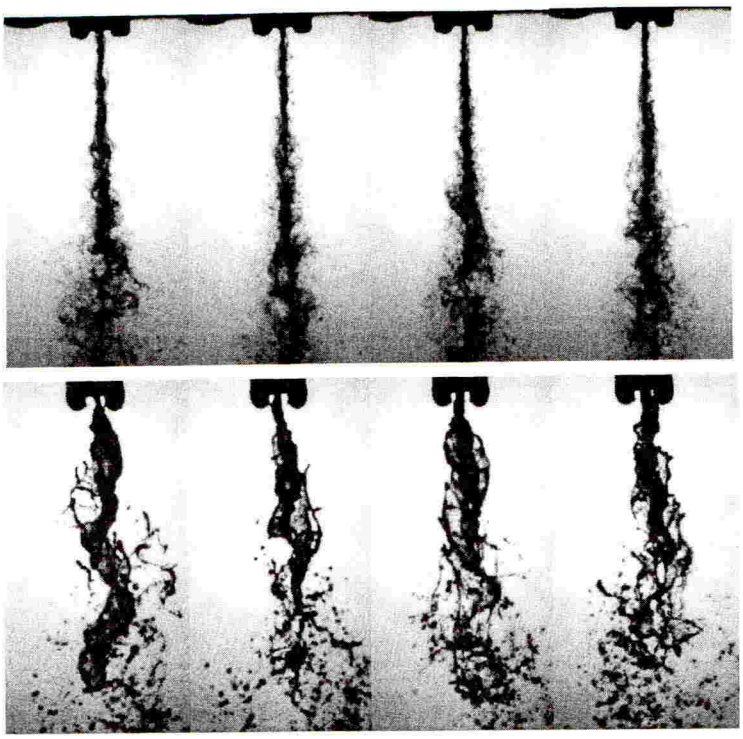

Figure 4. Sequences of side views of atomisation from 110-03 standard (top) and air-induction (bottom) flat-fan nozzles operated at 3 bar pressure. Images taken at 250 frames/s. Image height ca 44 mm.

## DISCUSSION

### Imaging

The quality of digital images rarely approaches those from conventional photography, as can be seen by comparing Figure 4 with images in Butler Ellis *et al.* (1997). However, the ease by which sequences of images are obtained makes the use of the PDIA a powerful tool for spray diagnostics. Animated sequences of spray break-up can show processes that are not apparent even with good quality single photographs. They have highlighted processes such as flow instabilities, bubble separation from the spray sheet, and spray sheet folding.

### Droplet Sizing

All droplet sizing techniques suffer to some extent when the drops are non-spherical. However, this imaging based technique appears to have the advantage of providing clear images of the droplets being sized, so that the user is aware that there may be a problem. The optical bench configuration of the PDIA makes it a more flexible instrument than earlier imaged based systems. It appears capable of generating a grid that is suitable for classifying nozzles under both under the original BCPC and the International schemes. Comparing the schemes, the Coarse / Very Coarse boundary with the International scheme appears to be a lot higher. Whereas with the BCPC scheme the widths of the categories were similar, with the

International scheme the Coarse category is now wider that the other two. The Medium / Coarse boundary nozzle caused some difficulties because of the larger fan angle of the reference nozzle. The preferred approach was to scan each half of the spray plume and sum the results. The values for the boundary VMDs established for the PDIA are likely to be coarser than those obtain using Malvern particle size analysers given the differences in sensitivity to fine sprays between the two techniques (Arnold, 1987).

## CONCLUSIONS

The PDIA is a useful tool for research into agricultural sprays. Its imaging technique is particularly suitable for droplet sizing with air-included sprays. Although not providing such high quality images as conventional high-speed photography, its imaging sequencing capabilities make it a powerful diagnostic tool for investigating the break-up process. Suitable classification grids can be generated from droplet size results for the original BCPC and International classification schemes. Directly comparative measurements between instruments are required to assess differences in classification. The problem of classifying air-induction and twin-fluid nozzles remains to be solved but this technique should provide a useful tool to assist with this characterisation.

## ACKNOWLEDGEMENT

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# Enhancement of sulfosulfuron activity by a new additive

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## ABSTRACT

A new additive consisting of the pyrimidine allopurinol and molybdenum trioxide, formulated in 50% monolaurate, was found to boost the activity of sulfosulfuron used mainly for the control of wild oats and rye grass in wheat. This increased activity led to the effective control of wild oat populations which are not normally controlled by the herbicide. The mixture of allopurinol and molybdate has previously been found to inhibit the synthesis of ABA, and it is presumed that at least part of the synergism shown between the additive and ALS-inhibiting herbicides involves lowering the ABA levels in the target plants, resulting in a lowering of their resistance to the herbicide.

## INTRODUCTION

Resistance to the ACCase-inhibiting grass weed herbicide diclofop-methyl was first reported in the Western Cape region of South Africa by Cairns & Hugo (1986). Resistance also developed to the new ACCase-inhibiting herbicides as fast as they came on the market. With the discovery of the ALS-inhibiting herbicides it was hoped that this new mode of action would halt the spread of the ACCase-resistant grass weeds. However, during the first season of the commercialisation of the ALS-inhibiting herbicides, producers began to report cases of less-than-optimum performance. Some four seasons after the registration of the ALS-inhibiting herbicides several cases of full-blown resistance had been reported. The degree of cross-resistance between the ACCase-resistance biotypes and the ALS-resistant biotypes was unknown. One of our studies examined the cross-resistance to ACCase- and ALS-inhibiting herbicides. During the course of these investigations, we found that the performance of sulfosulfuron could be greatly enhanced by the addition of a new growth regulator *cum* herbicide additive we have been working with for some time. This growth regulator consisted of the pyrimidine allopurinol and molybdate. Allopurinol is an inhibiter of aldehyde oxidase which catalyses the penultimate step in the biosynthesis of ABA. Cowan *et al.* (1999) found that the combination of allopurinol and molybdate increased the ABA metabolism of avocado fruit mesocarp tissue leading, ultimately, to a lower concentration of ABA in the fruit. It has been known for some time that ABA protects the plant from herbicides (Devine *et al.*, 1995). We, thus, postulated that the addition of this mixture to herbicides would reduce resistance of the plant to these herbicides by interfering with the normal mechanism by which plants cope with stress *viz.* synthesis of ABA. The mixture of

allopurinol and ammonium molybdate/molybdenum trioxide, as well as the two products on their own, were tested as additives for sulfosulfuron on a number of wild oat (*Avena fatua*) populations known to be resistant to at least one wild oat herbicide. The aim of the study was to find out if these additives could improve control of grass weeds in wheat by sulfosulfuron. This herbicide was chosen due to its sensitivity to the addition of additives. This paper reports on the results of three pot trials designed to evaluate whether the additives led to improved control of two difficult-to-control wild oat biotypes and a ripgut brome (*Bromus diandrus*) biotype.

## METHODS AND MATERIALS

Experiments were carried out on the M4 and #10 wild oat biotypes and a ripgut brome biotype. The M4 biotype had previously been shown to be 85-90 % resistant to sulfosulfuron at registered dosage rates (30 g a.i./ha). However this population could be controlled by the registered dosage (50 g a.i./ha) rate of the ACCase-inhibitor, clodinafop-propargyl. The #10 wild oat biotype was previously found to be resistant to clodinafop-propargyl, but to be reasonably controlled by sulfosulfuron. The ripgut brome biotype had not previously been tested for resistance. Four replicated pots each containing two plants were employed for each treatment. The plants were sprayed at the 4 - leaf stage with 30 g a.i./ha sulfosulfuron and either a standard silicon-based wetter or various combinations of monolaurate, allopurinol and molybdenum (Table 1).

Seed samples of the abovementioned populations from the Western Cape were set to germinate at 15$^0$C in the dark. Uniform seedlings were subsequently planted out in 15 cm pots filled with a fertilized potting mixture based on pine bark. The plants were grown in a polycarbonate-clad water-cooled tunnel in Pietermaritzburg, Natal (29' 38" S, 30' 28" E; altitude: 631 m). The temperature in the tunnel varied between 15 and 33 $^{o}$C and no artificial lighting was used. The herbicides were applied in May and June 2001. Herbicide application took place at the 3 - 4 leaf stage. Herbicide treatments were applied with a custom-made pot spraying apparatus delivering 200 litres/ha through a 80-02 flat fan nozzle. Evaluation of the experiments was done by visually assessing the number of surviving plants. The dry mass of surviving plants was also determined. The stock solution of allopurinol consisted of a 0.5M solution of allopurinol [4,6-dihydroxypyrazolo (3,4-D) pyrimidine] made up in 50 % monolaurate. Allopurinol was, thus, always applied together with the monolaurate. Molybdate ($MoO_3$) was applied as molybdic trioxide at different concentrations. Treatment numbers throughout the text are as given in Table 1.

Table 1. Treatment list and enhancement of the activity of sulfosulfuron by combinations of monolaurate, allopurinol and molybdenum as evaluated on two semi-resistant *Avena fatua* biotypes and a *Bromus diandrus* biotype.
note - data values as used in Figure 1.

| Treatment | | | % Control | | |
|---|---|---|---|---|---|
| number | a.i + additives | additive amount | *A. fatua* (M4) | *A. fatua* (#10) | *B. diandrus* (97) |
| 1 | (none) Control | - | 0 | 0 | 0 |
| 2 | Sulfosulfuron + wetter | 0.25 % | 0 | 25 | 0 |
| 3 | Sulfosulfuron + monolaurate | 0.5 % | 0 | 100 | 100 |
| 4 | Sulfosulfuron + monolaurate | 1.0 % | 0 | 100 | 100 |
| 5 | Sulfosulfuron + Allopurinol | 5mM | 0 | 100 | 100 |
| 6 | Sulfosulfuron + Allopurinol | 10mM | 33 | 100 | 100 |
| 7 | Sulfosulfuron + Allopurinol + $MoO_3$ | 5mM 50µM | 67 | 100 | 100 |
| 8 | Sulfosulfuron + Allopurinol + $MoO_3$ | 10mM 50µM | 0 | 100 | 100 |

## RESULTS

Sulfosulfuron at 30 g a.i./ha with a standard commercial wetter did not give good control of any of the wild oat biotypes or ripgut brome (Figure 1). Control of the M4 wild oat population was poor throughout and only sulfosulfuron with 10mM allopurinol (treatment 6) or 5mM allopurinol + 50µM $MoO_3$ (treatment 7) gave any control at all. However, dry matter reduction in all the other treatments - with the exception of sufosulfuron with 0.5 % monolaurate (treatment 3) - gave a significantly higher reduction in dry mass production compared to the herbicide with the standard wetter (results not shown).

Control of the clodinafop-propargyl resistant #10 wild oat population was significantly improved by the addition of all the experimental additives and combinations to sulfosulfuron (Figure 1). The performance of sufosulfuron in controlling ripgut brome was vastly improved by the addition of all the experimental additive combinations (Figure 1).

The abovementioned treatments were applied on two other problematical wild oat populations. In the case of wild oat biotype 00/14, which was resistant to several ACCase-inhibiting herbicides, the monolaurate/allopurinol/molybdenum mixtures greatly improved control by sulfosulfuron. However, no improvement could be observed in the control of the 00/15 wild oat biotype by sulfosulfuron. This biotype had been previously found to be resistant to both ALS and ACCase-inhibiting herbicides (results not shown).

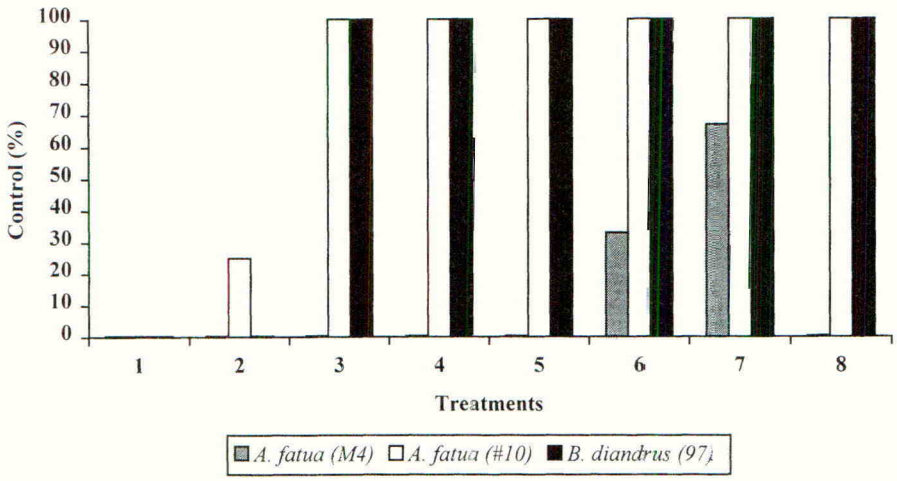


Figure 1. Enhancement of the activity of sulfosulfuron applied at 30 g a.i./ha by combinations of monolaurate, allopurinol and molybdenum as evaluated on two semi-resistant *Avena fatua* biotypes and a *Bromus diandrus* biotype.
Treatments as in Table 1.

## DISCUSSION

The control of ripgut brome and several (but not all) herbicide-tolerant wild oat populations by sulfosulfuron was significantly improved by the addition of allopurinol and molybdenum. However, one of the biggest surprises of this project was that monolaurate on its own as an additive with sulfosulfuron gave significantly better control than any of the registered additives. When this project was initiated we looked for an emulsifier to use for the formulation of a concentrated stock solution of allupurinol, which is sparingly soluble in water. After a number of candidate compounds were evaluated a monolaurate proved to be by far the best, although by no means ideal. All trials conducted with allopurinol and molybdenum thus included treatments with monolaurate on its own as an additive to sulfosulfuron at the same concentration as was present in the allopurinol-containing treatments. In all cases, and throughout a number of experiments (>10), the monolaurate on its own performed far better than the recommended non-ionic wetting agent, and even better than the latest silicon-based wetting agents. Even given the fact that the concentration of monolaurate used in these trials (up to 1 %) was far higher than the rate used for conventional additives, the improved degree of control by sufosulfuron was very significant.

The additives have also been found to be strongly synergistic with paraquat in the control of perennial grasses such as *Cynodon dactylon* (Bermuda grass) and *Pennesetum cladistinum* (Kikuyu). Paraquat normally only burns the tops off these grasses, which subsequently regenerate from stolons. Complete control of these grasses (pot-grown, but well established) was achieved with paraquat plus the additive combination (results not shown). A possible explanation for the synergistic effect of the additives on paraquat activity may be found in the work of Bethke & Jones (2001) who showed that ABA-treated cells metabolised $H_2O_2$ more efficiently than GA-treated ones. The improved control of weeds by the additives is assumed to be the result of their effect on the metabolism of ABA either by reduced sythesis and/or increased breakdown of the phytohormone as indicated by Cowan *et al.* (1999).

Monolaurate, apart from its action as an emulsifier, is also, presumably, increasing uptake of the herbicide. Whether this compound has an additional physiological role remains to be seen. Allopurinol is a known inhibitor of enzymes that have a molybdenum co-factor i.a. aldehyde oxidase, xanthine dehydrogenase, nitrate reductase, nirtrogenase and sulphite oxidase. Allopurinol works by binding tightly to the reduced molybdenum component of these enzymes (Massey *et al.*, 1970). If the amount of molybdenum is increased relative to that of allopurinol the mixture loses its potency as an additive. Indeed if too much molybdenum is added to allopurinol (greater than 1:1 on a molar basis) the mixture becomes antagonistic to the activity of herbicides (results not shown).

Allopurinol is also known to alter blue light effects in plants (Deng & Roenneberg, 1997). Blue light controls stomatal movement by inducing the extrusion of protons from the guard cells (Zeiger, 2000). Although stomatal aperture measurements were not taken, it would appear from photographs taken that stomatal opening in allopurinol-treated

plants was increased significantly (results not shown). Increased stomatal aperture would presumably lead to increased herbicide uptake.

All three components used as additives are relatively non-toxic. Allopurinol has been used as a pharmaceutical product for almost 40 years to treat hyperuricaemia and various other aliments. Molybdenum is an element which is essential for both animal and plant growth. Although high doses of the microelement can have various detrimental effects (such as an induced copper deficiency), the miniscule amount used in the mixture is unlikely to have any toxic effect. The monolaurate used is also extensively used in the pharmaceutical industry, and is one of the least toxic surfactants on the market.

## CONCLUSION

The mixture of allopurinol, molybdate and monolaurate has been shown in a pot trial study to be a very useful additive for use with sulfosulfuron, especially on weeds which show a certain degree of tolerance to the herbicide. The additive combination is currently undergoing field evaluation in the Western Cape and will be tested more extensively on other herbicide groups.

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# Adjuvant affects cuticular waxes and penetration of glyphosate

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## ABSTRACT

The study was conducted to examine the effect of glyphosate (± surfactant) applied to *Commelina communis* and *Solanum nigrum* plants over 48 h. Uptake and translocation of $^{14}$C-glyphosate were significantly higher in *S. nigrum* than *C. communis* possibly because of *S. nigrum's* smooth leaf surface and the presence of large amounts of non-polar waxes on its leaf surface. $^{14}$C-glyphosate uptake and control of *C. communis* were better under shade than under light. The reverse was found in *S. nigrum*. Shaded and moist conditions favored growth of *C. communis*. Uptake and translocation of $^{14}$C-glyphosate were significantly higher with surfactant than with glyphosate alone in both species. Significantly higher uptake and translocation of $^{14}$C-glyphosate was recorded up to 48 h. Uptake and translocation values of $^{14}$C-glyphosate were consistent with the percent control of these two weeds by glyphosate (± surfactant). Relative comparison of scanning electron micrographs also showed that the application of glyphosate with surfactant either disrupted or dissolved the waxes present on the leaf surface thus allowing for increased penetration of glyphosate a.i. into the plant.

## INTRODUCTION

Glyphosate is a non-selective, systemic, broad spectrum, post-emergence (POST) herbicide. Its activity depends on environmental factors such as light, temperature, humidity and soil moisture (Caseley & Coupland, 1985). Deposition, distribution, and retention characteristics of spray droplets constitute major influences on herbicide penetration into a leaf (Kirkwood, 1991). The chemical constituents of epicuticular wax and surface characteristics of the leaf surface affect these parameters. High levels of micro roughness may result in air/liquid and solid/liquid interfaces that inhibit herbicide penetration (Holloway, 1970). Improved herbicide efficacy with adjuvants has been attributed to the increase in leaf wettability and penetration resulting from reduced surface tension and contact angle of spray droplets on the leaf surface (Sharma *et al.*, 1996 and many other researchers).

*Commelina communis* (dayflower) is an annual plant often found in moist habitats (Murphy, 1996) and occurs in dense populations under citrus trees (personal observation). It is a difficult-to-control weed by glyphosate. Therefore, it was important to study the effect of light/shade on glyphosate absorption in *C. communis*. *Solanum nigrum* (black nightshade) is an annual plant with a smooth leaf surface, which contains a high percentage of non-polar (88 %), and only 11 % of polar, waxes (Harr *et al.*, 1991), and is controlled effectively by glyphosate. The effects of glyphosate alone, or formulated with surfactant, applied to *C. communis* and *S. nigrum* (with different surface properties) were examined. Treated leaf surfaces of *C. communis* were examined with a scanning electron microscope. The effect of light and shade conditions on the distribution and efficacy of glyphosate also was examined.

## METHODS AND MATERIALS

*C. communis* seedlings at the 2 to 3 leaf stage collected from a citrus grove were transplanted and *S. nigrum* seeds were sown in potting mix in plastic pots (15 cm). Each pot contained only one seedling of either plant and kept in a greenhouse maintained at 25/16 °C (± 0.5 °C) day/night temperatures with 70 % (± 5 %) r. h. under normal daylight.

Artificial shading was created in the greenhouse by suspending black plastic sheet 3 ft above the bench. One week prior to herbicide application, plants were acclimatized to the artificially shaded growth environment. Photosynthetic photon flux density (PPFD) was measured by a photometer (Quantum) at 4 different times during the day, and average PPFD values of 4.2 $\mu E/m^2/s$ under shade and 450 $\mu E/m^2/s$ under light conditions at the canopy level, were recorded during the experiment period.

Glyphosate at 560 g a.i./ha (as Rodeo formulation) ± 0.25 % v/v surfactant (Kinetic, a blend of organosilicone and nonionic surfactant) was used in the study. $^{14}$C-glyphosate with a specific activity 23.87 mCi/mmol was used for the radioassay studies. The solubility of glyphosate (N-(phosphonomethyl) glycine) isopropylamine salt is 900 g/litre at pH 7 and water temperature of 25 °C; $K_{ow}$ is 0.0006-0.0017. In the bioefficacy study, glyphosate (± surfactant) treatments were applied to both weeds at the 4 to 5 leaf stage (i.e. sufficient for uniform spray contact). Spraying used an air pressured chamber track sprayer delivering 189 litres/ha at 138 kPa via a flat fan nozzle. Control ratings from 0 to 100 % were made weekly up to four weeks after treatment (WAT); 0 indicating normal plant/no damage and 100 indicating complete death of the plant foliage as approved by the Weed Science Society of America (Frans *et al.*, 1986).

Prior to $^{14}$C-glyphosate application, the leaf to be treated was carefully covered with aluminum foil. The plants were sprayed with glyphosate (± surfactant) and transferred under controlled environmental conditions. Aluminum foil was removed, and well mixed $^{14}$C-glyphosate (± surfactant) 5 x 2 µl droplets (18000 to 20000 dpm) were applied to a discrete area on the adaxial surface at the middle part of the 3$^{rd}$ fully expanded leaf of the 4-leaf stage plant. The quantity of $^{14}$C applied to the leaves was calculated by dispensing similar number of droplets directly into 5 ml scintillation cocktail. Treated plants were harvested at 0.25, 1, 6, 24, and 48 h after herbicide application. Plants harvested at 0.25 and 1 h were kept on the bench in the laboratory while the remaining plants were placed in the appropriate controlled environment. At harvest, treated leaves were excised and washed with 2 x 4 ml water + ethanol (1:1 by volume) to recover unabsorbed $^{14}$C-glyphosate and then rinsed twice with 3 ml of ethanol solution. A 200 µl sub-sample from each washing was dispensed to vials containing 7 ml of scintillation liquid and then radio-assayed by liquid scintillation counter (LSC). The plants were dissected into: treated leaf (a), shoot section above treated leaf (b), shoot section below treated leaf (c), and roots (d). Plant samples were oven-dried at 50 °C for 48 h and combusting using a biological oxidizer to determine radioactivity present. Activity was quantified by LSC to determine absorption and translocation. The foliar uptake of $^{14}$C-glyphosate was defined as the sum of fractions a, b, c, and d, and total translocation as the sum of b, c, and d was calculated as a percentage of the applied dose. Summation of all radioassayed fractions from plants resulted in an overall $^{14}$C-glyphosate recovery of 95-98 %.

Scanning electron microphotographs (SEM) of treated and untreated *C. communis* were examined to determine changes in the epicuticular surface by herbicide ± surfactant treatments. SEM examination of *S. nigrum* was not examined because of its lower polar waxes and was easily controlled by glyphosate. Treatment solutions of glyphosate (± surfactant) or surfactant alone were applied as 2 µl droplets on the 3rd leaf of the four fully expanded leaves of *C. communis* at four places. Samples were taken using a 5 mm cork borer at different time intervals coincided with the harvesting time of the $^{14}$C-glyphosate study. Samples were fixed in 3 % glutaraldehyde in 0.066 M phosphate buffer at pH 6.8 for 1.5 to 2 h (Brlansky *et al.*, 1985). The samples were washed in 0.066 M phosphate buffer for 15 min three times, followed by post-fixation with 1 % $OsO_4$ in 0.066 M phosphate buffer pH 6.8 for 2 h at room temperature and washed in 0.066 M phosphate buffer for 15 min three times. Samples were placed on a spot of "Elmer's glue" on plastic petri dishes and allowed to air dry. The tissue samples on the glue were removed from the dish and mounted onto aluminum SEM stubs. Samples were then coated with 20 nm of gold-palladium (60:40 by wt) using a sputter coater. Photographs were taken of representative areas using SE microscope.

$^{14}$C-glyphosate studies were conducted as a factorial design with the three factors being light conditions, surfactant and time of harvest, with true three replications. The bioefficacy study was conducted as complete randomized block design with four true replications. Each weed was analyzed as separate experiment. The data were subjected to ANOVA using Agricultural Research Manager software (Gylling Data Management, Inc., Brookings, SD, USA) after performing an arc-sine transformation, but are presented in the original form for clarity. Means separation was based on Student-Newman-Keuls test (P = 0.05).

Table 1. Factors influencing on $^{14}$C-glyphosate distributions in *C. communis* and *S. nigrum*

| Treatments | --Uptake (% of applied)-- | | Translocation (% of applied) | |
|---|---|---|---|---|
| | *C. communis* | *S. nigrum* | *C. communis* | *S. nigrum* |
| (a) Light and Shade: | | | | |
| Light | 24 | 36 | 4 | 24 |
| Shade | 28 | 44 | 6 | 15 |
| LSD (P ≤ 0.05) | 1 | 1 | 0.4 | 1 |
| (b) Light condition ± surfactant: | | | | |
| Light (- surfactant) | 15 | 23 | 2 | 6 |
| Shade (- surfactant) | 13 | 37 | 5 | 7 |
| Light (+ surfactant) | 33 | 49 | 6 | 41 |
| Shade (+surfactant) | 44 | 51 | 7 | 24 |
| LSD (P ≤ 0.05) | 2 | 2 | 1 | 1 |

## RESULTS AND DISCUSSION

### Effect of light, shade and surfactant on $^{14}$C-glyphosate distributions

The data were averaged across these effects, as there was no significant interaction. Maximum uptake was only 28 % in *C. communis* and 44 % in *S. nigrum* under shaded condition. Translocation of $^{14}$C-glyphosate was significantly higher under shaded than under light in *C. communis*, while this observation was reverse in *S. nigrum* where translocation was greater under light (Table 1a). Regardless of the light levels, uptake and translocation values were significantly higher in *S. nigrum* than in *C. communis*. Significantly higher uptake occurred under shade in both species with $^{14}$C-glyphosate + surfactant than with glyphosate alone (Table 1b). In case of *S. nigrum* there was no difference in the uptake of $^{14}$C-glyphosate with surfactant under shade (51 %) or light (49 %) while translocation was significantly higher under light (41 %) than under shade (24 %). Translocation of $^{14}$C-glyphosate with surfactant in *C. communis* was very low under both shade and light conditions (Table 1b). The reason for higher uptake of glyphosate in *C. communis* under shade conditions may be its better growth habit under shade and moist conditions (Personal observation). Generally, an increase in soil moisture (Waldecker & Wayse, 1985) facilitates foliar uptake of glyphosate, as does high relative humidity (Caseley & Coupland, 1985). Possibly given a higher water potential in *C. communis* because of its moist and shade habitats, there might also be a higher uptake of glyphosate.

Table 2. Influence of surfactant and harvest time on $^{14}$C-glyphosate distributions

| Harvest time (h) | ----Uptake (% of applied)---- | | | | -Translocation (% of applied)- | | | |
|---|---|---|---|---|---|---|---|---|
| | *C. communis* | | *S. nigrum* | | *C. communis* | | *S. nigrum* | |
| | - S* | + S* | - S | + S | - S | + S | - S | +S |
| 0.25 | 2 | 20 | 6 | 26 | 1 | 2 | 1 | 14 |
| 1 | 6 | 27 | 9 | 35 | 2 | 3 | 2 | 23 |
| 6 | 14 | 35 | 24 | 54 | 2 | 5 | 4 | 37 |
| 24 | 21 | 51 | 51 | 66 | 4 | 6 | 9 | 43 |
| 48 | 26 | 58 | 59 | 68 | 9 | 16 | 17 | 46 |
| LSD (P≤0.05) | 3 | 3 | 2 | 2 | 1 | 1 | 2 | 2 |

* - S no surfactant; + S surfactant

### Effect of surfactant and time of harvest $^{14}$C-glyphosate distribution

In both species uptake and translocation of $^{14}$C-glyphosate increased significantly with time when surfactant was added, with the highest values at 48 h harvest time (Table 2). The rate of uptake of $^{14}$C-glyphosate was significantly higher up to 24 h than 48 h in both species. This indicated that uptake and translocation processes were more active up to 24 h, which is in agreement with previous studies where > 50 % of applied $^{14}$C-glyphosate was absorbed within 15 min in *Bidens frondosa* and the foliar penetration was enhanced up to 24 h (Sharma & Singh, 2000). Many researchers have reported that different nonionic surfactants increased cuticular penetration of the a.i. as a result of complex interactions between a.i., surfactant and target species (e.g. Stock & Holloway, 1993; Sharma *et al.*, 1996). Also, the addition of surfactant to herbicide may affect spray retention and penetration, and could act as a humectant (Kirkwood, 1991) and a co-solvent (Wyrill & Burnside, 1977).

## Bio-efficacy study

Application of glyphosate with surfactant recorded a significant increase in the control of *C. communis* (89 % ± 4.8) particularly under shade over 16 % (± 2.5) with no surfactant. Under light it was only 21 % (± 2.5) with surfactant over 12 % (± 2.4) with no surfactant. No significant difference in control between light (99 % ± 2.5) and shade (98 % ± 2.9) was obtained in *S. nigrum* with glyphosate + surfactant. However, these values were significantly higher than those obtained under light (80 % ± 4.1) and shade (74 % ± 2.5) with glyphosate alone. These results were similar to uptake and translocation of $^{14}$C-glyphosate in both species under light and shade conditions. In general, uptake, translocation and control were significantly higher in *S. nigrum* than in *C. communis*. This could be related to differences in epicuticular surface properties (Holloway, 1982).

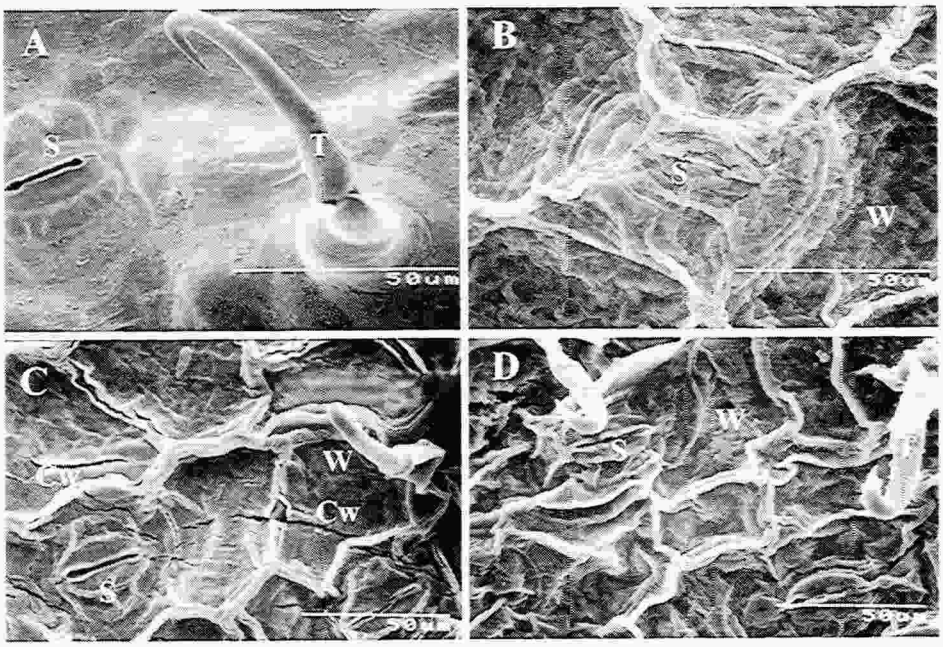


Figure 1. SEM of adaxial leaf surface of *C. communis* (A) untreated, (B) treated with glyphosate, (C) treated with surfactant and (D) treated with glyphosate + surfactant.
Letters denote: S-stomata, T- trichomes, W- cuticular waxes, and Cw- cracks in the wax layer

## Scanning Electron Micrographs (SEM)

SEM photomicrograph of the untreated adaxial leaf surface of *C. communis* (Figure 1A) shows stomata, trichomes and epicuticular waxes. Non-polar waxes were disrupted by glyphosate alone, which increased with time, but waxes remained intact to the cuticle. Wax granules were apparent on the leaf surface (Figure 1B). These may be remnants of polar waxes. With surfactant alone, no effect was seen on the stomata, but waxes looked dissolved and appeared dried and, later, cracks developed in the wax layers (Figure 1C). With glyphosate + surfactant, the waxes were disrupted and dissolved, trichomes ruptured from the base and stomata were disrupted at all sampling times (Figure 1D). Apparently, the presence of surfactant in glyphosate aided in this disruption, and/or in the dissolution of different

cuticular waxes, and also increased the area for a.i. penetration, and hence higher absorption of glyphosate in *C. communis*. The difference in leaf morphology, wax content, stomata, and trichomes are very important for effective herbicide absorption. Certainly the choice of surfactant should be considered in order to achieve more evenly spread of water-based sprays on hydrophobic leaf surfaces.

## ACKNOWLEDGEMENTS

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# Interactions between glyphosate formulations and organosilicone surfactants on perennial grasses

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## ABSTRACT

The effect of two glyphosate formulations (R and RII) applied alone or in combination with organosilicone surfactants was studied on three grass species in the glasshouse. Without surfactants, the efficacy of glyphosate-R on perennial ryegrass (*Lolium perenne*) and prairie grass (*Bromus willdenowii*) was significantly higher than glyphosate-RII. Addition of organosilicone surfactants decreased the herbicidal effect of glyphosate in most cases. Retention of glyphosate on foliage varied with grass species and formulation. Experiments conducted with $^{14}$C-glyphosate investigated the effect of formulation and organosilicone surfactants on uptake and translocation in perennial ryegrass and prairie grass over a 72-h time-course. $^{14}$C-glyphosate concentrations in young plant tissues and roots were also measured. There were no significant differences between the two formulations in uptake and translocation. Adding organosilicone surfactants antagonized uptake and translocation, decreasing the concentration of $^{14}$C-glyphosate in young plant tissues.

## INTRODUCTION

Enhanced uptake of glyphosate into plants when organosilicone surfactants are present is well documented: it is primarily due to their ability to spread extensively and reduce the surface tension and contact angle of the spray droplets. Organosilicone surfactants are also known to enhance stomatal infiltration (e.g. Stevens *et al.*, 1991), but they have also been reported to reduce the uptake of glyphosate into a variety of grasses (e.g. Gaskin & Stevens, 1993). Further, the addition of a range of organosilicone surfactants has been shown to adversely affect glyphosate activity in several species (Baylis & Hart, 1993; Jermyn, 1993). Efforts have been made to elucidate the mechanism of this organosilicone-induced antagonism, but results are limited and vary with surfactant and plant species.

Formulation is an important factor affecting herbicide uptake and activity (e.g. Feng *et al.*, 1998). Research is necessary on plant responses to different herbicide formulations, especially for newly developed formulations. A new glyphosate formulation, RII, (Roundup GII (G2); glyphosate, isopropylamine, 360 g a.i./litre; Monsanto, New Zealand) is claimed to have a better environmental profile in terms of aquatic life, but little

information is available on its performance on grasses. The present study compared the effects of RII and Roundup (R ) on three grass species, and examined the influence of two organosilicone surfactants on glyphosate performance, retention, uptake and translocation.

## METHODS AND MATERIALS

Perennial ryegrass (*Lolium perenne* L. cv. Grasslands Greenstone), tall fescue (*Festuca arundinacea* Schreb. cv. Grasslands Roa) and prairie grass (*Bromus willdenowii* Kunth. cv. Grasslands Matua) plants were glasshouse-grown from seed in standard potting mix in 5x5x8 cm plastic pots (day 24-28 °C, night 14-18 °C, 14 h daylength, 70-80 % r.h.). All plants were at the 4-leaf and 1-tiller stage at the time of treatment.

R and RII were used for all the experiments. The main surfactant in R is a tallowamine: the surfactant in RII is undisclosed. [Methyl-$^{14}$C] glyphosate was used for the uptake and translocation study. The organosilicone-based surfactants used were Silwet L-77 (S77) and Silwet S-800 (S800), both at 0.1 % v/v.

In fresh-weight reduction experiments, plants were cut to 8-cm height five days prior to spraying with glyphosate solutions at 360 g a.i./ha. A $CO_2$-pressurized knapsack sprayer fitted with two Teejet 8001 nozzles which delivered 300 litres/ha at a nozzle pressure of 300 kPa was used. Fresh weight of shoots was measured three weeks after spraying and used to calculate percent reduction from an untreated control.

Foliar retention of spray droplets of glyphosate formulations at 360 g a.i./ha with or without surfactants was measured on the three grass species. Spray solutions all contained fluorescein dye at 0.05 g/litre. After 30 min (when dry) plants were cut at ground level, the dye washed off in 30 ml of 5 mM sodium hydroxide and a sample from each washing used for fluorimetry (Shimadzu RF-360 spectrofluorophotometer, excitation and emission wavelengths 495 nm & 515 nm, respectively). The amount of dye in the wash solutions was determined from a standard curve. Foliage leaf area was measured using an area meter (Lambda LI 3100) and the leaves oven dried at 70 °C for 48 h to determine dry-weights.

Only perennial ryegrass and prairie grass were used in uptake and translocation experiments. Solutions of $^{14}$C-glyphosate in R and RII formulations, with or without surfactants, were prepared and approximately forty 10 µl droplets (equivalent to 180 g a.i./ha) applied to the upper surface of the second leaf of each plant with a microsyringe 3 h after the start of the photoperiod. Uptake and translocation of $^{14}$C-glyphosate into plants were measured 24 and 72 h after treatment (HAT). Treated leaves were excised, washed with 20 ml surfactant solution (Citowett, 0.25 ml/litre), and 1ml of the wash-off was added to 10 ml of Bray's scintillation cocktail. The radioactivity was determined by liquid scintillation spectrometry (LSS, Phillips PW 4700). Foliar uptake was quantified as the difference between the amount of applied and recovered radioactivity, expressed as a percentage of the applied radioactivity. The rest of the plant was divided into leaf 1, young tissues and roots; all were freeze-dried, weighed and kept frozen (-18 °C) until combusted in a sample oxidizer (R.J. Harvey, OX-300, calibrated by combustion of standards, recovery >92 % with no carry-over to successive samples). $^{14}$C-glyphosate in plant tissue was

completely oxidized to $^{14}CO_2$ which was trapped in a liquid scintillation cocktail and measured by LSS. The total amount of $^{14}$C-glyphosate translocated out of the treated area was calculated as a percentage of the amount applied according to the equation:
Translocation = [Applied dpm- (wash off dpm + treated leaf dpm)]*100/Applied dpm

Experiments were arranged in a completely randomized design with six replicates; typical results are presented. Data were subjected to analysis of variance (Minitab) where significant effects were indicated by an F-test. Means were compared using Duncan's Multiple Range Test. All data were transformed to square root prior to analysis, but since transformation did not affect the results the untransformed data are presented.

## RESULTS AND DISCUSSION

### Fresh-weight reduction

Prairie grass has been reported to be more tolerant to R than tall fescue or perennial ryegrass (Dastgheib & Field, 1995a). In these experiments, prairie grass was also the most tolerant to both R and RII (Table 1). Without surfactant R was more effective than RII on perennial ryegrass and prairie grass; both formulations were similar on tall fescue (Table 1).

Table 1. Control of three grass species with R or RII (360 g a.i./ha) with or without organosilicone surfactants (0.1 % v/v) 24 d after spraying. Values are % reduction in fresh weight compared to control. Means in each column with the same letter - not significantly different at 5% level according to Duncan's Multiple Range Test.

| Treatment | Perennial rye grass | Tall fescue | Prairie grass |
|---|---|---|---|
| R | 93.7 a | 83.9 a | 75.1 a |
| R + S77 | 87.0 a b | 65.2 b | 66.3 a b |
| R + S800 | 86.0 a b | 70.1 b | 66.6 a b |
| RII | 75.3 b | 80.2 a | 55.5 b |
| RII + S77 | 76.1 b | 69.4 b | 45.3 bc |
| RII + S800 | 43.8 c | 67.3 b | 54.0 b |

Addition of organosilicone surfactants had, in most cases, a negative effect on glyphosate performance for both R and in RII (Table 1). In perennial ryegrass, both surfactants caused a small and non-significant reduction in the efficacy of R. The efficacy of RII on perennial ryegrass was not affected by S77, but was significantly reduced by S800. In tall fescue the efficacy of both R and RII was significantly reduced by both surfactants. In prairie grass, addition of organosilicone surfactants caused only small and non-significant reductions in R and RII performance. Previously, organosilicone surfactants were reported to improve the performance of R in the same species at a rate of 180 g a.i./ha, but showed no effect at the higher rate (as used here) of 360 g a.i./ha (Dastgheib & Field, 1995a). Moreover, there was up to 6.4 times increase in the control of tall fescue with S77 if simulated rain followed

herbicide application (Dastgheib & Field, 1995a). This indicates that the benefit from additional surfactants is greater under sub-optimal or unfavourable conditions.

**Spray retention**

Retention results were similar whether expressed in terms of dry weight or leaf area, so only the latter are presented. When R or RII was applied alone, foliar retention varied with species. The order of retention for R was perennial rye grass > tall fescue > prairie grass (Table 2). This is consistent with a previous data (Dastgheib & Field, 1995a). Retention of R by perennial ryegrass was significantly higher than that for RII (2.71 and 1.33 $\mu l/cm^2$, respectively). No significant differences were found between R and RII retention on tall fescue and prairie grass (Table 2).

The effect of organosilicone surfactants on retention varied with species and formulation. Addition of S77 and S800 to R decreased retention on perennial ryegrass, but increased it on tall fescue. This may be due to leaf surface characteristics: tall fescue has a more highly ridged, trichomeous leaf surface than perennial ryegrass, which leads to a better response to surfactants (Jermyn, 1993). Further, the upper surfaces of perennial ryegrass leaves are covered with a dense arrangement of crystalline wax platelets (Baylis & Hart, 1993) which can result in run-off of solutions containing organosilicone surfactants. Addition of S77 and S800 to RII had no effect on retention on perennial ryegrass and tall fescue, but greatly increased retention on prairie grass. Differences in retention between R and RII probably reflect the different surfactants used in these formulations.

Table 2. Retention of R and RII (360 g a.i./ha) applied alone or with organosilicone surfactants (0.1 % v/v) by grass species. Values are $\mu l/cm^2$. Means in each column with the same letter - not significantly different at 5% level according to Duncan's Multiple Range Test.

| Treatment | Perennial ryegrass | Tall fescue | Prairie grass |
|---|---|---|---|
| R | 2.71 a | 1.93 b | 0.70 a b |
| R + S77 | 1.57 b | 3.59 a b | 0.92 a |
| R + S800 | 1.57 b | 5.31 a | 0.67 a b |
| RII | 1.33 b | 2.40 b | 0.52 b |
| RII + S77 | 1.48 b | 2.92 b | 0.71 a b |
| RII + S800 | 1.74 b | 3.23 b | 0.88 a |

**Uptake and translocation**

In the absence of organosilicone surfactants, there was no significant difference in the uptake of glyphosate from R and RII by perennial ryegrass. In prairie grass uptake of glyphosate from R was lower than from RII at 24 HAT, but similar at 72 HAT (Table 3).

Addition of organosilicone surfactants decreased uptake of glyphosate in both grasses. For example, in prairie grass 72 HAT uptake for R with S77 or S800 was only 29.0 and 30.7 %

of the applied amount, respectively; less than half the value for R alone. Previously, S77 reduced the uptake of glyphosate in a variety of grasses (Gaskin & Stevens, 1993).

Translocation of $^{14}$C-glyphosate in the two grasses followed a similar pattern to uptake. R and RII gave similar translocation values in perennial ryegrass at both assessments, and in prairie grass at 72 HAT. Previous studies found differences in uptake and translocation between other glyphosate formulations (Feng *et al.*, 1998). The presence of organosilicone surfactants antagonized $^{14}$C-glyphosate translocation significantly. This may be a consequence of reduced uptake leading to a smaller concentration gradient between source and sink in the plant. However, the interaction of organosilicone surfactants with $^{14}$C-glyphosate and/or additives present in the formulations cannot be ruled out.

Table 3. Uptake, translocation and concentration in young tissues of $^{14}$C-glyphosate applied as R or RII (180g a.i./ha) with or without organosilicone surfactants (0.1 % v/v) in perennial ryegrass (PR) and prairie grass (PG). Means in each column followed by the same letter - not significantly different at 5% level according to Duncan's Multiple Range Test.

| Treatment | Uptake (% of applied ) | | Translocation (% of applied) | | Concentration (dpm/g.dry weight) | |
|---|---|---|---|---|---|---|
| | PR | PG | PR | PG | PR | PG |
| **24 HAT** | | | | | | |
| RII | 72.4 a | 63.7 a | 45.0 a | 49.1 a | 91.8 b c | 100.6 a |
| R | 78.4 a | 48.4 b | 46.7 a | 27.9 b | 201.3 a | 98.7 a |
| R + S77 | 44.7 b | 30.3 c | 33.6 b | 25.4 b c | 86.1 b c | 48.5 b |
| R + S800 | 45.3 b | 22.7 c | 24.8 b | 21.8 c | 136.8 b | 43.9 b |
| **72 HAT** | | | | | | |
| RII | 80.8 a | 58.8 a | 57.5 a | 43.8 a | 144.3 b | 96.2 b |
| R | 81.2 a | 67.5 a | 60.8 a | 51.6 a | 218.9 a | 198.7 a |
| R + S77 | 48.2 b | 29.0 b | 39.9 b | 28.6 b | 57.6 c | 23.20 c |
| R + S800 | 42.9 b | 30.7 b | 37.2 b | 27.7 b | 68.3 c | 36.2 c |

$^{14}$C-glyphosate concentration in different fractions of the plants was measured. Concentration values in young tissues and roots were similar, so only young tissue data are presented (Table 3). $^{14}$C-glyphosate concentration in young tissues of perennial ryegrass was much higher with R than RII at both assessments. At 24 HAT concentrations in prairie grass were not significantly different for R and RII, but at 72 HAT concentrations in R were significantly higher than in RII (198.7 and 96.2 dpm/g dry weight, respectively).

Consistent with uptake and translocation, addition of organosilicone surfactants significantly decreased $^{14}$C-glyphosate concentrations in young tissues, with greater decline in concentration as HAT increased (Table 3). This mechanism should be studied further.

## CONCLUSIONS

This study found that when R or RII were applied alone, efficacy on grass species had a positive correlation with herbicide retention on the foliage. In tall fescue, the two formulations were similar in their phytotoxicity as well as their retention on the foliage. RII was less effective than R in controlling perennial ryegrass and prairie grass. RII also gave lower retention than R for both species.

The activity of R was much higher than that of RII on perennial ryegrass and prairie grass. Uptake and translocation of $^{14}$C-glyphosate were similar for the two formulations in these grasses, although the concentration of $^{14}$C-glyphosate in young tissues was significantly higher for R. This highlights the importance of distribution within the plant to the final effect of glyphosate (e.g. Dastgheib & Field, 1995b).

Adding organosilicone surfactants decreased the efficacy of glyphosate (regardless of foliar retention) on grass species for both formulations. This antagonism may be due to several steps after retention which have resulted in a lower concentration of glyphosate in young tissues. More research is needed to elucidate the mechanisms involved in such antagonism.

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# Product Integrity: a scientific approach to preventing cross-contamination at product change-overs

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## ABSTRACT

Product change-overs in multi-product crop protection chemical facilities require that the equipment be cleaned to a level at which residues of the active ingredients and other extraneous substances originating from the previous product will not cause any adverse biological, toxicological, or ecological effects, nor regulatory issues in the next product. To ensure that cross-contamination incidents are avoided, it is necessary to determine acceptable concentration levels (ACLs). The ACLs are based on NOELs of the previous a.i. for the most sensitive crop on which the following product is registered. If the ACL is higher than the legal limit dictated by legislation in the country where the subsequently produced product is sold, then the legal limit is applied.

## INTRODUCTION

The risk of cross-contamination of crop protection chemicals is best minimized by manufacturing the a.i.s and formulating and packaging the final product in dedicated plants. However, the economic forces within our industry often dictate the use of multi-product facilities. The consequence of this approach is that every time there is a switch from one product to the next, there is a potential risk that the residual concentration of the previous a.i. in the next product could cause damage to the crops on which the latter product is used. In the same manner, the residual concentration of previous "inert" ingredients could adversely influence the formulation properties of the following product. In other words, the use of non-dedicated plants could result in a costly cross-contamination incident.

Manufacturers who do not have dedicated facilities at their disposal, as well as the contract (toll) manufacturers used, must have appropriate processes in place to ensure that these incidents do not happen. Dow AgroSciences (DAS) has developed a Product Integrity (PI) policy requiring that scientifically determined cleaning levels be in place before a product change-over is allowed to take place. DAS refers to these cleaning levels as Acceptable Concentration Levels (ACLs), whilst a number of European companies use the term Acceptable Residual Impurity Level (ARIL). An ACL is defined as: "The concentration of the previous active ingredient, or any other extraneous substance, below which it will not cause adverse biological, toxicological, ecological effect or regulatory issues in the next product."

The US Environmental Protection Agency (EPA) has issued Guidelines on the Toxicologically Significant Levels of Contamination (TSLCs) of the previous product in the next one (EPA, 1996). DAS is of the opinion that this guideline provides a first tool to determine ACLs, but that additional data sources need to be consulted. The guideline is applicable to products manufactured and used in the US, whilst government agencies in other countries generally allow for self- regulation of ACLs by the crop protection industry, provided that the limits do not infringe the applicable plant protection legislation. A downside of universal implementation of the TSLCs is that they may be too high and could still lead to PI incidents, e.g. a TSLC for an insecticide or fungicide in another insecticide of 1000 ppm (Table 1) can be much too high if the insecticide that is the following product is used in an insecticide bait for social insects. Factors like repellency could come into play in those situations, which could prevent the "worker insects" from transporting the bait to the colony. TSLCs may also be too high in the case of highly active, low application rate herbicides like the sulfonylureas or the triazolopyrimidine sulfonanilides.

The purpose of this paper is to describe the techniques used to determine ACLs. Examples of ACL calculations are used to comment on its implementation.

## METHODS AND MATERIALS

The categories used by the EPA to determine the toxicologically significant levels are listed in Table 1. For U.S. products, the EPA guideline should be the first tool to determine the category in which the ACL has to fall, for under all conditions the ACL should be in compliance with this guideline. Contaminants are defined as an a.i. which is not listed as an impurity of the a.i. of the following product or in that product's Confidential Statement of Formula (the document submitted to the regulatory authorities). Contaminants in EPA Categories 7, 8 and 9 include all herbicides which are ALS inhibitors like the imidazolinones, triazolopyrimidine sulfonanilides and sulfonylureas.

Once a request for an ACL of an a.i. in the next product has been received, one must determined the crops this product is registered on and which application methods are used. Dose/response studies allowing the calculation of $ED_{10}$ value(s) (the dose/application rate causing a 10% adverse effect) and determination of the NOEL(s) for the a.i.(s) in the previous product are determined on the registered crops of the following product either by using post-em. foliar applications or by applying the a.i. to the soil using pre-plant incorporation (ppi) to ensure even distribution in the soil and availability to the plant. The application method chosen depends on that used for the following product.

In the case of post-em. applications, eight to nine application rates are used, typically with ten replicates. The spray volume is equivalent to 200 litres spray solution/ha. The application rate increases in ten-fold increments in rate finding studies, whilst in studies with known a.i.s the application rate increases in two-fold steps. A crop oil concentrate is added to all spray solutions at a concentration of 1.25 % v/v, because the next product in which the a.i. could be a contaminant may have a high adjuvant loading, thus enhancing phytotoxicity of the potential contaminant.

Table 1. Toxicologically significant levels of contamination: US EPA Categories.

| Category | Type of contaminant | Type of pesticide that is contaminated | Toxicologically Significant level mg/litre (ppm) |
|---|---|---|---|
| 1 | Insecticide, fungicide, molluscicide, or nematicide in..... | Any insecticide, fungicide, molluscicide, nematicide, herbicide, plant growth regulator, defoliant or desiccant | 1000 |
| 2 | Herbicide, plant growth regulator, defoliant or desiccant in..... | Any pesticide where the contaminant is accepted for use on all sites for which the product is labelled | 1000 |
| 3 | Any pesticide other than a low application rate herbicide in..... | An antimicrobial pesticide | 1000 |
| 4 | Normal rate herbicide, plant growth regulator, defoliant or desiccant in..... | Any herbicide, plant growth regulator, defoliant or desiccant | 250 |
| 5 | Any pesticide in..... | A pesticide applied to the human body | 100 |
| 6 | Normal rate herbicide, plant growth regulator, defoliant or desiccant in..... | Any insecticide , fungicide, molluscicide, or nematicide | 100 |
| 7 | Low application rate herbicide[1] in..... | A low application rate herbicide[1] | Level of quantification or 100 ppm whichever is higher |
| 8 | Low application rate herbicide[1] in..... | A normal rate herbicide, plant growth regulator, defoliant or desiccant | Level of quantification or 20 ppm whichever is higher |
| 9 | Low application rate herbicide[1] in..... | A pesticide other than a herbicide, plant growth regulator, defoliant or desiccant | Level of quantification or 1 ppm whichever is higher |

(1) A low application rate herbicide has an application rate lower than 0.5 lb a.i./acre ( 560 g a.i./ha)

The NOELs for soil-applied products (either pre-em. or ppi) are determined using ppi applications on a mineral soil medium with 0.5 % o.m., which have proved to generate more reproducible data and are considered to represent a worst case scenario.

The use of $ED_{10}$ values for calculating ACLs is considered more scientifically correct, as it is independent of the interval between the application rates. Nonetheless, depending on the symptoms caused by the potential contaminant, it may be more prudent to use the NOEL as basis for the calculations. If the potential contaminant would only affect the plant size without causing other symptoms, $ED_{10}$ values may be used. However, if the contaminant would produce visually striking symptoms (e.g. chlorotic spots) at low application rates (< $ED_{10}$), then caution dictates that the NOEL forms the basis for the calculation of the ACLs.

## DISCUSSION

### Examples of an ACL calculation

***Metosulam in haloxyfop-P-methyl***

This example has been chosen to demonstrate that application of the earlier mentioned guideline developed by the EPA does not always guarantee a safety margin high enough to take the biological properties of the previous a.i. into account for certain product change-overs.

The selected product change-over is from the highly active triazolopyrimidine sulfonanilide metosulam (Snel *et al.*, 1993) to haloxyfop-P-methyl (GALLANT 535 herbicide, 104 g a.i./litre). The haloxyfop-P-methyl formulations are registered for post-em. control of annual and perennial grasses in broad-leaved crops; viz. sugar beet, oilseed rape, sunflower, peas, potatoes, tomatoes, cucurbits, medicinal plants and several vegetables. It is critical to determine which crop species, of those on which the haloxofop-P-methyl products are registered is most sensitive to metosulam. In this particular case, soil applications can be ignored because the product is applied post-em. The crop most sensitive to metosulam is oilseed rape, on which metosulam has a NOEL of 0.002 g a.i./ha. The formula used to calculate the ACL is as follows:

[1000] x ( [NOEL g a.i./ha] / [safety factor] )
divided by
the highest application rate of the next product (litre formulated product/ha)

= ACL in mg/litre or ppm.

This approach results in the following calculation for metosulam in haloxyfop-P-methyl formulations, which are registered at a maximum application rate of 2.0 litres formulated product/ha. A safety factor of ten is used (this is discussed later):

ACL = 1000 x (0.002/10) / 2.0 = 0.1 mg/litre (0.1 ppm or 100 ppb).

If the EPA guidelines were followed in this example, this change-over would have fallen in Category 7 (Table 1): a low application rate herbicide in a low application rate herbicide,

which allows the use of an ACL of 100 ppm. At the highest application rate of the haloxyfop-P-methyl product, this could result in an application of 200 mg of metosulam/ha: 100-fold above the NOEL on oilseed rape.

***Cyhalofop-butyl in haloxyfop-P-methyl***

In cases where the potentially contaminating herbicide is selective on the crop on which the following product is applied, the biologically defendable ACL can be considerably higher than the level permitted by the EPA as shown in the following example. Using the same criteria as in the example above, the ACL calculation for cyhalofop-butyl in haloxyfop-P-methyl is as follows:

The NOEL of cyhalofop-butyl on the most sensitive crop on which haloxyfop-P-methyl is registered (soya bean) is > 400 g a.i./ha. The maximum application rate of the haloxyfop-P-methyl product on this crop is 2.0 litre product/ha. A safety factor of ten is used:

ACL = 1000 x (400/10) / 2.0 = 200000 mg/litre (20000 ppm).

If the EPA guidelines were followed in this example, the change-over would also have fallen in Category 7, viz. an ACL of 100 ppm would apply. In order to meet the regulatory requirements, the PI policy of DAS stipulates that in those areas where the EPA guidelines are not applied, the highest ACL will never exceed 1000 ppm. In this example, this would still mean that the concentration of cyhalofop-butyl in haloxyfop-P-methyl could be 10-fold higher than the ACL, which would have resulted from implementation of the EPA guidelines. This difference in ACL might reduce the cleaning time and the amount of contaminated waste.

**Safety factor**

Application of a safety factor is considered necessary in the calculation of an ACL for the following reasons:

- The dose/response studies are carried out in the glass house with constant temperatures, humidity and light regimes.
- Overlapping and variation when applying crop protection chemicals under field conditions is often unavoidable. This results in local doubling of the application rate.
- The test plants are kept under optimal conditions under glass house condition with regards to watering and fertilisation regime and lighting/day length conditions. Under these conditions the plants are less likely to be in stress conditions.
- The test plants are normally smaller than under application conditions in the field, viz. they will intercept less spray solution than in the field, e.g. in the case when grapes or apples are the test organisms, seedlings have to be used.
- The tests are carried out with the equivalent of a spray volume of 200 litres/ha, whilst modern field sprayers operate with lower spray volumes per ha resulting in spray droplets with a higher concentration of the product and the potential residues of the previous a.i. This difference is especially critical when the previous product is a contact herbicide that causes necrotic spots on the foliage.

The size of the safety factor used is a decision that is company specific.

**Comments**

- As shown in the example where production of a metosulam product precedes a graminicide registered in broad-leaved crops, it is necessary to depend on a science-based programme to determine the ACL suitable for this product change-over.
- Cleaning down to an ACL of 100 ppb presents challenges in terms of equipment design, cleaning technology, volume of contaminated waste, as well as analytical capabilities. DAS makes the utmost effort to avoid this type of product sequencing, and the most likely scenario would have been to follow metosulam with a crop "compatible" product, i.e. a product, which is registered for use on the same crops as metosulam, before the change-over to haloxyfop-P-methyl. A fluroxypyr-meptyl formulation would have presented such an opportunity. To ensure that the cleaning levels are achieved when switching to the broad-leaved crop herbicide, the cleaning level for metosulam in the crop "compatible" product is set well below the actual ACL for metosulam in that product. The manufacturing plant will not be released for the change-over to haloxyfop-P-methyl if the cleaning level for metosulam is not below 100 ppb. If this end point for the low application, highly active, non-compatible a.i. is not achieved after a production run of a crop "compatible" product, a further run of product crop "compatible" with metosulam will be required. It goes without saying that cleaning to the ACL for fluroxypyr-meptyl in haloxyfop-P-methyl must also be achieved. The crop "compatible" product acts in these situations as additional cleaning solvent for metosulam.
- ACLs are only valid for a given geography because the ACLs are based on the most sensitive crop on which the next product is registered in that particular geography. Additional crops and /or higher registered application rates in other geographies could alter the ACL. A global ACL can be achieved at a cost, for this would have to be based either on the application rate on the most sensitive crop on which the product is registered globally or on the ACLs dictated by the strictest legislation. This could result in a considerably lower ACL, which means additional cleaning costs and generation of contaminated waste.

## CONCLUSION

The use of ACLs based on biological criteria provides a robust tool for preventing cross-contamination incidents and for reducing cleaning costs and the generation of contaminated waste in product change-overs.

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# POSTER SESSION 8E

# WEED MANAGEMENT IN UNCROPPED LAND

Session Organiser: D V Clay
*Avon Vegetation Research, Bristol, UK*

Poster Papers: 8E-1 to 8E-6

# Non chemical weed control in urban areas

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## ABSTRACT

A significant transfer of residues to the water environment can be caused by the use of herbicides on impermeable surfaces in urban areas (roads, pavements, footpaths, patios...). Not enough research work has been done to study these displaced chemicals and, considering their environmental impact, non-chemical weed control methods deserve more evaluation. Experiments were carried out on the control of weeds in gutters, on pavements and over paved surfaces as part of a regional programme to restore water quality in Brittany. Alternatives tested included: flame gas-fired weeding, rotary wire brushes, periodic mechanised sweeping, calibrated mechanised sweeping, steam weeding and weeding with glyphosate herbicide. Economic and technical assessment of each method is focussed on the growth levels of weed, the botanical species observed, the number of operations required and their cost. Best results were obtained with steam weeding and calibrated mechanised sweeping in spite of difficulties encountered with respect to hardy, firmly-rooted perennial weeds, such as *Plantago* spp.

## INTRODUCTION

The task of monitoring the quality of the untreated water supply, under the aegis of *la Cellule d'Orientation Régionale pour la Protection des Eaux contre les Pesticides (CORPEP, 2000)* and the programme *Bretagne Eau Pure (BEP)*, has demonstrated the importance of the contamination from pesticides used to control weeds in urban areas (DIREN 2000; CORPEP 2000). To limit this contamination, urban areas have been divided into two categories - of high and low risk - according to their pesticide contamination capacity (Clisson *et al.*,1999). Low risk areas may be treated chemically. High risks accrue where the surfaces is impermeable, where there is an adjacent water supply facility and where there is a direct connection to rainwater drains. Use of chemicals is prohibited for this type of surface although these areas of course need to be maintained.

In this situation, mechanical and thermic alternatives to the use of chemicals are solutions which merit investigation. The object of this study was, firstly, to assess the agronomic efficiency of non-chemical alternatives and, secondly, to evaluate their practical and economic feasibility.

## METHODS AND MATERIALS

Two different sites were chosen to investigate the agronomic efficiency of mechanical and thermic weed control techniques. These sites corresponded to the two types of surface representative of the majority of impermeable surfaces encountered during weed control operations at a municipal level :

- the test site at Plélan-le-Grand for weeding gutters and pavements.
- the test site at Rennes' central market place for weeding paved areas.

For each site the same experimental system is employed with three replicates of each method and the control plot randomised in the group. Each tested surface was 10 x 1 m. The trials were carried out during an 8 month period from April to November 1999.

### Weeding of gutters and pavements

For the weeding of gutters and pavements, five weed control methods were compared to reference plots treated chemically and an unweeded control treatment (Table 1). Efficiency assessment for the different techniques involved counting the number of weeds present as well as assessing the degree of weed cover for each plot.

To assess results, different categories of weed were distinguished. The first three corresponded to the three main species or groups of species observed. They were toadgrass *(Juncus bufonius)*, common pearlwort *(Sagina apetala)* and meadow-grasses *(Poa* spp.*)* represented essentially by annual meadow-grass *(Poa annua)*. The fourth category contains the remaining weeds observed (mostly dicotyledons).

For gutters and pavements, the vegetation was recorded in and around joins being the only area favourable to plant development. Five types of join have been observed (Figure 1): pavement joins; kerbstone joins; gutter-kerbstone joins; gutter joins and roadside joins

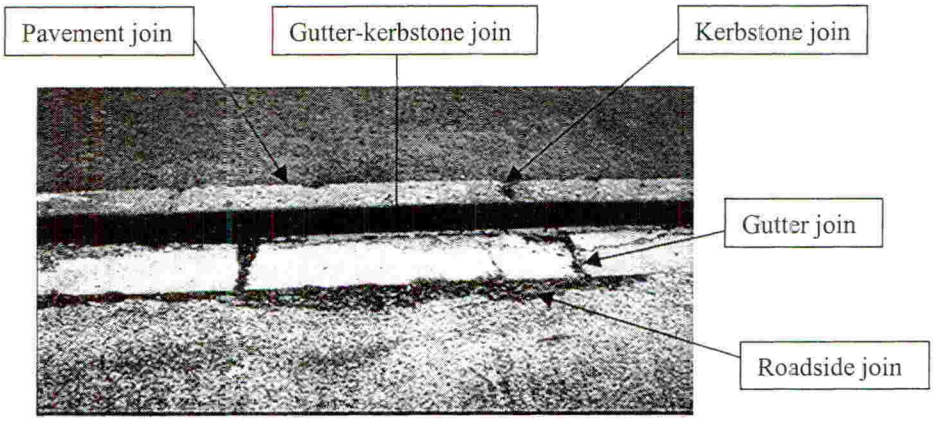


Figure 1. Different types of pavement joins

To assess the overall agronomic value of each technique, an efficacy percentage was attributed, every 2 to 3 weeks, to each tested surface (i.e. by comparing the weeded plots with the amount of vegetation covering the control sample). Every time a threshold efficacy of less than 70 % was observed, an additional treatment was prescribed. Thus the comparison of the different techniques was achieved by adding up the number of operations needed to maintain a measure of efficacy above 70 %. For Method 4 (periodic sweeping), no threshold was fixed, the treatment being performed on a fortnightly basis during the spring then once a month subsequently.

Estimates for speed of operation, reckoned in metres/hour, were also recorded (Table 2).

**Weeding of paved areas**

For the weeding of paved areas, three weed control strategies were compared to reference plots treated chemically and an unweeded control (Table 1). For the efficacy assessment the number of weeds present was counted as well as the degree of weed cover for each tested area. As with the treatments on gutters and pavements, an efficacy of less than 70 % necessitated a supplementary treatment. The overall efficacy was thus determined by the number of treatments required to maintain efficacy above the 70 % benchmark. A productivity evaluation was likewise been carried out.

**Description of weeding methods**

The reference chemical technique involved the use of glyphosate (as Roundup biovert, 360 g a.e./litre, Monsanto) applied at 5 litres/ha product using an ATH experimental sprayer (Tecnoma). The equipment was fitted with flat-fan TeeJet nozzles (XR 80015VS), nozzles operating at to 2.5 bars pressure and giving a spray volume rate of 500 litres/ha. A one metre span with four nozzles was used for paved areas and a hose with one nozzle was used for gutters and pavements.

A HOAF WM50 weeder was used for gas-fired flame treatment.

For mechanised sweeping, a motorized roadsweeper was coupled to a rotary high speed wire brush. The machine was driven slower than would be the case with normal roadsweeping. Given the technical specifications of this method of weed control, the vehicle was not used on pavement or on the paved areas, only on the gutters of the first test site. In contrast to periodic mechanised sweeping (see above), the number of interventions of calibrated mechanised sweeping was determined by the efficacy of the last intervention.

Steam weeding was carried out using the Weedcleaner system with a one metre span for the paved areas and, for the gutters and pavements, the hose attachment designed expressly for this purpose (Figure 2). The equipment was powered to generate steam at a temperature of 120°C.

Weeding with rotary wire brushes was achieved using a machine from the Lipco-Agria range.

Figure 2. Steam weeding, Weedcleaner system

## RESULTS AND DISCUSSION

This paper only sets out the results of overall weeding efficacy (number of weeding operations required) and estimates the cost of each technique. Table 1 indicates the number of interventions necessary for each technique to obtain an efficacy rate above 70 %.

### Weeding of gutters and pavements

Despite being straightforward and manoeuvrable to use, gas-fired flame weeding needed a high number of treatments which showed poor efficacy. It should also be noted that there is a fire hazard when used in proximity to motor vehicles (leaking fuel). For weed sweeping the calibrated approach, adjusted according to the density of weed cover, gave a reduction of weeding operations from 10 to 7. Overall, weed sweeping displays a high efficacy and did not damage road surfaces. Moreover sweeping also had the advantage of tidying up the highway. However pavements cannot be reached (in the absence of an articulated brush). The rotary wire brushes required the same number of operations as the chemical spray, but this technique is not recommended as the road surface deteriorates badly through its action.

Table 1. Number of operations needed for each technique to maintain 70 % efficacy on gutters/pavements and paved areas.

| Mode of intervention | Number of interventions | |
|---|---|---|
| | Gutters/pavements | Paved areas |
| (1) Control | 0 | 0 |
| (2) Glyphosate (reference) | 3 | 4 |
| (3) Flame gas-fired weeding | 8 | 8 |
| (4) Periodic sweeping | 10 | - |
| (5) Calibrated sweeping | 7 | - |
| (6) Rotary wire brushes | 3 | 5 |
| (7) Steam weeding (Weedcleaner) | 3 | 4 |

In the last analysis, the two techniques worth considering are as follows :

- Calibrated sweeping; this, of necessity, requires repeated interventions. It can be, however, organised as part of a contracted-out service.
- Steam-weeding (Weedcleaner system); this is the only technique needing as few operations as the chemical sprayer and without major drawbacks. The use of the hose attachment gives easy access to pavements (Figure 2). Attention should be drawn, nevertheless, to the inferior efficiency of this method with respect to the control of plantains (*Plantago* spp.).

**Weeding of paved areas**

Steam weeding (Weedcleaner) seemed the best method for weeding paved areas and required as few operations as chemical treatment. Weed control with the rotary wire brushes had to be done one more time than the chemical treatment. Moreover this technique damaged appreciably the joins and rendered paved surfaces slippery when the ground was wet. As with the gutters and pavements, the gas-fired flame treatment was ineffective. It only checked weed growth temporarily.

**Estimated costs for each technique**

Table 2 . Cost assessment for each method employed, including expenses for labour, consumables and the depreciation of equipment.

| Type of weeding | Chemical (glyphosate) | Thermic Weeding | Rotary wire brushes | Mechanised sweeping | Steam weeding |
|---|---|---|---|---|---|
| Yields for linear trials (metre/hour) | 2300 | 2 000 | 2 000 | 2 600 | 1 600 |
| Yields for surface area trials (metre²/hour) | 1700 | 1 000 | 1 000 | Not assessed | 1 000 |
| Cost of use (Francs/km/year)* | 590 | 2 280 | 2 550 | 890 | 810 |
| Cost of use (Francs/25 km highway) | 29 500 | 114 000 | 127 000 | 44 500 | 40 500 |
| Cost of use (Francs/1 000 m²/year)* | 800 | 1 520 | 960 | Unassessed | 850 |

*Taking account of the number of interventions required during the trials.

According to initial assessments, chemical treatment costs 590 F/km/year to maintain gutters and pavements and 800 F for the upkeep of paved surfaces.In one year, for an average size municipality with 25 km of highways to maintain (weeding of gutters and pavements), the cost of treatment with a chemical is approximately 30 000F per annum. Compared to which, weed control techniques involving flame weeders or rotary wire brushes incur significant extra expenditure (of the order of between 80 000F and 100 000F per annum respectively). These extras are due to the need for repeated operations for the flame weeding method and the need to sweep up after the rotary brushes have been in action. On the other hand, techniques like sweeping and steam weeding seem of interest economically (for 25 km of highway these cost 44 000 F and 41 000 F respectively). Although the cost annually of mechanised sweeping is higher than the use of a chemical, this technique incorporates the bonus of cleaning streets as well as weeding them. As for steam weeding, although the initial investment seems significant (80 000 F) and its cost is higher than the outlay for the spraying

of a chemical (810F/km/year as opposed to 590 F/km/year), the technique remains nonetheless affordable.

## CONCLUSIONS

This study demonstrated the value of new methods of weed control in areas where impermeable surfaces impose a very high risk of the displacement of pesticides into drainage water. For the control of weeds in paved areas, steam weeding seems the best alternative approach. To tackle the weeding of gutters and pavements, two solutions may be adopted: sweeping (without effect upon the pavement) or a combination of sweeping and steam weeding.

Observations made during the year's weeding experiments reveal significant differences in the efficacy of techniques. Monitoring over a period of several years would help to resolve certain questions such as whether, in the course of time, the number of operations with mechanical sweeping can be reduced and the long-term effect of the limited efficacy of steam weeding on plantains (*Plantago* spp.). Moreover, given the paucity of information relating to the use of these new weed control techniques in high risk areas, municipalities would need technical assistance to accompany their development. This new approach to weed control management combines the possibility of effective weed control on impermeable surfaces and restoring the quality of water.

## ACKNOWLEDGEMENTS

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# Vegetation changes in abandoned fields

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## ABSTRACT

The main objectives of this study were vegetation changes in fields abandoned after the termination of agricultural production. Species and seedbank changes and the amount of biomass produced were studied by sampling fields for 6 years after cropping was finished. The termination of cultivation led to the emergence of plant communities characterized by a large proportion of annual species in the initial years. The length of the period where annual species were abundant depended, in many cases, on the spread of *Elytrigia repens* and *Cirsium arvense* in the fields. Weed seed density in the ploughed layer (30 cm) of abandoned fields amounted to 157–666 seeds/$m^2$, with 75% of the seeds being produced by annual weeds. The amount of biomass in abandoned fields depended on the texture of the soil and the age of the plant community, being 31 t/ha at the maximum.

## INTRODUCTION

Estonian fields are becoming increasingly weed infested due to a number of factors. The main cause is a difficult economic situation resulting from the ending of large collective farms and the establishment of numerous small farms. In many cases, fields have been abandoned and become weed infested areas (about 25% of the cultivated land). In this situation, it is necessary to assess the areas where agricultural production has finished and to find out the most rational ways of managing them in order to alleviate future damage to the economy.

The emergence of new plant communities in unused fields is a rapid process. From abandoned fields, weeds spread to fields still cultivated, complicating weed control problems. The main objective of this study was to investigate the changes occurring in plant communities and the development of the predominant species in the communities and their effect on the soil.

## METHODS AND MATERIALS

The study was based on data gathered in 1995–2000 from areas where plant cultivation was discontinued for various reasons and at various times. In each field under study, a 100 x 100 $m^2$ observation plot was marked out. In these plots, observations and plant identifications were carried out each year in the second week of July. The observation plots were established on soils with different texture. The species composition of a plant community was established on a 0.25 $m^2$ piece of land in ten replications. From the plot soil samples of 0.2 x 0.2 x 0.3 m were taken in 10 cm layers to 30 cm depth. From these samples the number and the viability of seeds was determined in the laboratory. On the basis of the plant and soil samples obtained from the observation plots, the biomass produced by the plant community was determined.

Plant dry matter, above-ground residues (organic debris on the soil surface) and the weight of rhizomes and roots in the 0–30 cm soil layer was assessed. The assessment of the viable seedbank was performed in accordance with the methods of the laboratory of the Department of Field Crop Husbandry of the Estonian Agricultural University. The soil samples, 5 cm deep, were kept in moderately moist conditions at 18–22 $^{0}$C. Germinated plants were counted upon the emergence of the first true leaves, followed by the determination of their species.

This paper is based on the data obtained from observation plots on soils with three different textures (sand, sandy loam and clay), which have been analyzed more thoroughly. In drawing conclusions and making numerical generalizations, data from other observation plots (7–11, depending on the observation year) were also taken into account.

## RESULTS AND DISCUSSION

### Changes in plant communities

The ratio of annual species to perennial species depended on how long fields had been abandoned (Table 1).

Table 1. The density of annual (A) and perennial (P) weeds (plants/m $^{2}$) in abandoned fields of different soil textures.

| Year | Weed group | Sand | Sandy loam | Clay | V% |
|---|---|---|---|---|---|
| 1995 | A. | 48 | 204 | 21 | 96.6 |
| | P | 437 | 352 | 399 | 10.7 |
| 1996 | A | 20 | 70 | 0 | 94.2 |
| | P | 582 | 422 | 476 | 16.5 |
| 1997 | A | 44 | 66 | 8 | 74.4 |
| | P | 704 | 754 | 362 | 35.2 |
| 1998 | A | 38 | 20 | 0 | 98.3 |
| | P | 1156 | 754 | 688 | 39.2 |
| 1999 | A | 12 | 8 | 0 | 91.6 |
| | P | 674 | 1105 | 1524 | 38.6 |
| 2000 | A | 72 | 44 | 0 | 93.9 |
| | P | 628 | 1050 | 624 | 45.8 |

V% –Coefficient of variation

The discontinuation of cultivation resulted in the emergence of plant communities, which were characterized for the first 1-2 years by a considerable proportion of annual species (5–56% of the total number of plants). The total number of species in each community was relatively small, normally less than 15. Of annual species, *Tripleurospermum inodorum* proved to be the most common (21–39%), while *Chenopodium album, Scleranthus annuus, Polygonum lapathifolium, Viola arvensis, Centaurea cyanus, Vicia hirsuta* and *Spergula arvensis* occurred less frequently.

The length of the period with annual species having a large representation in a community depended in many cases on the spread of *Elytrigia repens (Agropyron repens)* in the abandoned fields. In land left idle for 5–6 years, perennial species supplanted annual species. Apart from *E. repens*, aggressive species turned out to be *Cirsium arvense* and *Artemisia vulgaris* (predominantly scattered all over the field). A general reason for perennial weeds becoming predominant was their stored reserves enabling a more rapid growth compared to the majority of the annual weeds thus giving advantages in root and shoot competition. The development of plant communities and the variation of predominant species in abandoned fields were influenced by various factors:

1) Weed invasion of crops before the termination of cultivation, and the specific composition and incidence of weeds. Following cereal crops there was a high incidence of *T. inodorum, P. lapathifolium, V. hirsuta., C. album.*, and, of perennial weeds, *E. repens.*

2) Following root crops (potato) the perennial weed species *C. arvense* and *Sonchus arvensis* increased with the potential for them to become predominant. The predominance of annual species in the plant communities of these abandoned fields was limited to 1–2 years or virtually non-existent (only a few species and specimens observed).

3) With perennial grass crops no period of annual species predominance was observed since the resultant community was composed of perennial species, with the predominance of either *Taraxacum spp, Agrostis tenuis, Poa trivialis* or, in wet areas, *Agrostis gigantea.*

4) The greater the proportion of perennial weed species in a particular cropping system, the more rapid the decline of annual species in abandoned fields. The presence of *E. repens*, and to a lesser extent *C. arvense* and *A. vulgaris*, influence this process.

5) Following the termination of cultivation, soils tending to be excessively wet contained *Tussilago farfara.* and *Equisetum arvense*, in addition to the perennial species mentioned above.

6) Four to six years after the termination of plant crop cultivation, the dominance of *E. repens was* reduced by the development of other invasive species such as *C. arvense, A. vulgaris, Taraxacum spp., Potentilla argentea* and *Rumex acetosella.*

The development of plant communities in abandoned fields suggest an increase in intraspecific and interspecific competition at both the root and the shoot level. In abandoned fields, the typical species appeared to *E. repens.* A frequency of around 1000 shoots/m $^2$ appeared to be the point at which the decrease in bioproduction, began. The growth and development of *E. repens* was impeded by the spread of *C. arvense.* The negative effect of the weather on the spread of *C. arvense* during the observation period was fairly small but its spread was inhibited on sandy soil. The soil surface residue present had no effect on the spread of *C. arvense.* The spread of this species was mainly ascribable to vegetative reproduction.

*A. vulgaris* had stable representation and considerable density in abandoned fields. Periodic fluctuations in its density resulted from environmental factors and intraspecific competition. Populations were largest on sandy soils but on loamy soils and soils tending to be excessively wet, the species was infrequent. A prerequisite for a rapid spread of *A. vulgaris* was a soil covered with little or no organic residue.

As competitors, *C. arvense, A. vulgaris* and *S. arvensis* had an advantage over the other species in the plant community due to their big biomass. However, on sandy soils and lands abandoned for a longer time, *S. arvensis* was not represented in the observation period. A deterioration in environmental conditions particularly soil compaction led to the supplantation of *S. arvensis* by other perennial species. The representation of *Taraxacum officinale* in abandoned fields

was largely confined to first-year plants. *T. officinale* was mainly introduced from neighboring habitats by wind. The intensity of its spread depended, as a rule, on the organic residue covering the soil – the thicker the layer, the less frequent the species. The spread of *T. officinale* was impeded by an increase in *E. repens* in the community and enhanced by a reduction in herbage density.

**Soil weed seedbank**

The weed seedbank was often irregularly placed in the ploughed layer of abandoned fields. This variability was due to many factors including application of manure abounding in weed seed, composts of irregular quality, variable location of weed species, the different seed-producing capacities of different species, irregular quality of soil cultivation methods. Therefore a major factor in the formation of soil weed seedbank was the quality of soil cultivation before the abandonment of the fields. The size of the seedbank in the topsoil layer was dependent on the seed-producing capacity of both the weed(s) present in the last crop and the subsequent plant community. Weed seed density in the ploughed layer (30 cm) of abandoned fields amounted to 157-666 seeds/m $^2$, with the upper 10 cm layer accommodating up to 51% of the total seedbank (Lauringson *et al.*, 2000). In the first 3 years following the termination of cultivation, the weed seedbank increased in the topsoil layer but decreased in later years (Table 2).

Table 2. Total weed seedbank in an abandoned field with sandy loam texture (seed/m$^2$)

| Soil layer | 1996 | 1997 | 1998 | 1999 | 2000 |
|---|---|---|---|---|---|
| <10 | 33 150 | 57 200 | 83 400 | 83 200 | 70 200 |
| 10–20 | X | 89 700 | 87 000 | 75 400 | 75 400 |
| 20–30 | X | 68 900 | 71 300 | 68 700 | 68 000 |
| Total | X | 215 800 | 241 700 | 227 300 | 213 600 |

Planting of abandoned fields with mixed crops of perennial grasses forming a solid turf helped to reduce the number of annual weed seeds in the upper soil layers ( Lauringson & Kuill, 1997). Although the vegetation in the abandoned fields is currently dominated by perennial plant species the soil seedbank is dominated by the seeds of annual species, constituting approximately 70–75% of the total viable seedbank. The deeper the soil layer, the smaller the density of viable seeds. The most frequently occurring species were *V. arvensis, Capsella bursa - pastoris, T. inodorum, A. vulgaris, Veronica spp, Thlaspi arvense* and *C. album.*

**Changes in organic matter levels in abandoned fields**

The above-ground mass of plant stalks and leaves and residues as well as the underground mass of plant roots and rhizomes was studied. The biomass produced by plants depended on the texture of the soil (Figure 1) and the time lapse since cultivation (Figure 2).

Total shoot biomass fluctuated from year to year, averaging 4.3 t/ha in 1998, 4.5 t/ha in 1999 and 3.2 t/ha in 2000. There was regularly less organic matter produced on the sandy soil. A high density in plant communities of the perennial species *C. arvense., S. arvensis.* and *A. vulgaris,* which inhabit the middle and top storeys, and the annual species *V. hirsuta* and *Galeopsis* spp. led to an increase in shoot biomass.

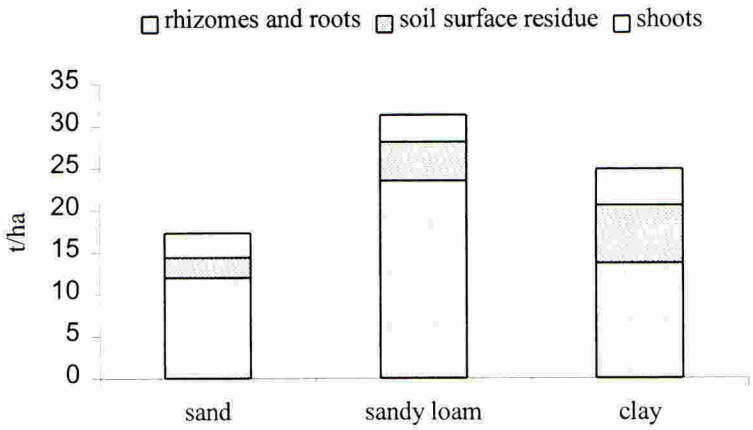


Figure 1. Dry matter (t/ha) of organic material in abandoned fields in 2000.

The accumulation of organic matter on and in the soil is a positive development in abandoned fields. Abundant residue contributes to the improvement of the physical and mechanical properties of the topsoil layer, reducing soil bulk density and decelerating soil compaction. The surface residue is a favorable environment for soil fauna. In 1998–2000, the average rate of residue formation for all observation plots was 3.5–4.0 t/ha, or 13.1–16.8% of the total organic matter (Fig. 2). The formation and development of residue is primarily contingent on the species composition in the field. *E. repens* accelerates the depositing of residue on the soil in an even layer but a high density in consecutive years decelerates the rate of residue deposited. The tall species with lignified stalks (*C. arvense, A. vulgaris, S. arvensis.)* do not form a residue layer of even thickness on the soil, despite their greater above-ground biomass. A residue layer evenly covering the soil reduces the establishment of wind-seeded species in abandoned fields.

The bulk of the biomass produced in abandoned fields is located in the ploughed layer. The average dry weight of organic matter in the 0–30 cm layer for all observation plots was 19 t/ha in 1998, 17.4 t/ha in 1999 and 16.6 t/ha in 2000, constituting 70.8%, 67.2% and 69.7% of the total biomass, respectively. The differences in the results for different fields were great, with the variation coefficient being 21%, 23.8% and 22.8% in the three consecutive years. Soil organic matter content and its changes depended on the mechanical composition of the soil. In a sandy soil, organic matter was accumulated in fairly small amounts. The highest concentration of organic matter was measured in the upper 10 cm layer. The mass of rhizomes and roots in a 0–10 cm layer constituted 86.4–87.5% of the respective figure for a 0–30 cm soil horizon on average. The effect of the density of the short shoots and stalks of *A. repens* on organic matter formation in topsoil layer as measured by the correlation coefficient "r" was

0.2–0.69, depending on the activity of the species' life cycle and the intraspecific competition. The main sources for organic matter at a depth of 10–20 cm were *A. vulgaris, C. arvense*, and, to a small extent, *E. repens* and *T. officinale*. A depth of 20–30 cm predominantly accommodated the roots of *C. arvense*.

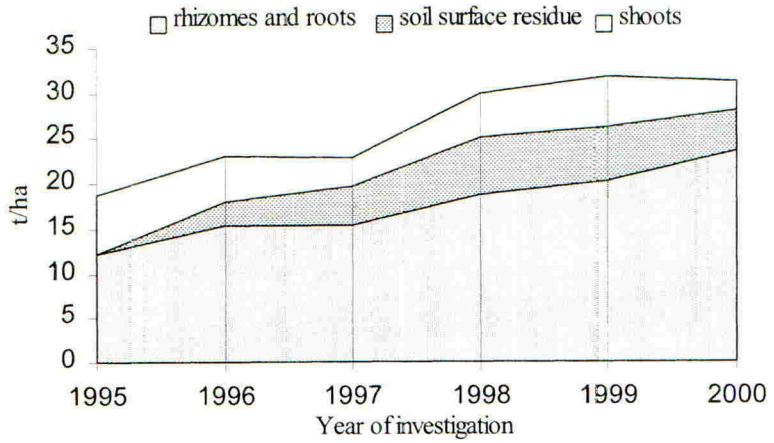


Figure 2. Dry matter (t/ha) of organic material in the abandoned field wi th sandy loam texture.

## CONCLUSIONS

The decision of whether it is practicable to resume agricultural production in abandoned areas or use other methods of management (forestation, growing shrubs for energy, etc.) has to be based on the fertility of the soil and the composition of the plant community formed in a particular field.

The termination of plant crop cultivation led to the emergence in abandoned fields of plant communities characterized by a considerable proportion of annual species in the first two years.

Four to six years after the termination of cultivation, *E. repens, C. arvense* and other perennial species become predominant in the fields. It is possible to recultivate abandoned areas, although the process is complicated by the presence of a large number of rhizomes and roots of perennial weeds and a high weed seed density in the soil.

Fields abandoned for a longer time often overgrew with shrubs. The resumption of agricultural production in these areas would require great expenditure.

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# Effect of synthetic and natural-product herbicides on *Senecio jacobaea* (common ragwort)

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## ABSTRACT

Three field experiments investigated the effectiveness of a range of treatments for the control of *Senecio jacobaea.* In the first, weed wiper applications of glyphosate and clopyralid applied in both May and June and glyphosate applied as a conventional spray in June killed all plants. In experiment 2, 2,4-D applied in May was marginally more effective at reducing the number of flowering plants compared with clopyralid, but clopyralid appeared to reduce the number of plants germinating the following year. The third experiment showed that the natural-product herbicide, citronella oil had a more rapid effect than either clopyralid or 2,4-D especially when applied to smaller plants in March. However clopyralid was the most effective treatment especially when applied in April and May.

## INTRODUCTION

*Senecio jacobaea* (common ragwort) is widely distributed throughout the UK; because of its toxicity to livestock, in particular cattle and horses, it is classified as a noxious weed and listed in the 1959 MAFF Weeds Act (MAFF 1999). As such, it is necessary to control *S. jacobaea* and prevent it from seeding and spreading onto agricultural land. Although hand pulling is a technique frequently used to control *S. jacobaea* especially along road verges and in horse paddocks care must be taken to ensure that the plants are removed and disposed of as its palatability to animals increases when it is dried (Clay 2000). Another problem with hand pulling is that regrowth from root fragments can occur (Simpson 1993). Chemical methods are available for the control of *S. jacobaea* but are not suitable or acceptable in some situations, therefore an alternative natural-product herbicide would be desirable.

In this paper the results of three field experiments are discussed. The first experiment compared conventional sprays of clopyralid, 2,4-D, triclopyr, fluroxypyr + triclopyr and glyphosate with weed wiper applications of glyphosate and clopyralid at two dates of application. The second experiment compared the efficacy of conventional spray applications of 2,4-D and clopyralid and the third investigated the efficacy of a natural-product herbicide (citronella oil) compared with clopyralid and 2,4-D at three application dates. Citronella oil, obtained from the grass *Cymbopogon winteranius,* is a ready to use herbicide for the control of *S. jacobaea* (B. B. 2000)

## MATERIALS AND METHODS

The three experiments described in this paper were all field trials sited on uncropped land at Failand, near Bristol on a silty loam soil.

**Experiment 1**

*S. jacobaea* plants were raised from seed sown on 21 August 1998 in trays of peat-based compost and transplanted one per 9cm diameter pot in early September. Seedlings were grown under cool glass and then moved outside to harden off. *S. jacobaea* plants were planted out into 1 x 3m plots in mid-November 1998; there were 12 plants per plot with plants 0.5m apart and two rows at 0.5m spacing. Experimental treatments of clopyralid (Dow Shield; 20% SL: Dow AgroSciences) at 0.2 kg a.i./ha, 2,4-D amine (Agricorn D; 50% SL: Farmers Crop Chemicals Ltd) at 2.3 kg a.e./ha, triclopyr (Garlon 4; 48% EC: Dow AgroSciences) at 1.92 kg a.e./ha, fluroxypyr + triclopyr (Evade; 2:6% EC: Dow AgroSciences) at 0.4 kg a.e./ha and glyphosate (Roundup Pro Biactive; 36% SL: Monsanto (UK) Ltd) at 1.44 kg a.e./ha were sprayed using an Oxford Precision Sprayer (OPS) fitted with one 11002 flat fan nozzle at a pressure of 98 kPa and a spray volume of 250 litres/ha. Weed wiper applications of glyphosate (33.5% product solution) and clopyralid (50% product solution) were made using a hand-held, rope, wick applicator (Weed Wiper Mini). Herbicide applications were made at two growth stages: rosettes 15 – 20 cm in diameter with extension growth just starting on 8 May 1999 and to plants 40 – 85 cm tall with flower buds present on16 June 1999. There was one untreated control per replicate and three replicates of each treatment. Assessments were made of % green cover and health of the plants at regular intervals throughout the summer, the number of plants that flowered were recorded at the end of August and shoot fresh weight recorded in early September.

**Experiment 2**

Herbicide treatments of clopyralid at 0.2 kg a.i./ha and 2,4-D at 2.3 kg a.e./ha were sprayed on 9 May 2000, on large plots 2.5 x 45m containing a natural population of *S. jacobaea* growing amongst other herbaceous vegetation, using an OPS fitted with five 11003 low pressure flat-fan nozzles at a pressure of 140 kPa and in a spray volume of 250 litres/ha. There were four replicates of each treatment and one untreated plot per replicate. Plant numbers per plot were counted at the time of application. The number of flowering plants present in July, August and September were counted and removed at the time of assessment and the number of plants present in April 2001 was also counted.

**Experiment 3**

Small plots, 1 x 2m, were laid out on a natural population of *S. jacobaea* in uncropped land. Herbicide treatments of a 'ready-to-use' formulation of citronella oil (Barrier H; 22.9% EC: Barrier Biotech Ltd) at 1500 litres/ha, 2,4-D at 2.1 kg a.e./ha and clopyralid at 0.2 kg a.i./ha were sprayed at three application dates. On the first date on 19 March 2001 plants were small to large compact rosettes; at the second date on 17 April, plants were actively growing but not extending and at the third application date on 23 May shoots were up to 35 cm tall but with no flower buds. All applications were made using an OPS fitted with two 11003 flat fan nozzles. 2,4-D and clopyralid were applied at a spray pressure of 126 kPa and in a volume rate of 340 litres/ha, whilst the citronella oil treatment was applied undiluted at a pressure of 140 kPa. The number of plants present on each plot and the % green cover were recorded at the time of spraying. Plant health was assessed visually using a score 0 – 7; where 0 = dead, 4 = 50% reduction in growth compared with the best untreated and 7 = as best untreated and % green cover was recorded at regular intervals throughout the summer. Plant numbers and the number of flowering plants present per plot were recorded at the end of the experiment.

## RESULTS

### Experiment 1

The data collected from Experiment 1 are presented in Table 1. The weed wiper applications were the most effective achieving complete kill of all plants at both herbicide dates, as did the later spray application of glyphosate. Initially all the other treatments resulted in statistically significant (P = 0.05) reductions in growth 1 month after treatment compared with the untreated plots. However in the longer term, only the early spray applications of clopyralid, triclopyr and fluroxypyr + triclopyr, and the later spray applications of triclopyr and glyphosate maintained statistically significant reductions in growth. With the exception of the late application of glyphosate, which killed all the plants, flowering was also only reduced by the early applications of clopyralid and triclopyr.

Table 1. Effect of herbicide treatments on the growth and flowering of *S. jacobaea*

| Herbicide | Date of Application | 14 June 99 cover | 16 July 99 cover | 26 August 99 cover | 26 August 99 No. Flowering | 2 Sept 99 Fr. Wt. (g) |
|---|---|---|---|---|---|---|
| Clopyralid | 8 May 99 | 27.5 | 8.5 | 40.0 | 5.5 | 1374 |
| 2,4-D | 8 May 99 | 37.5 | 27.5 | 67.5 | 9.0 | 2842 |
| Triclopyr | 8 May 99 | 17.5 | 20.0 | 30.0 | 5.5 | 1039 |
| Fluroxypyr + triclopyr | 8 May 99 | 55.0 | 80.0 | 65.0 | 11.0 | 2281 |
| Glyphosate | 8 May 99 | 17.5 | 27.5 | 65.0 | 10.5 | 2941 |
| Glyphosate w/w | 8 May 99 | 0.0 | 0.0 | 0.0 | 0.0 | 0 |
| Clopyralid w/w | 8 May 99 | 0.0 | 0.0 | 0.0 | 0.0 | 0 |
| Clopyralid | 16 June 99 | | 42.5 | 50.0 | 11.5 | 3763 |
| 2,4-D | 16 June 99 | | 47.5 | 45.0 | 8.5 | 2822 |
| Triclopyr | 16 June 99 | | 45.0 | 32.5 | 8.5 | 2202 |
| Fluroxypyr + triclopyr | 16 June 99 | | 47.5 | 75.0 | 11.5 | 4206 |
| Glyphosate | 16 June 99 | | 7.5 | 2.5 | 1.0 | 0 |
| Glyphosate w/w | 16 June 99 | | 1.0 | 0.0 | 0.0 | 0 |
| Clopyralid w/w | 16 June 99 | | 5.0 | 0.0 | 0.0 | 0 |
| Untreated | | 100.0 | 100.0 | 82.5 | 12.0 | 4298 |
| S.E.D. | | 3.27 | 6.28 | 9.62 | 1.19 | 839.4 |
| Df | | 7 | 14 | 14 | 14 | 14 |
| **l.s.d** | | **7.74** | **13.46** | **20.64** | **2.55** | **1800** |

### Experiment 2

Both of the herbicides significantly delayed the onset of flowering compared with the untreated control plots (Table 2). They also appreciably reduced the overall percentage of

plants that flowered throughout the whole flowering period of July to September. By the end of the season 2,4-D was slightly more effective than clopyralid. A count of the total number of plants on each plot the following April, almost one year after treatment showed that there were considerably fewer plants present on the plots previously treated with clopyralid than on either the 2,4-D treated plots or the untreated plots.

Table 2. Effect of 2,4-D and clopyralid application 9 May 2000 on flowering of *S. jacobaea* and on seedling numbers 1 year after application (plant number per plot)

| Treatment | No. plants at spraying | No. of flowering plants 2000 * | | | % plants flowered | 3 April 01 Plant number |
|---|---|---|---|---|---|---|
| | | 5 July | 1 Aug | 11 Sept | | |
| 2, 4-D | 198 | 0.0 | 33.3 | 16.0 | 27.4 | 121 |
| Clopyralid | 155 | 0.0 | 42.5 | 25.8 | 46.1 | 17 |
| Untreated | 159 | 106.5 | 37.6 | 9.6 | 105.2 | 116 |

* plants removed when counted

**Experiment 3**

Due to the natural variation in the population of *S. jacobaea* across the experimental area plant numbers and consequently % green cover was variable at the time of spraying. Plot data at later assessments have therefore been expressed as green cover as a % of initial cover; the number of flowering plants in July are expressed as a % of the total number of plants present before the plots were sprayed (Table 3).

Initially, all the treatments reduced the health of *S. jacobaea* considerably, with the treatments with citronella oil being the most effective causing rapid necrosis of most of the green leaf, thus resulting in considerably reductions in % green cover. However these effects were largely transient and within four weeks of treatment many of the larger plants which had not been killed, were recovering, this was especially evident from the later spraying dates where the treated plants were much larger. By July there was no difference in the percentage of plants flowering from the two early spray applications of citronella compared with the untreated plots, and although the third date did reduce the overall percentage this was not statistically significant ($p = 0.05$). The treatments with 2,4–D were slower acting than the citronella and mainly caused shoot distortion, and therefore although significantly reduced the health of the treated plants within one week of treatment only reduced % green cover at the April application date. However the symptoms continued to develop over time and by 8 weeks after treatment had resulted in considerable reductions in green cover with many plants dead or severely deformed. By July 2,4-D at all application dates had significantly reduced the total percentage of plants which flowered. Clopyralid was overall the most effective treatment, especially when applied in April and May. Clopyralid, like 2,4–D, was slower acting than the citronella oil but more effective in the longer-term, with the April and May applications resulting in 90 and 54% reductions in green cover respectively 8 weeks after treatment and with no flowering shoots produced in July.

Table 3. Effect of application date on the % green cover and health (Score 0 – 7) on *S. jacobaea* 1, 4 and 8 weeks after treatment (W.A.T.)

| | | 1 W.A.T | | 4 W.A.T | | 8 W.A.T | | July 01 |
|---|---|---|---|---|---|---|---|---|
| Treatment | Date | % reduction in green cover | Health (0-7)* | % reduction in green cover | Health (0-7)* | % reduction in green cover | Health (0-7)* | % plants flowered |
| citronella | March | 90.7 | 3.0 | 46.7 | 7.0 | 31.7 | 5.0 | 79.7 |
| 2, 4 – D | March | 0.0 | 6.0 | 41.1 | 4.0 | 65.1 | 3.3 | 22.3 |
| clopyralid | March | 0.0 | 6.0 | 33.3 | 4.0 | 70.6 | 2.7 | 31.1 |
| citronella | April | 78.3 | 3.3 | 33.3 | 5.0 | 10.0 | 5.0 | 71.8 |
| 2, 4 – D | April | 28.3 | 4.0 | 42.8 | 3.7 | 84.4 | 2.3 | 13.6 |
| clopyralid | April | 16.7 | 4.0 | 38.1 | 3.3 | 90.0 | 1.3 | 0.0 |
| citronella | May | 65.6 | 3.0 | 57.1 | 4.5 | 26.5 | 7.0 | 41.7 |
| 2, 4 – D | May | 15.3 | 4.0 | 32.8 | 3.5 | 63.3 | 3.0 | 20.3 |
| clopyralid | May | 5.6 | 4.0 | 6.7 | 3.0 | 53.9 | 2.3 | 0.0 |
| Untreated | | 0.0 | 7.0 | 31.1$^{+}$ | 7.0 | 27.8$^{+}$ | 7.0 | 69.0 |
| S.E.D. (df = 18) | | 12.40 | 0.149 | 16.95 | 0.257 | 8.17 | 0.50 | 20.65 |
| **Lsd** | | **26.05** | **0.313** | **35.61** | **0.540** | **17.15** | **1.05** | **43.39** |

* Health score: 0 = dead, 4 = 50% reduction in growth and 7 = best untreated
$^{+}$ = increase in % green cover

## DISCUSSION

*S. jacobaea* is a prolific weed which spreads rapidly from seed and therefore an important measure in its control is to minimise seed production, even if the plants are not killed.

The weed wiper applications of both clopyralid and glyphosate were found to be very effective, showing that selective application to control *S. jacobaea* may be feasible in areas where overall applications are either unacceptable or impracticable for economic reasons. Both herbicides have Approval for use in this way (Whitehead 2001), weed wiper application using hand-held equipment are feasible on plants at the rosette stage. For plants with extending shoots a tractor-trailed applicator has been developed (Bacon 1991). Where a non-selective treatment is used, glyphosate is clearly more effective applied to extending shoots in early summer than on rosette-stage *S. jacobaea* in spring. In this experiment the only other treatments, which statistically significantly reduced flowering, were the May applications of clopyralid and triclopyr. Although the applications of 2,4–D reduced overall growth, flowering was not significantly reduced and consequently many seeds would have been shed.

In all the experiments clopyralid and 2,4-D amine were relatively slow-acting but gave appreciable reduction in growth and flowering. In general efficacy of both herbicides was similar except in Experiment 3 where the April and May clopyralid treatments completely prevented flowering. Efficacy of the two herbicides in Experiment 2 may have been reduced by other vegetation shielding the *S. jacobaea,* and reducing the dose received. With 2,4-D,

June spraying of *S. jacobaea* when shoots are extending is recommended (Fryer & Makepeace 1978). Clopyralid has been shown to be very effective applied to *S. jacobaea* rosettes in autumn and spring (Clay 2000) and is recommended for use in grassland conservation areas (Crofts & Jefferson 1999). Clopyralid is damaging to a much smaller range of plant species than 2,4-D so its use would be advantageous where greater floral diversity is important. Results from Experiment 2 where seedling numbers on clopyralid-treated plots the spring after treatment were much lower than on the 2,4-D treated or untreated plots, suggest that treatment with clopyralid may inhibit subsequent germination of *S. jacobaea* seedlings. This might result from the release of clopyralid from decaying vegetation on the plots (Dow 2001). The citronella oil product has considerable potential where non-synthetic herbicides are required. It has a very rapid scorching effect compared with other treatments (Table 3). However it was less effective in this trial than when it is used as a ready-to-use spot application where small rosette plants are killed by one application (B. B. 2000). For control of larger plants a second application to regrowth 4 weeks after the first is recommended (B. B. 2000). The reduced efficacy in this trial may have resulted from a lower application rate than used with the spot-treatment method.

These trials have shown the relative efficacy of a number of treatments for *S. jacobaea* control in uncropped land and the value of weed wiping treatments.

## ACKNOWLEDGEMENTS

We would like to thank Barrier Biotech Ltd for supplying the sample of citronella oil used in Experiment 3.

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# Increasing botanical diversity and reducing weed abundance in degraded hedge-bases

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## ABSTRACT

An arable field hedgerow with a basal-flora harbouring pernicious weeds, notably *Galium aparine* and *Anisantha sterilis*, was selected for study. Our objectives were to develop practical methods to restore the hedge-base vegetation to a diverse perennial flora and reduce weed abundance. The management regimes imposed on the hedge-base included cutting, herbicide applications, sowing a native perennial seed-mix and excluding fertiliser. Results showed that sowing created the most botanically diverse plots, effectively reduced annual weeds and beneficially altered species composition, compared with unsown plots. Selective herbicide applications or excluding-fertiliser also increased plant diversity. Herbicide treatments suppressed weeds initially but *A. sterilis* re-infested plots the following year, whereas spring-cutting each year gave continued suppression.

## INTRODUCTION

The hedge-base flora of arable field boundaries is particularly vulnerable to inaccurate or excessive fertiliser treatments, herbicide drift or direct applications and disturbance from cultivation. These activities encourage aggressive nitrophilous weeds, such as *Galium aparine* and *Anisantha sterilis*, that thrive with high soil fertility and reduced competition from the natural perennial vegetation (Boatman *et al.*, 1994). Research has shown sowing wild-herb/grass seed mixtures in field boundaries can improve vegetation diversity and reduce the ingress of field margin weeds, compared with natural re-vegetation (West *et al.*, 1997). We designed two separate field experiments in arable land to compare the effects of various management regimes on the flora of degenerate hedge-bases and to determine whether vegetation diversity and composition are improved and weed abundance reduced. Preliminary results from one of these experiments were reported previously (West *et al.*, 1999). This paper uses data for plant diversity and cover collected over three years, from the other arable field experiment, to describe the establishment and development of vegetation under the different management regimes and their effect on the abundance of *G. aparine* and *A. sterilis*.

## MATERIALS AND METHODS

An arable field-boundary hedgerow at Long Ashton Research Station was selected for investigation. After cultivation of the field for drilling (winter wheat) in September 1996, the

hedgerow was marked into 18, 20 m lengths, allowing a one metre strip in front of the hedge for vegetation to regenerate. Six management treatments were imposed on this hedge-base flora as follows:- A) autumn-cut, B) spring-cut, C) total-herbicide, D) selective-herbicides, E) sown with a perennial hedgerow seed mix, F) exclude-fertiliser. The experiment was a randomised block design with three replicates of each treatment, two blocks having a south-east aspect and one block a north-east aspect. In October 1996 the hedge-base flora contained an abundance of *G. aparine* and *A. sterilis*. At this time vegetation was removed from the E plots by applying the total herbicide glyphosate at 1.44 kg a.e. $ha^{-1}$, and sowing a perennial seed mixture for hedgerows (Emorsgate EH1) at 4g $m^{-2}$ in early November. All other plots remained unsown and allowed to revegetate naturally through the winter. In March 1997, the herbicide quinmerac at 0.5 kg a.i.$ha^{-1}$ was applied to the D plots for control of *G. aparine*, and, in April, the graminicide fluazifop-P-butyl at 0.125 kg a.i $ha^{-1}$ was applied to the same plots for control of *A. sterilis*. In early April 1997, glyphosate at 1.08 kg a.e. $ha^{-1}$ was applied to the C plots to simulate a sterile strip and the vegetation in the B plots was cut and cuttings removed. Fertilisers were excluded from the F plots by covering the hedge-front and verge with a plastic sheet during application and removing the collected granules. No treatment was required during spring 1997 on the unsown (A) plots or the sown (E) plots. Vegetation in all plots, apart from the B (spring-cut) plots, was cut and removed in September each year. April cutting of B plots and fertiliser-exclusion from F plots were the only treatments repeated in spring 1998 and 1999.

Plant communities, within the 1 m strip along the hedge-base, were assessed in spring and summer of 1997, 1998 and 1999. Each species present was recorded and given a modified Braun-Blanquet cover abundance score (0-9), which was subsequently converted to a percentage value as follows 0=0, 1=0.25, 2=0.5, 3=1, 4=2, 5=5, 6=12.5, 7=25, 8=50, 9=75%. The converted values for all species present in a plot were summed to give an estimate of total percentage cover. Some plots had a total cover in excess of 100%, due to overlapping foliage of individual species, and gives an indication of differences in vegetation architecture. Species numbers and total estimated percentage cover were analysed without transformation. For the weed species of interest (*G. aparine* and *A. sterilis*) the percentages were transformed to $\log_e(\%+0.25)$ prior to analysis. In order to study differences between plant communities, possibly associated with management treatments, the plant data abundance scores (0-9) were entered into a detrended correspondence analysis (DCA) using the CANOCO 4 (CANOnical Community Ordination) software (ter Braak & Smilauer, 1998).

## RESULTS

### Botanical diversity (species numbers)

During the establishment period, in 1997, sown plots contained a significantly greater number of plant species compared with all unsown plots and remained the most diverse plots in 1998 and 1999 (Table 1). Plots having vegetation cut in autumn or spring remained the least diverse, after the first assessment, while those plots having either a selective-herbicide application or fertilisers-excluded showed some enhanced diversity by the third year. The number of plant species was initially severely reduced by glyphosate treatments, but plots recovered their diversity at subsequent assessments.

Table 1. Number of species in the verge flora (values are means of three replicate 20 m plots)

| | May 1997 | July 1997 | May 1998 | July 1998 | May 1999 | July 1999 |
|---|---|---|---|---|---|---|
| A – Autumn-cut | 22.3 | 25.3 | 20.3 | 20.3 | 21.0 | 19.3 |
| B – Spring-cut | 21.0 | 26.7 | 16.7 | 23.0 | 18.7 | 19.3 |
| C – Total-herbicide | 5.3 | 25.7 | 23.3 | 23.7 | 24.7 | 21.0 |
| D – Selective herbicides | 20.0 | 28.7 | 28.3 | 27.3 | 26.0 | 25.3 |
| E – Sown | 30.7 | 38.3 | 28.0 | 27.0 | 32.0 | 28.3 |
| F – Exclude-fertiliser | 22.0 | 31.0 | 22.3 | 24.7 | 25.3 | 25.0 |

SED(46df) = 2.63 (between treatments) SED(60df) = 2.24 (within treatments)
LSD (p = 0.05) = 5.30 (between treatments) LSD(p = 0.05) = 4.50 (within treatments)

**Total vegetation cover**

By May 1997 (Table 2) greatest vegetation cover had developed on the autumn-cut and sown plots (>100%) while cover was significantly inhibited on plots treated with selective-herbicides (59%), and severely restricted on plots treated in April with glyphosate (2%). By July 1997 there was substantial vegetation cover on all plots apart from the glyphosate treated plots, on which cover was still significantly reduced (58%). In 1998 and 1999, as expected, there was significantly less vegetation cover on April-cut plots at the May assessments, but a considerable cover (>100%) on all other plots. Plots having much overlapping vegetation, and hence the most diverse structures, are indicated by the greatest percentage covers.

Table 2. Total % cover of verge flora (values are means of three replicate 20 m plots)

| | May 1997 | July 1997 | May 1998 | July 1998 | May 1999 | July 1999 |
|---|---|---|---|---|---|---|
| A – Autumn-cut | 109.8 | 124.5 | 116.6 | 101.1 | 136.8 | 102.6 |
| B – Spring-cut | 79.2 | 105.8 | 82.2 | 110.5 | 77.8 | 81.6 |
| C – Total-herbicide | 2.0 | 58.4 | 121.4 | 89.7 | 122.5 | 108.2 |
| D – Selective herbicides | 59.1 | 109.4 | 143.6 | 131.1 | 119.8 | 111.6 |
| E – Sown | 107.2 | 124.6 | 122.0 | 132.2 | 138.4 | 114.2 |
| F – Exclude-fertiliser | 80.7 | 97.2 | 104.5 | 110.8 | 116.9 | 101.8 |

SED(60df) = 15.61 (between treatments) SED(60df) = 14.45 (within treatments)
LSD (p = 0.05) = 31.22 (between treatments) LSD (p =0.05)= 28.91(within treatments)

**Cover of *Galium aparine* and *Anisantha sterilis***

In May 1997 (Table 3) cover of *G. aparine* was most extensive in the autumn-cut plots (32%) but also had a considerable presence in sown plots (25%) and those with fertiliser-excluded (15%). In comparison, cover of *G. aparine* was severely reduced on plots that had either vegetation cut or herbicides applied in April 1997. In May 1998, although the overall abundance of *G. aparine* was low, there was significantly more cover on plots treated with

selective or total-herbicides the previous year compared with all other plots, on which cover was negligible (<1%). In May 1999 cover of *G. aparine* was low on all plots and completely absent from those with the vegetation cut in April.

Table 3. Percentage cover of *G. aparine* log(%+0.25) in hedge-base flora (values are means of three replicate 20 m plots with back-transformed % means given in parentheses)

| | May 1997 | | May 1998 | | May 1999 | |
|---|---|---|---|---|---|---|
| A – Autumn-cut | 3.46 | (32) | -0.12 | (<1) | 1.12 | (3) |
| B – Spring-cut | -0.12 | (<1) | -1.39 | (0) | -1.39 | (0) |
| C – Total-herbicide | -1.39 | (0) | 1.95 | (7) | 1.01 | (3) |
| D - Selective herbicides | 1.95 | (7) | 1.31 | (3) | 0.53 | (2) |
| E – Sown | 3.23 | (25) | -0.12 | (<1) | 0.39 | (1) |
| F – Exclude-fertiliser | 2.71 | (15) | -0.25 | (<1) | 0.70 | (2) |

SED(66df) = 0.601 (between treatments) SED(60df) = 0.582 (within treatments)
LSD (p = 0.05) = 1.199 (between treatments) LSD (p =0.05)= 1.163(within treatments)

In May 1997 (Table 4) *A. sterilis* was absent from plots receiving an April application of total-herbicide and extremely low (<1%) on the selective-herbicide treated plots. There was significantly more cover on all other plots, the sown (13%) and autumn-cut plots (9%) having the greatest abundance. However, by May 1998 there was a significant increase of *A. sterilis* cover on plots treated with herbicides the previous year and on those from which fertiliser had been excluded. Cover remained at a similar level to the previous year on autumn-cut and sown plots, with only a sparse cover on April-cut plots. By May 1999 an extensive cover of *A.sterilis* (40%) had developed on plots sprayed with the total-herbicide in 1997 and a considerable presence (16-20%) remained on plots that were autumn-cut, unfertilised or previously treated with selective-herbicides. However, cover of *A. sterilis* on sown plots (5%) was significantly reduced from the 1997 level and cover remained consistently low (4%) on the April-cut plots.

Table 4. Percentage cover of *A. sterilis* log(%+0.25) in hedge-base flora (values are means of three replicate 20 m plots with back-transformed % means given in parentheses)

| | May 1997 | | May 1998 | | May 1999 | |
|---|---|---|---|---|---|---|
| A – Autumn-cut | 2.25 | (9) | 2.25 | (9) | 2.77 | (16) |
| B – Spring-cut | 1.38 | (4) | 1.39 | (4) | 1.48 | (4) |
| C – Total-herbicide | -1.39 | (0) | 3.23 | (25) | 3.69 | (40) |
| D – Selective herbicides | 0.17 | (<1) | 2.77 | (16) | 2.77 | (16) |
| E – Sown | 2.55 | (13) | 2.25 | (9) | 1.66 | (5) |
| F – Exclude-fertiliser | 1.48 | (4) | 3.23 | (25) | 3.01 | (20) |

SED(66df) = 0.530 (between treatments) SED(60df) = 0.479 (within treatments)
LSD (p = 0.05) = 1.062 (between treatments) LSD (p =0.05)= 0.957(within treatments)

## Detrended correspondence analysis (DCA)

The DCA ordination diagrams (Fig. 1) indicated the sown (E) plots had a distinctly different plant composition from all unsown plots, shown by the separation along Axis 1 in 1997 and 1998. Separation between unsown plots along Axis 2 in 1998 suggests differences of plant composition between spring-cut (B) plots and those treated with herbicides (C & D). In May 1999 a generally similar distribution was found to that in 1998 (not shown).

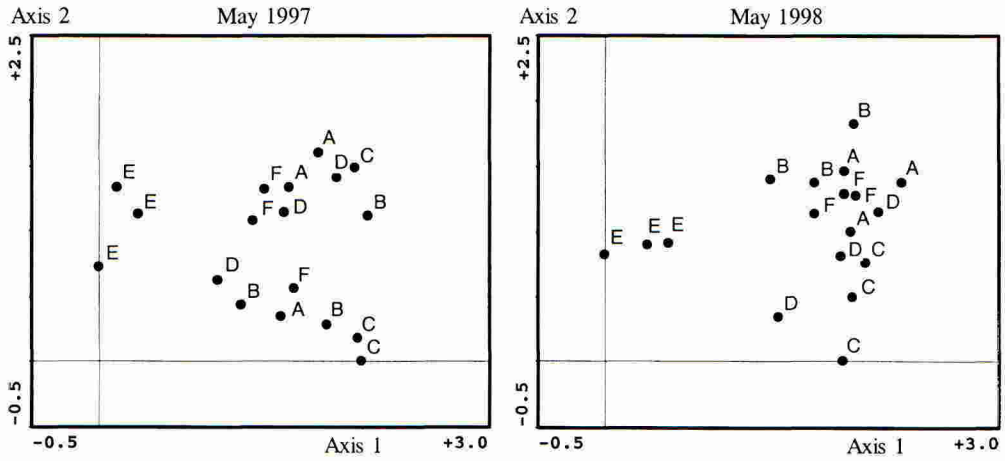


Figure 1. DCA ordination for plant communities in plots with various management treatments

## DISCUSSION

Results showed that sowing a perennial seed-mix proved the most successful, of the methods tested, for creating a botanically diverse hedge-base habitat that, once established, effectively inhibited re-invasion by annual weeds. Selective herbicides or excluding fertiliser were also useful for maintaining diversity on unsown plots. Herbicide treatments gave a temporary control of annual weeds but spring-cutting each year gave continued suppression. The ordination methods highlighted beneficial differences of species composition in sown compared with unsown plots and indicated changes in composition between management types within the unsown plots. The sown plots developed a stable, structurally diverse, perennial flora with a paucity of weed species. In contrast unsown plots, particularly those initially treated with herbicides, contained a weedy more transient plant community of annuals and perennials. Spring-cutting each year reduced the propagation of annual weeds.

Our results suggest establishing a stable perennial flora is the more permanent solution for suppressing annual weeds (West *et al.*, 1997) but selective herbicides may be useful in the initial restoration of weedy hedge-bases. Quinmerac was effective for controlling *G. aparine* and had little effect on non-target flora. Fluazifop controls weed grasses, without damage to broad-leaved herbs, but the recovery of *A. sterilis* suggests that repeat applications would be needed to prevent re-infestation. The resurgence of *A. sterilis* on plots treated with glyphosate in 1997 indicates this approach to weed management in field boundaries is not sustainable. Likewise, sterile strips may not reduce this weed. Unsown or sown hedge-bases may benefit

from cutting the vegetation in spring-only (removing cuttings), as this will help development of wild-herbs and reduce annual weeds (Marshall & Nowakowski, 1995). However, spring-cutting may not be a practical option for farmers with autumn-sown crops. These results corroborate some of the findings from our other arable hedge-base study (West *et al.*, 1999).

In conjunction with these studies Maudsley *et al.* (1998) reported that diversity of insect fauna in the hedge-base was reduced as the cover of aggressive annual weeds, particularly *G. aparine*, increased; while Cormie (1998) reported that cutting in spring-only provided a more suitable habitat for overwintering invertebrates. Thus, vegetation changes in hedge-base habitats have important implications for associated invertebrates.

In conclusion, various management techniques, especially sowing a native perennial seed-mix, have the potential to re-create hedge-base vegetation with both agronomic and ecological benefits, and further work to investigate combinations of these techniques is warranted. However, once achieved, successful maintenance of the vegetation diversity and structure will only remain sustainable alongside the sensitive management of adjacent field operations.

## ACKNOWLEDGEMENTS

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# An investigation into the effect of florasulam, fluroxypyr and metsulfuron-methyl when applied to newly-planted and established hedgerow species

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## ABSTRACT

Florasulam, fluroxypyr and metsulfuron-methyl are all spring-applied cereal herbicides with activity on broad-leaf weeds. A single replicated trial was established in the spring of 2000 to evaluate what effect these products would have on both newly-planted and established hedgerow species. Applications were made to hawthorn (*Crataegus monogyna*), blackthorn (*Prunus spinosa*), dog rose (*Rosa canina*), grey willow (*Salix cinerea*), white poplar (*Populus alba*), silver birch (*Betula pendula*), Norway spruce (*Picea abies*) and hazel (*Corylus avellana*). Florasulam was applied at a range of rates from 15 g a.i./ha (twice the recommended rate) down to 0.045 g a.i./ha – (used to mimic drift situations) - and was compared to fluroxypyr and metsulfuron-methyl, both at recommended rates. The aim was to identify if florasulam would remain selective to hedgerow species in drift situations and, if the outcome was positive, identify whether florasulam had potential for use in hedgerow establishment situations and/or forest nursery beds. The first application was made approximately 2 months after transplanting the saplings, with the second application, to previously untreated plants, in May 2001. Following the first application, florasulam proved selective (<10% injury) to Norway spruce, hazel, hawthorn, grey willow and white poplar at all rates tested. Metsulfuron-methyl proved selective to Norway spruce and hazel, whilst the application of fluroxypyr was not selective. The second application proved more injurious, with Norway spruce being the only species to be selective to all rates of florasulam. At 15 g a.i./ha, florasulam proved non-selective to silver birch, hazel and white poplar, whilst the 15 and 7.5 g rates were non-selective to hawthorn, blackthorn, dog rose and grey willow. Applications of metsulfuron-methyl proved selective to Norway spruce only and, as in timing A, fluroxypyr proved non-selective. There is potential for florasulam to be used in areas of hedgerow establishment and forest nursery beds.

## INTRODUCTION

Florasulam (tradenames Boxer/Primus) is a herbicide of the triazolopyrimidine group of herbicides (Lepiece *et al.* 1998). It is an inhibitor of acetolactate synthase (ALS) for use in cereals for the control of *Galium aparine* (cleavers) and a number of other key dicotyledonous weeds. As part of the continued development process, research was carried out into the effects of florasulam against non-target plants and to compare it to currently available products that are recommended for the control of dicotyledonous weeds. The aim of this work was twofold: a) to identify areas where drift – if it occurred – would be a problem and b) to establish if florasulam could be used in hedgerow establishment situations. The movement of crop protection materials away from their intended target poses several problems for all farmers. Besides the economic damage to nearby susceptible crops, possible

problems include reduced efficacy of plant protection products, airborne contamination of watercourses, and the social and financial costs resulting from the accidental damage that drift can cause. The impact upon important habitats, such as hedgerows, can be significant. The contamination by herbicides, either from drift or direct application, may kill a wide range of naturally occurring wild plants, or may create conditions for the establishment of aggressive weed species that could later invade crops. Few studies exist, where the total drift is estimated as a percentage of the amount sprayed. Maybank (1978) states that 1-8% of the sprayed amount are deposited outside the sprayed area. In most studies, the drift is estimated in different distances from the sprayed area. In the European context, the study by Ganzelmeier *et al.* (1995) is considered the best source of data concerning field spraying of annual crops under optimal conditions. Approximately 0.1% (0.03-0.3%) of the sprayed amount is registered in 10 metres distance from the sprayed area. However these modelling studies have assumed a given structure for ditches when calculating the contamination of surface water due to drift deposition. Some work has been conducted to examine spray drift collected on different plant species growing in boundaries (Haughton *et al.* 1998) and the results have shown lower levels of deposit in wider buffer strips.

This paper summarises data obtained over a 2 year period from a trial established in the United Kingdom in 2000/2001 in which florasulam was compared in terms of selectivity to commonly occurring hedgerow species (see Table 1) with metsulfuron-methyl (as Ally/Lorate 20DF) and fluroxypyr (as Starane 2). The study was designed to produce a direct comparison of the selectivity of the substances at two different growth stages of the hedgerow species. Metsulfuron-methyl and fluroxypyr were applied at recommended rates in comparison to florasulam applied at twice label rate, label rate, 0.1% label rate and rates reflecting drift expected at 1 metre and 5 metres from the target site (see Table 2).

## MATERIALS AND METHODS

The trial was established on a sandy clay loam in Oxfordshire (UK), in spring 2000 and was of a randomised complete block design with four replicates. Two applications were made the first being on the 12th May 2000 - 76 days after the saplings were transplanted - (to ensure adequate root development) and were in full leaf at the time of spraying (Table 1). The second application was made one year later on the 8th May 2001 to previously untreated trees. The herbicides were applied through Lurmark 03-F110 flat fan nozzles delivering 200 l/ha spray volume in a 2 metre band over the top of the trees using a backpack sprayer. Selectivity was assessed as visual percent injury in comparison to the untreated (0%).

Table 1 Hedgerow species present in trial

| Common name | Latin name | Height at application (cm) | |
|---|---|---|---|
| | | 2000 | 2001 |
| Hawthorn | *Crataegus monogyna* | 20-40 | 50-60 |
| Blackthorn | *Prunus spinosa* | 20-40 | 50-60 |
| Dog Rose | *Rosa canina* | 20-40 | 50-60 |
| Grey willow | *Salix cinerea* | 60-90 | 70-100 |
| White poplar | *Populus alba* | 60-90 | 70-100 |
| Silver birch | *Betula pendula* | 20-40 | 35-60 |
| Norway spruce | *Picea abies* | 15-30 | 20-35 |
| Hazel | *Corylus avellana* | 20-40 | 40-60 |

Table 2 Treatments applied to hedgerow species in May 2000 and May 2001

| Treatment | Rate (a.i. $ha^{-1}$) | Formulation |
|---|---|---|
| Florasulam | 15 g | 50 g/l SC |
| Florasulam | 7.5 g | 50 g/l SC |
| Florasulam | 0.75 g | 50 g/l SC |
| Florasulam | 0.375 g | 50 g/l SC |
| Florasulam | 0.045 g | 50 g/l SC |
| Metsulfuron-methyl | 6 g | 200 g/kg WG |
| Fluroxypyr | 200 g | 200 g/l EC |
| Untreated | - | - |

## RESULTS AND DISCUSSION

The effect of treatments on each hedgerow species is presented in turn following application at both timing A and timing B (see Figures 1-7) with the exception of Norway spruce, because all treatments apart from fluroxypyr showed low injury levels. The level, at which point a product was deemed selective was injury of 10% and less, observed at any point for the duration of the trial. If a product caused injury above this point at any assessment it was deemed non-selective. Following the first application, florasulam proved selective (<10% injury) to Norway spruce, hazel, hawthorn, grey willow and white poplar at all rates tested. The 15 g a.i./ha rate of florasulam proved non-selective to silver birch, whilst the 7.5 and 15 g rates were non-selective to blackthorn and dog rose. Metsulfuron-methyl proved selective to Norway spruce and hazel, whilst the application of fluroxypyr was not selective to any species. The second application proved more injurious to the hedgerow species in general with Norway spruce being the only species to be selective to all rates of florasulam. When applied at 15 g a.i./ha, florasulam proved non-selective to silver birch, hazel and white poplar, whilst the 15 and 7.5 g rates were non-selective to hawthorn, blackthorn, dog rose and grey willow. It was also noted that an atypical result of 13% injury was observed following the application of 0.75 g of florasulam against grey willow. Applications of metsulfuron-methyl proved selective to Norway spruce only and, as in timing A, fluroxypyr proved non-selective.

The increase in injury observed at the second timing could be explained by the weather conditions experienced in the UK during the spring of 2001. Cold and very wet conditions preceded a period of warmer weather that resulted in rapid growth of the hedgerow species around the time of the second application. This potentially facilitated enhanced uptake of herbicide and hence greater injury because the species were under greater stress. It was noted that the application of florasulam at the two rates intended to mimic drift resulted in negligible injury to any species.

In light of these results, there is potential for florasulam to be used in areas of hedgerow establishment and forest nursery beds (the herbicide market for Industrial, Amenity and Forestry Use is valued at approximately £11 million (CPA, 2001)). Although there are products available for use in these areas, they can have certain restrictions. The key weeds of concern in these areas are annual dicotyledons, annual grasses, cleavers and groundsel.

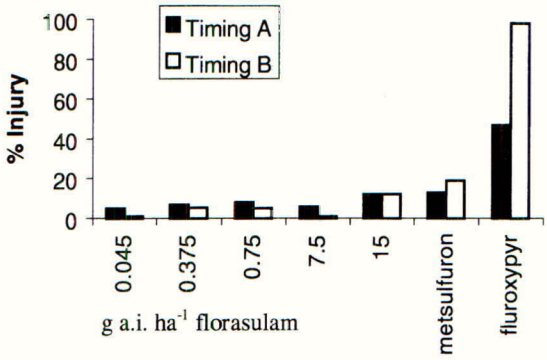


Figure 1. Injury to silver birch following application A & B

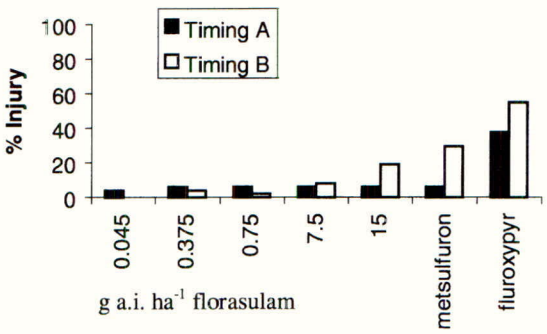


Figure 2. Injury to hazel following application A & B

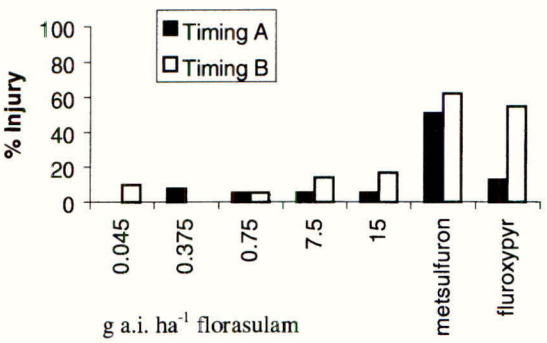


Figure 3. Injury to hawthorn following application A & B

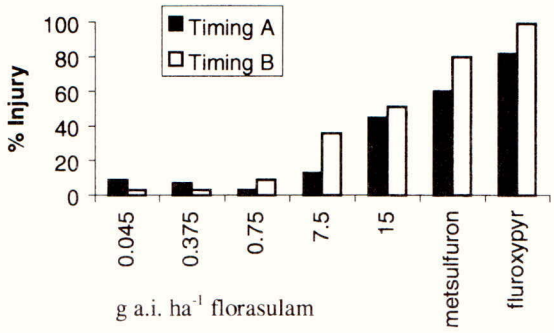


Figure 4. Injury to blackthorn following application A & B

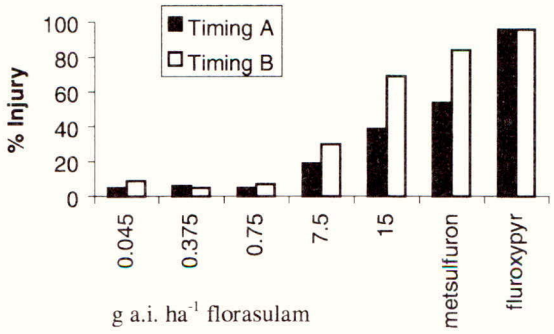


Figure 5. Injury to dog rose following application A & B

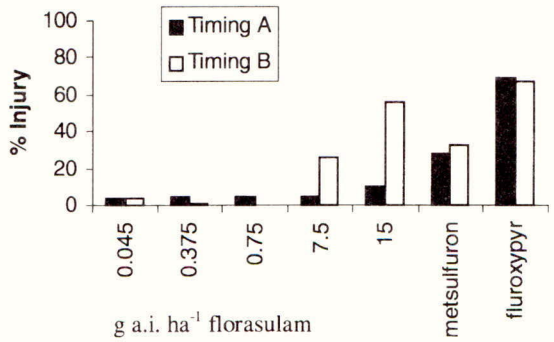


Figure 6. Injury to grey willow following application A & B

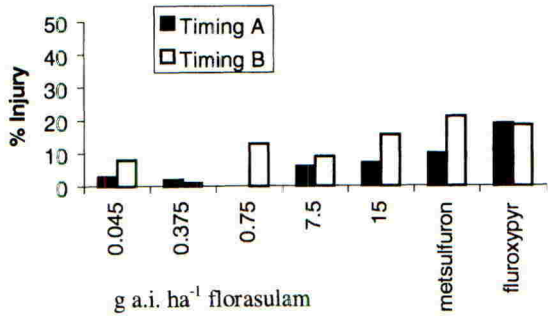


Figure 7. Injury to white poplar following application A & B

## CONCLUSIONS

Conclusions drawn from this trial are tentative because it was a single trial. Data indicated that florasulam was selective to Norway spruce, silver birch, hazel, hawthorn, grey willow and white poplar when applied at label rate (7.5 g a.i./ha) at the first timing (sapling stage). When applied to hedgerow species at one year of age, florasulam was selective to Norway spruce, silver birch, hazel and white poplar at label rate.

The application of florasulam at those rates deemed to mimic drift at 1 metre from target site and 5 metres from target site proved selective to all species at both timings. Florasulam proved more selective to the hedgerow species than metsulfuron-methyl or fluroxypyr.

Further research is needed to assess the effect on hedgerow species and to evaluate the potential use of florasulam in industrial, amenity and forestry use.

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# Development of ground flora during establishment of commercial short-rotation coppice (SRC) plantations

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## ABSTRACT

Short-rotation coppice (SRC) growing for energy production is an expanding fledgling industry in the UK. As part of the UK's commitment to international agreements concerning climate change a national flagship project (Project Arbre) encompassing the complete cycle of energy production from willow growing through to the sale of the electricity produced was initiated in Yorkshire. As a part of this initiative the environmental effects of commercial-scale coppice production are being assessed. Part of the monitoring consists of assessing key wildlife indicators including use of coppice by songbirds, butterflies, other canopy invertebrates and a detailed assessment of ground flora. In order to understand the potential effects of coppice on the local environment, a comparison with existing neighbouring crops is also undertaken. Results from the first year of the ground-flora-monitoring programme are presented here. They indicate that during establishment willow SRC contains more weed species than conventional arable crops but that a more stable ground flora with less competitive plants is beginning to colonise after just one year's growth. Headlands of SRC plantations contained fewer potentially harmful weed species in comparison to those associated with arable crops.

## INTRODUCTION

Short-rotation willow coppice (SRC) is an arable crop unlike others in that it is perennial in nature being harvested on a 3 to 4 year rotation instead of on an annual basis. The crop itself is non-competitive during establishment and therefore requires considerable weed control during soil preparation and initial stages of growth. Once established however, the crop required very little maintenance and few herbicide applications. Intensive research work has been conducted into the ecology of SRC plantations (Coates & Say 1999, Sage 1995, Sage & Tucker 1998, Tucker & Sage 1999). Much of this work have focused on small trial sites throughout the country and showed that in general SRC appears to be beneficial to a wide variety of wildlife. With less soil disturbance throughout the growing cycle, there is the opportunity for a more stable floral community to develop within the crop hence fewer weeds will be present that may negatively interfere with crop growth. In April 2000, ecological monitoring of commercial sites commenced. The main aim of the monitoring is to compare the ecological effects of SRC with the previous land use i.e. arable farmland. In addition to other key wildlife

indicators, the project monitors the ground flora both within and adjacent to the SRC crops and in equivalent arable plots in the same area.

## MATERIALS & METHODS

Ground flora monitoring was undertaken at 12 of the Project Arbre sites, half of which were planted with willow coppice in 1999 and half in 2000. Thus findings are representative of establishment phases of the SRC growth cycle. In addition to the SRC plantations, 12 arable fields grown with conventional arable crops such as wheat and barley were monitored in the same areas (control plots). The sites are located throughout the Yorkshire region within 60km of the power station. The previous land use of SRC plantations was arable, mainly cereals. The plantations consisted of a mixture of six different willow (*Salix viminalis*) varieties in various proportions these being Tora, Jorunn, Jorr, Orm Ulv and Bowles Hybrid. Table 1 shows the herbicide applications to the SRC sites during ground preparation and establishment 1999/2000.

Table 1. Herbicide applications to SRC monitoring sites during ground preparation and establishment 1999/2000.

| | Site | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Herbicide | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| 'Weedazol' | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × | × | × | × |
| 'Shield' | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × | × | × | × |
| 'Laser' | ✓ | ✓ | ✓ | × | ✓ | × | × | × | × | × | × | × |
| 'Codazole' | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × | × | × | × | × |
| 'Stomp' | ✓ | ✓ | × | ✓ | × | ✓ | × | ✓ | ✓ | × | ✓ | ✓ |
| 'Flexidor' | ✓ | ✓ | ✓ | ✓ | × | ✓ | × | ✓ | ✓ | × | ✓ | ✓ |
| 'Round-up' | × | × | ✓ | ✓ | × | × | ✓ | × | × | × | × | × |
| 'Gramoxone' | × | × | × | × | × | × | × | × | × | ✓ | × | × |

× = no herbicide applied ✓ = herbicide applied

Within selectively placed quadrats (1 x 10m), the percentage vegetative cover of plant species (in addition to the crop species) was recorded. Quadrats were placed parallel and including the non-cropped field headland. The quadrats were located on the edge of the crop (1m) and at 4m, 15m, 60m and 100m from this edge (Figure 1). These quadrats were positioned along one of two parallel transects running perpendicular to one of the field edges. Thus in total for each site 12 quadrats were sampled. In total, 288 quadrats covering an area of 2880m$^2$ were sampled across all sites including control plots. Plots were surveyed during the spring/summer (May-July).

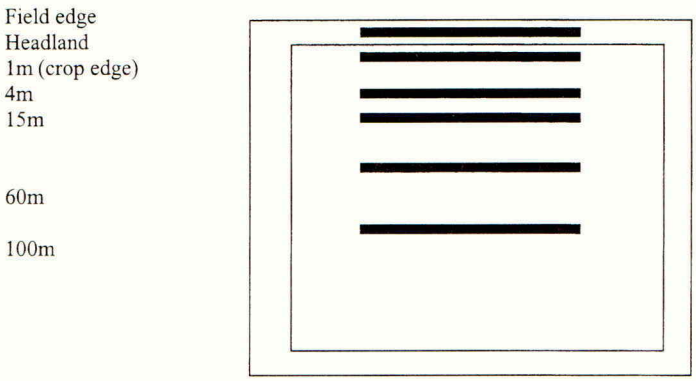


Figure 1. Diagram showing the locations of quadrats within a stylised field

## RESULTS

The main findings from our monitoring during 2000 were that SRC during establishment phases contained slightly greater weed cover than the arable fields sampled ($F_{1,41}$=4.55 P<0.05, Figure 2.).

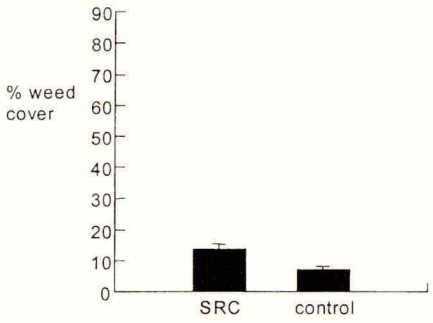


Figure 2. Mean percentage weed cover in SRC and Control plots, May-July 2000

The vegetative cover in the SRC plots consisted mainly of grasses although less invasive long-lived perennials also comprised a considerable part of this vegetation. This suggests that even within one year of growth the vegetation within SRC crops begins to develop from tall competitive and predominantly annual communities to more stable long lived perennial communities.

Within conventional arable crops weed cover declined with distance into the crop ($F_{4,105}$ = 3.31 P<0.05), however this was not the case in SRC plantations where weed cover did not decline with distance into the crop (Figure 3).

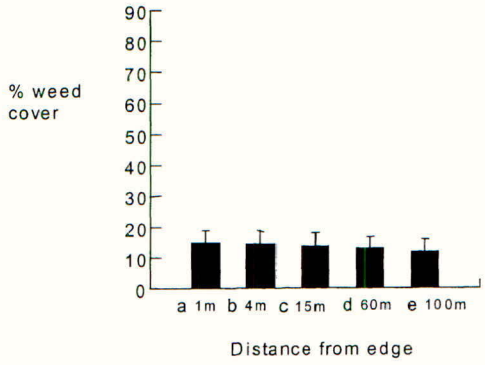


Figure 3. Mean percentage weed cover at various distances from the SRC crop edge.

More weeds were found in sites planted during 1999 than those planted during 2000 ($F_{1,22}$=36.08 P<0.001, Figure 4).

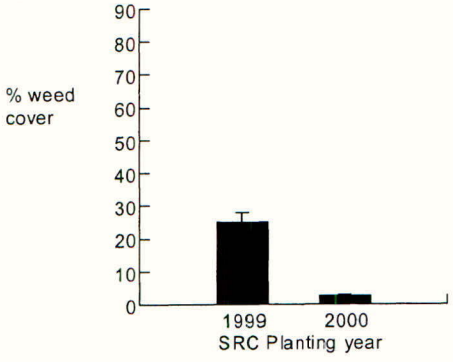


Figure 4. Mean percentage weed cover in SRC plantations established in 1999 and 2000.

Non-cropped headlands of fields contained a greater diversity and a more stable vegetative cover than within the crops of both conventional crops and that of SRC. Within the headlands, those surrounding arable crops contained more weeds than those surrounding SRC crop ($F_{1,38}$=13.99 P<0.001, Figure 5).

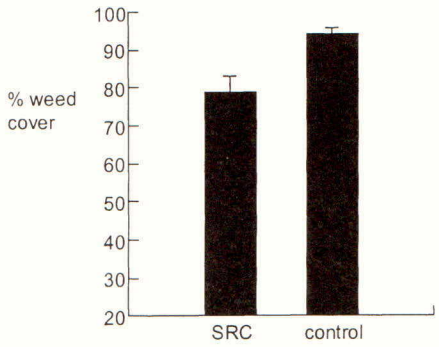


Figure 5. Mean percentage weed cover in non-cropped headlands of SRC and Control plots.

There was a significant effect of shading within headlands ($F_{3,19}$=5.14 $P<0.05$). Headlands with southerly orientation and hence shaded by the surrounding hedgerow had less vegetative cover, (Figure 6).

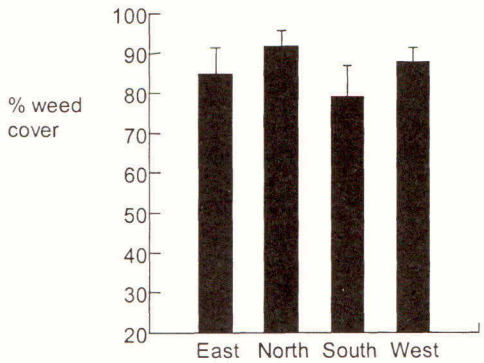


Figure 6. Mean percentage weed cover in headlands of both SRC and Control plots at different orientations.

## DISCUSSION

The results from the first year of ecological monitoring of Project Arbre sites suggest that during establishment SRC does contain greater weed cover (in addition to the crop) than conventional arable crops. After just one growing season there is already a tendency for the flora to develop into a more stable plant community which poses less of a threat to the

economic value of the crop itself. Initial and thorough treatment of the seedbed prior to planting is important in establishing the crop. Most Project Arbre sites were treated with herbicide prior to planting and received further spot applications throughout the first year (Rich *et al.*, 2000). Other benefits of SRC over conventional crops in terms of weed management are that overall, fewer chemicals are required, only really having to be applied to a specific problem. The more stable ground flora will encourage insect predators of potential pests to the crop and also provide habitats and nectar sources for other invertebrate life, thus enhancing the SRC's biodiversity. As the SRC is on a three/four year rotation, there will be fewer disturbances of the soil and greater shading hence less opportunity for invasive weed species to become established (Sage & Tucker 1998). Monitoring of the Project Arbre sites will continue for the first full cycle of the willow coppice growth (3-4 years), hence we will be able to evaluate the full ecological potential of the crop in comparison to the previous land use.

## ACKNOWLEDGEMENTS

The work described in this paper has been carried out under contract as part of the New and Renewable Energy Programme, managed by ETSU on behalf of the Department of Trade and Industry. Project Arbre is an initiative of Arbre Energy Ltd and we would like to acknowledge their assistance. The Central Science Laboratory has contributed to other aspects of the Project Arbre ecological monitoring programme.

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# SESSION 9A

# HERBICIDE RESISTANT WEEDS (RISK ASSESSMENT, BASELINE SENSITIVITY AND MANAGEMENT)

Chairman: J H Orson
*Morley Research Centre, Wymondham, UK*

Session Organiser: S Cranwell
*DuPont, Stevenage, UK*

Papers: 9A-1 to 9A-4

# Impact and management of herbicide-resistant weeds in Canada

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## ABSTRACT

The most abundant herbicide-resistant (HR) weed biotypes in Canada are ACCase inhibitor-HR wild oat (*Avena fatua*), and ALS inhibitor-HR common chickweed (*Stellaria media*), kochia (*Kochia scoparia*), and pigweed (*Amaranthus*) spp. *A. fatua* populations with multiple-group resistance have the greatest immediate economic impact on cropping systems in western Canada. Proactive or reactive management of HR weeds should (1) consider the relative risks of herbicides of different modes of action to select for resistance and the differing propensity of herbicides to be metabolized in HR biotypes when rotating among herbicides, (2) meet basic criteria for effective herbicide mixtures, and (3) incorporate agronomic practices in cropping systems that help reduce weed seed production and spread.

## INTRODUCTION

Thirty-eight herbicide-resistant (HR) weed biotypes occur in Canada (Figure 1). Fourteen spp. are resistant to ALS inhibitors, 11 to triazines, four to auxinic herbicides, two to ACCase inhibitors, two to phenylureas, two to bipyridyliums, and one each to triallate/difenzoquat, dinitroanilines, and flamprop. Although similar number of HR biotypes occur in western and eastern Canada, they are collectively most widespread and abundant in the prairie provinces (Alberta, Saskatchewan, Manitoba) of western Canada. This region accounts for 75% of national herbicide use. Most biotypes in eastern Canada evolved resistance to the triazine herbicides in the 1970s and early 1980s. Following the introduction of ACCase and ALS inhibitor herbicides in the early 1980s, the number of HR biotypes has increased rapidly in western Canada. Herein, we outline the occurrence of HR weed biotypes in Canada, estimate their economic impact on crop production systems, and assess tactics for their management.

## OCCURRENCE OF HERBICIDE-RESISTANT WEED BIOTYPES IN CANADA

### Group A/1: ACCase inhibitors

These herbicides are applied frequently to crops grown in western Canada to control the two most abundant weeds, *A. fatua* and green foxtail (*Setaria viridis*) (Figure 2). Group A-HR *A. fatua* is the most common biotype in Canada, occurring in c. one-half of fields surveyed in

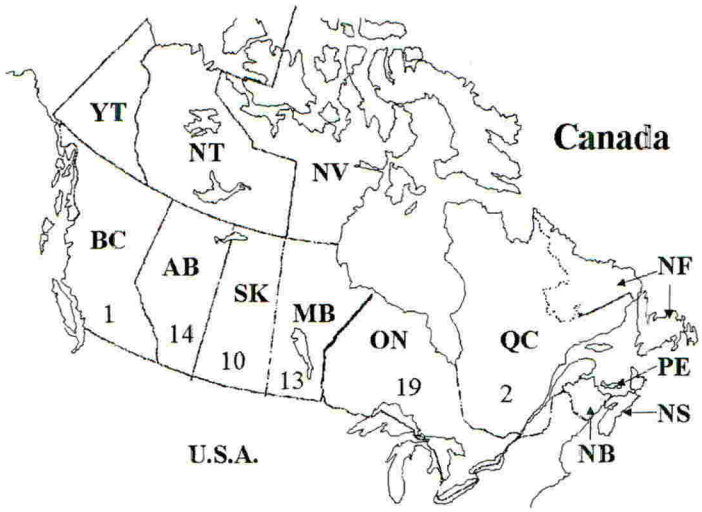


Figure 1. Number of herbicide-resistant weed biotypes in Canada, by province: BC, British Columbia; AB, Alberta; SK, Saskatchewan; MB, Manitoba; ON, Ontario; QC, Quebec; NB, New Brunswick; NS, Nova Scotia; PE, Prince Edward Island; NF, Newfoundland (YT, Yukon Territory; NT, Northwest Territories; NV, Nunavut).

each of the three prairie provinces; in Saskatchewan alone, 2.4 million ha were estimated to be infested (Beckie *et al.*, 1999c). Occurrence was directly related to frequency of Group A herbicide use. Crop rotations have little influence on Group A herbicide-use patterns, because these products are registered for use in cereal, oilseed, and annual legume crops (Légère *et al.*, 2000), which dominate cropping systems in the prairies.

Similar to *A. fatua*, resistance in *S. viridis* to Group A herbicides is prevalent in Saskatchewan. A field survey determined that one in every 20 fields (1 million ha) had Group A-HR *S. viridis*; 83% of grain elevators had screenings, which originated from fields located within the service area, containing seeds of this HR biotype (Beckie *et al.*, 1999b). We speculate this HR biotype is more abundant and widespread in Manitoba than in Saskatchewan because of the greater relative abundance of this sp. combined with frequent Group A herbicide use. For both *A. fatua* and *S. viridis*, incidence of aryloxyphenoxypropionate (APP) resistance in HR biotypes is markedly higher than cyclohexanedione (CHD) resistance (Beckie *et al.*, 1999b, c). Thus as a short-term tactic, CHDs may have a higher probability of success in proactive or reactive management of Group A-HR biotypes of these spp.

**Group B/2: ALS inhibitors**

ALS inhibitor resistance has been documented in nine broadleaf weed species in western Canada (Beckie *et al.*, 2001). These spp. include numerous populations of *S. media* reported since 1988 in Alberta, over 50 populations of *K. scoparia* in semi-arid regions of Saskatchewan and Alberta documented over the past five years, a biotype of false cleavers (*Galium spurium*) identified in 1996 in Alberta (Hall *et al.*, 1998), and a biotype of wild

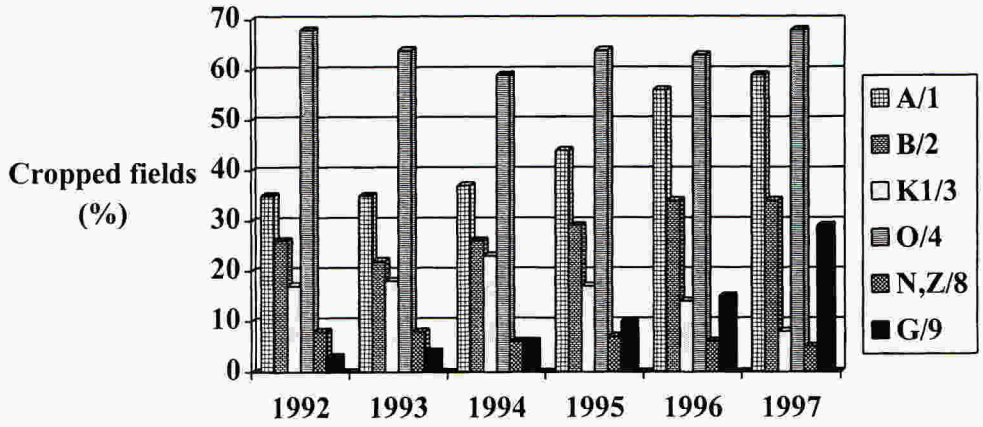


Figure 2. Herbicide group use in the prairie provinces (Alberta, Saskatchewan, Manitoba) in the 1990s (reconfigured from Beckie *et al.*, 1999c, 2001).

mustard (*Sinapis arvensis*) reported in Manitoba in 1992 and another from Alberta in 1993. Two populations of field pennycress (*Thlaspi arvense*) were recently confirmed in Alberta by the authors. In 1997 in Ontario, resistance was documented in eight Powell amaranth (green pigweed) (*Amaranthus powellii*) and four redroot pigweed (*Amaranthus retroflexus*) populations (Ferguson *et al.*, 2001). Group B resistance has been confirmed recently in one population of eastern black nightshade (*Solanum ptycanthum*) and four populations of common ragweed (*Ambrosia artemisiifolia*) in Ontario (F Tardif, unpubl. data).

Patterns of herbicide use contributed to the selection for Group B resistance in broadleaf weeds. Over 30% of fields in the prairies received a Group B application in 1996 and 1997 (Figure 2). In Ontario, *Amaranthus* HR biotypes were selected primarily in soybean (*Glycine max*); in 1997, more than 75% of the crop was treated with at least one ALS inhibitor.

Group B resistance in *A. fatua* has been documented in western Canada. In a survey of fields in two randomly selected townships (144, 64-ha fields each) in 1997, 20 to 30% of fields had populations exhibiting Group B resistance (Beckie *et al.*, 2001). In a survey in Saskatchewan that year, 23% of grain elevators had Group B-HR *A. fatua*; in Manitoba in 1997, this biotype was found in 21% of cereal fields sprayed with imazamethabenz (Beckie *et al.*, 1999c).

**Group K1/3: Dinitroanilines**

Despite extensive and sustained use of dinitroanilines during the past 30 years in western Canada, only one weed sp. has evolved resistance. Dinitroaniline resistance in *S. viridis*, discovered in 1988 in Manitoba, typically developed after 15 to 20 applications. The persistence of trifluralin resistance between 1988 and 1995 in fields infested with HR *S. viridis* suggests no apparent fitness penalty. In southwestern Manitoba, one in four fields is estimated to have dinitroaniline-HR *S. viridis*. In a field survey in Saskatchewan in 1996, this biotype occurred in 11% of fields; most fields occurred in the region with the highest relative abundance of this sp. (Beckie *et al.*, 1999b).

**Group O/4: Auxinic herbicides**

In 1990, populations of *S. arvensis* resistant to various auxinic herbicides were discovered in Manitoba, after selection with a mixture of MCPA, mecoprop, and dicamba for 10 consecutive years in addition to auxinic herbicides used previously. Resistance to dicamba was conferred by a single, completely dominant, nuclear allele (Jasieniuk *et al.*,1995). This simple inheritance, which facilitates rapid resistance evolution, was not expected because of the low incidence of HR biotypes despite long-term and widespread use of these herbicides (e.g., Figure 2). In 1998 in Alberta, a common hempnettle (*Galeopsis tetrahit*) biotype resistant to dicamba and MCPA was reported (Heap 2001).

**Group C1/5: Photosystem II inhibitors (triazines)**

The greatest number of weed biotypes in eastern Canada are resistant to the triazines. Resistance was first documented in common lambsquarters (*Chenopodium album*) in 1974 in Ontario. Since then, a total of 10 spp. have evolved triazine-HR biotypes in the province. Of all the spp., *C. album* and *Amaranthus* spp. are most widespread. Resistance to triazines has also been found in Quebec and in the Maritime region (Beckie *et al.*, 2001). In 1994, a metribuzin-HR biotype of *S. arvensis* was found in a field in Manitoba where the herbicide had been applied frequently (Beckie *et al.*, 2001).

**Group C2/7: Photosystem II inhibitors (phenylureas)**

In carrot (*Daucus carota*), linuron is one of the few broadleaf weed herbicides available. Two cases of linuron-selected resistance have recently been reported in eastern Canada from fields that were in carrot production. *A. artemisiifolia* populations from southwestern Quebec (Saint-Louis *et al.*, 2000), and a biotype of *A. powellii* in Ontario are resistant to linuron; the latter biotype exhibits cross-resistance to atrazine and prometryn (Beckie *et al.*, 2001).

**Group N, Z/8: Triallate and difenzoquat**

Resistance in 15 *A. fatua* populations to triallate was originally confirmed in Alberta in 1990 (O'Donovan *et al.*, 1994). These biotypes were also resistant to the chemically unrelated herbicide, difenzoquat, even through little history of use in infested fields was evident. Most of these fields were in monoculture barley (*Hordeum vulgare*) or wheat (*Triticum aestivum*) production. In 1997, about 15% of fields and 24% of grain elevators in Saskatchewan and 19% of fields in Manitoba had Group N, Z-HR *A. fatua* (Beckie *et al.*, 1999c, 2001).

There is little difference in fitness between HR and herbicide-susceptible (HS) biotypes. However, seeds from HR populations are less dormant than those from HS populations, which may at least partially explain a general decline observed in the level of HR:HS *A. fatua* from 1990 to 1997 in fields in Alberta (O'Donovan *et al.*, 2000). Greater and more rapid emergence of HR individuals compared to HS individuals may be potentially exploited for selective HR biotype control prior to seeding.

**Group D/22: Photosystem I inhibitors (bipyridyliums)**

Resistance to paraquat has been documented in horseweed (*Conyza canadensis*) and Virginia

pepperweed (*Lepidium virginicum*) (Smisek *et al.*, 1998). These HR biotypes occurred in fruit orchards in Ontario where paraquat was used intensively (three to five times a year for at least 10 years) to control weeds between trees. Growers have switched to using glyphosate to manage these HR populations, which has effectively contained resistance.

### Multiple-group resistance

Three *A. fatua* populations in northwestern Manitoba in 1994 were discovered to be resistant to fenoxaprop-P (Group A), imazamethabenz (Group B), and flamprop (Group Z/25) (Friesen *et al.*, 2000). In a field survey in Saskatchewan in 1996, 20% of Group A-HR populations were also resistant to ALS inhibitors, even though a field history indicated these herbicides were not used frequently (Beckie *et al.*, 1999c). In a survey of two townships in Saskatchewan in 1997, double- and triple-group resistance were exhibited in populations in 30 to 40% of fields in both townships (Beckie *et al.*, 2001). In Manitoba in 1997, 27% of cereal fields surveyed had *A. fatua* resistant to herbicides from more than one group; four populations were resistant to all herbicides registered for use in *T. aestivum* (Groups A, B, N, Z) (Beckie *et al.*, 1999c). Similar to the multiple-group populations discovered in 1994, the fields had a history of Group A herbicide use only. An additional five quadruple-group HR populations from northwestern Manitoba have since been confirmed (Beckie *et al.*, 2001). The likely mechanism conferring multiple-group resistance in these biotypes is enhanced metabolism by cytochrome P450 oxygenases (Friesen & Hall, 2000).

Group A- and K1-HR *S. viridis* in Manitoba and Saskatchewan (Beckie *et al.*, 1999b) is likely due to two resistance mechanisms within individuals. HR biotypes were initially selected with Group K1 products; subsequent control of these HR biotypes with Group A herbicides selected for multiple-group HR biotypes. Similarly, resistance to ALS inhibitors and to the synthetic auxin, quinclorac, in a biotype of *G. spurium* is likely due to two mechanisms. ALS resistance in this biotype is due to target site insensitivity, whereas the mechanism of quinclorac resistance is unknown (Hall *et al.*, 1998). One *A. powellii* population in Ontario with triazine and imazethapyr resistance was confirmed recently (Beckie *et al.*, 2001). Triazine resistance in this population is conferred by a mutation in the *psbA* gene, whereas imazethapyr resistance is due to mutation in the ALS gene.

## IMPACT OF RESISTANCE ON CROP PRODUCTION SYSTEMS

The immediate, direct economic impact of HR biotypes on cropping systems in Canada depends on the availability of cost-effective alternative herbicides with a different mode of action in the major crops grown. The estimated impact of the most frequently-occurring HR biotypes on crop production systems is summarized in Table 1, using the procedure described in Beckie *et al.* (1999c). The impact of HR biotypes is somewhat ameliorated by the availability of canola (*Brassica napus*), *G. max*, and corn (*Zea mays*) varieties resistant to non-selective herbicides. To date, there are no reports of resistance to Group G or H. In western Canada, control of Group A-HR *A. fatua* will increase herbicide costs in broadleaf crops (*B. napus*; flax, *Linum usitatissimum*; field pea, *Pisum sativum*) , whereas control of Group B-HR *A. fatua* will increase costs in cereal crops (*T. aestivum*, *H. vulgare*). Multiple group-HR *A. fatua* will increase weed control costs in all these crops. Depending on their cross-resistance pattern, there may be no alternative herbicides for their control in *T. aestivum*. As a consequence, the affected growers are likely to plant more HR *B. napus*,

Table 1. The estimated immediate economic impact of the most common herbicide-resistant (HR) biotypes on cropping systems in Canada

| HR biotype | Impact rating | Alternative products and their cost-effectiveness |
|---|---|---|
| Gp A/1 *A. fatua* | High | Gp B/2, K1/3, N,Z/8, G/9*, H/10*; increased cost in broadleaf crops |
| *Gp B/2 A. fatua* | High | Gp A/1, K1/3, N,Z/8, G/9*, H/10*; increased cost in cereal crops |
| Gp N,Z/8 *A. fatua* | Low | Gp A/1, B/2, K1/3, G/9*, H/10*; one or more alternative herbicides are cost-effective |
| Multiple gp *A. fatua* (e.g., A/1+B/2) | Very High | Gp G/9*, H/10*, few effective selective herbicides; increased cost in all crops. Fewer spring cereals, more HR *B. napus* & perennial crops |
| Gp A/1 *S. viridis* | High | Gp B/2, K1/3, O/4, C2/7, N,Z/8, G/9*, H/10*; increased cost in broadleaf crops |
| Gp K1/3 *S. viridis* | Moderate | Gps A/1, B/2, O/4, C2/7, N,Z/8, G/9* or H/10*; increased cost in *Triticum aestivum* |
| Gps A/1, K1/3 *S. viridis* | High | Gp B/2, O/4, C2/7, N,Z/8, G/9*, H/10*; increased cost in *T. aestivum* & broadleaf crops |
| Gp B/2 *S. media* | High | Gp K1/3, O/4, C1/5, C3/6, C2/7, N,Z/8, G/9*, H/10*; increased cost in cereal crops |
| Gp B/2 *K. scoparia* | Moderate | Gp K1/3, O/4, C3/6, C2/7, G/9*, H/10*; increased cost in cereal crops |
| Gp B/2 *Amaranthus* spp. | High | Gp K1/3, O/4, C1/5, C3/6, C2/7, G/9$^{+}$ or H/10$^{+}$; increased cost in non-HR *Glycine max* |
| Gp O/4 *S. arvensis* | Moderate | Gp B/2, C1/5, C3/6, C2/7, G/9*, H/10*; increased cost in all crops |
| Gp C1/5 spp. | Low | Gp B/2, K1/3, O/4, C3/6, C2/7, G/9$^{+}$ or H/10$^{+}$; one or more alternative herbicides are cost-effective |

*Registered for in-crop use in HR *Brassica napus* only.
$^{+}$Registered for in-crop use in HR *Glycine max* or *Zea mays* only.

fall cereals, or perennial crops. Biotypes of *S. viridis* have a moderate to high impact on weed control costs in broadleaf or cereal crops. Control of Group A-HR biotypes of this sp. will increase herbicide costs in broadleaf crops, similar to that of *A. fatua*. Group K1-HR biotypes will increase costs in *T. aestivum*, whereas control of biotypes resistant to both A and K1 herbicides will increase costs in *T. aestivum* and broadleaf crops. In *H. vulgare*, propanil is the sole remaining herbicide for control of these multiple-resistant biotypes.

Group B-HR *S. media* and *K. scoparia* biotypes increase weed control costs in cereal crop production, whereas Group O/4-HR *S. arvensis* biotypes increase costs in both cereal and broadleaf crops. In eastern Canada, the economic impact of ALS inhibitor resistance in *Amaranthus* spp. is high; control of these biotypes in non-HR *G. max* will increase herbicide costs. In contrast, triazine resistance in various spp. has minimal impact on herbicide costs in *Z. mays*, *G. max*, and *T. aestivum*-based cropping systems.

## RECOMMENDED MANAGEMENT TACTICS

Growers have been reluctant to proactively manage weeds to delay the selection for herbicide resistance. Selection pressure can be reduced or varied by the use of herbicide rotations, mixtures, and altering time of application, i.e., pre-seeding, in-crop, pre- and post-harvest. Although growers are increasingly practicing herbicide group rotation, the level of adoption is still relatively low (37% of growers in 1998 in Saskatchewan) (Beckie *et al.*, 1999a). Herbicide rotations, mixtures, or sequences generally have the greatest effect in delaying resistance when the mechanism conferring resistance is target site-based, target weed species are highly self-pollinated, and seed spread is restricted. If mixing partners do not meet the criteria of similar persistence and efficacy but different propensity for selecting for resistance in target species, the effectiveness of mixtures for delaying resistance will be reduced and may inadvertently accelerate evolution of multiple resistance. Metabolism-based resistance conferring resistance to herbicides of different modes of action will clearly limit the effectiveness of herbicide group rotation as a tool to delay resistance. Guidelines for rotating herbicides with different propensity to be metabolized need to be developed to combat increasing cases of metabolism-based HR grass weed populations. Herbicides that are not readily metabolized are unlikely to select for metabolism-based resistance. Herbicides that are detoxified via pathways different than that mediated by cytochrome P450 oxygenases or that are not metabolized will lessen the chance of selecting for multiple-group (metabolism-based) HR grass weed populations.

Not all herbicides have the same proclivity for selecting for resistance in weeds. We recommend to growers that the higher the risk of a herbicide mode of action of selecting for resistance, the less often herbicides from that group should be applied (Beckie *et al.*, 2001). It is widely agreed that Group A and B herbicides pose a high risk (i.e., generally less than 10 applications) for selecting HR biotypes relative to herbicides from other groups. Lower risk non-selective herbicides, Group G or H, should be used in-crop, or Group D or G should be used pre-seeding to reduce the number of weeds selected with higher risk, in-crop herbicides.

With the exception of frequency of fallow in the rotation, resistance development in *A. fatua* was little affected by cultural practices used by growers (Légère *et al.*, 2000) and we predict a similar response with other HR weeds. Although consistency and efficacy of cultural practices pale in comparison to herbicide performance, synergies can be realized which provide opportunities to reduce weed populations and therefore selection. Unfortunately, the increasing size of farms with concomitant limited labor and time availability has reinforced a heavy reliance on herbicides. Although containment of HR patches at early stages of development by herbicides or non-chemical methods is recommended and research shows it to be effective (H J Beckie, unpubl. data), most growers fail to detect small patches. Field scouting after in-crop herbicide application is not convenient because of the large cropped acreages. In western Canada, seed spread of HR *A. fatua* and *S. viridis* from patches within and among fields has been documented (Andrews *et al.*, 1998; Beckie *et al.*, 2001). Management practices that limit the spread of HR seed can slow the occurrence of resistance. Growers who reported practicing weed sanitation (e.g., cleaning equipment when moving between fields, tarping grain trucks, mowing or spraying ditches or uncontrolled weed patches, etc.) were less likely to have HR *A. fatua* than those who were less careful (Légère *et al.*, 2000). If the HR population covers a wide area across the field, management should focus on reducing seed return by using lower risk herbicides in conjunction with cultural practices, such as silaging or growing competitive crops.

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# Situation and management of *Avena sterilis* ssp *ludoviciana* and *Phalaris paradoxa* resistant to ACCase inhibitors in Italy

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## ABSTRACT

All enquiries made to Syngenta about poor control of *Avena sterilis* ssp *ludoviciana* and *Phalaris paradoxa* were monitored over three years. More than 100 seed samples were collected from the affected fields and tested with one DIM and two FOP herbicides. All seed samples came from fields cultivated with durum wheat in central and southern Italy. Several populations of *A. sterilis* ssp *ludoviciana* and *P. paradoxa* proved to be resistant to one or more ACCase inhibitor herbicides. Two fields where the resistance situation was particularly serious were chosen for field experiments. Three years of experiments testing the efficacy of chemical and agronomic means for controlling resistant *A. sterilis* and *P. paradoxa* are presented. The emergence dynamics of the two resistant species was also determined in the same fields. For both species the situation appears to be evolving slowly. No single chemical or agronomic treatment solved the problem of resistant populations. False seedbed preparation and some alternative herbicides proved to be useful in controlling resistant populations. Integrated weed management appears to be the best solution.

## INTRODUCTION

Herbicide resistance in Italy has evolved more slowly than in other developed countries and until the early nineties, only three species had become resistant to triazine (Porceddu *et al.*, 1997). The reasons for this seem to be related to the higher diversity that still characterised much of Italian cropped land. However, since then several species have become resistant to various chemical classes of herbicides (Sattin *et al.*, 1999; Sattin *et al.*, 2000; Bravin *et al.*, 2001). As expected, resistance developed in the two cropping systems where diversity is more limited: rice and durum wheat (*Triticum durum*) monoculture or, even where durum wheat is rotated with tomato or sugarbeet, herbicides with the same mode of action are used in these crops. The problem of resistance appears to be particularly serious for durum wheat, where there are no alternative herbicides for controlling monocots satisfactorily, as well as no alternative cash crops in the very dry and poor soils of central and southern Italy. In Italy, durum wheat covers an area of about 1.6 million ha, of which about 500,000 are treated with graminicides.

The aims of this study were: to confirm the presence of resistance to ACCase inhibitors in *Avena sterilis ssp ludoviciana* (hereafter called *A. ludoviciana*) and *Phalaris paradoxa* infesting durum wheat fields; to verify the extent of resistance; to gain information on the

pattern of cross-resistance to ACCase inhibitors and investigate the possible chemical and agronomic means for controlling resistant populations.

## MATERIALS AND METHODS

Seeds of *A. ludoviciana* and *P. paradoxa* were collected for three years (1998-2000) from fields where enquiries had been made to Syngenta regarding poor control of the two weeds in durum wheat crops in Italy. Syngenta covers more than 70% of the graminicide market in Italy. Historical records of herbicide use and other agronomic techniques used in the sampled fields were collected from the farmers. Seed samples were cleaned and stored at ambient temperature.

### Pot experiments - Screenings

To break dormancy, seeds were vernalised (seeds of *A. ludoviciana* were also dehulled) for 8 days at 4 °C in petri dishes on wet filter paper in the dark; they were then placed in other petri dishes on agar medium (0.6 %) with the addition of 0.2% $KNO_3$ and placed in a germination cabinet at 12-25 °C night/day with a 12 h photoperiod. After six days, seedlings were transplanted into 16 cm diameter pots filled with a substrate (silty loam soil 60%, sand 30% and peat 10% by volume). Eight and twelve seedlings per pot of *A. ludoviciana* and *P. paradoxa*, respectively, were transplanted and kept in a greenhouse at Legnaro (45° 21' N, 11° 58' E) After a week, plants were thinned to six and eight per pot for *A. ludoviciana* and *P. paradoxa*, respectively.
The experimental layout was a completely randomised design with two replicates of three pots (i.e. 18 and 24 plants) for each population. Populations were screened at the field dose (1x) and three times that (3x) with the following herbicides: diclofop-methyl (1x: 710 g a.i./ha), clodinafop-propargyl (1x: 60 g a.i./ha) and tralkoxydim. Given that the latter herbicide showed a much higher efficacy in the greenhouse, the first experiments did not provide any useful information because the label field dose (425 g a.i./ha) killed all the plants. The discriminating dose was then determined by means of a dose-response experiment that included susceptible checks of both species. The experimental procedure was similar to that used for the screenings except that there were three replicates. The 7 doses of tralkoxydim ranged from 13.3 to 425 g a.i./ha. A log-logistic equation was fitted to the data (Seefeldt *et al.*, 1995). $ED_{95}$ was considered as an adequate herbicide efficacy and the adopted doses were obtained rounding up the $ED_{95}$ (Table 1).

Table 1. Tralkoxydim $ED_{95}$, relative standard error (SE) and greenhouse discriminating doses based on plant survival.

| | $ED_{95}$ (g a.i./ha) | SE | Greenhouse dose (g a.i./ha) |
|---|---|---|---|
| *A. ludoviciana* | 29.8 | 0.61 | 30.0 |
| *P. paradoxa* | 90.3 | 18.00 | 92.5 |

In each screening experiment of *A. ludoviciana*, a susceptible check coming from the same area as the samples was included; in the *P. paradoxa* experiments, it was not possible to find a susceptible population from untreated areas so a susceptible check from Herbiseed (No. 9527 in the 1999-2000 catalogue) was included.

Plants were sprayed using a precision bench sprayer delivering 300 L/ha, at a pressure of 215 kPa, and a speed of about 0.75 m/sec, with a boom equipped with three flat-fan (extended range) hydraulic nozzles (TeeJet, 11002). Plants were treated at two-three leaves, corresponding to growth stage 12 of the Extended BBCH Scale (Hess *et al.*, 1997).
The number of surviving plants was recorded 18-20 days after herbicide application. Plants that showed no active growth, regardless of colour, were considered to be dead. The experiments were carried out during autumn/winter/spring, so light was supplemented using 400 W metal-halide lamps, which provided a Photosynthetic Photon Flux Density (PPFD) of about 150 μmol/$m^2$ s and a 14-hour photoperiod. According to the number of surviving plants, populations were classified into three categories of resistance: RR = >20% survival at dose 1x and >10% at dose 3x; R = >20% survival at dose 1x; SR = survival between 5 and 20% at dose 1x; S = survival <5% at dose 1x.

**Dose-response experiment**

Given the screening results, populations 53, 56 and 54 (susceptible check) of *A. ludoviciana* were tested in an outdoor pot dose-response experiment. Herbicides used were diclofop-methyl (1x: 852 g a.i./ha) and clodinafop-propargyl (1x: 60 g a.i./ha) for populations 54 and 56 only.
Doses ranged from 1/2 to 8 times and from 1/16 to twice the field dose for resistant and susceptible populations, respectively. The number of plants surviving the treatments and shoot fresh weight were recorded 20 days after herbicide treatments. The experimental layout was a completely randomised design with four replicates, each of two pots. A similar procedure to that for the screenings was followed, except that there were five plants per pot. A log-logistic equation was fitted to the data (Seefeldt *et al.*, 1995).

**Field studies - Spray experiments**

During 3 seasons (98-99/99-00/00-01), 7 field trials on durum wheat were done at 2 locations in the Apulia region (loc. Bari 41.1° N - 16.0' E; loc. Foggia 41.6° N - 15.5' E), one infested with resistant populations of *A. ludoviciana* (4 trials) and the other with resistant *P. paradoxa* (3 trials). These 2 sites were chosen according to field history, results of greenhouse experiments, high infestation of resistant species and low pressure of other weeds.
The treatments differed not only by the 2 weed species, but also over the years for the same species. While in the first year all products available on the market with some efficacy against the two weed species were tested (although only the most interesting treatments are presented), in the following years only the more promising products and spray programmes were considered. In 1999 two trials were done on *A. ludoviciana*, one with normal sowing time and the other with false seedbed preparation and sowing delayed by more than a month.
All the trials were conducted according to the manual for field trials in plant protection (Ciba-Geigy, Third edition, 1992). The experimental layout was a completely randomised design with three replicates. Two longitudinal untreated check strips of 1.5 m between the blocks and one transversal check strip of 1.5 m every two plots were included (in this way every plot had at least 2 checks on 2 sides), the plot size was 15 $m^2$ (3 m x 5 m). The applications were made with a portable plot sprayer equipment fitted with a gas cylinder ($CO_2$), a pressure gauge and a 3 m boom. The nozzle type was FLAT-FAN / Teejet 80015, the spray volume was 300 L/ha and the pressure was 250 kPa. Further experimental details are given in Table 2.
The evaluation of crop tolerance was estimated using a percent rating scale from 0 (no effect) to 100 (complete kill). Efficacy was evaluated through a visual estimate of the biomass

reduction of the target weed in the treated plots in relation to the untreated control strip. All the data were statistically evaluated by the Tukey test at p=0.05.

Table 2. Sowing and herbicide application dates of field trials. Location Bari: wheat plus *A. ludoviciana*; location Foggia: wheat plus *P. paradoxa*.

| | | BBCH stage | | 1998-1999 | | 1999-2000 | | 2000-2001 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Crop | Weed | Bari | Foggia | Bari | Foggia | Bari | Foggia |
| Soil tillage | | | | - | - | 6/11<br>22/12 | 18/11 | 11/11 | 11/11 |
| Sowing: | normal | - | - | 7/12 | 16/11 | 14/11 | 2/12 | 25/11 | 14/11 |
| | delayed | - | - | - | - | 23/12 | - | - | - |
| Application : | a: pre-em. | 01 | 01 | 09/12<br>- | 18/11<br>03/02 | 14/11<br>23/12 | 3/12<br>- | 25/11<br>- | 17/11<br>- |
| | b: early-post | 13-21<br>- | 11-21<br>- | 16/02<br>- | 10/03<br>- | 19/01<br>31/1 | 1/2<br>- | 4/01<br>- | 14/01<br>- |
| | c: normal-post | 23-30 | 15-31 | 10/03 | - | 8/03 | 3/3 | 2/03 | 19/02 |
| | d: late-post | 31-35 | 31-35 | 16/04 | | - | - | - | - |

**Real flora**

During the 99-00 and 00-01 seasons the dynamics of emergence of the resistant weeds was determined on the same sites as the field experiments. Six steel rectangular quadrates (12 cm x 50 cm) had been randomly fixed on the ground of each field, along the check strips and across the rows of wheat. New seedlings within each quadrate were counted and removed every 15-20 days starting from mid November (just before usual weed emergence begins) to tillering stage (BBCH-scale 22-28).

## RESULTS AND DISCUSSION

**Pot experiments**

More than 90 populations have been screened and about 25% of these have proved to be resistant to at least one ACCase inhibitor (Table 3). The type of sampling was able to highlight the worst cases where resistance was already well evolved, but probably missed most of the situations where resistance had just begun to develop. The number of new cases seems to be fairly stable and the evolution of resistance in the two species appears, at the moment, to be fairly slow. This is probably due to their reproductive system.

Table 3. Number of populations RR and/or R to at least one herbicide.

| Species | 1998 | 1999 | 2000 | Total no. of screened populations |
|---|---|---|---|---|
| *A. ludoviciana* | 9 | 2 | 5 | 52 |
| *P. paradoxa* | 2 | 2 | 2 | 39 |

Table 4. Results of screenings of the two species. Number of RR, R and SR populations sampled in 1998, 1999 and 2000.

| *Resistance categories* | RR | | | R | | | SR | | |
|---|---|---|---|---|---|---|---|---|---|
| Year | 98 | 99 | 00 | 98 | 99 | 00 | 98 | 99 | 00 |
| *A. ludoviciana* | | | | | | | | | |
| Diclofop-methyl | 8 | 0 | 4 | 1 | 2 | 0 | 2 | 4 | 1 |
| Clodinafop-propargyl | 2 | 0 | 2 | 4 | 1 | 3 | 0 | 0 | 0 |
| Tralkoxydim | - | - | 2 | - | - | 2 | - | - | 1 |
| *P. paradoxa* | | | | | | | | | |
| Diclofop-methyl | 2 | 0 | 1 | 0 | 2 | 0 | 0 | 0 | 0 |
| Clodinafop-propargyl | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| Tralkoxydim | - | - | 0 | - | - | 1 | - | - | 1 |

Altogether, 16 populations of *A. ludoviciana* and 6 of *P. paradoxa* have proved to be resistant to ACCase inhibitors (Table 3). Most of the *A. ludoviciana* populations came from Apulia, but more recently a population from Sicily and one from Basilicata have shown resistance and resistant populations of *P. paradoxa* exist in Apulia, Marche (near Ancona) and south of Maremma (north of Rome). Most of the resistant populations have been proved to be resistant to diclofop-methyl, resulting from the longer use of this herbicide (Table 4). A few populations of both species are cross-resistant to tralkoxydim. Some populations coming from Apulia show a high level of resistance with very few plants killed at dose 3x. This is confirmed by the results of the dose-response experiments on *A. ludoviciana* (Table 5) and by other recent experiments on *P. paradoxa* (Lucchesi & Sattin, unpublished data). The resistant ratios in Table 5, in terms of both survival and fresh weight, for both FOPs are high and support the hypothesis that the resistant mechanism involved might be a target site.

Table 5. Herbicide dose that causes 50% reduction of surviving plants and shoot fresh weight relative to untreated controls ($ED_{50}$ and $GR_{50}$ and their standard error – SE) of ACCase-susceptible (S) and -resistant (R) populations of *A. ludoviciana*.

| Herbicide | Pop. | $ED_{50}$ (g a.i. /ha) | SE | $ED_{50}$ ratio | $GR_{50}$ (g a.i. /ha) | SE | $GR_{50}$ ratio |
|---|---|---|---|---|---|---|---|
| Diclofop-methyl | 54 (S) | 168 | 3.4 | | 233 | 12 | |
| | 56 (R) | 6133 | 638 | 36 | 5294 | 869 | 23 |
| | 53 (R) | >3408 | | >20 | >3408 | | >15 |
| Clodinafop-propargyl | 54 (S) | 7.4 | 0.11 | | 5.6 | 0.05 | |
| | 56 (R) | 152 | 8.0 | 20 | 111 | 19 | 20 |

Field histories highlight that where resistance developed, fields had had at least 5 treatments with ACCase inhibitors. Often, but not always, the cropping system was a wheat monoculture. However, where rotation was adopted, the other crops (i.e. autumn sown sugarbeet, clover) were often treated with an ACCase inhibitor. Until the mid-nineties diclofop was the usual selecting agent while later, fenoxaprop and especially clodinafop were often used.

**Field studies - Actual flora**

The dynamics of weed emergence was similar in the two years for both species (Figure 1),

with a higher no. of seedlings emerged during the second season: 1283±39 seedlings/$m^2$ during the season 00/01 vs. 983±13 seedlings/$m^2$ during 99/00 for *A. ludoviciana*; 1176±36 seedlings/$m^2$ during the season 00/01 vs. 883±25 seedlings/$m^2$ during 99/00 for *P .paradoxa*. The shift of about two weeks between the two years for *A. ludoviciana* is likely to be due to the different rainfall patterns. The peak of emergence of *P. paradoxa* appears to be later than that of *A. ludoviciana*, but more than 70% of the seedlings of both species emerged within a month from the last tillage operation and by the end of December most of the emergence fluxes were over.

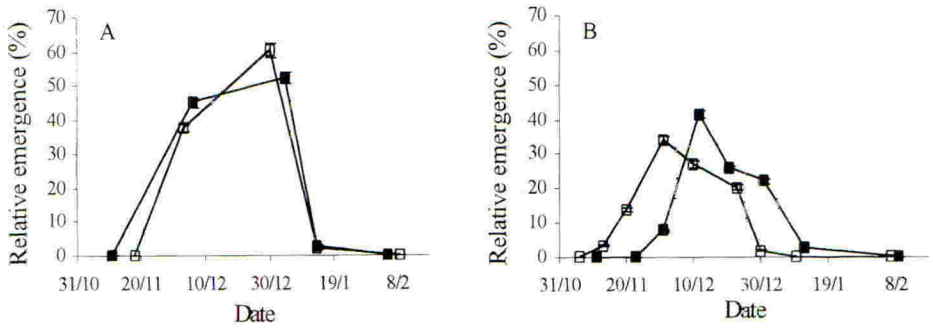


Figure 1. Dynamics of the percentage of weed emergence relative to the total number of emerged seedlings for the seasons 99-00 (□) and 00-01 (■). A = *P. paradoxa* and B = *A. ludoviciana*. Vertical bars represent SE.

**Spray experiments**

The level of ground cover by the resistant *A. ludoviciana* was very high in all three years (on average above 90%), however delaying sowing by more than a month caused a drastic reduction of the weed pressure (13% of ground cover) (Table 6). All treatments with only one product in post-emergence were insufficient, even at four times the maximum label rate (data not shown). All the ACCase inhibitors used alone gave very poor control. Two applications of a single a.i. were always better than one application of two tank-mixed a.i. (some data not shown). The herbicides that gave a significant effect were: imazamethabenz, chlorotoluron and glyphosate. Chlorotoluron showed a very good efficacy, but strongly influenced by the rainfall during the month after application, when there is little rainfall efficacy is lower. When imazamethabenz was included in a spray programme the level of efficacy improved, but without resolving the problem. Adding L-flamprop-isopropyl resulted in a poor incremental control in spray programmes. None of the treatments showed any problem related to crop tolerance. The best results in terms of both efficacy and consistency over the years was obtained by a spray programme including chlorotoluron+glyphosate followed by an early post-emergence treatment with clodinafop+oil. Good results were also obtained with a pre-emergence treatment with chlorotoluron+clodinafop+oil. In terms of weed control strategy, delaying sowing by more than a month gave equal or better results than any spray solution applied in the normal sowing time experiment. Combining delayed sowing with the best spray programmes was therefore the best solution.

Table 6. Efficacy and timing of application (a = pre-em, b = early post-em., c = normal post-em.) of some herbicide treatments against *A. ludoviciana*. Spray programmes are separated by horizontal lines. 99-00ds = delayed sowing. The data relative to the untreated check (in bold) are the percentages of ground cover. Figures without common letters differ significantly according to Tukey's test at p=0.05.

| | Rate a.i./ha | Type of application | | | Relative efficacy (%) | | | |
|---|---|---|---|---|---|---|---|---|
| | | 98-99 | 99-00 | 00-01 | 98-99 | 99-00 | 99-00ds | 00-01 |
| **Untreated** | | | | | **93%** | **98%** | **13%** | **83%** |
| Chlorotoluron | 1750 | a | a | - | 86 ab | 45 c | 70 b | - |
| Chlorotoluron+ Clodinafop+oil | 1750+60+1000* | a | a | a | 96 a | 88 a | 92 a | 48 bc |
| Chlorotoluron | 1750 | - | a | a | - | 75 ab | 85 ab | 75 a |
| Clodinafop + oil | 60+1000* | - | b | b | | | | |
| Glyphosate | 1050 | - | - | a | - | - | - | 65 ab |
| Imazamethabenz | 576 | - | - | b | | | | |
| Glyphosate | 1050 | - | - | a | - | - | - | 72 a |
| Clodinafop + oil | 60+1000* | - | - | b | | | | |
| Chlorotoluron + Glyphosate | 1750+1050 | - | a | a | - | 89 a | 95 a | 82 a |
| Clodinafop + oil | 60+1000* | | b | b | | | | |
| Imazamethabenz | 576 | b | b | b | 78 c | 38 c | 71 b | 79 a |
| Clodinafop + oil | 60+1000* | c | c | c | | | | |
| Chlorotoluron | 1750 | - | a | a | - | 65 b | 77 b | 78 a |
| Imazamethabenz | 576 | - | b | b | | | | |
| Chlorotoluron+Imazamethabenz | 1400+576 | b | - | - | 53 d | - | - | - |
| Chlorotoluron+Imazamethabenz | 1400+576 | b | - | - | 40 d | - | - | - |
| Clodinafop + oil | 60+1000* | c | - | - | | | | |
| Chlorotoluron+Imazamethabenz | 1400+576 | b | - | - | 43 d | - | - | - |
| L-flamprop-isopropyl | 728 | d | - | - | | | | |
| Clodinafop + oil | 60+1000* | b | - | - | 13 e | - | - | - |
| L-flamprop-isopropyl | 728 | d | - | - | | | | |
| Clodinafop + oil | 60+1000* | b | b | b | 25 e | 5 d | 49 cd | 26 c |
| Tralkoxydim + additive | 382+1000* | c | c | - | 0 f | 0 d | 46 d | - |
| Diclofop | 852 | c | c | - | 12 ef | 0 d | 41 d | - |
| Fenoxaprop | 79 | c | c | - | 3 f | 0 d | 40 d | - |
| L-flamprop-isopropyl | 728 | d | - | - | 38 ed | - | - | - |

*= formulated product

The infestation of resistant *P. paradoxa* was also high in all the experiments, with an average ground cover of 86% (Table 7). Only terbutryne applied alone or in mixture with other herbicides gave good efficacy on this resistant weed, although some problems of crop tolerance at the higher herbicide rate may appear. The best solution was a pre-emergence treatment with terbutryne followed by an early post-emergence with clodinafop.

The results show that no single chemical or agronomic treatment resolved the problem, but integrated weed management appears to be the best solution to control the two ACCase resistant grasses. In particular, it appears to be crucial to control, or reduce the impact, of the December emergence flux, in order to reduce the weed pressure. The limit of this technique is its feasibility in the field (because the soil is often too wet in December) and the likely yield reduction due to a shorter crop cycle. A key point would be to be able to rotate both crop and herbicide mode of action.

Table 7. Selectivity and efficacy of herbicide treatments against *P. paradoxa* herbicide. For table legend see table 6.

| Herbicide treatments | Rate a.i./ha | Type of application | | | Crop tolerance (%) | | | Relative efficacy (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 98-99 | 99-00 | 00-01 | 98-99 | 99-00 | 00-01 | 98-99 | 99-00 | 00-01 |
| **Untreated check** | | | | | | | | **88%** | **92%** | **79%** |
| Terbutryne | 1165 | a | a | a | 10 a | 0 b | 10 a | 75 c | 80 b | 83 a |
| Terbutryne | 1631 | a | a | - | 15 a | 10 a | - | 90 ab | 93 a | - |
| Terbutryne | 1165 | a | a | a | 10 a | 0 b | 12 a | 92 ab | 85 b | 93 a |
| Clodinafop + oil | 60+1000* | b | b | b | | | | | | |
| Terbutryne | 1631 | a | a | - | 15 a | 0 b | - | 96 a | 87 ab | - |
| Clodinafop + oil | 60+1000* | b | b | - | | | | | | |
| Terbutryne | 1165 | a | a | a | 10 a | 0 b | 14 a | 88 bc | 88 ab | 92 a |
| Chlortoluron + Chlorsulfuron | 1400+11.25 | b | b | b | | | | | | |
| Terbutryne + Glyphosate | 1165+1050 | - | a | a | - | 0 b | 10 a | - | 92 a | 84 a |
| Terbutryne + Glyphosate | 1165+1050 | - | a | a | - | 0 b | 10 a | - | 94 a | 94 a |
| Clodinafop + oil | 60+1000* | - | b | b | | | | | | |
| Chlortoluron + Chlorsulfuron | 1400+11.25 | - | b | - | - | 0 b | - | - | 32 cd | - |
| Clodinafop + oil | 60+1000* | - | c | - | | | | | | |
| Clodinafop + oil | 60+1000* | b | b | b | 0 b | 0 b | 0 b | 17 ed | 20 cd | 0 c |
| Tralcoxydim + additive | 382+1000* | c | - | - | 0 b | - | - | 0 e | - | - |
| Diclofop | 852 | c | c | - | 0 b | 0 b | - | 0 e | 0 d | - |
| Fenoxaprop | 79 | c | c | - | 0 b | 0 b | - | 0 e | 0 d | - |
| Imazamethabenz+ Clodin. + oil | 576+60 | b | b | b | 0 b | 0 b | 0 b | 50 d | 85 ab | 25 bc |
| Imazamethabenz+ Clodin. + oil | 576+60+1000* | - | b | b | - | 0 b | 0 b | - | 95 a | 45 b |
| Clodinafop + oil | 60+1000* | - | c | c | | | | | | |

* = formulated product


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# Resistance risk analysis – florasulam, a case study

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## ABSTRACT

Resistance risk analysis has been a necessary component of the registration process for new products within the EU since EU Directive 91/414/EEC. Guidance on how to implement the requirements of the directive only became fully available with the publication of EPPO guideline PP 1/213(1). Using florasulam, a broad-leaved weed herbicide for use in cereals as a case study, the paper examines the risk analysis carried out whilst the EPPO guideline was still being developed. The inherent and agronomic risks for the use of florasulam are considered and a risk assessment made. A resistance management strategy is proposed along with an outline of the pre- and post launch monitoring programmes. Finally the paper discusses the difficulties of conducting a risk analysis and the need for further guidance on some aspects of resistance risk analysis for the future.

## INTRODUCTION

European Union Commission Directive 93/71/EEC amending Council Directive 91/414/EEC "concerning the placing of plant protection products on the market" requires that applicants registering plant protection products evaluate the risk of resistance development and propose management strategies to address such risks. At the time Council Directive 91/414/EEC was introduced, guidelines for addressing resistance risk and resistance management did not exist and although Directive 93/71/EEC indicates that, where available, EPPO guidelines should be used to fulfil efficacy data requirements, it was only in 1999 that such guidelines became available (OEPP/EPPO, 1999).

During the period between the introduction of the EU directives and the publication of the EPPO guideline it was difficult for registrants to know exactly what procedures they should follow and what information they should provide. Equally, registration authorities were uncertain what data to demand and how to evaluate a registrants application.

During this time Dow AgroSciences was in the process of developing a new dicotyledon herbicide compound for use in cereals. Primus and Boxer herbicides contain the active ingredient florasulam, a triazolopyrimidine sulfonanalide, acetolactate synthase (ALS) inhibitor, which has activity against a number of key dicotyledon weeds including *Galium aparine*, *Stellaria media*, *Matricaria* sp. and *Papaver rhoeas*. As part of the Dow AgroSciences stewardship programme for new products it was decided that the company should as far as possible follow the EPPO guideline as it went through its various drafts. Also, the general principles of carrying out an assessment of risk based on the

recommendations of the Resistance Action Committees and described by Jutsum *et al.* (1998) would be followed. UK PSD had already developed a number of initiatives (Furk, 2000) and along with other regulatory authorities as members of the EPPO panel designing the guideline, were in a good position to advise on data requirements.

## RESISTANCE RISK

The EPPO standard divides the risk factors contributing to the risk of resistance as falling into two categories – those inherent in the compound and its effects on the pest and those that might result from an agronomic use pattern.

### Inherent risk

The target species for florasulam are a range of dicotyledon weed species that are common in cereal crops throughout Europe, as well as occurring in a wide range of crops in the arable rotation. ALS inhibitors are used in a number of these crops, in particular cereals, and so there is a risk of resistance development in these target species. The first recorded instances of ALS resistance in broad-leaved species were in 1987 in *Kochia scoparia* and *Lactuca serriola* in the U.S. (Heap, 2001). Extensive use of ALS products globally since the 1980's has led to the development of resistance to ALS products in 50 species belonging to 17 different families, however, more importantly the areas affected outside the U.S. are relatively small. Instances of ALS inhibitor resistance to florasulam targeted species have been limited to *S. media* and *P. rhoeas* with no instances in *G. aparine* or *Matricaria* sp. (Table 1.)

Table 1. Instances of ALS resistance in *S. media* and *P. rhoeas*.

| | Country | Year first recorded | No. of sites | Estimated ha infested |
|---|---|---|---|---|
| *S. media* | Canada, Alberta | 1988 | 101-500 | 400-4,000 |
| | Denmark | 1991 | 1 | 1-2 |
| | New Zealand | 1995 | N/r | N/r |
| | Sweden | 1995 | 1 | 1-2 |
| | Ireland | 1996 | N/r | N/r |
| | UK | 2000 | 1 | N/r |
| *P. rhoeas* | Spain[+] | 1993 | 51-100 | 4,000-40,000 |
| | Greece | 1998 | 1 | 4,000-40,000 |
| | Italy | 1998 | 2-5 | 40-200 |

N/r – not recorded + cross resistance to synthetic auxins

The low incidence of resistance to ALS products despite extensive global use suggests a low resistance risk. The target species themselves also show a low predisposition to the development of resistance in general with no cases of resistance to any herbicide recorded for *G. aparine*. Instances of resistance in *P. rhoeas* are confined to those in Table 1. This is similar to *S. media* where the only cases are those in Table 1 and instances of triazine resistance in Germany in 1978 and synthetic auxin resistance in the UK in 1985.

*Matricaria* sp. have only shown resistance to synthetic auxins in France and the UK in 1975 and to triazines in the UK in 1989 (Heap, 2001).

There is only one example of recorded cross resistance within dicotyledon weed species, between ALS inhibitors and other modes of action although this is not clearly defined, as confirmed by laboratory studies. Data generated in 1998 from glasshouse testing of a Spanish biotype of *P. rhoeas* with known resistance to tribenuron-methyl and 2,4-D (Table 2, Spain 1) showed that although sensitivity to florasulam was reduced, cross resistance to other ALS-inhibiting herbicides did not necessarily extend to florasulam. Additionally a further biotype (Spain 2) with resistance to ALS inhibitors only, showed a reduced tolerance to florasulam but not definitive cross-resistance.

Table 2. $GR_{80}$ values (g ai/ha) with 95% confidence limits for control of three *P. rhoeas* biotypes

| | Susceptible | Spain 1 | Spain 2 |
|---|---|---|---|
| Florasulam | 3.7 (2.7-5) | 9.1 (6-13) | 8.3 (4.6-14.7) |
| Metsulfuron-methyl | 4.8 (3-7.6) | >12 | >12 |
| Tribenuron-methyl | 8.9 (5.8-13.6) | >30 | >30 |
| 2,4-D | 361 (266-491) | 1500 (618-3650) | 345 (256-463) |

Susceptible – supplied by Herbiseed Ltd.
Spain 1 – known resistance to tribenuron methyl
Spain 2 – collected from florasulam field trial

Taken as a whole this shows the low propensity for these target species to develop herbicide resistance. This coupled with the low resistance rate for ALS herbicides in general, within Europe, determined that the inherent risk from florasulam and its target species is low. This is a good example of a situation where resistance may have previously occurred in a target species but there continues to be a low risk of resistance development – it depends on an assessment of the complete package of information in the context of the planned use of the herbicide in question.

**Agronomic risk**

Factors considered to increase the risk of resistance development associated with the particular characteristics of the crop, the geographic area in which the product is applied and use pattern, are varied. In relation to the use of florasulam, it is a spring applied product that is recommended for application either as a single maximum dose or as a split application up to the maximum dose. ALS inhibitors are also used in other crops throughout Europe in normal agricultural rotation such as on sugar beet. In addition, cereals can also be grown in a monoculture regime thus increasing the resistance risk as an ALS inhibitor could then be used each year in the rotation. On balance, the risk due to use pattern is not considered to be high, although there may be specific local exceptions such as use in rice monoculture, or in some areas of Spain where no other herbicide with a different mode of action is used.

Other risk factors that were considered as part of the risk analysis appear to have little applicability to florasulam. There is no lack of alternative active substances with at least 10 other classes of compounds with different modes of action available for dicotyledon weed

control in cereals. There are also a number of cultural control measures that can be applied, such as cultivation and utilising stale seed beds.

All these factors show a low risk of resistance development for florasulam. However, even with what might at first sight appear to be a simple case, the complexity of multiple products in the same usage pattern demands a close analysis.

## RESISTANCE RISK MANAGEMENT

Resistance risk management refers to the process of deciding if the risk is acceptable and then, where necessary, applying conditions of use that specifically minimise or delay the appearance of resistance in the field – referred to as modifiers.

As the risk assessment for florasulam was considered low, it was considered that the overall risk was acceptable. Accordingly, strict adherence to the EPPO guideline could at this point remove the need for any modifiers. Even so, registrants, who have invested considerable resource in developing new products want to sustain the return on their investment by prolonging the commercial life of their product. This can be best done by avoiding the development of resistance.

## REGISTRATION REQUIREMENTS

### Resistance Management Strategy

To enable registration authorities to assess the risk of resistance development, a variety of information is to be provided, as laid out in the EPPO guideline. Much of the information centers around subjects already discussed earlier in this paper, such as mode of action, target species, use patterns, etc. Since the overall risk was considered low and therefore acceptable for florasulam, a management strategy is not strictly necessary. However, for the reasons described above and because there is an underlying desire by regulatory authorities to see a resistance management strategy in place from the first registration of a product, the following conditions were applied to florasulam:-

- single application to each crop (although it can be applied as a split dose)
- not used in rotational dicotyledon crops
- only available in mixture with 2,4-D in areas with known *P. rhoeas* resistance to ALS inhibitors – there may be an issue with dual mode of action cross resistance, however, these biotypes have been shown to have no definite cross resistance to florasulam.
- Recommend mixtures/alternations with other compounds exhibiting a different mode of action.

### Baseline Monitoring

It is incumbent on the registrant to establish the baseline sensitivity of key target species. Although this is not necessary to carry out the initial risk assessment, it does enable an understanding of the natural variation in sensitivity. This is critical for use in future monitoring and in conducting the overall risk analysis.

Programmes to establish the baseline sensitivity of the four key target weeds for florasulam were put in place prior to the commercial product launch and are reported elsewhere (Paterson *et al.* 2001). These programmes have established the natural variation in target weed sensitivity to florasulam across key countries in Europe. As expected there is considerable natural variation, of approximately 2-4x, between the most sensitive and the most tolerant biotypes tested. The variability is quite normal but does highlight the difficulty in detecting any subsequent shift in sensitivity which might indicate resistance development. Whilst for target site resistance a significant increase in sensitivity may be seen, if metabolic resistance were to develop, the shift may be more gradual and so much more difficult to detect. There are also practical difficulties in conducting the testing; for example, seed collection of some species and determining the number of sites that should be tested, per species, across Europe.

As part of the ongoing monitoring of the product post launch, product use complaints will also be monitored and reported to regulatory authorities. Where other factors can not adequately explain product failure, a glasshouse study of the sensitivity of the biotype to florasulam will be conducted. Use will also be made of field trials that will continue to be conducted each year to look for shifts in response, however, frequency analysis shows that there is considerable natural variation shown year on year (Fig. 1) which makes the detection of any shift due to resistance development more difficult.

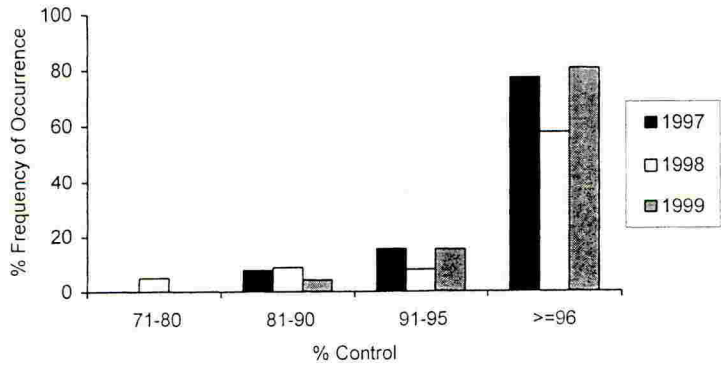


Figure 1. Variability in response of *G. aparine* to florasulam in field trials applied at 5 g ai/ha, 1997-99 (no. trials in EU countries, 1997, 39; 1998, 138; 1999, 92)

## REGISTRATION EVALUATION

Although the final guideline was not available for much of the time that the evaluation took place, the fact that PSD and other country regulatory authorities were aware of, and as members of the EPPO panel had access to, the draft guideline meant they were able to use it as a basis for their evaluation. In most instances, they agreed with the applicants risk analysis and did not impose extra modifiers. They also agreed with the monitoring programme of continued population testing, evaluation of trials and complaint monitoring. However they

did ask for the inclusion of *S. media* and *Matricaria* sp. as extra species beyond what was originally planned, and for annual (UK) or biennial (France) notification of resistance development (even if none has occurred). Overall, early dialogue with the regulatory authorities ensures that the overall risk analysis can be conducted in an accurate and balanced manner.

## SUMMARY

Since the EPPO guideline was published applicants and registration authorities have sought clarification on specific aspects of the guideline. In particular, clarification was sought with respect to the number of species and sample numbers for baseline monitoring, and the definition of what is an acceptable risk.

A workshop involving regulators and applicant companies was held in Poznan followed by a Panel meeting in Paris. Meeting such as these should help to ensure that the guideline remains a vibrant and evolutionary document for use by all EU member states, all 43 member countries of EPPO, and any other countries globally that may choose to use it as a model.

Although the registration of florasulam occurred at a time when the guideline was still being developed, its general principles were followed by the applicant and regulatory authorities. Although florasulam may be considered a relatively simple example for the analysis of resistance risk, it still raised a number of issues that ultimately involve a subjective assessment with few quantitative criteria. It is clear that more complex risk evaluations will pose greater challenges.

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# Baseline sensitivity to herbicides: a guideline to methodologies

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## ABSTRACT

Baseline sensitivity is now specifically referred to in the resistance risk analysis section of the EPPO 'Guideline for the Efficacy Evaluation of Plant Protection Products'. Baseline sensitivity testing is one component of the registration procedure for new active ingredients, but there are widely differing views on how best to satisfy registration requirements. This paper suggests approaches both for species in which resistance has, and has not yet been demonstrated. The approach comprises glasshouse dose response studies in which the new active ingredient is applied to a range of populations of each of the major target weeds, and/or those weeds which are thought to have the greatest innate capacity to develop resistance. An example of a baseline sensitivity study involving response of nine populations of *Stellaria media* to imazamox is presented. The procedures suggested do not constitute an EPPO agreed protocol but should assist registrants in satisfying the requirement for baseline sensitivity data.

## INTRODUCTION

Baseline sensitivity (or background monitoring) is now specifically referred to in the resistance risk analysis section of the European and Mediterranean Plant Protection Organisation's (EPPO) 'Guideline for the Efficacy Evaluation of Plant Protection Products' (EPPO, 1999a, b). This is also available on the EPPO website (www.eppo.org) under 'EPPO standards'. The rationale behind the resistance risk evaluation process, which led to the production of the EPPO guideline, has been described in detail by Leonard (2000), and an overview of EPPO by Roy and Smith (1999). The guideline sets out to communicate to both registration authorities and applicants, what their obligations are with respect to assessing resistance risk and developing appropriate management strategies.

There are widely differing views on the value of baseline sensitivity evaluation for new active ingredients and what information is required. Although it is unlikely that all 43 EPPO member countries, (mainly but not exclusively European), will adopt a unified approach, registrants will be required to adhere to the EPPO Guidelines unless they can give good reasons for not doing so. Consequently, within most member countries there is likely to be a ***requirement*** to submit baseline sensitivity data with submissions for new active ingredients where a risk of resistance has been identified. The situation for re-registration of old active ingredients is less clear, but it is likely that applicants will be required to bring their active ingredient dossiers up to the standard expected for new active ingredients. Data requirements for re-registration are likely to depend on the incidence of resistance that exists at that time.

The aim of this paper is not to present a protocol for generating baseline sensitivity data that will become a requirement for registration purposes, rather to suggest approaches which, in the author's view, are appropriate, balanced and achievable.

## AIM OF TESTING FOR BASELINE SENSITIVITY TO HERBICIDES

Natural genetic variation means that populations of organisms are likely to vary in their sensitivity to toxic substances. This variation may be small or large, and is generally impossible to predict. In relation to weeds, different populations of an individual species may vary in their response to a new herbicide even before it has been used commercially. This variation in response need not necessarily impact on herbicide efficacy in the field, as this will depend on the dose used relative to the intrinsic sensitivity of the weed to the herbicide.

The aim of baseline sensitivity testing is to establish the scale of variation in herbicide response between weed populations, prior to the introduction of the new herbicide. Consequently, any subsequent changes in sensitivity of a weed to the herbicide, after it is introduced commercially, should be detected more reliably. The establishment of a good baseline is particularly important when resistance evolves by a gradual, progressive decrease in population sensitivity, rather than by an increase in the proportion of highly resistant individuals. A good baseline will enable any cases of evolved herbicide resistance to be identified promptly and unequivocally.

### Is there a need for baseline sensitivity testing in weeds where no resistance has ever been demonstrated?

Possibly, depending on the assessment of resistance risk. The aim is not to try to find resistant populations (which is likely to be impossible) but to establish a good, well characterised baseline against which to measure any future changes in sensitivity. The EPPO Guidelines recognise that it would be impractical to generate baseline data for a large number of different weeds listed on a product label. In such cases the data can be limited to the most important weeds, especially those judged to have the greatest capacity to develop resistance.

### What about weeds where resistance is already widespread?

The aim here is rather different, and should be to establish whether the new active ingredient is affected by existing mechanisms of resistance and cross-resistance. This is not easy to predict, as even herbicides active at the same target site will not necessarily all be affected by target site resistance to the same degree. For example, weeds with ALS target site resistance may show resistance to one, or to several of the different classes of ALS inhibitor (e.g. sulfonylureas, imidazolinones, triazolopyrimidines) (Devine & Preston, 2000).

Enhanced metabolism affects herbicides to varying degrees, and this is dependent more on the molecular structure of a compound, and the ease to which it is metabolised, rather than on its mode of action. This mechanism tends to confer a variable degree of cross-resistance to a wide range of different chemical classes. If enhanced metabolism resistance is known to exist, baseline sensitivity testing can be useful in establishing the range of responses that already occur. In some cases it may be possible to predict the vulnerability of a new herbicide to existing resistance mechanisms but in most cases empirical testing will be essential.

## SUGGESTED METHODOLOGY FOR BASELINE SENSITIVITY TESTING

### A: In species in which resistance has <u>not</u> yet been demonstrated

- Conduct dose response studies using the new active ingredient on a *minimum* of 10 populations of each of the major target weeds, and/or those weeds which are considered to have the greatest innate capacity to develop resistance.
- Populations should be representative of typical agricultural situations, rather than from non-agricultural areas, and obtained from a wide geographical area within the country.
- It may be sensible to include some populations from field trial sites where both good and unsatisfactory control has been achieved. The more critical glasshouse comparisons, conducted under standard conditions, will enable a better determination of whether such differences are due to genetic differences (i.e. baseline differences) or to climatic, environmental or other factors, which may warrant further investigation.
- Not all species included on the label will need to be assessed. In some cases it may be best to evaluate a larger number (≈25) of populations of one or two key species, while in other cases a smaller number of populations (≈5) of more species may be preferable. For herbicides active against broad-leaved weeds, chickweed (*Stellaria media)* is considered to be one of the best standard weeds to use, provided it is listed on the herbicide label.
- At least 6 doses, and preferably more (8 - 10), should be used, and usually it will be appropriate to use increments in which each dose is twice the preceding dose in the range. Doses should include those below and above the field recommended rate, although herbicide activity is likely to be greater under glasshouse conditions than in the field. Preliminary tests may be required to determine the most appropriate dose range.
- Studies will normally be conducted in the glasshouse, but outdoor studies in pots/containers with a standard soil would be an alternative, and might permit the use of fewer doses and provide information more relevant to field conditions.
- Herbicidal effects are best recorded as foliage fresh weights once full symptoms have developed, often 2-4 weeks after spraying in glasshouse conditions. Longer times may be necessary with slower acting herbicides. This assessment is more objective than visual assessments alone, which are more subjective. However, visual assessments may be useful if herbicidal symptoms vary between populations.
- Dose response data should be analysed so that $ED_{50}$ values (the dose required to reduce foliage fresh weight by 50%, relative to untreated controls) can be estimated and compared using appropriate statistical techniques.
- If a wide variation in response is obtained, perhaps more than a four-fold difference in $ED_{50}$ values between populations, additional populations should be considered for assay and other more critical test methods considered.
- Seeds of some of the baseline populations *must* be stored and maintained for future use, should concerns about the development of resistance occur after the introduction of the new active ingredient. It is be best to concentrate on maintaining good representative standards, rather than trying to maintain all the baseline populations.

Notes:

1. In the author's opinion, results from field experiments alone do not provide adequate baseline data, unless results are very consistent. So many factors (e.g. soil, climate, spray timing, weed emergence patterns and growth stage, infestation level, crop shielding) can influence herbicide performance in the field that it is impossible to separate the genetic from the other effects on herbicide performance. Results from field trials should be seen as being complementary to, rather than as a substitute for, more detailed appraisals. See approach suggested above.
2. It may be possible to use populations from several countries to obtain baseline data applicable to a wider area. In this case a greater number of populations need to be used, otherwise there is a risk that populations from one country may appear to respond differently to populations from another country simply because the sample size is inadequate. Such a finding might well generate a requirement from the registration bodies for additional data.
3. Obtaining suitable seed samples needs careful planning and the time needed should not be underestimated. Some species are much easier to collect than others, and some are more amenable to glasshouse studies (e.g. seeds which lack dormancy and plants which are easier to establish and maintain).

**B: In species in which resistance <u>has</u> been demonstrated (irrespective of whether resistance is to the same, or different, mode of action as the new active ingredient)**

- The same procedure outlined above should be adopted but using *known resistant and susceptible populations*, rather than a random selection. The aim should be to establish whether existing resistant populations show cross-resistance to the new active ingredient.
- The susceptible standards used must be representative, and not atypically susceptible to herbicides. Use of more than a single susceptible standard is recommended.
- The populations used should include ones with different resistance mechanisms, if more than a single mechanism has been identified. Ideally they will all have been characterised at the biochemical, as well as the whole plant, level.
- If well characterised resistant populations are used, then relatively few populations may be needed in any evaluation (e.g. two susceptible and four resistant populations). If resistance due to enhanced metabolism exists, using several such populations is advisable as the enzyme systems responsible may well differ between populations.
- If no resistance to the new active ingredient is found, then the range of populations can be used as part of a baseline against which to measure any future changes in response, although it would be desirable to test additional, randomly collected populations.
- If any evidence for resistance to the new active ingredient is found in glasshouse experiments, further studies may be justified in order to determine the impact of resistance under field conditions. Such resistance profiling studies may include outdoor container and true field experiments. All these approaches have their own advantages/disadvantages:

    - Glasshouse experiments allow differences in response between many populations to be determined rapidly but the differences found may not relate directly to the field.
    - Outdoor container studies can simulate field conditions and permit comparison of

herbicide performance on several populations under identical soil and climatic conditions. However, unless a series of experiments is conducted, comparisons are made under a single soil/climatic condition. Crop competition can only adequately be simulated in large containers, which limits the number which can managed.

- True field experiments are essential, but it is impossible to entirely separate the effects of climate, environment and resistance on herbicide performance, especially when resistance is partial rather than absolute. Using the same populations (where possible) in each of the experimental situations allows a much more comprehensive appraisal to be made.

## EXAMPLE OF A BASELINE SENSITIVITY STUDY

A glasshouse experiment was conducted at IACR-Rothamsted to determine the response of nine populations of *Stellaria media* (chickweed) to the imidazolinone herbicide imazamox. The populations originated from arable fields over a very wide geographical area within the United Kingdom, from Caithness in the north of Scotland to Hampshire in the south, Hereford in the west to Cambridge in the east of England. In addition two samples from a commercial supplier of weed seeds ('Herbiseeds') were included which originated from fields in southern England and the former Yugoslavia.

The dose responses were similar for all populations (Figure 1). The calculated $ED_{50}$ values (dose required to reduce foliage fresh weight by 50% relative to the untreated controls for the same population) ranged from 5.52 g/ha (Cambridge) to 8.03 g/ha (Leicester). There were no statistically significant differences ($P \leq 0.05$) between $ED_{50}$ values.

It was concluded that this range of populations formed a good UK baseline for response of *Stellaria media* to imazamox against which to measure any future changes. As the responses were similar, there would be little advantage in maintaining seed supplies of all populations. Ensuring continuing availability of perhaps two or three populations should be acceptable.

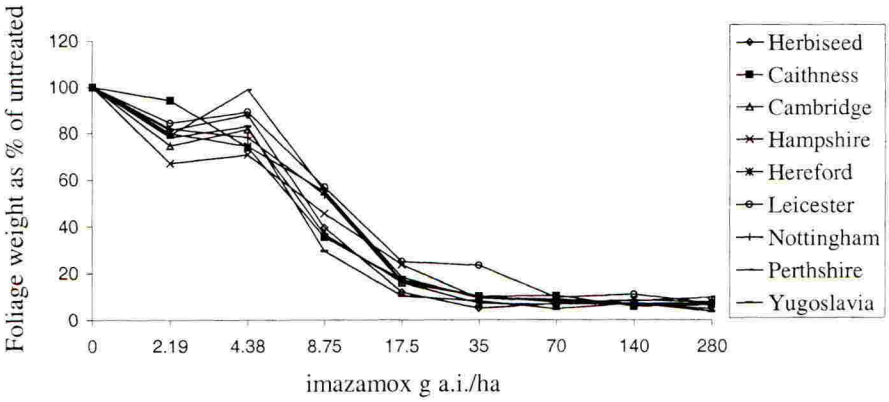


Figure 1. Response of nine *Stellaria media* populations to imazamox (reproduced with permission of BASF plc)

## CONCLUSION

The implementation of EPPO's resistance risk assessment guideline means that companies will increasingly need to analyse resistance risk, and modify use patterns or develop management strategies, at an early stage in product development. Resistance risk evaluation will become an integral part of the registration decision making process (Leonard, 2000). Each member country of EPPO will have to determine the information needed to satisfy that country's registration requirements for new active ingredients.

Baseline sensitivity is only one aspect of resistance risk assessment but has been one of the most controversial aspects due to widely different views on how best to satisfy registration requirements. The procedures suggested in this paper do not constitute an EPPO agreed protocol but will, I hope, assist registrants in satisfying the requirement for baseline sensitivity data. The procedures suggested may need to be modified to meet the specific requirements of different registration authorities.

Baseline sensitivity information is of little value unless an effective monitoring programme is initiated when the new active ingredient is commercialised (EPPO, 1999a, b). Ideally, monitoring will include sampling and testing surviving weeds in representative commercial crops, as well as investigating complaints of poor herbicide efficacy by growers. The prior establishment of a good baseline, and retention of seed samples, should make it easier to positively identify any cases of evolved herbicide resistance.

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