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Physics and Biology of Pesticide Application

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OPERATOR EXPOSURE STUDY WITH DEMETON-S-METHYL (DSM) APPLIED TO CEREALS.

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ABSTRACT

In 1994, Paragon Global Services conducted a GLP operator exposure study on behalf of United Phosphorus Ltd. to determine the dermal and inhalation exposure to, and absorption of, an emulsifiable concentrate formulation containing DSM by agricultural workers during a single ground application to cereals. The main source of potential exposure was on the hands during mixing and loading. The results obtained were much less than the Predictive Operator Exposure Model (POEM).

INTRODUCTION

DSM is a systemic and contact insecticide and acaricide commonly used for the control of aphids and red spider mites on certain agricultural and horticultural crops. Ground application to cereals is thought to be the most representative use pattern and the most appropriate for regulatory review as stipulated by the PSD.

Following assessment of operator risk by the UK Subcommittee on Pesticides and the Advisory Committee on Pesticides a recommendation for an operator exposure study was agreed by the six government departments responsible for pesticides. Using the Predictive Operator Exposure Model (POEM) (MAFF/BAA, 1986; Martin, 1990 and Hamey, 1992) to estimate the potential exposure to the approved formulation of DSM the Pesticide Safety Directorate (PSD) considered the Toxicity Exposure Ratio (TER) to be on the limit of acceptability, vindicating the requirement for operator exposure data (S. Norman, personal communication). The results from this study will show the exposure distribution and a comparison will be made with POEM.

MATERIALS AND METHODS

The study design was based on the U.S. E.P.A. Pesticide Assessment Guidelines, subdivision U, 1986 and GIFAP Technical Monograph No. 14, April 1990 - Monitoring Studies in the Assessment of Field Worker Exposure to Pesticides. In addition, detailed discussions were held with the PSD during protocol development.

Application

Six test subjects were used to monitor the exposure during mixing and loading, application and equipment cleaning during a typical working day using a hydraulic boom and closed cab tractor. An application time of 5.0 to 5.7 hours for each test subject was used and thought to be representative of normal practices from a survey conducted by the National Association of Agricultural Contractors.

Each test subject wore protective clothing according to the test chemical label as follows: coverall (all activities), protective gloves (all activities), faceshield (mixing only) and rubber boots (all activities). In addition, each test subject wore long sleeved cotton vests and briefs to help monitor the efficiency of the protective coveralls.

DSM was applied at a rate of 120-123g a.i./ha in 206-211 litres water/ha. Prior to each application, the sprayer output and tractor speed were calibrated.

During the application of each spray tank, samples of spray were taken from the nozzles of the spray boom to demonstrate achieved concentration and homogeneity of the tank mixes.

A computerised weather station was erected at each trial location to monitor wind speed, wind direction, humidity, air temperature and rainfall every 15 minutes during the application day.

Exposure sampling

All samples (except blood, urine and handwash) were placed in plastic bags and subsequently into residue bags prior to being immediately placed on dry ice in the field.

Subsequent to the application of the final spray tank and again after the equipment cleaning procedure, the coverall and undergarments were cut from each test subject. The coverall was sectioned into two arms, two legs, trunk and hood. In order to monitor the exposure during the separate activities of mixing and loading, application and equipment cleaning, both gloves were sampled upon completion of each activity by each test subject. Handwash samples were also taken after each activity, which involved each test subject washing his hands with 1 litre of water and a bar of soap. The rinsings were captured and extracted immediately in the field in a 1 litre separating funnel with 2 x 50ml dichloromethane and 10g sodium chloride.

Potential inhalation exposure was monitored by attaching a Casella AFC 123 personal air sampler to the neck of the coverall for each test subject (fitted with a Whatman® GF/A 3.7 cm glass microfibre filter). The air sampler was calibrated to give a flow rate of 2.0 l/min and switched on prior to the initial mixing/loading activity. The sampling matrix was removed after the equipment cleaning.

Biological monitoring was undertaken for each test subject by taking blood and urine samples. Blood samples were typically taken up to seven days prior to application, one day after application and three days after application. At each occasion both plasma cholinesterase and erythrocyte cholinesterase activities were measured. 24-hour urine samples were taken one day prior to application, the day of application and one, two and three days after application. The total volume of urine collected for each 24-hour period was recorded prior to subsampling. The urine was analysed for demeton-S-methyl sulphone and demeton-S-methyl sulphoxide metabolites of DSM.

Field spike sampling

Field spike samples were generated to assess the stability of the test chemical during the sampling day, shipment to the analytical laboratory and storage prior to analysis. Each matrix type was fortified with an acetone standard at three levels bracketing the anticipated exposure level (Table 1). Two sets of coverall, undergarment and glove field spikes were prepared. One of these sets was placed on dry ice as soon as possible in the field and the other set was left at ambient conditions for a period equivalent to the exposure period. One set of air filter field spikes was prepared and left at ambient conditions for a period equivalent to the exposure period before being placed on dry ice in the field. One set of urine field spikes was prepared and placed on dry ice as soon as possible in the field. One set of handwash field spikes was prepared at the laboratory by fortifying a previously prepared handwash solution and freezing as soon as possible.

With the exception of the handwash (which was prepared at the analytical laboratory), all the field spike samples were shipped and stored in the same manner and conditions as the exposure samples.

Table 1 Fortification levels for field spikes

Matrix	Fortification Level (mg a.i.)			
	Control	1	2	3
Coverall	0	0.05	0.5	5.0
Undergarments	0	0.005	0.05	0.5
Gloves	0	0.2	2.0	10.0
Handwash	0	0.02	0.2	2.0
Air Filter	0	0.005	0.05	0.5
Urine	0	0.00375	0.0375	0.375

Analytical method

The analytical method used was based upon the procedure described in method S16 of the DFG Manual of Pesticide Residue Analysis Volume 1 (1987). The method consisted of solvent extraction, oxidation and solvent partition, prior to concentration and analysis by GC with NPD detection. The analytical results were expressed as demeton-S-methyl sulphone.

Equipment and materials

DB Wax GC column, 15m x 0.53mm, 0.5µm film thickness (J & W Scientific)
SPB-35 column, 30m x 0.25mm, 0.25µm film thickness (Supelco Inc.)
HP5890 gas chromatograph fitted with a nitrogen-phosphorus detector and
HP7673 autosampler (Hewlett Packard, Wokingham, Berkshire)

Kontron MT2 data station for data collection and analyses (Kontron Instruments, Watford, Herts.).

Calibration curve

A calibration curve was prepared covering the range 0.5-30 μg/ml and chromatographed. There was a linear relationship between peak height and demeton-S-methyl sulphone concentration with a correlation coefficient of 0.9976. The limit of quantitation was set at 0.5 μg/ml, the lowest point on the calibration curve.

Method recovery

Coveralls, undergarments, gloves, air filters and handwash samples were fortified in triplicate at the same concentrations as the field spike samples. The mean recoveries were 87.0%, 87.9%, 54.4%, 90.9% and 51.4% respectively. The metabolites provided for urine analysis proved to be impure and thus not suitable for quantitative analysis. Qualitative validation was performed in order to demonstrate that the compounds could be recovered from urine and that they could be detected at the level specified in the protocol (nominally 0.075 μg/ml).

RESULTS AND DISCUSSION

Analysis of results indicated that the main source of potential exposure was on the hands during the mixing and loading activity. Table 2 indicates the main areas for potential exposure during a single day's application activities and how they compare with POEM. The results are expressed as the mean exposure for all six test subjects.

< 1% of the total demeton-S-methyl sulphone residues on the gloves was found in the handwash after the mixing and loading and application procedures (corrected for method recovery). Approximately 20% of the total demeton-S-methyl sulphone residues on the coveralls was found on the undergarments.

No demeton-S-methyl sulphone or demeton-S-methyl sulphoxide was found in the urine samples.

No effects of exposure to DSM were detected in either the plasma or erythrocyte cholinesterase activity of any of the test subjects.

The results from this study show a much reduced exposure on the protective clothing than that predicted by POEM (approximately 100 fold, including corrections for method recovery). The distribution of exposure throughout the whole day also differs from POEM, as shown in Table 2.

The majority of the field spike samples gave variable and generally low recoveries. This is probably the result of the physical properties of DSM. DSM is volatile and does not freeze at -20°C, the temperature at which the samples and field spikes were stored. The low recoveries may have been enhanced by the use of acetone for preparation of the spiking solutions. It is possible that the DSM residues incurred on the clothing may have been more stable than the field spikes due to the xylene formulation of the test material. However, preliminary stability tests performed with DSM prepared in xylene still indicated a stability problem (approximately 50% of the spiked DSM was lost after 3 weeks storage at -20°C). The volatility of DSM may account for some of the discrepancy between POEM and the results obtained in this study for exposure on the protective clothing.

Table 2 Distribution of total exposure corrected for method recovery.

Matrix	Activity	Actual Exposure (% of total)	Actual Exposure (µg)	POEM Prediction (% of total)	POEM Prediction (mg)
Gloves	Mixing/Loading	84.7	732	65	63.8
Gloves	Application	10.9	94	23	22.6
Gloves	Equipment Cleaning	2.0	17	-	
Coverall (arms)	Mixing/Loading/Application	1.2(a)	10	12(a)	12.18
Coverall (legs)	Mixing/Loading/Application	0.8(a)	7	-	
Coverall (legs)	Equipment Cleaning	<0.1	<1	-	
Coverall (hood)	Equipment Cleaning	<0.1	<1	-	
Coverall (arms)	Equipment Cleaning	<0.1	<1	-	
Coverall (hood)	Mixing/Loading/Application	<0.1(a)	<1	-	
Coverall (trunk)	Equipment Cleaning	Not Detected	Not Detected	-	
Coverall (trunk)*	Mixing/Loading/Application	Not Detected(a)	Not Detected	-	
Air Filter	All activities	Not Detected	Not Detected	-	

* Results obtained from 3 test subjects only.

(a) All results combined to compare with POEM.

CONCLUSION

The results indicate that the main source of potential exposure during a single days application activities occurs on the hands during mixing and loading and to a lesser extent during application. However, with suitable protective gloves, the actual exposure to the skin is negligible. Some potential exposure occurs on the legs and arms during the mixing and loading and application. It is thought that the exposure on the legs is due to the operator walking through the contaminated crop during the spraying activity (e.g. opening the spray boom, removing debris, etc.). Suitable protective coveralls will reduce the exposure on the undergarments. The air filter samples indicate that exposure via the inhalation route is not a major concern, however, this may be due to the volatility of the test material and the nature of sampling matrix.

Biological monitoring did not detect any effects of exposure to DSM on plasma or erythrocyte cholinesterase. No urinary metabolites of DSM were found in the urine.

Despite the questionable field spike samples, it is clear that if used according to the test chemical label, there is negligible risk to the operator during a single days application activities with DSM.

The residue data in this study have been both difficult to interpret (not supported by field recoveries) and to compare with POEM. This has emphasised that when dealing with volatile materials consideration should be given to dispensing with residue analysis and concentrating on the more meaningful biological monitoring. In all cases it is imperative that time be allocated to conduct stability work on the matrices of interest prior to conducting any field work.

REFERENCES

DFG, Deutsche Forschungsgemeinschaft (1987). *Manual of Pesticide Residue Analysis*, Volume **1**, S16.

GIFAP, International Group of National Associations of Manufacturers of Agrochemical Products (1990). Monitoring Studies in the Assessment of Field Worker Exposure to Pesticides. Technical Monograph No. 14. Brussels, Belgium.

Hamey, P Y (1992). Pesticides Safety Directorate UK Predictive Operator Exposure Model (POEM): A User's Guide V1.0 Pesticides Safety Directorate.

MAFF/BAA, Ministry of Agriculture, Fisheries and Food/British Agrochemical Association (1986). UK Predictive Operator Exposure Model (POEM): Estimation of Exposure and Absorption of Pesticides by Spray Operators. SC8001.

Martin, A D (1990). A Predictive Model for the Assessment of Dermal Exposure to Pesticides, Proceedings of Prediction of Percutaneous Penetration, Ed Scott R. C., Guy R., and Hadcraft J., pub, IBC Technical Services London.

U.S. Environmental Protection Agency (1986). Pesticide Assessment Guidelines, Subdivision U, Applicator Exposure Monitoring. Washington DC.

POTENTIAL OPERATOR EXPOSURE TO HERBICIDES: A COMPARISON BETWEEN KNAPSACK AND CDA HAND SPRAYERS

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ABSTRACT

Spray treatments with low volume CDA hand sprayers generally gave lower levels of operator contamination than high volume treatments with knapsack sprayers. The majority of contamination (80 -95%) occurs on the lower leg and feet irrespective of sprayer type. There is considerable scope to greatly reduce contamination with all sprayer types by changing spray practices and avoiding holding the spray lance in front of the operator. Use of a spray management valve (SMV) with the knapsack sprayer also reduced operator contamination.

INTRODUCTION

Chemical weed control with manually carried sprayers is practised throughout the world in a variety of crop and non-crop situations. In developed agriculture manually carried sprayers are often used around field margins, for spot treatments or general use on smallholdings. They are also widely used in the industrial and amenity sector. In many other parts of the world where agriculture is still labour intensive and non-mechanised, manually carried sprayers are frequently the sole means of applying herbicides. The use of herbicides in these situations is increasing as the time and effort involved in hand weeding has often been identified as a major constraint to agricultural productivity (Matthews and Thornhill, 1993).

The conventional lever operated knapsack (LOK) sprayer with hand lance is the most widely used sprayer for this purpose, although the necessity to fetch and carry large volumes of water for spraying is itself often time consuming and laborious. In some circumstances, particularly where water is scarce, low volume Controlled Droplet Application (CDA) sprayers have been used as an alternative. These sprayers use a spinning disc to control droplet size and reduce drift. Application volumes are typically 10-30l/ha offering significant logistical advantages over conventional spraying. Normally a more concentrated spray mix is used with low volume CDA treatments therefore comparisons were made with a conventional sprayer to assess the levels of operator contamination with each system. There are four potential sources of operator contamination during the spraying process:-

- Contact during mixing and filling
- Contact with airborne spray material
- Contact with treated vegetation
- Contact with leaking or contaminated sprayer parts

The objective of these trials was primarily to examine the levels of contamination occurring with each sprayer type from contact with airborne spray material during actual spraying. Spray operators were dressed in disposable 'Tyvec' spray suits and gloves and provided with face masks. A fluorescent tracer dye, sodium fluorescein, was added to the spray solution which could be recovered from suits, gloves and mask filters. The levels of contamination on various parts of the body could then be quantified with the aid of a spectrofluorimeter. The methods used were similar to those described by Merrit (1989). An attempt was also made to examine contamination occurring during the mixing and filling process. It was considered difficult to quantify the levels of contamination due to contact with vegetation or contaminated sprayer surfaces as these are largely dependent on the particular situation/sprayer under investigation. It was hoped, however, to obtain an indication of the degree of risk to operators when using herbicides with both conventional and CDA hand sprayers and suggest what measures may be most appropriate to minimise this.

MATERIALS AND METHODS

Sprayers

For conventional herbicide applications a Cooper Pegler 'CP3' knapsack sprayer was used fitted with a Lurmark 'An 2.0' flat fan deflector nozzle. Applications were made both with and without a Spray Management Valve (SMV) from Fluid Technology, set to regulate the pressure at the nozzle to 1 bar (100 kPa). As frequently, in practise, the same sprayer and nozzle are used for both herbicide and insecticide applications some spray treatments were also made with a hollow cone nozzle (Lurmark DC05/CR-45) without SMV.

Two types of low volume CDA sprayer were also evaluated: firstly a Micron 'Microfit Herbi' sprayer which produces a circular pattern of spray droplets, around 250µm in diameter in a 1.2m band, and secondly a Micron 'Microfit Herbaflex' producing droplets, around 200µm in diameter, which are directed in a fan pattern towards the ground for narrow band treatments. Both sprayers are normally held with the spray lance in front of the operator although they can also be held to the side or rear.

Field Methodology

The trial site was a relatively large open area of short grass cover around 5cm in height. Plot sizes measured 30m x 30m separated by a distance of 20m. Consecutive spray passes were made in parallel tracks across each plot with the spray released approximately 50cm and 20cm from the ground with the LOK and CDA sprayers respectively. Flow rates for the LOK sprayer were 1.1 l/min (with SMV), 1.3 l/min (without SMV) and 0.6 l/min with the hollow cone nozzle. With the 'Herbi' and 'Herbaflex' sprayers flow rates were around 0.08 l/min and 0.04 l/min respectively. Five replicates were normally made with each sprayer configuration. During spray treatments the wind speed and direction was recorded (2m above ground) with a portable field station (Vector Instruments) positioed in the middle of two adjacent plots. Temperature and humidity were also recorded and the total volume of spray material applied measured. Temperatures were around 19 -23^0 C with windspeeds between 0.2 and 1.2 m/sec which varied from perpendicular to near parallel to the direction of travel.

Dye solutions of sodium fluorescein were usually prepared on the same day as the spray treatments. High volume applications with the LOK sprayer used a concentration of 0.5 - 1.0 g/l of water with 0.1% 'Agral 90' surfactant. Applications at low volumes with the CDA sprayers used a dye concentration of 5-10 g/l. Spray treatments took around 5-10 minutes for each replicate and thereafter any spray deposit was allowed to dry on the 'Tyvec' suits before these were cut into sections and placed in labelled polythene bags. Samples were then stored in black plastic bags to minimise degradation of the fluorescein tracer by sunlight. A sample of spray liquid was also taken from each sprayer and, with a micropipette, 100µl of spray solution transferred onto an unsprayed piece of suit section. This 'reference' sample was then left in daylight for 10 minutes to dry before being placed into labelled bags with the other sample materials. These 'reference' samples were subsequently used to prepare the 'known standard' dye solutions for calibration of the fluorimeter.

In trials to examine contamination during mixing and filling, four operators dressed in 'Tyvec' suits with gloves and respirators, performed a standard routine using a 'concentrate' solution (10 g/l) of sodium fluorescein dye in water. The 'concentrate was supplied in a 5 litre 'Plysu Multigaurd' container and operators required to measure out 500ml of 'concentrate' and transfer this to the sprayer tank/bottle adding water as necessary. Tank lids or spray bottles were secured and the sprayers positioned ready for spraying. This routine was repeated ten times by each operator before the suits, gloves and mask filters were removed for analysis.

<u>Laboratory Methodology</u>

One litre of water containing 0.1% 'Agral 90' surfactant and 0.02 M NaOH solution was added to each plastic bag containing the suit section, gloves or mask filters to extract the tracer material. Samples were shaken and left to stand for a period of 1 hour being agitated routinely throughout. A sample of each solution was then transferred into a cuvette from which a reading could be taken with the spectrofluorimeter (Sequoia Turner model 450) to determine the concentration of tracer recovered. For both the LOK and CDA sprayers a calibration curve was plotted using known dilutions of the spray mix and thereafter any corrections to the readings made accordingly.

RESULTS

Results for operator contamination are expressed as the mean amount of spray material recovered from the various suit sections in µl per litre of spray applied. Expressing the contamination levels as a proportion of the spray applied allows for a direct comparison between sprayers irrespective of differences in volumes applied (refer to Table 1). From this an estimate of the quantity of active ingredient deposited can be calculated for a particular dosage rate usually expressed as mg/ha treated (refer to Figure 1 for a comparative example).

Results indicated that with all sprayers the majority of contamination occurs on the operators feet and lower leg (below the knee). Unfortunately measurements of contamination of the feet proved unreliable as spray deposits were brushed off the 'Tyvec' boots by the grass. With the LOK sprayer and deflector nozzle, around 80% of contamination occurred on the lower leg and 16% on the thighs. Some contamination was also found on the left hand which held the spray lance. The LOK sprayer with hollow cone nozzle provided some of the highest deposits on the lower leg but less on the thighs in comparison to the LOK with standard deflector nozzle. Otherwise the two treatments were comparable. Using the Spray Management Valve (SMV) reduced operator contamination. Spray treatments with the 'Herbi' sprayer held to the front provided significantly lower contamination levels than either the standard LOK with deflector or hollow cone nozzle, confirming earlier studies by de la Fuente (1991), but gave comparable results to those with the LOK fitted with SMV. The 'Herbaflex' gave the lowest levels of contamination of all treatments where the spray head/lance was held in front of the operator. Contamination was again largely confined to the lower leg. Where the 'Herbi' spray head was held to the side or rear this virtually eliminated any contamination with a 50 fold reduction in contamination of the lower leg. For all treatments spray deposits on the upper torso were negligible, as was the inhalable fraction of the spray which was at the limits of detection using this methodology. An ANOVA test confirmed highly significant differences were found between sprayers ($p<0.001$) and within different body areas ($p<0.001$).

Table 1. Operator contamination on different parts of the body * (µl/litre applied)

	Suit Section (area in cm^2)											
	Hood (1200)	Mask (172)	Ftorso (6250)	Rtorso (6250)	R arm (1350)	L arm (1350)	Glove (900)	R thigh (1900)	L thigh (1900)	R Leg (1250)	L Leg (1250	Total (23772)
LOK (F)												
mean	0.65	0.03	7.09	3.39	2.41	2.48	2.79	54.70	33.35	206.04	227.19	540.12
std deviation.	0.50	0.03	12.64	3.38	2.32	3.73	3.69	94.26	48.83	65.29	70.79	294.32
LOK SMV (F)												
mean	0.16	0.02	0.32	0.32	0.23	0.23	0.52	1.66	4.62	87.42	99.55	195.05
std deviation.	0.10	0.01	0.09	0.17	0.04	0.08	0.40	0.99	5.71	39.78	28.19	60.00
LOK HC (F)												
mean	0.26	0.05	1.60	1.02	0.30	0.39	0.80	4.60	2.55	175.18	294.60	481.35
std deviation.	0.07	0.06	0.78	0.69	0.09	0.20	0.24	2.58	1.29	102.51	167.36	267.77
HERBI (F)												
mean	0.71	0.03	3.40	0.49	0.69	0.96	1.48	1.74	1.79	77.38	86.63	175.62
std deviation.	0.45	0.04	1.76	0.41	0.66	0.41	1.29	0.91	0.91	86.06	54.26	140.98
HERBI (S)												
mean	0.24	0.00	0.84	0.11	0.44	0.06	0.86	0.26	0.62	0.68	0.50	4.68
std deviation.	0.19	0.00	0.50	0.11	0.27	0.11	0.44	0.33	0.41	0.63	0.86	2.25
HERBI (R)												
mean	0.00	0.00	0.77	0.00	0.00	0.97	0.58	0.19	0.19	3.95	5.17	11.82
HFLEX (F)												
mean	0.00	0.00	0.00	0.08	0.00	0.00	0.00	0.00	0.26	78.34	44.04	122.72

* mean of 5 replicates except for Herbi (R) and Herbaflex (F) with only 2 replicates each.

Key:		
	LOK	Lever operated knapsack with deflector nozzle
	LOK SMV	Lever operated knapsack with deflector nozzle and SMV set for 1 bar.
	HERBI (F) (S) (R)	Herbi with spray head held to the front (F), side (S) or rear (R)
	HFLEX (F)	Herbaflex held with spray head to the front
	LOK (HC)	Lever operated knapsack with hollow cone nozzle at around 2 bar

The results for contamination levels during the mixing and filling process are given in Table 2. These represent the mean contamination levels from four different operators performing the same mixing routine ten times expressed as µl/litre of 'concentrate'. On this occasion only one example of each sprayer type was examined as the mixing process is similar for the different knapsack configurations and similar for both the 'Herbi' and 'Herbaflex'. These results suggested that the gloves received the highest contamination during mixing and filling accounting for around 30% and 64% of the total contamination for the LOK and CDA sprayers respectively. Contamination levels were, however, much lower than reported elsewhere (Craig and Mbevi, 1993) and it is likely that this methodology underestimates contact with herbicides during mixing and filling. These tests were performed under laboratory conditions which is unlikely to accurately reflect the field situation.

Figure 1. Contamination on various parts of the body expressed as mg a.i./ha treated. (Assumes a dose rate of 500g a.i./ha)

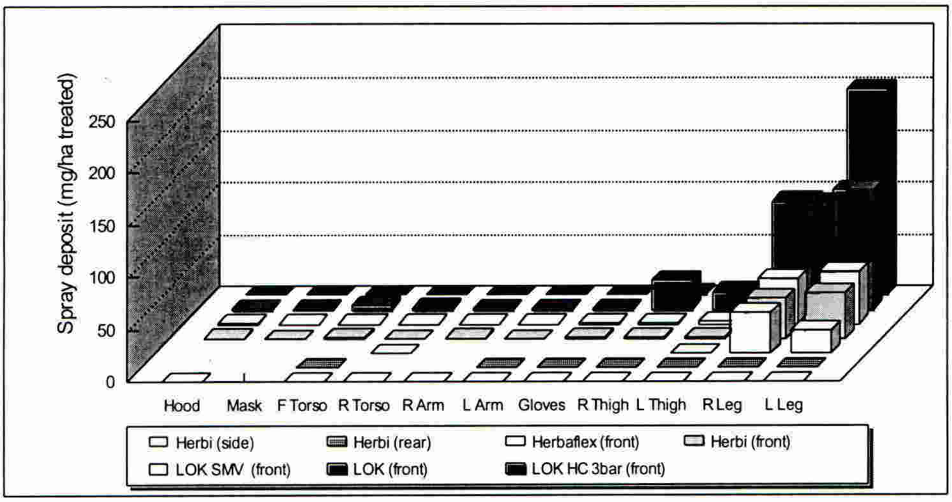

Table 2 Operator contamination during mixing and filling (µl/litre applied).

Sprayer	Hood	F Torso	R Torso	R Arm	L Arm	Gloves	R Thigh	L Thigh	R Leg	L Leg	Total
L.O.K. mean	0.22	3.98	2.74	1.34	1.4	8.52	2.58	3.34	3.24	1.92	29.28
HERBI mean	0.00	2.21	0.14	1.72	1.4	24.06	1.24	0.54	1.3	2.78	35.79

DISCUSSION

The contamination levels found in these trials represent the potential dermal exposure, as defined by Chester (1993), to herbicides by spray operators. Whilst these levels are unlikely to present any risk of acute dermal toxicity there is the possibility of some chronic effects such as skin irritation and sores with certain herbicides if spray treatments are made in bare feet or short trousers and operators do not wear gloves or wash their hands after handling the concentrate. Obviously such malpractices are to be discouraged but can and do occur in some situations where spray operators have not been trained, are unsupervised or do not have access to proper footwear or gloves. Training both by local extension services and agrochemical suppliers together with clear label instructions provide one of the most effective means of combating misuse of pesticides. Improved packaging and formulations can also reduce the risks to operators as well as encouraging the use of safer less hazardous products or actively prohibiting the use of some products. Where CDA sprayers have been used in small scale tropical agriculture these have generally been introduced through local extension services, agrochemical suppliers or into managed plantation estates, in part due to the requirement to train users in the use of such techniques. These types of applicator are also not recommended for use with toxic products, such as paraquat, at concentrations higher than those recommended on the label.

Irrespective of sprayer type, where boots, long trousers and gloves are worn there is likely to be little risk to spray operators when applying herbicides from contact with airborne spray material. The majority of contamination during herbicide treatments occurs due to the operator holding the spray lance to the front whilst walking forward and therefore deposits either impinge directly on the legs and feet or are transferred from the treated vegetation. Holding the spray head to the side of the operator can therefore significantly reduce any contamination simply by not walking through the area being sprayed. In these trials, the use of a spray management valve (SMV) also reduced the levels of contamination from the knapsack sprayer presumably by avoiding higher pressures during pumping and thereby the creation of smaller droplets which are more prone to displacement by the wind.

Experiments to evaluate the levels of contamination which may occur during mixing and filling were inconclusive due to the difficulty of simulating essentially chance accidents.

In these trials there was no attempt to assess the contamination occurring from leaking sprayers or contact with contaminated surfaces. Often poor quality materials or inappropriate designs can lead to significant operator contamination. One example is manufacturers omitting tank lid seals and non return air bleed valves for reasons of cost, which can lead to leakage on the operators back. Such a source of contamination should not be overlooked and may often exceed any contamination occurring during actual spraying (Turnbull 1985). Similarly transfer of spray deposits from treated vegetation can be a significant source and will largely be dependent on the height of the vegetation. Holding the spray head/nozzle as low as possible to the ground will reduce operator contamination although this may not always be possible in tall weeds and therefore extra care is required in such situations.

CONCLUSIONS

It can be concluded that with the CDA sprayers examined there was no increased risk to spray operators due to the higher concentration of active ingredient in the spray mix and in these trials the levels of contamination were lower than standard practices with the LOK sprayer. The majority of contamination will occur on the feet and lower legs irrespective of which type of sprayer is used when spraying in front of the operator therefore adequate footwear, long trousers and gloves are essential for safe application. There is considerable scope for reducing operator contamination simply by changing spray practices by holding the spray lance to the side or rear where possible.

REFERENCES

Chester, G (1993) Operator exposure to pesticides. In. *Application Technology for Crop Protection*, G. A. Matthews and E.C. Hislop (eds), CAB International, pp 123-141

Craig, I and Mbevi, C (1993) Hand contamination received by spray operators during pesticide preparation. *Pesticides News* 19, pp. 3-5.

de la Fuente, K H. (1991) A study of the effect of ambient wind conditions on the deposit patterns and contamination of operators using hand held and knapsack sprayers. MSc. thesis in Agrochemical application technology, Silsoe college, Bedfordshire.

Matthews, G A; Thornhill, E W. (1993) Herbicide applications: Equipment design for small-scale farmers. BCPC, Conf. Weeds. pp1171 - 1176

Merrit, C R (1989) Evaluation of operator exposure and spray displacement with hand operated herbicide application systems. BCPC conf. Weeds, pp623-630

Turnbull C J; Sanderson, D M; Crome, S J; (1985) Exposure to pesticides during application. In. *Occupational hazards of pesticide use*, C J Turnbull (ed) Taylor Francis, London.

OPTIONS FOR REDUCED VOLUME "COARSE" DROPLET SPRAYING

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ABSTRACT

Reduced volume spraying of crops has logistic advantages which should translate into more timely pesticide applications, facilitating dose reductions. Small flow rate hydraulic pressure nozzles generally produce many drift-prone small droplets making such applications dangerous. This preliminary study therefore examines spray retention of fluorescein on young wheat and rape plants using a variety of nozzles delivering *ca* 75 l/ha as fine to coarse sprays, compares their drift potential and attempts a correlation with the spray spectra produced. Results obtained suggest that a new type of anvil nozzle has the potential to safely achieve the objectives of even distribution and retention comparable to that from a standard fine nozzle. Rebound of large droplets from foliage was reduced substantially by inclusion of polymeric adjuvants in the spray solution.

INTRODUCTION

Many farmers apply near recommended doses of pesticides to crops in spray volumes of about 200 l/ha using hydraulic pressure nozzles. Biological efficacy is usually good, but the potential to increase efficiency by reducing doses in such applications is often limited by sub-optimal timing, due to the need to work safely in favourable weather. Increased work rates associated with the application of reduced spray volumes is therefore an attractive option for improving timing and efficiency. Fine quality sprays (Doble *et al.*, 1985) are often used in reduced volume applications to maintain droplet numbers and to provide good deposition on targets. However, safety is prejudiced if the nozzles produce a large proportion of small drift-prone droplets. Thus, spraying is often a compromise between the conflicting requirements of safety and efficiency. Our recent work has examined methods for improving the safety and efficiency of reduced volume, fine sprays using air-assistance (Hislop *et al.*, 1993; 1995) and electrostatic charging (Western *et al.*, 1994). Herein, we report preliminary experiments to determine the advantages and disadvantages of an unconventional approach to reduced volume spraying using coarse droplets and spray adjuvants.

MATERIALS AND METHODS

All nozzles used in this work (Table 1) originated from Spraying Systems Co., Wheaton, Illinois, USA. They were nominally 110 degree flat fan atomisers and all were operated at an appropriate pressure to deliver 0.45 l/min per nozzle. Extended pressure range (XR) 11001 and 015 nozzles, classified as producing fine sprays, and a Drift Guard (DG) 110015 pre-orifice nozzle producing a medium/coarse quality spray, served as standards. Newly introduced Turbo Teejet (TT) anvil-type nozzles 11001, 015 and 02 sizes, nominally producing sprays of medium, coarse and very coarse classification, respectively, (manufacturer's information) were used for comparison.

The droplet sizes produced by the nozzles spraying 0.45 l/min of 0.1% aqueous Agral (Zeneca plc) were measured with a phase/doppler particle analyser (Aerometrics Inc.,Sunnyvale, California, USA). Sprays were sampled 25 cm below the nozzles as 13 short axis scans each 6 cm apart. All measurements were replicated three times.

Spray drift and spray deposition on plants were measured in our wind tunnel *cum* spray chamber (Hislop, 1989). The procedure for drift measurement (three replicates) was as previously described (Western & Hislop, 1991; Miller *et al.*, 1993), using sodium fluorescein as the spray tracer and six horizontal collector strings spaced 7.5 cm apart, with the lowest 40 cm below nozzle height. Drift was sampled 2 m downwind of a single nozzle in a wind speed of 2.5 m/sec.

Spray deposited on spring wheat cv. Axona at *ca* GS13 (Tottman *et al.*, 1979), representing a near vertical target, and oilseed rape seedlings cv. Ariana with four true leaves, as a horizontal target, was also measured using the same tracer. These plants were grown in pots in a cool glasshouse. Each pot contained either four wheat or two rape plants in line. In each spray run, six pots of wheat and six of rape together with six horizontal polypropylene discs (d= 5.5 cm), were aligned in a row parallel to the direction of spraying and centrally between two spray nozzles spaced 50 cm apart on a boom. The discs and the top of each plant type were 40 cm below the nozzles. Spray speed throughout was 2.0 m/sec. The discs were used to measure the volume of spray falling in the sampling area, to take account of the differing overlap patterns from the various nozzles and spray solutions used. Deposits on discs were measured separately but the plants in a pot were bulked, providing six replicate measurements for each collecting surface. The procedure for the quantitative extraction, measurement and normalisation of deposits as ng tracer per g plant dry weight per g tracer applied per hectare (Hislop *et al.*,1993).

The base spray solutions used were fluorescein in tap water with or without 0.1% v/v Agral. In various spray runs these solutions were amended with one of the following adjuvants: A, 0.1% v/v Silwet L-77, an organosilicone surfactant (Newman Agrochemicals Ltd). B & C, 0.5% w/v polyvinyl alcohol, Mr wt. 30,000 - 70,000 and 70,000 - 100,000, respectively, designated PVA 1 and PVA 2 (Sigma Chemical Co). D, 0.025% AgRHO DR2000, a water-soluble non-ionic drift reducing agent of unknown composition (Rhône-Poulenc Surfactants & Specialities). E, 0.03% w/v Target, water-soluble polymers of acrylamide, acrylate and saccharides (Newman Agrochemicals Ltd). The adjuvant concentrations used were based on manufacturers recommendations, or on previous experience.

RESULTS

Mean droplet size and velocity data summarised in Table 1 differ from those supplied by Spraying Systems Co. because the spray solutions differed and sampling methods were almost certainly different. Since the replicates within measurements for each nozzle were so similar, most values quoted are statistically different ($P < 0.05$). Figure 1 shows the mean total drift on the collectors. Table 2 summarises the mean deposits on wheat and rape plants sprayed with the different nozzles, arranged in order of increasing droplet sizes (XR 11001-finest to TT 11002-coarsest). Statistical analysis of the latter data cannot be summarised briefly, because some spray runs were repeated a number of times with batches of plants which differed in size, while others were measured only once. However, a close approximation to the significant difference between any two means can be gleaned from the standard error values quoted, in the usual manner(+/- 2x SE).

Table 1. Droplet spectra from nozzles atomising 0.45 l/min 0.1% aqueous Agral

Nozzle	V(10)[a] (μm)	VMD[b] (μm)	V(90)[c] (μm)	NMD (μm)	% Volume <100μm	% Volume >350μm	Mean velocity (m/s)
XR11001	80.6	161.7	266.5	44.3	19	1.4	2.64
TT11001	100.2	191.6	315.1	53.9	11	4.1	2.04
XR110015	110.4	214.8	339.7	54.8	8.3	8.0	2.38
TT110015	158.3	293.8	447.6	87.5	2.5	30.4	1.77
DG110015	152.2	291.8	434.3	71.3	3.5	28.6	2.4
TT11002	194.7	362.2	572.7	104.4	1.4	52.3	1.71

[a] = the diameter in the droplet spectrum at which 10% of the spray volume is contained in smaller and 90% in larger droplets.
[b] = volume median diameter - the diameter in the droplet spectrum at which half the spray volume is contained in smaller and half in larger droplets.
[c] = the diameter in the droplet spectrum at which 90% of the spray volume is contained in smaller and 10% in larger droplets.

Figure 1. Total spray drift at 2 m in a wind speed of 2.5 m/s

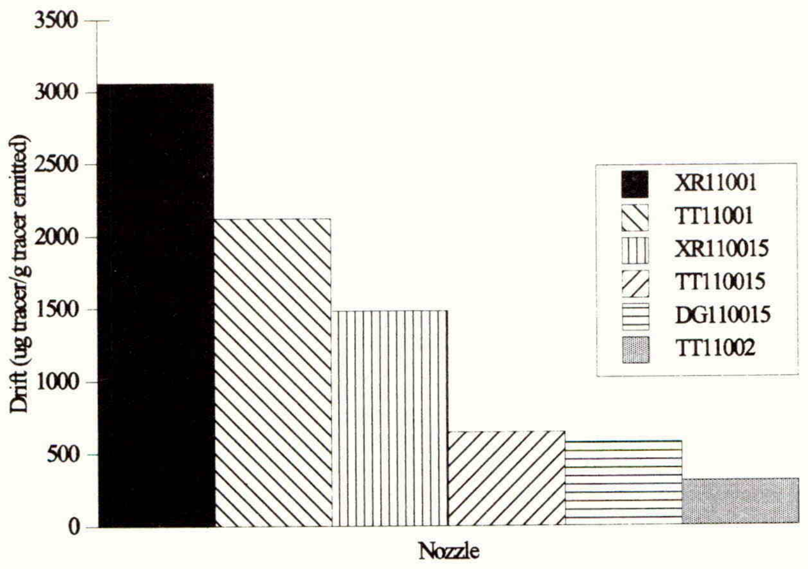

Table 2. Mean spray deposits [ng tracer/g plant dry wt/g tracer/ha; (s.e.)] on spring wheat and oilseed rape plants

Spray solutions*		Spray nozzles											
		XR11001		TT11001		XR110015		TT110015		DG110015		TT11002	
		- Agral	+ Agral	-	+	-	+	-	+	-	+	-	+
Base solution	Wheat	784	1159	617	1210	482	1089	328	921	300	735	229	618
		(23)	(34)	(32)	(51)	(26)	(36)	(17)	(61)	(11)	(26)	(9)	(25)
	Rape	1280	2412	756	2138	886	2868	626	1896	610	2343	423	1568
		(54)	(95)	(50)	(80)	(81)	(162)	(44)	(160)	(73)	(128)	(41)	(76)
+ L77	Wheat	1018	988	-	-	-	-	-	-	-	-	462	535
(0.1%)		(83)	(78)									(29)	(27)
	Rape	1840	2006	-	-	-	-	-	-	-	-	919	1482
		(141)	(75)									(54)	(100)
+ PVA1	Wheat	1487	1264	1205	1472	-	-	1044	930	-	-	837	690
(0.5%)		(80)	(80)	(132)	(178)			(51)	(40)			(85)	(49)
	Rape	2735	2950	2887	2196	-	-	1876	1680	-	-	1189	1630
		(213)	(164)	(226)	(273)			(218)	(101)			(80)	(136)
+ PVA2	Wheat	1478	1112	-	-	-	-	-	-	-	-	774	545
(0.5%)		(106)	(82)									(33)	(37)
	Rape	2098	2549	-	-	-	-	-	-	-	-	1074	1578
		(183)	(133)									(83)	(91)
+ Ag 2000	Wheat	1796	1007	1137	1099	-	-	1237	736	-	-	723	600
(0.025%)		(56)	(55)	(66)	(49)			(155)	(45)			(85)	(41)
	Rape	2211	2041	1579	2102	-	-	984	1480	-	-	730	2197
		(187)	(182)	(230)	(124)			(67)	(121)			(91)	(202)
+ Target	Wheat	598	1098	799	1076	-	-	723	1211	-	-	516	1464
(0.03%)		(23)	(53)	(20)	(82)			(97)	(79)			(46)	(168)
	Rape	928	2583	913	2107	-	-	797	2196	-	-	418	2548
		(64)	(148)	(56)	(102)			(121)	(223)			(62)	(399)

* See Materials and Methods   - indicates not measured

Table 3. Percentage spray retention on spring wheat and oilseed rape plants compared to the finest nozzle (XR11001) applying the base solution with Agral (=100%)

Spray solutions*		Spray nozzles											
		XR11001		TT11001		XR110015		TT110015		DG110015		TT11002	
		- Agral	+ Agral	-	+	-	+	-	+	-	+	-	+
Base solution	Wheat	68	100	53	105	42	94	28	80	26	64	20	53
	Rape	53	100	31	89	37	119	26	79	25	97	18	65
+ L77	Wheat	88	85	-	-	-	-	-	-	-	-	40	46
	Rape	76	83	-	-	-	-	-	-	-	-	38	61
+ PVA1	Wheat	128	109	104	127	-	-	90	80	-	-	72	60
	Rape	113	122	120	91	-	-	78	70	-	-	49	68
+ PVA2	Wheat	128	96	-	-	-	-	-	-	-	-	67	47
	Rape	87	106	-	-	-	-	-	-	-	-	45	65
+ Ag2000	Wheat	155	87	98	95	-	-	107	64	-	-	62	52
	Rape	92	85	66	87	-	-	41	61	-	-	33	91
+ Target	Wheat	52	95	69	93	-	-	62	105	-	-	45	126
	Rape	38	107	38	87	-	-	33	91	-	-	17	106

* See Materials and Methods

DISCUSSION

The droplet size data for the various nozzles used to spray aqueous Agral (Table 1) are logical, show the expected trends and correlate well with the drift results in Fig.1. The only possible anomaly is the slightly greater drift from the TT 110015 nozzle compared to the DG110015 (not significantly different) and lack of correlation with the percentage of spray volume in droplets <100 μm. However, this is well explained by the lower velocity of the droplets from the TT nozzle. Velocity data for droplets <100 μm are not presented, but they differed by a factor of slightly more than 2, compared to a total spectrum mean velocity difference of 1.4 (Table 1). However, our limited droplet spectra data suggest that the spray quality produced by the Turbo Teejet nozzles used might be finer than that suggested in the manufacturer's preliminary classification.

Interpretation of the deposit data in Table 2 is more difficult, but some noteworthy trends are evident from the percentage retention figures calculated taking the finest spray containing Agral as 100 for each plant species (Table 3). For example, there is a clear tendency for deposition to decrease with increasing droplet size, and all applications of aqueous fluorescein were poorly retained compared with the base solution containing Agral. In part the latter observation could be due to larger droplet sizes in sprays without surfactant but it is more likely to be due to poor wetting of the foliage. The organosilicone surfactant L77, having a lower static surface tension that Agral, was not very well retained on the plants but coverage was visibly good. In contrast, all of the polymeric adjuvants tended to increase retention, particularly so when applied in the absence of Agral and with the finest nozzle. An exception to this generalisation was Target (polyacrylamide), but as an addition to Agral its ability to increase deposition on both species sprayed with the coarsest nozzle was remarkable. This adjuvant, like Ag2000, increases the viscosity of the spray solutions, acting to increase spray droplet size and to reduce spray drift. The possible practical advantage of the latter material over some agents, is that viscosity is maintained after recirculation (confidential information) while materials like Target may be degraded (Chapple *et al*., 1992). However, both adjuvants have the practical disadvantage that they reduce the spray fan angle and thus produce less even patternation than solutions of low viscosity. Polyvinyl alcohol at 0.5% did not visibly increase the viscosity of the spray solutions although we know that it can increase spray droplet sizes to a small degree. Both molecular weight samples increased spray deposition from the finer nozzles but less so for the coarsest nozzle when used with Agral.

The spray retention data with the various nozzles and solutions are generally as expected. Large droplets are poorly retained on targets (Hartley & Brunskill, 1958) and surface-active agents, particularly those which migrate rapidly to droplet surfaces, can improve retention (Anderson & Hall, 1989). Laboratory work has demonstrated the capacity of viscosity modifiers to minimise droplet rebound (Crease *et al*., 1991) and a mechanism involving energy absorption has been proposed (Hartley & Graham-Bryce, 1980). The benefits of PVA have been demonstrated (Wirth *et al*., 1991) and increased surface elasticity invoked as a possible explanation (Hall *et al*, 1993; Holloway, 1994). All of these studies, together with the ones reported here, suggest that a suitable combination of atomiser and spray formulation could lead to safe and efficient reduced volume pesticide spraying of crops. The Turbo Teejet nozzles are a valuable addition to the range of nozzle options available, because they have orifices which are less prone to blockage than standard atomisers with similar flow rates and, unlike other anvil nozzles, they provide good distribution across the spray swath (data not included here, but confirm manufacturers claims).

Thus, some are very suitable for safe reduced-volume applications to moist soil. Whether or not they can provide good coverage and biological activity of foliage-applied agrochemicals remains to be determined.

ACKNOWLEDGEMENTS

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REFERENCES

Anderson, N H; Hall D J (1989) The role of dynamic surface tension in the retention of surfactant sprays on pea plants. In: *Adjuvants and Agrochemicals*. P N P Chow, C A Grant, A M Hinshalwood, E Simundsson (eds). CRC Press, **Vol 2**, pp. 51-62.

Crease, G J; Hall F R; Thacker J R M (1991) Reflection of agricultural sprays from leaf surfaces. *Journal of Environmental Science and Health*. **B26**, 383-407.

Chapple, A C; Downer R A; Hall F R (1992) Effect of pump type on atomization of spray formulations. In: *Pesticide Formulations and Application Systems*. L E Bode, D G Chasin (eds). ASTM **11**, pp. 193-205.

Doble, S J; Matthews G A; Rutherford, I; Southcombe, E S E (1985) A system for classifying hydraulic nozzles and other atomisers into categories of spray quality. *British Crop Protection Conference-Weeds*. **3**, 1125-1133.

Hall, F R; Chapple A C; Downer R A; Kirchner L M; Thacker J R M (1993) Pesticide application as affected by spray modifiers . *Pesticide Science*. **38**, 123-133.

Hartley, G S; Brunskill R T (1958) Reflection of water drops from surfaces. In: *Surface phenomena in chemistry and biology*. J F Danielli, K G A Parkhurst, A C Riddiford (eds) Pergamon Press, pp. 214-223.

Hartley, G S; Graham-Bryce I J (1980) *Physical Principles of Pesticide Behaviour, Vol. 2*. Academic Press.

Hislop, E C. (1989) Crop spraying under controlled conditions. In: *Comparing Laboratory and Field Pesticide Performance*. L R Copping, S B Wakerley, C R Merritt, R C Ready, B T Grayson (eds). Aspects of Applied Biology, **21**, pp. 119-120.

Hislop, E C; Western N M; Cooke B K; Butler R (1993) Experimental air-assisted spraying of young cereal plants under controlled conditions. *Crop Protection*. **12,** 193-200.

Hislop, E C; Western N M; Butler R (1995) Experimental air-assisted spraying of a maturing cereal crop under controlled conditions. *Crop Protection*. **14**, 19-26.

Holloway, P J. (1994) Physicochemical factors influencing the adjuvant-enhanced spray deposition and coverage of foliage-applied agrochemicals. In: *Interactions between Adjuvants, Agrochemicals and Target Organisms*. P J Holloway, R T Rees, D Stock (eds). Ernst Schering Research Foundation Workshop **12**, Springer-Verlâg, pp. 83-106.

Miller, P C H; Hislop E C; Parkin C S; Matthews G A; Gilbert A J (1993) The classification of spray generator performance based on wind tunnel assessments of spray drift. In: *ANPP-BCPC Second International Symposium on Pesticide Application Techniques*, Strasbourg, **1,** 109-116.

Tottman, D R; Makepeace R J; Broad H (1979) An explanation of the decimal code for the growth stages of cereals, with illustrations. *Annals of Applied Biology*. **93**, 221-234.

Western, N M; Hislop E C (1991) Drift of charged and uncharged drops from an experimental air-assisted sprayer. In: *Air-assisted Spraying in Crop Protection.* A Lavers, P Herrington, E S E Southcombe (eds). BCPC Monograph No.**46**, pp. 69-76.

Western, N M; Hislop E C; Dalton W J (1994) Experimental air-assisted electrohydrodynamic spraying. *Crop Protection*. **13**, 179-188.

Wirth, W; Strop S; Jacobsen W (1991) Mechanisms controlling leaf retention of agricultural spray solutions. *Pesticide Science*. **33**, 411-420.

AN INVESTIGATION OF ALTERNATIVE APPLICATION TECHNIQUES FOR THE CONTROL OF BLACK-GRASS

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ABSTRACT

The commercial formulation of clodinafop-propargyl, Topik 240EC, was assessed through a variety of application systems and techniques with a view to optimising its field performance. Applications to moderate infestations of black-grass (*Alopecurus myosuroides*) showed a tendency to quicker activity at lower spray volumes and finer quality sprays. All treatments gave 100% control at head count. The fluorescent tracer Uvitex OB was used to assess deposition from a variety of nozzle types and water volumes. Black-grass was analysed for deposition using the method described by B.K. Cooke & E.C. Hislop, 1993. Recovered fluorescence from specimens of black-grass indicated that higher deposition or retention of spray occurs with lower volumes of water with finer spray quality nozzles.

INTRODUCTION

Black-grass has long been a problem in cereal cultivation. It's pre adaptive method of crop mimicry makes the post-emergence timing of its treatment vital to achieve satisfactory control. Optimising the performance of graminicides used to treat black-grass could make a vital contribution to successful control. Failure to control black-grass may cause losses as high as £400/ha. This potential loss must be the keenest reason why decisive control is needed. Reducing dose rates of formulations, as is the current desire of many agronomists and farmers, seems imprudent as this increases the likelihood of poor control.

The advent of resistance in black-grass to certain graminicide groups may have resulted in places from indecisive treatment, where partial doses and continued use of the same product have occurred (Boothroyd, et al, 1993). Ineffective application of the product may well contribute to this.

In 1994 a study undertaken by Whittaker & Robinson (previously unpublished) concluded that the efficacy of clodinafop-propargyl was affected by the method of application (spray quality and volume of water per hectare). Finer spray qualities at lower volumes displayed greater efficacy. Efficacy was assessed for both the speed of activity and the overall level of control.

Literature indicates that this was a possible outcome. Western et al (1985), reported that reduced volume sprays display greater retention properties though less penetration of the crop occurs. Mabb & Hicks (1989), report that large drops are poorly retained on the target plant.

Considering a finer spray quality at lower water volumes, it is understandable that efficacy may be greater with this application method. The applied solution has a higher proportion of active ingredient, and finer sprays give better coverage and retention, when compared to coarser spray qualities. Quantifying deposition from the application methods tested with the fluorescent tracer Uvitex OB should allow any greater retention or deposition from the nozzles used to be detected.

METHODS AND MATERIALS

The field trial was carried out on 5.5.95 on a heavy land site at Elmdon , Cambridgeshire. The trial consisted of 9 treatments, with three repetitions of each. Plots were 20m x 6m. Treatment details are given in Table 1.0.

The growth stage of black-grass within the trial was assessed to be 31 to 55, ear emergence. The crop was at GS32. Mean wheat population was calculated to be 200 plants/m^2. Populations of black-grass were low, varying between 0-30 plants/m^2.

Chemicals used for the field trial

The formulation of clodinafop-propargyl "Topik" (Ciba Agriculture) was used in conjunction with the adjuvant oil "Output" (Zeneca Agrochemicals). The rate of use of clodinafop-propargyl was 30g/ha (125 ml of formulation), with 1 l/ha of adjuvant. Uvitex OB was used as the fluorescent tracer for recovery from the weed target, black-grass. 4g of Uvitex OB was dissolved in xylene. This solution was then added to 1 litre of the adjuvant.

Application Details

Table 1.0 Nozzle and calibration data for the Frazier Agribuggy

Treatment number	Volume (l/ha)	Nozzle type & Quality	Manufacturers name & product Code.	Application Preasure (Bar).
1	70	Airtec Fine	Cleanacres	2.5 liquid 20psi air.
2	70	Airtec Medium	Cleanacres	2.0 liquid 15psi air.
3	100	Fanjet Fine	Teejet 11002	2.4
4	100	Fanjet Medium	Teejet 11002DG	2.3
5	200	Fanjet Fine	Teejet 11003	2.4
6	200	Fanjet Medium	Teejet 11004XR	2.4
7	200	Fanjet Coarse	Teejet 11004 Turbo	2.7
8	400	Twinjet Fine	Teejet 11004	2.4
9	Control	Untreated	Control	Control

Field sampling procedure

Before any spraying was undertaken, control samples (three repetitions of ten plants) were taken from the entire trial site at random. Samples of black-grass were taken from the respective plots simultaneously, very shortly after the plot was sprayed. Ten samples were taken from the plot and placed in amber glass jars for storage until analysis. Before samples were analysed for fluorescence their fresh weight was recorded, to enable transformation of data to micrograms clodinafop per gram fresh weight of sample.

Method used for fluorescence analysis

Extraction of the fluorescent agent was done using 20ml of 90% hexane 10% acetone solution. This solution was applied to the black-grass sample and vigorously shaken. The resulting solution was passed through a Perkin Elmer LS30 fluorimeter for fluorescence measurement. Fluorescence was measured against fresh standard solutions in extraction fluid.

Control samples were examined for fluorescence also. The fluorescence produced by these was deemed to be background "noise". This was subtracted from the total reading for the treatment.

Scoring of trial for efficacy

An objective score was given to the plots that accounted for the visible symptoms the black-grass was displaying after treatment. The following scheme of symptoms in Table 2.0 was used for guidance.

Table 2 Details of observed symptoms used for efficacy scoring

Efficacy Score	Symptoms
100	Whole plant necrotic and collapsed
95	Flag leaf and all upper leaves necrotic/chlorotic
90	Flag and upper leaves chlorotic/purple.
85	Flag leaves purple.
80	Margin of flag leaves purple.

Visible symptoms of Clodinafop occur within 4 weeks death occurs within 10. The score given reflected the condition of the black-grass specimens in the entire plot.

RESULTS AND ANALYSIS

The fluorescence recovered for a treatment was summed, as was the weight of all the samples from the treatment. The recovered fluorescent material has then been converted to micrograms active ingredient deposited on the weed target . This is a robust assumption as detailed by Cooke & Hislop (1993). Table 3.0 gives the amount of clodinafop-propargyl deposited on the weed target per gram of fresh weight.

Table 3.0 The amount of clodinafop-propargyl (micrograms/g fresh weight) deposited on the weed samples collected from the treatments given. Background fluorescence has been deducted, at the levels obtained from the controls.

Treatment Nozzle Quality	Volume l/ha.	Clodinafop-propargyl deposited (micrograms/g freshweight)
Airtec Fine	70	68
Airtec Medium	70	56
Fine	100	60
Medium	100	48
Fine	200	34
Medium	200	33
Coarse	200	33
Fine	400	22
Control (background reading)		3.2

Fig. 1.0 Bar Chart Illustrating Micrograms Clodinafop Deposited On A. myoseroides Per Gram Fresh Weight

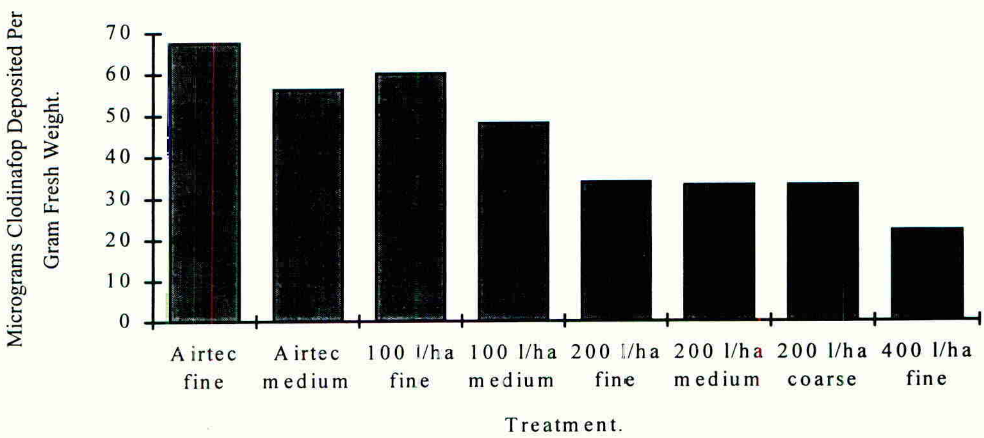


Figure 1.0 illustrates that the finer quality sprays at lower volumes give the greatest deposition. This agrees with the findings of Ayres et al (1985), who found that increased spray deposit occurred at lower volumes. Western et al (1985) report that smaller droplets have greater retention properties compared to larger droplets. The reduced volume treatments in this case appear to follow this pattern, improving deposition.The biological efficacy of the treatments was assessed as per the method. The mean results are given in Table 4.0.

Table 4.0 The mean of the results for each treatment on the date assessment was done.

Treatment	Date of Assessment	
	20.6.95	14.7.95
Airtec Fine	97.5	100
Airtec Medium	96.1	100
100l Fine	92.8	100
100l Medium	95.1	100
200l Fine	90	100
200l Medium	90	100
200l Coarse	90	100
400l Fine	92.5	100

The symptoms of black-grass chlorosis / necrosis at the first assessment were most marked in plots treated at reduced volume and finer spray quality. At the final assessment complete control was achieved by all treatments. These results correlate with previous work by Whittaker and Robinson, 1994, given in Figure 3.0.

Fig. 2.0 Bar Chart Illustrating Efficacy Score For Each Treatment On The Date Assessed.

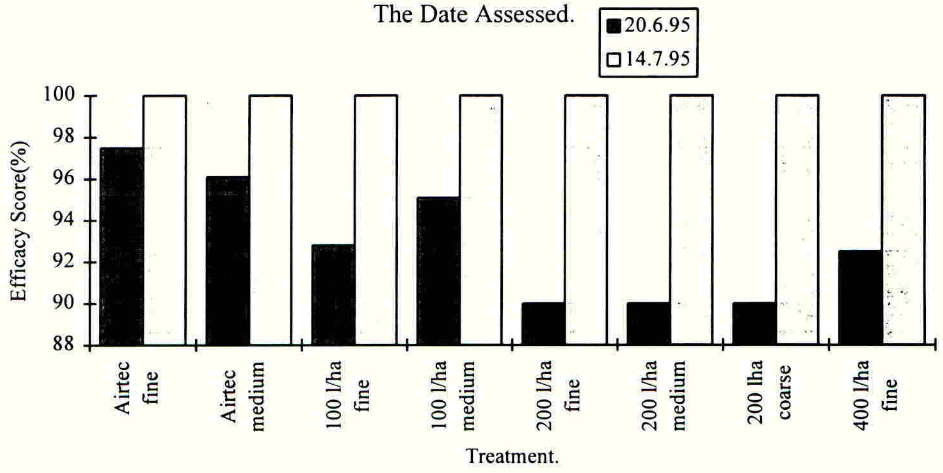
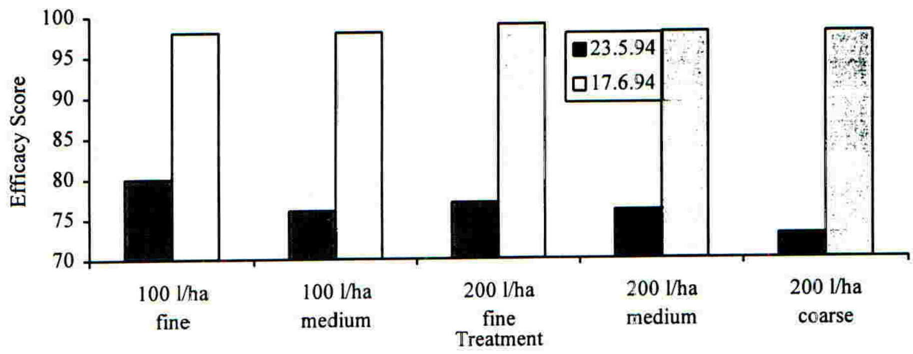


Fig. 3.0 Bar Chart Illustrating Efficacy Results From Preliminary Trials In 1994



DISCUSSION

All treatments gave complete control by the final assessment. However, a pattern is evident in the deposition and efficacy of the various application methods studied. Lower volume spray treatments with finer spray qualities achieved higher levels of clodinafop deposition on the target, black-grass. Smaller droplets show a greater tendency towards retention than larger ones (Western et al,1985). This property of retention may well be instrumental in improving deposition over coarser treatments. Finer droplets give more even coverage. A combination of these two factors appears to accelerate the symptoms of treatment on the target compared to the coarser treatments. The problem of drift with these treatment methods should be considered.

The Airtec spraying method delivered the highest overall deposition on the target. This may be simply a function of the reduced spray volume (70 l/ha), or a result of enhanced impaction due to the extra energy supplied by the air during the drop formation process. Finer drops do not penetrate the canopy as well as coarse ones (Western et al. 1985). Air assistance imparted by the twin fluid atomiser may have improved penetration, aiding deposition.

All 200 l/ha treatments show similar deposition and efficacy characteristics. The volume of application appears to have a greater effect on deposition than spray quality. This is similar to the findings of Ayres et al (1985) who found that deposits appeared to be more influenced by volume rate than spray quality. The 400 l/ha treatment emphasizes this point by delivering the lowest deposition with the highest volume rate. It is possible that the excellent coverage achieved with the 400 l/ha (fine) treatment, compensated for the reduced deposition to give a satisfactory performance in the field.

Fine quality sprays produce larger numbers of droplets of driftable size, a potentialdrawback to the methods found in this study to be most effective at achieving high deposits of clodinafop. Field experience from a wide variety of products has shown that timing is the most important factor when optimising application. The Airtec nozzle though is reported by several sources to produce significantly less drift than standard flat fan nozzles, Western *et al* (1989), Rutherford *et al* (1989). Similarly, low drift nozzles, while not necessarily offering the optimum spray recovery would allow a degree of compromise when spraying must be done. Timing of application is of paramount importance. In marginal conditions, the compromise of using a coarser spray is preferable to not spraying.

CONCLUSION

The eventual control of black-grass in all plots was complete. This study demonstrated that at low infestations of black-grass the symptoms of clodinafop-propargyl on the target weed occurred more rapidly at lower spray volumes and finer spray qualities. It is possible that the more rapid occurrence of the symptoms is due to the greater spray deposits and leaf surface coverage associated with these treatments. Further study is intended, to investigate the influence of different application techniques at alternative spray timings.

REFERENCES

Ayres, P; Taylor, W A; Cotterill, E G (1985) The Influence Of Cereal Canopy And Application Method On Spray Deposition And Biological Activity Of A Herbicide For Broad Leaved Weeds. Brighton Crop Protection Conference Weeds 1985

Boothroyd, D; Clarke, J; Ickeringill, D; Mackay, J *Alopecurus myoseroides* Control Using Fenoxaprop-Ethyl Dose Adjustments Adjuvants And Mixes. Brighton Crop Protection Conference Weeds 1993.

Mabb, L P; Hicks, W K (1989) Effects Of Formulation Changes On The Biological Efficacy Of CDA And Hydraulic Nozzle Applications Of Two Herbicides. Brighton Crop Protection Conference Weeds 1989.

Matthews, G A; & Hislop, E C Application Technology For Crop Protection, 1993, CAB International.

Western, N M; Hislop, E C; Herrington, P J; Woodley, S A(1985)Relationships Of Nozzle And Spinning Disc Spray Characteristics To Retention And Distribution In Cereals. Symposium On Application Biology 1985 BCPC. Monogram No. 28.

Whittaker, S & Robinson, T H 1994, Ciba Agriculture internal report.

Rutherford, I; Bell, G J; Freer, J B S; Herrington, P J; Miller, P C H (Dr) (1989) Brighton Crop Protection Conference Weeds 1989.

PREDICTION OF SPRAY DRIFT FROM FLAT-FAN HYDRAULIC NOZZLES USING DIMENSIONAL ANALYSIS.

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ABSTRACT

An empirical model, using dimensional analysis and based on data from wind tunnel experiments, has been developed to predict potential spray drift from flat-fan hydraulic nozzles. Nozzle parameters used in the model include output, and size. Operational parameters include nozzle height, nozzle orientation to the airstream, wind velocity, and downwind measurement distance. The experimental data was obtained using the protocol established by Miller *et al.* (1993). Spray movement was measured in a 2 m wide by 1 m high wind tunnel operating in the range 1 to 3 m s^{-1} windspeed. Sprays were traced using fluorescence and sampled downwind using 2 mm o.d. polyethylene tubes. The dimensional analysis model showed good correlation with experimental results and could be used to demonstrate the influence of nozzle parameters and operational conditions on potential spray drift.

INTRODUCTION

To date models describing the behaviour of spray drift have been based on diffusion calculations (Bache & Sayer 1975), random-walk computer simulations (Thompson and Ley 1983, Miller and Hadfield 1989), or computational fluid dynamic (CFD) codes (Weiner and Parkin 1993). Random-walk and CFD models can produce simulations that take into account most of the parameters that influence drift. However, even with modern processors, computer run times can be long since the paths of individual particles are followed and large numbers of particles are required for statistical accuracy. Validation of drift models requires extensive field experimentation, but many of the critical parameters that influence drift can be controlled and simulated in wind tunnels. For this reason wind tunnel simulations now play a major part validating drift models (Miller *et al.* 1993).

In this paper wind tunnel simulations have again been used to generate data but an alternative more simple approach to modelling has been adopted. Instead of the results being related to complex computer simulations, an empirical model based on dimensional analysis has been developed. Data has been obtained from experiments with flat-fan hydraulic nozzles. The nozzle parameters used in the model include nozzle output and size. Operational parameters include nozzle height, nozzle orientation to the airstream, wind velocity, and downwind measurement distance.

METHOD AND MATERIALS

Wind Tunnel

The Silsoe College low-speed suction wind tunnel was used in the experiments. Following earlier work defining the protocols for drift measurements in wind tunnels (Miller *et al.* 1993), the width of the tunnel was increased from 1 m to 2 m. The tunnel was 1 m high and 7 m long and the wind speed range was 1 - 3 m s^{-1}. Single spray nozzles were mounted on a boom 2 m downwind of the tunnel entrance. The floor was covered with artificial turf to prevent drop re-entrainment and the section directly under the nozzle was fitted with a small sump to collect the majority of the spray liquid leaving the nozzle. The spray system consisted of a 10 l pressurised container delivering liquid to the boom through a solenoid valve. The solenoid valve was operated by an electronic timer that was set to provide spray periods of 10 s.

Drift Measurement

The spray was traced using a standard fluorescence technique (Merritt 1989). The spray solution consisted of 0.1% (w/v) sodium fluorescein and 0.1% (v/v) Agral non-ionic surfactant. Airborne spray was sampled downwind of the spray nozzle using 2 mm o.d. polyethylene tubing stretched across the tunnel at 0.1 m height intervals. The fraction of the spray airborne downwind of the nozzle was calculated from the dimensions of the collectors, the height intervals between collectors, the duration of the spray, the discharge from the nozzle, and the amount of tracer collected on the sample. This fraction was referred to as the Drift Potential (*Dp*) and used as a basis for the model predictions.

Dimensional Analysis

Dimensional analysis is a useful technique for investigating problems in all sections of engineering (Taylor 1974) but has been particularly successful in fluid mechanics (Douglas *et al.* 1985). The theoretical basis of the technique was developed in the early part of this century by Buckingham (1914). The analysis is developed from a fundamental consideration of the parameters concerned in the problem. An equation is developed with a constant of proportionality and a series on non-dimensional groups each with its own index. The constant and the indices are then determined by fitting the experimental data to the model using statistical techniques. In the problem investigated here, the following parameters were identified and were incorporated into a dimensional analysis model.

Table 1: Parameters used in the simple dimensional analysis model

	Parameter	Symbol	Units
Operational Parameters	Nozzle Height	H	m
	Nozzle Orientation	θ	°
	Downwind Distance	S	m
	Wind Speed	U	m s^{-1}
Nozzle Design Parameters	Equivalent Diameter	D	m
	Discharge	Q	m^3 s^{-1}
	Coefficient of Discharge	C_d	

The form of the model was

$$Dp = k\,(C_d)^a \left(\frac{H}{D}\right)^b \left(\frac{H}{S}\right)^c (\theta)^d \left(H\sqrt{\frac{U}{Q}}\right)^e \qquad (1)$$

The orientation θ is the angle in the vertical plane that the spray nozzle makes to the airstream. Thus, when θ = 0 ° the nozzle is fully aligned with the airstream. The singularity in the model caused when θ = 0 ° can be avoided by using θ = 2 ° for this setting. This should not cause any significant loss of accuracy. The equivalent diameter of the orifice was calculated by

$$D = 2\sqrt{\frac{A}{\pi}} \qquad (2)$$

where A is the orifice area.

The discharge from an orifice (Q) is intrinsically linked to the pressure drop across the nozzle (ΔP), and the fluid density (ρ) by Equation 3. The coefficient of discharge (C_d) is ≤ 1 and is a measure of the energy loss through an orifice.

$$Q = C_d\, A\sqrt{\frac{\Delta P}{\rho}} \qquad (3)$$

It is therefore not necessary to include all the parameters in Equation 3 within the dimensional analysis model. The model has been developed without using the pressure drop across the nozzle (ΔP) as an input, The nozzle inputs are discharge (Q), nozzle size (D) and coefficient of discharge (C_d). Further parameters such as liquid properties influence drift but these were excluded in this simple model as the results apply only to the standard test liquid of water plus 0.1 % Agral.

Other Measurements

The model requires parameters that characterise the nozzle design. The equivalent diameters of the nozzles (D) were calculated using Equation 2 with the orifice areas (A) measured using a microscope fitted with a video camera and connected to an Optomax V image analysis computer. The coefficients of discharge (C_d) were calculated using data from discharge measurements and Equation 3.

Experimental Design

A split-block design was used with each test replicated three times. The variables examined were nozzle size, pressure, nozzle height, downwind distance and nozzle orientation. A set of nozzles from a single manufacturer was used. Unless otherwise stated, the standard operating conditions refer to a F110/0.8/03 nozzle (BCPC code - Doble *et al.* 1985), operating at 3 bar pressure, 0.5 m height, normal to an airstream of 2 m s^{-1}. Measurements of Drift Potential (Dp) were made 2 m downwind of the nozzle. Fifty one individual tests were carried out.

RESULTS

Experimental Results

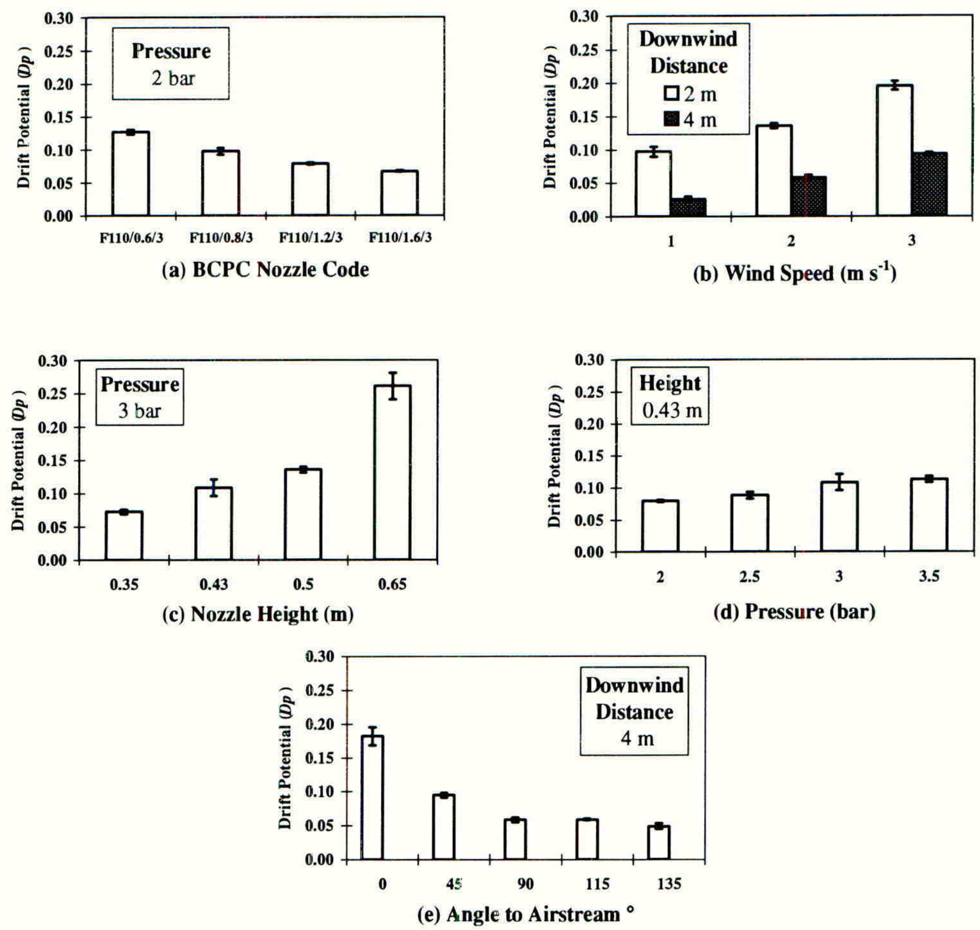

Figure 1: Variation of mean Drift Potential (*Dp*) with (a) nozzle size, (b) wind speed and downwind distance, (c) nozzle height, (d) spray pressure and (e) nozzle orientation to the airstream

The experimental results are summarised in Figures 1 (a-e). The error bars represent the 95 % confidence interval of the mean. Drift Potential (*Dp*) increased with increasing nozzle height and pressure, but decreased with increasing downwind distance and nozzle size. Drift potential also increased as the nozzle was aligned with the airstream.

Empirical Model

The above data were analysed using Genstat 5 ™ (1993) to establish the coefficient and indices in the model. These are shown below in Table 2. The variance in the data accounted for by the model is 95%. The large standard error in index *b* indicates that the model does not fully take into account the influence of nozzle size. In particular, it appears that data from the F110/1.6/3 nozzle is responsible for most of the large residual errors.

Table 2: Fitted values for the coefficients in Equation 1

Coefficient	Mean Value	Standard Error
k	0.001612	
a	5.973	0.776
b	-0.180	0.201
c	1.0451	0.0709
d	-0.2664	0.0167
e	1.618	0.156

To illustrate goodness of fit, the measured and predicted results are plotted on Figure 2. The error bars on the abscissa represent the 95% confidence interval of the mean.

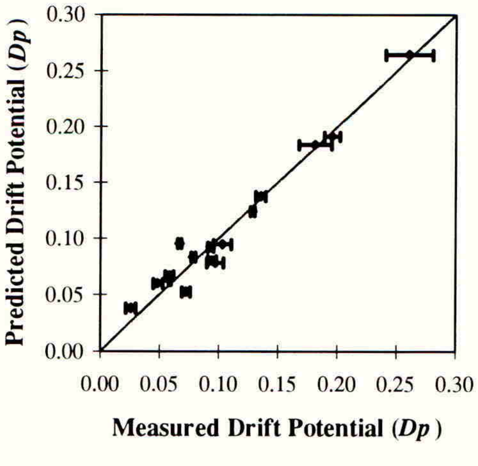

Figure 2: Scatter plot of predicted and measured Drift Potentials

As can be seen, the model significantly over and under-predicts drift potential at values of *Dp* < 0.1. Again, the data responsible for most of this variability comes from the largest nozzle tested (F110/1.6/3). However, since the model gives accurate estimates for *Dp* > 0.1, and higher values are more critical, the model appears to have enough reliability to be used formulate operational guidelines.

CONCLUSIONS AND FURTHER WORK

Although the scope of the model is limited to a specific range of nozzles and conditions, the nozzles selected are in common use and an accurate predictive model has been developed. The model has practical benefits and could be used to formulate guidelines for users. Further validation could be carried out by comparing the model with data from field experiments and results from more complex models. The model is currently being extended to include the effect of formulations (liquid properties) and reduced drift (pre-orifice) flat-fan hydraulic nozzles (Castell 1993). Dimensional analysis models are also being developed by the authors to predict drop size spectra using data from a laser-based probe.

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REFERENCES

Bache, D.H. and Sayer W.J.D. (1975) Transport of aerial spray I: A model of aerial dispersion. *Agricultural Meteorology*, **15**, p257-271

Buckingham (1914) On physically similar systems: Illustrations of the use of dimensional equations. *Physics Review*, **4**, p347-376

Castell, J.A., (1993) The development of drift reducing hydraulic fan spray nozzle. *Proceedings ANPP-BCPC 2nd International Symposium on Pesticide Application Techniques Strasbourg 22-24 September 1993*, p227-234

Doble, S.J., Matthews, G.A., Rutherford, I., and Southcombe, E.S.E. (1985) The British Crop Protection Council scheme for classification of pesticide application equipment by hazard. *BCPC Crop Protection Conference- Weeds*, **3**, p1125-1133

Douglas, J.F., Gasiorek, J.M., and Swaffield, J.A. (1985) *Fluid Mechanics*, 2nd edition, Longman Scientific & Technical, New York, 746p

Genstat 5 ™ (1993) *Genstat 5 Release 3 Reference Manual*, Edited by Genstat 5 Committee, Rothamsted Experimental Station, Clarendon Press, Oxford

Merritt, C.R., (1989) Evaluation of operator exposure and spray displacement with hand-operated herbicide application systems. *BCPC Crop Protection Conference- Weeds,* **6b-4**, p623-630

Miller, P.C.H. and Hadfield, D.J. (1989) A simulation model of the spray drift from hydraulic nozzles. *Journal of Agricultural Engineering Research,* **42**, p135-147

Miller, P.C.H., Hislop, E.C., Parkin, C.S., Matthews, G.A., and Gilbert, A.J. (1993) The classification of spray generator performance based on wind tunnel assessments of spray drift. *Proceedings ANPP-BCPC 2nd International Symposium on Pesticide Application Techniques Strasbourg 22-24 September 1993*, P109-116

Taylor, E.S. (1974) *Dimensional analysis for engineers*, Clarendon Press, Oxford, 162p

Thompson, N. and Ley, A.J., (1983) Estimating spray drift using a random-walk model of evaporating droplets. *Journal of Agricultural Engineering Research*, **28**, p419-435

Wiener, K.-L. and Parkin, C.S. (1993) The use of computational fluid dynamic code for modelling spray from a mistblower. *Journal of Agricultural Engineering Research,* **55**, p313-324

Session 6A

Management of Herbicide Resistance in Weeds

Chairman	Professor J Gressel
Session Organiser	Mr J C Caseley
Papers	6A-1 to 6A-6

STUDIES ON MECHANISMS AND GENETICS OF RESISTANCE: THEIR CONTRIBUTION TO HERBICIDE RESISTANCE MANAGEMENT

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ABSTRACT

Understanding the mechanisms and genetics of resistance can lead to more effective management of herbicide resistant weeds. This information is important in determining which herbicides should be used in combinations or rotations to prevent either target site or metabolism-based resistance. In addition, understanding the gene flow within and between populations aids in predicting the selection of resistance and in preventing the spread of resistance. New molecular genetic markers as well as other detection methods based on the mechanisms of resistance may be useful in detecting resistance within populations before the trait becomes widespread.

INTRODUCTION

Herbicide resistance is a major concern of the weed science community as evidenced by the numerous meetings, symposia, and publications devoted to this topic. The primary goal of these gatherings and publications is to develop more effective ways to manage the development and spread of resistant weed biotypes.

Much of the research on herbicide resistance focuses on the mechanisms, genetics, and, more recently, molecular genetics of resistance. Although this research can help us understand how resistance developed and spread, does this information contribute to more effective management of resistant weeds? The objective of this paper is to try to answer this question.

Knowledge about the mechanisms and genetics of resistance can help us determine the most effective management practices. However, if we wait until we understand all the mechanisms and genetics of herbicide resistance before we implement management practices, we commit ourselves to a *reactive* position on resistance management. A *pro-active* approach to herbicide resistance management is to assume that all herbicides have the potential to select for resistance and to implement integrated weed management programs with new and existing herbicides to prevent or delay the development of resistance as long as possible. In addition, as new herbicides are discovered, we may be able to use the information obtained in the laboratory to predict the mechanisms and genetics of resistance before it occurs in the field. This information can then be used to integrate the new herbicides into the weed management program of the farmer in a way that minimizes the chances for selecting resistant weed populations.

Table 1. Mechanisms and Genetics of Herbicide Resistance.

Herbicide Chemical Class	**Resistant Species**	**Resistance Mechanism**	**Inheritance of Resistance**	**Reference**
Triazine	Numerous	AS	1 chloroplast gene	Gronwald, 1994
	Abutilon theophrasti	AM	1 nuclear gene, Semi-dominant	Gronwald, 1994
Substituted Ureas	*Alopecurus myosuroides*	AM	Nuclear, 2 genes	Moss, 1990
Aryloxyphenoxy-propionate Cyclohexanedione	*Avena fatua*	AS	1 nuclear gene, Semi-dominant	Murray, *et al.*, 1995
	Lolium rigidum	AS	1 nuclear gene, Semi-dominant	Richter and Powles, 1993
Sulfonylurea	*Lactuca serriola*	AS	1 nuclear gene, Semi-dominant	Saari, *et al.*, 1995
	Kochia scoparia	AS	1 nuclear gene, Semi-dominant	Saari, *et al.*, 1995
Imidazolinone	*Xanthium strumarium*	AS	1 nuclear gene, Semi-dominant	Saari, *et al.*, 1995
Dinitroanilines	*Setaria viridis*	AS	1 nuclear gene, Recessive	Smeda and Vaughn, 1994
	Eleusine indica	AS	Nuclear, Multigenic	Smeda and Vaughn, 1994
Bipyridilium	*Conyza bonariensis*	AM	1 nuclear gene, Dominant	Darmency, 1994
	Hordeum glaucum	AU	1 nuclear gene, Semi-dominant	Darmency, 1994
Phenoxyacetic acids	*Brassica kaber*	?	1 nuclear gene Dominant	Jasieniuk, *et al.*, 1995

[a]AS-altered site of action; AM-altered metabolism, AU-altered uptake/translocation

MECHANISMS AND GENETICS OF RESISTANCE

There are at least 3 mechanisms of resistance to herbicides. These are alterations at the site of action, changes in the rate of detoxification of the herbicide and modifications in the uptake and translocation of the herbicide. All of these mechanisms have been found in resistant weed populations (Table 1). The inheritance of resistance has also been determined in many cases. In the majority of the cases that have been studied, the resistant trait is due to a semi-dominant or dominant nuclear gene. The exceptions to this are dinitroaniline resistance in *Setaria viridis*, which is a recessive trait; and triazine resistance, which is encoded on a chloroplast gene (Table 1).

MANAGEMENT OF HERBICIDE RESISTANT WEED POPULATIONS

The goal of herbicide resistance management is to prevent or delay the selection of herbicide resistant populations by reducing the selection pressure of a herbicide. To reduce this pressure an effective weed management program integrates the use of herbicides with mechanical, cultural, and biological control methods. The primary resistance management recommendations are summarized in Table 2. The question is: does understanding the mechanisms and genetics of resistance increase the effectiveness of these recommendations? To answer this question, let us consider how we can use information on the mechanisms and genetics of resistance for each of the recommendations listed in Table 2

Table 2. Herbicide Resistance Management Recommendations

1. Use historical weed densities or weed thresholds, as appropriate, to tailor the herbicide program to the weed spectrum. and weed pressure.
2. Use a diverse herbicide program that includes a tank-mix or sequential treatments with herbicides that have different modes of action and are effective on the same spectrum of weeds.
3. Use non-chemical weed control practices such as tillage or mowing in conjunction with herbicides whenever possible.
4. Rotate crops and use herbicides with different modes of action.
5. Discourage extended use of a single herbicide or herbicides with the same mode of action on the same field.
6. Use certified crop seeds, and clean equipment when moving from one field to another to prevent spreading of resistant weed seeds or plant material.

Knowledge of the weed species present in a field is critical for designing an effective weed management program. It is also needed for effective resistance management. The presence of an obligate outcrossing species, such as *L. rigidum*, may require additional measures for resistance management as compared to a predominantly selfing species, such as *S. viridis*. Holtum and Powles (1991) suggested that the many different mechanisms of resistance that have been selected in *L. rigidum* populations is due to the high genetic diversity of this species and its ability to exchange genetic information between populations. Thus, depending on herbicides alone for controlling this weed species has resulted in the selection of many resistant populations in Australia that contain multiple mechanisms of resistance. On the other hand, herbicide rotations or mixtures may be much more effective in limiting the development and spread of resistance in *S. viridis*.

So, if one knows the potential mechanisms of resistance to the herbicides being applied as well as the inheritance pattern for that resistance, more effective management strategies can be implemented. Unfortunately, our understanding of the biology and genetics of many weed species is limited, and this information may not be available, even if we know which weeds infest an area.

An effective resistance management program does not rely solely on one herbicide or herbicides with the same mode of action. One way to reduce reliance on a single herbicidal mode of action is to combine herbicides with different modes of action either in rotations, mixtures or sequential applications. However, to know what combinations of herbicides will be effective one must know the potential mechanisms of resistance. Triazine resistance is due to an alteration at the target site of the herbicide (Gronwald, 1994). Resistance has been selected where a triazine was the only herbicide used. In areas where the triazine was combined with another herbicide,with another mode of action, such as an acetanilide, resistance has not developed in those weeds that are susceptible to both of these herbicides (Stephenson, *et al.*, 1990). In these cases, herbicide mixtures have been an effective management practice to prevent the development of resistance.

However, if the primary mechanism of resistance is due to alteration of the metabolism of a herbicide, combinations of herbicides must include compounds that not only have a different mechanism of action but are detoxified by different metabolic routes. In the case of multiple resistant populations of *L. rigidum* and *A. myosuroides*, the resistant biotypes appear to have elevated levels of mixed function oxidases. Thus, an effective tank-mix partner herbicide should not be detoxified via mixed function oxidases.

Knowledge of the genetics of resistance can also help prevent the spread of resistant biotypes. If the resistant trait is inherited as a dominant, nuclearly-encoded trait, resistance can spread from a resistant population outside a field to populations within a field. Thus, to control the spread of resistance it is critical that farmers control the weeds around the edges of the field as well as within the field (Thill, *et al.*, 1994).

On the other hand, Maxwell, et al. (1990) suggested that farmers leave strips of susceptible weeds within the field to prevent the selection of resistance if the resistance is inherited as a recessive trait in an outcrossing species. Under these conditions, pollen from the susceptible weeds will prevent the resistance from being expressed. Ghersa, *et al.* (1994) found that the level of diclofop-methyl resistance in *Lolium multiflorum* could be decreased 6% per year by manipulating the pollen flow in the resistant population between susceptible and resistant populations.

As seen in Table 1, most of the cases of resistance appear to be due to target site alteration that is a single gene trait. One way to decrease the probability of selecting for this type of resistance is to decrease the rate and frequency of herbicide applications (Gressel and Segel, 1990). However, reducing the use rate may select for polygenic resistance (Gressel, 1994). If we do not know the genetics of resistance, then we cannot know what effect different weed management practices will have on the selection for resistance. Thus, it is important to understand the genetics of resistance to determine the selection pressure of different management practices.

Molecular biology has the potential to play a role in managing resistance if it can be used to determine the initial frequency of resistance in a population. If the resistant trait does not exist within a population, it cannot be selected. Gutierri *et al.* (1992) found an excellent relationship between the restriction fragment length polymorphisms (RFLPs) of polymerase chain reaction amplification products and sulfonylurea resistance in several *K. scoparia* populations. This occurred because the mutation conferring resistance to sulfonylureas also altered a restriction enzyme site in the gene. If a population contained these mutations, then RFLP analysis of that population would reveal it and one would know that resistance could rapidly increase in that population. However, this relationship between the changes in RFLP and ALS resistance did not hold up in a sulfonylurea-resistant *K. scoparia* collection where the mutation for resistance did not occur at this restriction site. As our knowledge of the molecular genetics of different resistant biotypes increases, there may be more application for this technology.

Information on the mechanisms and genetics of resistance is also important for predicting the development of resistance using various models (Maxwell, *et al.*, 1990; Gressel and Segel, 1990; Jasieniuk and Maxwell, 1994). These models are based on the initial resistance gene frequency, mode of inheritance of resistance, the gene flow and breeding system within a weed population, and the fitness of resistant versus susceptible populations. If this information is known, then these models can more accurately predict the development of resistance and help identify the most effective management strategies. However, in many cases this information is not available until after resistance has been selected in the field.

REACTIVE VERSUS PROACTIVE RESISTANT WEED MANAGEMENT

Although knowledge of the mechanisms and genetics of herbicide resistance can help us in our weed management strategies, this information is often not available until after resistance has occurred. If we continue to wait until resistance develops before obtaining this information, we will be forced to manage resistance in a reactive manner, giving up weed control on certain species and relying on the development of new techniques or management strategies to keep the problem from getting out of hand.

A better approach is to manage all herbicides as if they have the potential to select for resistance. We now have laboratory systems that can provide information on potential mechanisms of resistance that might develop for new herbicides as well as the genetic attributes of those mechanisms. Using this information should help us manage resistance in a more proactive way.

We can take advantage of the tools supplied by the many advances in plant tissue culture, molecular genetics and biochemistry to determine the site of action of new compounds and to select for resistant biotypes in the laboratory early in the development process of a new herbicide. In addition, the mechanism of crop selectivity of new herbicides will often tell us how the herbicide can be metabolically detoxified. This information, in turn, can be used to predict how rapidly and what mechanisms of resistance might develop as well as indicate which management practices will be the most effective in preventing resistance from developing.

Early in the development of the ALS and ACCase inhibitors, researchers selected for resistance to both of these classes of herbicides through cell culture selection (Saari, *et al.*, 1994; Devine and Shimabukuro, 1994). Analyses of resistant plants regenerated from these cultures showed that the mechanism of resistance was due to an alteration at the target site for the herbicides that was inherited as a single, semidominant trait (Saari *et al.*, 1994). Molecular genetic analysis of the ALS resistant biotypes showed there are at least 10 sites within the ALS genome that can contain a mutation which will make the enzyme resistant to the inhibitors (Saari *et al.*, 1994). As described above, mutations in the ALS gene have been responsible for the ALS inhibitor resistant weed populations that have been selected in the field.

The mechanism of crop selectivity of most herbicides is due to the ability of the crop to metabolically detoxify the herbicide. Continuous use of these herbicides has also selected weed biotypes that can detoxify the herbicides in a similar manner. For example, isoproturon-resistant *A. myosuroides* biotypes have the ability to detoxify this herbicide via mixed function oxidases (Hall, *et al.*, 1994). In the development process for a new herbicide, the metabolic pathway of that herbicide in the crop is often determined. This information could be used to determine which herbicides might be effectively mixed or rotated with the new herbicide and not select for the same metabolic detoxification pathway.

Molecular analysis of laboratory-generated-resistant biotypes may reveal exploitable molecular tags that could be used to screen weed populations for the presence of resistant biotypes. This approach has been successfully used in screening wild insect populations for the presence of a gene that is linked to resistance to pyrethroids (Taylor, *et al.*, 1993). If molecular markers for herbicide resistance can be found and used to screen weed populations, this information could be used to tailor a weed management program that will minimize the selection pressure on populations that contain the resistant trait.

CONCLUSION

To answer the question posed in the introduction: yes, understanding the mechanisms and genetics of herbicide resistance can aid in managing resistant weed populations more effectively. This information is vital for choosing which herbicides to tank mix or rotate in weed management programs in order to avoid herbicides with the same mode of action or which are detoxified via the same metabolic pathway. Knowledge of the inheritance of resistance and of the way genes flow within a weed population will increase the effectiveness of non-herbicidal weed control practices to minimize the spread of resistance into susceptible populations. However, it is important that farmers manage their weed problems so that they minimize selection for resistance. This means that all herbicides should be part of an integrated weed management program whether or not resistance has been selected.

REFERENCES

Burnet, M W M; Hart, Q; Holtum, J A M; Powles, S B (1994) Resistance to nine herbicide classes in a population of rigid ryegrass (*Lolium rigidum*). *Weed Science*. **42**, 369-377.

Darmency, H (1994) Genetics of herbicide resistance in weeds and crops. In: *Herbicide Resistance in Plants: Biology and Biochemistry,* S E Powles & J A M Holtom (eds), Boca Raton, FL : CRC Press, Inc., pp.263-298.

Devine, M D; Shimabukuro, R H (1994) Resistance to acetyl coenzyme A carboxylase inhibiting herbicides. In: *Herbicide Resistance in Plants: Biology and Biochemistry,* S E Powles & J A M Holtom (eds), Boca Raton, FL : CRC Press, Inc., pp.141-170.

Ghersa, C M; Marines-Ghersa, M A; Brewer, T G; Roush, M L (1994) Use of gene flow to control diclofop-methyl resistance in Italian ryegrass (*Lolium multiflorum*). *Weed Technology*. **8**, 139-147.

Gressel, J. (1994) Catch 22-mutually exclusive strategies for delaying/preventing polygentically vs. monogenetically inherited resistances. In: *Eight International Congress of Pesticide Chemistry: Option 2000,* N. N. Ragsdale, P. C. Kearney, & J. R. Plimmer (eds), Washington, D. C. American Chemical Society, pp.330-339.

Gressel, J; Segel, L A (1990) Modelling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. *Weed Technology*. **4**, 186-198.

Gronwald, J W (1994) Resistance to photosystem II inhibiting herbicides. In: *Herbicide Resistance in Plants: Biology and Biochemistry,* S E Powles & J A M Holtom (eds), Boca Raton, FL : CRC Press, Inc., pp.27-60.

Guttieri, M J; Eberlein, C V; Mallory-Smith, C A; Thill, D C; Hoffman, D L (1992) DNA sequence variation in domain A of the acetolactate synthase genes of herbicide-resistant and -susceptible weed biotypes. *Weed Science*. **40**, 670-676.

Guttieri, M J; Eberlein, C V; Thill, D C (1995) Diverse mutations in the acetolactate synthase gene confer chlorsulfuron resistance in Kochia (*Kochia scoparia*) biotypes. *Weed Science*. **43**, 175-178.

Hall, L M; Holtum, J A M; Powles, S B (1994) Mechanisms responsible for cross resistance and multiple resistance. In: *Herbicide Resistance in Plants: Biology and Biochemistry,* S E Powles & J A M Holtom (eds), Boca Raton, FL : CRC Press, Inc., pp.243-262.

Heap, I M (1994) Identification and documentation of herbicide resistance. *Phytoprotection*. **75**(suppl.), 85-90.

Holtum, J A M; Powles, S B (1991) Annual ryegrass: an abundance of resistance, a plethora of mechanisms. *Proceedings Brighton Crop Protecion Conference-Weeds 1991,* **3,** .pp. 1071-1078.

Jasieniuk, M; Maxwell, B D (1994) Population genetics and the evolution of herbicide resistance in weeds. *Phytoprotection*. **75**(suppl.), 25-36.

Jasieniuk, M; Morrison, I N; Brule-babel, A L (1995) Inheritace of dicamba resistance in wild mustard (*Brassica kaber*). *Weed Science*. **43**, 192-195.

Leah, J M; Caseley, J C; Riches, C R; Valverde, B (1995) Age-related mechanism of propnail tolerance in jungle-rice, *Echinochloa colona*. *Pesticide Science*. **43**, 347-354.

Matthews, J M (1994) Management of herbicide resistant populations. In: *Herbicide Resistance in Plants: Biology and Biochemistry,* S E Powles & J A M Holtom (eds), Boca Raton, FL : CRC Press, Inc., pp.317-336.

Maxwell, B D; Roush, M L; Radosevich, S R (1990) Predicting the evolution and dynamics of herbicide resistance in weed populations. *Weed Technology*. **4**, 2-13.

Morrison, I N; Devine, M D (1994) Herbicide resistance in the Canadian prairie provinces: five years after the fact. *Phytoprotection* **75**(suppl.), 5-16.

Moss, S R (1990) Herbicide cross-resistance in slender foxtail (*Alopecurus myosuroides*). *Weed Science*. **38**, 492-496.

Murray, B G; Morrison, I N; Brule-Babel, A L (1995) Inheritance of acetyl-coA carboxylase inhibitor resistance in wild oat (*Avena fatua*). *Weed Science*. **43**, 233-238.

Richter, J; Powles, S B (1993) Pollen expression of herbicide target site resistance genes in annual ryegrass (*Lolium rigidum*). *Plant Physiology*. **102**, 1037-1041.

Saari, L L; Cotterman, J C; Thill, D C (1994) Resistance to acetolactate synthase inhibiting herbicides. In: *Herbicide Resistance in Plants: Biology and Biochemistry,* S E Powles & J A M Holtom (eds), Boca Raton, FL : CRC Press, Inc., pp.83-140

Smeda, R J; Vaughn, K C (1994) Resistance to dinitroaniline herbicides. In: *Herbicide Resistance in Plants: Biology and Biochemistry,* S E Powles & J A M Holtom (eds), Boca Raton, FL : CRC Press, Inc., pp.215-228.

Stallings, G P; Thill, D C; Mallory-Smith, C A; Shafii, B (1995) Pollen-mediated gene flow in sulfonylurea-resistant Kochia (*Kochia scoparia*). *Weed Science*. **43**, 95-102.

Stephenson, G R; Dykstra, M D; McLaren, R D; Hamil, A S (1990) Agronomic practices influencing triazine-resistant weed distribution in Ontario. *Weed Technology*. **4**, 199-207.

Taylor, M F J; Heckel, D G; Brown, T. M; Kreitman, M E; Black, B (1993) Linkage of pyrethroid insecticide resistance to a sodium channel locus in tobacco budworm. *Insect Biochemistry and Molecular Biology*. **23**, 763-775.

Thill, D C; O'Donovan, J T; Mallory-Smith, C A (1994) Integrated weed management strategies for delaying herbicide resistance in wild oats. *Phytoprotection* **75**(suppl.), 61-70.

TECHNIQUES FOR DETERMINING HERBICIDE RESISTANCE

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ABSTRACT

Testing for resistance is a vital component for the rational implementation of integrated control strategies. Ideally, diagnostic tests for resistance should be rapid, accurate, cheap, readily available and give a reliable indication of the likely impact of resistance on herbicide activity in the field. This paper reviews techniques for determining resistance, with emphasis on procedures suitable for initial identification or confirmation of resistance, rather than research techniques, including: field observations and experimentation, whole plant studies, Petri-dish assays, chlorophyll fluorescence, leaf disc flotation and novel techniques such as pollen germination.

INTRODUCTION

The factors favouring the evolution of resistance are well documented (Maxwell & Mortimer, 1994), but our ability to predict resistance development on an individual farm scale remain poor. Resistance has usually developed in one, or at most a few, species within a weed community despite all being exposed to the same intensity of herbicide use.

Worldwide experience has been that farmers and growers tend to do little about resistance management until it has been detected on their own farm - or their neighbours. An essential pre-requisite for confirmation of resistance is a good diagnostic test. Ideally this should be rapid, accurate, cheap, readily available and provide a reliable indication of the likely impact of resistance on herbicide performance in the field.

This paper will review some of the techniques used in the determination of resistance. The emphasis will be on techniques used for the initial identification or confirmation of a suspected resistance problem, rather than research techniques such as enzyme assays used for understanding the nature of resistance mechanisms.

DETECTION OF RESISTANCE

The most important single factor determining the ease of identifying resistance is the *degree of insensitivity*. Small differences may have an appreciable effect on herbicide efficacy in the field so should not be discounted, but the detection and interpretation of relatively small effects is more difficult than situations where resistance is absolute.

The importance of differences in the resistance status of individual plants within a population should be recognised as it can affect the interpretation of any test result. Resistance may be due to a quantitative increase in level of resistance of all individuals within a population or an increase in the proportion of very resistant types.

1. FIELD OBSERVATION

Accurate field observation is important so that any reduction in herbicide efficacy can be detected. This *may* indicate developing resistance. However, many other factors, apart from resistance, may be responsible for poor herbicide performance. These include:

(a) *Herbicide application factors*: inappropriate choice of herbicide; dose rate too low; incorrect timing; wrong water volume rate; inadequate or faulty spraying equipment; poor application technique; omission of recommended adjuvants; use of non-recommended tank mixes or sequences; adverse environmental conditions at time of application.

(b) *Soil conditions*: soil moisture; pH; seedbed quality; adsorption due to high organic matter levels or the presence of surface trash.

(c) *Climatic conditions*: rainfall patterns; temperature.

(d) *Weed factors*: size of weeds; germination after spraying; depth of rooting; excessively high infestation level; inadequate target due to crop shielding.

Because so many factors may be responsible for inadequate herbicide performance it is often difficult or impossible to determine the exact cause of herbicide failure in the field. It is essential that resistance is not cited as a reason or excuse for herbicide failure without supporting evidence.

Initial suspicion of resistance will usually result from unsatisfactory weed control following a herbicide application in the field. The Herbicide Resistance Action Committee (HRAC) has produced a protocol for use when responding to weed control failures (HRAC, undated), and many of the points raised are detailed below. Resistance should not be assumed, and other possible reasons for failure will need to be considered. However, resistance must be considered as a possible cause, especially if all other factors have been eliminated. Although it is rarely possible to confirm resistance solely on the basis of field observation and consideration of field records, several factors will point in this direction.

These are: (a). *The level of weed control of other susceptible species*. If these have been controlled effectively, then resistance is a distinct possibility. (b). *The presence of alive plants adjacent to dead individuals*. This may indicate the presence of resistant individuals, although such situations can arise through variations in weed growth stage, incorrect application or through crop shielding. (c). *Past experience*. If the surviving species has been controlled successfully by the same treatment in the past, or a gradual decline in control has been noticed over a period of years, resistance may be responsible. (d). *Herbicide history*. The repeated annual use of the same herbicide, or herbicides with the same mode of action, favours selection for resistance. (e). *Occurrence of resistance in the vicinity*. If resistance in the same weed and involving the same herbicide has been positively identified in adjacent fields or farms, then there is a high probability that resistance is implicated. (f). *Cropping and cultural history*. Many cases of resistance are associated with intensive agricultural systems involving crop monoculture and minimum tillage. These systems appear more prone to the development of resistance.

2. FIELD EXPERIMENTATION

It may be possible to conduct a field experiment on a suspected resistant population in the same year as the reported herbicide failure. This has the advantages that the experiment can be sited precisely in the affected area, it is relatively easy to conduct, extra information is collected in the same growing season and the results can give practical information for use in the following crop. The limitations are that experiments established in the same crop year are often, by necessity, set up relatively late, so applications of herbicides may not be at the ideal timings. Also, unless crop damage is disregarded, the choice of herbicides will be limited to those that can be used within that crop. Using repeat applications or doses higher than those approved for use may be illegal unless an experimental permit is obtained and may involve destroying the crop. Alternatively, field experiments may be set up in the same field in a subsequent crop. This allows more flexibility in choice of herbicides and timings.

The value, and limitations of field trials can be demonstrated with the following example for a site where fluazifop-P-butyl failed to control *Avena fatua* (wild-oats) in a field of oil-seed rape in Kent, UK. An unreplicated trial was established by staff of Willmot Pertwee Ltd. and herbicides applied with recommended adjuvants on 6 April 1994 when wild-oats were at the 1-2 node stage. The herbicides included some not recommended for use in oil-seed rape, so the trial area was sprayed with glyphosate and not harvested. Herbicide activity was determined using a 1-10 score rating system on 16 May where 0=dead and 10=unaffected (Table 1). All the aryloxyphenoxypropionate herbicides gave no or minimal wild-oat control. In contrast the cyclohexanedione herbicides gave better control, especially cycloxydim which killed all plants. The other herbicides gave moderate levels of control. Such information is clearly of practical value, and the farmer concerned would be unwise to use aryloxyphenoxypropionate herbicides on that field as complete herbicide failure is likely.

However, what is the interpretation of the results for the herbicides giving intermediate levels of control? All these herbicides are capable of giving high levels of control of susceptible populations. Are the results due to the late application or is partial resistance responsible? Clearly there is no means of knowing from this data alone as no comparisons with a known susceptible standard can be made. Partial resistance to tralkoxydim, imazamethabenz and flamprop-M-isopropyl has been detected in another population in the UK (Moss & Clarke, unpublished), so it would be unwise to assume that partial resistance was not responsible.

While such field experiments can provide useful information of immediate practical use, they have the limitation that it is difficult to determine how much poor herbicide performance is due to resistance, and how much to other unrelated factors. This is a particular problem with cases of partial resistance and experiments involving soil acting herbicides in particular, as activity is greatly influenced by environmental conditions.

Table 1. Control of *Avena fatua* panicles on an unreplicated field trial. (Results reproduced with the permission of Willmot Pertwee Ltd.)

Treatment	Dose	Score (see text)
Untreated	-	10
Fluazifop-P-butyl	125 g/ha + wetter	10
Propaquizafop	100 g/ha	9
Quizalofop-ethyl	125 g/ha + oil	10
Fenoxaprop-P-ethyl	55 g/ha	9.5
Clodinafop-propargyl	30 g/ha + oil	10
Diclofop-methyl	1.14 kg/ha	10
Cycloxydim	200 g/ha + oil	0
Sethoxydim	338 g/ha + oil	3.5
Tralkoxydim	350 g/ha + oil + wetter	5
Difenzoquat	990 g/ha	4
Flamprop-M-isopropyl	600 g/ha + oil	3.5
Imazamethabenz	600 g/ha + wetter	5

3. WHOLE PLANT STUDIES

The most widely used test for resistance involves growing plants from seeds in glasshouse or controlled environment chambers. Typically plants are grown in pots of soil or nutrient medium and treated with herbicides applied either at a single discriminating dose, or more usually a range of doses. Assessments may involve visual assessments of mortality or vigour or measurements of fresh or dry weight of foliage.

With single dose assays the choice of dose is critical when resistance is partial. An essential component of all such tests is the inclusion of susceptible reference populations. With some forms of resistance, such as most cases of resistance to triazine herbicides, resistance tends to be absolute. For example Yaacoby *et al.* (1986) showed an absolute difference in response in three grass species treated with five doses of atrazine. All susceptible plants were killed by 0.25-1.0 kg/ha whereas all resistant plants survived up to 4 kg/ha. In such cases, resistance is easy to identify and choice of dose not critical - the same conclusion would have been made regardless of dose used.

This contrasts markedly with results from numerous studies with *Alopecurus myosuroides* (black-grass) populations from the UK where resistance is not absolute, and the level of control can vary markedly with dose used and between populations. A * rating system has been devised which encompasses the concept of varying degrees of resistance at the population level (Clarke & Moss, 1989). This * rating system describes different degrees of resistance to chlorotoluron based on a comparison with the % reduction in foliage weight values of three reference populations. Typical values for these three populations treated with 2.5-2.75 kg chlorotoluron/ha were: Rothamsted (susceptible) 93%;

Faringdon (partially resistant) 78%; Peldon (resistant) 33%. The inclusion of the reference populations is crucial as it enables comparisons to be made between experiments conducted at different times and at different locations. This * rating system has recently been updated for classifying populations for their degree of resistance to both chlorotoluron and fenoxaprop-ethyl (Clarke *et al.*, 1994).

Although single dose assays can be successful it is preferable to use a range of doses to obtain a response curve. This enables the degree of resistance to be better quantified by calculating the ratio of doses required to produce the same effect in the resistant and susceptible population. Usually the dose required to give a 50% reduction in the measured parameter, usually foliage weight or number of surviving plants, relative to the untreated control is determined. Ratios of these estimates, (variously termed ED_{50}, GR_{50}, LD_{50} or I_{50}), relative to that of a susceptible population provide a resistance index (RI) which enables the degree of resistance to be described relatively simply. To obtain a good estimate of ED_{50} the dose range should be relatively wide and usually at least six doses are needed. With highly resistant populations it may not be possible to obtain an ED_{50} value and so a precise resistance index cannot be calculated.

It is sometimes suggested that ED_{90} values are a better basis for comparison because these are closer to the level of control expected in the field. If dose response curves have the same asymptotic upper and lower limit and the same slope they are said to be parallel and the resistance index (or relative potency) is independent of response level (Streibig, 1992). However, parallelism cannot be assumed and in most cases ED_{50} values form the best basis for comparison because they can usually be fitted with greater precision than ED_{90} values.

Predicting the likely impact of resistance on field performance is difficult, unless resistance is absolute. Although pot evaluations have limitations they are capable of mimicking field applications and detecting resistance regardless of mechanism. This is a very positive attribute. The main limitation is the necessity of collecting seeds which may have innate dormancy, the time taken to get results and having a relatively high labour and glasshouse space requirement.

4. PETRI-DISH ASSAYS

Petri-dish assays have been used successfully for evaluation of resistance to a range of herbicides, including triazines, dinitroanilines and ACCase inhibitors. In most tests, seeds are germinated on filter paper or agar in the presence of herbicide and some growth parameter such as shoot or root length is assessed after one-three weeks. Clay and Underwood (1990) compared the response of resistant and susceptible biotypes of four weed species to simazine. The method distinguished between the resistant and susceptible biotypes of the four species but took 25 days to give a clear result.

Beckie *et al.* (1990) described a rapid bioassay for trifluralin-resistant *Setaria viridis* (green foxtail) based on measurement of radicle growth of seedlings exposed to trifluralin. Root length assessments were a more useful parameter for determining resistance than shoot length. In contrast Moss (1990) found that differences in root length between populations of *Alopecurus myosuroides* (black-grass) were much less pronounced than differences in shoot length in Petri-dish assays involving pendimethalin and trifluralin. Subsequent experiments indicated that the resistance mechanisms in the two species were different, which may account for this.

Heap and Knight (1986) described a germination test for evaluating resistance of *Lolium rigidum* populations in Australia to aryloxyphenoxypropionate herbicides. This tests involved measuring coleoptile length after 7 days and was capable of distinguishing resistant and susceptible biotypes. Gill (1990) evaluated this test and found that the assay tended to underestimate the level of herbicide resistance in comparison to pot experiments. He stressed that caution was needed not only in choice of herbicide dose but also in the interpretation of the results.

Smeda *et al* (1995) described a bioassay using seeds of *Sorghum halepense* (Johnsongrass) for evaluating resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides. In this test, herbicides were incorporated into the agar medium and fresh weight of seedlings were recorded after nine days. A good correlation between results from the Petri-dish bioassay and the more lengthy greenhouse studies was obtained.

Petri-dish assays take less time than pot tests and require little space, especially if conducted in incubators. However, such techniques will not be applicable to all forms of resistance. Interpretation of the results needs to be done with care, as herbicides are applied in a manner completely different to conventional field applications and this will affect method and speed of uptake. No single assessment will be appropriate for all assays and studies will be required to correlate results with whole plant responses. Another major limitation is that innate seed dormancy may severely reduce the potential advantage of this technique for rapid evaluation of fresh seed samples.

5. CHLOROPHYLL FLUORESCENCE IN INTACT LEAVES

Although many standard procedures for measuring photosynthesis are available, experiments tend to be complex, only a few samples can be examined simultaneously and many of the methods require considerable technical skill and expensive equipment (Truelove & Hensley, 1982). Consequently indirect methods of assessing photosynthetic activity, as detailed here and in section 6 have been devised.

Fluorescence has been used to study the mode of action of triazine resistance at the molecular level involving isolated chloroplasts but such studies go beyond the scope of this review. Fortunately fluorescence induction can be measured in intact leaves and this technique has been used in many studies of resistance to photosynthetic inhibiting herbicides, especially the triazines.

Changes in fluorescence signals give an indirect measure of photosynthetic activity in a much simpler way than measuring photosynthesis directly. Various types of equipment have been used, varying considerably in sophistication. The basic method involves the illumination of a dark adapted leaf pre-treated with herbicide, and then determining the emitted fluorescence transient (Rubin, 1995). Differences in the emitted fluorescence signal indicate the photosynthetic capacity of the leaves which gives an indication of their resistance status. The exact fluorescence parameters used vary considerably between authors, and are partly dependent on the sophistication of the equipment used, but the basic principle remains the same. Initially most of these studies involved resistance to triazine herbicides due to chloroplastic resistance which was usually absolute. Resistance due to enhanced metabolism is likely to be more difficult to detect with fluorescence because the degree of resistance is much smaller.

However, chlorophyll fluorescence has shown promising results when used for determination of quantitative resistance in plants conferred by enhanced metabolism (van Oorschot & Van Leeuwen, 1992; Rubin, 1995). At Rothamsted we have investigated the use of a commercial portable fluorescence meter ("Hansatech Plant Efficiency Analyser") for resistance detection in *Alopecurus myosuroides* (black-grass). The parameter used was area over the induction curve, in arbitrary units. The values are measured and displayed directly on the equipment. More sophisticated measurements are possible by linking to a computer such that the complete induction curve can be displayed, but for routine testing purposes the area measurement seems appropriate and necessitates no computer link up. An example of the results obtained, and a comparison with ED_{50} values obtained from glasshouse dose response experiments is shown in Table 2. Detached leaves were placed in tubes of chlorotoluron solution for three hours, transferred to tubes of water and readings taken 24 hours later. There was a good correlation between results from fluorescence and a conventional pot assay.

Table 2. Resistance evaluation of three *Alopecurus myosuroides* populations using chlorophyll fluorescence and pot assays.

	Fluorescence area measurements			Pot test
	Nil	Mean of three chlorotoluron concentrations[1]	% reduction in area	% reduction in foliage weight[2]
Rothamsted (susc.)	537	39	93%	92%
Faringdon	547	319	42%	67%
Peldon (resistant)	574	509	11%	6%

[1] = 4×10^{-4}, 1×10^{-3}, 5×10^{-3} M. [2] = Three weeks after spraying 2.75 kg chlorotoluron/ha.

6. LEAF DISC FLOTATION

Hensley (1981) described a simple bioassay for identifying triazine resistant and susceptible biotypes. Leaf discs from the tested plants were vacuum infiltrated in a phosphate buffer solution, causing the leaf discs to sink. The vacuum was then released, a bicarbonate solution added and on exposure to light sufficient oxygen was generated in intracellular spaces of photosynthesising discs to cause them to float to the surface. In the presence of atrazine, a photosynthetic inhibitor, discs from susceptible plants failed to float whereas those from resistant plants floated to the surface within one hour. Clay and Underwood (1990) successfully used this technique to compare response of three species to simazine. This technique can be conducted directly with leaves from suspect plants, but not all plant species can be used successfully.

An attempt was made to modify this test for detecting differences in response to the substituted-urea herbicide chlorotoluron in *Alopecurus myosuroides* which is resistant due to enhanced metabolism (Kemp *et al.*, 1990). Leaf sections about 4mm in length were cut from very young plants and 15 were added to test tubes containing 10 ml of a phosphate buffer solution. Chlorotoluron was added to the buffer to create the following concentrations (ppm) 0, 20, 50, 100. Plants from four populations were used. The sets of tubes were placed in a vacuum desiccator and a vacuum applied for 20 minutes. On release of the vacuum the leaf sections sank. The buffer was poured off and replaced with fresh buffer containing sodium bicarbonate at 2000 ppm (w/v) without herbicide. Tubes were placed about 30 cm under a 150 watt domestic table lamp and the number of leaf sections floating in each tube was determined at half hour intervals for three hours. The maximum % floating during this period was used as the assessment criteria.

Table 3. Resistance evaluation of four *Alopecurus myosuroides* populations using a leaf flotation method and a pot assay.

	Leaf section flotation experiment: maximum % leaf sections floating in 4 hours; chlorotoluron concentration (ppm)					Pot test
	0	20	50	100	mean	% reduction in foliage weight[1]
Rothamsted (susc.)	100	9	0	10	6	90%
Faringdon	100	40	36	0	25	70%
Peldon A1 (resist.)	100	77	86	50	51	9%
Peldon B2 (resist.)	100	78	54	70	56	1%

[1]= Three weeks after spraying 3.5 kg chlorotoluron/ha.

The results demonstrate that there was a good correlation between resistance detected in pot tests and that determined from the floating leaf assay, and it was possible to discriminate between different degrees of resistance (Table 3). These results show that leaf flotation systems are capable of detecting metabolism based resistance. The degree of resistance imposed by enhanced metabolism in *Alopecurus myosuroides* is far less than for most examples of chloroplastic resistance. It should be noted that the technique was laborious and it was concluded that it was not applicable for routine screening purposes.

7. NOVEL TECHNIQUES

Richter and Powles (1993) demonstrated that biotypes of *Lolium rigidum* (annual rye-grass) resistant to ALS and ACCase inhibiting herbicides expressed this resistance in the pollen. In the presence of herbicides, pollen from resistant biotypes germinated well whereas that from susceptible biotypes was inhibited. This technique may form the basis for a rapid screen for certain target-site based herbicide-resistance mechanisms.

Gerwick *et al* (1993) described a method for rapid diagnosis of ALS resistant weeds based on the differential accumulation of acetoin in the presence and absence of an ALS inhibiting herbicide. Inhibition of ALS in susceptible plants prevents the build up of acetoin and forms the basis for distinguishing between sensitive and resistant biotypes. Further development of this technique may allow the production of a kit for field use.

Immunological (ELISA) and DNA analysis techniques may become realistic options for routine testing for herbicide resistance in future, but are initially more likely to have greater impact in the research field.

CONCLUSIONS

A crucial difference between herbicide and other types of resistance is the potential to grow plants from seeds of resistant plants. Most weed seeds can be stored for long periods in dry conditions, often for several years. This is a major advantage compared to many plant pathogens or insects, where long term storage of material for bioassays may be difficult or impossible.

The glasshouse pot assay is likely to remain the most appropriate single test for resistance as herbicide application and activity mimic what happens in the field. The relative simplicity of such a test is a major advantage but the time consuming nature and delay in obtaining a result are major constraints. More specific resistance assays may be quicker and more precisely identify the mechanisms responsible, but their very precision may be a limitation, especially where multiple mechanisms of resistance exist.

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REFERENCES

Beckie, H J; Friesen, L F; Nawolsky, K M; Morrison, I N (1990) A rapid bioassay to detect trifluralin-resistant green foxtail (*Setaria viridis*). *Weed Technology*. **4**, 505-508.

Clarke, J H; Moss, S R (1989) The distribution and control of herbicide resistant *Alopecurus myosuroides* (black-grass) in central and eastern England. *Proceedings of the Brighton Crop Protection Conference - Weeds*, 301-308.

Clarke, J H; Blair, A M; Moss, S R (1994) The testing and classification of herbicide resistant *Alopecurus myosuroides* (black-grass). *Aspects of Applied Biology* **37**, *Sampling to Make Decisions*, 181-188.

Clay, D V; Underwood, C (1990) The identification of triazine- and paraquat-resistant weed biotypes and their response to other herbicides. *EUR 11561 - Importance and Perspectives on Herbicide-resistant Weeds*, R Cavalloro; G Noye, (eds), Office for Official Publications of the European Communities, Luxembourg, pp. 47-55.

Gerwick, B C; Mireles, L C; Eilers, R J (1993) Rapid diagnosis of ALS/AHAS-resistant weeds. *Weed Technology*. **7**, 519-524.

Gill, G S (1990) Evaluation of the Petri dish assay for screening diclofop-methyl resistance in partially resistant populations of annual ryegrass, *Lolium rigidum*. *Proceedings of the 9th Australian Weeds Conference*, 220-223.

Heap, I; Knight, R (1986) The occurrence of herbicide cross-resistance in a population of annual ryegrass, *Lolium rigidum*, resistant to diclofop-methyl. *Australian Journal of Agricultural Research*. **37**, 149-156.

Hensley, J R (1981) A method for identification of triazine resistant and susceptible biotypes of several weeds. *Weed Science*. **29**, 70-73.

Herbicide Resistance Action Committee (HRAC) How to minimize resistance risks and how to respond to cases of suspected and confirmed resistance. Leaflet, 10 pp.

Kemp, M S; Moss, S R; Thomas, T H (1990) Herbicide resistance in *Alopecurus myosuroides*. In: *Managing Resistance to Agrochemicals: from Fundamental Research to Practical Strategies*, M B Green, H M LeBaron, H M & W K Moberg (eds), Washington: American Chemical Society, pp. 376-393.

Maxwell, B D; Mortimer, A M (1994) Selection for herbicide resistance. In: *Herbicide Resistance in Plants - Biology and Biochemistry*, S B Powles & J A M Holtum (eds), Florida: CRC Press, pp. 1-26.

Moss, S R (1990) Herbicide cross-resistance in slender foxtail (*Alopecurus myosuroides*). *Weed Science*. **38**, 492-496.

Richter, J; Powles, S B (1993) Pollen expression of herbicide target site resistance genes in annual ryegrass (*Lolium rigidum*). *Plant Physiology*. **102**, 1037-1041.

Rubin, B (1995) Monitoring and identification of herbicide-resistant weeds. *International Symposium on Weed and Crop Resistance to Herbicides*, Cordoba, Spain, April 1995 (in press).

Smeda, R J; Barrentine, W L; Snipes, C E; Rippee, J H (1995) Identification of Johnsongrass resistance to graminicides and alternative control methods. *International Symposium on Weed and Crop Resistance to Herbicides*, Cordoba, Spain, April 1995 (in press).

Streibig, J C (1992) Quantitative assessment of herbicide phytotoxicity with dilution assay. Department of Agricultural Sciences, Royal Veterinary and Agricultural University, Copenhagen. 98 pp.

Truelove, B; Hensley, J R (1982) Methods of testing for herbicide resistance. In: *Herbicide Resistance in Plants* H M LeBaron & J Gressel (eds), Wiley-Interscience, pp. 117-131.

Van Oorschot, J L P; Van Leeuwen, P H (1992) Use of fluorescence induction to diagnose resistance of *Alopecurus myosuroides* Huds. (black-grass) to chlorotoluron. *Weed Research*. **32**, 473-482.

Yaacoby, T; Schonfeld, M; Rubin, B (1986) Characteristics of atrazine-resistant biotypes of three grass weeds. *Weed Science*. **34**, 181-184.

MANAGING WEED RESISTANCE : THE ROLE OF THE AGROCHEMICAL INDUSTRY

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ABSTRACT

During the last fifty years, the agrochemical industry has produced a range of herbicides of increasing sophistication which have been adopted globally. However, heavy reliance on chemical weed control linked to practices such as continuous cropping has resulted in over one hundred weed species developing resistant biotypes. The agrochemical industry responded during the last decade by forming the Herbicide Resistance Action Committee (HRAC) which has been involved in verifying resistance cases, proposing management strategies, organising education initiatives, setting up monitoring programmes, and sponsoring fundamental research. Legislation is also being introduced in different countries to delay resistance and aid the management of resistant weeds. Management techniques advocated by the HRAC include the use of mixtures, alternating modes of action and adopting specific cultural practices to improve the longevity of current herbicides. Key to the success of the chemical strategy is a knowledge of the modes of action of products available. This approach has been pioneered in Australia and the HRAC has now developed a guide and is working with groups such as the WSSA to develop common, acceptable guidelines for global use.

INTRODUCTION

Modern crop production is highly dependent on agrochemicals, and abundant, sustained food production is attributable, in part, to the use of herbicides. During the latter half of the twentieth century, the agrochemical industry has produced a range of increasingly sophisticated herbicides which have been adopted enthusiastically on a global basis. However, heavy reliance on chemical weed control with a limited number of active ingredients, linked to practices such as continuous cropping, has resulted in a number of cases of weeds developing resistance to herbicides. This paper reviews the current herbicide market and the incidence of weed resistance, and focuses on key elements of industry's response to managing herbicide resistance.

CURRENT HERBICIDE MARKET

Global herbicide sales in 1994 were $12.995 billion (Wood Mackenzie, 1995) and accounted for nearly half of all agrochemicals used, with 65% of the market being in North America and

Western Europe (Figure 1).

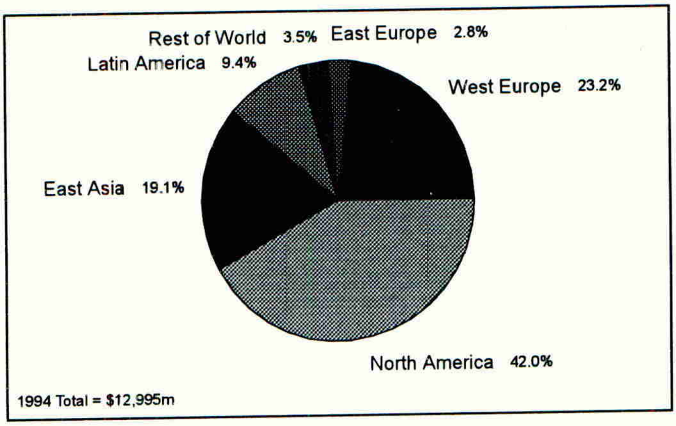

Figure 1. Herbicide sales by region
(from Wood Mackenzie, 1995)

In terms of crop use, the major herbicide sectors were cereals, maize, rice, soya, and fruit and vegetables which accounted for nearly three quarters of the market. Details of herbicide use by crop is shown in Figure 2.

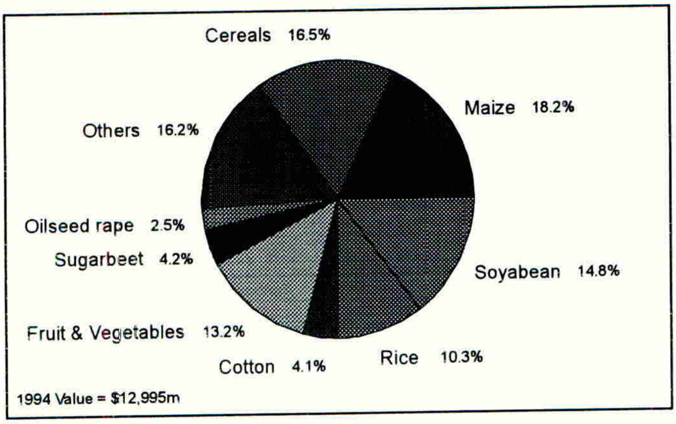

Figure 2. 1994 Herbicide Sales by Crop
(from Wood Mackenzie, 1995)

Key herbicide groups currently used include triazines, amides, carbamates, ureas, toluidines, diazines, diphenyl ethers and hormone weed killers, but the chemical groups showing increased growth in the past few years are sulfonyl ureas, imidazolinones, aryloxyphenoxy propanoates, glyphosate and paraquat. In terms of future prospects, Wood Mackenzie (1995) are predicting an annual growth rate for herbicide sales of 1.4% p.a., with the bulk of the growth expected in North America, Latin America and East Asia.

HERBICIDE RESISTANCE

Definition of terms

It is critical to have accepted definitions for resistance if the phenomenon is to be managed effectively, as a very large number of cases reported by farmers and growers are not confirmed as they are attributable to factors such as misapplication. The following definitions are accepted by the agrochemical industry (see Rubin, 1991; O'Keeffe et al, 1993).

Resistance is the naturally occurring inheritable ability of some weed biotypes within a population to survive a herbicide treatment that would, under normal conditions of use, effectively control that weed population. Selection of resistant biotypes may eventually result in control failures.

Cross-resistance is where a weed biotype is resistant to two or more herbicides due to the presence of a single resistance mechanism.

Multiple resistance refers to situations where resistant plants possess two or more distinct resistance mechanisms.

Confirmed cases of herbicide-resistant weeds

The first case of a weed species resistant to herbicides was observed in the 1960s, when *Senecio vulgaris* was confirmed to be resistant to triazines (Ryan, 1970). Since then, there has been a steady increase in the number of resistant species and classes of herbicides to which resistance has evolved. Le Baron (1991, 1992) reviewed the extent of herbicide resistance at the beginning of the 1990s and concluded that there were over one hundred grass and broadleaved weed species which had developed resistant biotypes. In total, these biotypes had been found in over forty countries. The resistance is reported to cover fifteen different modes of action, including inhibitors of photosynthesis at photosystem II such as triazines, the ACCase (acetyl CoA carboxylase) inhibitors - aryloxyphenoxy propanoates and cyclohexanediones, and the ALS (acetolactate synthase) inhibitors - sulfonylureas, imidazolinones, sulfonamides and triazolopyrimidines. The results of Le Baron's survey are summarised in Table 1.

Table 1 - Occurrence of resistance to different herbicide groups (modified from Le Baron 1991, 1992)

Herbicide group	Example	Number of resistant biotypes	Number of countries in which resistant biotypes have been reported
Triazine	Atrazine	58	22
Bipyridiliums	Paraquat	16	9
ALS-inhibitors* (e.g. sulfonyl-ureas; imidazolinones)	Chlorsulfuron	8	4
Phenyl-ureas	Chlortoluron	6	3
Phenoxy-alkanoic acids	MCPA, mecoprop	6	5
ACCase inhibitors** (e.g. cyclohexanediones; aryloxyphenoxypropanoates)	Diclofop-methyl	4	5
Dinitroanilines	Trifluralin	3	2
Amides	Propanil	2	2
Triazoles	Aminotriazole	2	2
Uracils	Bromacil	2	1
Carbamates	Phenmedipham	2	1
Pyridazines	Chloridazon	1	3
Nitriles	Bromoxynil	1	1
Organoarsenicals	Sodium hydrogen methylarsonate (MSMA)	1	1
Unclassified	Picloram	1	1
	Total :	113	

*ALS, acetolactate synthase (also referred to as AHAS, acetohydroxyacid synthase)
** ACCase, acetyl-coenzyme A carboxylase

Since the survey was completed, new cases of resistance have been confirmed, including the first case of resistance to ALS-inhibitors in Europe - reported in Denmark for *Stellaria media*. No comprehensive survey has been completed since the study by Le Baron, but industry has cooperated with the Weed Science Society of America (WSSA) in generating up-to-date information on the extent of resistance in 1995 and results should be published imminently. However, it is worth stressing that with the exception of triazines, only a very small proportion of agricultural land has resistant weed problems.

THE ROLE OF THE AGROCHEMICAL INDUSTRY IN MANAGING HERBICIDE RESISTANCE

Organisation

During the 1980s, in response to an increase in reported cases of weed resistance, the agrochemical industry formed an international industry-led Herbicide Resistance Action Committee (HRAC). This committee consists of technical representatives from the major herbicide producing companies, and together with the Insecticide, Fungicide and Rodenticide Resistance Action Committees (IRAC, FRAC and RRAC) functions under the auspices of the International Group of National Associations of Agrochemical Manufacturers (GIFAP). The companies currently represented on the HRAC are listed in Table 2.

Table 2. HRAC Membership

Company	Representative
AgrEvo	Mr Sam Howard
BASF	Dr Helmut Walter
Bayer	Dr Robert Schmidt
Ciba	Dr David Neville
Cyanamid	Dr Zia Rafii
DowElanco	Mr Antony Straszewski
DuPont	Dr Len Saari
Monsanto	Dr James Graham (Chairman)
Rhone-Poulenc	Mr Gordon Flemons
Rohm & Haas	Mr Steve Connor
Sandoz	Mr Richard Hess
Tomen	Mr Roger Gaillot
Zeneca	Dr Alan Jutsum

Additional companies attending Work Group Meetings : Kumiai, Nissan.

The objective of the HRAC is to delay and manage effectively herbicide resistance in weeds in order to minimise the impact of resistance on agricultural production. The committee liaises with universities, advisory and extension services, farmers, distributors and governments, and is involved in formulating and communicating resistance management strategies both in open meetings and directly to the farmer. At the outset, the HRAC set up three Work Groups to focus on resistance to ALS inhibitors, triazines and grass herbicides (Jutsum & Shaner, 1992). These groups were very effective in verifying resistance, proposing management strategies, organising education initiatives, setting up monitoring programmes, sponsoring fundamental research, and guiding the activities of various national sub-groups. During the nineties, the HRAC has spent over $300,000 supporting herbicide resistance work. Expenditure has been primarily on academic research to verify resistance and its mechanism of action, and spread under field conditions. Funds have also been used for disseminating information, including the production of monographs on grass weed resistance and herbicide mode of action, and sponsoring various scientific weed resistance meetings. However, during the last year, the industry has agreed that resistance demands have changed and that Work Groups can discharge their accountabilities more effectively if they focus on specific geographical regions. The current groups are displayed in Figure 3.

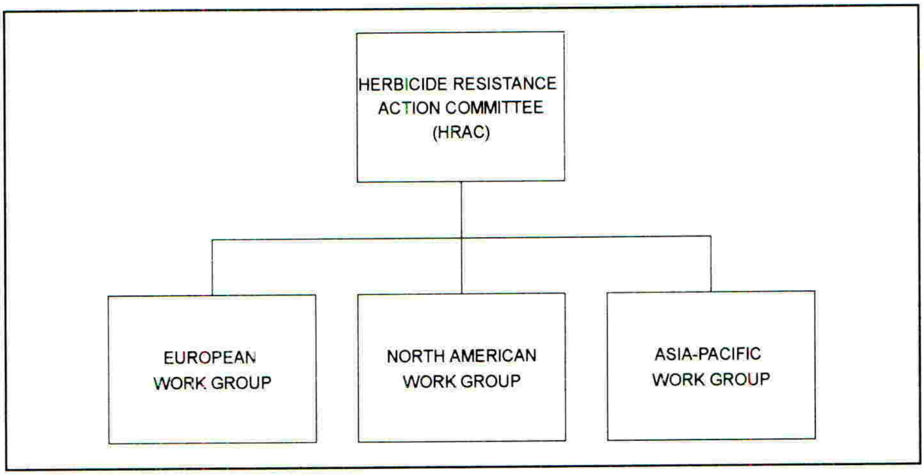

Figure 3. Organisation of HRAC Work Groups

Development of legislation

Effective weed management relies on a knowledge of which combinations of herbicide and weed could lead to resistance and also on the reliable, early detection of resistant populations.
When resistance is suspected by a farmer or grower, seed samples should be collected and evaluated using a whole plant bioassay in growth rooms or possibly in the field. These assays can be used to verify resistance by comparing complete dose response curves for the population suspected of being resistant with a reference susceptible population, and are preferable to the use of in vitro techniques. This area has been reviewed recently by Heap (1994) and Moss (1995).

Legislation regarding the assessment of resistance risk, resistance identification, and the management of resistance is being introduced in some countries, such as Italy and Holland, and the HRAC and the agrochemical industry want to work closely with those developing guidelines. However, the HRAC, along with the other Resistance Action Committees, have provided considered, agreed inputs on resistance management for the European Union Registration Directives, 91-414 EEC and 93/71/EEC, but unfortunately our advice seems to have been disregarded. Nonetheless, the stance of the HRAC is clear. We strongly support the concept of protecting valuable herbicides by preventing weed resistance problems, and our aim is to ensure that the end user manages herbicides in a way to gain maximum value without harming the environment, himself, or creating weed resistance problems. With these objectives in mind, we are providing advice to individual members of the EU who seek our guidance. However, it is imperative that the HRAC works with regulatory bodies, as the agrochemical industry supports strong product stewardship, but must ensure that research expenditure is allocated to generating information that prevents or solves weed resistance problems.

Weed management practices

Management techniques advocated by the HRAC include the careful selection of an appropriate herbicide, the use of mixtures, alternating modes of action, and adopting specific cultural practices to improve the longevity of current herbicides. Weed resistance can build up more rapidly if the selection pressure is continually present; so, taking into account the agronomic requirements, herbicides with short persistence should be favoured. However, even a product of short persistence might induce resistance if it is applied so frequently that it is, in effect, providing a persistent selection pressure.

Mixing products with different modes of action should maintain control for longer periods than for either product used alone. This is because the chances of an individual weed being simultaneously resistant to both components is low. Use of herbicide mixtures is already common, but is usually employed to broaden the spectrum of weeds controlled. To prevent resistance, both mixture components should control the same spectrum of weeds and have a similar biological persistence, yet have different target sites and be detoxified in a different manner (Wrubel & Gressel, 1994). In addition, rotating the product type applied to control a weed, within and between seasons, will slow the build-up of populations resistant to the different herbicides used. However, this can be achieved more readily by crop rotations, which allow a broader range of products to be used against a given weed than in continuous monoculture.

Cultural practices, such as mechanical cultivations, like ploughing and hoeing, provide an alternative means of controlling resistant weed biotypes, and can be used in or between crops, with herbicides reserved for the stages at which they are most beneficial.

Mode of action classification and labelling

One factor critical to the success of using herbicides effectively, alone or in mixtures, is a knowledge of the mode of action of individual products, and uniform, accurate guidelines must be introduced globally. For such a strategy to be implemented, herbicide users must be able to recognise and record herbicide modes of action. This is not always evident with the current labelling practices and it can be difficult for a user to recognise and record herbicide modes of action easily. What is required is that herbicide labels present a clear, 'user friendly' symbol which identifies the mode of action, but the creation of such a system and its implementation requires the co-operation of manufacturers and the public sector.

In Australia, a herbicide mode of action labelling system has been developed in a collaborative venture between the local HRAC and academia, and this system has now been implemented such that it is mandatory for all herbicide labels in Australia to carry a large alphabetical symbol identifying the herbicide mode of action. Alphabetical symbols were chosen after lengthy debate within industry in which a colour based system was rejected (because many individuals are colour-blind) and a numerical system was rejected because a similar system is used to indicate the poison schedule of particular products. The system is 'user friendly' in that the user needs only to recognise and record that a different alphabetical symbol indicates a different mode of action. The aim is to encourage users to recognise and record these alphabetical symbols by having a simple classification system with a minimum of complexity. The introduction of this system in Australia is being accompanied by a wide-ranging extension programme to educate users and informationproviders in using this classification system as a part of resistance management. All sectors of the industry have enthusiastically supported the introduction of this mandatory system in Australia.

Other nations (eg. USA) are in various stages of designing systems for herbicide mode of action classification, and Canada has introduced one, but so far only Australia has a mandatory system in place. The HRAC's objective, though, is to achieve international standardisation rather than a range of different systems.

The HRAC and the Weed Science Society of America (WSSA) are both developing a herbicide mode of action classification system based on the pioneering Australian proposal. All three systems use an alphabetical symbol and show substantial similarity. Groups A and B are identical, but from Group C onwards there is divergence between the systems. The HRAC now hopes to work with the WSSA to develop common, acceptable guidelines. A summary of the proposed HRAC classification by mode of action is shown opposite.

The introduction of a mode of action classification on herbicide labels will be invaluable in managing weed resistance. However, it is imperative that industry is involved in the initiative and it is supported by the major agrochemical producers. The agrochemical industry and governments on a local basis, must also ensure that small generic producers adhere to these guidelines, as it is known that a few local companies producing herbicides which are out of patent, for example in India, are exacerbating resistance problems by simply recommending higher rates to increase their sales.

Overall, label guidelines reflecting herbicide mode of action will form a significant step forward in combatting herbicide resistance and should allow chemicals to fill a vital role in integrated weed management practices.

CONCLUSIONS

The agrochemical industry has a significant role to play in managing weed resistance and is represented effectively by the HRAC. This group is instrumental in verifying resistance cases, proposing management strategies, organising education initiatives, setting up monitoring programmes, and sponsoring fundamental research. The HRAC is also providing inputs to legislators, and advocating management techniques based on herbicide mode of action, and to this end has produced a herbicide mode of action classification which it hopes to introduce globally in conjunction with the WSSA.

Table 3. Classification of herbicides by mode of action

Group	Principal mode of action	Chemical family
A	Inhibitors of acetyl CoA carboxylase (ACCase)	aryloxyphenoxy-propanoates, cyclohexanediones
B	Inhibitors of acetolacate synthase (ALS)	sulfonylureas, imidazolinones, triazolopyrimidines, pyrimidinyl-(thio)ethers
C	Inhibitors of photosynthesis at photosystem II	triazines, triazinones, phenyl ureas, nitriles, benzothiadiazoles, acetamides, uracils, pyridazinones, phenyl-pyridazines
D	Inhibitors of tubulin formation	dinitroanilines, pyridazines
E	Inhibitors of mitosis	carbamates, thiocarbamates, organophosphates
F	Inhibitors of carotenoid biosynthesis	nicotinanilides, triazoles, pyridazinones, isoxazolidinones
G	Inhibitors of protoporphyrinogen oxidase	diphenyl ethers, oxadiazoles, N-phenylphthalimides
H	Inhibitors of plastoquinone biosynthesis	triketones
I	Disrupters of plant cell growth (hormone mimics)	phenoxys, benzoic acids, pyridine carboxylic acids
J	Inhibitors of cell wall synthesis	benzamides, dichlobenil
K	Herbicides with diverse sites of action	chloroacetamides, aminopropionates, benzofurans, phthalamates, nitriles, quinoline carboxylic acids, carbamates
L	Inhibitors of photosynthesis at photosystem I	bipyridyls
M	Inhibitors of EPSP-synthase	glyphosate
N	Inhibitors of glutamine synthetase	glufosinate
O	Uncouplers of energy transfer	organoarsenicals

REFERENCES

Heap, I M (1994) Identification and documentation of herbicide resistance. Phytoprotection, 75 (Supplement), 85-90

Jutsum, A R; Shaner, D (1992) Herbicide Resistance: The Stance of the Agrochemical Industry. Proceedings of the First International Weed Control Congress, **2**, 244-246

Le Baron, H M (1991) Distribution and seriousness of herbicide-resistant weed infestations worldwide. In: *Herbicide Resistance in Weeds and Crops*, J C Caseley, G W Cussans & R K Atkin (eds), Oxford: Butterworth-Heinemann, 27-43.

Le Baron, H M (1992) Herbicide resistance in crops and weeds and its management. Proceedings of the 3rd Tropical Weed Science Conference, Kuala Lumpur, Malaysia, 1990.

Moss, S R (1995) Techniques for determination of herbicide resistance. Brighton Crop Protection Conference - Weeds, 1995 (in press)

O'Keeffe, M G; Graham, J C; Jutsum, A R (1993) Herbicide Resistance Action Committee 1993. Pesticide Outlook, 15-19

Rubin, B (1991) Herbicide resistance in weeds and crops; progress and prospects. In: *Herbicide Resistance in Weeds and Crops*, J C Caseley, G W Cussans & R K Atkin (eds), Oxford: Butterworth-Heinemann, 387-414.

Ryan, G F (1970) Resistance of common groundsel to simazine and atrazine. Weed Science, **18**, 614-616.

Wood Mackenzie (1995) Agrochemical Service, June 1995.

Wrubel, R P; Gressel J (1994) Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study. Weed Technology, 8, 635-648.

APPROACHES TO MANAGING ACCase INHIBITOR RESISTANCE IN WILD OAT ON THE CANADIAN PRAIRIES

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ABSTRACT

Since ACCase inhibitor resistance was first reported in wild oat (*Avena fatua*) in western Canada in 1990, hundreds of resistant populations have been identified, the majority in Manitoba. To date, the mainstay of resistance management has been herbicide rotation. Field surveys confirm that a "1 year-in-3" rotation is adequate to slow resistance evolution. Surface applications without immediate incorporation of soil-applied herbicides and the recent registration of herbicide-tolerant, transgenic canolas have expanded the rotational options for farmers. However, the recent identification of multiple resistance in wild oat underscores the need to adopt more holistic approaches to resistance management in the long term. These include using chaff collectors on combine harvesters to minimize weed seed spread, promoting vigourous crop growth to outcompete weeds, growing highly competitive crops such as fall rye or winter wheat, and using forage crops in rotations.

INTRODUCTION

Wild oat resistance to acetyl coenzyme A carboxylase (ACCase) inhibitors was first confirmed in western Canada in 1990 when three populations from Manitoba and one from Saskatchewan proved resistant to diclofop methyl and fenoxaprop-P-ethyl, both aryloxyphenoxypropionates (Heap & Morrison, 1991; Heap *et al.*, 1993). Three of the four were also resistant to sethoxydim, a cyclohexanedione. Since then hundreds of additional populations from the three Prairie Provinces have been confirmed to be resistant to these chemical families, the majority occurring in Manitoba (Figure 1).

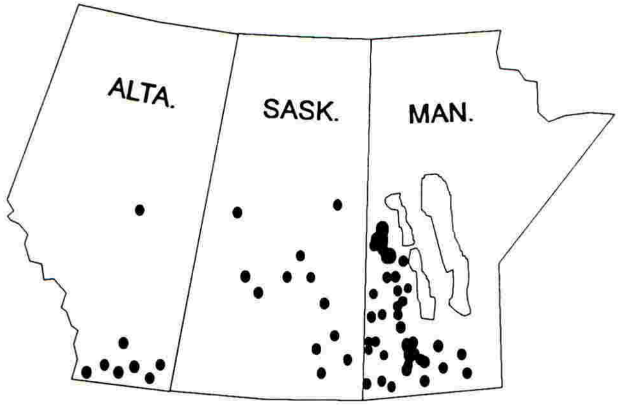

Figure 1. Distribution of ACCase inhibitor (Group 1) resistance in Alberta, Saskatchewan and Manitoba (adapted from R. Pidskalny, Cyanamid Canada Inc.).

The origins of the problem date back to 1976 when diclofop methyl was first commercialized, followed later by sethoxydim in 1983. Virtually all of the fields where resistant wild oat was first identified had histories of eight or more years treatment with these herbicides over the previous twelve years. Diclofop methyl was the popular choice in cereal crops, whereas sethoxydim was used in broadleaf crops such as flax and oilseed rape.

In addition to the two chemicals mentioned above, there are six other ACCase inhibitors currently registered in western Canada. Four new active ingredients have been registered since 1990 and several new commercial products either representing new formulations or packaged mixtures have been introduced into the marketplace (Table 1).

Table 1. ACCase inhibitor (Group 1) herbicides registered for use in western Canada.

Chemical Family/ Common Name	Trade Name	Registration Year	Major Use
Aryloxyphenoxypropionates			
diclofop methyl	Hoe-Grass	1976	wheat, barley, canola, flax
fluazifop-P-butyl	Fusilade Venture	1984 1994	canola, flax, lentils, peas
+ fenoxaprop-P-ethyl	Fusion	1993	canola, flax, lentils, peas
fenoxaprop-P-ethyl	Puma	1993	wheat
+ MCPA/thifensulfuron	Triumph Plus	1990	wheat
+ MCPA/thifensulfuron/2,4D	Champion Plus	1994	barley
quizalofop ethyl	Assure	1991	canola, flax
clodinafop-propargyl	Horizon	1995	wheat
Cyclohexanediones			
sethoxydim	Poast	1983	canola, flax, lentils, peas, sugarbeets
tralkoxydim	Achieve	1992	barley, wheat
+ clopyralid/MCPA	Prevail	1994	barley, wheat
clethodim	Select	1992	canola, flax

In order to simplify discussion of the resistance problem with farmers, the "group" concept was developed (Heap & Morrison, 1991). Both the aryloxyphenoxy-propionates and cyclohexanediones were placed in Group 1. This is because nearly all wild oat populations tested exhibited some level of cross-resistance to herbicides in both chemical families. In the same way, the sulfonylureas and imidazolinones (acetolactate synthase or ALS inhibitors) were assigned to Group 2, and the dinitroanilines to Group 3. Two chemically unrelated wild oat herbicides, triallate ('Avadex BW') and difenzoquat ('Avenge'), were placed in Group 8 based on evidence that resistance to one also resulted in resistance to the other (O'Donovan et al. 1994). Flamprop methyl ('Mataven') was included in a group of its own, as were chemicals such as glyphosate ('Roundup'). Collectively these were referred to as 'others'.

To avoid or delay the onset of new cases of resistance an extension campaign focusing on herbicide rotation was launched in all three prairie provinces. Farmers were informed about the resistance phenomena and encouraged to rotate herbicide usage among the different groups. In Manitoba a "1-in-3" rule was adopted. The rule states that herbicide groups should be rotated so that no product (or product from the same group) is used on a field more often than once in three years. The rule was derived after various scenarios were assessed using the rotational model of Gressel and Segel (1990). While quantitative data for most parameters was (and still is) missing, the model was run using best "guesstimates" (M. Goodwin, personal communication) of factors such as initial gene frequency (10^{-6}), relative fitness (1.0) and effective kill (95%). The idea was to extend the effective lifetime of commercial wild oat herbicides over a 15 year time span. The main appeal of the "1-in-3" rule was that it provided farmers with a hard and fast guideline of what, and what not, to do.

RISK AREAS

As the risk of selecting resistant weed populations is closely linked with the frequency of herbicide application, herbicide use histories obtained from the Manitoba Crop Insurance Corporation were used to define areas of Manitoba at low, medium and high risk for resistance. The database contained field-by-field information on herbicide use and other agronomic practices since 1981 for over 700 townships (36 sq. mi.) where arable agriculture is practised. A low risk township was defined as being one where less than 30% of the fields within the township were treated annually with a Group 1 herbicide since 1981. Medium and high risk townships were those where Group 1 products were used on 30 to 50% and over 50% of the fields, respectively.

Data for the province as a whole indicated that since 1981 the use of Group 1 products had increased four-fold such that by 1991 over 50% of the sprayed fields were treated with these products (Figure 2). By 1993 over half the townships in the province were judged to be in a high risk category.

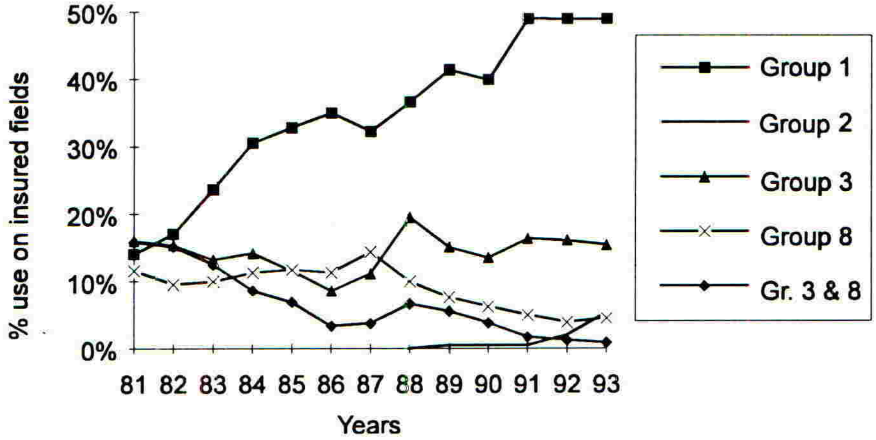

Figure 2. Proportion of sprayed fields in Manitoba treated with various wild oat herbicide groups, 1981-1993 (MCIC database).

While the delineation of risk areas was extremely useful in identifying townships where the problem was most likely to occur, the actual occurrence of resistant weeds within risk areas was unknown. In response to this, two surveys were conducted in one high risk township in 1993. The first consisted of a roadside survey in which seed samples were collected from wild oat patches visible from a car travelling along north-south roadways. Subsequent laboratory assays indicated that resistance occurred in about 1 in 6 fields. While this in itself was alarming, a more detailed, systematic survey of 30 wheat fields selected at random from within the same township indicated that resistant wild oat occurred in 20 of these fields! This survey entailed sampling wild oat on a grid pattern at 80 m intervals in the field. The severity of resistant wild oat in affected fields ranged from a few, isolated plants to large, heavily infested patches covering several hectares. Prior to the survey, none of the landowners claimed to have suspected that they had a resistance problem.

In 1994 another roadside survey was conducted in 5 low, 5 medium and 6 high risk townships. In total 533 wild oat samples were collected. The least and most number of samples collected were 12 in one low risk township and 61 in a medium risk township. Upon screening these collections using a seed bioassay procedure to distinguish resistance to either fenoxaprop or sethoxydim, just 8 samples out of 304 (2.5%) from low and medium risk townships proved to be resistant. In contrast, among the 229 samples collected from high risk townships, 43, or approximately 20%, were resistant.

While the survey did not differentiate between low and medium risk townships, it clearly confirmed that the problem was much worse in high risk areas characterized by heavy, ongoing reliance on Group 1 herbicides. Furthermore, it proved that resistance evolution is slowed under reduced selection intensity and indirectly upheld the "1-in-3" rule as an effective short- to medium-term way of reducing the chances of selecting for resistant wild oat populations.

In the five years since herbicide rotation has been widely promoted as a cornerstone in resistance management, the concept has been widely adopted by farmers. A recent mail-in survey conducted by Manitoba Agriculture (D. Kelner, personal communication) indicated that over 75% of respondents in those townships where the 1994 roadside survey was conducted could identify a "1-in-3" rotation. Regardless of what risk category farmers were in, about half considered that their risk of developing resistance was low. When asked to rate the seriousness of the problem to their own operation on a scale ranging from a) very serious, b) moderately serious, c) a concern, d) of little importance, to e) not a concern, 17% indicated that the problem was very serious. Sixty percent of respondents in the high risk townships rated the problem as "a concern", compared to 50% in the low and medium risk townships.

While most farmers claimed to be practicing a "1-in-3" rotation on most fields, those who were not indicated that the main reason was because "it doesn't always fit their crop rotations". Somewhat alarmingly, a substantial number (15 to 25%) indicated that it was not necessary to rotate chemicals since they didn't have the problem. Apparently these farmers didn't consider rotation as an avoidance strategy but rather as a means of remediating an existing problem.

EXPANDING ROTATIONAL OPTIONS

The widespread use of Group 1 herbicides through the 1980's and early 90's was a testament to their excellent performance in a broad range of cereal and broadleaf crops including wheat, barley, flax, canola, peas, lentils and sugarbeets. The increase in market share of the Group 1 herbicides was primarily at the expense of other products which traditionally held a large share of the wild oat market. These included triallate, alone or formulated with trifluralin ('Fortress') for use in cereals, and trifluralin for use in oilseeds and pulses (Fig. 2).

One of the primary reasons for the changes in market share was related to the fact that the Group 1 herbicides are all post-emergence products whereas both triallate and trifluralin are soil-applied chemicals requiring thorough soil incorporation for optimal activity. For the past 15 years in western Canada there has been a marked trend toward reduced or minimum tillage systems where postemergence herbicides typically have a better fit than preplant or pre-emergence incorporated products. For minimum or zero-till farmers restricted to using post-emergence products, there was no option of using products such as triallate, trifluralin or ethalfluralin ('Edge') in a herbicide rotation. This imposed a serious limitation on these farmers.

Surface applications

Throughout the 1980's it was generally recommended that triallate granules be applied after September 15 prior to freeze up with one incorporation with a disc or cultivator within 48 hours after application, and another later in the fall or in the spring before seeding. A reevaluation of the necessity of fall incorporation of granules initiated by Kirkland (1994) resulted in a change in recommended practice such that the chemical could be applied late in the fall when soil temperatures are less than 4°C, with incorporation delayed until spring.

In 1994 the product label was amended to include this "fall surface application" and in 1995 the concept extended to include registration of triallate in minimum tillage systems. This use provides for triallate granules to be applied late in the fall or early in the spring and incorporated with one "high disturbance" tillage operation 10 to 14 days after application. According to the company, rotary harrowing, light tine or spring-tooth harrowing, and direct seeding with a discer or air seeder with sweeps constitutes a high disturbance operation. This revised method of application now provides a welcome opportunity for minimum till farmers to use triallate in their rotation.

While not yet recommended, surface application of ethalfluralin is also being evaluated for use in minimum or zero-tillage systems. In fields where minimum tillage had been practiced for three or more years, satisfactory control of wild oat was attained where granular ethalfluralin was applied in the fall with no incorporation. Greater than 85% control of wild oat has been observed using this method in canola and peas (Irvine et al. 1994). Should the method prove reliable and become a recommended practice, farmers would have the additional option of including a Group 3 product in direct seeding systems.

<u>Role of transgenics</u>

In early 1995, two transgenic herbicide-resistant canola varieties were registered in Canada. The area planted to these varieties was restricted to less than 20,000 ha but many farmers, including those already with a resistant weed problem, are anxiously waiting for wider release of these varieties, or their successors, next year. One variety, Innovator, is resistant to glufosinate ammonium ('Liberty'); the other to glyphosate. Both herbicides provide effective wild oat and broad-spectrum weed control in canola. The option of having two distinctly different "new" modes of action to include in a herbicide rotation where canola is grown has great appeal. Advancement of this new technology should reduce farmers' reliance on Group 1 products and provide an effective means of combatting the problem where it has developed already.

MULTIPLE RESISTANCE

The recent identification of two wild oat populations resistant to the ALS inhibitor imazamethabenz ('Assert') has confounded the prevalent notion that herbicide rotation alone will take care of the resistance problem. One population was collected in a field where the herbicide was applied in 1993 and the other in 1994. In both cases it was the first time that imazamethabenz had been used in the affected fields. Neither field had been sprayed with another Group 2 product for wild oat control. Even though other herbicides had been used during the preceding four years, the wild oat problem had continually worsened. Subsequent growth room experiments confirmed that these populations were not only resistant to imazamethabenz but to flamprop methyl and fenoxaprop-P-ethyl as well. Field trials conducted during the 1995 growing season at one of the sites confirmed these observations but also showed that recommended rates of other ACCase inhibitors, including fluazifop-P-butyl and clethodim, were effective in controlling the weed.

Many questions remain unanswered. For example, it is not known if there is one or more mechanism(s) conferring resistance to these populations. It is also not known if the populations have always been resistant to imazamethabenz, or if resistance to this chemical was somehow selected through repeated use of other products. In one field four different wild oat herbicides with four different modes of action had been applied from 1983 to 1993.

What is clear, however, is that the farmers who identified the problem were rotating products with the expectation that they could avert the resistance problem on their farms. Herbicide rotation in itself did not provide the intended result. This does not invalidate the practice as a means of avoiding or delaying the evolution of single, target site resistance, but it does serve as a reminder that herbicide rotation may only be a stop-gap, short- to medium-term solution to the problem. The occurrence of this new form of multiple resistance in Canada underscores the necessity of developing longer-term, more integrated approaches to weed management.

PATCH WATCH

Based on field survey results indicating a high, but hitherto undetected, incidence of resistant wild oat where there has been heavy reliance on Group 1 herbicides, a major focus of provincial extension specialists is now on a 'Patch Watch" program. The program is based on the premise that new cases of resistance may be contained by managing them separately from the rest of a field. Once a suspicious patch is identified farmers are advised to mow, cultivate or spot spray the affected areas to prevent seed production.

At harvest, patches are to be harvested separately from the rest of the field to curtail seed spread both in the direction of movement and laterally. This is becoming increasingly important as more farmers are equipping their harvesters with straw choppers and chaff spreaders which spread residues over widths of up to 12 m behind the combine harvester.

In high risk areas, resistant gene flow via seed movement is now seen to be a more likely source of a new infestations than independent selection of resistant mutants from within a population. Studies are currently underway at the University of Manitoba to determine the effect of different harvesters, used with and without chaff collection wagons, on the population dynamics and spread of wild oat (S. Shirtliffe and M. Entz, personal communication). While the practice of using chaff wagons in the current age of modern agriculture is uncommon, some producers are expressing a "renewed" interest in the practice and units are available from a agricultural machinery manufacturer in Saskatchewan (Redekop Industries, Saskatoon).

CULTURAL CONTROLS

With the introduction of new and increasingly effective herbicides during the 1970's and 80's, a generation of farmers came to rely almost exclusively on chemicals to control weeds. Compared to earlier years, the importance of judiciously planning crop rotations and utilizing cultural practices to control weeds diminished. The problem of herbicide resistance coupled with other issues relating to the long-term sustainability of present-day production systems has prompted a renewed interest in cultural methods of weed control (Morrison and Kraft, 1994). Some of these methods are directly relevant to the topic of managing herbicide resistance, as they either reduce the selection intensity imposed by herbicides and/or contribute to an overall reduction in weed densities and fecundity. Others destabilize weed communities which have evolved in a monoculture system where tillage practices, seeding, spraying and harvesting have been conducted in more or less the same way for most field crops commonly grown on the prairies.

Various cultural options for delaying herbicide resistance in wild oat have recently been reviewed by Thill *et al.* (1994) who concluded that the objective of any successful control program should be to prevent wild oat seed return to the soil. Hence any economically and environmentally acceptable practice that reduces weed establishment, competitiveness, seed production, seed shed or migration is applicable.

Adoption of a combination of chemical and cultural control tactics to combat resistance evolution is defined as being "Weed Smart", an expression coined in Australia. Being "Weed Smart" means to maximize crop competition by selecting appropriately competitive crop kinds/cultivars and ensuring rapid, uniform emergence by planting good quality seed into a firm, well-prepared seedbed. It means using seeding equipment that bands fertilizer close to the seed rows which are spaced close enough together to ensure quick canopy closure. It also means keeping records of previous cropping practices and herbicide use histories, scouting fields regularly, and making decisions on whether or not to spray based on economic thresholds.

Growing winter cereals such as fall rye or winter wheat and forages in rotations is also "Weed Smart" as these crops typically will outcompete weeds such as wild oat which emerges in the spring. As stated in a recent editorial (Morris, 1995) in a major farm paper:

> "The threat of herbicide resistance - which is greatest in Manitoba - means farmers will need more arrows in their weed control quiver. One of those is throwing the weeds out of balance by growing an entirely different type of crop than the ones seeded in the spring. That means forages or winter crops. Development of new winter wheat varieties and new markets for them is one of the most important priorities in post-resistance .... Western Canada."

Drawing on studies compiled in the early 1960's on the effects of including forages in rotations (Siemens, 1963), Ominski *et al.* (1994) recently concluded a survey in which weed numbers were determined in fields where wheat was grown after alfalfa, and where wheat was grown after wheat. The mean field density per m^2 and frequency of wild oat in wheat after alfalfa were 1.3 and 27.2 as compared to 46.4

and 83.3, respectively, in wheat after wheat. These findings clearly indicate that it is "Weed Smart" to include alfalfa in a rotation for control of weeds like wild oat.

One of the current limitations to growing winter wheat on the eastern Prairies is that there are no cultivars with high levels of disease resistance adapted to the region. The crop, which must be planted into standing stubble to ensure good winter survival, has enormous potential in an integrated weed management system. Breeding programs currently underway at the University of Manitoba are expected to produce adapted cultivars within the next three to five years (A. Brûlé-Babel, personal communication).

SUMMARY

Herbicide resistance in wild oat is one of the major production problems facing farmers and the agrichemical industry in western Canada. This has been precipitated by heavy reliance on ACCase inhibitors in a wide spectrum of cereal, oilseed and grain legume crops over the past 15 years. In the eastern Prairies, most notably in parts of Manitoba, over half the arable land is considered to be at high risk for developing resistance. In order to preserve the use of ACCase inhibitors in crops where there are few effective alternatives, most farmers have accepted the wisdom of herbicide rotation. For many this has meant using herbicides with different modes of action in their cereal crops, and rotating back to ACCase inhibitors in crops like flax, peas and canola. In the long term it is imperative that farmers reduce their near-total reliance on herbicides to control weeds and take extra precautions to avoid transporting weed seeds from one site to another. The issue of herbicide resistance is forcing changes in the production system to include more holistic approaches to weed management. In essence it is compelling farmers to "do all the right things for the wrong reasons".

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REFERENCES

Gressel, J; Segel L A (1990) Modelling the effectiveness of herbicide rotations and mixtures as strategies to delay or preclude resistance. *Weed Technology*. **4**, 186-198.

Heap, I M; Morrison I N (1991) Herbicide Resistant Weeds. Manitoba Agriculture, Agdex No. 818. 4 p.

Heap, I M; Murray B G; Loeppky H A; Morrison I N (1993) Resistance to aryloxyphenoxypropionate and cyclohexanedione herbicides in wild oat (*Avena fatua*). *Weed Science*. **41**, 232-238.

Irvine, A; Beauchesne G; McGregor W R; Hare D; Koebernick D; Anderson J (1994) Surface applied ethalfluralin for weed control in direct seeding systems. Abstract.

Expert Committee on Weeds (Western Canada Section). December 5-8, 1994. Saskatoon.

Kirkland, K J (1994) Efficacy of fall incorporated and non-incorporated granular triallate on wild oat (*Avena fatua*) and wheat (*Triticum aestivum*). *Weed Technology*. **8**, 607-611.

Morris, J W (1995) Move it east. Manitoba Cooperator. **52(4)**, 4.

Morrison, I N; Heap I M; Murray B (1992) Herbicide resistance in wild oat - the Canadian experience. In: *Wild Oats in World Agriculture*, A R Barr & R W Medd (eds), Proceedings Fourth International Oat Conference Vol. II, pp. 36-40.

Morrison, I N; Devine M D (1994) Herbicide resistance in the Canadian Prairie Provinces: Five years after the fact. *Phytoprotection*. **75 (Suppl.)**, 5-16.

Morrison, I N; Kraft D (eds) (1994) Sustainability of Canada's agri-food system: a prairie perspective. International Institute for Sustainable Development, Winnipeg. 192 p.

O'Donovan, J T; Sharma M P; Harker K N; Maurice D; Baig M N; Blackshaw R E (1994) Wild oat (*Avena fatua*) populations resistant to triallate are also resistant to difenzoquat. *Weed Science*. **42**, 195-199.

Ominski, P; Entz M H; Goodwin M D (1994) Using alfalfa to control weeds: a comparative study. Proceedings Manitoba Agri-Forum. January 5-6, 1994. Winnipeg. pp. 170-173.

Siemens, L B (1963) Cropping Systems: An Evaluation of the Literature. Technical Bulletin No. 1, Faculty of Agriculture and Home Economics. University of Manitoba.

Thill, D C; O'Donovan J T; Mallory-Smith C A (1994) Integrated weed management strategies for delaying herbicide resistance in wild oats. *Phytoprotection*. **75 (Suppl.)**, 61-70.

A REVIEW OF PROPANIL RESISTANT *Echinochloa crusgalli* IN ARKANSAS AND FIELD ADVICE FOR ITS MANAGEMENT IN DRY SEEDED RICE

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ABSTRACT

Since the initial identification of propanil-resistant barnyardgrass *Echinochloa crusgalli* (L.) Beauv. in Arkansas in 1990, it has now been confirmed in 152 populations in 16 counties. Survey information on the resistant populations confirms a heavy, often sole dependence upon propanil for weed control for a long time, and also shows the problem to occur more rapidly where rice is grown every year or every other year as opposed to every third year. Commercial formulations of propanil containing carbaryl (Super Wham) or molinate (Arrosolo) are more effective than propanil alone but, do not provide reliable control of propanil resistant *E. crusgalli*. Propanil formulations tank mixed with quinclorac, thiobencarb or pendimethalin are very effective for controlling resistant and susceptible biotypes when applied post-emergence while quinclorac and mixtures of quinclorac with pendimethalin and thiobencarb are very effective when applied preemergence. Resistant and susceptible biotypes are controlled in rotational crops by trifluralin, pendimethalin, metolachlor, alachlor, dimethenamid, clomazone and the postemergence graminicides such as clethodim.

INTRODUCTION

Rice is grown on approximately 0.6 million ha in Arkansas, making it the largest rice producing state with over 40% of the U.S. production. In 1962, propanil was introduced into the United States rice market (Brandes, 1962). Thereafter, rice yields in the U.S. increased 34 to 74% (Smith, 1965) and both the acreage and production of rice have continued to increase. Because of the effectiveness of propanil, it has been used on most fields every year since its introduction, often as multiple applications. Since the acreage expansion in the 1970s, rice has often been grown either continuously or every other year as opposed to the more traditional rotation of rice every third year. A typical grower program for weed control since the introduction of propanil has been two preflood applications of propanil for annual grass control followed by a post-flood application of 2,4,-D for broadleaf and aquatic weed control.

In the late 1980s, farmers began reporting poor control of barnyardgrass with propanil. Initially this was attributed to factors such as poor timing and application accuracy, unfavorable environmental conditions or a combination of these factors. However, evaluation of seed from a problem field in the greenhouse in 1990 showed it to be resistant to propanil at rates as high as 11 kg/ha. (Baltazar & Smith, 1994) and subsequent field studies confirmed the resistance.

DISTRIBUTION OF PROPANIL RESISTANT BARNYARDGRASS IN ARKANSAS AND THE POTENTIAL FOR INCREASE

Since 1991, rice growers in Arkansas have been encouraged to collect mature seeds samples from problem fields and submit them for propanil-resistance testing (Carey, 1995). The response of these seedlings to propanil is compared with known susceptible and resistant populations under greenhouse conditions. Rice production practices associated with each sample were obtained through an accompanying questionnaire.

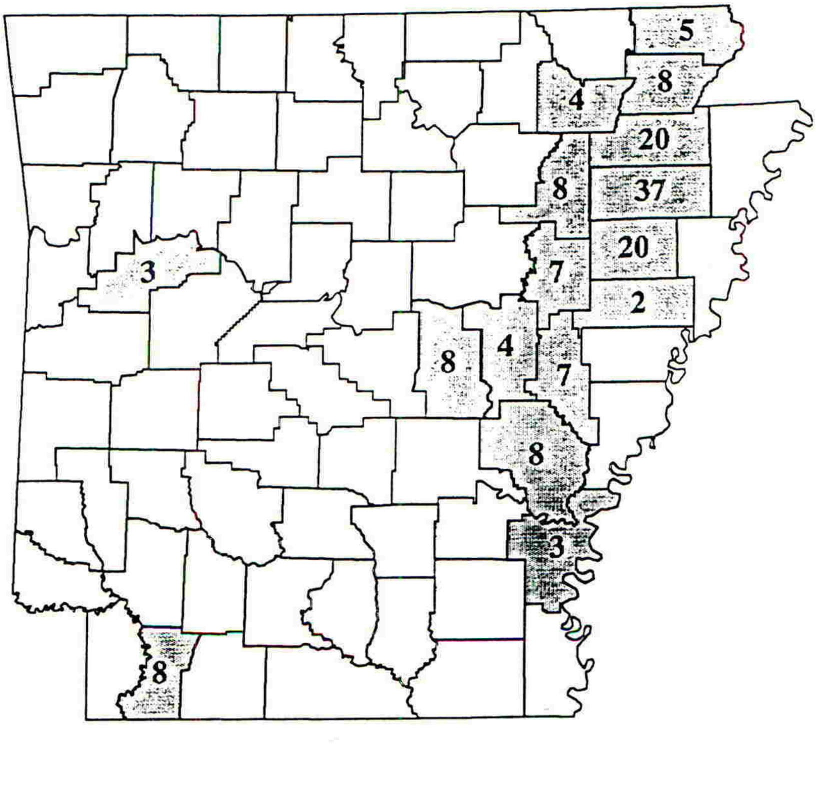

Figure 1. Number of propanil-resistant barnyardgrass populations confirmed by county in Arkansas (1991-1994)

A total of 152 populations of propanil-resistant barnyardgrass have been confirmed from seed samples submitted by Arkansas rice growers: 67 in 1991, 51 in 1992, 16 in 1993, and 18 in 1994. The number and distribution of propanil-resistant populations in Arkansas is shown in Figure 1. Of 143 growers responding in 1991 and 1992: 1) 80% ranked barnyardgrass as their main weed problem; 2) 100% had applied propanil every year the field had been in rice production; 3) 82% used propanil in combination with another herbicide; 4) there was significantly better control of barnyardgrass in fields rotated out of rice for 2 of 3 years than in rice fields with less rotation; and 5) 90% used certified rice seed (Carey, 1995).

Experiments were conducted at Main Agricultural Experiment Station, Fayetteville, to study the population dynamics of two resistance categories of barnyardgrass: (shown in Table 1) which were selected by spraying a 4.5 kg/ha rate of propanil on progeny of seed collected from grower fields, and visually rating their susceptibility. In 1993, propanil at 0, 4.5, 13.5, and 28 kg/ha plus crop oil concentrate was applied to plants in each resistance category. Seeds from surviving plants in each plot were harvested in 1993 and evaluated for resistance to propanil using the greenhouse assay procedure with propanil doses as above. Progeny of moderately resistant plants previously treated with the different rates of propanil were then highly resistant to propanil at the same rates. Data from the 4.5 kg/ha rate are shown as an example in Table 1 below. Propanil treatment apparently removed the susceptible plants from the population and the progeny were then all resistant, suggesting that the moderately resistant barnyardgrass populations are a mixture of susceptible and resistant biotypes.

Table 1. Glasshouse response of two populations of barnyardgrass to selection pressure from propanil at 4.5 kg ai/ha (% control)

Classification	Parent[a]	Progeny[b]
susceptible	85	87
moderately resistant	51	10
LSD .05	23	15

[a]Percent control of plants grown from original seed collected from grower fields, treated with 4.5 kg/ha propanil.

[b]Percent control of plants grown from seed collected from surviving plants in (a) above.

CONTROL OF PROPANIL-RESISTANT BARNYARDGRASS IN ROTATED CROPS

It is recommended in Arkansas that, where possible, rice only be grown one year out of three. As a worst case, it should only be grown one year out of every two. Soybeans are the primary rotated crop and alternatives include corn, grain sorghum and cotton. A large number of effective herbicides are available for barnyardgrass control in each of these crops. These include trifluralin, pendimethalin, clomazone, metolachlor and the postemergence graminicides, such as fluazofop, quinclorac, sethoxydim and clethodim, for soybeans and cotton; metolachlor, alachlor and atrazine in corn and grain sorghum; and dimethenamid in soybeans and corn. Field experiments conducted to date have shown no cross or multiple resistance, and have shown no differences in control among resistant and susceptible biotypes to any rotational crop herbicide evaluated. Glyphosate applied to glyphosate-tolerant soybeans will be an effective rotated crop treatment beginning in 1996.

CONTROL OF PROPANIL RESISTANT BARNYARDGRASS IN RICE

Field research by Baltazar & Smith (1994) was begun in 1991 in rice fields near Harrisburg, Arkansas to confirm the barnyardgrass resistance and to compare herbicide treatments for control of propanil resistant barnyardgrass. Resistant plants survived rates of propanil up to 11 kg/ha. Emulsifiable propanil frequently controlled resistant barnyardgrass better than a dry flowable formulation. In more recent research by Helms (unpublished data) and Talbert (1993), a newly developed flowable formulation of propanil (Super Wham) and an emulsifiable formulation of propanil and molinate (Arrosolo) have provided better control of resistant barnyardgrass than emulsifiable propanil alone. However, sequential applications of all of these formulations, when used alone, have failed to provide an acceptable level of control even at above normal use rates.

Research by Talbert *et al.* (1995) has shown that the insecticide carbaryl can be used to enhance the activity of propanil on resistant barnyardgrass. When propanil has been applied following higher rates of carbaryl, both biotypes of barnyardgrass have been controlled. This would appear to confirm that the mechanism of resistance in the resistant biotypes in Arkansas is an increase in the aryl acylamidase enzyme responsible for propanil tolerance in rice. This would be consistent with the findings of Leah *et al.* (1994), for *E. colonum.* Research to determine if applications of low rates of carbaryl tank-mixed with propanil provide commercially acceptable control of resistant barnyardgrass without a corresponding increase in rice injury continues. Early results show promise but they must be confirmed over a range of environmental conditions, and soil textures.

Recent laboratory analyses by Lavy *et al.* (pers. comm. 1995) at the University of Arkansas have identified low levels of carbaryl in the flowable propanil formulation Super Wham. This could explain why it has shown more activity on resistant barnyardgrass compared to other propanil formulations. There have been field reports of excessive rice injury when this formulation of propanil has been applied to the rice

Table 2. A comparison of various herbicide treatments for control of propanil resistant barnyardgrass

Treatment	Rate kg ai/ha	Timing	*E. crusgalli* % control	Rice yield kg/ha
Untreated control			0	470
Propanil 4 EC	4.48	2-3 LF S	68	4705
Propanil 4 EC	4.48	4-5 LF		
Propanil 4 EC	4.48	2-3 LF TM	95	7561
Thiobencarb 8 EC	3.36	2-3 LF		
Propanil 4 EC	4.48	2-3 LF TM	91	7556
Pendimethalin 3.3 EC	1.12	2-3 LF		
Propanil 4 EC	4.48	2-3 LF TM	95	7717
Quinclorac	0.28	2-3 LF		
Quinclorac 75 DF	0.43	DPRE	95	8379
Quinclorac 75 DF	0.22	DPRE TM	95	8327
Pendimethalin 3.3 EC	1.12	DPRE		
Quinclorac 75 DF	0.22	DPRE TM	95	8002
Thiobencarb 8 EC	2.24	DPRE		
Quinclorac 75 DF	0.43	DPRE TM	95	8314
Thiobencarb 8 EC	2.24	DPRE		
Pendimethalin 3.3 EC	1.12	DPRE	93	6802
		LSD (0.5) =	13	1597

DPRE = delayed preemergence
S = sequential
TM = tank mix

Table 3. A comparison of three propanil formulations for control of resistant *E. crusgalli*

Treatment	Rate kg ai/ha	Timing	*E. crugalli* % control	Rice yield kg/ha
Untreated control			0	290
Propanil 4 EC (Stam M4)	4.48	2 LF S	28	674
Propanil 4 EC (Stam M4)	4.48	4 LF		
Propanil 4 F (Super Wham)[1]	4.48	2 LF S	74	3847
Propanil 4 F (Super Wham)	4.48	4 LF		
Propanil + molinate 6 EC (Arrosolo)	3.36 + 3.36	2 LF S	75	3913
Propanil + molinate 6 EC (Arrosolo)	3.36 + 3.36	4 LF		
Propanil 4 EC	4.48	2 LF TM	95	8047
Quinclorac	0.43	2 LF		
		LSD (0.05)	21	1716

[1]Crop oil concentrate - Trade name Agridex - added at 2.3 l/ha added to these treatments

S = sequential

TM = tank mix

plant under adverse conditions.

Greenhouse and field research by Baltazar & Smith (1994) indicated that acceptable control of resistant barnyardgrass could be achieved when propanil was tank mixed with either quinclorac, thiobencarb or pendimethalin and these treatments are widely used by growers. Other effective treatments included quinclorac or pendimethalin applied alone, quinclorac + thiobencarb and quinclorac + pendimethalin applied as delayed preemergence treatments. With the latter, the herbicide is applied 3 to 7 days after the rice has been dry seeded, and the soil has been sealed by a rainfall or irrigation flush, but before emergence of the rice. This approach is finding increased favor with Arkansas rice growers.

Of the single herbicides, quinclorac has been the most consistent. It is effective against propanil resistant barnyardgrass at doses as low as one-half the labeled rate when tank mixed with any of the propanil formulations and applied postemergence, or tank mixed with pendimethalin or thiobencarb and applied delayed preemergence. Because of the obvious economic benefit, these treatments have quickly been accepted by Arkansas rice growers.

Supporting data for the key points in this section, are presented in Tables 2 and 3. Data from selected treatments in an applied research and demonstration trial conducted at the Rice Research and Extension Center, Stuttgart, Arkansas are presented in Table 2. All herbicide treatments were applied either as delayed preemergence treatments (dpre) or at the 2-3 leaf stage (except one application of propanil and one of propanil + molinate at 4-5 leaf stage) of the dry seeded rice. The barnyardgrass was a mixture of susceptible and resistant biotypes, with the susceptible biotype being native and the resistant biotype overseeded. Barnyardgrass control ratings were made at 35 days after the 2-3 leaf treatments and rice grain yields were recorded. A similar study was conducted in the same area to compare propanil formulations for control of a heavy infestation of resistant barnyardgrass. All treatments were either applied at the 2-leaf or 4-leaf stage prior to flooding of dry seeded rice.

GROWER ADVICE

In summary, propanil resistant barnyardgrass has become a common problem and the potential exists for its continued rapid spread. Because of this, it is equally important for the rice grower who does not yet have the problem to manage for prevention, as it is for the grower with a resistant population to manage for control. However, barnyardgrass resistance to propanil is only one example of resistance of a major weed to one of the primary herbicides in a major crop in Arkansas. Other examples include cocklebur (*Xanthium sp.*) and pigweed (*Amaranthus sp.*) to the ALS inhibitors and johnsongrass (*sorghum halepense (L.) pers.*) to the ACCase inhibitors. With the discovery of these herbicide resistant weeds, an extensive grower awareness programs was begun by the University of Arkansas. Methods used to accomplish this included a section on resistance management added to the annual revision of *Recommended Chemicals for Weed and Brush Control in Arkansas* (Baldwin, *et al.* 1995), the major weed control publication in the state. Other methods included popular press articles, slide sets for county agent use, and including resistance management as a subject in all grower meeting presentations. In addition, the University of Arkansas provides free weed resistance testing to growers in the state. Grower awareness of propanil resistant barnyardgrass in Arkansas has increased rapidly. The decrease in the number of samples sent in for resistance confirmation the past two years indicates growers are aware that they need to manage the crop for both prevention and control.

Advice to growers is to understand the major factors that promote resistance. These included an over-reliance on herbicides in some cases, relying on a single herbicide or mode of action over a sustained period, and sequential applications of the same herbicide or mode of action.

The keys to managing or preventing herbicide resistance include: rotating crops where possible, using mechanical tillage and other cultural practices where possible, rotating herbicide having different modes of action, using tank mixtures of herbicides having different modes of action, avoiding sequential applications of the same herbicide or herbicides having the same mode of action, and mechanically controlling weeds when fields are fallow. A number of practices are useful for preventing and controlling propanil resistant barnyardgrass, including the use of effective grass herbicides to other crops in rotation, the use of alternatives to propanil for grass control in rice, and the use of certified rice planting seed. The Arkansas State Plant Board in 1994 ruled that barnyardgrass is a noxious weed in seed rice, and none is allowed in any certified rice seed.

Rotate to an alternate crop at least every other year and preferably two years out of every three. Make sure that excellent barnyardgrass is achieved in the rotated crop. However, effective crop rotation is often difficult for growers because all of the land on the farm may not be suitable for rice production.
When rice is grown, substitute pendimethalin, quinclorac or thiobencarb for propanil or tank mix these with propanil. Since pendimethalin, quinclorac and thiobencarb each have different modes of action, it is recommended that they be rotated to prevent development of cross-resistance to herbicides other than propanil.

Propanil continues to be heavily used, even where resistance has developed, due to the excellent control it provides of other weeds. If a grower uses the proper practices to prevent the occurrence of propanil resistance, then propanil remains a viable option for barnyardgrass control. However, once the problem develops, it is very difficult to completely eliminate the propanil resistant biotypes.

REFERENCES

Baldwin, F L; Boyd, J W; Guy, C B (1995) *Recommended Chemicals for Weed and Brush Control in Arkansas, MP-44*. University of Arkansas Cooperative Extension Service, p.15. Miscellaneous Publication.

Baltazar, A M; Smith Jr, R J (1994) Propanil-resistant barnyardgrass (*Echinochloa crus-galli*) control in rice (*Oryza sativa*). *Weed Technology* **8**, 576-581.

Brandes, G A (1962) Control of grasses and weeds in rice with Stam F-34 (3,4 dichloropropionanilide). *Rice Journal* **65**, 37-39.

Carey III, V F; Hoagland, R E; Talbert, R E (1995) Verification and distribution of propanil-resistant barnyardgrass (*Echinochloa crusgalli*) in Arkansas. *Weed Technology* **9**, 366-372.

Carey III, V F; Talbert, R E; Baltazar, A M; Smith Jr, R J (1992) Propanil barnyardgrass in Arkansas. *Proceedings of Southern Weed Science Society* **45**; 296.

Leah, J M; Caseley, J C; Riches, C R; Valverde, B (1994) Association between elevated activity of aryl acylamidase and propanil resistance in jungle-rice, *Echinochloa colonum. Pesticide Science* **42**, 281-289.

Smith Jr, R J (1965) Propanil and mixtures with propanil for weed control in rice. *Weeds* **13**, 236-238.

Talbert, R E, Carey III, V F; Kitt, M J; Helms, R S; Black, H L (1995) Control, biology and ecology of propanil barnyardgrass resistant. Arkansas Rice Research Studies 1994, Arkansas Agricultural Experiment Station, July 1995, Research Series **446**, 23-31.

CREEPING RESISTANCES: THE OUTCOME OF USING MARGINALLY-EFFECTIVE OR REDUCED RATES OF HERBICIDES

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ABSTRACT

There is increasing field epidemiological evidence for the evolution of non-target site resistances where the magnitude of resistance of most individuals in the population continues to creep up with each selection cycle. This has happened both to older, probably multisite inhibitors as well as to single site inhibitors, especially where low dose rates are used. This trend, probably due to polygenically-inherited metabolism, is likely to increase as pressures to reduce herbicide inputs increase.

INTRODUCTION

It would wise to ask how the trend to greatly reduce herbicide use will affect the evolution of resistance and its management. Reduced herbicide use can come about by either: (a) more frequent abstinence; (b) by using the same herbicides at the same frequency but at cut rates, or; (c) by using more potent, newer, single-target herbicides used at lower rates. We have become used to the appearance of target-site resistances to potent mono-site herbicides, inherited in a monogenic fashion (Shaner, 1995) and will probably see more of them. These resistances typically appear where herbicides exert strong selection pressures, i.e. where herbicides are potent and persistent enough to control nearly all individuals in all flushes of a weed throughout a season. This happened with the most persistent inhibitors of photo-system II and acetolactate synthase as well as with the potent but less persistent inhibitors of acetylCo-A carboxylase, in grass weeds that germinate in a single flush.

There are a recent paucity of cases of non target site resistances appearing in some of the major crops; e.g. wheat and rice. These latter cases are typified by a slow, incremental, creeping increases in the LD_{50} of the whole population as a function of repeated treatments. This was well documented by Heap (1988) for diclofop-methyl resistance in field populations of *Lolium rigidum* in Australia, where low rates (375g/ha) are typically used. The first populations found did not have target resistance. In Canada, where three times this rate of diclofop-methyl are used, a *Lolium* sp. evolved only target-site resistance (Morrison, 1995), and resistant individuals were totally resistant to much higher levels of the herbicide, without any change in the LD_{50} with repeated treatments. Creeping resistances have been found earlier (cf. Holliday & Putwain, 1988, Gressel *et al.*, 1982) but their incidences have been overshadowed by the target site resistances, until the rampant creeping resistances covered much of Australian wheat fields (Powles & Matthews, 1992). Many herbicides have been considered to be immune to the evolution of resistance, with resistant populations appearing after only 20-40 repeated uses in monoculture. This seems to have been true for the phenoxy and chloroacetamide herbicides, as well as glyphosate. Thus, it has been disturbing to see resistances creeping within these groups following recurrent selection (Huang & Lin, 1993, Duncan & Weller, 1987, Boerboom *et al.*, 1991). This is a field problem in *Echinochloa crus-galli* with butachlor in rice (Huang & Lin, 1993). The most worrisome case of late has been a creeping non-target site resistance of *Phalaris minor* to isoproturon (Malik & Singh, 1995). This resistant weed now covers over half a million ha of green revolution wheat in India. The use of isoproturon is de-registered for 1995/6 in the Karnal and Kurukshetra regions of Haryana State due to nearly complete loss of effect. The resistant biotypes are cross-resistant to diclofop-methyl and pendimethalin, even though they had rarely been used (R K Malik, pers. comm., 1995). An informal field epidemiological survey showed that resistance typically appeared after 10-15 repeated isoproturon treatments when Indian farmers underdosed the herbicide by either: (a) purposefully using low doses; (b) used heavily adulterated herbicide; (c) lost much herbicide to binding to burnt rice-straw carbon; (d) by treating the weed at too late, less susceptible stages, and/or; (e) non-uniform hand broadcasting the herbicide (Gressel *et al.*, 1995). We should expect many more such cases, with regulation or market economy controlled cut backs in herbicide use.

POSSIBLE OUTCOMES OF HERBICIDE ABSTINENCE

Total abstinence from herbicide use will totally prevent herbicide-selected evolution of resistance. That is axiomatic. What about occasional abstinence? Will it delay resistance for as many treatment cycles as were missed? The answer probably depends upon what the farmer allows to happen. If the farmer uses good monitoring techniques and abstains from herbicide use when there was little or no reason for chemical weed control, there should be mainly positive, resistance-delaying effects. This is especially so when individuals resistant to the last-used herbicide are less fit, and will be competed away by more fit, susceptible individuals. Conversely, abstinence that allows a huge build up of weed seed populations can be very negative. Evolution is a quantitative selection process; the more individuals to choose from in a field, the more likely there will resistant ones in that field, and the more likely outcrossing weeds will be near to mates. Field epidemiology has shown time and again that resistance is most likely to appear first in weeds with heavy seed infestations; *Amaranthus, Chenopodium, Lolium, Kochia,* etc., spp.

OUTCOMES OF RATE CUTTING

Theory, supported by field epidemiology, has suggested that lowering the selection pressure (by lowering herbicide persistence and/or rates) delays the evolution of monogenic, mainly target site resistances. Now we find that substantially lowering rates to the minimum effective levels enhances the rapidity of evolution of multigenic (or multi-changes in a gene)-inherited resistances that are mainly due to increased herbicide metabolism. This is clearly a biological "Catch 22" when it comes to designing resistance management strategies (Gressel, 1995a). The theoretical explanation of this enhanced creepy evolution at low dose rates is as follows: there are many alleles that can mutate, and each confers enough resistance to overcome a small increment of herbicide. An unlikely confluence of many such mutations have to be present to confer resistance to higher doses. As there are many such alleles compared to the rarer alleles for target site resistance, it is more likely that low, marginally-effective doses will select for these ubiquitous minor mutations. Different minor mutations will accumulate in the population under repeated selection, conferring higher and higher levels of resistance, especially when the dose is gradually increased after signs of incipient resistance become apparent to the farmer. Such sequential selections have also been shown to select for polygenic resistances (or gene amplifications, or changes within a gene) in laboratory selections for resistance to chlorsulfuron (Caretto *et al.*, 1994, Mackenzie *et al.*, 1995) and glyphosate (Suh *et al.*, 1993). Increments of glyphosate resistance in plants have also come from metabolism (Komossa *et al.*, 1992), enhanced transcription of mRNA for (Holländer-Czytko *et al.*, 1992), or changes in (Forlani *et al.*, 1992), the target enzyme. Cross resistance to glyphosate occurred when selecting for antibiotic resistance (Peñaloza-Vásquez *et al.*,1995). Recurrent selection could select for combinations of such genes, with glyphosate resistance levels creeping above field rates.

MODELLED STRATEGIES TO OBVIATE THE EFFECTS OF RATE CUTTING

Models using a cycles with a sequence of a few low doses followed by a moderate dose have been elucidated and propounded (Gressel, 1995a; Gardner, Mangel and Gressel, in preparation). The moderate dose is chosen to be sufficient to control individuals that have already accumulated a few polygenes for resistance. If the models are as effective in the field as they are on paper, their use would delay resistance for a longer period than either the use of low or high dose alone. This remains to be tested.

MEANINGFUL ROTATIONS-STILL THE BEST RESISTANCE PREVENTION

The best time-proven resistance delaying tactic has been to rotate crops and herbicides in such a way that weed seed banks are kept suppressed, and that different modes of action and modes of crop selectivities are used. The use of meaningful herbicide mixtures (Wrubel & Gressel, 1995) and synergies (Gressel, 1990) can also be of value. This is easier said than done in many agricultural ecosystems. Too many areas can only support one type of crop, e.g. the otherwise marginal lands where much of the world's wheat is cultivated, and wheat seems to have but one mechanism of herbicide detoxification (Gressel, 1988). In general, the variety of herbicide chemistries available for such rotations is decreasing instead of increasing, due to the greater rate of deregistering older herbicides than the rate of registering new chemistries/modes of action. Thus, genetic engineering to introduce new modes of herbicide resistance into crops such as wheat seems to be imperative (Gressel, 1995b), as long as the resistances are not to resistance-prone or already heavily used herbicides.

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REFERENCES

Boerboom, C M; Ehlke, N J; Wyse D L; Somers D A (1991) Recurrent selection for glyphosate tolerance in birdsfoot trefoil. *Crop Science* **31**, 1124-1129.

Caretto, S; Giardina, M C; Nicolodi, C; Mariotti, D (1994) Chlorsulfuron resistance in *Daucus carota* cell lines and plants: involvement of gene amplification. *Theoretical and Applied Genetics* **88**, 520-524.

Duncan, C N; Weller S C (1987) Heritability of glyphosate susceptibility among biotypes of field bindweed. *Journal of Heredity* **78**, 257-260.

Forlani, G; Nielsen, E; Racchi, M L (1992) A glyphosate-resistant EPSP-synthase confers tolerance to a maize cell line. *Plant Science* **85**, 9-15.

Gressel, J (1988) Multiple resistances to wheat selective herbicides: New challenges to molecular biology. *Oxford Surveys of Plant Molecular and Cellular Biology* **5**, 195-203.

Gressel, J (1990) Synergizing herbicides. *Reviews in Weed Science* **5**, 49-82.

Gressel, J (1995a) Catch 22 - Mutually exclusive strategies for delaying/preventing polygenically vs. monogenically inherited resistances. In: *Options 2000* N N Ragsdale, P C Kearney & J R Plimmer (eds), Washington, D.C.: American Chemical Society, pp. 330-349.

Gressel, J (1995b) The potential role of herbicide resistant crops in world agriculture. In: *Herbicide Resistant Crops: Agricultural, Economic, Environmental, Regulatory and Technological Aspects.* S O Duke, (Ed.) Boca-Raton: Lewis Publishers, pp. 231-250.

Gressel, J; Ammon, H U; Fogelfors, M; Gasquez, J; Kay, Q O N; Kees, H (1982) Discovery and distribution of herbicide resistant weeds outside North America. In: *Herbicide Resistance in Plants.* H Le Baron & J Gressel, (eds), New York: Wiley, pp. 31-55.

Gressel, J; Gianessi, L; Darby, C P; Saari, L; Seth, A (1994) *Herbicide-resistant weeds: a threat to wheat production in India.* Discussion Paper PS-94-3, Washington D.C.: National Center for Food and Agricultural Policy, 37 pp.

Heap, I (1988) *Resistance to Herbicides in Annual Ryegrass (Lolium rigidum)*, Ph.D. Thesis, Waite Agr. Inst., Univ. of Adelaide.

Holländer-Czytko, H; Sommer, I; Amrhein, N (1992) Glyphosate tolerance of cultured *Corydalis sempervirens* cells is acquired by an increased rate of transcription of EPSP-synthase as well as by reduced enzyme turnover. *Plant Molecular Biology* **20**, 1029-1036.

Holliday, R J; Putwain, P D (1980) Evolution of herbicide resistance in *Senecio vulgaris*; variation in susceptibility to simazine between and within populations. *Journal of Applied Ecology* **17**, 799-808.

Huang, B Q; Lin, S X (1993) Study on the resistance of barnyardgrass to butachlor in paddy fields in China (in Chinese, English abstract), *Journal of the South China Agricultural University* **14**, 103-108.

Komossa, D; Gennity, I; Sandermann, H Jr (1992) Plant metabolism of herbicides with C-P bonds: glyphosate. *Pesticide Biochemistry and Physiology* **43**, 85-94.

Mackenzie, R; Mortimer, A M; Putwain, P D (1995) The inheritance of chlorsulfuron resistance in perennial ryegrass: strategic implications for management of resistance. *This Volume*.

Peñaloza-Vásquez, A; Oropeza, A; Mena, G L; Bailey A M (1995) Expression of hygromycin B phosphotransferase gene confers tolerance to the herbicide glyphosate. *Plant Cell Reports*, **14**, 482-487.

Powles, S B; Matthews, J M (1992) Multiple herbicide resistance in annual ryegrass (*Lolium rigidum*): A driving force for the adoption of integrated weed management. In: *Achievements and Developments in Combating Pesticide Resistance*, I Denholm, A L Devonshire & D Hollomon (eds), London: Elsevier, pp. 75-87.

Shaner, D (1995) Studies on the mechanisms and genetics of resistance - their contribution to herbicide resistance management. *This Volume*.

Suh, H; Hepburn, A G; Kriz, A L; Widholm, J M (1993) Structure of the amplified EPSP-synthase gene in glyphosate-resistant carrot cells. *Plant Molecular Biology* **22**, 195-205.

Wrubel, R P; Gressel, J (1994) Are herbicide mixtures useful for delaying the rapid evolution of resistance? A case study, *Weed Technology* **8**, 635-648.