SESSION 4C

CHARACTERISATION AND CONTROL OF HERBICIDE RESISTANT WEEDS

Session Organiser	MR D V CLAY *Avon Vegetation Research, Bristol, UK*
Poster Papers	4C-1 to 4C-10

HERBICIDE RESISTANCE AND GENE FLOW IN BLACK-GRASS (*Alopecurus myosuroides*) AND WILD-OATS (*Avena* spp.)

G CAVAN, S R MOSS
IACR-Rothamsted, Harpenden, AL5 2JQ, UK

ABSTRACT

The process of resistance evolution to fenoxaprop-P-ethyl was investigated in the cereal weeds black-grass (*Alopecurus myosuroides*) and wild-oats (*Avena fatua* and *Avena sterilis* ssp. *ludoviciana*) at a number of locations where distinct patches occur within fields. Genetic fingerprints produced using PCR-based techniques did not provide evidence that resistance had spread from one patch to the others. Herbicide-resistant *Avena* patches contained on average less genetic diversity than herbicide-sensitive counterparts but all *A. myosuroides* patches contained similar diversity.

INTRODUCTION

The emergence of herbicide resistance in black-grass (*Alopecurus myosuroides*) and wild-oats (*Avena* spp.) threatens cereal production throughout north-west Europe. At some sites, resistant weeds are present in a number of discrete patches in different fields on the same farm. It is not clear if each patch has evolved through independent mutations or whether resistance gene-flow has occurred by the movement of pollen or seeds. One method to distinguish these hypotheses is to assess the degree to which individual plants are related by common descent, using anonymous DNA markers. In addition, analysis of the markers allows an estimation of the genetic diversity within each patch and examination of the relationship between genetic diversity and the evolution of resistance.

The polymerase chain reaction (PCR) can be used to generate individual-specific banding profiles ('genetic fingerprints') by a variety of techniques. For example, inter-simple sequence amplification, ISSA (Zietkiewicz *et al.*, 1994) uses primers whose sequence is based on core motifs found in repetitive DNA such as minisatellites (Wong *et al.*, 1986) or microsatellites; random amplification of polymorphic DNA, RAPD (Welsh & McClelland, 1990; Williams *et al.*, 1990) uses decamer primers of random sequence. Individuals related by descent would be expected to show similarities in their banding profiles; and so ISSA or RAPD profiles can be used to estimate the genetic distance between any two individuals, the diversity within a patch and the average genetic distance between plants in any two patches (Nei, 1973; 1978; Lynch & Milligan, 1994).

MATERIALS AND METHODS

A. myosuroides seed was sampled from farm Notts A (Moss & Clarke, 1995); *Avena fatua* and *Avena sterilis* ssp. *ludoviciana* from Essex 1 (Moss et al., 1994) during July 1993. *Avena* seeds were incubated dry at 30°C in the dark for 3 weeks then pricked with a needle. All seeds were then incubated with distilled water on moist filter paper at 11°C, 10 hours light/ 14 hours dark until coleoptiles were emerged (*Avena* spp. after 2-4 days, *A. myosuroides* after 5-8 days). Germinated seeds were then sown in compost, singly in 5cm square pots. Seedlings were grown in a heated greenhouse (10 hours light, min. 10°C/ 14 hours dark, min. 8°C) to the three to four leaf stage. One leaf was removed from each plant for DNA analysis, frozen in liquid nitrogen and stored at -20°C. Five days later the plants were treated with fenoxaprop-P-ethyl ('Cheetah Super' EC) at 69 g a.i.ha^{-1} and returned to the heated greenhouse. Individual plants surviving beyond a further 7 weeks were scored as resistant.

Genomic DNA was extracted from 24 seedlings from each patch as in Sharp *et al.* (1988) and 50 ng was added in a total PCR reaction volume of 20μl on ice (10mM Tris-Cl pH8.3, 50mM KCl, 2-4mM $MgCl_2$, 0.2 mM dNTPs, 0.4 U Taq polymerase (Boehringer Mannheim)), mixed and transferred to 94°C on an Hybaid OmniGene thermal cycler (Life Sciences International UK, Basingstoke). ISSA primers were included at 1μM; $MgCl_2$ at 2mM and the reaction program was 40 cycles of: 94°C for 1 minute, 52°C for 1 minute, 72°C for 1 minute; plus a final incubation at 72°C for 5 minutes. RAPD primers were included at 0.4μM with either 3mM or 4mM $MgCl_2$ according to the individual primer and the program was 40 cycles of: 94°C for 1 minute, 36°C for 1 minute, 72°C for 1 minute; plus a final incubation at 72°C for 5 minutes. Reaction products were electrophoresed (10-16 V.hr.cm^{-1}) through 2% agarose/1XTBE stained with 100ng.ml^{-1} ethidium bromide and photographed on an ultra-violet transilluminator. One to five clearly polymorphic bands were scored present or absent for each individual and used as markers and input to the POPGENE program (Yeh & Boyle, 1996) for estimation of genetic distances and diversity.

RESULTS

Three ISSA primers (5'-$(CA)_8$(A/G)(C/T)-3', 5'-$(GA)_8$(A/C)(G/C)-3' and 5'-$(CT)_8$(A/G) (G/C)-3') used singly and in combination generated a total of 20 markers. Banding profiles were more conserved in the *Avena* sampled: for a large number of primers bands were present in all individuals of both species. Three ISSA primers (5'-$(CT)_8$(A/G)(G/C)-3'), 5'-$(GT)_8$(A/C)(G/C)-3' and 5'-ACAGGGGTGTGGGG-3') used singly generated a total of seven markers; three RAPD primers (5'-ACGGATCCTG-3', 5'CCGATATCGG-3', and 5'-GGCTGCAGAA-3') were used with 4mM $MgCl_2$ and a further one (5'-CCAAG CTTCC-3') with 3mM $MgCl_2$; the RAPD primers generated a total of nine markers.

The genetic diversities within each patch (equivalent to the expected heterozygosities under Hardy-Weinberg equilibrium, calculated as in Lynch and Milligan, 1994) are shown in Table 1. Herbicide-resistant *Avena* patches contained on average less genetic diversity (0.18) than herbicide-sensitive counterparts (0.27, SE(8df)=0.034), but all *A. myosuroides* patches contained similar diversity (both averaged 0.34, SE(4df)=0.008).

When total species diversity was partitioned into that between or within patches (Table 2), differences between patches accounted for a greater proportion of total diversity in *Avena* (0.36 overall, 0.30 in *A. fatua* and 0.26 in *A. sterilis.* ssp. *ludoviciana*) than in *A. myosuroides* (0.13).

For both *A. myosuroides* and *Avena* spp., genetic distances were estimated between all pairs of patches and neighbour joining used to place patches in a dendrogram (Fig. 1). Both resistant *A. myosuroides* from patch 2 and resistant *A. fatua* from patch 3 were genetically more similar to sensitive plants from those same patches than to more distant resistant plants.

Table 1. Average gene diversity within patches. The average values are given for resistant and sensitive patches (R and S)

	Alopecurus		*Avena* all markers		*Avena* ISSA		*Avena* RAPDs	
	R	S	R	S	R	S	R	S
Gene Diversity	0.34	0.34	0.18	0:27	0.12	0.27	0.22	0.27

Table 2. Partition of total intraspecific diversity (Ht) into that within patches (Hs) and between patches (Gst)

	Alopecurus	*Avena* overall	*Avena* ISSA	*Avena* RAPDs	*Avena fatua*	*A. sterilis.* ssp. *ludoviciana*
Ht	0.37	0.38	0.38	0.38	0.37	0.29
Hs	0.32	0.22	0.20	0.24	0.26	0.19
Gst	0.13	0.36	0.43	0.31	0.30	0.26

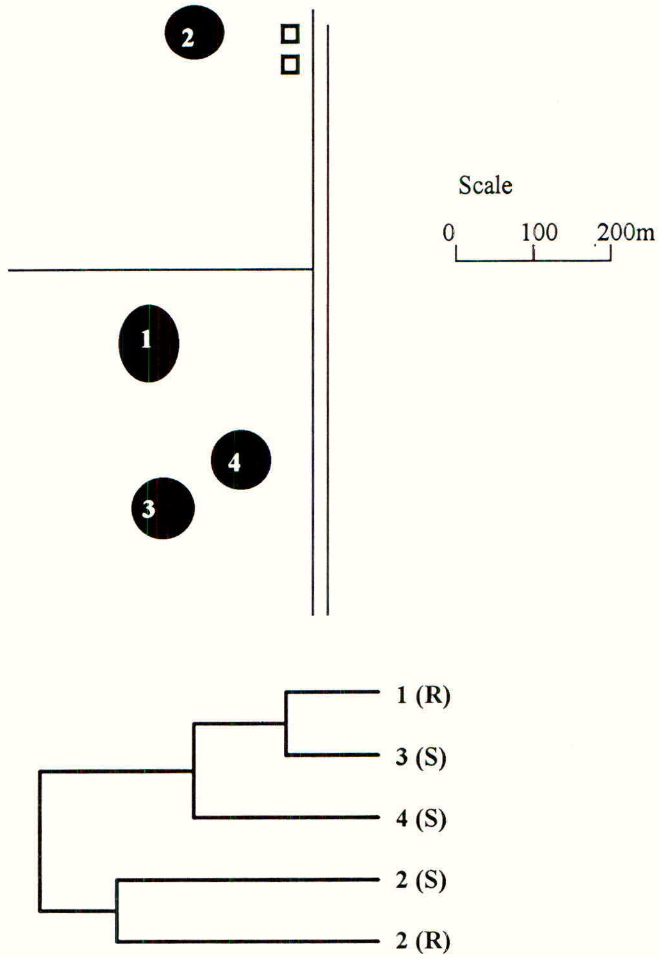
DISCUSSION

The proportion of variation accounted for by differences between the *A. myosuroides* patches (0.13) is six times greater than that estimated using seven aloozyme loci in a survey of more widely separated populations (Chauvel & Gasquez, 1994) but is considerably lower than the figure for either *Avena* species as would be expected from their contrasting breeding systems (Warwick, 1990): *A. myosuroides* is predominantly outcrossing whereas both *Avena* species show high rates of self-fertilisation. The similar level of diversity found in all *A. myosuroides* patches, whether resistant or sensitive also corresponds to the allozyme study. The contrasting situation in *Avena* where variation is markedly lower in resistant than in sensitive patches suggests that herbicide selection pressure reduces diversity only when applied to self-fertilising species.

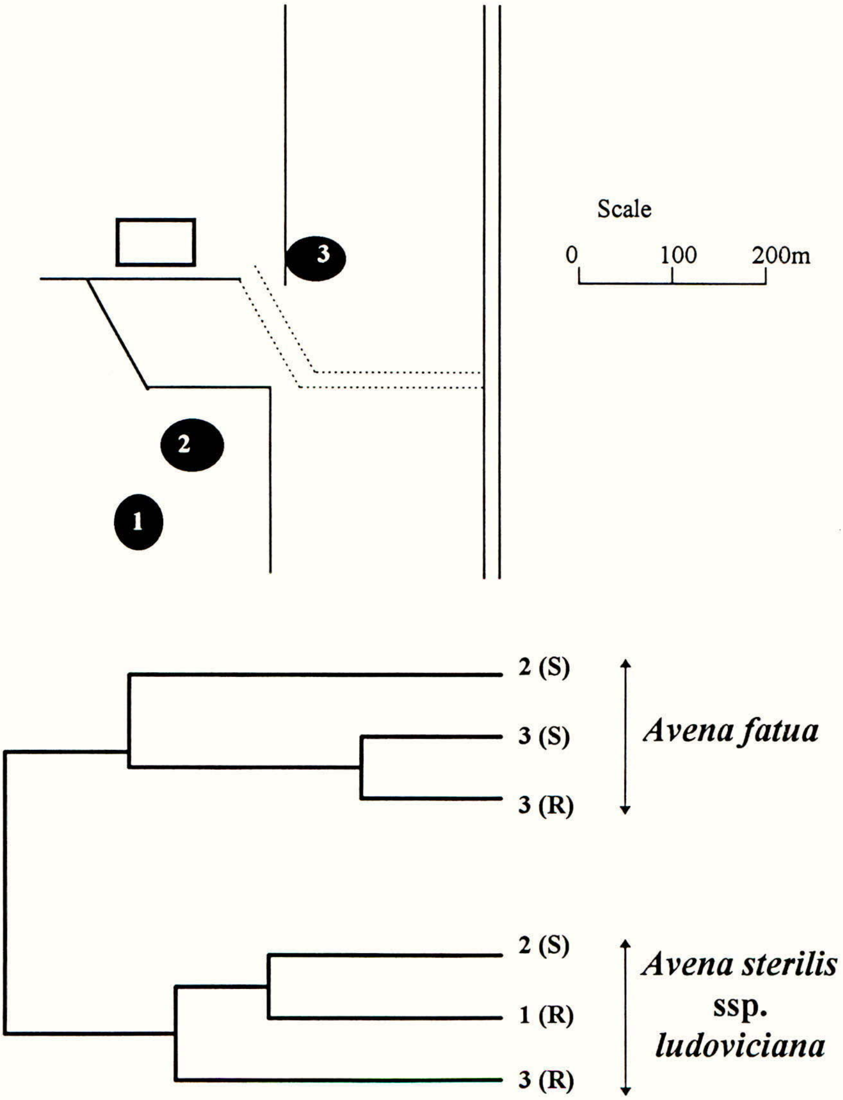
In both the *A. myosuroides* and the *Avena* spp. studied, a close genetic relationship between resistant and sensitive plants from the same patch implies that resistance has evolved by mutation independently within each patch, rather than by the movement of pollen or seed from more distant resistant plants in the same or nearby fields.

Figure 1. Map with the infestations of resistant and sensitive weeds shown as black patches, with dendrograms showing the genetic relationships between them and resistance status (R=resistant, S=sensitive).

1a. *A. myosuroides* patches on farm Notts A (Moss & Clarke, 1995). Patch 2 contains both resistant and sensitive plants.



1b. Map of *Avena* patches on farm Essex 1. Patches 2 & 3 contain both species; there are both resistant and sensitive *A. fatua* in patch 2. The dendrogram includes resistant patches from elsewhere in Essex (4 is from farm Essex 12, 5 from Essex T11) and additional sensitive reference patches (6 if Long Ashton *A. fatua*, 7 is Long Ashton *A.sterilis* ssp.*ludoviciana*) (Moss et al., 1994).



Conversely, it could be argued that where herbicide use is insufficient to kill all sensitive plants they could outcross with a resistant plant, and within two to three generations resistant descendants would have a banding profile more typical of their sensitive ancestors; this could be more reasonably argued for *A. myosuroides* than *Avena* spp. where the high Gst values and reduced diversity in resistant patches are consistent with a low rate of outcrossing. Markers tightly linked to the resistance genes could provide more useful data (Guillemaud *et al.*, 1996).

REFERENCES

Chauvel B, Gasquez J (1994) Relationships between genetic polymorphism and herbicide resistance within *Alopecurus myosuroides* Huds. *Heredity*, **72**, 336-344.

Guillemaud T, Rooker S, Pasteur N, Raymond M (1996) Testing the unique amplification event and the world-wide migration hypothesis of insecticide resistance genes with sequence data. *Heredity*, 77, 535-543.

Lynch M, Milligan B G (1994) Analysis of population genetic structure with RAPD markers. *Mol. Ecology*, **3**, 91-99.

Moss S R, Richardson A, Clarke J (1994) Variation in sensitivity to herbicides in UK wild-oat populations. Project Report, Herbicide Resistance Action Committee.

Moss S R, Clarke J H (1995) Inheritance of herbicide resistance in black-grass (*Alopecurus myosuroides*) and responses of the weed to a range of herbicides. Project Report 116, Hone Grown Cereals Authority, London.

Nei M (1973) Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci.* USA, **70**, 3321-3323.

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics.* **89**, 583-590.

Sharp P J, Kreis M, Shewry P R, Gale M D (1988) Location of b-amylase sequences in wheat and its relatives. Theor. Appl. Genetics, **75**, 286-290.

Warwick S L (1990) Genetic variation in weeds. In Biological approaches and evolutionary trends in plants, (ed S Kawano), pp.3-18. Academic Press, London.

Welsh J; McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res.* **18**, 7213-7218.

Williams J G K; Kubelik A R; Livak K J; Rafalski J A; Tingey S V (1990) DNA polymorphisms amplified with arbitrary primers are useful as genetic markers. *Nucleic Acids Res.* **18**, 6531-6535.

Wong Z, Wilson V, Jeffreys A J, Thein S L (!986) Cloning a selected fragment from a human DNA fingerprint - isolation of an extremely polymorphic minisatellite. *Nucleic Acids Res.* **14**, 4605-4616.

Yeh F C; Boyle T (1996) POPGENE version 1.1: Microsoft window-based software for population genetics analysis. http://www.rr.ualberta.ca/profs/yeh.htm

Zietkiewicz E; Rafalski A; Labuda D (1994) Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics.* **20**, 176-183.

SULFONYLUREA RESISTANCE IN A BIOTYPE OF *MONOCHORIA KORSAKOWII*, AN ANNUAL PADDY WEED IN JAPAN

G X WANG
Tohoku National Agricultural Experiment Station, Omagari, Akita 014-01, Japan

H KOHARA
Hokkaido Central Agricultural Experiment Station, Iwamizawa, Hokkaido 069-03, Japan

K ITOH
National Institute of Agro-Environmental Sciences, Tsukuba, Ibaraki 305, Japan

ABSTRACT

A biotype of *Monochoria korsakowii* resistant to bensulfuron-methyl has been discovered in Hokkaido rice fields that have been treated with bensulfuron-methyl-based herbicides for 5 consecutive years. In vinyl- and greenhouse tests, the biotype was also resistant to both pre-em. and post-em. herbicides with the same mode of action including pyrazosulfuron-ethyl, imazosulfuron and cyclosulfamuron. The resistant biotype was about 100 times more resistant to all sulfonylurea herbicides used in these tests than the susceptible one. This was the first confirmed occurrence of herbicide resistance resulting from the use of a sulfonylurea herbicide in Japan. Herbicides with different modes of action, including pretilachlor, pyrazolate and thenylchlor effectively controlled the resistant biotype on the experimental farm.

INTRODUCTION

Monochoria korsakowii is an annual paddy weed in Hokkaido Prefecture, Japan (Wang & Kusanagi, 1994; Wang *et al.*, 1996). Bensulfuron-methyl (DPX-84) was the first sulfonylurea (SU) herbicide used widely in this area for broadleaf weed control and proved highly effective in controlling *M. korsakowii*. SU herbicides control or suppress broadleaf and some grass weeds by inhibiting acetolactate synthase (ALS), thereby decreasing biosynthesis of branched-chain amino acids (Ray, 1984).

In 1994, over 160,000 ha of rice fields in Hokkaido Prefecture, Japan were treated with SU-based herbicides. But the failure of SU herbicides to control *M. korsakowii* in 1994 at Hokkaido Prefecture after several years of successful use led to an investigation into the possibility of resistance. In this paper, we report the occurrence of *M. korsakowii* biotypes with resistance to bensulfuron-methyl as well as other herbicides with the same mode of action, and indicate what alternative herbicides should control it.

MATERIALS AND METHODS

Field survey

A field survey was conducted in September 1995 on the Ishikari, Sorachi, Iburi, Shiribeshi and Hidaka districts of Hokkaido to determine the proportion of the resistant biotype in the area where the rice fields were treated with SU-based herbicides in May.

Response to sulfonylurea herbicide applications

Experiment 1: This experiment was conducted outdoors in 1995 in 15.8 cm diameter pots filled with gray lowland soil. The resistant seeds were collected in October, 1994 from plants that had survived SU-based herbicide treatments in a rice field of Naganuma Town, Hokkaido Prefecture. Susceptible seeds were collected from an untreated area near Omagari City, Akita Prefecture. Seeds were sowed on May 26, 1995. Seedlings were thinned to about thirty per pot at the 1-leaf stage on June 12 and were treated with bensulfuron-methyl (DPX-84) and pyrazosulfuron-ethyl (NC-311), respectively, under the submerged condition with 3 cm water depth. Herbicide concentrations used on two replicate pots of each biotype are shown in Table 1 with the standard concentrations. Plant length was measured 21 days after the treatment and growth was observed up to flowering time.

Experiment 2: Seeds were obtained in October, 1994 from two sites: a rice field at Naganuma Town, Hokkaido Prefecture that had been successively treated with SU-based herbicides during the 5 previous years and an untreated site near Naganuma Town. Seeds were planted in 11.4 cm diameter pots filled with gley soil in greenhouse with three replications and grown under submerged condition with 3 to 5 cm water depth. Seedlings at the 1-leaf stage were treated with bensulfuron-methyl at 3.13, 6.25, 12.5, 25, 50, 100, 200 and 400 g a.i./ha, pyrazosulfuron-ethyl at 0.66, 2.63, 10.5, 42 and 168 g a.i./ha and imazosulfuron as TH-913 at 2.82, 11.25, 45, 180 and 720 g a.i./ha, respectively. Of these, 25, 10.5 and 45 g a.i./ha were the standard concentrations, respectively. All herbicides were applied as granules. The number of surviving plants was recorded 1 month after treatment.

Experiment 3: Seeds of the same biotypes as experiment 1 were sowed in 15.8 cm diameter pots filled with gray lowland soil and placed outdoors on May 17, 1996 with three replications. Herbicides and doses (g a.i./ha) applied pre-em. (immediately after the sowing) and post-em. (at 1-leaf stage, on May 31) were shown in Table 1. Three plants of each pot were harvested and fresh weight was measured on July 17. 50% growth reduction (GR_{50}) was computed.

Table 1. Doses (g a.i./ha) used in the experiment 3.

Herbicide	R-type	S-type
Bensulfuron-methyl	18.8, 75, 150, 300, 600	0.75, 2.5, 7.5, 18.8, 75
Imazosulfuron	22.5, 90., 180, 360, 720	0.9, 3, 9, 22.5, 90
Cyclosulfamuron*	15, 60, 120, 240, 480	0.6, 1.98, 6, 15, 60

* Chemical name: 1-[[o-(cyclopropylcarbonyl) phenyl] sulfamoyl]-3-(4, 6-dimethoxy-2-pyrimidinyl) urea.

Response to other herbicides

Eleven herbicides having modes of action different from the SU herbicides were tested for their effect on the SU-resistant *M. korsakowii*. The herbicides tested were shown in Table 2. CG-113 granule, SW-751 granule, NSK-855 SC, NC-311KP granule, NC-329 granule, TH-913ADS(L) granule, HSW-941 granule, DPX-84T granule, DPX-84SC granule and TDS-888 SC were applied alone 5 days after rice planting, or sequentially applied with molinate SM granule 20 days after rice planting, respectively, at their standard concentrations. This study was conducted at the Experiment Plot of Hokkaido Central Agricultural Experiment Station with two replications. The number of plants surviving each herbicide treatment was recorded 47 days after rice planting.

Table 2. Herbicides used in this study with modes of action different from the sulfonylureas

Herbicide	Formulation
CG-113	Pretilachlor (4.0%)
SW-751	Pyrazolynate (10.0%)
NSK-855	Bensulfuron-methyl (1.4%) / thenylchlor (5.0%)
NC-311KP	Pyrazosulfuron-ethyl (0.3%) / pentoxazone* (3.9%)
NC-329	Pyrazosulfuron-ethyl (0.3%) / pretilachlor (4.5%) / dimethametryn (0.6%) / esprocarb (15.0%)
TH-913ADS (L)	Imazosulfuron (0.9%) / pretilachlor (4.5%) / dimethametryn (0.6%) / daimuron (15.0%)
HSW-941	Pretilachlor (3.0%) / pyrazolynate (18.0%) / benfuresate (3.0%) / dimethametryn (0.6%)
DPX-84T	Bensulfuron-methyl (0.75%) / mefenacet (10.0%)
DPX-84SC	Bensulfuron-methyl (0.75%) / esprocarb (21.0%)
TDS-888	Bensulfuron-methyl (1.4%) / pyributicarb (12.0%)
Molinate SM	Molinate (8%) / simetryn (1.5%) / MCPB (0.8%)

* Chemical name: 3-(4 -chloro-5-cyclopentyloxy-2-fluorophenyl)-5-isopropylidene-1,3-oxazolidine-2,4-dione.

RESULTS AND DISCUSSION

Field survey

Resistant biotypes were observed at 30 of 55 sites (cities, towns or villages) surveyed on Ishikari (9 sites), Sorachi (10), Iburi (4) and Hidaka (7) districts. No resistant biotype was observed on Shiribeshi districts of Hokkaido, Japan (Fig. 1).

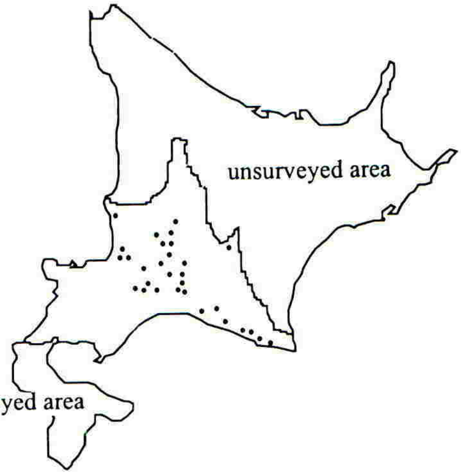


Fig. 1. The sites (•) where the SU-resistant biotype of *Monochoria korsakowii* were first observed at Hokkaido Prefecture, Japan in 1995.

Response to sulfonylurea herbicide applications

Experiment 1: The plants of *M. korsakowii* from a rice field of Naganuma Town, Hokkaido Prefecture that had been successively treated with SU-based herbicides during the five previous years exhibited a high level of resistance to both bensulfuron-methyl and pyrazosulfuron-ethyl. Though the 2× and 4× rates of standard concentration were used, grewth, flowering and fruiting were identical to control plants (Table 3). Even at the 8× rate, a certain number of plants survived. In contrast, all the plants from susceptible population died or the growth was controlled.

Experiment 2: Response to the standard and higher herbicide doses is shown in Fig. 2. Data for lower doses is not shown: plants of susceptible biotypes were all killed and resistant biotypes unaffected by these doses. The resistant biotypes treated at the recommended rate of bensulfuron-methyl, pyrazosulfuron-ethyl and imazosulfuron applications at the 1-leaf stage, 25 g a. i. / ha, 10.5 g a. i. / ha and 45 g a. i. / ha, respectively, and all rates up to 2× recommended rate, showed no mortality. Even at 4×, a substantial number of plants (97%, 90% and 100%, respectively) survived and grew normally. In contrast, all the plants from the susceptible population died at all doses of bensulfuron-methyl, pyrazosulfuron-ethyl and imazosulfuron.

Experiment 3: The response of the resistant and susceptible biotypes varied among SU herbicides (Table 4). The GR_{50} values for the resistant biotype treated pre-em. with

Table 3. Plant length (% of control) of *Monochoria korsakowii* treated with sulfonylurea herbicides.

Bensulfuron-methyl			Pyrazosulfuron-ethyl		
Dose	Biotype		Dose	Biotype	
(g a.i./ha)	Resistant	Susceptible	(g a.i./ha)	Resistant	Susceptible
6000.00	0.0	—			
3000.00	0.0	—	1260.00	0.0	—
1500.00	2.5	—	420.00	0.0	—
600.00	12.5	—	168.00	3.0	—
300.00	22.5	—	84.00	17.5	—
150.00	50.0	2.0	42.00	45.0	0.5
75.00 *	75.0	4.0	21.00 *	78.5	3.5
37.50	90.0	5.0	10.50	93.5	6.5
18.80	95.0	9.0	5.25	95.0	6.5
7.50	100.0	22.5	2.10	100.0	20.0
3.80	—	26.5	1.05	—	42.5
0.75	—	72.5	0.21	—	100.0
0.38	—	100.0	0.10	—	100.0
Control	100.0	100.0	Control	100.0	100.0

* Standard concentration.

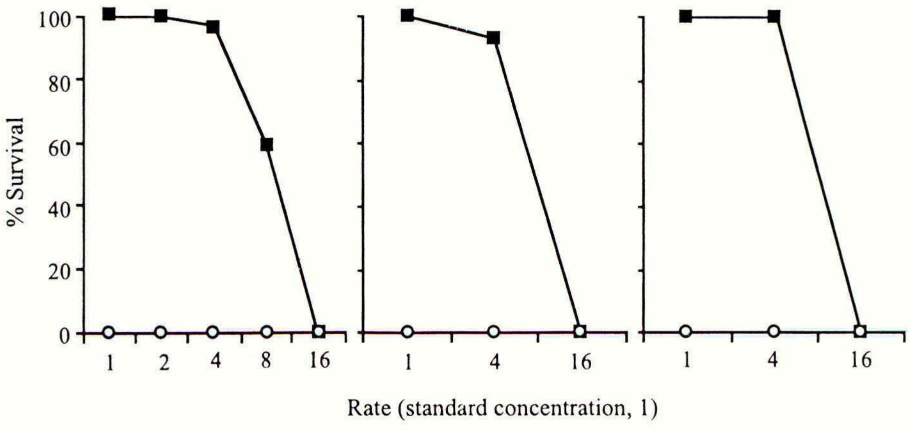


Fig. 2. Effect of bensulfuron-methyl (left), pyrazosulfuron-ethyl (middle) and imazosulfuron on survival of *M. korsakowii* from resistant (—■—) and susceptible (—○—) populations.

bensulfuron-methyl, imazosulfuron and cyclosulfamuron were more than 126, 107 and 83 times the GR_{50} for the susceptible biotype, respectively. When treated post-em. the GR_{50} values were more than 100, 129 and 95 times, respectively. The GR_{50} values for both the resistant and susceptible biotypes treated post-em. (at 1-leaf stage) were more than those treated pre-em.

Table 4. Response of *Monochoria korsakowii* to several SU herbicides.

SU herbicide	GR_{50} (50% growth reduction)					
	Treated pre-em.			Treated post-em.		
	R-type [1] (g a.i./ha)	S-type [2] (g a.i./ha)	R/S	R-type (g a.i./ha)	S-type (g a.i./ha)	R/S
Bensulfuron-methyl	130.21	1.03	126	148.45	1.48	100
Imazosulfuron	183.78	1.72	107	258.58	2.01	129
Cyclosulfamuron	50.32	0.61	83	90.65	0.95	95

[1] Resistant biotype; [2] Susceptible biotype.

Table 5. Effect of several herbicides on the survival of *Monochoria korsakowii* from SU-resistant population.

Product	Product rate (per ha)	Single application [1]	Sequential application [2]
CG-113	10 kg	0 [3]	0
SW-751	30 kg	0	0
NSK-855	5 *l*	0	0
NC-311KP	10 kg	0	0
NC-329	10 kg	0	0
TH-913ADS(L)	10 kg	0	0
HSW-941	10 kg	0	0
DPX-84T	10 kg	5	0
DPX-84SC	10 kg	8	0
TDS-888	5 *l*	7	2
Control	—	9	—

[1] Single applications were conducted five days after rice planting.

[2] Molinate SM granule was applied 15 days after single application.

[3] The number showed the surviving plants per pot 47 days after rice planting.

Response to other herbicides

Plants from the SU resistant populations of *M. korsakowii* were effectively killed by the herbicides, including pretilachlor (CG-113), pyrazolate (SW-751), thenylchlor and pentoxazone applied at recommended rates (Table 5). In order to control the plants from the resistant population effectively, it was also practicable to apply DPX-84T and DPX-84SC sequentially with molinate SM (Table 5). These tests suggest that control of SU-resistant *M. korsakowii* is possible with herbicides having a different mode of action applied at the early stage and middle stage of growth.

These experiments showed the *M. korsakowii* biotype from a rice field of Naganuma Town, Hokkaido Prefecture was resistant to sulfonylurea herbicides. This biotype showed cross resistance to the four sulfonylurea herbicides tested but was controlled by herbicides with a different mode of action.

This is the first report of naturally occurring resistance due to selection by the sulfonylurea herbicides in Japan. Since our discovery, four more resistant species or varieties, *Lindernia micrantha* D. Don., *L. dubia* var. *dubia* Pennell, *L. dubia* var. *major* Pennell and *L. pyxidaria* Pennell have been identified at the northeastern districts of Japan (Itoh & Wang, 1997).

The major factors controlling the evolution of herbicide resistance in weeds are the selection pressure and the initial frequency of resistant genes in the population (Gressel & Segel, 1978). The initial frequency of genes for herbicide resistance in weed populations is initially low. Therefore the nature and extent of the selection pressure is important in determining the rate at which weed populations become resistant (Gressel & Segel, 1978). The resistant biotype of *M. korsakowii* has become evident (Fig. 1) following application of SU herbicides year after year from 1988 as the main means of weed control on rice field that has been the selection pressure. Moreover the mating systems are also important in determining the rate at which *M. korsakowi* populations become resistant. *M. korsakowii* have somatic enantiostyly, i. e., each plant bears two morphs of flowers, left- and right-handed flowers, with the style deflection to the left and right, respectively (Wang *et al.*, 1995). Enantiostyly has been interpreted as a mechanism which promotes cross-pollination (Bowers, 1975, Ornduff & Dulberger, 1978, Dulberger & Ornduff, 1980, Webb & Lloyd, 1986), and in the case of *M. korsakowii*, the mean of outcrossing rates was 72.3% when the flowers were visited by both *Apis cerana japonica* and *Xylocopa circumvolans*, and the mean of outcrossing rates was 49.2% by only *Apis cerana japonica* (Wang *et al.*, non published). Wang *et al.* (non published) carried out the study on the inheritance of SU resistance in *M. korsakowii* and the results provided a good evidence that a single dominant gene controls the SU resistance in *M. korsakowii.* Therefore the resistant plants are developed not only from the spread of SU-resistant seeds from the original site to the non-resistant site following the movement of soil adhered on the farm machinery but also from cross-pollination with resistant pollens transported by various insects. The above results will is helpful to manage the SU-resistant *M. korsakowii*.

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REFERENCES

Bowers K A W (1975). The pollination ecology of Solanum rostratum (Solanaceae). *Ameican Journal of Botany.* **62**, 633-638.

Dulberger R & Ornduff R (1980). Floral morphology and reproductive biology of four species of *Cyanella* (Tecophilaeaceae). *New Phytologist.* **86**, 45-56.

Gressel J & Segel L A (1978). The paucity of plants evolving genetic resistance to herbicides: Possible reasons and implications. *Journal of Theoretical Biology.* **75**, 349-371.

Itoh K & Wang G X (In press). An outbreak of sulfonylurea herbicide resistance in Scrophulariaceae paddy weeds in Japan. *16th Asian-Pacific Weed Science Society Conference Proceedings*, Kuala Lumpur.

Ornduff R & Dulberger R (1978). Floral enantiomorphy and reproductive system of *Wachendorfia paniculata* (Haemodoraceae). *New Phytologist.* **80**, 427-434.

Ray T B (1984). Site of action of chlorsulfuron. Inhibition of valine and isoleucine biosynthesis in plants. *Plant Physiology.* **75**, 827-831.

Wang G X & Kusanagi T (1994). Distributions of *Monochoria korsakowii* and *M. vaginalis* in Japan, and the scientific name of the Japanese paddy weed, Konagi. *Weed Research, Japan.* **39** (Supplement I) 226-227.

Wang G X, Miura R & Kusanagi T (1995). The Enantiostyly and the Pollination Biology of *Monochoria korsakowii* (Pontederiaceae). *Acta Phytotaxonomica et Geobotanica.* **46**, 55-65.

Wang G X, Kusanagi T & Itoh K (1996). Isozyme variation in *Monochoria korsakowii* and *M. vaginalis* (Pontederiaceae). *Weed Research, Japan.* **41**, 255-263.

Webb C J & Lloyd D G (1986). The avoidance of interference between the presentation of pollen and stigmas in angiosperms. *New Zealand Journal of Botany.* **24**, 163-178.

CHLORSULFURON CROSS-RESISTANCE IN A CHLOROTOLURON-RESISTANT BIOTYPE OF *ALOPECURUS MYOSUROIDES*

J M MENENDEZ, R DE PRADO
Departamento de Química Agrícola y Edafología. E.T.S. Ingenieros Agrónomos y de Montes, Apdo. 3048, 14080-Córdoba, Spain

M D DEVINE
Department of Crop Science and Plant Ecology, University of Saskatchewan, Saskatoon, Saskatchewan, Canada S7N 5A8

ABSTRACT

A chlorotoluron-resistant biotype of *Alopecurus myosuroides* found in Spain exhibited cross-resistance to the acetolactate synthase (ALS)-inhibiting herbicides chlorsulfuron and imazamethabenz. Herbicide rates that inhibited shoot growth by 50% (ED_{50}) values were determined for several ALS inhibitors, including both sulfonylureas and imidazolinones. For chlorsulfuron and imazamethabenz, the ED_{50} values for the resistant biotype were 35 and 27 times higher than that observed in the susceptible biotype, respectively. In addition, this resistant *A. myosuroides* biotype displayed a lower but significant increase in tolerance to triasulfuron, the other sulfonylurea tested. There were no significant differences between the resistant and the susceptible biotypes in ALS sensitivity to chlorsulfuron and imazamethabenz. Therefore, a mutation at the target site is not the resistance mechanism. Preliminary metabolism studies revealed that both biotypes were able to metabolize chlorsulfuron to the same polar compounds. However, the resistant biotype metabolized the herbicide faster and to a greater extent than the susceptible biotype. Cytochrome P450-mediated herbicide metabolism has been previously characterized as the mechanism of resistance to chlorotoluron and diclofop-methyl in this biotype. We suggest that chlorsulfuron resistance in the resistant biotype of *A. myosuroides* is due to its greater ability to metabolize this herbicide to non toxic metabolites.

INTRODUCTION

Alopecurus myosuroides is a cross-pollinating weed present in Europe and occurring in winter wheat and legume crops. In the last twenty years, several *A. myosuroides* populations from Germany, UK, Israel, Holland and France have developed resistance to herbicides following selection with these chemicals (Niemann & Pestemer, 1984, Moss & Cussans, 1985, Yaacoby *et al.*, 1986, Smant, 1991; Gasquez, 1995). In 1991, a chlorotoluron-resistant (R) biotype of *A. myosuroides* was detected in northeastern Spain (De Prado *et al.*, 1991). This biotype exhibits varying degrees of cross-resistance to phenylurea, aryloxyphenoxypropionate and cyclohexanedione herbicides (Menendez *et al.*, 1994, Menendez & De Prado, 1996) used specifically to control this grass weed. Previous studies revealed that chlorotoluron and diclofop-methyl resistance in the R biotype was due to enhanced detoxification, this process being catalyzed by cytochrome P450 monoxygenases (Menendez & De Prado, 1996, 1997). As the presence of this mechanism of resistance often confers a moderate, broad-spectrum cross-resistance to other herbicides from different chemical families, cross-resistance to graminicides different from those previous studied would not be surprising. Sulfonylurea and imidazolinone herbicides such as chlorsulfuron and imazamethabenz are potent inhibitors of acetolactate synthase (ALS; EC 4.1.3.18), a key enzyme in the biosynthesis of branched-chain amino acids. These herbicides, used pre-em. and post-em., are recommended for broadleaf and grass weeds control in cereals. However, despite the relative newness of these herbicides, chlorsulfuron-resistant weed biotypes have been already described, some of them with enhanced metabolism as the mechanism of resistance.

This study was conducted to: investigate the whole plant response of resistant *A. myosuroides* to several sulfonylurea and imidazolinone herbicides under growth room conditions; compare the susceptibility to ALS inhibitors of the resistant and susceptible biotypes at the enzyme level; and determine whether enhanced metabolism is the basis of chlorsulfuron resistance in the R biotype.

MATERIALS AND METHODS

Chemicals and plant material

[^{14}C]chlorsulfuron (specific activity, 0.42 MBq mg^{-1}) were kindly supplied by E.I. du Pont de Nemours and Co. Commercial formulations of chlorsulfuron ('Glean' 75% *wt/wt* MG), triasulfuron ('Logran 20' 20% *wt/wt* WG), imazapyr ('Arsenal' 25% *wt/v* SL) and imazamethabenz ('Assert' 30% *wt/v* SC) were supplied by Du Pont Iberica S.A., Novartis and Cyanamid Iberica S.A. All other chemicals used were in analytical grade. Seeds from both resistant and susceptible *A. myosuroides* were germinated and planted in 6.5 cm diameter, 7 cm high plastics posts as previously described (Menendez *et al.*, 1994; Menendez & De Prado, 1996). All plants were grown in a grown chamber at 24°C light and 18°C dark periods, 16-h photoperiod, 350 μmol m^{-2} s^{-1} light intensity. Relative humidity was a constant 80%. Plants were watered as needed.

Growth assays

In pre-em. treatments (triasulfuron), pregerminated seeds were planted in pots sprayed with commercial formulation of triasulfuron at several concentrations using a laboratory track sprayer equipped with flat-fan nozzles (Albuz) delivering 200 litres ha^{-1} at 250 kPa. In post-em. treatments, resistant and susceptible plants at the two- to three-leaf stage were sprayed with commercial formulations using the same track sprayer and conditions. In both pre-em- and post-em assays, treatments were replicated three times (five plants per replicate) in a completely randomized design, and shoot fresh weight was recorded after 21 days for each treatment. Concentrations of herbicide that caused a 50% decrease of growth with respect to the control (ED50) were determined for each biotype and herbicide as previously described (Menendez *et al.*, 1994). For these and the ALS experiments, Newman-Keuls' LSD tests were used to detect differences between resistant and susceptible biotypes.

ALS assays

Acetolactate synthase extraction and assays were performed following the protocol outlined by Devine *et al.* (1991). The enzyme was extracted from 3 g of leaf tissue, precipitated by ammonium sulphate fractionation, and desalted on a PD-10 Sephadex G-25 column. 50 µlitre samples of the eluted protein solution were assayed in triplicate at final chlorsulfuron and imazamethabenz concentrations of 10^{-9} to 10^{-4} M. Results were expressed as I_{50} values (concentration of herbicide required to reduce enzyme activity by 50%). Experiments were replicated five times. Imazamethabenz (i.e. the free acid) was produced as previously described (Devine *et al.*, 1991).

Metabolism experiments

Metabolism of [^{14}C]chlorsulfuron was examined in leaf and root tissue from plants at the three-leaf stage grown as above. [^{14}C]chlorsulfuron was diluted with the commercial herbicide to a final specific activity of 4800 Bq mg^{-1}. The final chlorsulfuron concentration was 250 mg litre^{-1}. 2400 Bq were applied to the adaxial surface of the third leaf on each plant in four 0.5 µlitre droplets with a microapplicator. Plants were harvested 24 and 48 h after herbicide treatment. The treated leaves were washed with 1 ml 80% acetone to remove unabsorbed radiolabel. Aliquots of the leaf wash solution were assayed for radioactivity. The rinsed, treated plants were excised in shoots and roots for separate extraction. Plant tissue (shoots or roots) was pulverized in liquid nitrogen using a mortar and pestle. The powder was extracted at 4°C with 80% acetone as described in Menendez & De Prado (1996). Chlorsulfuron and its metabolites in the extract were separated by tlc on 250-µm silica gel plates and a chloroform: acetic acid (19:3, *v/v*) mobile phase. The radioactive zones were detected with a radiochromatogram scanner and [^{14}C]chlorsulfuron was identified by comparing the Rf from the radioactive spots with the Rf obtained for cold chlorsulfuron. For quantitative determinations, the radioactive zones were scraped off, extracted with 80% acetone and measured by liquid scintillation counting. The experiment was repeated three times.

RESULTS

Growth assays

In the dose-response experiments, both chlorsulfuron and imazamethabenz failed in controlling resistant *A. myosuroides* at usual field rates (Table 1). The R biotype showed ED_{50} values of 69.4 and 3032 g a.i. ha^{-1} for chlorsulfuron and imazamethabenz, respectively, this biotype being 35.6 and 27 times more resistant than the susceptible biotype to these two herbicides. In contrast, both biotypes were susceptible to the non-selective imidazolinone herbicide imazapyr (Table 1). Data obtained from triasulfuron assays could suggest that there are no differences in response to this herbicide between R and S plants. However, although both biotypes showed similar decrease of weight at the lower herbicide rates tested, S plants displayed a 100% mortality at triasulfuron doses greater than 5 g a.i. ha^{-1}, while R plants survived herbicide rates of 30 g a.i. ha^{-1} (data not shown).

Table 1. Effect of several sulfonylurea and imidazolinone herbicides on growth of chlorotoluron-resistant and -susceptible biotypes of *A. myosuroides*.

	ED_{50} (g a.i. ha^{-1})[a]		
Herbicides	Resistant	Susceptible	Resistance factor[b]
Sulfonylureas			
Chlorsulfuron	69.4a	1.9b	35.6
Triasulfuron	0.8a	0.7b	1.2
Imidazolinones			
Imazamethabenz	3032.1a	111.8b	27.1
Imazapyr	18.7a	11.1a	1.7

[a] ED_{50}, herbicide concentration causing 50% decrease in shoot fresh weight.

[b] Resistance factor, $ED_{50}(R)/ED_{50}(S)$.

Note: Values are means of three experiments. Means followed by the same letter within rows are not significantly different according to Newman-Keuls' LSD test.

ALS assays

No significant differences were found between the R and S biotypes in terms of ALS sensitivity to chlorsulfuron and imazamethabenz (Figure 1). I_{50} values for chlorsulfuron ranged from 10.2 (resistant) to 14 nM (susceptible), while ALS activity was less susceptible to imazamethabenz, with I_{50} values ranging from 5.4 (susceptible) and 5.8 μM (resistant). The values obtained are in the same range of those observed in other grasses with sensitive ALS and discard an alteration of the target site as possible mechanism of resistance.

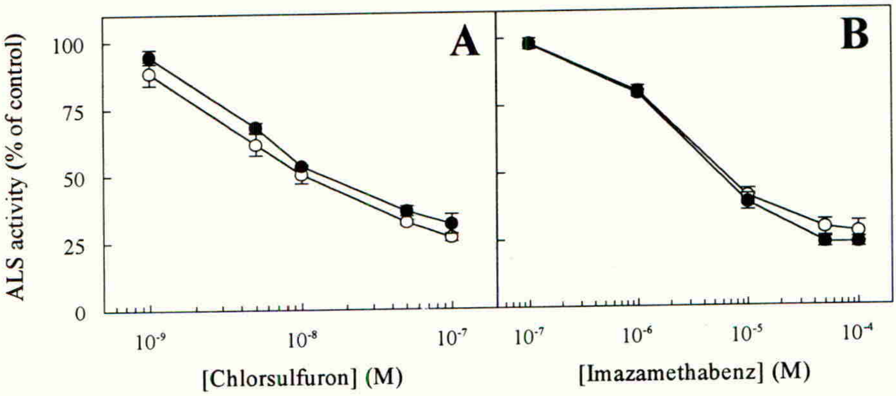

Figure 1. Inhibition of ALS activity in a chlorotoluron-resistant (open symbols) and -susceptible (closed symbols) biotype of *A. myosuroides* by chlorsulfuron (A) and imazamethabenz (B). Vertical bars represent standard errors of the means.

Metabolism studies

Applied [^{14}C]chlorsulfuron was not mobilized from treated leaves to roots during the experiment, as no appreciable radioactivity was extracted from the excised roots at any of the harvest times. In shoots, the same chlorsulfuron derivatives (namely chlorsulfuron and several more polar unknown metabolites referred to as Fraction metabolite(s) I, II and III) were found in both R and S biotypes of *A. myosuroides* (Figure 2). However, chlorsulfuron was metabolized much more rapidly in resistant plants. Therefore, only 10% of the extracted radioactivity was quantified as chlorsulfuron in the R biotype 48 h after treatment. In contrast, almost 40% of the radioactivity present in S extracts was the parent herbicide at the same period of incubation (Figure 2). The nature of Fractions I, II and III is still unknown, the order of polarity being Fraction I > Fraction II > Fraction III > chlorsulfuron. The R biotype formed about 50% more Fraction I metabolite(s) than the S biotype 48 after treatment, with corresponding decreases in chlorsulfuron fractions as the polar compound increased. Fraction II values were similar during all the experiment and Fraction III was detectable only at the first harvest time (Figure 2).

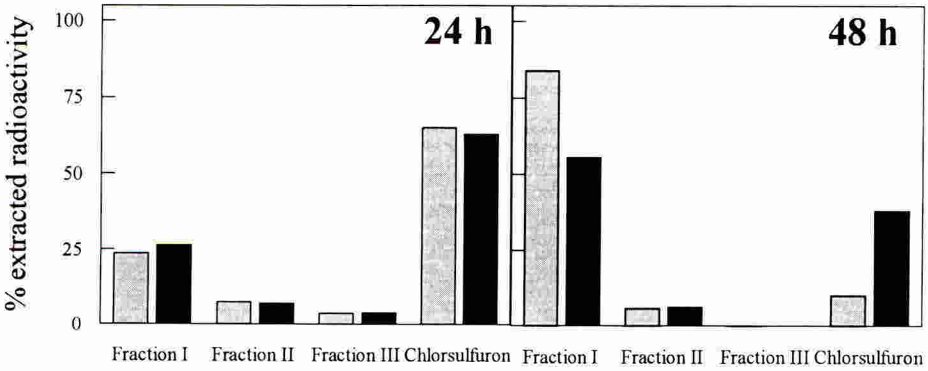

Figure 2. Percentage of extracted radiolabel found as chlorsulfuron (Rf: 0.79), Fraction I (Rf: 0.0), Fraction II (Rf: 0.10) and Fraction III (Rf: 0.62) in leaf extracts of chlorotoluron-resistant (grey bars) and -susceptible (black bars) biotypes of *A. myosuroides* 24 and 48 h after treatment with [^{14}C]chlorsulfuron. Data for a typical experiment is presented.

DISCUSSION

The chlorotoluron-resistant biotype of *A. myosuroides* exhibited cross-resistance to the sulfonylurea herbicide chlorsulfuron and the imidazolinone herbicide imazamethabenz. As previously described for phenylurea, aryloxyphenoxypropionate and cyclohexanedione herbicides (Menendez *et al*., 1994, Menendez & De Prado, 1996), this cross-resistance phenomenon is not related to specific chemical families but to specific herbicides. Thus, the R plants were susceptible and only slightly resistant to imazapyr and triasulfuron, respectively, two herbicides belonging to the same chemical families. Resistance to these herbicides is not due to a mutation at the target site, as both biotypes show similar chlorsulfuron- and imazamethabenz-sensitive ALS activity. In addition, as differences in herbicide absorption, penetration and translocation did not contribute to chlorotoluron or diclofop-methyl resistance (Menendez *et al*., 1994, Menendez & De Prado, 1996), these processes are not likely to be the mechanisms of resistance to chlorsulfuron and imazamethabenz. Enhanced detoxification is probably the basis for chlorsulfuron resistance in the R biotype, as this herbicide was metabolized and conjugated with polar compounds faster and to a greater extent in the resistant biotype than in the susceptible biotype. Although the nature of the unknown metabolites must be ascertained, these data are consistent with those observed in chlorotoluron and diclofop-methyl metabolism, where the resistant biotype mimics wheat's ability to metabolize selective graminicides, these process being catalyzed in the resistant biotype by cytochrome P450 enzymatic systems (Menendez & De Prado, 1996, 1997). The mechanism of resistance to chlorotoluron developed in *A. myosuroides* also confers resistance to diclofop-methyl and, probably, to chlorsulfuron and imazamethabenz. Therefore, the moderate, broad-spectrum cross-resistance conferred by enhanced detoxification threatens to severely limit the usefulness of the selective graminicides available, making complete control of this biotype extremely difficult.

REFERENCES

De Prado, R; Menendez, J; Tena, M; Caseley, J; Taberner, A (1991) Response to substituted ureas, triazines and chloroacetanilides in a biotype of *Alopecurus myosuroides* resistant to chlorotoluron. *Proceedings Brighton Crop Protection Conference - Weeds*. 1065-1070.

Devine, M D; Marles, M A S; Hall, L M (1991) Inhibition of acetolactate synthase in susceptible and resistant biotypes of *Stellaria media*. *Pesticide Science*. **31,** 273-280.

Gasquez, J (1995) Etat de la résistance aux herbicides chez les adventices. *XVIeme Congrès COLUMA Reims*, pp. 201-210.

Menendez, J; De Prado, R (1996) Diclofop-methyl cross-resistance in a chlorotoluron-resistant biotype of *Alopecurus myosuroides*. *Pesticide Biochemistry and Physiology*. **56,** 123-133.

Menendez, J; De Prado, R (1997) Detoxification of chlorotoluron in a chlorotoluron-resistant biotype of *Alopecurus myosuroides*. Comparision between cell cultures and whole plants. *Physiologia Plantarum*. **99,** 97-104.

Menendez, J; Jorrin, J; Romera, E; De Prado, R (1994) Resistance to chlorotoluron of a slender foxtail (*Alopecurus myosuroides*) biotype. *Weed Science*. **42,** 340-344.

Moss, S R; Cussans, G W (1985) Variability in the susceptibility of *Alopecurus myosuroides* (black-grass) to chlorotoluron and isoproturon. *Aspects of Applied Biology*. **9,** 91-98.

Niemann, P; Pestemer, W (1984) Resistenz verschiedener Herkünfte von Acker-Fuchsschwanz (*Alopecurus myosuroides*) gegenüber Herbizidbehandlungen. *Nachrichtenblatt des Deutschen Pflanzenschutzdienstes (Braunschweig)*. **36,** 113-118.

Smant, G (1991) Eerste vaststelling van resistentie bij duist tegen chloortoluron in Nederland. *Gewasbescherming*. **22,** 62-64.

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

DEVELOPMENT OF NEW RELIABLE QUICK TESTS AND STATE OF GRASS-WEED HERBICIDE RESISTANCE IN FRANCE

A LETOUZÉ, J GASQUEZ, D VACCARA
Institut National de la Recherche Agronomique, BV 1540, 21034 Dijon Cedex France

D ORLANDO
Institut Technique des Céréales et des Fourrages, 91720 Boigneville France

JL LETERRIER
AgrEvo France, Les Algorithmes, St Aubin, 91197 Gif/Yvette Cedex France

C ROY, E BOUVARD-DERIEUX
NOVARTIS, 14 Bd. Richelieu, TSA 30002, 92845 Rueil-Malmaison Cedex France

ABSTRACT

Since 1993, a working group and a network of companies and technical institutes have found in France populations of blackgrass (*Alopecurus myosuroides*), rye grass (*Lolium rigidum*) and wild oats (*Avena fatua*) which are resistant to aryloxyphenoxypropionic acids (APP) and to phenylureas. Among resistant populations some survive more than 100 times the lethal dose (LD 100) suggesting that several resistance mechanisms may be involved. Almost all the resistant blackgrass populations are distributed in northern France. There are still very few resistant wild oat populations. Up to now, the resistance to APP has been usually checked by spraying seedlings with herbicide. This spray technique is time consuming and not very accurate. Therefore we have developed three efficient, rapid and portable tests which allow easy detection of the APP resistant blackgrass in order to check their frequency within a population. These tests use either seedlings, young tillers or pollen grains of blackgrass.

INTRODUCTION

Since the first compilation on herbicide resistances in 1982 (Bandeen *et al.* 1982), the number of herbicide resistant species is regularly increasing : in 1982 only about 30 species were reported, about 100 species in 1990 (LeBaron, 1991) and more than 160 nowadays (I.Heap personal com.). Fortunately the number of resistant species in France is very limited. For more than twenty years only triazine resistant species have been reported. This resistance is still present in maize fields and vineyards. Nevertheless farmers are still using atrazine, the herbicide responsible for the selection of the resistance. In 1993 we invited representatives from the INRA, technical institutes, companies and cooperatives to form a working group (within the Association Nationale pour la Protection des Plantes) in order to search for new resistances in every major crop in France. Blackgrass and rye grass were the only reported species which were hard to control and problems were only met in winter cereals with phenylureas and aryloxyphenoxypropionic acids (APP). Furthermore it was rather difficult to detect these two kinds of resistance. There is no test like fluorescence used to detect resistance to triazine. APP resistant plants were usually detected by spraying plants at the three leaf stage. This method has disadvantages in that it is time consuming (several weeks), expensive, not very accurate, susceptible plants are killed and heterozygous and homozygous resistant plants can not be distinguished. Therefore we tried to find some reliable tests which were easier to use and quicker.

SURVEY OF WEED RESISTANCE

Materials and Methods

Inquiries were made through the networks of several companies. They searched for farmers who were complaining about a poor weed control. Thanks to a simple questionnaire they checked the potential for an effective resistance in the field. They took into account the variation of the crop rotation, the number and the type of herbicides used and the quality of the weed control. Seeds were colllected from fields where the poor control was certainly not a occasional failure of the herbicide treatments. Seeds were sown in the greenhouse in shallow trays filled with soil (2/3 of clay and 1/3 of sand) and kept in greenhouse (18 °C by day / 10 °C by night). Plants were thinned to leave 25 seedlings in each tray. Four trays were sprayed per dose using a laboratory track sprayer giving a volume rate of 300 litres/ha. The principle of our greenhouse spray test is to determine for each herbicide and for susceptible plants of each species the lethal dose (LD 100) through a range of doses. Then each population is sprayed with one dose right above the LD 100 previously determined. The surviving plants are counted and represent the rate of resistant plants within each population. Blackgrass populations were tested with 800 g/ha isoproturon as « IP Flo » 500 g a.i./litre SC and 69 g/ha fenoxaprop-P-ethyl as « Puma S » 69 g a.i./litre EW. Rye grass populations were sprayed with 2500 g/ha chlorotoluron as « Dicuran » 500g a.i./litre SC and 1800 g/ha diclofop methyl as « Illoxan CE » 360 g a.i./litre EC. Wild oat populations have only been tested with 69 g/ha fenoxaprop-P-ethyl.

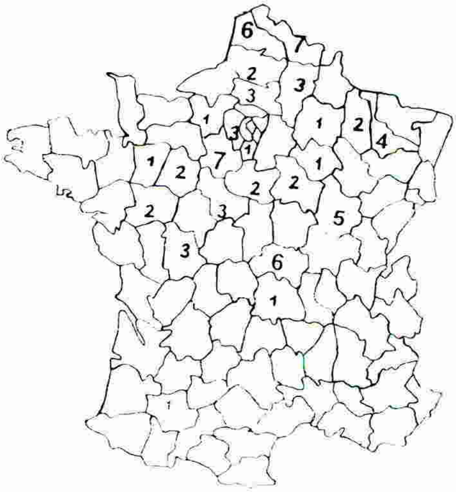

Fig 1. Distribution of resistant blackgrass populations (number found in each department)

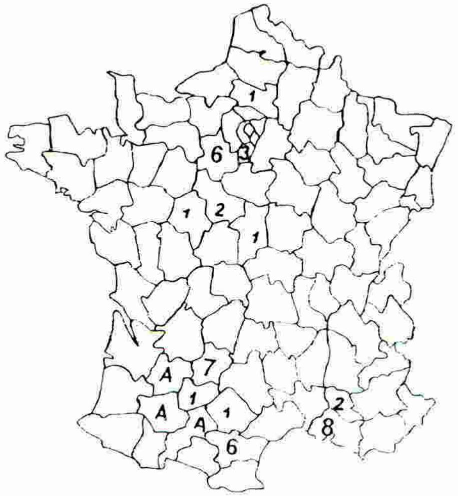

Fig 2. Distribution of resistant rye grass and wild oat populations (A)

Results

Since 1993 we have found many APP resistant populations of blackgrass and rye grass (Orlando *et al.* 1995). The 83 APP resistant populations of blackgrass (Table 1) were collected in 25 departments, mainly in northern France (Fig. 1). The porportion of APP resistant plants within a population has increased from 10-40% in 1993 to 100% in 1996 (Gasquez, 1996). In 1995, we already pointed out that the APP resistant populations of blackgrass are a mixture of susceptible and resistant plants (even several resistance mechanisms in the same population) (Gasquez, 1995). In addition all the 83 APP resistant blackgrass populations were sprayed with higher doses of fenoxaprop-P-ethyl, 15 populations did not survive 138 g/ha but the other ones survived up to 6900 g/ha. Assuming an empirical correlation between target-mutation and resistance to high doses, we may postulate that these very resistant plants to APP herbicide must have an acetyl CoA carboxylase (ACCase) mutation. Besides only two populations of blackgrass survived the treatment with isoproturon (Table 1). These populations are also APP resistant surviving 6900 g/ha.

The 49 APP resistant populations of rye grass were confined to 12 departments in the centre and south of France (Table 1 and Fig. 2). All the rye grass populations were susceptible to phenylureas (Table 1). The resistant populations survived diclofop treatment up to 1800 g/ha and two survive more than 10000 g/ha. We checked that one of these populations has a mutated ACCase.

The few APP resistant populations of wild oats (either *Avena fatua* or *Avena sterilis*) (Table 1) were collected in 1996. They were found in the south-west of France, one of the remaining regions where wild oats are still a major problem (Fig. 2).

Table 1. Distribution of grass-weed populations in France resistant to isoproturon (IPU), fenoxaprop (fenox.), chlorotoluron (chlor.), diclofop (dic.)

	Blackgrass		Rye grass		Wild oat
	IPU	fenox.	chlor.	dic.	fenox.
1st year of record	1993	1993	-	1993	1996
No.of departments	2	25	0	12	3
No. of resistant populations	2	83	0	49	4

TESTS

In this work, three tests using either seedlings, tillers or pollen grains were developed to allow quick detection of resistant plants in order to determine their frequency within a blackgrass population. The first test compares growth of the resistant and susceptible coleoptiles in a herbicide solution. The second compares the response of young resistant and susceptible tillers to high herbicide concentrations. The last test compares germination of resistant and suceptible pollen grains in a medium supplemented with herbicide.

Materials and Methods

Three biotypes of blackgrass from Dijon area (France) were used in this study : one biotype resistant to high concentrations of fenoxaprop-P-ethyl, another one susceptible to field dose of fenoxaprop-P-ethyl and a last one coming from a natural population where about 20% are resistant to 138 g/ha fenoxaprop-P-ethyl. These three biotypes were used as standards to set up experimental conditions for a quick screening of APP resistant plants.

Seedling Test : Seeds from the three biotypes were germinated in glass petri dishes on a moist filter paper laid on small glass tubes. Petri dishes were filled with a nutrient solution. After 3 days, seeds with rootlets (3-5 mm long) were transferred onto a filter paper within a closed bottle and different solutions of racemate fenoxaprop-P acid added to each bottle. They were maintained under a 18-h, 20°C light / 6-h, 22°C dark regime. The herbicide effect was then recorded by measuring the coleoptile length at different times.

Tiller test : Seeds from the highly resistant and the susceptible biotypes were sown in a soil mixture (coarse sand and peat 1 : 3, v/v) and grown in a greenhouse. Vegetative tillers at the three leaf stage (third leaf not fully developed) were selected. Roots were carefully removed and tillers were kept in high concentrations of racemate fenoxaprop-P acid. After 48 hours, the herbicide effect was then assessed by comparing the third leaf necrosis degree.

Pollen Test : Seeds from the highly resistant and the susceptible biotypes were grown as in the tiller test. Inflorescences with anthers just extruded from the glumes were excised and transfered in a beaker of water. In order to induce release of sufficient amount of pollen, the material was kept at about 20 cm from a cold light. Pollen was shaken onto a 0.25% solid agar medium supplemented with different concentrations of racemate fenoxaprop-P acid. This medium was poured into plastic petri dishes and incubated under humid conditions at 30°C for 2 hours. Pollen germination was assessed using a microscope (×200). Pollen was scored as germinated if the pollen tube was at least half the size of the pollen grain.

Results

Seedling Test : The coleoptile growth of susceptible seedlings was strongly inhibited by increasing concentrations of racemate fenoxaprop-P acid. In contrast, except at the highest doses the herbicide had little or no effect on the coleoptile growth of resistant seedlings.

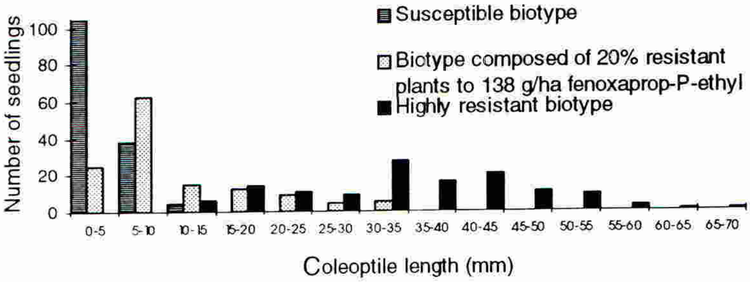

Figure 3. Distribution of coleoptile lengths of three *Alopecurus myosuroides* biotype seedlings after 6 days growth in 6 mg/l racemate fenoxaprop-P acid.

The best compromise between time and dose factors to distinguish resistant seedlings from the susceptible ones is a 6 days growth in 6 mg/l racemate fenoxaprop-P acid (Fig. 3). Furthermore, the biotype composed of 20% resistant plants to 138 g/ha fenoxaprop-P-ethyl could be distinguish from the susceptible and the highly resistant ones because its coleoptile length distribution was in between the two other ones (Fig. 3).

Tiller Test : At lower doses, even after several days, it was difficult to distinguish the resistant tillers from the susceptible ones. On the other hand, at higher doses the third leaf of resistant and susceptible tillers became necrotic almost at the same time. According to these observations, 200 mg/l racemate fenoxaprop-P acid was the only herbicide concentration where the youngest leaf of susceptible tillers was completely necrotic 48 hours after treatment, while the resistant leaf was not yet affected (data not shown).

Pollen Test : Pollen grains of the suceptible biotype were more inhibited by increased concentrations of racemate fenoxaprop-P acid than these of the resistant one (Fig. 4). Thus this experiment showed that ACCase may be expressed in pollen grains (Richter and Powles, 1993). Pollen germination of the susceptible biotype was inhibited to 50% by 45 µM racemate fenoxaprop-P acid, while this concentration had no effect on resistant pollen grains germination. 50% inhibition of resistant pollen grains germination was caused by 180 µM racemate fenoxaprop-P acid, four times as much as for susceptible pollen grains. This dose reponse experiment showed that the optimal herbicide concentration to detect resistant pollen grains was 200 µM.

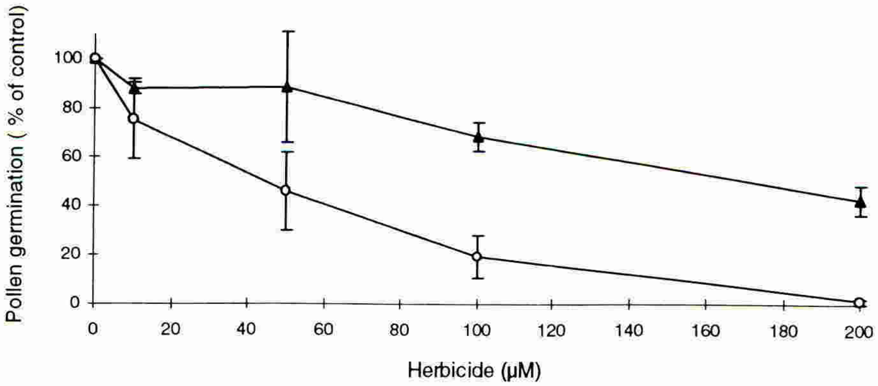

Figure 4. Effect of increasing concentrations of racemate fenoxaprop-P acid on the germination of pollen from susceptible (o) and highly resistant (▲) *Alopecurus myosuroides* biotypes. Data are means of four replicates. Vertical bars : 95% confidence interval

DISCUSSION AND CONCLUSIONS

Although resistant populations of the three species are rather scarce, their number is regularly increasing and this resistance is becoming a major concern for weed control in France. As far as we know, and at least for blackgrass, the resistant populations appeared in fields where, for many years, farmers have been using very simple crop rotations (generally winter crops), no ploughing and always the same APP herbicide for grass weed control (Orlando *et al.* 1995).

Another reason for the development of APP resistant blackgrass is a poor control of grass weeds in some crops i.e. oilseed rape. This leads quickly to a drastic increase in the number of blackgrass seedlings up to 1000/m^2. Within such a huge number of plants a resistant mutant (frequency i.e. 10^{-7}) will be definitively present in a three hectares field whatever the cultural practices and resistant blackgrass will spread after a few APP treatments. Therefore, one of the objectives of the ANPP working group is to give information on the resistance mechanisms and on the spread of resistant populations. The aim of this group is also to help farmers manage the resistance with non herbicide weed control and try to prevent (or at least to delay) the resistance development (Thomas *et al.* 1995).

Up to now, the only test used to detect APP resistant plants was a simulated field treatment in less variable conditions (temperature, soil and seedling stage are controlled) but involving a post-emergence spray of a commercial formulation. In this test the herbicide effect is clear only after four or five weeks.The three tests developed in this work use the technical acid form of APP herbicides. This form is much more water soluble and allows the use of high herbicide concentrations directly absorbed by young seedling roots, tillers or pollen grains. These three tests allow easy detection of the resistant blackgrass. They are rapid, portable and do not kill the susceptible plants. Nevertheless, each one has its particular uses and advantages. The tiller test does not require any special conditions or equipment. We developed this test as a practical field test to check the resistance of vegetative plants at the end of winter. The pollen germination test is the quickiest and could allowed to distinguish homozygous from heterozygous resistant plants provide the genetic determinism is semidominant. Moreover this method being non destructive the resistance of the same plant to several herbicides can be checked. While the coleoptile test is the most time consuming (6 days), this method allows to distinguish different levels of resistance.

We are currently finalizing these tests for others herbicides and for rye grass and wild oat.

REFERENCES

Bandeen J D; Stephenson G R; Cowett E R (1982) Discovery, Development, and Distribution of Triazine-Resistant Weed Biotypes. In *Herbicide Resistance in Plants*, (eds H M LeBaron & J Gressel), pp.9-31.Wiley-Interscience, United States of America.

Gasquez J (1995) Etat de la résistance aux herbicides chez les adventices. *Seizième conférence du COLUMA Reims.* **1**, 201-211.

Gasquez J (1996) Etat de la résistance aux herbicide chez les adventices en France. *Xe Colloque International sur la biologie des mauvaises herbes Dijon.* 173-181.

LeBaron H M (**1991**) Distribution and seriousness of herbicide-resistant weed infestations worlwide. In *Herbicide Resistance in Weeds and Crops*, (eds J C Caseley, GW Cussans & RK Atkin), pp.27-45. Butterworth Heinemann, Great Britain.

Orlando D; Dapilly P; Bertin G; Leterrier J L; Gasquez J (1995) Mise en évidence des graminées adventices résistantes aux antigraminées dans les céréales. *Seizième conférence du COLUMA Reims.* **1**, 221-225.

Richter J; Powels SB (1993) Pollen Expression of Herbicide Target Site Resistance Genes in Annual Ryegrass (*Lolium rigidum*). *Plant Physiology.* **102**, 1037-1041.

Thomas G; Cagnac B; Rollin F; Leterrier JL (1995) Face à un risque de résistance quelle stratégie adopter? *Seizième conférence du COLUMA Reims.* **1**, 225-235.

EFFICACY OF DICLOFOP-METHYL AGAINST ISOPROTURON - RESISTANT *PHALARIS MINOR* IN RELATION TO WHEAT CULTIVAR AND SPACING.

L.S.BRAR, BHAGWANT SINGH
Department of Agronomy, PAU Ludhiana - 141 004 , Punjab, India

ABSTRACT

The bread wheat cultivars owing to their greater height, dry matter production and number of mature tillers gave more supression of *P.minor* than durum wheat, resulting in 23.4 and 19.1 % higher grain yield . Closer plant spacing obtained by narrower row spacing or sowing rows at right angles also reduced dry matter of *P.minor* by 36.2 and 30.3 per cent respectively compared with normal row spacing and hence produced significantly higher grain yield. Combining cultivars with either closer row spacing or cross sowing supressed *P.minor* more effectively than normal row spacing . It was interesting to note that closer sowing though gave no supressing effect on the weed compared to cross sowing and the latter gave slightly higher grain yield. These studies also showed that use of bread wheat cultivars with a lower diclofop-methyl dose gave similar yields as obtained from durum wheat with a higher dose of diclofop-methyl.

INTRODUCTION

The cultivation of short statured high yielding and input responsive wheat cultivars resulted in severe infestations of grass weeds viz. *Phalaris minor* and *Avena ludoviciana* (Gill & Brar,1975). Further the adoption of a rice - wheat rotation coupled with irrigation and fertilizer use produced ecological conditions favouring *P.minor* growth and development which thus became a serious problem in the wheat crop. Assuming 15 % reduction in grain yield, Punjab alone suffers annual losses to the tune of 2.0 million tonnes (Brar, 1994).

To combat the problem of *P.minor* wheat growers adopted a large scale use of herbicides, especially isoproturon, which by and large provided good control of this weed for over one decade. Owing to continuous use of isoproturon, *P.minor* has acquired resistance against isoproturon (Malik and Singh, 1993; Walia *et al.*,1997). In this situation integrated weed management needs to be accorded a top priority; reduction in herbicide use through compensatory increase in the non-chemical control needs to be explored to sustain crop productivity.

MATERIALS AND METHODS

A field study was conducted at the research farm of Punjab Agricultural University, Ludhiana (India) situated at an altitude of 247 m above sea level, 30°56'
North latitude and 75°52' East longitude. The experiment was laid out in split plot design with four replicates having cultivars and planting pattern as main plots and herbicide treatments as sub-plots (Table 1). The soil was a loamy sand having 80.0% sand, 16% silt and 3.2% clay (in top 15 cm), low in organic matter (0.30%), available nitrogen and medium in available phosphorus. Soil reaction and electrical conductivity were normal. Three wheat cultivars, namely HD 2329, WH 542 bread wheats and PBW 34 durum wheat were sown on Nov 14, 1994 with row to row distances of 15.0 cm, 22.5 cm and, for rows sown at right angles, 22.5x22.5cm. A seed rate of 100 kg/ha was used for cultivars HD 2329 and PBW 34, whereas for WH 542 it was 87.5 kg/ha. Nitrogen was applied at the rate of 125 kg/ha; half of the nitrogen and all of P (60 kg P_2O_5/ha) was applied at sowing time and the remaining nitrogen along with the first irrigation was given 28 days after sowing; in all four irrigations were given. Diclofop-methyl (280g/litre EC) was used at three different doses i.e. 0.5 kg/ha, 0.75 kg/ha and 1.0 kg/ha as post-emergence spray (35 days after sowing). Herbicides were sprayed with a knapsack sprayer fitted with a flat fan nozzle delivering 500 litres /ha volume rate at a pressure of 420 kPa. The height of 10 wheat plants/plot and number of mature tillers/m^2 were recorded at harvest. Dry weight of weed and crop samples were recorded after oven drying at 70°C for 48 h. Net plot size harvested for grain yield was 9 m^2.

RESULTS AND DISCUSSION

Effect of cultivars

The study showed that bread wheat cultivars caused significant reduction in the dry matter accumulation of *P. minor* right from the initial stage of crop growth and this effect persisted till harvest of the crop. At crop harvest, dry matter accumulation by *P. minor* growing among cultivars WH 542 and HD 2329 was of the order of 30.0 and 23.9 % less than that obtained in the durum wheat cultivar PBW 34 (Table 1). An edge in the competitiveness of cvs.WH 542 and HD 2329 over PBW 34 was reflected in the dry matter accumulation by the crop as these cultivars accumulated 49.4 and 45.7 % more dry matter respectively, compared to PBW 34 (Table 4) ; thus the more vigorous growth of the bread wheat cultivars exerted more canopy pressure over *P.minor* compared to the durum wheat cultivar. The observations on the number of mature tillers also indicated the competitive ability of bread wheat cultivars over the durum wheat (Table 4). The vigorous growth of these cultivars intercepted more photosynthetically-active radiation (PAR) recorded at 90 days GS of the crop (data not presented).

Effect of planting pattern

Narrow row spacing (15 cm) and bi-directional sowing of wheat (22.5 cm x 22.5 cm) significantly increased grain yield by 10.3 and 14.9 %, respectively over the row spacing of 22.5 cm. The increase in grain yield due to narrow row spacing and bi-directional sowing may be attributed to a significant reduction in the dry matter accumulation of *P.minor* compared to normal spacing (Table 3). At crop harvest narrow and bi-directional sowing registered 36.2 and 30.3 % reduction in the dry matter of *P. minor* over the normal crop spacing, because of production of more dry matter and mature tillers per unit area by the crop compared to normal spacing. Similar results were also reported by Singh *et al.*, 1995; Galichenko, 1995; Parihar & Singh, 1995. It was also shown that narrow row spacing with application of diclofop-methyl at 0.5 kg/ha gave dry matter accumulation by *P.minor* statistically comparable to that of the normal spacing with application of diclofop-methyl at a higher dose (0.75 kg/ha) (Table 2).

Effect of herbicide

The weed control potential of diclofop-methyl increased with dose as shown by the reduction in *P.minor* dry matter (Table 2). With each increment in the dose of diclofop-methyl, mature tillers /unit area, dry matter production and grain yield all increased significantly over the previous dose (Table 3, 4). The studies further show that there was a differential response between the different cultivars in relation to the application of diclofop-methyl. The increase in the dose of herbicide from 0.50 to 1.0 kg/ha gave a significant increase in the yield of each cultivar (Table 3). On the other hand , it was observed that the cvs. HD 2329 and WH 542 proved superior to PBW 34, with the successive increase in the dose of diclofop-methyl. It was interesting to note that the post-emergence application of diclofop-methyl 0.50 kg/ha on bread wheat cv.WH 542 gave statistically comparable yield to application at 1.0 kg/ha in durum wheat cv.PBW 34. Similarly, cv.HD 2329 treated with 0.75 kg/ha diclofop-methyl provided a comparable yield to cv. PBW 34 sprayed with 1.0 kg/ha diclofop-methyl (Table 3).

Table 1. Interaction between sowing pattern and wheat cultivars on dry matter accumulation (q/ha) of *P.minor* at crop harvest stage

Cultivars	Dry matter accumulation (quintals/ha) Pattern of sowing (row spacing,cm)		
	15.0	22.5	22.5x22.5
PBW 34	3.83* (14.66)**	4.28 (18.32)	3.91 (15.28)
HD2329	3.22 (10.37)	3.98 (15.84)	3.32 (11.02)
WH542	2.89 (8.35)	3.99 (15.92)	3.11 (9.67)

L.S.D (P<0.05):

Sowing pattern x Cultivar = 0.38

*Square root transformation values (Stat. Analysed)

**Original values in parentheses

Table 2 . Interaction effects on dry matter (q/ha) of *P.minor* at harvest.

Sowing pattern/ Row spacing (cm)	Unweeded	Diclofop-methyl (kg/ha)		
		0.5	0.75	1.0
15.0	4.78* (22.85)**	3.66 (13.40)	2.80 (7.84)	2.01 (4.04)
22.5	5.99 (35.88)	4.68 (21.90)	3.35 (11.22)	2.43 (5.90)
22.5x22.5	4.86 (23.91)	3.87 (14.98)	2.95 (8.70)	1.98 (3.92)

L.S.D (P<0.05)

Sowing pattern x Weed control : 0.38

* Square root transformed values (statistically analysed)

** Original values in paremntheses

Table 3 . Effect of different treatments on grain yield (kg/ha) of wheat.

Cvs.	Pattern of sowing / row spacing			Unweeded	Diclofop-methyl (kg/ha)			
	15.0	22.5	22.5x22.5		0.5	0.75	1.0	Mean
PBW34	2885	2768	3014	1820	2761	3282	3693	2889
HD2329	3523	3115	3684	2662	3396	3729	4002	3441
WH542	3660	3243	3794	2758	3489	3847	4168	3565
Mean	3356	3042	3497	2412	3207	3619	3995	

LSD (P<0.05)	
Cultivars	206
Sowing pattern	206
Weed control	122
Cultivars x weed control	211
Other interactions	NS

Table 4 . Effect of different treatments on growth and development of wheat.

Treatments	Final plant height (cm)	Mature tillers/m^2	Final dry matter (q/ha)
Cultivars			
PBW 34	76.1	271	109.1
HD 2329	78.0	299	158.9
WH 542	78.2	302	162.9
LSD (P<0.05)	1.7	12	11.4
Sowing pattern			
15.0 cm	78.0	294	142.6
22.5 cm	77.0	275	131.2
22.5x22.5 cm	78.1	303	147.2
LSD (P<0.05)	NS	12	11.4
Weed control			
Unweeded check	78.9	247	94.4
Diclo. 0.5 Kg/ha	78.0	280	139.7
Diclo. 0.75 Kg/ha	77.6	308	155.2
Diclo. 1.0 Kg/ha	76.7	326	171.9
LSD (P<0.05)	1.6	16	12.8

Interactions = NS

REFERENCES

Brar, L S (1994) Weed management in rice - wheat system. Paper presented at *The National Conference on Sustaining Rice - Wheat Cropping System in Indo-Gangetic Plains* held at Lucknow, U.P.(India), 8-9 Sept.

Galichenko, I I (1995) Which sowing method is best for winter crops. *Field crops Abstract*. **48** , 4766.

Gill, H S ; Brar, L S (1975) Importance of herbicides in agriculture of Punjab and Haryana. *Pesticides*. **9**, 20-24.

Malik, R K ; Singh, S (1993) Evolving strategies for herbicide use in wheat : Resistance and Integrated Weed Management. In : *Integrated weed management for sustainable agriculture*. Proceedings International Symposium, CCS HAU, Hissar, India. **1**,225-236.

Parihar, G N ; Singh, R (1995) Response of wheat (*Triticum aestivum*) genotypes to seed rates and sowing methods under western Rajasthan conditions. *Indian Journal of Agronomy* . **40**, 97-98.

Singh, G ; Singh, O P ; Yadav, R A ; Singh, R S ; Singh, B B (1995) Effect of seeding methods, seed rates and fertility levels on yield and economics of late sown wheat after rice in flood prone area. *Annals of Agricultural Research*. **15**, 448-451.

Walia, U S ; Brar, L S ; Dhaliwal, B K (1997) Resistance to isoproturon in *Phalaris minor* Retz. In Punjab. *Plant Protection Quarterly* **12**, 138-140.

ACTIVITY OF JV 485, A PROTOPORPHYRINOGEN OXIDASE INHIBITOR, ON HERBICIDE-RESISTANT BLACK-GRASS (*ALOPECURUS MYOSUROIDES*)

S R MOSS
IACR-Rothamsted, Harpenden, Herts AL5 2JQ, UK

M S ROOKE
Department of Genetics and Microbiology, University of Liverpool, Liverpool L69 3BX, UK

ABSTRACT

Experiments were conducted to determine the efficacy of JV 485, (proposed common name = isopropazol), a new protoporphyrinogen oxidase (PPO or Protox) inhibiting herbicide, on six populations of black-grass (*Alopecurus myosuroides*) with contrasting resistance characteristics. In glasshouse dose response assays there was no evidence that JV 485 was affected by resistance to any appreciable degree. In an outdoor container experiment, JV 485 at 175 g a.i./ha applied pre-emergence gave consistently good control (98.8-99.4% reduction in foliage weight) of all populations. In nearly all cases JV 485 gave levels of control at least as good as, and often better than pendimethalin, isoproturon and clodinafop+oil. In a field trial with a heavy infestation of fenoxaprop-resistant black-grass (untreated = 1146 heads/m^2), pre-emergence applications of JV 485 at 175 g a.i./ha gave excellent control, achieving a 99% reduction in head numbers. It was concluded that JV 485 is not affected by any of the resistance mechanisms so far detected in UK black-grass populations.

INTRODUCTION

JV 485 (proposed name = isopropazol) is a protoporphyrinogen oxidase inhibiting herbicide being jointly developed for use in wheat in Europe by Bayer and Monsanto (Prosch *et al.*, 1997; Bolton *et al.*, 1997). One of the main target weeds for this herbicide is black-grass. No herbicides with this mode of action are currently available for black-grass control in cereal crops in the UK.

Herbicide-resistant black-grass occurs on over 750 farms in England and in several other European countries. A major concern is the degree of cross-resistance to herbicides with different modes of action, which appears to be a consequence of multiple-resistance mechanisms (Moss & Clarke, 1995). It is difficult to predict to what extent a new herbicide will be affected by resistance, so experiments were conducted to evaluate JV 485 activity on resistant black-grass.

MATERIALS AND METHODS

The six black-grass populations used were: Rothamsted, Peldon, Faringdon, Lincs. E1, Oxford AA1, Lincs. K1. The range of populations tested encompass all the known mechanisms of resistance present in UK black-grass (Table 1). These are summarised below and further details are given in Hall *et al.*, (1994), Moss & Clarke, (1995) and references therein.

Table 1. Resistance mechanisms in populations used in glasshouse assay and outdoor containers.

	Enhanced metabolism	Target site (ACCase)	Other mechanisms (uncharacterised)
Rothamsted	S	S	No
Peldon	✓✓✓	No	No?
Faringdon	✓✓	No	?
Lincs. E1	✓✓	in 5% plants	Yes
Oxford AA1	✓	in 85% plants	No?
Lincs. K1	✓✓	No	?

S = susceptible. ✓, ✓✓, ✓✓✓= show enhanced metabolism to a low, medium and high degree.

Glasshouse dose response assay

JV 485 was applied pre-emergence at eight doses (range = 11 - 350 g a.i./ha) to pots (9 cm) containing pre-germinated seeds (10 per pot) of the six black-grass populations sown in Kettering loam. A randomised block design was used with six replicates and included untreated pots. Treatments were applied pre-emergence using a laboratory sprayer on 2 October 1996, one day after sowing pre-germinated seeds. On 7-8 November 1996, about 5 weeks after spraying, herbicide activity was recorded by assessing the foliage fresh weight for each individual pot. Dose response data were analysed using a logistic relationship between foliage fresh weight and $\log_{10}$ dose, and ED_{50} values (herbicide rate required to reduce fresh weight by 50% relative to untreated) determined.

Outdoor container experiment

The response of the six black-grass populations to JV 485, pendimethalin, isoproturon and clodinafop was determined in outdoor containers to simulate field conditions (Moss & Clarke, 1995). Seeds (200-350 per container) were incorporated into the top 5 cm of a silty loam/grit mix in separate plastic containers (27x18x10 cm deep) on 25 September 1996 and placed in an outdoor sand bed. The experiment comprised a randomised block design with three replicates and two untreated containers per replicate for each population. Details of herbicide doses are given in Table 3.

JV 485 and pendimethalin were applied pre-emergence on the 30 September 1996 (5 days after sowing) using a laboratory sprayer delivering 250 litres water/ha at 224 kPa through a single Lurmark 01-F110 nozzle. The other herbicides were applied post-emergence on the 4/5 November 1996 when black-grass growth stage was 3 - 4 leaves with 1st tiller emerging.

Herbicide activity for pre-emergence treatments (JV 485 and pendimethalin) was recorded on the 2-4 December 1996, approximately 2 months after spraying, by assessing foliage fresh weight per container. The isoproturon and clodinafop treatments were assessed in the same manner on 20 February 1997.

Field experiment

A trial was established in a field at Rothamsted heavily infested with black-grass. This population was highly resistant to fenoxaprop (4*) but only marginally insensitive to chlorotoluron (1*) (See Moss & Clarke, (1995) for explanation of * rating system).

The experiment comprised a randomised block design with four replicates. Plot size was 3 x 12 m and the treatments, which included JV 485 and other herbicides, are listed in Table 4. Winter wheat (cv. Mercia) was drilled on 17 October 1996 and pre-emergence treatments applied 5 days later. The very early post-emergence application of trifluralin was applied on 8 November when black-grass plants were < 1 leaf, and other post-emergence treatments on 3 March 1997 when black-grass was at the 3 leaf stage. Tri-allate granules were applied by hand-held applicator. Other herbicides were applied in 220 litres water/ha at 230 kPa using a small plot sprayer with a 3 m boom. The trial received 196 kg N/ha applied on 11 April 1997.

Black-grass plant populations were assessed on 18 December 1996 on plots treated pre-emergence by counting all plants in 10 random 0.1 m^2 quadrats. Black-grass head densities were determined in four 0.25 m^2 quadrats per plot on 3 June 1997.

Screening of black-grass populations for resistance to chlorotoluron, fenoxaprop and JV 485.

Twenty-nine populations, mainly collected from field trial sites, were evaluated in a standard glasshouse screening assay (Moss & Clarke, 1995). Pots were treated pre-emergence with JV 485 at 87.5 g /ha or post-emergence with chlorotoluron (2.75 kg/ha) or fenoxaprop-P-ethyl (68.75 g/ha). Foliage weight was recorded as a measure of herbicide activity.

RESULTS

Glasshouse dose response assay

Table 2. Response of six black-grass populations to JV 485 in a dose response assay

Population	$Log_{10} ED_{50}$	ED_{50} (g a.i./ha)	Resistance Index
Rothamsted	1.332	21.5	1.0
Peldon	1.560	36.3	1.7
Faringdon	1.473	29.7	1.4
Lincs. E1	1.400	25.1	1.2
Oxford AA1	1.479	30.1	1.4
Lincs. K1	1.417	26.1	1.2
S.E. ±	0.071	-	-
L.S.D. ($P \leq 0.05$)	0.205	-	-

Resistance Index is the ratio of ED_{50} values relative to the susceptible Rothamsted.

JV 485 showed high levels of activity against black-grass in the glasshouse and gave similar levels of activity on all six populations. (Table 2). The only statistically significant difference ($P \leq 0.05$) was between the Rothamsted susceptible standard and the Peldon population. However, this difference (a resistance index of 1.7) was much smaller than that found by Moss & Clarke (1995) for many other herbicides in previous studies with this population (e.g. chlorotoluron 27.6; fenoxaprop 3.9; pendimethalin 13.7). Thus there was no evidence that JV 485 was affected by resistance to any appreciable degree.

Outdoor container experiment

JV 485 at 175 g a.i./ha (the proposed recommended rate) gave excellent control (>98% reduction) of all populations (Table 3). Control of the populations known to be resistant to many other herbicides was similar to that of the susceptible standard, Rothamsted. At the lowest rate (75 g a.i./ha) activity was still good (>85%) on all populations except Oxford AA1 (74%). The significantly ($P \leq 0.05$) poorer control of the Oxford AA1 population at this lowest rate was a consistent effect over all replicates. There is no obvious explanation for this as this trend was not evident at the higher rates or in the glasshouse dose response experiment.

In contrast, the performance of pendimethalin at 2 kg a.i./ha was poorer, especially on resistant populations (42-64% control). Isoproturon and clodinafop+oil applied post-emergence gave excellent control of some populations but poor control of others. JV 485 at 175 g a.i./ha gave consistently good control of all populations and in nearly all cases gave levels of control at least as good as, and usually better than that obtained from these other standard herbicide treatments.

Table 3. Efficacy of herbicides applied under simulated field conditions (outdoor containers)

	% reduction in foliage fresh weight relative to untreated controls					
	Roth.	Peldon	Faring.	Lincs. E1	Oxf. AA1	Lincs. K1
Pre-emergence						
JV 485 (75 g/ha)	89.2	85.7	90.9	90.5	74.0	93.0
JV 485 (125 g/ha)	97.5	96.0	97.5	96.0	96.8	98.5
JV 485 (175 g/ha)	98.8	99.2	99.4	98.8	99.3	99.8
Pendimethalin (2 kg/ha)	92.1	41.7	55.0	59.3	64.2	60.2
S.E. ±			3.85			
L.S.D. ($P \leq 0.05$)			10.95			
Post-emergence						
Isoproturon (1.5 kg/ha)	100	38.2	92.1	97.3	92.7	83.3
Isoproturon (2.5 kg/ha)	100	46.6	96.4	97.6	99.1	89.9
Clodinafop (30 g/ha) + oil ('Actipron')	99.9	99.1	98.2	73.7	33.8	96.1
S.E. ±			4.45			
L.S.D. ($P \leq 0.05$)			12.78			

Field experiment

JV 485 applied pre-emergence at 175 g a.i./ha gave excellent control of black-grass plants. In December 1996, two months after application, the number of surviving plants/m^2 for pre-emergence treatments was: JV 485 - 11; tri-allate - 267; trifluralin - 195. There were 752 black-grass plants/m^2 on untreated plots, so JV 485 gave 99% control compared with 65% for tri-allate and 74% for trifluralin. At this assessment time the early post-emergence application of trifluralin gave only 26% control.

JV 485 decreased head numbers by 99.4% (Table 4). This was better than any of the other herbicide treatments. The very poor performance of fenoxaprop-P-ethyl supported the screening assessment of the degree of resistance to this herbicide. Clodinafop and the formulated mixture of trifluralin and clodinafop both gave substantially better control than fenoxaprop, indicating that clodinafop tends to be less affected by resistance than fenoxaprop. Despite screening tests indicating only marginal insensitivity to urea herbicides, isoproturon gave poor control of black-grass at both rates when applied alone. This was almost certainly a consequence of the very dry soil conditions present following application. Sequences of pre-emergence tri-allate or trifluralin followed by isoproturon at 1.5 kg/ha gave 89% - 92% control, which was much better than the control achieved by isoproturon mixtures applied post-emergence.

Thus on this field infested with fenoxaprop-resistant black-grass, the best control was achieved by a single pre-emergence application of JV 485 at 175 g a.i./ha. However, this was a single trial in one year, so it will be important that the efficacy of JV 485 on resistant black-grass is assessed in other fields and years to determine its consistency under differing soil and climatic conditions.

Table 4. Efficacy of herbicides on a fenoxaprop-resistant black-grass population in a field trial

Pre-emergence or (Very early post-em.)	Post-emergence	% reduction in head nos. (Nils = 1146 heads/m^2)
JV 485 (175 g/ha)	-	99
-	Isoproturon (2.5 kg/ha)	52
-	Isoproturon (1.5 kg/ha)	29
Tri-allate (2.25 kg/ha)	Isoproturon (1.5 kg/ha)	92
Trifluralin (0.96 kg/ha)	Isoproturon (1.5 kg/ha)	89
(Trifluralin (0.96 kg/ha))	Isoproturon (1.5 kg/ha)	80
-	Trifluralin (0.96 kg/ha) + Isoproturon (1.5 kg/ha)	39
-	Pendimethalin (1.32 kg/ha) + Isoproturon (1.5 kg/ha)	46
-	Fenoxaprop-P-ethyl (68.75 g/ha)	17
-	Clodinafop (30 g/ha) + oil ('Actipron')	89
-	Trifluralin (0.96 kg/ha) + Clodinafop (30 g/ha) + oil	87
S.E.±		4.2
L.S.D. ($P \leq 0.05$)		12.2

Screening of black-grass populations for resistance to chlorotoluron, fenoxaprop and JV 485.

The large differences between populations in response to chlorotoluron (1 - 95% reductions in foliage weight) and fenoxaprop (10 - 95%), demonstrated that some populations were resistant to these herbicides. All plants emerging from pots treated with JV 485 showed severe phytotoxic symptoms, but some plants did recover and the final % reduction figures for JV 485 ranged from 71-99 %. These values were much more consistent between populations than were those for chlorotoluron and fenoxaprop. Single dose screening tests have limitations, but in this test there was no clear evidence that any of the 29 populations showed resistance to JV 485.

DISCUSSION

Although several crop and weed species have natural resistance to protoporphyrinogen oxidase inhibiting herbicides, there are no verified cases of evolution of resistance due to selection by herbicides with this mode of action (Dayan & Duke, 1997). In this series of experiments there was no evidence that JV 485 was affected by resistance. It is concluded that JV 485 is not affected by any of the resistance mechanisms so far detected in UK black-grass populations.

It would be unwise to conclude that resistance cannot evolve to herbicides with this mode of action, as they appear to have a single site of action (Dayan & Duke, 1997). It would be sensible to adopt integrated strategies, which include cultural methods and herbicides with different modes of action, in order to minimise the risk of resistance evolving.

ACKNOWLEDGEMENTS

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REFERENCES

Dayan, F E; Duke, S O (1997) Phytotoxicity of protoporphyrinogen oxidase inhibitors: phenomenology, mode of action and mechanisms of resistance. In *Herbicide Activity: Toxicology, Biochemistry and Molecular Biology*, (eds R M Roe, J D Burton & R J Kuhr), pp. 11-35. IOS Press, Amsterdam.

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*, (eds S B Powles & J A M Holtum), pp.243-261. CRC Press, Boca Raton, Florida.

Moss, S R; Clarke J H (1995) Inheritance of herbicide resistance in black-grass (*Alopecurus myosuroides*) and responses of the weed to a range of herbicides. *Home-Grown Cereals Authority Project Report No.* **116**, 100pp.

Prosch, S D; Ciha, A J; Grogna, R; Hamper, B C; Feucht, D; Dreist, M (1997) JV 485: a new herbicide for pre-emergence broad spectrum weed control in winter wheat. *Proceedings 1997 Brighton Crop Protection Conference - Weeds.*

Bolton, B J G; Rose, P W; Norton, A J (1997) JV 485 - an advanced pre-emergence herbicide for the control of *Alopecurus myosuroides* and broad-leaved weeds in UK winter wheat. *Proceedings 1997 Brighton Crop Protection Conference - Weeds.*

AN INTEGRATED STRATEGY FOR THE SUCCESSFUL MANAGEMENT OF HERBICIDE RESISTANT *Alopecurus myosuroides* (BLACK-GRASS) IN THE UK

M A READ, J J PALMER, S HOWARD
AgrEvo UK Limited, East Winch Hall, East Winch, King's Lynn, Norfolk, PE32 1HN, UK

ABSTRACT

Trials carried out on confirmed populations of resistant *Alopecurus myosuroides* (black-grass) over a number of years in the UK show the benefits of early herbicide treatment, pre-treatment with tri-allate or trifluralin and mixtures with isoproturon. Glasshouse resistance tests confirmed the high cross-resistance of fenoxaprop-p-ethyl resistant populations to other 'fops' (aryloxyphenoxyproprionates) and to tralkoxydim. Cross-resistance to isoproturon was low and re-affirms the useful role this product can play as part of an overall resistance management strategy. The introduction of genetically modified crops tolerant to glufosinate-ammonium offers another product group for control of resistant *A. myosuroides*. By careful combination of cultural, cropping and chemical tools, UK farmers can prevent or manage populations of resistant black-grass such that they can continue to farm cereals profitably.

INTRODUCTION

Black-grass, *(Alopecurus myosuroides)*, is one of the most important grass weeds of winter cereals. The enormous potential for this weed to drastically affect the yield of winter cereals was indicated by researchers before intensive cereal production was started in the UK (Long, 1929). It is also a species which is ideally suited to the build-up of resistance, being a prolific producer of seed with low dormancy and favouring heavier soils which are more difficult to plough.

Resistance to chlorotoluron was first detected in a UK population of *A. myosuroides* in 1982 (Moss and Cussans, 1985). Resistant black-grass populations are now present on around 750 farms throughout England (Moss and Albertini, 1996). The majority of these populations are resistant to 'fop' herbicides (aryloxyphenoxproprionate group) but cross-resistance to other herbicides is also common.

In 1989, AgrEvo began looking at the control of chlorotoluron resistant black-grass with their new graminicide, fenoxaprop-ethyl (trade name 'Cheetah R'). It soon became clear that a programmed approach was required to manage this problem effectively and to prevent further resistance build-up in other populations. Trials have been carried out in most years since then.

Clarke and Moss (1991) published one of the first reports on strategies for the control of resistant *A. myosuroides* in the UK. In 1993 the UK Weed Resistance Action Group (WRAG) published its first edition of Guidelines for the prevention and control of Herbicide-

Resistant black-grass (Moss and Clarke, 1994). This has recently been updated and the revised edition was issued in September 1997.

In addition, AgrEvo also undertook a large programme of resistance and cross-resistance testing of *A. myosuroides* from UK, from 1994 onwards, in order to provide advisers and farmers with information on which to base their resistance-management decisions.

This paper summarises data from field-trials and also the results of cross-resistance testing. This, together with experience from overseas and working in conjunction with WRAG has led to the formulation of the AgrEvo strategy which has been published in the booklet, 'Grass Weed Management' (AgrEvo, 1996).

MATERIALS AND METHODS

All trials were carried out on commercial crops of winter wheat in England, covering a range of locations, soil types and cultivars. Sites were chosen where enhanced metabolism resistance to 'fops' had been confirmed by testing in previous seasons.

Small-plot trials

Small-plot trials were designed as randomised blocks with three or four replicates and a plot size of 15-40 m^2. Applications were made using pressurised knapsack small-plot sprayers at a pressure of 250 kPa delivering 200 l/ha through four flat fan nozzles spaced 25 cm apart on 2 m spray booms.

Large-scale trials

Large-scale trials were unreplicated with a plot size of at least 600 m^2 and treatments were applied through conventional farm sprayers at 200 l/ha.

Commercial products, available in the UK, were used at the dose rates mentioned below.

A. myosuroides was assessed by counting seed heads, as soon as all had emerged, in random quadrats which varied in size from 0.1 to 0.5 m^2 according to the density of *A. myosuroides.*

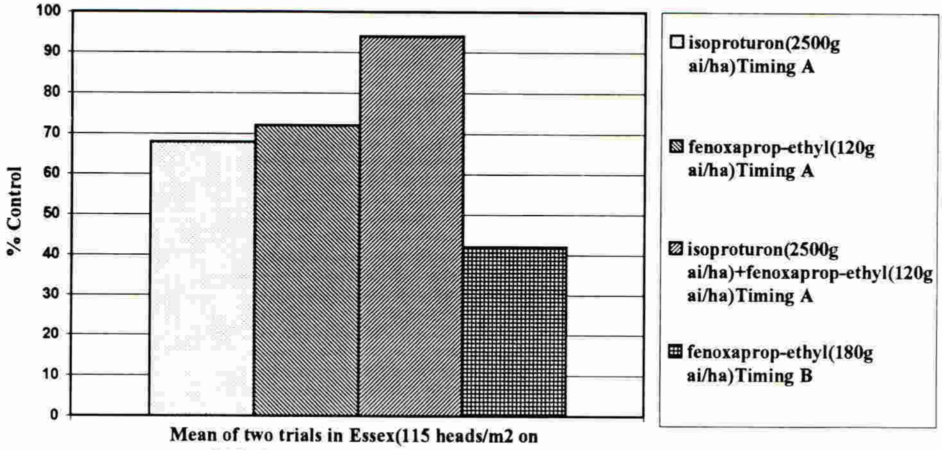
Cross-resistance tests

These were carried out in the Biology Department of AgrEvo in Frankfurt, Germany. Samples found to be resistant to fenoxaprop-p-ethyl were treated with auxillin and grown in pots in the glasshouse under controlled conditions. At GS 12-14 (Zadoks *et al,* 1974) the plants were treated with the test herbicides using a single Teejet nozzle (8002E) at 140 kPa in 300 l/ha of water. Four replicates were used and the percentage level of control was assessed visually 14 and 28 days after application. Results were compared to both known susceptible and resistant standards and the populations were categorised as susceptible (S), partially resistant (PR) or resistant (R). Treatments applied were as follows:-
fenoxaprop-p-ethyl at 110 g and 220 g a.i./ha;tralkoxydim (+ oil) at 140 g and 280 g a.i./ha; clodinafop-propargyl (+ oil) at 30 g and 60 g a.i./ha; isoproturon at 1250 g and 2500 g a.i./ha

RESULTS AND DISCUSSION

Results of small-plot work in 1989 (Figure 1) indicated the benefit of applying treatments to small black-grass (GS 11-13). Late applications of fenoxaprop-ethyl to larger weeds (GS 30-31) gave poorer results. The trials also indicated the importance of mixing isoproturon with fenoxaprop-ethyl.

Figure 1 Control of resistant *A. myosuroides* with isoproturon and fenoxaprop-ethyl at two timings(1989)



Mean of two trials in Essex(115 heads/m2 on untreated).Timing A was 22/12/89 at GS 11-13 of the weeds.Timing B was 4/4/90 at GS 30-31 of the weeds

Integration of different chemical groups into a programme, and the importance of pre-treatment with either tri-allate or trifluralin was shown in a large-scale unreplicated field-trial in 1991 (Table 1). Tri-allate pre-treatment enhanced the performance of both isoproturon and fenoxaprop-p-ethyl applied post-emergence in a programme. Trifluralin applied pre-emergence also increased the level of control achieved by isoproturon and fenoxaprop-p-ethyl, but to a lesser extent. This trial also showed the importance of isoproturon as it gave excellent control of ‘fop’-resistant black-grass. The significance of ploughing, in order to reduce the weed-seed population was also illustrated.

Pre-treatments have been shown to be an effective part of a strategy but they need to be applied at their optimum timing. Table 2 shows the results of four trials in 1995 in which trifluralin, applied pre-emergence, gave better control of *A. myosuroides* than when applied post-emergence, with or without the addition of mineral oil. Higher doses of trifluralin pre-emergence also gave better control but no trifluralin treatment was sufficient on its own.

Table 1 Programmes for the control of resistant *A. myosuroides* (farmer applied trial in Lincolnshire, 1991)

			% Control					
Treatment / Timing	**Rate g a.i./ha**	**G.S of weed at appln**	**Alone**		**+ trifluralin (1200 g a.i./ha)**		**+ tri-allate (2250 g a.i./ha)**	
			min. cultn	**plough**	**min. cultn**	**plough**	**min. cultn**	**plough**
isoproturon / A	2500	12-13	95	97	92	97	99	100
fenoxaprop-p-ethyl / B	55	21-26	50	58	73	75	90	92
Untreated (heads/m2)	-	-	480	350	400	350	480	350

Timing A was applied on 05/11/91. Timing B was applied on 04/03/92.

Table 2 Control of resistant *A. myosuroides* with trifluralin at different timings

			% Control	
Treatment	**Rate g a.i./ha**	**Timing**	**Mean**	**Range**
trifluralin	960	pre-emergence	57	33-68
trifluralin	1200	pre-emergence	70	56-82
trifluralin	960	GS 12-13	25	0-47
trifluralin + oil	960 + 990	GS 12-13	43	16-64
Untreated (heads/m2)	-	-	547	125-1077

Four trials, Essex (2), Suffolk (1), Linconshire (1).

The importance of application of herbicides to small weeds in the autumn / winter was further shown in 1995 (Table 3) where in three trials several different treatments were applied to *A. myosuroides* at GS 12 or GS 23. In all cases the earlier application gave superior control. In some trials excellent levels of control were achieved. Following this result and commercial use, emphasis on timing in future trials was limited to GS 11-13. These trials again showed that mixes of isoproturon + fenoxaprop-p-ethyl were useful in a management strategy.

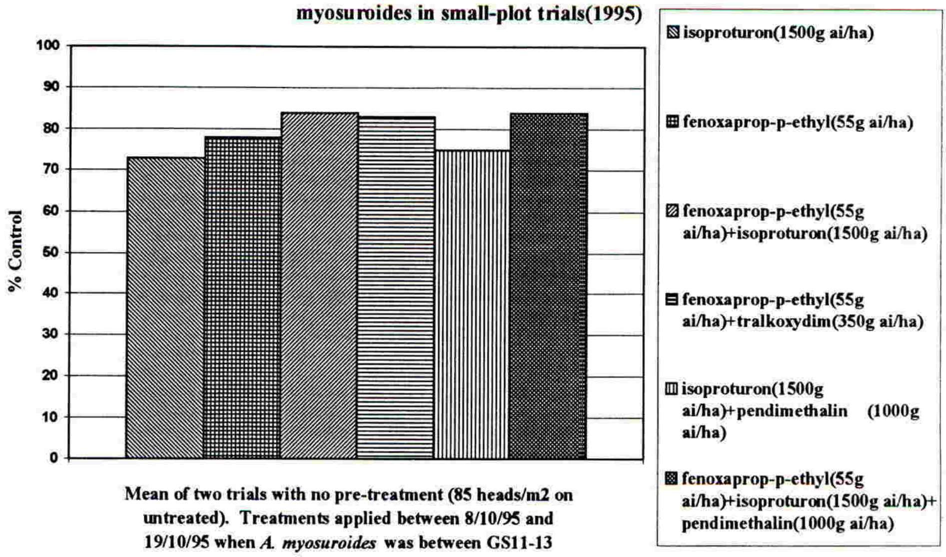
Table 3 Comparison of early / late application on control of resistant *A. myosuroides*

		% Control			
		'Early' (GS 12)		'Late' (GS 22-23)	
Treatment	**Rate g a.i./ha**	**Mean**	**Range**	**Mean**	**Range**
isoproturon	2500	82	79 - 85	46	29 - 73
fenoxaprop-p-ethyl	55/82.5	70	48 - 97	47	29 - 76
fenoxaprop-p-ethyl + isoproturon	55 + 1500 / 82.5 + 1500	86	76 - 99	51	34 - 84
fenoxaprop-p-ethyl + isoproturon	55 + 2500 / 82.5 + 2500	89	81 - 100	57	42 - 84
clodinafop-propargyl + oil	30 + 990	-	-	52	32 - 76
Untreated (heads/m^2)	-	688	142 - 1077	688	142 - 1077

Three trials; Essex (2), Suffolk (1).

Following the recommendations of the Isoproturon Review (MAFF 1995) which was instigated following concerns about isoproturon being found in drinking water, lower doses of isoproturon, (1500 g a.i./ha), and other residual products e.g pendimethalin (Figure 2) were evaluated. No pre-treatments were applied and the results show at 1500 g a.i./ha isoproturon alone was inadequate. The addition of fenoxaprop and pendimethalin increased performance but not to a sufficient level. Neither did a mixture of fenoxaprop-p-ethyl + tralkoxydim.

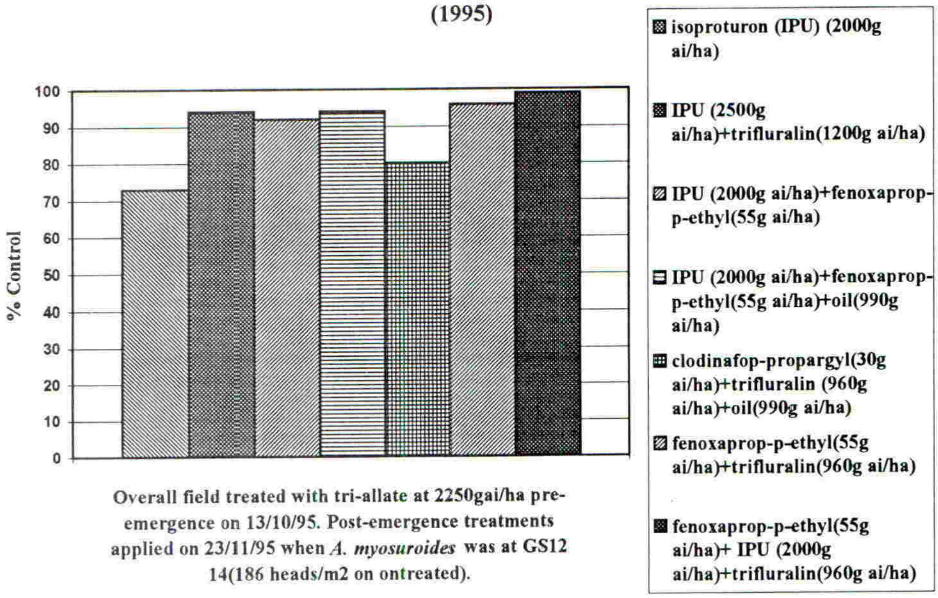
Figure 2 Comparison of various mixtures for the control of resistant A. myosuroides in small-plot trials(1995)



Mean of two trials with no pre-treatment (85 heads/m2 on untreated). Treatments applied between 8/10/95 and 19/10/95 when *A. myosuroides* was between GS11-13

Combining tri-allate pre-treatments, early applications and herbicides with different modes of action in an integrated programme was tested in 1995 and 1996 (Figure 3 and Table 4). In the large-scale farmer trial, in Norfolk, the best post-emergence treatment was fenoxaprop-p-ethyl + isoproturon + trifluralin. Fenoxaprop-p-ethyl + isoproturon and fenoxaprop-p-ethyl + trifluralin also performed well. Clodinafop-propargyl + trifluralin performed less well at this site.

Figure 3 Control of resistant *A. myosuroides* in a farmer-applied trial (1995)



Overall field treated with tri-allate at 2250gai/ha pre-emergence on 13/10/95. Post-emergence treatments applied on 23/11/95 when *A. myosuroides* was at GS12 14(186 heads/m2 on ontreated).

Table 4. Control of resistant *A. myosuroides* from programmes in small-plot trials

Treatment	Rate (g a.i./ha)	Timing	% Control Mean	 Range
trifluralin	1200	A	41	0-71
isoproturon	2500	B	86	77-94
trifluralin followed by	1200	A	97	94-99
isoproturon + fenoxaprop-p-ethyl	2500 + 55	B	97	94-99
isoproturon + fenoxaprop-p-ethyl	2500 + 55	B	94	89-98
(isoproturon / trifluralin) + fenoxaprop-p-ethyl	(1800/1200) + 55	B	97	92-100
clodinafop-propargyl + trifluralin + oil	30 + 960	B	93	80-100
Untreated (heads / m^2)	-	-	179	103-190

Mean of three trials in Oxon, Norfolk and Lincolnshire pre-treated with tri-allate.
Timing A was applied pre-emergence. Timing B was applied at GS 11-13 of the weed.

In small-plot work (Table 4) the best results were given by a mixture or programme of isoproturon + trifluralin with fenoxaprop-p-ethyl.

In the future, the use of glufosinate-ammonium in tolerant crops will introduce a new herbicide group. Glufosinate-ammonium gave excellent control of resistant *A. myosuroides* at a site in Wiltshire (Table 5), where a genetically modified crop of winter oil seed rape tolerant to this specific herbicide was grown in 1996.

Table 5 Control of resistant *A. myosuroides* in a transformed crop of winter oilseed rape

Treatment	Rate (g a.i./ha)	% Control Mean
quizalofop-p-ethyl	37.5	17
+ oil	+ 990	
glufosinate-ammonium	800	100
Untreated (heads / m^2)	-	95

Treatments were applied on 13/11/96 when the weed was at GS 13-23.

The performance of different active ingredients in the field indicated a difference in susceptibility and different cross-resistance patterns in resistant *A. myosuroides* populations. Glasshouse work in 1994 and 1995 confirmed that in fenoxaprop-p-ethyl resistant populations there is high cross-resistance to tralkoxydim and other 'fops' e.g clodinafop-propargyl but low cross-resistance to isoproturon (Table 6).

Table 6 Cross-resistance patterns for samples confirmed as resistant to fenoxaprop-p-ethyl (1994 and 1995)

Harvest Year	No of Samples	clodinafop-propargyl %		tralkoxydim %		isoproturon %	
		PR	R	PR	R	PR	R
1994	110	0	88	0	62	32	2
1995	169	4	95	2	95	2	63

CONCLUSIONS

Prior to drilling the cereal crop, ploughing is a very useful means of reducing the weed-seed population in the soil and thus reducing pressure on the subsequent herbicides. The trials have shown that pre-treatment of resistant *A. myosuroides* with either tri-allate or trifluralin is a good start to the herbicide programme. Trifluralin works better pre-emergence of the *A. myosuroides* and is most effective at the full dose of 1200 g a.i./ha.

Irrespective of whether there has been any pre-treatment, post-emergence herbicides should be applied at GS 11-13 of the resistant *A. myosuroides*. Later applications to well tillered plants, particularly in the Spring, were much less effective.

For a long term strategy, and in order to reduce selection pressure from individual herbicides, it is important to integrate herbicides with different modes of action. 'Fop' resistant populations of *A. myosuroides* in the UK appear to have low cross-resistance to isoproturon and since this product has very useful residual activity it remains a vital component in the herbicide programme.

In the future, genetically modified crops tolerant to the herbicide glufosinate-ammonium will be introduced. This herbicide has been shown to control 'fop' resistant *A. myosuroides* and so this product will offer a new, alternative group for the control of *A. myosuroides* in some broad-leafed crops.

The prevention or management of resistant *A. myosuroides* requires a planned, long-term strategy incorporating cultural, cropping and chemical programmes.

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REFERENCES

AgrEvo (1996) Grass Weed Management. AgrEvo UK Crop Protection Ltd, King's Lynn, Norfolk, 25-28.

Clarke, J H; Moss S R (1991) The occurrence of herbicide resistant *Alopecurus myosuroides* (black-grass) in the United Kingdom and Strategies for its control. *Proceedings 1991 Brighton Crop Protection Conference - Weeds 3*, 1041 - 1048.

Long, H C (1929) Weeds in Arable Land. HMSO, London 127.

MAFF (1995) MAFF Review of Isoproturon. *Pesticide Register* **7**, ii.

Moss, S R; Albertini, A (1996) Occurence of Herbicide-Resistant Black-Grass by 1995: A Compilation of Results from all Organisations and Companies which Conducted Screening Assays. *33rd Annual Review of Weed Control.* BCPC, 5-9.

Moss, S R; Clarke, J H (1994) Guidelines for the prevention and control of Herbicide resistant black-grass. *Crop Protection* **13,** 230 - 234.

Moss, S R; Cussans, G W (1985) Variability in the susceptibility of *Alopecurus myosuroides* (black-grass) to chlorotoluron and isoproturon. *Aspects of Applied Biology* **9,** 91-98.

Zadoks, J C; Chang, T T; Konzak, C F (1974) A decimal code for the growth stages of cereals. *Weed Research* **14**, 414-421.

THE ROLE OF PROPYZAMIDE IN MANAGEMENT OF HERBICIDE RESISTANT BLACK-GRASS IN OILSEED RAPE

J EDMONDS
Rohm and Haas (UK) Ltd., Lennig House, 2 Mason's Avenue, Croyden, CR9 3NB

J C CASELEY
IACR-Long Ashton Research Station, Long Ashton, Bristol BS18 9AF, UK

ABSTRACT

Black-grass (*Alopecurus myosuroides*) infestation in arable land in the UK has become an increasing problem and has received much attention in recent years, particularly the control of herbicide resistant biotypes. The use of mixtures and sequences of herbicides with different modes of action is recognized as an important component of herbicide resistance management. Results from pot experiments showed propyzamide controlled chlorotoluron and fenoxaprop-P resistant black-grass and the importance of the soil water regime for propyzamide activity was demonstrated.

INTRODUCTION

Propyzamide is a selective systemic herbicide which is used for the control of grasses, both annual and perennial and some broad-leaved weeds in many crops including oilseed rape. It is now widely accepted that integrated approaches to weed control are required to manage herbicide resistance and the use of herbicide mixtures and sequences is a key component of this strategy (Jutsum and Graham 1995). Propyzamide has an unknown mode of action and provides an alternative to widely used contact graminicides in the cereal / oilseed rape rotation. The aim of this work was to evaluate propyzamide against fenoxaprop-P and chlorotoluron resistant and susceptible black-grass biotypes and to investigate some of the factors that influence propyzamide activity including soil moisture regime, depth of seed in the soil profile and the foliage compared to root uptake.

MATERIALS AND METHODS

General

The biotypes used were:

LARS -	known to be susceptible to chlorotoluron, fenoxaprop-P and propyzamide
Peldon -	high R to chlorotoluron, moderate R to fenoxaprop-P
Bucks -	high R to chlorotoluron, moderate R to fenoxaprop-P
Lincs E1 -	slight R to chlorotoluron, high R to fenoxaprop-P
Faringdon -	partial R to chlorotoluron and fenoxaprop-P
Notts A1 -	high R to 'fop' chemistry e.g. fenoxaprop-P, high R to 'dim' chemistry e.g. cycloxydim and slight R to chlorotoluron

Seeds of the above biotypes were placed in a cool glasshouse (14/10 $^{+}$- 4^{O}C) on moist filter paper in a plastic tray and covered with clear plastic. After incubation for 5 days, germinated seeds with 0.5 - 1.0 mm of emerged radicle were placed on moist sandy loam soil contained in 10 cm diameter plastic pots. For the pre- and post-emergence studies eight and five germinated seeds respectively were sown per pot. Seeds were then covered with 0.5 mm of 1:1 soil/sand mix, and each pot was watered from above for 1 second with a hose fitted with a fine rose. The pots were then placed on capillary matting in a cool glasshouse with supplementary lighting.

All sprayed herbicide treatments were applied with a laboratory pot-sprayer fitted with a SpraySystems 8001 nozzle, calibrated to deliver 200 l/ha at 210 kPa pressure at the appropriate growth stage under investigation.
All the treatments and untreated controls were arranged in a randomised block design consisting of four replicates. Fresh weight of the foliage was determined as the final assessment.

Pre-emergence activity of propyzamide against fenoxaprop-P and chlorotoluron resistant and susceptible *A. myosuroides*

Propyzamide and chlorotoluron at the doses shown in Table 1, were sprayed onto the soil surface on the day following planting. Pots were then placed on capillary matting in a cool glasshouse with supplementary lighting. One day after spraying and every three to four days thereafter, pots were watered from above with 13 ml water applied to the soil surface with a dispenser. This amount of water is equivalent to 2 mm hr $^{-1}$ of rain for 1 hour and was applied to move the herbicide down the soil profile into the root zone. This would also reduce photodegradation of the propyzamide.

The route of uptake of propyzamide into *A. myosuroides* ; shoot versus root

For this experiment LARS and Peldon biotypes were used. For root uptake studies, seeds were sown at depths of 0.5 and 1.5 cm, with the latter sown one week before the others so that all treatments could be sprayed the same day. The two different seed depths were used to simulate seed germination from different positions in the soil profile. Immediately after sowing, each pot was watered from above for one second with a hose fitted with a fine rose. Seedlings were thinned to one or three plants per pot at the one leaf growth stage for the foliage and root uptake experiments respectively.
For the root uptake experiment, chlorotoluron and propyzamide at the doses shown in Table 2 were applied with a laboratory pot-sprayer at the 2-3 leaf growth stage.

After spraying, sets of pots were divided into seeds planted at 0.5 and 1.5 cm depth and subjected to the following watering regimes

1. Sub-irrigation only via capillary mat
2. Sub-irrigation plus 26 ml twice per week applied to the soil surface
3. No sub-irrigation, 13 ml twice per week applied to the soil surface
4. No sub-irrigation, 13 ml four times per week applied to the soil surface
5. No sub-irrigation, 26 ml twice per week applied to the soil surface

After 10 days, water applications to the soil surface were increased from 13 and 26 ml to 20 and 40 ml respectively for all regimes to avoid water stress in plants that were not sub-irrigated.

For the foliage uptake study, propyzamide (400 g a.i./l) was made up at a concentration equivalent to 1100 g a.i./ha in 200 l of water and chlorotoluron at 2800 g a.i./ha in 200 l of water was used as a reference treatment.
Herbicide was applied to plants of *A. myosuroides* at the three leaf growth stage using a Burkard micro applicator. Three 2 microlitre drops were applied to the centre of the last fully expanded leaf with the leaf supported in a horizontal position to prevent drops from rolling into the leaf axil. On another set of plants, three 2 microlitre drops were applied close to the ligule and then the leaf axil was pulled gently open to draw the herbicide into the axil between the 2nd and 3rd leaves.

RESULTS

Pre-emergence activity of propyzamide against fenoxaprop-P and chlorotoluron resistant and susceptible *A. myosuroides*

The lowest dose of chlorotoluron (1.4 kg a.i./ha) killed the LARS and Notts. A1 biotypes, but the other four biotypes survived, with the Peldon and Bucks attaining around half the untreated control fresh weight following treatment with 5.6 kg a.i./ha. Propyzamide at the lowest dose of 100 g a.i./ha, reduced the weight of all the biotypes by 40-50% and seedlings of all the biotypes were killed by 500 g a.i./ha (Table 1)

Table 1 - The effect of chlorotoluron and propyzamide applied pre-emergence to six biotypes of *A. myosuroides* 19 DAT. Foliage fresh weight as % of untreated control.

g. a.i./ha	LARS	Peldon	Bucks	Lincs E1	Faringdon	Notts A1
chlorotoluron						
1400 gai ha^{-1}	18	82	73	35	42	16
2800gai ha^{-1}	11	67	61	21	20	10
5600 gai ha^{-1}	10	59	45	16	25	4
propyzamide						
100 gai ha^{-1}	58	55	60	59	52	49
300 gai ha^{-1}	19	8	11	5	15	14
500 gai ha^{-1}	16	7	7	8	8	4
700 gai ha^{-1}	7	6	5	6	7	4
900 gai ha^{-1}	6	2	3	4	10	4
1100 gai ha^{-1}	7	3	4	4	3	8
control	100	100	100	100	100	100
fresh weight (g)	0.274	0.255	0.271	0.286	0.306	0.285

Effect of soil water regime and seed planting depth on propyzamide activity against LARS and Peldon biotypes of *A. myosuroides*

Although sowing depth had no effect on foliage fresh weight of controls, there were significant interactions when herbicide was applied. Chlorotoluron significantly reduced fresh weights of plants of the susceptible biotype LARS compared with the untreated controls for all watering regimes except for plants sown at 1.5 cm and watered by sub-irrigation 13/26 ml applied to the soil surface twice weekly. In contrast, chlorotoluron only reduced the weight in the resistant Peldon biotype in plants which were sown shallowly at 0.5 cm and given the wettest watering regime (sub-irrigation plus 26/40 ml twice weekly). For both biotypes, the only watering regimes which gave a significant shoot weight decrease with increasing dose of propyzamide were the two wettest regimes with surface irrigation (sub-irrigation plus 26/40 ml twice weekly at both sowing depths, and the 26/40 ml twice weekly at 0.5 cm sowing depth). The strongest decreases were for the wettest watering regime (sub-irrigation plus 26/40 ml twice weekly) at the shallowest sowing depth. Under the best watering regime of sub-irrigation plus 26/40 ml twice a week, Peldon was significantly more susceptible than LARS to 300 g a.i./ha of propyzamide, but with 500 g a.i./ha the reverse was true. A similar situation was seen with the less favourable watering regime of 13/20 ml four times per week for propyzamide at the two higher doses. For all other treatment combinations (and the means of all propyzamide doses) there was no significant difference between the two biotypes.

Table 2. The effect of soil water regime and seed planting depth on propyzamide activity against LARS and Peldon biotypes of *A. myosuroides* at the 2-3 leaf stage 28 DAT. Fresh weight of shoots (g).

			chlorotoluron	propyzamide g a.i./ha				
Regime	Depth	Control	2800	300	500	700	900	1100
LARS biotype								
sub-irrigation	0.5 cm	9.96	4.56	9.39	10.06	9.68	8.78	9.06
	1.5 cm	8.97	8.56	9.10	11.49	8.30	10.83	9.66
13/20 ml 2 x week	0.5 cm	6.85	0.52	5.4	5.53	5.51	5.7	5.63
13/20 ml 4 x week	0.5 cm	7.57	0.2	7.22	7.3	4.68	3.69	7.79
26/40 ml 2 x week	0.5 cm	8.46	7.04	6.96	5.42	5.27	6.66	3.9
sub-irrigation + 26/40 ml 2x week	0.5 cm	11.7	0.11	10.06	1.79	2.65	3.24	2.21
	1.5 cm	14.75	0.41	13.92	10.08	12.51	7.48	5.35
Peldon biotype								
sub-irrigation	0.5 cm	10.10	9.90	8.72	7.88	8.12	6.85	8.44
	1.5 cm	10.41	9.85	9.37	8.32	9.62	9.43	9.10
13/20 ml 2 x week	0.5 cm	5.24	4.99	4.69	4.59	4.97	4.74	4.92
13/20 ml 4 x week	0.5 cm	6.52	6.68	6.33	6.73	5.68	6.44	3.53
26/40 ml 2 x week	0.5 cm	8.46	7.04	6.96	5.42	5.27	6.66	3.9
sub-irrigation + 26/40 ml 2 x week	0.5 cm	10.98	6.74	7.27	3.94	3.41	5.15	1.87
	1.5 cm	11.37	10.63	11.55	10.24	11.62	9.00	7.39

LSD ($p = 0.05$) 2.05

Foliage uptake

Applications of propyzamide to the mid lamina, inner second leaf sheath of the second leaf of plants at the two to three leaf stage had no effect on the shoot fresh weight of the biotypes Peldon or LARS at 27 DAT.
Applications of chlorotoluron to the same areas of the Peldon biotype, which is resistant to chlorotoluron, also had no effect on the fresh weight. However, although chlorotoluron had a limited effect on the sensitive LARS biotype when applied to the mid-laminar region of the second leaf, there was a pronounced effect when it was applied to the second leaf sheath, causing a 40% reduction in fresh weight (Table 3).

Table 3. The effect of placement of chlorotoluron and propyzamide on the mid-lamina and inner sheath of LARS and Peldon *A. myosuroides* at the 2 leaf growth stage 27 DAT. Shoot fresh weight as % of untreated control

Applied to mid-lamina				Applied to inner leaf sheath			
Propyzamide		Chlorotoluron		Propyzamide		Chlorotoluron	
P	L	P	L	P	L	P	L
110	109	110	96	101	102	95	60

Fresh weight of control (g) Peldon (P) 3.14, LARS (L) 2.94

DISCUSSION

Pre-emergence activity of propyzamide against fenoxaprop-P and chlorotoluron resistant and susceptible *A. myosuroides*

Pre-emergence treatment with chlorotoluron of the different biotypes produced varying results which divided into the expected patterns, i.e. highly resistant Peldon and Bucks C1 were least controlled, moderately resistant biotypes Faringdon and Lincs E1 were the next level with the susceptible biotypes controlled best of all.

Propyzamide, when applied pre-emergence, was equally effective against all the fenoxaprop - P and chlorotoluron resistant and susceptible biotypes. Although emergence was relatively unaffected, subsequent growth was substantially inhibited, with propyzamide giving good control at 300 g a.i./ha and complete kill at 700 g a.i./ha.

The effectiveness of pre-emergence propyzamide in controlling all the fenoxaprop-P and chlorotoluron biotypes suggests that this herbicide can play an increasingly important role in herbicide resistance management programmes involving oilseed rape in the rotation.

Effect of soil water regime and seed planting depth on propyzamide activity against LARS and Peldon biotypes of *A. myosuroides*

The results from both the susceptible LARS and resistant Peldon biotypes showed the trend that the higher levels of soil moisture create the best environment for propyzamide activity. However, sub-irrigation alone on a capillary matting which maintained the soil very close to field capacity resulted in low propyzamide activity. This is probably due to water moving up the soil profile and thus minimising contact of the herbicide with the black-grass roots. A key factor determing propyzamide activity is that sufficient water is applied to the soil surface to move the herbicide down the soil profile to the root zone. Removal of propyzamide from the soil surface reduces photodegradation of the herbicide.

The depth of germination also provides a further crucial factor influencing propyzamide activity. Higher levels of control are achieved when black-grass germinates near the soil surface as this is where the highest concentrations of propyzamide are located. In this experiment, control was better of black-grass when seed was planted at 0.5 cm compared to 1.5 cm. The shoot fresh weight results (Table 2) indicate there are no differences of practical significance between the Peldon and LARS biotypes treated with propyzamide

Foliage uptake

The results from applying propyzamide to either the second leaf or second leaf sheath confirms that the uptake of propyzamide is by the roots only, as there is no difference between treated and control plants. In contrast, chlorotoluron has some foliar activity, the difference, as highlighted by the effects seen against the resistant (Peldon) and sensitive (LARS) biotype.

General discussion

In the field situation, the results suggest that pre-emergence and very early post-emergence activity will be good as long as the soil is moist at the time of application and there is rain thereafter. Rain has the following beneficial effects on propyzamide

1. before spraying, rain moistens the soil and water occupies potential binding sites for propyzamide
2. following application it washes active ingredient off target and non-target foliage, which is beneficial as here is no foliage uptake and the propyzamide is deposited on the soil.
3. rain dissolves the propyzamide on the soil surface and moves it down the soil profile into the root surface where uptake into the plant occurs.

The excellent control of the black-grass biotypes of known herbicide resistance following pre-emergence application and good early post-emergence activity obtained under several of the wetter soil moisture regimes suggests propyzamide has a valuable role to play in a structured management strategy for the control of herbicide resistant *A. myosuroides*.

REFERENCES

Jutsum A R; Graham J C (1995) Managing weed resistance: the role of the agrochemical industry. *Brighton Crop Protection Conference - Weeds 557-566.*

NEW MANAGEMENT APPROACHES FOR ISOPROTURON-RESISTANT *PHALARIS MINOR* IN INDIA

S SINGH, R C KIRKWOOD
Department of Bioscience and Biotechnology, University of Strathclyde, Glasgow, G4 0NR

G MARSHALL
Plant Science Division, Scottish Agricultural College, Auchincruive, Ayr, KA6 5HW

ABSTRACT

Phalaris minor resistance to isoproturon in wheat is increasing rapidly in the rice-wheat cropping zones of north-west India. The resistant biotypes of *P. minor* require an 8-18 times higher dose of isoproturon for the same level of control to that of the susceptible biotypes. Consequently, the higher dose is phytotoxic to wheat and results in a 30-80 % reduction in yield and complete crop failure under heavy infestations. The physiological and biochemical basis of resistance is a consequence of enhanced metabolic degradation of isoproturon in resistant species. Recommendations for weed management include crop rotations which include competitive crops, herbicide rotations and introducing alternative herbicides such as chlorotoluron and other graminicides e.g. tralkoxydim. The integration of agronomic practices, including manipulating the seed bank reserves of *P. minor*, are also discussed in relation to minimising the impact of isoproturon-resistant *P. minor* in wheat production.

INTRODUCTION

India is the third largest producer of wheat in the world. The north western Indian states are the grain bowl of the country where rice-wheat is the main crop rotation system. The increased yield of these crops over the last four decades has sustained the 'green revolution' in India. The adoption of these fertiliser-responsive, high yielding dwarf wheat varieties suffered a serious setback owing to their poor ability to compete with grass weeds notably wild oat (*Avena ludoviciana*) and littleseed canarygrass (*Phalaris minor*) during the 1970s. Increased use of nitrogenous fertilisers and optimum irrigation favoured these vigorously competiting weeds (Singh & Malik, 1992). Isoproturon, metoxuron, chlorotoluron and methabenzthiazuron were recommended two decades ago for the control of *P. minor* in wheat (Gill *et al.*, 1978), the first one being used predominantly. However, increased selection pressure due to continuous use of isoproturon over the last 15 years in the same cropping system of rice-wheat resulted in the evolution of resistant (R) biotypes of *P. minor* (Malik & Singh, 1993, 1995, Singh *et al.*, 1993). The resistance was so serious that many farmers were forced to plough up the wheat fields or to harvest it as a green fodder (Malik & Singh, 1993).

CURRENT STATUS

Overall, the national production of wheat increased by 18% from 1985-1989 compared to only 8% from 1989-1994, despite being an increase of 4% in area grown, during the latter period (Anon. 1996). The resistance problem has greatly increased in area and is causing a serious challenge for management of *P. minor*. Failure to control the R biotypes has resulted in a 30-80 % yield loss and complete crop failure under severe infestations. While the affected area is increasing annually, the resistance problem has been more or less confined to those areas with the same rotations of crop and herbicide; resulting in withdrawal of isoproturon for use in the affected areas. Given only one herbicide choice for effective weed management in wheat, farmers were extremely vulnerable in economic terms in the resistance-prone areas. Though the economic returns for wheat production decreased in 1990s compared to 1980s, the use of rotational crops still provides lower margins.

RESEARCH: A RATIONAL BASIS FOR RESISTANCE MANAGEMENT

To avoid the recurrence of resistance to other herbicides and for efficient management of R biotypes of *P. minor* it is imperative to understand the underlying mechanism of resistance to isoproturon as summarised in Table 1.

Table 1. Mechanism of isoproturon resistance in *P. minor*

Research approach	Conclusions	Reference
Physiological studies	The R biotypes required a significantly higher dose of isoproturon compared to S biotypes for the same level of control	Singh *et al*., 1995c Singh *et al*., 1996 Singh *et al*., 1997
	No alteration at target site observed in photosynthetic and chlorophyll fluorescence studies	Singh *et al*., 1995b Singh *et al*., 1997
Uptake/translocation studies	No differences between R & S biotypes in uptake/translocation of ^{14}C isoproturon	Singh *et al*., 1996
Metabolism: general	Enhanced metabolism in the R biotype, primary determinant of resistance	Singh *et al*., 1996
Metabolism: inhibitor studies	Pathway of degradation involves hydroxylation and demethylation, ABT (1-aminobenzotriazole) and PBO (piperonyl butoxide) inhibited these degradation pathways. Mixed function oxidase inhibitors (ABT and PBO) enhanced isoproturon activity and resulted in loss of resistance in the R biotype.	Singh *et al*., 1997

From these results it was deduced that resistance in the R biotypes of *P. minor* is due to enhanced degradation by cytochrome P-450 monooxygenases.

RECOMMENDATIONS OR OPTIONS FOR RESISTANCE MANAGEMENT

Enhanced metabolic resistance to isoproturon or chlorotoluron has also been recorded with other grass weeds including *Alopecurus myosuroides* in the UK and *Lolium rigidum* in Australia (Powles & Preston, 1995). Both species have shown multiple resistance to herbicides with different modes of action and this kind of resistance is very difficult to control. Suggestions for the management of resistance are as follows:

Crop Rotation

The change of crop will result in a rotation of herbicides and possibly the rotational crop may provide a competitive effect on the resistant weeds. A survey of the affected area during 1993 revealed that the occurrence of resistance was only 8-16% where wheat was rotated with sugarcane/vegetables, pigeonpea, clover or sunflower compared to 67% under a rice-wheat cropping system (Malik & Singh, 1995). Alternate crops like sugarcane (long duration) and sunflower (short duration) with their ultimately aggressive vegetative growth may have a suppressing effect on *P. minor*. Coupled with the use of triazine or dinitroaniline herbicides, effective weed control can be achieved (Singh *et al.*, 1995a). Berseem (*Trifolium alexandrium*) used as a green fodder for cattle can be used successfully to control *P. minor*. Similarly vegetable cash crops also help in checking the multiplication of *P. minor*. Potato, winter maize, oilseeds and pulses are potential crops which can be successfully rotated with wheat. However, due to the small land holding of the majority of farmers, only a limited area under wheat cultivation can be rotated. It is imperative that farmers obtain a remunerative price for the produce from alternate crops to encourage the use of crop rotation.

Herbicide Rotation

The rotation of herbicides with different modes of action may be important in avoiding the evolution of resistance. Chlorotoluron, which was used initially for the control of *P. minor*, has been found to provide excellent control of the R biotypes (Singh *et al.*, unpublished data). Chlorotoluron was found to be more active on the S biotype than isoproturon. While *A. myosuroides* and *L. rigidum* have evolved resistance to chlorotoluron, this does not mean it cannot be used as a substitute for a similar herbicide in a totally different situation. A resistant biotype of *P. minor* from Israel has been reported to be resistant to fenoxaprop-P but not to isoproturon or methabenzthiazuron (Tal *et al.*, 1996). This biotype has also exhibited cross-resistance to tralkoxydim, sethoxydim and cycloxydim whereas the Indian biotypes of resistant *P. minor* are sensitive to these herbicides (Singh *et al.*, 1995a). Both biotypes, however, are similarly cross-resistant to diclofop-methyl (Singh *et al.*, 1995a; Tal *et al.*, 1996). Cross-resistance to clodinafop-propargyl has yet to be confirmed, though differential responses were observed in the R biotypes following treatment of *P. minor* in a pot study and nutrient solution treatments in this laboratory (Singh *et al.*, unpublished data). Under field evaluation trials in the resistant affected areas in India, however, clodinafop and Mon 37500 (a sulfonylurea herbicide) were reported to provide 70% control of R biotypes of *P. minor* (Malik, R.K.; 1997 *pers. comm*). The relatively high dose of clodinafop-propargyl required to control the R biotypes under pot studies raised doubts regarding its long-term success in the field conditions. Other herbicides which have shown promising results against the R biotypes both under pot and field experiments are tralkoxydim, terbutryne, pendimethalin, trifluralin, fenoxaprop-P-ethyl (Singh *et al.*, unpublished data; Singh *et al.*, 1995a; Malik, R.K. 1997 *pers. comm*) and propachlor, metazachlor and atrazine in pot experiments (Singh *et al.*, 1995a). However, emergence of wheat was reduced by 50 % by trifluralin in the field and there is a need to increase the seed rate or manipulation in depth of sowing/herbicide incorporation to reduce crop phytotoxicity (Singh *et al.*, unpublished data).

Certain of these herbicides are not active against broadleaf weeds, and a suitable mixture of two or three herbicides may need to be used for efficient grass and broadleaf weed control. A tank mixture of isoproturon with fenoxaprop-P-ethyl or atrazine was not found effective (Singh *et al.*, 1995c and Singh *et al.* unpublished data). Tralkoxydim has less effect on *P. minor* than isoproturon, while a tank mixture of both provided higher grain yield of wheat. Mixtures of chlorotoluron and tralkoxydim hold promise in the resistance-prone areas. Similarly, herbicide formulation can be important. However, the additive (Silwet L-77, 0.05%) has not been shown to enhance efficacy (Singh *et al.*, 1995c).

Cytochrome P-450 inhibitors have been used largely to elucidate the mechanism of resistance but only a few compounds have shown field utility. PBO increased the activity of isoproturon against the R biotypes but increased phytotoxicity to wheat. More research is needed to identify P-450 inhibitors that can selectively work against R weeds.

Weed Biology and Agronomic Practices

Agronomic factors can contribute to the growth and competitive behaviour of weeds and crops. Small seeded weeds like *P. minor* which germinate from upper soil layers can be buried by deep ploughing. Germination of *P. minor* is increased by the late planting of wheat in December at lower soil temperatures (Malik & Singh, 1993). Since *P. minor* germinates in two to three flushes, this germination behaviour can be exploited in exhausting the soil seed bank under rotational crops planted in January. The germinated seeds are destroyed during field preparation for late sown crops or can be killed by a contact herbicide. Though variations have been observed in respect of germination and growth of R & S biotypes, the role of temperature may be of primary importance. Early and rapid germination of the R biotype (KR-1) under lower temperatures could be exploited by selectively cultivating or spraying out this biotype before sowing of the crop.

Effective weed control in wheat crops may be influenced by a number of factors including: (1) the selection of varieties with early vigour and canopy cover, (2) increased seed rates with sowing rows at right angles (narrow spacing) and (3) an optimum fertiliser rate. These measures not only favour crop competition against grass weeds but also increase herbicide efficacy (Malik & Singh, 1993). Dwarf wheat varieties are more vulnerable to competition with *A. ludoviciana* and *P. minor* compared to tall growing varieties. The R biotypes appear to be equally competitive with the isoproturon susceptible biotype. The methods and timing of herbicide application also influence efficacy. For example, the activity of isoproturon was found to be higher at the 2-3 leaf stage of *P. minor* and *A. ludoviciana* compared to later growth. Clearly, it is the integration of agronomic practices with herbicide treatments which is the foundation for improved management of the herbicide resistant biotypes of *P. minor*.

FUTURE PROSPECTS

It would be unwise to think that *P. minor* will not evolve resistance against new herbicides. Resistance to sulfonyl urea herbicides was quick to evolve and both *A. myosuroides* and *Avena fatua* were found to have only moderate sensitivity against Mon 37500 (Parrish *et al*., 1995). In Israel, *P. minor* has been shown to have cross-resistance to clodinafop (Tal *et al*., 1996). Resistance to many of the above mentioned herbicides has evolved and these herbicides need to be used judiciously for effective management of R biotypes of *P. minor*. Lower resistance to isoproturon than chlorotoluron in *A. myosuroides* and *L. rigidum* was postulated due to differences in their molecular structure and greater vulnerability of oxidative breakdown of the latter by the R biotypes, however, complete loss of resistance to chlorotoluron in isoproturon R biotypes of *P. minor* indicate involvement of different enzymes in the breakdown of these herbicides.

Herbicide resistant transgenic wheat could be another option for managing resistant weeds including *P. minor*, but this would be adopted only where the herbicide and seed costs form part of a package which is economically viable. Alternative solutions are required to meet current problems.

Farmer awareness and proper education about resistance is necessary to cope with the present situation. They have to be convinced that the rotation of crops and herbicides is beneficial in the long term and essential for efficient crop production. Failure to use the optimum herbicide dose and proper application method is another factor which is partly responsible for isoproturon resistance in *P. minor*. The vigilance and integrity of enforcement agencies to ensure quality control of herbicides is also vital in the resistance management strategy. It will not be easy to produce enough wheat to feed the ever burgeoning human population of India if resistance is not properly managed. The stakes are high and failure to solve the problem may result in the loss in food sufficiency and loss of national pride.

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REFERENCES

Anonymous (1996). *Statistical Yearbook for Asia and the Pacific*. United Nations. pp. 180-181.

Gill H S; Walia U S; Brar L S (1978). Control of *Phalaris minor* Retz. and wild oats in wheat with new herbicides. *Pesticides* **12**, 55-56.

Malik R K; Singh S (1993). Evolving strategies for herbicide use in wheat: Resistance and integrated weed management. *Proceedings Indian Society of Weed Science, International Symposium on Integrated weed Management for Sustainable Agriculture*, Hisar, India, Nov. 18-20, **1**, 225-238.

Malik R K; Singh S (1995). Littleseed canarygrass (*Phalaris minor*) resistance to isoproturon in India. *Weed Technology*. **9**, 419-425.

Parrish S K; Kaufmann J E; Croon K A; Ishida Y; Ohta K; Itoh S (1995). Mon 37500: A new selective herbicide to control annual and perennial weeds in wheat. *Proceedings Brighton Crop Protection Conference - Weeds*. **1**, 57-63.

Powles S B; Preston C (1995). *Herbicide cross resistance and multiple resistance in plants*. Herbicide Resistance Action Committee, Monograph No. 2, Brussels, Belgium.

Singh S; Malik R K; Malik Y P; Garg V K (1993). Resistance of some *Phalaris minor* biotypes to isoproturon but not to pendimethalin. *Proceedings Indian Society of Weed Science, International Symposium on Integrated weed Management for Sustainable Agriculture*, Hisar, India, Nov. 18-20, **2**, 125-130.

Singh S; Malik R K (1992). Weed management and fertiliser utilisation. *Fertiliser News*. **37**, 59-63.

Singh S; Kirkwood R C; Marshall G (1995a). Evaluation of isoproturon resistant Littleseed canary grass (*Phalaris minor*) to a range of graminicides. *Weed Science Society of America, Annual Meeting,* Seattle, Jan. 30- Feb. 2, Abstract, **162**, pp. 54.

Singh S; Kirkwood R C; Marshall G (1995b). The effect of isoproturon on photosynthesis of resistant and susceptible biotypes of *Phalaris minor* Retz. *Proceedings International Symposium on Weed and Crop Resistance to Herbicides*, 3-6 April, Cordoba, Spain, **1**, 115-117.

Singh S; Kirkwood R C; Marshall G (1995c). The effect of Silwet L-77 or fenoxaprop-P-ethyl on the efficacy of isoproturon applied to isoproturon resistant *Phalaris minor*. *Proceedings Brighton Crop Protection Conference - Weeds* **1**, 231-236.

Singh S; Kirkwood R C; Marshall G (1996). Uptake, translocation and metabolism of isoproturon in wheat, susceptible and resistant biotypes of *Phalaris minor*. *Proceedings Second International Weed Control Congress,* Copenhagen (Denmark), 25-28 June, 1996, **2**, 529-534.

Singh S; Kirkwood R C; Marshall G (1997). Enhanced degradation by cytochrome P-450 is the mechanism of resistance in *Phalaris minor* (Retz.) (Littleseed canarygrass) biotypes. *Weed Science Society of America's Annual Meeting*, Orlando, Florida, Feb. 3-6, Abstract **167**, p. 65.

Tal A; Zarka S; Rubin B (1996). Fenoxaprop-P resistance in *Phalaris minor* conferred by an insensitive acetyl coenzyme A carboxylase. *Pesticide Biochemistry and Physiology* **56**, 134-140.

INCREASING THE NUMBER OF MECHANISMS OF ACTION OF HERBICIDES FOR MANAGEMENT OF WEED RESISTANCE

R A VIDAL, N G FLECK
Faculdade de Agronomia, UFRGS, Caixa Postal 776, 90001-970, Porto Alegre, RS, Brazil

N A OLIVEIRA, R STRELLO, F B GUIMARAES, N G SILVA
ZENECA Brasil Ltda, Caixa Postal 55094, 04799-970, São Paulo, SP, Brazil

ABSTRACT

One of the strategies to control resistant weeds is to use herbicides with different mechanisms of action. Research was conducted in southern Brazil to evaluate control strategies for *Euphorbia heterophylla* resistant to ALS inhibitors. Treatments were organised in a two-way factorial experiment, where factor A consisted of weed control practices before soybean emergence, involving treatments with up to three mechanisms of action, and compared with zero to two tillage operations; and factor B consisted of weed control after soybean emergence, involving treatments with one or two mechanisms of action. *E. heterophylla* control ranged from 50 to 90%. The number of herbicide mechanisms of action used before crop emergence did not affect weed control, having a similar effect to mechanical weed control. However, the use of two herbicides with different mechanisms of action after soybean emergence resulted in weed control at least 25% greater than control observed with the use of only one.

INTRODUCTION

Euphorbia heterophylla has widespread distribution in central-southern Brazil and causes from 20 to 50% soybean yield loss at densities of 12 to 54 plants/m^2 (Chemale & Fleck, 1982). In soybeans, selective post-emergence control of *E. heterophylla* is achieved with acetolactate synthase (ALS) and protoporphyrinogen oxidase (PROTOX) inhibitors (Lorenzi, 1994). The recent development of *E. heterophylla* resistance to ALS inhibitors (Vidal, 1997) has been an added burden to farmers, specially those in soybean mono-cropping, because of limited control options. In this case, *E. heterophylla* control with PROTOX inhibitors is limited because, when sprayed on plants at early growth stages, they allow time for reinfestation, and, when applied on plants at late growth stages, they only kill the apical meristem and plants regrow from adventitious buds (Willard & Griffin, 1993).

Resistance occurs as a result of high selection pressure from the herbicide on a weed population over several years. The strategies suggested to manage weed resistance include: rotation of herbicides from different mechanisms of action (MOA); crop rotation; mixture of herbicides with different MOA; and integration of mechanical and cultural practices with chemical weed control (Vidal, 1997). In soybean mono-cropping, management options for resistant *E. heterophylla* are limited to the last two of these strategies. However, Vidal *et al.* (1997) observed resistant *E. heterophylla* was not cross resistant to herbicides from different MOA such as 2,4-D (2,4-dichlorophenoxy)acetic acid), glyphosate trimesium (trimethylsulfonium salt of N-phosphonomethyl)glycine), and paraquat (1,1'-dimethyl-4,4'-bipyriginium ion), which are widely used for weed management in no-till areas. The objective of this work was to develop *E. heterophylla* management strategies integrating chemical and mechanical methods of weed control.

MATERIALS AND METHODS

One experiment was conducted in 1996-97 near Passo Fundo city, state of Rio Grande do Sul, Brazil. The soil is classified as an Oxissol, with pH 6.1 and 3.9% o.m. The experimental area had been in no-tilled wheat-soybean rotation for several years. Preliminary experiments at the area indicated that 40% *E. heterophylla* were resistant to ALS inhibitors.

The experiment was initiated 23 November 96, after wheat harvest with a combine. Split-plot treatments were organised in a randomised complete block design with four replicates and sub-plot size 2 x 6 m. The main plots had different weed control practices before soybean emergence, whereas the sub-plots had different post-emergence herbicides (Table 1). Additionally, two control treatments were added to the design: weedy the whole season, and 200 g/ha of paraquat as 'GRAMOXONE' applied 2 days after planting (DAP). Weed control in treatments before soybean emergence consisted of: a) tandem disk used twice 21 days before planting and once at planting time; b) tandem disk twice at 21 days before planting and paraquat at 200 g/ha two DAP; c) glyphosate trimesium as 'ZAPP' at 396 g/ha 21 days before planting and paraquat (200 g/ha) 2 DAP; and d) glyphosate trimesium at 330 g/ha plus 2,4-D

Table 1. Treatment list for *Euphorbia heterophylla* control.

Treatments		Sub-treatments		Number of MOA[1]		
20 DBP[2]	at planting	22 DAP[2]	37 DAP	before[3]	after[3]	total
tandem disk	tandem disk	fomesafen	-	0	1	1
tandem disk	tandem disk	imazethapyr	-	0	1	1
tandem disk	tandem disk	fomesafen + imazet[4]	fomesafen	0	2	2
tandem disk	paraquat	fomesafen	-	1	1	2
tandem disk	paraquat	imazethapyr	-	1	1	2
tandem disk	paraquat	fomesafen + imazet	fomesafen	1	2	3
glyphosate[5]	paraquat	fomesafen	-	2	1	3
glyphosate	paraquat	imazethapyr	-	2	1	3
glyphosate	paraquat	fomesafen + imazet	fomesafen	2	2	4
glyphosate + 2,4-D	paraquat	fomesafen	-	3	1	4
glyphosate + 2,4-D	paraquat	imazethapyr	-	3	1	4
glyphosate + 2,4-D	paraquat	fomesafen + imazet	fomesafen	3	2	5
control 1:	paraquat					
control 2 :	weedy the whole season					

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[1] MOA = mechanism of action

[2] DBP = days before planting; DAP = days after planting

[3] before = before soybean emergence; after = after soybean emergence

[4] imazet = imazethapyr

[5] glyphosate = glyphosate trimesium

as ‘DEFERON’ at 400 g/ha 21 days before planting complemented with paraquat at 200 g/ha 2 DAP (Table 1). Adjuvants were added to each herbicide according to the manufacturers’ recommendations.

Herbicide treatments were applied with a CO_2 sprayer with four 11002 XR nozzles, at 150 l/ha diluent volume and 275 kPa pressure. Soybean variety ‘BR-16’ was planted at 65 kg/ha on 14 December 96. Weed control in post-emergence treatments consisted of: a) fomesafen (5-[2-chloro-4-(trifluoromethyl)phenoxy]-N-(methylsulfonyl)-2-nitrobenzamide) as ‘FLEX’ at 250 g/ha 22 DAP; b) imazethapyr (2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1H-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid) as ‘PIVOT’ at 100 g/ha 22 DAP; and c) fomesafen at 100 g/ha plus imazethapyr 40 g/ha at 22 DAP, complemented with fomesafen at 100 g/ha 37 DAP (Table 1). Adjuvants and equipment for post-emergence treatments were as described for treatments before soybean emergence.

E. heterophylla control was estimated at 45 and 80 DAP through a visual assessment using a 0-100 scale, where 0 was no weed control and 100 was complete weed control. *E. heterophylla* density was measured at 45 DAP, in 4 625 cm^2 quadrats, randomly allocated in the middle of each plot. All data were subjected to ANOVA. To accommodate heterogeneity of variance, ANOVA was also performed on control data transformed by sin(x+1), and weed count transformed by log(x+1). Fisher’s protected LSD ($P = 0.05$) was used to compare means. Correlations were performed between weed density and visual control ratings.

RESULTS AND DISCUSSION

E. heterophylla control

Weed control decreased from first to second assessment dates, mainly when post-emergence treatments consisted of one, compared to two MOA. Overall, *E. heterophylla* control was 70 and 50%, for the first and second assessment dates, respectively (Tables 2 and 3). Both the pre-emergence herbicides and the mechanical method gave similar results in control of the weed (Tables 2 and 3). This result was expected because the different strategies for weed management in pre-emergence were designed to control several weed fluxes (Table 1).

Performance of post-emergence treatments depended on pre-emergence treatments (Tables 2 and 3). At 45 DAP, imazethapyr was less efficient when no herbicide was used for *E. heterophylla* control in pre-emergence (Table 2). However, imazethapyr efficacy was intermediate when one or two MOA were used in pre-emergence. Furthermore, fomesafen and imazethapyr were similar when three MOA were used in pre-emergence. In all pre-emergence treatments, best *E. heterophylla* control was obtained when two MOA were used in post-emergence.

Again, at 80 DAP, best *E. heterophylla* control was obtained when herbicides from two MOA were used in post-emergence for all pre-emergence treatments (Table 3). Fomesafen and imazethapyr had similar performance when either none or one MOA were used in pre-emergence. However, when two or three MOA were used pre-emergence, imazethapyr gave better *E. heterophylla* control than fomesafen.

As rainfall was sporadic during 1996/97 crop season, disking the soil for weed control at pre-emergence may have increased water evaporation from the soil. As a consequence, weeds probably were stressed during post-emergence application, which may have reduced absorption and efficacy of post-emergence herbicides. Treatments with two MOA in post-emergence received an additional application of fomesafen 15 days after the first spraying, which may have helped to control plants that were recovering from the first application.

Table 2. *Euphorbia heterophylla* control (%) at 45 DAP with treatments applied before and after soybean emergence.

Treatments before soybean emergence	Treatments after soybean emergence Fomesafen	 Imazethapyr	 Fom+Imaz/Fom[1]
0 MOA[2]	71	56	91
1 MOA	58	68	88
2 MOA	51	65	87
3 MOA	62	63	83
LSD 5% (within post[3])	----------------	8	----------------
LSD 5% (within pre[3])	----------------	ns	----------------

[1] Fomesafen + imazethapyr at 22 DAP + fomesafen at 37 DAP

[2] Number of herbicide mechanism of action for the treatment tested

[3] post = treatments after soybean emergence; pre = treatments before soybean amergence

Table 3. *Euphorbia heterophylla* control (%) at 80 DAP with treatments applied before and after soybean emergence.

Treatments before soybean emergence	Treatments after soybean emergence Fomesafen	 Imazethapyr	 Fom+Imaz/Fom[1]
0 MOA[2]	32	36	81
1 MOA	34	37	83
2 MOA	31	50	79
3 MOA	28	54	79
LSD 5% (within post[3])	----------------	6	----------------
LSD 5% (within pre[3])	----------------	ns	----------------

[1] Fomesafen + imazethapyr at 22 DAP + fomesafen at 37 DAP

[2] Number of herbicide mechanism of action for the treatment tested

[3] post = treatments after soybean emergence; pre = treatments before soybean amergence

E. heterophylla density

Average *E. heterophylla* density in weedy treatment was 71 plants/m^2 and in the paraquat treatment was 178 plants/m^2. No interaction was observed between pre- and post-emergence treatments. No differences were observed for weed management strategies pre-emergence (Table 4). When averaged for post-emergence herbicides, pre-emergence treatments reduced *E. heterophylla* by 75%, compared to the paraquat control.

Similar *E. heterophylla* infestation was observed in either post-emergence treatment with only one MOA (Table 4). Plants in plots sprayed with fomesafen alone had regrown from earlier injury, whereas *E. heterophylla* in plots sprayed with imazethapyr alone were growing without apparent injury, suggesting that only plants insensitive to this herbicide escaped from control.

E. heterophylla density was lowest in plots receiving two different post-emergence herbicide treatments (Table 4). By the time of the second application, in these treatments, some weeds had regrown from lateral buds. There were two kind of regrowth patterns observed: plants with fully developing branches, and plants with very small and weak branches. These symptoms suggested that those plants were ALS resistant and ALS susceptible, respectively. There was also some new *E. heterophylla* infestation. In fact, Brecke (1995) reported *E. heterophylla* seeds can germinate in a wide range of soil depths, and under several temperature and moisture conditions. The subsequent application with fomesafen eliminated most of those plants. These results and the symptoms observed on plants in this research suggest that best weed control obtained in post-emergence treatments with two MOA occurred, in part, because of using herbicides with different MOA, and in part due to the latter application.

Correlations between *E. heterophylla* control and density were significant ($P > 0.05$), suggesting visual ratings adequately assessed herbicide efficacy. A higher correlation coefficient was found when the weed count was performed the same day of visual ratings ($r^2 = 0,70$), compared to those performed with latter visual ratings ($r^2 = 0,61$). Visual ratings were more sensitive than weed density for detecting differences between treatments and factors tested (Tables 2 and 3, compared to Table 4). This higher sensitivity may occur, at least in part, because visual impact of weed control depend on a combination of factors, such as: weed number, size, leaf area, and canopy development. Therefore, visual ratings are advantageous because they are precise, reliable, quick to perform, and less expensive.

CONCLUSIONS

The number of herbicides with different mechanisms of action used before crop emergence did not affect *E. heterophylla* control, having a similar effect to mechanical weed control. However, use of herbicides combining two mechanisms of action after soybean emergence resulted in weed control at least 25% better than control observed with the use of only one.

Table 4. *Euphorbia heterophylla* density (plants/m^2)at 45 DAP with treatments applied before and after soybean emergence.

Treatments before	Treatments after soybean emergence		
soybean emergence	Fomesafen	Imazethapyr	Fom+Imaz/Fom[1]
0 MOA[2]	27	68	6
1 MOA	62	60	17
2 MOA	96	30	41
3 MOA	57	38	19
Overall	60	49	21
LSD 5% (within post[3] averages)		23	
LSD 5% (within pre[3])		ns	

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[1] Fomesafen + imazethapyr at 22 DAP + fomesafen at 37 DAP

[2] Number of herbicide mechanism of action for the treatment tested

[3] post = treatments after soybean emergence; pre = treatments before soybean amergence

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REFERENCES

Brecke, B J (1995) Wild poinsettia (*Euphorbia heterophylla*) germination and emergence. Weed Science. **43**, 103-106.

Chemale, V M & Fleck, N G (1982) Avaliação de cultivares de soja (*Glycine max* (L.) Merrill) em competição com *Euphorbia heterophylla* L. sob três densidades e dois períodos de ocorrência. *Planta Daninha*. **2**, 36-45.

Lorenzi, H (1994) *Manual de identificação e controle de plantas daninhas: plantio direto e convencional*. (4 ed.) pp. 146-147. Plantarum, Nova Odessa.

Vidal, R A (1997) *Herbicidas: mecanismos de ação e resistência de plantas*, pp. 19-130. Palotti, Porto Alegre.

Vidal R A; Fleck N G; Theisen G; Neves R; Petry L (1997) Picão-preto e leiteira resistentes aos inibidores de ALS não apresentam resistência cruzada aos herbicidas com diferentes mecanismos de ação. *Resumos do Congresso Brasileiro da Ciência das Plantas Daninhas*. p. 465.

Willard, T S & Griffin, J L (1993) Growth response of wild poinsettia (*Euphorbia heterophylla*) following foliar herbicide applications. *Weed Technology*. **7**, 190-195.

SESSION 5A

APPLICATION AND FORMULATION PARAMETERS: THEIR INFLUENCE ON PRODUCT PERFORMANCE AND SAFETY

Chairman and Session Organiser	MR T ROBINSON *Novartis Crop Protection UK Ltd, Cambridge, UK*
Papers	5A-1 to 5A-4

THE INTERNATIONAL (BCPC) SPRAY CLASSIFICATION SYSTEM INCLUDING A DRIFT POTENTIAL FACTOR

E S E SOUTHCOMBE,
BCPC Applications Committee, c/o AgrEvo UK Limited, Chesterford Park, Saffron Walden, Essex, CB10 1XL, UK

P C H MILLER,
Silsoe Research Institute, Wrest Park, Silsoe, Bedford, MK45 4HS, UK

H GANZELMEIER,
Biologische Bundesanstalt fur Land- und Forstwirtschaft, Messeweg 11/12, D-38104 Braunsweig, Germany

J C VAN DE ZANDE
Instituut voor Milieu- en Agritechniek (IMAG-DLO), Mansholtlaan 10-12, 6700-AA Wageningen, The Netherlands

A MIRALLES
ANPP / CIETAP, c/o Cemagref, 361 rue J F Breton, F-34033 Montpellier, France

A J HEWITT
Stewart Agricultural Research Services Inc., PO Box 509, Macon, MO 63552, USA

ABSTRACT

The system for classifying sprays and atomizers used to apply pesticide products introduced by BCPC in 1985 has been extended. In additon to the existing categories of spray quality, a new set of categories classifying sprays by their drift potential, as measured in comparative wind tunnel tests or comparable procedures, has been introduced. The two components will be combined to provide a more comprehensive means to describe sprays and atomizers. Benefits of the classification system are to allow suppliers and users of pesticide products to match their spraying equipment more closely to the requirements of biological performance and environmental protection. The procedures and protocols used to make the measurements of droplet size spectrums and drift potential will be issued as standards in due course.

INTRODUCTION

The spraying devices used to apply crop protection and other pesticide products employ a variety of nozzles to atomise the spray mixture into a spray of droplets which is then directed towards the target. Instructions given on the labels of crop protection products indicate clearly the dose of product and the volume of spray mixture, usually in water, to be applied to the target. However, information on the nature of the spray to be used is often missing or unclear.

In 1985, the British Crop Protection Council (BCPC) proposed a system for classifying sprays and the nozzles producing them into categories of spray quality. (Doble *et al.*,1985, Southcombe, 1988a,b). The technical basis of this system was simple using analysis of droplet size spectrum compared to a set of standard reference nozzles. Later, BCPC recognised the need to develop the technical basis to include consideration of the drift potential of sprays. To meet the increasing interest in the system from a number of other countries, a meeting of interested parties from many European countries and the USA was held in Rotterdam in October 1994, and agreement was reached on most of the issues related to spray classification.

This paper outlines the new spray classification system that has been proposed by two Working Groups established at Rotterdam and which is now being presented as an international system to all regions and countries interested in adopting a means to advise on, or to control the use of spraying equipment.

OBJECTIVES

Classification of sprays and nozzles serves two main functions, namely:

(i) to define the spray quality most appropriate to the product, pest and target that can be communicated on the product label, and

(ii) 2. to enable the use of sprays likely to be environmentally unacceptable to be avoided.

The original BCPC classification system relied on an analysis of the droplet size spectrum or 'Spray Quality'. Three broad categories covered most of the nozzles commonly found. The terminology used to describe them - 'Fine', 'Medium', 'Coarse', etc. - was deliberately practical to appeal to the end-user.

In developing this new approach to spray classification, we have maintained a practical approach based on a scientific background. We recognised that to evaluate all the causal parameters relating to droplet production, droplet life, transport, impact and off target drift was beyond the resources available to this project. We therefore concentrated our efforts on developing a means to define the effects of these parameters in terms of a spray quality as previously, and with an additional element relating to the potential within a spray for some of its components to be displaced in a wind, which we have termed the Drift Potential factor. This allows a difference to be made between sprays with the same nominal descriptor such as volume median diameter or spray quality category, but of very different widths in their droplet size spectrums. We have also recognised that a practical approach with relevance and appeal to the end-user is needed, and that the following criteria need to be met in a successful classification system :-

- It may need a pragmatic approach where scientific solutions are not easily available.
- It must be acceptable to Regulatory, Health & Safety and other authorities in the countries adopting it and to the suppliers and users of atomizers and spraying equipment.
- It should be flexible and cover all reasonable atomizer and spray types

- The methods and protocols must be standardised and usable by all interested parties.
- It must be presented to the end-user in simple, meaningful and understandable terms when used on labels and in literature.

SPRAY QUALITY

Most atomizers produce a range of droplet sizes by virtue of their atomization process. Some well designed rotary atomizers produce a narrow range; most hydraulic nozzles produce a wider range of sizes. In the original system, to classify a nozzle at a particular pressure, a reference chart was constructed by plotting the droplet spectrums of four Reference Nozzles. These were, with one exception, *characteristic* of the three categories of spray quality (Table 1). The threshold boundaries between the categories were determined by interpolation mid-way between the adjacent characteristic curves. The perceived benefit of this was to allow some latitude in deciding which category to place test nozzles that coincided with category thresholds. The droplet spectrums for test nozzles must be measured with the identical equipment setup and conditions at the same time as the reference nozzles.

In the new system, the same basic principles have been retained except that the Reference Nozzles now define the *thresholds* between categories. This change reflects the opinion of a number of organisations consulted who required a positive demarcation between categories.

Table 1. Reference Nozzles

Category	**Threshold Nozzles**	**Characteristic Nozzles**
'Very Fine' / 'Fine'	F110 / 0.45 / 4.5 (11001)	
'Fine'		F110 / 0.85 / 3.5 (11002)
'Fine' / 'Medium'	F110 / 1.18 / 3.0 (11003)	
'Medium'		F110 / 1.44 / 2.5 (11004)
'Medium' / 'Coarse'	F110 / 1.93 / 2.0 (11006)	
'Coarse'		F110 / 2.58 / 2.0 (11008)
'Coarse' / 'Very Coarse'	F80 / 2.88 / 2.5 (8008)	

Note : Nozzles given in BCPC Nozzle Code - (angle) / (litre/min) / (bar)
followed by typical manufacturers' code

One of the major advantages of this classification scheme is that it facilitates the use and comparison of droplet size data measured using different particle size analysers and sampling techniques. Despite considerable advances in the design of instruments to measure droplet sizes, numbers and velocities, there are still significant differences between the results produced, making direct comparisons and the use of absolute figures difficult.

The Working Groups have devised, in collaboration with other groups studying the measurement of agricultural sprays, a series of protocols covering the most commonly used laser based droplet sizing instruments. In addition, several nozzle manufacturers are supplying sets of standardised reference nozzles, which will be validated by an independent laboratory and lodged at selected organisations in those countries operating the system.

There are some types of atomizers that do not easily fit into a comparison with the hydraulic reference nozzles. The three most important types are rotary, twin-fluid and air-inclusion nozzles. Rotary atomizers normally produce a narrow droplet size spectrum and can therefore be easily classified by a specific parameter such as the volume median diameter.

Nozzles in which air is used as part of the spray formation process often produce droplets containing air inclusions. Twin-fluid types are fed with air under pressure, whilst other design types draw air in using a Venturi principle. Each droplet size analyser treats these droplets differently and they produce results that cannot be used to classify the nozzles. It is known that the actual size is larger than those from an equivalent flat fan nozzle with water, but that the density is lower than for a water droplet. The presence of the air bubbles undoubtedly affects both droplet transport and deposition patterns. It is recognised that the protocols currently available will not be able to effectively categorise the sprays produced by this type of nozzle because they are physically very different from those of the Reference Nozzles. Work will continue to seek ways in which an effective definition of spray quality can be used in such cases.

DRIFT POTENTIAL FACTOR

The recognition that the risk of spray drift was not a function only of the droplet size distribution meant that test methods were needed to establish the risk of drift associated with different nozzles operating on boom sprayers. Wind tunnel tests provide one way in which the risk of drift from given nozzle conditions can be quantified but it is accepted that the use of field measurements and modelling approaches are also valid in determining a relative drift risk factor.

Wind tunnel approaches

Initial studies with a range of single nozzles spraying in a wind tunnel have shown that differences in the risk of drift could be related to measures of the airborne spray profile (Helck *et al.*, In preparation, Helck *et al.*, 1997, Miller *et al.*, 1989) or the spray deposition on the floor of the tunnel downwind of the nozzle (Young, 1991; Miralles & Bogliani, 1993).

A comparative study involving five different research facilities in the UK examined the measurement of airborne drift profiles from a range of nozzle types operating in different wind tunnel configurations and using a number of different sampling methodologies. Results from this work showed relatively good agreement between the quantities of airborne drift measured in the different conditions particularly when these were normalised using results for the original BCPC reference flat fan nozzles (Miller *et al.*, 1993). The agreement was closest for wind speeds in the range 2.0 to 2.5 m/s and was further improved by taking results from tunnels which met defined criteria in terms of the minimum dimensions of the cross-section (Parkin, Wheeler, 1996). An outline test protocol for use when conducting wind tunnel tests to assess the risk of drift from different nozzles systems was proposed as a result of this work (Miller *et al*, 1993).

Subsequent collaborative work at the BBA in Braunsweig, Germany and at Silsoe Research Institute in the UK showed that there were some limitations to this proposed outline protocol. Measured airborne profiles from different nozzle systems, when plotted as airborne spray volumes at different heights, gave characteristic curves that overlapped. (Helck & Herbst, 1997). The reference nozzles were used to define characteristic curves of cumulative airborne spray volume against distance below the nozzle and to define classes of drift risk assessment (Miller *et al*, 1995). However results from a series of tests with different nozzle systems gave characteristics which did not have the same form as those for the reference nozzles. This was particularly the case for spinning discs and some cone nozzles with relatively low initial droplet velocities. The wind speed of 2.0 to 2.5 m/s was shown to be critical with respect to the degree that an air flow would penetrate the spray fan from conventional pressure nozzles of different flow.rate capacities operating with the spray fan at right angles to the air flow. Substantial differences were then observed between the volumes and airborne distribution of spray liquid detrained from single nozzles and from multiple nozzles mounted on a boom because of the change in air flow patterns around and through the spray structure (Miller *et al*, 1995)

It was therefore recognised that any comparative analysis of the airborne spray profiles downwind of a test nozzle in a wind tunnel needed to take account of the total volume and the vertical distribution of airborne spray. Two possible approaches have been identified for use in a standardised protocol, namely:

(i) to make measurements at a distance that is far enough away downwind from the nozzle such that the effects due to spray structure and droplet size distribution within the spray have settled; or

(ii) to make measurements closer to the nozzle and use a comparative method of analysis which accounts for the total airborne spray volume and its vertical distribution.

Method (i) above has advantages in terms of a simplified analysis and a result that can be closely related to the field performance of a nozzle or a boom sprayer. However, it requires a relatively large wind tunnel facility, sampling typically 5 metres from the nozzle and may not adequately address the assessment of drift risk close to the sprayer. Method (ii) can be used with a smaller tunnel system and work is now in progress to finalise the methods by which results from such tests can be used to define a comparative drift potential factor. This again will use the reference nozzles to define the categories for this factor. A comparative scale will be established based on a calculation of the first moment of the airborne drift profile measured at a distance of 2.0 metres downwind of the nozzle. This will be calculated as follows :

$$DPF = \sum V_n \frac{\sum V_n h_n}{\sum V_n} = \sum V_n h_n$$

where V_n is the volume of airborne spray collected at height h_n , and DPF is the drift potential factor. A drift potential factor will then be compared with the equivalent results obtained with the appropriate reference nozzles.

Work at Cemagref, Montpellier France, has involved macroscopic evaluations of the wind effects on sprays emitted by nozzles in the laboratory. The technique is based on the comparison between the liquid distributions obtained on a patternator when the spray is subjected or not to a wind. To measure the wind effect from the liquid distributions, two notions have been considered :

(i) the distribution displacement corresponding to the natural displacement on the soil, and

(ii) the drift which is the water leaving the patternator.

The displacement is calculated from the equation :

$$A = \frac{\sum_{i=1}^{i=N} (i-0.5)\, v_i}{\sum_{i=1}^{i=N} v_i}\, e$$

where "A" represent the displacement (m), "i" the test-tube index, "N" the total number of test-tube, "v_i" the water quantity collected in the test tube (mL/min) and "e" the collector channel wide (0.05 m for the Cemagref patternator).

The total drift is calculated from the equation :

$$D = 100\left(1 - \frac{\sum_{i=1}^{i=N} v_i}{Q_0}\right)$$

where "D" represents the drift (%),"i" the test-tube index, "N" the total number of test-tube, "v_i" the water quantity collected in the test tube (ml/min) and Q_0 the nozzle flow rate at the same pressure.

This methodology has been employed on single nozzle (Miralles, 1992, 1993, 1994) to compare the drift potential of nozzles at different pressures, heights and nozzle orientation to the air stream including flat fan nozzles with the spray perpendicular or parallel to the air flow. Again, comparison of the results obtained with test nozzles with those from the reference nozzles will form the basis for determining the drift potential factor.

Drift models

A number of models exist to predict the movement of spray droplets and hence the risk of drift. Two examples are mentioned here to show how characteristics of the sprays are used in different ways. Future work will need to refine the relationships between such models and the principles of spray quality and drift potential described in this paper. It is likely, however, that the use of models, in conjunction with the appropriate experimental data, will provide an alternative approach for determining the drift potential factor.

The drift model IDEFICS developed at IMAG-DLO, Wageningen in the Netherlands simulates the paths through air of drops starting at the nozzle outlet and calculates downwind deposits on the ground (Holterman, Van de Zande, 1996). The model simulates the spraying process of a conventional boom sprayer in a cross wind, accounting for sprayer related parameters (such as nozzle characterisation), crop height and atmospheric conditions. The simulation method differs from the wind tunnel approach in that the nozzle is placed in a cross wind, yet accounts for a head wind contribution due to driving speed. These simulations are closely linked to single nozzle experiments outdoors where a single nozzle moves on a track in a cross wind situation. These experiments show a much higher reproducibility than drift measurements with a more practical setup using a real sprayer in a real crop. The single nozzle experiments are primarily used for validating the drift models, but they also offer a good perspective to investigate spray drift for nozzle classification.

Working under a co-operative research and development agreement, the Spray Drift Task Force (SDTF), United States Environmental Protection Agency and United States Dept. of Agriculture have developed a computer model, AgDRIFT® for predicting pesticide movements and deposition (Hewitt, 1997). One of the most important of the input parameters is the droplet size spectrum of the spray. An option for describing the emission droplet size spectrum is the use of categories of spray quality. In addition to selection of the droplet size spectrum in terms of spray quality category (for example from a catalogue/ applicator handbook or other source), AgDRIFT® allows data to be input from various sources including measured data and data generated from an empirical atomisation model, DROPKICK®, developed by the SDTF. The physical properties of the tank mix, such as surface tension, density, shear and extensional viscosity can be input to DROPKICK® to produce a model-predicted droplet size spectrum.

The description of droplet size in terms of spray quality category provides an excellent tool for making decisions on application practices. It is likely that increasing numbers of pesticide labels in the USA may describe droplet size requirements for spray applications based on classification schemes. For example, buffer zone recommendations may be based on spray droplet size category and other factors such as spray release height, boom length and meteorological conditions such as wind speed. Based on deposition rate predictions using measured or predicted droplet size data, the label for a particular product might indicate that a nozzle classified as no finer than a specified category should be used to achieve acceptable spray coverage and efficacy and minimal drift potential for specific non-target entities at defined distances downwind of the application area.

TERMINOLOGY

This new classification system has two components - spray quality and drift potential. Spray quality terms are well established and accepted as the series ‘Very Fine’, ‘Fine’, ‘Medium’, ‘Coarse’ and ‘Very Coarse’. It is not expected that the new classification system will significantly change the categories already applied to nozzles at different working pressures.

Drift potential terms will be related to the percentage reduction in drift to a defined reference nozzle. This will be the threshold reference nozzle at the finer boundary of the test nozzle's spray quality category. This will mean that most drift potential terms will refer to a reduction in drift potential for that spray quality category. so avoiding terms such as 'High Drift Potential' which in practice are not acceptable. Some proposed terms, which have not yet been formally adopted, might be :-

Drift reduction %:	< 0	0-25	25-50	50-75	> 75
Drift potential term :	Higher	Normal	Low	Double low	Triple low

It is clear that for any spray quality category there will be a limited number of Drift Potential categories which in practice will be applicable. For example a 'Fine' spray is unlikely to have a 'Double Low' Drift Potental category.

Examples of the use of the two components on a product label for application by ground sprayer are :-

(i) A product which poses no significant threat to neighbouring areas might be :
"Apply as a 'MEDIUM' spray with 'NORMAL' drift potential"

(ii) A product which must not be allowed to drift onto neighbouring areas might be :
"Apply as a 'MEDIUM' spray with 'LOW' drift potential"

It is expected that both these terms will also be incorporated into the performance tables supplied by nozzle manufacturers so that product suppliers, advisors and users can select nozzles or other atomizers that satisfy the requirements for both biological performance and environmental protection.

DISCUSSION

The principle of spray and nozzle classification has been embraced by organisations in a number of countries. An improved and extended system is now proposed which enables classification to be made from two perspectives - spray quality and drift potential. This allows a more accurate and comprehensive way to characterise the spray produced by nozzles and other atomizers, and a more flexible way to indicate desirable or mandatory spray characteristics to the end-user. This might be to give optimum biological performance with adequate environmental protection, or to ensure the highest level of environmental protection, for example where no-spray or buffer zones have to be enforced.

The methods and protocols needed to operate the system are being prepared and are expected to be issued in due course as standards. The sets of reference nozzles are also being manufactured and validated (at the time of preparing this paper) and will be lodged with selected institutions in participating countries.

It will have been noted that no reference has been made to the spray liquid. The Working Groups have agreed that the test fluid will be water, unless the atomizer depends on a modified water or oil for its correct operation. As the effects of product formulations on spray characteristics are complex and often not easily predicted it is not possible to cover all possibilities with a single test fluid.

BCPC and the "Rotterdam" Working Group are also developing a system for classifying the potential hazard of all types of pesticides application techniques and equipment. (Parkin *et al.*, 1994). This is now known as the Pesticide Application Safety Scheme (PASS) and again involves a multi-national collaboration. Spray classification will eventually form a component of the PASS scheme.

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REFERENCES

Doble S J, Matthews G A, Rutherford I, Southcombe E S E (1985). A System for Classifying Hydraulic and Other Atomizers Into Categories of Spray Quality. Proceedings *British Crop Protection Conference - Weeds*, **3**, 1125-1133.

Helck C, Herbst A (1997). Drift Potential Index - Eine Neue Kennzahl zur Beurteilung von Pflanzenschutzduesen Hinsichtlich Ihres Abtriftpotentials. To be published in Nachrichtenblatt des Deutschen Pflanzenschutzdienstes, Stuttgart

Helck C, Herbst A, Ganzelmeier H (1997). New approaches to determine drift potential for nozzles. *Spray and nozzle classification: meeting of the reference nozzle and driftability Working Groups* – Silsoe April 1997 (unpublished*)*

Helck C, Herbst A, Ganzelmeier, H (In preparation) Drift potential index – a new parameter to characterise the drift risk from agricultural nozzles. To be submitted to *The Journal of Agricultural Engineering Research*

Hewitt A J (1997). Droplet Size and Agricultural Spraying, Part 1. *Atomization & Sprays,* **7**, (3), 253-244

Holterman H J, van de Zande J C (1996). Drift Reduction in Crop Protection: Evaluation of Technical Measures Using a Drift Model. Proceedings *British Crop Protection Conference - Pests & Diseases.* 111-116

Miller P C H, Hislop E C, Parkin C S, Gilbert A J. (1993). The Classification of Spray Generator Performance Based on Wind Tunnel Assessment of Spray Drift. In Proc. *ANPP/BCPC Second International Symposium on Pesticide Application,* Strasbourg.

Miller P C H, Mawer C J, Merritt C R (1989). Wind Tunnel Studies of the Spray Drift from Two Types of Agricultural Spray Nozzle. *Aspects of Applied Biology.* **21.** 237 - 238

Miller P C H, Smith R W, Tuck C R, Walklate P J. (1995). The Classification of Agricultural Sprays Based on Droplet Size Distributions and the Results from Wind Tunnel Tests. Proc.*British Crop Protection Conference - Weeds,* 1125-1134.

Miralles A, (1992). Simulacio de Deriva en Barres de Polvoritzacio: Documenyt de Cours Réalisé pour la Formation Continue Organisée par La Estacion Maquinaria Agricola (Lerida). 18p.

Miralles A, Bogliani M (1993) Evaluation Macrooscopique du Vent sur un Jet de Pulverisation. In Proceedings *ANPP/BCPC Second International Symposium on Pesticide Application,* Strasbourg. **1,** 117 – 124.

Miralles A, Boisgontier D, Bouvier J L (1994). Comparison de Dérive de Différents Produits Phytosanitaires. Rapports confidential No.46, 130 p.

Parkin C S, Gilbert A J, Southcombe E S E, Marshall C J (1994). The British Crop Protection Council Scheme for the Classification of Pesticide Application Equipment by Hazard, *Crop Protection,* **13** (4), 281-285

Parkin C S, Wheeler P N. (1996). Influence of Spray Induced Vortices on the Movement of Drops in Wind Tunnels. *Journal of Agricultural Engineering Research* **63,** 35-44

Southcombe E S E, (1988a). The BCPC Nozzle Selection System. Proceedings *International Symposium on Pesticide Application*, Paris. 71-78.

Southcombe E S E, (1988b). The Coding and Selection of Agricultural Spraying Nozzles. Proceedings US Agricultural Research Institute Workshop on *Improving On-target Placement of Pesticides*, Reston, VA, USA.

Young B W, (1991). A Method for Assessing the Drift Potential of Hydraulic Nozzle Spray Clouds, and the Effect of Air Assistance. In *Air-Assisted Spraying in Crop Protection.* BCPC Monograph No.46 (eds. Lavers, Herrington, Southcombe) 77 - 86.

THE INFLUENCE OF SPRAY DISTRIBUTION AND DROP SIZE, ON THE DOSE RESPONSE OF HERBICIDES

P ENFÄLT, A ENQVIST, P BENGTSSON
Department of Agricultural Engineering, SLU, PO Box 7033, S-750 07 Uppsala, SWEDEN

K ALNESS
Swedish Farmers Supply & Crop Marketing - SLR, S:t Göransgatan 160 A, Box 30192, S-104 25 Stockholm, SWEDEN

ABSTRACT

The shape of the dose response curve is influenced by many parameters, both biological and technical. The uniformity of spray distribution and droplet sizes used for application are examples of technical parameters. How great the influences are, depends on the chemical used and the prevailing circumstances. These factors influence the shape of the dose response. A steep dose response curve is more sensitive to variations than a more flat response. An uneven spray distribution tends to flatten the dose response and a higher dose is needed to obtain full effect of the treatment. Under perfect weather conditions larger droplets will give a flatter dose response than the small ones do.

INTRODUCTION

The aim with crop protection is to achieve a good effect at low cost, without a negative influence on the environment. Optimising the usage of pesticides is important as doses both too low and too high give environmental and economical disadvantages. It is essential to know how different parameters influence the dose response when optimising treatment and application methods. An example of adaptation through dose response is in precision farming. To be able to do such adaptation one has to know the prevailing circumstances, biological as well as technical ones, and how they will influence the result of the treatment. The biological ones that have been studied include. chemical substance, crop variety and different meteorological parameters.

From the technical point the spray problems are related to spray distribution uniformity, droplet sizes and drift etc. Regarding non uniform spray distribution it can be caused by boom movements, wear of nozzles, driving speed and wind etc. Spray distribution can be described in two ways stationary and dynamic. The dynamic spray distribution is measured under a moving boom and the stationary one measured on a patternator, the dynamic distribution is influenced by air (Young 1990) and boom movements (Langenakens & Ramon 1993). In this paper the spray distribution refers to dynamic distribution. The droplet size is linked to the problem of drift, evaporation and coverage etc.

This paper will present results from field trials and modelling work that describes the influence of spray distribution uniformity, droplet sizes and time of application on the dose response behaviour including different shapes of the dose response curve.

MATERIAL AND METHODS

The effect on the dose response of an uneven spray distribution will be explained with a model. Measured data of dose response and spray distributions will be used with this model to show the effect of an irregular spray distribution. The influence of droplet sizes on the dose response will be shown with results from field experiments.

Field trials with linear change of dose

All field trials described in this paper are made with the dose linearly changed in the field trial plot (Alness et. al. 1995). With this method the doses are changed while driving in the trial plot, instead of using several plots with a fixed dose in each plot. Figure 1 shows a sketch of a plot for field trials with linear change of dose. By using this experimental technique the whole dose response behaviour can be studied in one single plot. This reduces the influence of the variations in the field on the results, through smaller areas being used. Another advantage is that the doses used, do not have to be fixed before the grading of the trials are made i.e. it will be more easy to find the interesting part of the dose response though any dose can be chosen at grading time. This can be a problem when one fixed dose per plot is used. In these kind of experiments the dose has to be fixed before spraying when we do not know exactly how the chemical will work. The system used for linearly changed doses includes a injection system, a boom with the same reaction time to all nozzles and a computer. The computer will control the injection and also collect data as speed, flow rate and position in the plot while driving, this information will then be used to find the exact dose in the plot and small errors can be corrected afterwards.

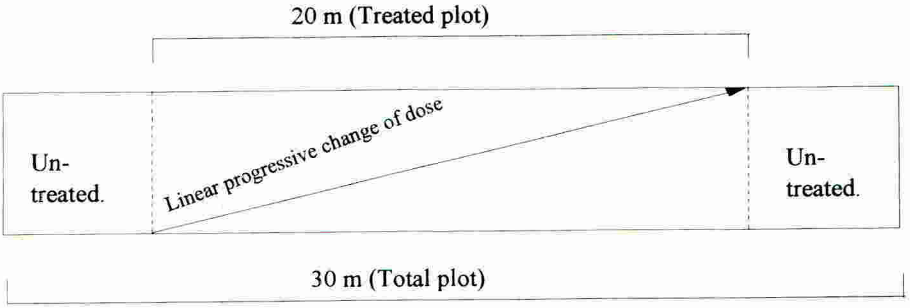


Figure 1. A schematic sketch of a field trial plot for linearly change of the dose

In the experiments the liquid rate was 200 l/ha and two chemicals have been used 1. Express 50 (tribenuronmetyl 500g/kg DuPont) + Starane 180 (fluroxipyr 180g/l DowElanco) with a maximum dose of 3.75 g tribenuronmetyl and 90 g fluroxipyr per ha. 2. Ariane S (MCPA 200g/l, klopyralid 20g/l, fluroxipyr 40g/l DowElanco) with a maximum dose of 350 g MCPA /ha, 35 g klopyralid /ha and 70 g fluroxipyr /ha. The driving speed was 3.6 km/h and the wind during the application was negligible. Three different nozzles were used TeeJet® 110015 XR VS at 3 bar pressure giving a (volume mean diameter) VMD of 200 µm, TeeJet® 11002 XR VS at 1.8 bar pressure giving a VMD of 240 µm and TeeJet® 11002 TT VP at 2.0 bar pressure giving a VMD of 395 µm.

The experiments were assessed 30 days after spraying, by examining the wet weight of the weeds. This were done at ten places in each trial plot including two places in the untreated area.

Calculations

The experimental data from the field experiments is described with a four parameter logistic function (1) (Ratkowsky 1990), which has been fitted to the data using the function *fmins* in MATLAB®.

$$g(x) = A + B - \left(\frac{A - B}{1 + \left(\frac{x}{c} \right)^{d}} \right) \qquad (1)$$

where:

g(x)	effect of dose x
A	maximum effect
B	minimum effect

c describes the inflexion point and *d* the slope of the curve around *c*. $c>0$

To calculate the mean effect of a single dose when affected by an uneven spray distribution, the effect of the herbicide/pesticide at different doses, i.e. the ideal dose response, and the distribution of the spray liquid needs to be known. The effect of the treatment in the dose response relationship can be expressed in different terms, depending of the aim of the study. It can for instance be a percentage of weed control, absolute or relative increase in the harvest yield, or percentage of healthy plants resulting and so on. Spray distribution can denote either the liquids distribution on a surface, or at different heights in a plant stand. The calculations can be made with discrete variables or continuously varying functions. Knowing the frequency at which the doses are distributed at, the different dose intervals and also the effects of these doses, the mean effect of a treatment can then be described mathematically as equation (2) (Hoel 1994).

$$\overline{E}_m = \sum_{k=1}^{n} f_k e_k \qquad (2)$$

$\overline{E}_m$	effect of dose m
f_k	frequency of dose k
e_k	biological effect of the non-distributed dose k

where: $$\sum_{k=1}^{n} f_k = 1 \qquad (3)$$

In the continuous case mathematical functions are used for both the non distributed dose response function and the density distribution function, and integration is used instead of summation.

$$E(m) = \int_0^{\infty} f(m,x)g(x)dx \qquad (4)$$

E(m) effect of dose m

$f(m,x)$ density function for the distribution with mean (m)

$g(x)$ dose-response function

where: $$\int_0^\infty f(m,x)dx = 1 \qquad (5)$$

The mean effect will hereby not necessarily be equal to the effect of the mean dose. By calculating the mean effect of several doses, a new dose-response relationship is obtained.

Spray distribution patterns

For the modelling work three dynamical spray distributions collected from a test where 24 sprayers were tested at farm level (Enfält et. al. 1997) are used. These are shown in figure (2). The coefficient of variation, CV for the three dynamic spray distributions was, 6, 30 and 66 %.

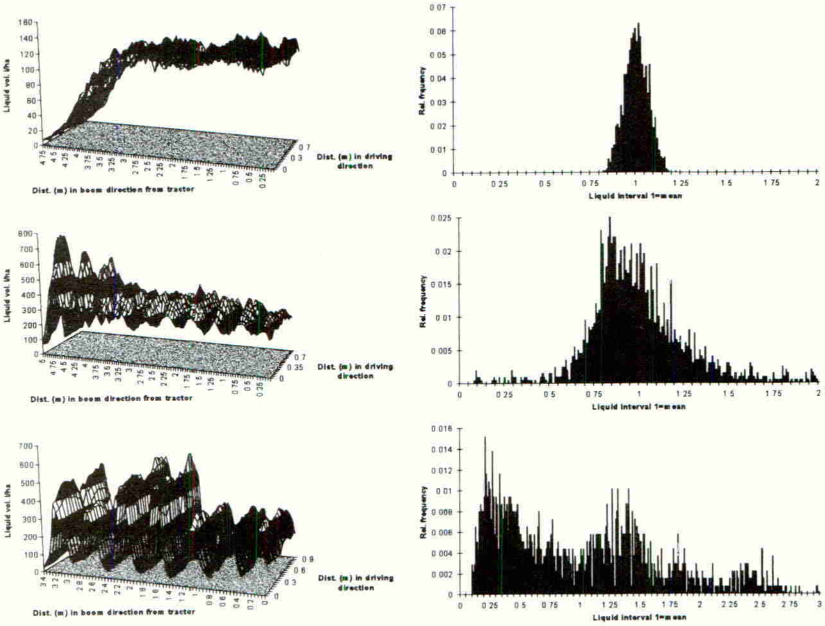


Figure 2. The spray distribution achieved with three different sprayers (Enfält et. al. 1997). From the top, CV 6%, CV 30% and CV 66%. On the right side, the density distribution of the liquid volumes of the three spray liquid distributions.

For the continuous calculations using eqn. (4) a log normal distribution function (6) has been used to describe the density distribution data. This distribution has been chosen though its low level is zero, which is not the case with the normal distribution function.

$$f(x,\sigma,\mu) = \frac{1}{\left(x\varepsilon\sqrt{2\pi}\right)} * e^{\frac{-(\ln x-\lambda)^2}{2\varepsilon^2}} \qquad (6)$$

where: $f(x) =$ frequency of dose x

$$\alpha = \ln\mu - \frac{1}{2}\ln\left(\frac{\sigma^2 - \mu^2}{\mu^2}\right) \text{ and } \beta = \sqrt{\ln\left(\frac{\sigma^2 - \mu^2}{\mu^2}\right)}$$

α and β are dummy variables, μ (mean value), σ^2 (variance)

To calculate a certain log normal density distribution the CV value have been used from the measured data, though CV includes μ and σ.

RESULTS

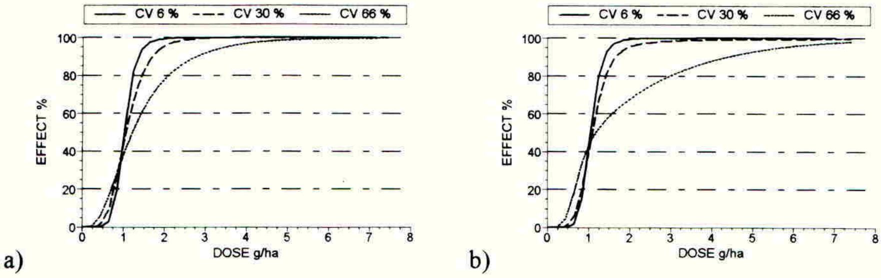
The results from the regression of the field experimental data to equation (1) are shown in table (1). Though all weed weights are related to the untreated area the A and B variable in the equation becomes 100 respectively 0.

Table 1. Values for the parameters in eqn (1) describing the dose response when fitted to experimental data

		Parameters in eqn (1)			
Chemical	**Droplet size VMD (µm)**	**A**	**B**	**c**	**d**
MCPA + klopyralid + fluroxipyr	200	100	0	0.3456	2.6452
	240	100	0	0.3019	1.7086
	395	100	0	0.3429	1.7697
tribenuronmetyl + fluroxipyr	200	100	0	0.9587	8.1257
	240	100	0	0.8257	4.0729
	395	100	0	0.7096	1.1657

Spray distribution uniformity

Calculations have been made using eqn. (3) and the three spray distributions in figure (1) both with the measured density distribution and the log normal distribution function using the CV values. The dose responses for the two chemicals, sprayed with the droplet size 200 µm, have been used as a base for the calculations. The results are shown in figure (3) as new dose response relationships and table (2) shows the doses needed to obtain specific responses.



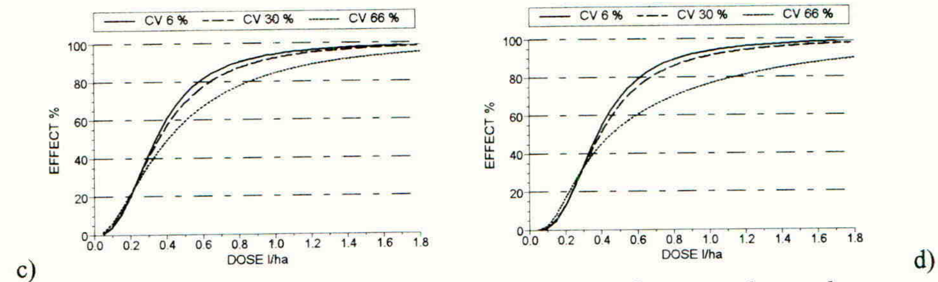


Figure 3. Relationship between dose and average biological response for weed control with tribenuronmetyl + fluroxipyr (a & b) and weed control with MCPA + klopyralid + fluroxipyr (c & d), situation using the discrete calculations (b & d) and continuous calculations (a & c) and the three different spray liquid distributions (CV 6%, 30% & 66%).

Table 2. Results of the calculated doses needed to obtain four different effects for different qualities of spray liquid distribution for both chemicals used in weed control, calculated with the discrete formula and the continuous way of calculation, compared to the optimal dose-response.

Treatment	**Recommended dose (Product)**	**CV, %**	**Effect, %**	**Dose needed** **Optimal CV = 0%**	**Discrete values**	**Continuous function**
MCPA + klopyralid + fluroxipyr	1.75 l/ha	6	75	0.52	0.52	0.53
			85	0.66	0.66	0.67
			95	1.05	1.05	1.06
			98	1.50	1.50	1.52
		30	75	0.52	0.56	0.58
			85	0.66	0.74	0.75
			95	1.05	1.25	1.21
			98	1.50	1.94	1.75
		66	75	0.52	0.91	0.75
			85	0.66	1.38	1.04
			95	1.05	2.64	1.85
			98	1.50	4.04	2.82
tribenuronmetyl + fluroxipyr	7.5 g/ha	6	75	1.16	1.16	1.17
			85	1.25	1.26	1.27
			95	1.46	1.47	1.48
			98	1.64	1.65	1.66
		30	75	1.16	1.30	1.36
			85	1.25	1.46	1.55
			95	1.46	1.89	1.94
			98	1.64	2.59	2.27
		66	75	1.16	2.47	1.88
			85	1.25	3.52	2.37
			95	1.46	5.61	3.51
			98	1.64	7.34	4.60

Influence of droplet sizes

In figure (4) the dose response relationships are shown for the two chemicals and the different droplet size spectrum used. Table (3) contains the dose needed to obtain a specific response of a treatment.

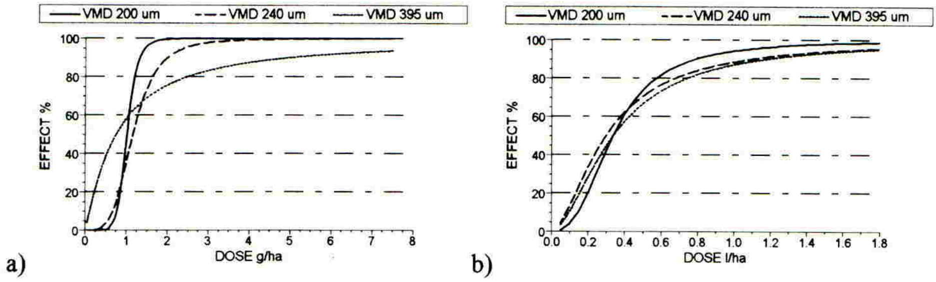


Figure 4. Relationship between dose and average biological response for weed control with tribenuronmetyl + fluroxipyr (a) and weed control with MCPA + klopyralid + fluroxipyr (b), when using different dròplet sizes, nozzles and pressures. Each line represent different droplet sizes used.

Table 3. Comparison of the dose needed to obtain specific responses in the droplet size trial.

Treatment	**Recommended dose (Product)**	**Droplet size (µm)**	**Effect %**	**Dose needed**
				(l/ha)
MCPA +	1.75 l/ha	200	95	1.05
klopyralid +			85	0.66
fluroxipyr		240	95	1.69
			85	0.83
		395	95	1.81
			85	0.91
				(g/ha)
tribenuronmetyl	7.5 g/ha	200	95	1.38
+ fluroxipyr			85	1.19
		240	95	1.7
			85	1.26
		395	95	8.90
			85	3.14

DISCUSSION

These experiments and calculated examples show that the droplet size and the uniformity of the spray liquid distribution will influence the dose response characteristics when spraying agrochemicals. How great the influence will be depends on the chemical used but also on the prevailing circumstances at spraying time. Notice the similarities between the shape of the curves when using different droplet sizes and when affected by an irregular spray distribution. When using smaller droplets the effect of the wind has to be considered. At high wind speeds the final effect may be more influenced by the irregular spray distribution caused by the wind than on the droplet size and the advantage of using small droplets can be lost. The results described in this paper also show that a sharp dose response is more sensitive to irregularities than a more flat one. But in the case where there is a sharp dose response, the dose can be reduced without losing effect if correctly sprayed, but at a certain dose all effect can be lost. With a more flat dose response the effect decreases when trying to reduce the dose, but in the other hand no drastic reductions of the effect will occur.

By using the measured density distribution of the dynamical spray distribution and calculating a new response curve more information about the spray distribution quality is given instead of only the CV value. This way of calculating may be valid not only for weed control, but also for fungi treatments.

When dealing with dose response curves all kind of variations in the experiments, biological as well as technical ones tend to level out the shape of the dose response curve. This was also found by (Ridout & Fenlon 1991). One error that probably occurs frequently, is that the speed or flow rate will vary. This will, if not noticed translate the dose response curve on the dose axis, and if many trial plots, some applied with too high and some with too low a speed or spray volume, are used to define a dose response, the result will be a more flat curve. Therefore it is important when making field trials to log these parameters during the application, to give a good control of the actual result.

At the end it is the biological effect of the treatment that counts and new techniques should be evaluated with biological trials as well as the more technical ones such as distribution, coverage and deposit etc. The whole dose response relationship should be studied and compared in these tests. So when biological field experiments are carried out to screen new chemical products or find the different relationships between the different parameters mentioned above, the result will be influenced by distribution, uniformity and the droplet size distribution used. The technique with linearly changed doses in the field trial plot has shown to be well suited for this purpose.

REFERENCES

Alness, K., Bengtsson, P., Engqvist, A. & Enfält, P. A field experimental system for developing methodology for selective plant protection. Proceedings, Tenth International Conference on Mechanization of Field Experiments (IAMFEE/FRANCE '96), Paris/Versailles, France 137-144, 1996

Enfält, P., Engqvist, A. & Alness, K. Assesment of the dynamic spray distribution on a flat surface using image analysis. Aspects of Applied Biology 48, Optimising pesticide applications. 1997, 17-24

Hoel, P. G. Introduction to Mathematical Statistics. Wiley & Sons, New York, USA, 1984.

Langenakens, J. J. & Ramon, H. The effect of tyre pressure on the dynamic behaviour of sprayer booms. Proceedings Second International Symposium on Pesticides Application Techniques, 1993

Ratkowsky, D. A. Handbook of Nonlinear Regression Models. New York, USA, 1990.

Ridout, MS. & Fenlon, JS. Analysing dose-mortality data when doses are subject to error. Annals of Plant Physiology 1991, 119, 191-201

Young, B W. Droplet dynamics in hydraulic nozzle spray clouds. In: Pesticide Formulations and Application Systems: 10th Volume ASTM STP 1078, American Society for Testing and Materials, Philadelphia, 1990.

FATTY ACID DERIVATIVES AS CO-FORMULANTS FOR HERBICIDES

P LANGLOIS, C GAUVRIT
Laboratoire de Phytopharmacie, INRA, BV 1540, F-21034 Dijon Cedex, France

Z MOULOUNGUI
Laboratoire de Chimie Agro-Industrielle, UA INRA/INPT 31A1010, Ecole Nationale Supérieure de Chimie de Toulouse, 118, route de Narbonne, F-31077 Toulouse Cedex, France

F DARCHY
Formulation, Rhône Poulenc Agro, 14/20 rue Pierre Baizet, F-69009 Lyon, France

ABSTRACT

Fatty acid derivatives were assessed as co-formulants for herbicides. The series studied were N-propylene amides of C10 to C18 fatty acids ; ethers of oleic and stearic acids ; tetraoxyethylene esters of oleic and undecylenic acids ; 1-glyceryl esters of heptanoic, undecylenic, oleic, linoleic and erucic acids. The ethers enhanced the foliar penetration of phenmedipham but the amides had a low or nil influence. In the tetraoxyethylene and 1-glyceryl series, the most lipophilic derivatives promoted the foliar penetration of phenmedipham to the greatest extent. This was particularly clear-cut for monoglycerides. In the latter case, the requirement for lipophilicity was less stringent for 2,4-D. Tetraoxyethylene undecylenate and glyceryl undecylenate exhibited surfactant properties in both static and dynamic tests. Biological assays were done with quizalofop-P-ethyl formulated with methyl oleate and tetraoxyethylene undecylenate and showed that the biological efficacy of the latter formulation could compare with a commercial formulation.

INTRODUCTION

Increasing concerns about the environmental impact of some adjuvants have prompted research for more benign replacements. An example is the use of esterified vegetable oils instead of mineral oils as adjuvants for specific graminicides. The same trend is followed for adjuvants in formulations of crop protection chemicals, although it is less documented for confidentiality reasons. For example, alkyl polyglucosides were assessed as spray deposition agents in this view (Hoyle & Holloway, 1996). Fatty acid esters are finding increasing industrial applications because of their environmental innocuity, their biodegradability (Cornish *et al.*, 1993), the absence of irritating and toxic properties (Bogaerts, 1991), and in some cases their chemical reactivity and their sophisticated chemical structure.

We previously described eco-compatible methods (without solvents or polluting effluents) for the synthesis of 1-monoglycerides and tetraoxyethylene esters of fatty acids (Mouloungui & Gauvrit, 1977). The first objective of the present study was to assess as adjuvants the latter series and two new series based on amide and ether functions. The compounds were examined as enhancers of herbicide penetration, surface-active agents and solvents. The second objective was to select the most promising compounds to devise a herbicide formulation and to compare it with a commercial counterpart. In the series studied, the fatty acids were oleic, linoleic and erucic acids which are abundant for example in rapeseed, sunflower and *Crambe abyssinica* Hochst ex Fries oils, respectively. We also used heptanoic and undecylenic acids which are produced during the cracking of castor bean oil.

MATERIALS AND METHODS

Fatty acids derivatives belonged to the following series :

Amides **:** R-CO-NH-CH_2-CH=CH_2

	R
AC 10:0	C_8H_{16}-COOH
AC 11:1	C_8H_{16}-CH=CH_2
AC 12:0	$C_{11}H_{23}$
AC 14:0	$C_{13}H_{27}$
AC 16:0	$C_{15}H_{31}$
AC 18:0	$C_{17}H_{35}$
AC 18:1	C_7H_{14}-CH=CH-C_8H_{17}

Ethers : R_1-O-R_2

	R_1	R_2
THE1	$C_{16}H_{33}$	C_4H_9
THE3	$C_{18}H_{37}$	(2-C_2H_5)-C_6H_{13}
THE4	C_7H_{14}-CH=CH-C_8H_{17}	(2-C_2H_5)-C_6H_{13}
THE5	C_7H_{14}-CH=CH-C_8H_{17}	C_8H_{17}

TFE1 and TFE2 were fusel ethers with 7 and 9 carbon atoms, respectively.

Monoglycerides : CH_2OH-CHOH-CH_2-O-CO-R

	R
MGC 7:0	C_6H_{13}
MGC 11:1	C_8H_{16}-CH=CH_2
MGC 18:1	C_7H_{14}-CH=CH-C_8H_{17}
MGC 18:2	C_7H_{14}-CH=CH-CH_2-CH=CH-C_5H_{13}
MGC 22:1	$C_{11}H_{22}$-CH=CH-C_8H_{17}

Tetraoxyethylene esters : R_1-(O-CH2-CH2)$_4$-O-CO-R_2

	R_1	R_2
MUTEG	H	C_8H_{16}-CH=CH_2
DUTEG	R_2-CO	C_8H_{16}-CH=CH_2
MOTEG	H	C_7H_{14}-CH=CH-C_8H_{17}
DOTEG	R_2-CO	C_7H_{14}-CH=CH-C_8H_{17}

The synthesis procedures were described in Mouloungui & Gauvrit (1997). The compounds were > 97 % pure, the tetraoxyethylene derivatives were monodisperse.

[^{14}C] phenmedipham (648 MBq mmol^{-1}, AgrEvo, Germany), and [^{14}C] 2,4-D (2.07 GBq mmole^{-1}, Amersham, UK) were uniformly labelled on the phenyl ring. Their radiochemical purity was higher than 98 %. Pilot® is a commercial EC containing 50 g L^{-1} quizalofop-P-ethyl. It will be hereafter referred to as Formulation A. Formulation B contained 51.65 g L^{-1} quizalofop-P-ethyl, 105.0 g L^{-1} MUTEG, 45.0 g L^{-1} Rhodacal 70 (calcium dodecylbenzene sulfonate) and 698.35 g L^{-1} methylated rapeseed oil. Rhodacal 70 was omitted from Formulation C which contained 60 g L^{-1} quizalofop-P-ethyl, 122 g L^{-1} MUTEG and 816 g L^{-1} methylated rapeseed oil.

Uptake experiments on barley were done with 4 replicates when the plants were at the 1-2 leaf stage. Barley (cv. Plaisant) were grown under controlled conditions in vermiculite at 23/19 °C (light/dark), 16 h photoperiod (fluorescent light delivering 220 µE m^{-2} s^{-1} P.A.R.), 70 (±10) % relative humidity. Twenty *c.* 0.2-µL droplets of ^{14}C-labelled phenmedipham or 2,4-D (10 mM in acetone-water (19:1), *c.* 170 Bq µL^{-1}) were deposited with a microsyringe onto the upper third of the adaxial surface of the first leaf. When present, fatty acid derivatives were also 10 mM. The plants were then returned to the same conditions as during their growth. Penetration was determined 0, 6, 24 and 72 h after treatment and evaluated by washing the treated area of each leaf with 0.5 mL acetone. The radioactivity in surface washes was determined by liquid scintillation counting (l.s.c.). The solvent-washed treated leaf and the rest of the aerial parts were then combusted in oxygen for radioactivity assessment as $[^{14}C]CO_2$. This amount of radioactivity was considered to have penetrated. Radioactivity found in the roots was negligible (data not shown). Radioactivity recovery was > 90 %.

Static surface tension was measured by the method of de Nouy (tensiometer Krüss, model K12). Dynamic surface tension was measured by the bubble pressure method (tensiometer Krüss, model BP12). Wheat (cv. Darius) was grown in a greenhouse. Contact angles (θ) were determined by measuring the basis (Δ) and the height (h) of 1-µL droplets deposited on the adaxial surface of the first leaf of wheat plants ; $\theta = 2 \text{ Arctan } (2 h/\Delta)$ (Foy & Smith, 1964). Spray retention was determined after adding 5 g L^{-1} of the dye Eurogran Carmoisine® (Warner Jenkinson, France) to the preparation under study. It was sprayed (250 L ha^{-1}) on four pots containing ten wheat plants. After the deposits had dried the shoots were washed with a known volume of water and the dye concentration was determined by spectroscopy at 515 nm. This enabled us to calculate the amount of spray liquid retained on wheat shoots. It was checked that 5 g L^{-1} of the dye did not influence the surface tension of water.

Biological assays were done on barley plants grown under controlled conditions in vermiculite at 20/10 °C (light/dark), 16 h photoperiod (fluorescent light delivering 280 µE m^{-2} s^{-1} P.A.R.), 70/80 (±10) % relative humidity. Eight plants were sown per pot and were thinned to five on the eve of the treatment. They were treated (five doses) when the second leaf was 2-4 cm long. Treatment was done by application of a single 1-µL droplet at the lower third on the adaxial surface of the first leaf. Fifteen plants (three pots) were treated for each dose of each formulation. Plants were harvested 14 days after treatment and dried for 24 h at 60 °C before dry weight determination of the plants harvested in each pot. The dose-response curves were fitted by non-linear regression with the assumption that at zero and infinite doses biomass was equal for the different treatments. The experiment was repeated once over time and yielded comparable results.

RESULTS

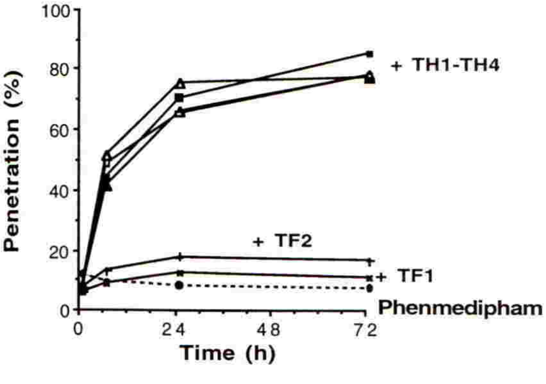


Figure 1. Penetration of phenmedipham into barley leaves as affected by ether derivatives. Penetration is expressed as % of radiolabel applied. SEM = 2.4 (56 d.f.).

The penetration of phenmedipham and 2,4-D without fatty acid derivatives present into barley leaves was low : after 72 h, less than 10 % of the applied label was absorbed (Figs. 1-3).

Penetration increased in the presence of some of the fatty acid derivatives tested. All ethers of the TH series increased phenmedipham penetration, to 40-50 % after 6 h, to 80-90 % after 72 h (Fig. 1). No significative difference was found between them. On the contrary, fusel ethers (TF1 and TF2) had a limited influence (less than 20 % penetration after 72 h). The same was true for compounds of the amide series. The highest penetration figure for phenmedipham was obtained in the presence of AC11:1 and did not exceed 30 % after 72 h (data not shown). With the other amides phenmedipham penetration was not affected.

Monoglycerides increased the foliar penetration of 2,4-D, to the highest extent in the cases of MGC18:1, MGC18:2 and MGC22:1, and to the lowest in the case of MGC7:0, MGC11:1 being intermediate (Fig. 2A). The influence of MGC18:1, MGC18:2 and MGC22:1 was comparable to that of methyl oleate. MGC18:1, MGC18:2 and MGC22:1 enhanced the foliar penetration of phenmedipham, although to a lower extent than methyl oleate (Fig. 2B). The influence of MGC11:1 was limited and that of MGC7:0 was nil.

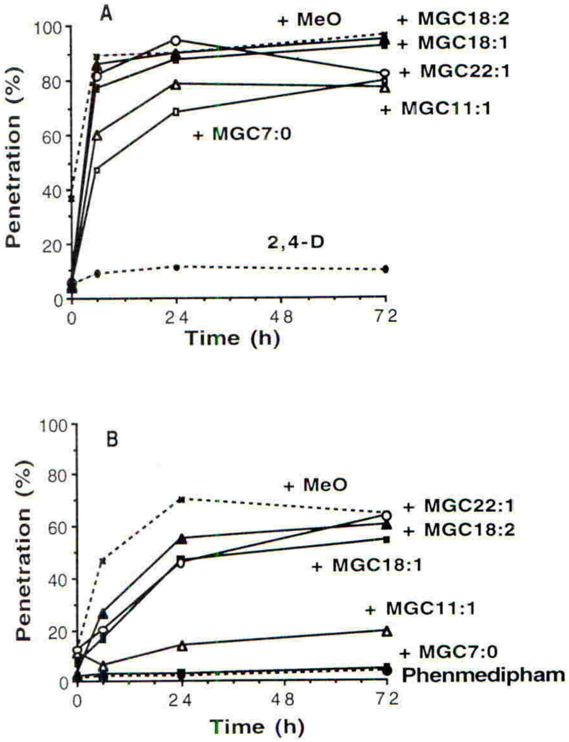


Figure 2. Penetration of 2,4-D (A) and phenmedipham (B) into barley leaves as affected by monoglycerides. MeO = methyl oleate. Penetration is expressed as % of radiolabel applied. SEM = 2.9 (A) and 3.6 (B) (56 d.f.).

The tetraoxyethylene derivatives also enhanced phenmedipham penetration. MOTEG, DOTEG and DUTEG had a similar influence (around 80 % penetration after 24 h), whereas MUTEG induced lower penetration rates of phenmedipham (50 and 58 % after 24 and 72 h, respectively) (Fig. 3). The same observation was made with 2,4-D since its foliar penetration in the presence of MOTEG, DOTEG and DUTEG was around 90 % after 6 h, as compared to 75 % in the presence of MUTEG (data not shown). The differences were reduced but still significant after 24 and 72 h.

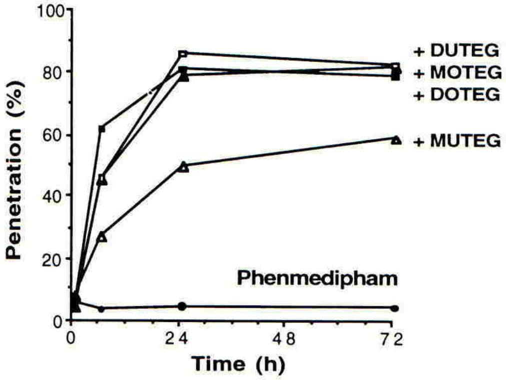


Figure 3. Penetration of phenmedipham into barley leaves as affected by tetraoxyethylene derivatives. Penetration is expressed as % of radiolabel applied. SEM = 2.1 (40 d.f.).

The tetraoxyethylene derivatives and the monoglycerides exhibited surface properties. When present at 1 g L^{-1}, they decreased the static surface tension of water (read at 0 Hz) to values around 30 mN m^{-1} (Table 1). However, most of them had a limited influence on dynamic surface tension (read at 2-10 Hz) except MGC11:1 and MUTEG, which lowered it to 55 and 45 mN m^{-1} at 10 Hz, respectively (Table 1). These values were similar to those brought about by Silwet L77 (organosilicone) and Soprophor 860 (branched 1-tridecanol hexaethoxylate), respectively.

Table 1. Surface tension of water (mN m^{-1}) as affected by tetraoxyethylene derivatives and monoglycerides (1 g L^{-1}) as a function of bubble frequency.

Frequency (Hz)	0	2	5	10
Compound				
MUTEG	30.0	33	36	45
MOTEG	30.3	67	71	70
DUTEG	29.5	47	72	71
DOTEG	29.9	56	73	72
MGC7:0	31.8	58	60	61
MGC11:1	26.1	34	41	55
MGC18:1	31.6	68	68	70
MGC18:2	28.0	73	73	73
MGC22:1	32.2	73	73	73
Silwet L77	20.9	34	52	59
Soprophor 860	26.3	31	36	45

Contact angles of water droplets on wheat leaves were decreased by the tetraoxyethylene derivatives and the monoglycerides (Table 2). Like Silwet L77 and Soprophor 860, MGC11:1 gave contact angles lower than 10 °. MUTEG was more effective than MOTEG, DOTEG and DUTEG in decreasing contact angles.

Table 2. Contact angles of water droplets on wheat leaves as affected by tetraoxyethylene derivatives and monoglycerides (1 g L^{-1}).

	Contact angle (°)	CI
MUTEG	21	3
MOTEG	54	10
DUTEG	41	19
DOTEG	52	8
MGC7:0	64	3
MGC11:1	< 10	nd
MGC18:1	42	18
MGC18:2	50	10
MGC22:1	63	5
Silwet L77	< 10	nd
Soprophor 860	< 10	nd

CI = confidence interval at $P = 0.05$ probability level. nd, not determined.

Retention of water sprays on wheat leaves was also affected by MGC11:1 and MUTEG. Under our experimental conditions, spray retention without fatty acid derivatives present was around 45 µl g^{-1} dry weight. Addition of 1 g L^{-1} monoglycerides or DUTEG and MOTEG in water increased it to values ranging from 55 to 110 µL g^{-1} dry weight (Fig. 4). In the presence of MUTEG spray retention reached 250 µL g^{-1} dry weight, compared to 180 and 200 µL g^{-1} dry weight for Silwet L77 and Soprophor 860, respectively.

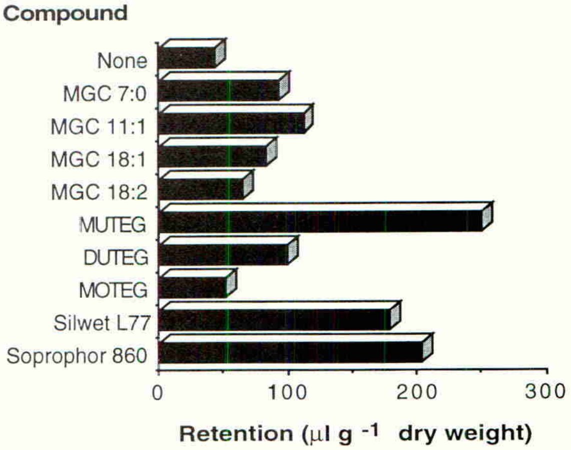


Figure 4. Retention of water sprays on wheat leaves as affected by tetraoxyethylene derivatives and monoglycerides. SEM = 13 (30 d.f.)

The fatty acid derivatives which were liquid at room temperature were assayed as solvents for phenmedipham. None of them was a good solvent for this herbicide (data not shown).

Quizalofop-P-ethyl decreased the biomass of barley at doses > 40 ng $plant^{-1}$ (Fig. 5). The dose-response curves of Formulations A, B and C were similar and no statistical differences were found between the parameters defining the curves. ED_{50} (CI) were 54 (±8), 48 (±7) and 52

(±7) ng plant^{-1}, and ED_{90} (CI) were 110 (±16), 106 (±12) and 106 (±15) ng plant^{-1} for Formulations A, B and C, respectively.

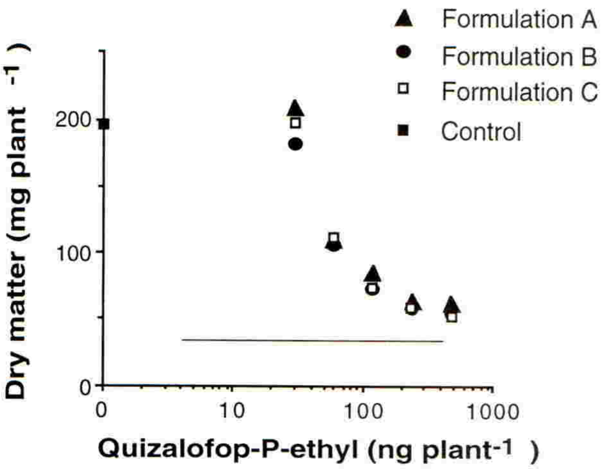


Figure 5. Response of barley plants treated with three different formulations of quizalofop-P-ethyl at five doses. The horizontal line indicates dry weight on the day of treatment. Means of three replicates ; indication of variability is given in the text.

DISCUSSION

As far as herbicide penetration is concerned, amides gave the poorest results. An explanation may be that since they are solid at room temperature, they may prevent the herbicide in the deposit from reaching the cuticle. In spite of being also solid, monoglycerides such as MGC18:1, MGC18:2 and MGC22:1 enhanced phenmedipham penetration. However, they have a pasty appearance and this physical state may not be an hindrance to herbicide diffusion. We made a similar observation with octadecanyl oleate (pasty solid) which enhanced the foliar penetration of phenmedipham and quizalofop-P-ethyl (Serre *et al.*, 1993). Compounds of the ether series promoted phenmedipham penetration with the exception of the fusel ethers. However, due to their low number of carbon atoms (7 and 9), the latter are expected to be volatile under conditions of droplet application (Briggs & Bromilow, 1994). Among tetraoxyethylene esters and monoglycerides, the most lipophilic compounds were the best enhancers of herbicide penetration. In the case of monoglycerides it was particularly clear-cut with the more lipophilic compound, namely phenmedipham, in good agreement with the views of Stock & Holloway (1993). For example, phenmedipham penetration was not affected by MGC7:0, whereas that of 2,4-D (less lipophilic) was enhanced ; an analogous observation was made with MGC11:1. However, increasing chain length from MGC18:1 to MGC22:1 did not result in an increase in herbicide penetration. Hence, it may not be useful to look for oils containing fatty acids with more than 18 carbon atoms. From these observations, we can infer that fatty acid derivatives can yield penetration enhancers, provided the rules already established concerning volatility, physical state and lipophilicity are respected.

Among the compounds studied, only tetraoxyethylene esters and monoglycerides were expected to be surface active. Indeed, all of them decreased the static surface tension of water to values around 30 mN m^{-1} and gave low values for contact angles on the difficult-to-wet surface of wheat leaves. However, only MUTEG and MGC11:1 were active on dynamic surface tension, lowering it to 45 and 55 mN m^{-1}, respectively. These values are comparable to those we obtained with wetters used in agrochemical formulation. Under our conditions, MUTEG increased spray retention on wheat to a greater extent than the wetters used as reference. Hence, the present study indicates that MUTEG is worth of interest as a surface-active agent derived from vegetable oil.

We failed to obtain a good solvent of phenmedipham from the series we studied. Hence, to experiment on formulations we had to resort to the methyl ester derivative of rapeseed oil, since we had already observed that methyl oleate is a good solvent of quizalofop-P-ethyl (Serre *et al.*, 1996). Due to the low quantities of fatty acid derivatives synthesized, we had not enough MUTEG to formulate the quantities of quizalofop-P-ethyl required to spray plants. Hence, herbicide application was done by means of a microsyringe. The experiments indicated that the biological efficacy of the two formulations based on fatty acid derivatives was comparable to the commercial formulation. However their stability when emulsified was far lower (minutes as compared to hours). Hence, under our conditions there was no relation between emulsion stability and biological efficacy. Finally, since in the present study the formulations based on fatty acid derivatives were very simple and not yet optimized for stability, further research may provide entirely vegetable formulations for practical use.

ACKNOWLEDGEMENTS

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REFERENCES

Bogaerts, L (1991) Esters : performance oleochemicals for food and industrial usage. In *Proceedings of the World Conference on Oleochemical : Into the 21st Century*, (ed T H Applewhite), pp. 251-255. A.O.C. Press, Champaign, Illinois.

Briggs, G G; Bromilow, R H (1994) Influence of physicochemical properties on uptake and loss of pesticides and adjuvants from the leaf surface. In : *Interactions between Adjuvants, Agrochemicals and Target Organisms*. P J Holloway, R T Rees & D Stock (eds), Berlin : Springer-Verlag, pp. 1-26.

Cornish, A; Battersby, N S; Watkinson, R J (1993) Environmental fate of mineral, vegetable and transesterified vegetable oils. *Pesticide Science* **37**, 173-178.

Foy, C L; Smith, L W (1964) Surface tension lowering, wettability of paraffin and corn leaf surfaces, and herbicidal enhancement of dalapon by seven surfactants. *Weeds* **13**, 15-19.

Hoyle, E R; Holloway, P J (1996) Performance of alkyl polyglucosides as spray deposition agents. *Brighton Crop Protection Conference - Pests & Diseases*, **4D-18**, 441-442.

Mouloungui, Z; Gauvrit, C (in press). Synthesis and influence of fatty acid esters on the foliar penetration of herbicides. *Industrial Crops and Products*

Serre, I; Cabanne, F; Gauvrit, C (1993) Influence of methyl to octadecyl oleates on the penetration of herbicides through various plant cuticles. *Mededelingen van de Faculteit Landbouwwetenschappen Rijksuniversiteit Gent* **58/3a**, 795-802.

Serre, I; Cabanne, F; Gauvrit, C (1996) Behaviour of alkyl oleates on leaf surfaces in relation to their influence on herbicide penetration. *Brighton Crop Protection Conference-Weeds*, **7B-3**, 807-812.

Stock, D; Holloway, P J (1993) Possible mechanisms for surfactant-induced foliar uptake of agrochemicals. *Pesticide Science* **38**, 165-177.

THE EFFECTS OF FORWARD SPEED ON THE DRIFT FROM BOOM SPRAYERS

P C H MILLER, R W SMITH
Silsoe Research Institute, Wrest Park, Silsoe, Bedford, MK45 4HS

ABSTRACT

The effects of forward speed on the drift from boom sprayers was assessed in a series of wind tunnel and field experiments in which the mean wind direction was at right angles to the direction of travel. In the field experiment, drift was measured 5.0 m downwind of the end of of a 12 m boom fitted with F110/0.6/3.0 nozzles operating at a constant pressure of 3.0 bar. Results showed that in a mean wind speed of 10.0 km/h, drift increased by approximately 51% for a forward speed increase from 4.0 to 8.0 km/h and by 144% when the speed was further increased to 16.0 km/h. Experiments in the wind tunnel measured drift at different forward speeds, in different wind speeds and at different nozzle pressures. The results also showed that higher forward speeds gave higher levels of drift and that changes in forward speed had a greater effect on drift than changes in nozzle pressure in the range 2.0 to 4.0 bar.

INTRODUCTION

The requirement to achieve a timely application of pesticide encourages operators of boom sprayers to spray at higher forward speeds, with lower volume rates and often relatively fine sprays. The objective of the work reported in this paper was to quantify the effects of sprayer speed on drift and to determine the extent to which changes in spray pressure with conventional flat fan nozzles can lead to an additional risk of drift. Many boom sprayer control systems adjust spraying pressure with changes in forward speed so as to maintain a constant application rate such that higher speeds involve higher spraying pressures and an increased risk of spray drift.

Although there is considerable published data relating to the effect of spray quality and wind speed on the drift from boom sprayers (e.g. Miller *et al.*, 1991, Gilbert & Bell, 1988), relatively few studies have examined the effects of forward speed. Taylor *et al.* (1989) measured the drift from boom sprayers at forward speeds of 4.0, 7.0 and 10.0 km/h and found an increase in airborne spray downwind of the machine of approximately 4% as speed increased from 4.0 to 7.0 km/h and 90% for a speed increase from 7.0 to 10.0 km/h. This rapid increase in drift with increasing forward speed suggests that further work is required to quantify the effects and identify the factors causing the increase. A measured increase in drift with increasing nozzle pressure has been reported by Norby and Skuterud (1974) and by Bode *et al.* (1976). Bode *et al.* (1976) showed that increasing pressure from 1.76 to 2.76 bar when travelling at a constant forward speed of 2.5 km/h increased drift by 72%.

Wind tunnel techniques have now become an accepted way of studying the drift characteristics of boom spraying configurations (Western *et al.*, 1989, Miller *et al.*, 1993). Such approaches enable studies to be conducted under repeatable and controlled conditions that can be directly related to field performance.

MATERIALS AND METHODS

Field experiments

Drift was measured 5.0 m downwind from the end of a 12 m mounted boom sprayer that made multiple passes along a track arranged at right angles to the mean wind direction. Airborne spray was collected on two lengths of 1.98 mm diameter polythene line supported vertically to a height of 9.0 m. The spray liquid contained 0.25% of a soluble tracer dye (Green S, Warner Jenkinson Ltd.) and 0.1% of a non-ionic surfactant. Drift deposits were quantified by spectophotometry using reference solutions collected from the spray nozzles. Meteorological conditions were recorded continuoulsy during the experiments using sensors mounted on a 10.0 m mast with a vane anemometer positioned at the top, and five cup anemometers and temperature sensors attached at heights of 0.7, 1.6, 3.4, 6.1, and 10 m from the ground. Reference wind speeds were taken from the wind velocity profile, interpolated to a height of 2.0 m.

The boom height was set 0.8 m above a grass stubble surface (height approximately 100 mm) and F100/0.6/3.0 nozzles at 0.5m spacings along the boom were operated at a pressure of 3.0 bar. Measurements were made in a range of wind speed conditions at forward speeds of 4.0, 8.0, 12.0 and 16.0 km/h. Forward speed for each spray passed was checked by timing between two markers 50 m apart.

Wind tunnel experiments with a moving nozzle

The wind tunnel had a working cross-section 2.0 m wide and 1.5 m high, and had a rail attached to the roof which was used to transport a single test nozzle at a constant speed across the tunnel. The nozzle was suspended from the rail, 0.5 m above the floor and orientated so that the spray fan was aligned normal to the direction of nozzle movement. The tunnel floor was covered with an artificial grass matting to minimise droplet bounce, and the downwind airborne spray was collected on five horizontal 1.98 mm diameter polythene sampling lines spaced at 100 mm centres up to the nozzle height. The distance between the nozzle and collector lines was 2.25 m.

The nozzle sprayed into collection tubes placed at the start and end of each traverse to minimise the quantity of spray that could become airborne prior to the nozzle being moved across the tunnel. The spray liquid contained a lower concentration of tracer dye than used in the field experiments (0.05% of Green S, Warner Jenkinson) as the volume of airborne spray passing the samplers was expected to be greater. Dye deposits were recovered from the middle 1.0 m length of each sampling line to avoid possible edge effects associated with start and stop times of the nozzle movement and were quantified by spectrophotometry. The number of passes made by the test nozzle during each experiment varied between 5 and

15 depending on the nozzle speed and air speed along the tunnel in order to obtain a measurable deposit. Air speed was measured using a Gill Instruments 3-dimensional ultrasonic anemometer located at the nozzle height within the entry section of the tunnel.

Measurements were made with a single F110/0.6/3.0 travelling across the tunnel at speeds of 1.8, 5.4 and 9.0 km/h in wind speed of 7.2, 12.0 and 21.0 km/h down the tunnel. The effect of nozzle pressure was studied in a wind speed down the tunnel of 7.2 km/h using two sizes of nozzle (F110/0.6/3.0 and F110/1.6/3.0). Pressures in the range 1.0 to 4.0 bar were used for both nozzle sizes and nozzles were moved across the tunnel at five speed settings in the range 1.8 to 10.5 km/h. All experimental measurements were replicated in a random sequence.

RESULTS

Field measurements

The measured spray drift values at the four sprayer speeds are plotted in Figure 1. The captured spray volumes have been expressed as a percentage of the output from the sprayer so as to provide a basis for comparison independent of the effects of the lower volume application rates that were associated with the higher forward speeds. A linear regression analysis has been used to distinguish trends at the four forward speed settings (Gilbert & Bell, 1988, Miller, 1993)

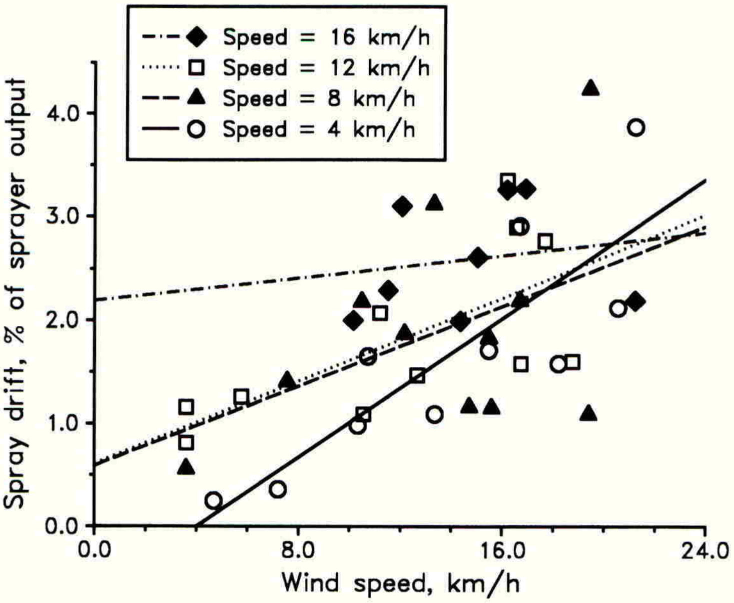

Figure 1. Field measurements of spray drift at four different forward speeds

The results show considerable scatter as is typical of data from many field measurements of spray drift. The form of the linear relationship at the lowest speed is consistent with published data (Miller, 199) with an intercept on the x-axis at approximately 3.8 km/h. At the highest forward speed of 16.8 km/h, the lowest wind speed for which measurements were made was 10.1 km/h and further data is required to justify the extension of the linear regression analysis over the full range of wind speeds. The analysis showed no difference in the measured spray profiles at sprayer speeds of 8 and 12 km/h.

Wind tunnel experiments

The measured airborne spray volumes from the F110/0.6/3.0 nozzle operating in different wind speeds and at different forward speeds are plotted in Figure 2. At each forward speed, the volume of airborne spray increased approximately linearly with wind speed in agreement with the results from previous wind tunnel drift studies reported by Western *et al.* (1991), Miller *et al.* (1993) and Smith and Miller (1994).

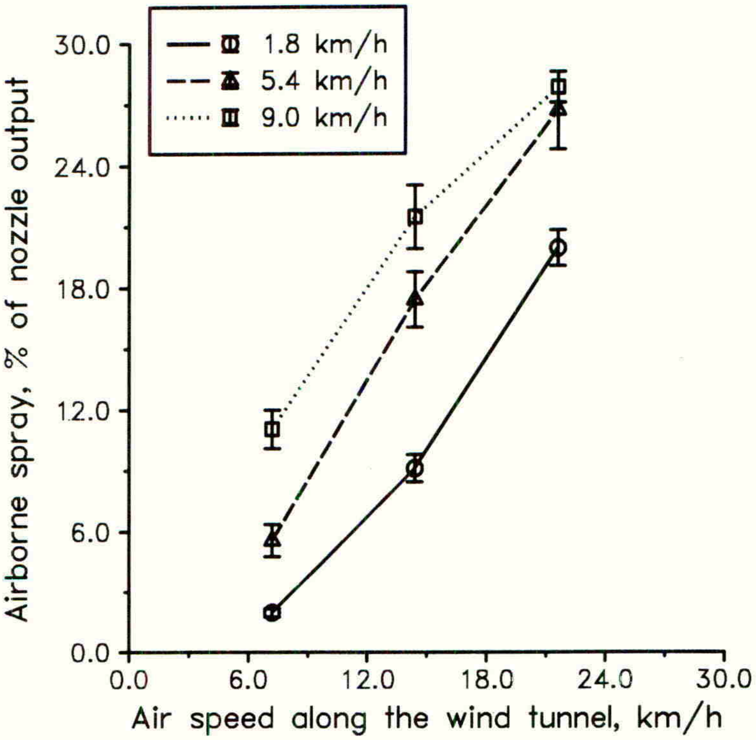

Figure 2. Measured airborne spray downwind of a F110/0.6/3.0 nozzle moving across a wind tunnel at different forward speeds and wind speeds

The rate of increase of airborne spray with wind speed shown by the gradients of the lines on Figure 2 was approximately the same at each of the three forward speeds. The effect of increasing forward speed was to displace the measured airborne spray volumes to higher levels. For example, increasing forward speed from 1.8 to 5.4 km/h increased the volume of airborne spray by 3.9, 8.3 and 6.8% of nozzle output in wind speeds of 7.2, 14.4 and 21.6 kh/h respectively and, at a wind speed of 14.4 km/h, represented an increase in airborne spray volume of 104%. Results from replicated measurements were in good agreement and gave relatively small standard errors as plotted on Figure 2.

The effects of varying both nozzle speed and pressure for the F110/0.6/3.0 and F110/1.6/3.0 nozzles operating in a wind speed of 7.2 km/h is shown in Figures 3 and 4 respectively.

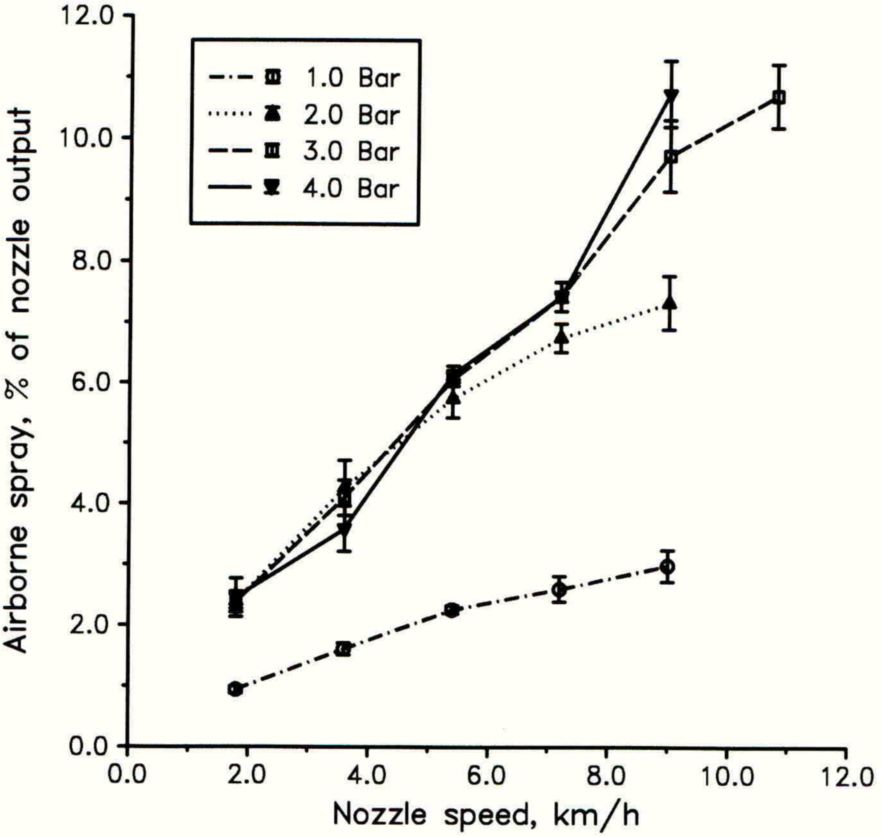

Figure 3. Measured airborne spray downwind of a F110/0.6/3.0 nozzle operating in a wind tunnel at different pressures and speeds in a wind speed of 7.2 km/h

With the smaller size of nozzle, Figure 3, increasing the forward speed increased the measured airborne spray volume approximately linearly but there were no differences in the measured values for pressure of between 2.0 and 4.0 bar for speeds up to to 7.2 km/h. There was some evidence that, at the higher speeds (e.g. 9.0 km/h), increasing pressure in the range 2.0 to 4.0 bar did result in increased airborne spray volumes as expected. Measured airborne spray volumes at a pressure of 1.0 bar were less than 50% of those at the other pressures and it is likely that the nozzle was not forming an adequate spray at this pressure (Miller and Smith, in preparation). For the larger nozzle size, Figure 4, similar trends were observed but with a greater dependence upon pressure with a pressure of 2.0 bar giving 30% lower airborne spray volumes at forward speeds of above 4.0 km/h. There were no differences in airborne spray volumes measured at operating pressures of 3.0 and 4.0 bar.

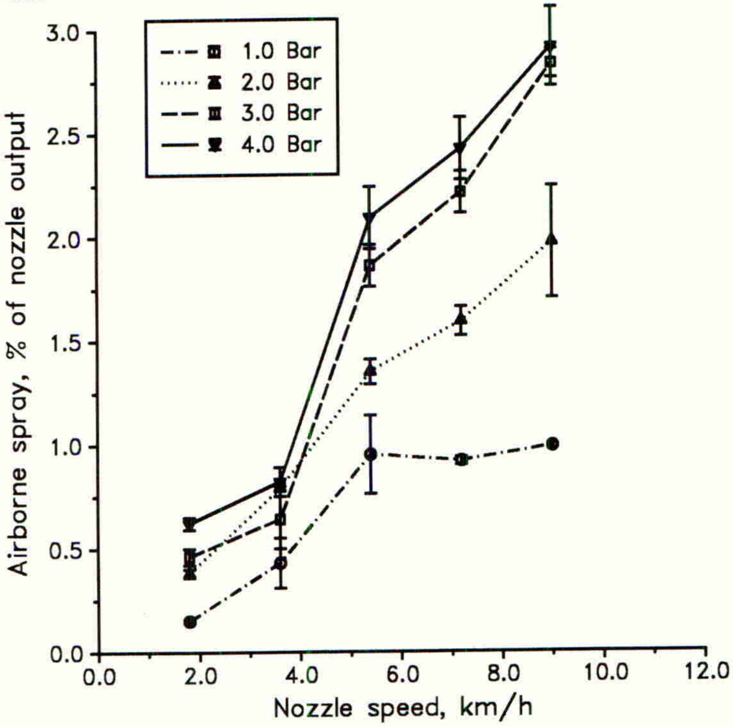

Figure 4. Measured airborne spray downwind of a F110/1.6/3.0 nozzle operating in a wind tunnel at different pressures and speeds in a wind tunnel speed of 7.2 km/h

For all measurements at different forward speeds and in different wind speeds replicated results were in reasonable agreement and the standard errors plotted on Figures 3 and 4 are relatively small.

DISCUSSION

The results from both the field and wind tunnel studies reported here have shown that increasing the forward speed of boom sprayers increases spray drift. The results also provide evidence to show that the effective air flow due to the forward motion of the nozzle is responsible for the detrainment of droplets from the spray structure. The wind tunnel measurements of airborne spray volumes with moving nozzles operating at a range of pressure gave values that were directly comparable with data for static nozzles operating at comparable pressure settings and with the spray fan at right angles to the direction of the air flow (Miller *et al.,* 1993, Miller and Smith, in preparation). The measurements have also shown that in wind speed conditions that are defined as acceptable for spray application with boom sprayers, increasing the forward speed by, for example, 4.0 km/h has a similar effect on spray drift to operating in a mean wind speed that is 4.0 km/h higher. This result has implications for codes of practice relating to the operation of boom sprayers where recommendations to reduce forward speeds in higher wind conditions or when control of spray drift is particularly important should be included.

The observed differences between the airborne spray measured from the two sizes of flat fan nozzle operating in the wind tunnel were consistent with previous work (Ghosh and Hunt, In press, Miller *et al.*, 1995) which has shown that the penetration of an air flow into a spray structure, and hence drift, is determined by the ratio between the strength of the external air flow and the entrained air velocity within the spray structure. Entrained air velocities are a function of nozzle flow rate with higher flow rates entraining more air and increasing entrained air velocities. Higher flow rate nozzles may therefore be more suited to operation at higher forward speeds where the spray fan structure can provide some control of small droplet movement. Such a strategy is consistent with maintaining a constant application rate over a range of operating speeds.

Increasing nozzle pressure increases the percentage of spray volume in the smaller droplet sizes and hence is generally expected to lead to an increase in spray drift. However, increasing pressure also increases droplet velocities, flow rates and, to a limited extent, entrained air velocities so reducing the risk of drift. The results from the work reported in this paper suggest that for pressures in the range up to 4.0 bar, air flow interactions around the spray are more important in terms of spray drift than those due to spray pressure. This has implications for the design and operation of sprayer control systems in which pressure is adjusted to change nozzle flow rates and hence keep application rates constant over a defined speed range. Spray pressure has previously been shown to have a substantial effect on drift based on field experiments. The dual tracer system used by Bode *et al.* (1976) measured drift with spray pressures of 1.76 bar and 2.76 bar. These pressures would also be expected to show an increase in airborne spray in this work since a spray pressure of 1.0 bar gave a low level of airborne spray. Norby and Skuterud (1974) used pressures of 2.5 and 10.0 bar, but their experiments were based on a single measurement with one pass of the spray vehicle which might not account for fluctuations in wind speed influencing their results.

The results from the field experiment suggested that the largest effects due to sprayer forward speed were in low wind speed conditions. These trials were conducted with a nozzle representative of those producing fine quality sprays. It is likely that at the higher wind speeds, the air flow due to the wind alone is sufficient to penetrate the spray structure with this nozzle and therefore the effects on drift due to forward speed are small.

Results from both the field and wind tunnel experiments show consistent trends. The maximum forward speeds that could be used in the wind tunnel experiment were limited by the performance of the nozzle transporter that was required to accelerate and decelerate the nozzle to a constant speed in a short distance.

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REFERENCES

Bode, L E, Butler, B J, Goering, C E (1976) Spray drift and recovery as affected by spray thickener, nozzle type, and nozzle pressure. *Transactions of the American Society of Agricultural Engineering.* **19 (2):** 213-218

Ghosh, S, Hunt, J C R (In press) Spray jets in a cross flow. Submitted to Journal of Fluid Mechanics

Gilbert, A J, Bell, G J (1988) Evaluation of drift hazards arising from pesticide spray application. *Aspects of Applied Biology* **17:** 363-375

Miller, P C H (1993) Spray drift and its measurements. In Application Technology for Crop Protection (eds G A Matthews or E C Hislop) *C.A.B. International*, 101-122

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

Miller, P C H, Smith, R W (In preparation) The effect of forward speed on the drift from boom sprayers. To be submitted to Crop Protection.

Miller, P C H, Smith, R W, Tuck, C. R, Walklate, P J (1995) The classification of agricultural sprays based on droplet size distributors and the results from wind tunnel test. *Proceedings Brighton Crop Protection Conference - Weeds,* 1125-1134

Miller, P C H, Tuck, C R, Gilbert, A J, Bell, G J (1991) The performance characteristics of a twin fluid nozzle sprayer: BCPC Monogram No. 46. *Air-assisted Spraying in Crop Protection,* 97-106

Norby, A, Skuterud, R (1974) The effects of boom height, working pressure and wind speed on spray drift. *Weed Research* **14:** 385-395

Smith, R W, Miller, P C H (1994) Drift predictions in the near nozzle region of a flat fan spray. *Journal of Agricultural Engineering Research* **59:** 111-120

Taylor, W A, Anderson, P G, Cooper, S (1989(The use of air assistance in a field crop sprayer to reduce drift and modify drop trajectories. *Proceedings Brighton Crop Protection Conference - Weeds,* 631-639

Western, N B, Hislop, E C, Herrington, P J, Jones, E I (1989) Comparative drift measurements for BCPC reference nozzles and for an Airtec twin-fluid nozzle under controlled conditions. *Proceedings British Crop Protection Council Conference - Weeds,* 641-648