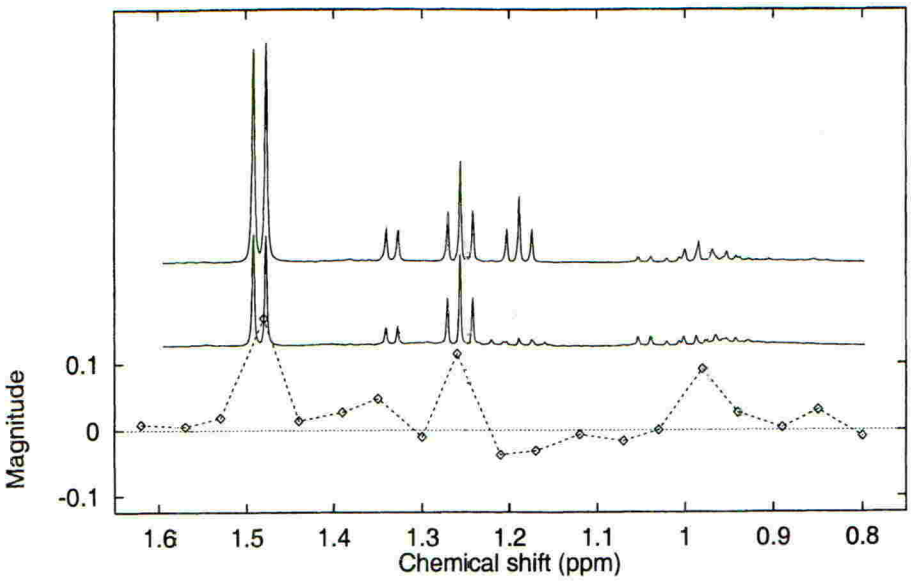

Figure 3: Parts of proton NMR spectra of sulfometuron-methyl treated (top) and control (middle) plants, together with the PLS-DA loadings for data-reduced spectra of sulfometuron treated plants (bottom). The triplet at 0.98ppm is only observed in sulfometuron treated plants.

This work shows that NMR is an effective method for metabolite profiling, and that metabolite profiling has the potential to identify the MOA of herbicides whether the MOA is known or new. We are now investigating other methods of quantifying metabolites to see whether they can further improve the throughput of the metabolite profiling technique.

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Mechanism of action of sulcotrione in mature plant tissues

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ABSTRACT

Sulcotrione acts by inhibiting 4-hydroxyphenyl pyruvate dioxygenase (HPPD) an early step in the biosynthesis of plastoquinone (PQ). Kim *et al.* (1999) proposed that, especially in developed tissues, the ultimate herbicidal effect of sulcotrione might result more from indirect inhibition of photosynthetic electron transport than of carotenoid biosynthesis. Here it is shown that treatment of mature cucumber cotyledons with, alternatively, diuron, fluridone, or sulcotrione, resulted in distinct perturbations in the relative levels of chlorophyll and of total carotenoids. Four days after herbicide treatment, the residual chlorophyll/carotenoid ratios were 5.24, 7.02 and 5.88, respectively. Sulcotrione caused a much more rapid decrease in the *in planta* quantum yield of photosystem II (PS II) as monitored by chlorophyll fluorescence than did fluridone. Furthermore, measurements of 2,6-Dichlorophenolindophenol reduction in extracted thylakoids indicated that sulcotrione was a more effective inhibitor of the Hill reaction in cucumber, a herbicide sensitive species than in corn, a herbicide-insensitive species. Overall, these results are consistent with the notion that the major herbicidal effect of sulcotrione in mature green tissue is to indirectly inhibit electron transport *via* a reduction in PQ levels.

INTRODUCTION

Sulcotrione is one representative of a new class of herbicides which act by inhibition of HPPD (Secor 1994). This causes significant reductions in the PQ and tocopherol contents of treated plants, which further leads to decreases in phytoene desaturase activity and in the carotenoid content of chloroplast (Prisbylla *et al.*, 1993). Loss of carotenoid leads to bleaching and plant death.

However the pattern of plant bleaching associated with inhibition of HPPD is distinctive and is not identical to that observed with herbicides such as norflurazon and fluridone which inhibit phytoene desaturase directly (Mayonado *et al.*, 1989; Sandman *et al.*, 1990).

This difference may be significant and indicative of some further underlying difference in mode of action. Kim *et al.* (1999) proposed that the reduction in PQ levels caused by sulcotrione treatment should lead to a decrease not only in phytoene desaturase activity but also to a decrease in photosynthetic electron transport and especially so in mature green tissues. Here, this suggestion is further investigated.

MATERIALS AND METHODS

Measurements of the *in planta* content of photosynthetic pigments and of the quantum yield of photosynthetic electron transport

Cucumber seeds were planted in a pot and grown in a greenhouse for 8 d. Cotyledons at 80% of full growth were treated with three different herbicides at rates calculated to give approximately equivalent levels of herbicidal effect e.g. 30 g a.i./ha of diuron, 100 g a.i./ha of fluridone, and 500 g a.i./ha of sulcotrione. The treated plants were grown for 4 d in a controlled growth chamber at 25°C with a photoperiod of 14 h and light intensity of 55 µmol $m^{-2}s^{-1}$. Photosystem II (PS II) quantum yield was measured at 24 h intervals, using a pulse amplitude modulation fluorimeter (PAM-2000, Walz, Effeltrich, Germany). Chlorophyll and carotenoids were extracted 4 d after herbicide treatment with methanol and quantitated by the method of Lichtenthaler (1987).

Herbicidal activity after foliar treatment

350 cm^2-area pots were filled with sterilized, sandy loam soil (pH 6.0) including 1.0% organic matter and appropriate amount of fertilizers. Cucumber (*Cucumis sativa* L.) and corn (*Zea mays* L.) were sown and grown in a greenhouse at 30/20 °C (day/night) using a 14 h-photoperiod. Herbicides were dissolved in acetone and diluted with water including a nonionic surfactant (Tween 20) and sprayed, 10 d after inoculation, at 4,000 liter/ha. Final concentrations of acetone and of Tween-20 in the solutions were 50% and 0.1%, respectively. The biological activity of herbicides was rated visually 6 d after treatment using a scale between 0 and 100 where '0' represented no herbicidal effect and '100' represented complete death.

Inhibition of the Hill reaction

2,6-Dichlorophenolindophenol reduction assays were conducted according to the method of Anderson *et al.* (1994) at a final volume of 10 ml.

RESULTS AND DISCUSSION

Herbicide-induced changes in the contents of photosynthetic pigments and in the quantum yield of photosynthetic electron transport

In order to better understand the mode of action of sulcotrione its effects on the pigment content and photosynthetic quantum yield of mature cucumber cotyledon tissue were compared with those of other herbicides yielding visually similar symptoms such as fluridone, a known inhibitor of phytoene desaturase and diuron, a known inhibitor of electron transport in PS II. Experiments were carried out under low light conditions in order to better clarify the causes of bleaching. After 4 days all herbicide treatments resulted in decreases in the contents of both chlorophyll and carotenoids but to differing and distinct extents (Table 1). Sulcotrione treatment resulted in a chlorophyll/ carotenoid ratio of 5.88, diuron treatment resulted in a ratio of 5.24 (the same as in untreated controls) whilst fluridone resulted in a relatively greater reduction in carotenoids and yielded a final ratio of 7.02 Qualitatively, by this measure, sulcotrione is more 'diuron-like' than 'fluridone-like'. Furthermore, analysis of

herbicide-induced changes in chlorophyll fluorescence indicated that sulcotrione exerted a much more rapid inhibitory effect on the quantum yield of PSII than did fluridone (Figure 1). The rate of inhibition of quantum yield by the three herbicides paralleled the extent to which they reduced the chlorophyll content *relative* to carotenoids (Table 1). Thus, although carotenoid contents were less diminished four days after treatment with sulcotrione than with fluridone, PS II quantum yield was, nevertheless, inhibited more rapidly following treatment with sulcotrione.

It has been reported that indirect inhibition of PS II by fluridone can be related to an effect of the carotenoid deficiency which follows inhibition of phytoene desaturase (Sandman *et al.*, 1996; Trebst & Depka, 1997). If sulcotrione-mediated inhibition of PS II were mediated solely by a such a mechanism then it would be expected that sulcotrione should inhibit PS II less than fluridone. However, as indicated in Figure 1, the converse was observed.

Table 1. Changes in photosynthetic pigment contents of cucumber cotyledons 4 d after treatment of with diuron, fluridone and sulcotrione.

Herbicides	Pigment contents (μg/ml)		
	Chlorophylls (A)	Carotenoids (B)	A/B
Control	115.8±5.9	19.7±1.1	5.88
Diuron	81.7±1.6	15.6±0.4	5.24
Fluridone	89.9±2.2	12.8±0.2	7.02
Sulcotrione	96.8±4.5	16.5±0.8	5.88

Data represent the mean±SE from three replicates.

Relation between herbicidal activity *in vivo* and inhibition of the Hill reaction

The herbicidal activity of sulcotrione observed in the greenhouse was compared with the extent of inhibition of the Hill reaction. Consistent with the relative tolerance of corn to herbicide treatment. Following treatment with sulcotrione the Hill reaction was much less inhibited in thylakoids extracted from corn than in cucumber thylakoids (Figure 2). These results are consistent with the major effect of sulcotrione in mature tissue being related to inhibition of electron-transport in PSII. although other explanations are also possible.

Considering that PQ is a mediator of photosynthetic electron transport as well as an essential cofactor for phytoene desaturase (Norris *et al.*, 1995), it is to be expected that sulcotrione should, indirectly, affect both processes. *A priori* it would seem most likely that sulcotrione should exert a relatively greater effect on photosynthetic electron transport in mature green tissues in which rates of *de novo* carotenoid biosynthesis are relatively low but rates of photosynthesis remain relatively high. This notion is consistent with the observation that the typical bleaching (whitening) symptoms induced by sulcotrione which correspond to carotenoid deficiency-induced degradation of chlorophyll (Sandmann *et al.*, 1990; Böger, 1996) are observed in the developing rather than mature tissues of treated plants.

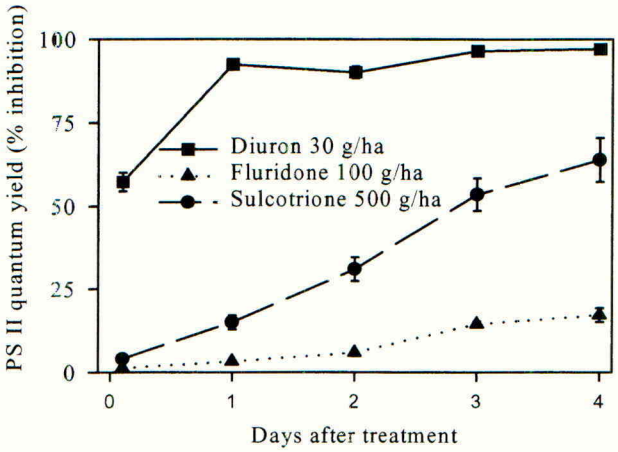

Figure 1. Inhibition of PS II quantum yield determined by chlorophyll fluorescence in cucumber cotyledons treated with diuron, fluridone and sulcotrione.

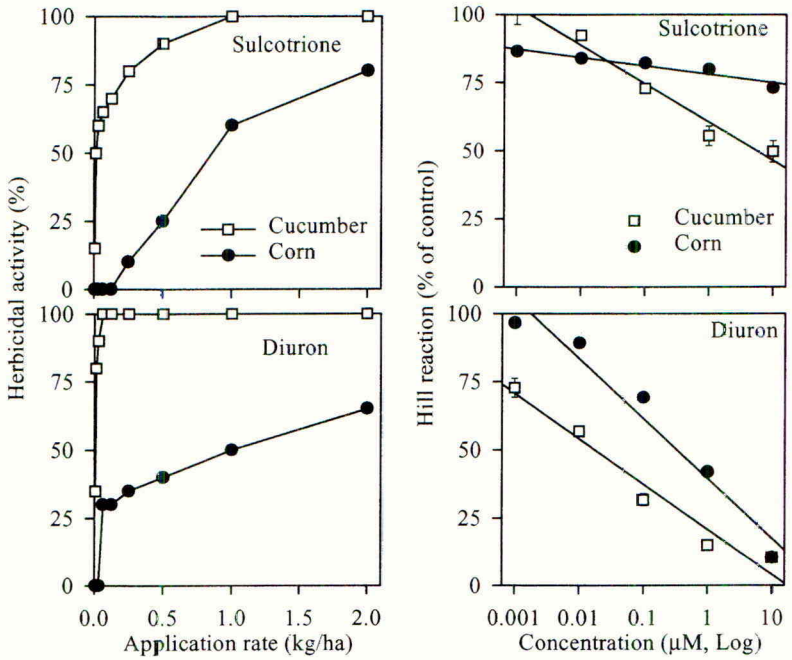

Figure 2. Effects of sulcotrione and diuron on herbicidal activity and on the Hill reaction in thylakoid membrane extracted from cucumbe or corn. Herbicidal activity was determined at 6 d after application.

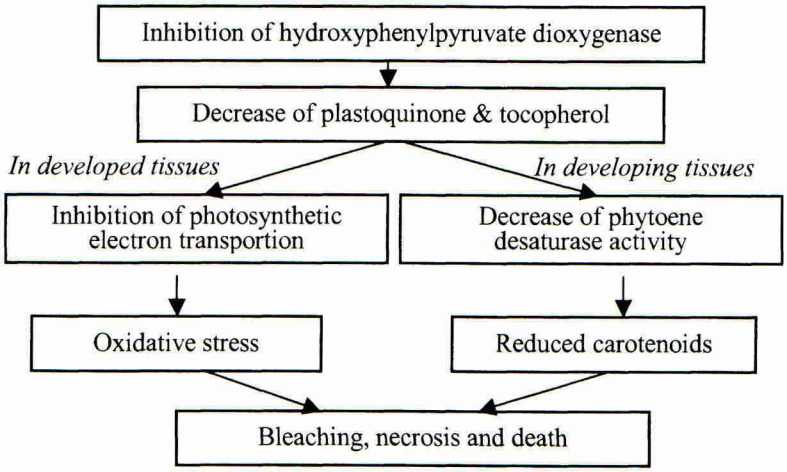

Figure 3. Possible action mechanism of sulcotrione herbicide in different developmental stages of plants

In conclusion, we propose that the major herbicidal consequences of sulcotrione inhibition of HPPD differ according to the developmental stages of plant tissues. In developing tissues such as new leaves the major effect is on inhibition of carotenoid biosynthesis whereas in older leaves and other mature tissues the major effect is on inhibition of photosynthetic electron transport (Figure 3).

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Mesotrione: Mechanism of herbicidal activity and selectivity in corn

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ABSTRACT

Mesotrione, a herbicide that acts by inhibition of the 4-hydroxyphenylpyruvate dioxygenase (HPPD) step in plastoquinone biosynthesis, is highly effective for broad-leaved weed control in maize. The basis of its efficacy at low use rates is shown here to lie in its exquisite potency (e.g. K_d 15 pM) and slow rate of dissociation (t ½ c. 2d) from its HPPD target site in dicotyledonous weeds. Safety to corn is shown to derive not only from a relatively low rate of uptake but also from a relatively fast rate of cytochrome P450 catalysed 4-hydroxylation. The significance of this to the prevention of corn injury is underscored by the observation that sweetcorn lines, which are impaired in their ability to perform this hydroxylation, either by mutational variation or by addition of malathion, a cytochrome P450 inhibitor, exhibit significantly increased susceptibility to mesotrione damage. Mesotrione is also a much less potent inhibitor of the HPPD in monocotyledonous plants than of that in dicotyledonous plants. It is, for example, several hundred fold weaker an inhibitor of HPPD from wheat (K_d 7 nM, t ½ for dissociation of the enzyme/ inhibitor complex c. 10 min) than of HPPD from *Arabidopsis thaliana*. Tobacco, a species normally damaged by less than 2 g/ ha of mesotrione, is rendered entirely insensitive to the application of several hundred g/ ha after it is transformed to express the HPPD gene from wheat. This illustrates that inherent resistance at the level of the monocot HPPD target enzyme has an important role in determining the robust safety of mesotrione to corn.

INTRODUCTION

Mesotrione (Figure 1) is the active ingredient of Callisto, a herbicide recently developed by Syngenta for the selective pre- and post-emergence control of mainly broad-leaved weeds in maize (Wichert *et al.*, 1999). It is a member of the benzoylcyclohexane-1,3-dione family of herbicides, which are chemically derived from a natural phytotoxin obtained from the Californian bottlebrush plant, *Callistemon citrinus*. Mesotrione acts by competitive inhibition of the enzyme HPPD, a component of the biochemical pathway that converts tyrosine to plastoquinone and α-tocopherol (Prysbilla *et al.*, 1993).

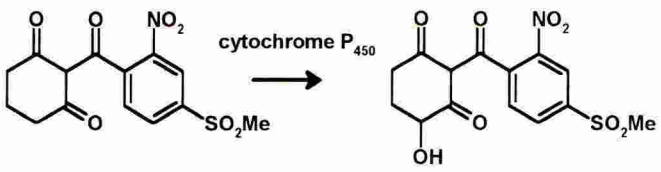

Figure 1. Structure of mesotrione and of its 4-OH metabolite

The observed crop selectivity of mesotrione may be due, at least in part, to foliar uptake being slower into maize than into weed species (Mitchell *et al.*, 2001). Mesotrione is a weak acid, with a dissociation constant (pK_a) of 3.12 at 20°C resulting in a pH dependency for water solubility ideal for uptake into, and translocation within plants. Comparative data regarding the extent of uptake of ^{14}C mesotrione, into the foliage of treated leaves of a range of weed species indicated relatively rapid uptake (c. 75-90% after 6 hours) relative to maize (c. 50%). Here we explore further aspects of the interaction of mesotrione with HPPD and the mechanisms that underpin its high degree of safety for use in corn.

MATERIALS AND METHODS

Mesotrione was prepared > 95% pure and uniformly ^{14}C-labelled to a specific activity of 1.12 GBq/ mmol (Wichert *et al.*, 1999). *Arabidopsis thaliana* and wheat HPPD genes were obtained *via* 'One-step' RT-PCR (Qiagen) of mRNA purified using the Oligotex method (Qiagen) from total RNA prepared by Tri-Zol extraction (Life Technologies) of 5d old seedlings. Primers to obtain full length coding sequences were designed based upon the known DNA sequences (Norris *et al.*, 1998; Derwent Geneseqn accession AAA29169) and included 5' *Nde*1 and 3' *Bam* H1 restriction enzyme sites. The authentic products obtained were cloned into pCR2.1TOPO (Invitrogen), excised using *Nde* 1 and *Bam* H1 (where the *Nde*1 site corresponds to the first codon), ligated into similarly restricted pET-24a (Novagen) and transformed into *E.coli* BL21(DE3). Cells were grown in a fermenter in L-broth containing 100 µg/ ml kanamycin and induced for expression for 2h before harvest, washing, resuspension in ice-cold 0.1M Hepes/ KOH buffer at pH 7.5 and disruption in a Constant Systems Basic Z cell disrupter. The resultant extracts were clarified by centrifugation for 2h at 40000 *g* and the supernatant exchanged down Sephadex G25 into the same buffer. For tobacco transformation, the *Nde*1-*Bam*H1 wheat HPPD fragment was first cloned into vector pMCJA, identical to pMJB1 (Thompson *et al.*, 1998) but with the *Nco*1 site at the translation initiation codon replaced by *Nde*1. The thus created expression cassette, comprising a double enhanced 35S cauliflower mosaic virus promoter/ tobacco mosaic virus translational enhancer/ wheat HPPD coding sequence/ nopaline synthase 3' terminator region was excised as a *Hind*III/ *Eco*R1 partial digest, cloned into pBIN19 (EMBL accession PPU09365), transformed *via Agrobacterium tumefaciens* LBA4404 into plant tissue of tobacco variety Samsun, selected and regenerated into plants as described by Thompson *et al* (1998). PCR positive primary transformants , single-copy segregants from later generations and control plants were grown in small pots in the glasshouse and, at the 5-7 leaf stage, were sprayed with 300 g/ ha mesotrione formulated in 0.5% Turbocharge ™ surfactant/ water at 200 litre/ ha (Figure 3). The extent of damage visible as bleaching of meristems, bleaching of leaves, necrosis and stunting of growth was assessed visually at 18 DAT by reference to unsprayed controls.

Wheat HPPD and Arabidopsis HPPD extracts exhibited K_m values of 10.1 µM and c. 3.5 µM hydroxyphenylpyruvate and, based on measured titres of active site ^{14}C inhibitor-binding, k_{cat}/K_m values of 1.1 x 10^6 and 1.35 x 10^6/ s/ M, respectively. Inhibitor binding experiments were all carried at 25°C in Bis-Tris propane buffer at pH 7.0 containing 25 mM sodium ascorbate and 2 µg/ ml of highly pure catalase (Sigma C3155). HPPD assays were carried out in 50 mM Bis-Tris propane buffer at pH 7.0 as described by Viviani *et al.* (1998) with initial rates obtained by curve fitting to time points taken at 10s intervals up to 70s. HPPD active-site titres in extracts were calculated from the ratio of bound inhibitor/ amount of extract as derived

from titration (between 0 and c. 0.9 μM) versus a fixed concentration of ^{14}C-inhibitor (c. 0.3 μM). Bound and free inhibitor were rapidly separated by gel filtration and quantitated by scintillation counting. Calculated titres accounted for the fact that inhibitors bind rapidly to half the available HPPD sites (Garcia *et al.*, 2000) and then slowly with the remainder. Values of HPPD/ inhibitor dissociation rates, k_{off}, were obtained from the rate of the exchange reaction observed (Figure 2) after addition of 50 μM of unlabelled inhibitor (I) to c. 0.35 μM HPPD/ ^{14}C inhibitor complex (EI*) preformed after reaction for > 3 h at 25°C between HPPD (E) and a slight excess of ^{14}C inhibitor (I*). Aliquots of bound and free ^{14}C label were removed at different times, separated by gel filtration and counted. The data obtained were fitted numerically to the scheme EI* + I ⇆ EI + I* to obtain best fit values to the rate constant, k_{off}, governing the dissociation EI* → E + I* (Schloss 1989). Values of HPPD/ inhibitor association rates, k_{on}, were a) similarly obtained but using unlabelled excess mesotrione to rapidly quench the binding reaction between HPPD and ^{14}C mesotrione or b) calculated from the rate of decline in enzyme activity at 25°C of HPPD reacted for different times with different concentrations of mesotrione and then simultaneously trapped and assayed by rapid quenching *via* 10 fold dilution into 150 μM HPP.

For metabolism studies, c. 1 μg of ^{14}C mesotrione was spotted as microdroplets (20 x 0.2 μl) to the middle of fully expanded leaves of 18 d old soybean (*Glycine max.*) and sweetcorn plants (*Zea mays* cv. 'Landmark' or 'Merit', as indicated). At 6 and 24 h time points, the treated area was excised and compound on the leaf surface removed into 2 ml of acetone. The leaf segment was powdered in liquid nitrogen, extracted into 10 ml of acetone, lyophilized, resuspended in acetonitrile and applied to a 2D Sorbsil C-30 tlc plate run with (15:15:5:1) chloroform/ ethyl acetate/ methanol/ formate in the first dimension and (20:8:1) chloroform/ methanol/ ammonium hydroxide in the second dimension. Radioactive spots were identified by reference to the R_f values of authentic mesotrione and 4-OH mesotrione in the first dimension of 0.25 and 0.50 and, in the second, of 0.56 and 0.9, respectively.

RESULTS AND DISCUSSION

Following foliar uptake, ^{14}C-mesotrione is translocated both acropetally and basipetally with some metabolism occurring in all plant species but particularly rapidly in maize (Mitchell *et al.*, 2001). Here, the possibility that metabolic inactivation of mesotrione is a major determinant of safety in corn was further pursued through comparative study of the early metabolism of mesotrione in "Landmark" corn and soybean, a highly sensitive species. A single leaf of each species was treated with radiolabelled mesotrione at a rate equivalent to 72 g ha^{-1}, and the extract of the treated leaf was analysed by tlc at 6 and 24h. After 24 h, less than 40% of the parent mesotrione was found in corn whereas > 85% remained in treated soyabean (Table 1). Mesotrione was not only taken up less rapidly into corn but was also metabolised much faster than in soybean. The major early metabolite formed was 4-hydroxy-mesotrione (Figure 1). Overall, the combined effect of these differences in uptake and rate of metabolism between the two species would be to significantly reduce the relative amount of mesotrione in the sensitive tissues of corn plants.

'Merit' is a line of sweetcorn, which is peculiarly susceptible to mesotrione injury. It is about 6-7 fold more susceptible than typical corn lines, exhibiting an equivalent level of transient injury at c. 70 g/ ha as, for example, 'Landmark' at 500 g/ ha of mesotrione. Comparative studies of uptake and metabolism, similar to table 1, indicated that, whereas there was no

significant difference in uptake, Merit was comparatively deficient in its ability to form the 4-OH derivative of mesotrione with c. 60% survival of parent and only c. 5 % conversion to 4-OH after 24h as compared to c. 13 and c. 40%, respectively in Landmark corn.

Table 1. Comparative uptake and metabolism of mesotrione in soybean and corn.

Sample/ Leaf Content	After 6h	After 24 h
Soya/ total(pmol)	283	256
Soya/ % parent	78	66.5
Soya/ % 4-OH	0	2
Corn/ total(pmol)	80	125
Corn/ % parent	55	13.5
Corn/ % 4-OH	13	40

In a further experiment to investigate the importance of mesotrione hydroxylation to crop safety, ^{14}C mesotrione was applied in droplet form, at a rate equivalent to 70g/ ha, to leaves of Landmark and Merit corn lines after first pre-spraying with 400 or 800 g/ ha of malathion, a known inhibitor of cytochrome P450 hydroxylase enzymes. Analysis by tlc confirmed that malathion did indeed inhibit the formation of the 4-OH metabolite of mesotrione in Landmark corn. Individually, neither malathion nor mesotrione caused injury to Landmark corn; however the combination caused Landmark to suffer mesotrione injury to a similar extent as the Merit susceptible line (c.10-15 % damage at 14 DAT with obvious partial leaf bleaching). Thus oxidative conversion of mesotrione to 4-OH mesotrione is probably cytochrome P450 hydroxylase catalysed and is an important determinant of mesotrione safety to corn.

Mesotrione is generally more active against broad leaved than against grass species. Further experiments were carried out to understand whether some difference in inherent susceptibility at the level of the HPPD target enzyme might contribute to the relative safety of mesotrione in corn. Figure 2 depicts data from one experiment to measure the dissociation rates of the complexes formed between mesotrione and HPPD from wheat, a representative monocotyledonous plant, and between mesotrione and HPPD from *Arabidopsis thaliana*. The experiment shows that mesotrione forms a much less stable complex with HPPD from wheat (t ½ c. 10 min, k_{off} c. 1 x 10^{-3}/ s) than it does with HPPD from *Arabidopsis thaliana* (t ½ c. 2 d, k_{off}, 3.3 x 10^{-6}/s). Dissociation rate is an important component of herbicidal activity since it determines how long the inhibitor can 'stick' to its target and maintain an effective block after spraying. It may be noted that, in Figure 2, c. 25% of the mesotrione initially bound to *Arabidopsis thaliana* HPPD exchanged relatively rapidly. In separate experiments we showed this fraction to represent inactive enzyme and it is disregarded in the determination of k_{off} that is calculated from the major slow phase. In this context it should be noted that, since the wheat enzyme was highly active and, moreover, since HPPD inhibitors with high levels of graminicidal activity similar to 2-[2-chloro-4-methanesulphonylbenzoyl]-4,4,6,6-

tetramethylcyclohexane-1,3,5-trione (Mitchell *et al.*, 2001) exchanged only slowly (<1.5 x 10^{-5}/s) from wheat HPPD, there was no question of the fast exchange of mesotrione from wheat HPPD being an artefact of enzyme inactivation. Mesotrione bound about equally rapidly to *Arabidopsis thaliana* and wheat HPPD with k_{on} values of 2.5 x10^5/ s/ M and 1.8x10^5/ s/ M, respectively. Overall K_d values were thus, c. 15 and 7000 pM, representing a > 400 fold difference in inherent susceptibility to inhibition by mesotrione. Even allowing for the c. 3 fold difference in Km value such a large difference in susceptibility to inhibition could readily explain why mesotrione is predominantly a broad leaved weed herbicide.

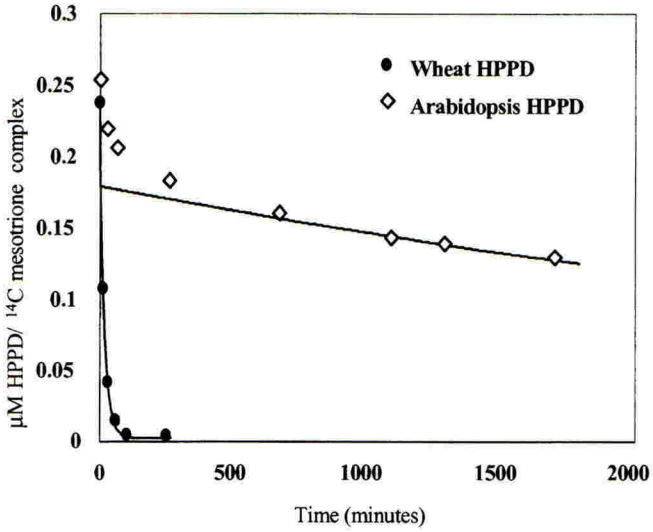

Figure 2. Exchange of ^{14}C mesotrione from wheat and from *Arabidopsis* HPPD

A further experiment (Figure 3) dramatically illustrated the contribution of intrinsic activity to crop safety. Tobacco plants were transformed to express wheat HPPD to c. 0.1% of the total tobacco leaf protein (estimated by Western analysis). At spray rates up to 300 g/ha as many as a third of primary transformants showed no visible signs of damage in contrast to untransformed tobacco which showed significant damage at less than 2 g/ ha. Resistance was even higher in homozygous plants of later generations. In comparable experiments wherein the *Arabidopis thaliana* HPPD gene (Norris *et al.*, 1998) was similarly expressed, but in *Arabidopsis*, we also observed an enhancement in resistance to mesotrione but relatively modest and < c. 8 fold in even the best individual events.

In summary, mesotrione is an effective herbicide because of its very high affinity for its HPPD target site. It is exceptionally safe to corn not only because 1) it is taken up more slowly into corn than into the majority of target weeds but also because 2) at least judged from wheat, the HPPD target enzyme of monocotyledonous plants is several hundred fold inherently less sensitive to mesotrione than that in broad leaved species and 3) corn has the ability to rapidly metabolise mesotrione to inactive derivatives and, in particular, *via* P450 catalysed 4-hydroxylation at the 4-position of the cyclohexanedione unit.

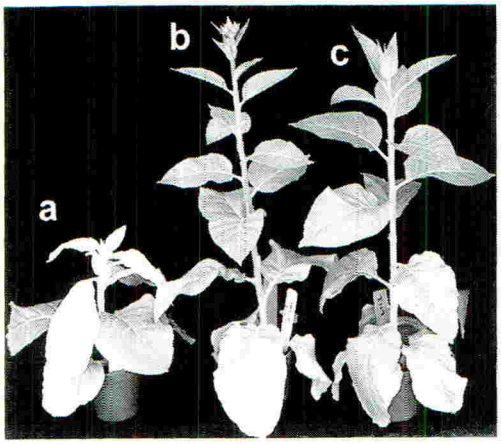

Figure 3. Control (a) and transgenic (c) tobacco expressing wheat HPPD 18 DAT after treatment with 300 g/ ha mesotrione as compared with unsprayed control tobacco (b).

ACKNOWLEDGEMENTS

The authors especially thank Peter Sutton and Matt Cordingley in weed biology and the radiochemistry teams at both Jealott's Hill and the Western Research Center, CA, USA

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Histological investigations into the mode of action of the novel grass herbicide oxaziclomefone

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ABSTRACT

Oxaziclomefone is a new grass herbicide offering excellent control of *Echinochloa crus-galli* and other grass weeds in rice. Preliminary investigations into its mode of action have shown it to have no inhibitory effects on established herbicide target sites and therefore a novel mode of action is implicated. Histological analysis shows that the mode of action of oxaziclomefone can be distinguished from those of dichlobenil and oryzalin, but there are similarities to cinmethylin. However, initial studies have shown oxaziclomefone not to share the biochemical site of action of cinmethylin.

INTRODUCTION

Oxaziclomefone (MY-100; 3-[1-(3,5-dichlorophenyl)-1- methylethyl]-2,3 dihydro-6-methyl-5-phenyl-4*H*-1,3-oxazin-4-one) (Figure 1) is a novel herbicide developed by Aventis CropScience for the control of *Echinochloa* spp. in paddy rice and transplanted rice (Jikihara *et al.*, 1997). In combination with a number of sulfonylurea herbicide partners, oxaziclomefone has been launched this year in Japan in the one-shot application market for weed control in rice, this sector accounting for 70% of the weed control market for rice in Japan. Oxaziclomefone offers growers season-long residuality, a wide window of application, low application rates and excellent selectivity for the control of *Echinochloa* spp. plus sedges and certain broad-leaved weeds, when applied pre- to early post-emergence.

Of special interest is the fact that oxaziclomefone brings a new type of mode of action to this market, complementary to that of sulfonylureas. Oxaziclomefone causes stunting and chlorosis of weeds in the greenhouse but clearly possesses a mode of action new and distinct from that of established herbicide classes. We have undertaken a histological and microscopic characterisation of the action of this herbicide and here present the results in comparison to certain growth-inhibiting reference herbicides *viz.* cinmethylin, dichlobenil and oryzalin. These reference herbicides are reportedly a pro-inhibitor of asparagine synthetase (Romagni *et al.*, 2000), and inhibitors of cellulose biosynthesis and mitosis respectively.

MATERIALS AND METHODS

Treatment of wheat tissues with herbicide

Wheat seeds were germinated in the dark until the root system was 20 - 30 mm long. Seedlings were then grown hydroponically in Long Ashton mineral nutrient solution

under standard glasshouse conditions, keeping the solution continually aerated and maintaining the roots in the dark and the shoots in the light. When plants reached the two-leaf stage they were treated with the herbicide under investigation by adding it to the nutrient solution. The concentration of herbicides used were such that the wheat plants showed symptoms three to four days after treatment yet remained alive for at least eleven days after treatment.

Light microscopic analysis of tissues

Sections of root tip were fixed in 2 % glutaraldehyde for two hours at room temperature. The sections were then infiltrated with methacrylate and embedded in resin. Longitudinal sections 4 µm thick were cut and stained with either Toluidene Blue, which stains cellulose cell walls, cytoplasm and nuclei blue, or Ruthenium Red which stains pectin red.

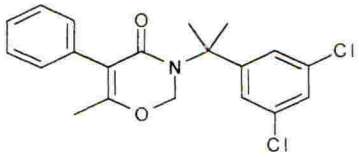

Figure 1. Structure of oxaziclomefone.

RESULTS

When wheat plants at the two-leaf stage were treated with 24 nM oxaziclomefone root and shoot growth ceased although the plants remained alive (Figure 2b). Protuberances resembling developing lateral roots appeared in the region 0 – 5 cm back from the root tip three days after treatment. As treatment continued these became more obvious although ceased to enlarge further after eleven days following treatment (Figure 2d). No discoloration of these 'lateral-root-like' protuberances was observed. Untreated control plants showed continued root and shoot growth and no root abnormalities developed (Figure 2a and 2c).

Microscopic analysis of sections from control roots showed no cellular abnormalities. The cells at the root tip were dense with the degree of vacuolation increasing as the cells aged (Figure 3a). Nuclei and nuceoli were present and all stages of mitosis were clearly visible in the zone of cell division (Figure 3b and 3d). No stages of mitosis were found in comparable regions of the root when plants were treated with 24 nM oxaziclomefone for 24 h although nuclei and nucleoli were present (Figure 4b). There were no differences in the shape of the roots of oxaziclomefone-treated plants (Figure 4a) compared with the control plants (Figure 3a).

When stained with Toluidene Blue and Ruthenium Red, the cell wall of control plants stained as a solid boundary (Figure 3b and 3c). In root sections from oxaziclomefone-treated plants, there was little or no staining of the wall with Toluidene Blue (Figure 4b). Staining of the cell walls of oxaziclomefone-treated plants by Ruthenium Red gave thin

lines of stain around the perimeter of the cells, the remainder of the cell wall showing no staining (Figure 4c).

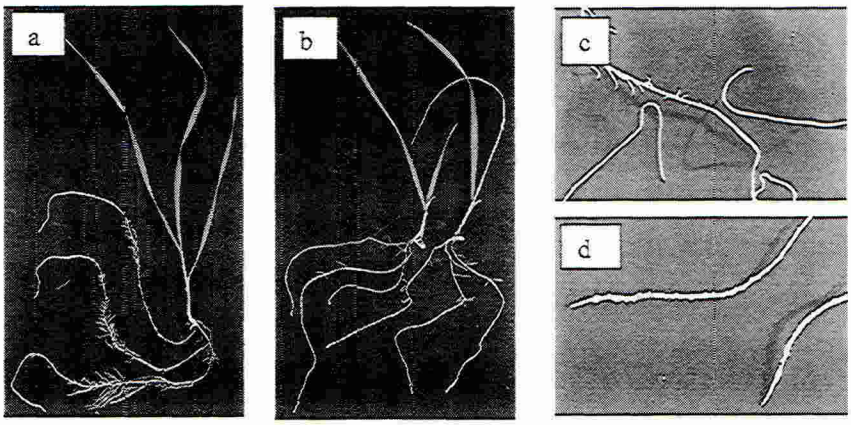

Figure 2. Cessation of root and shoot growth and root abnormalities caused by 24 nM oxaziclomefone. Wheat plants were treated via the nutrient solution at the two-leaf stage and grown in herbicide-amended media for eleven days. (a) And (c) are control plants; (b) and (d) were treated with oxaziclomefone.

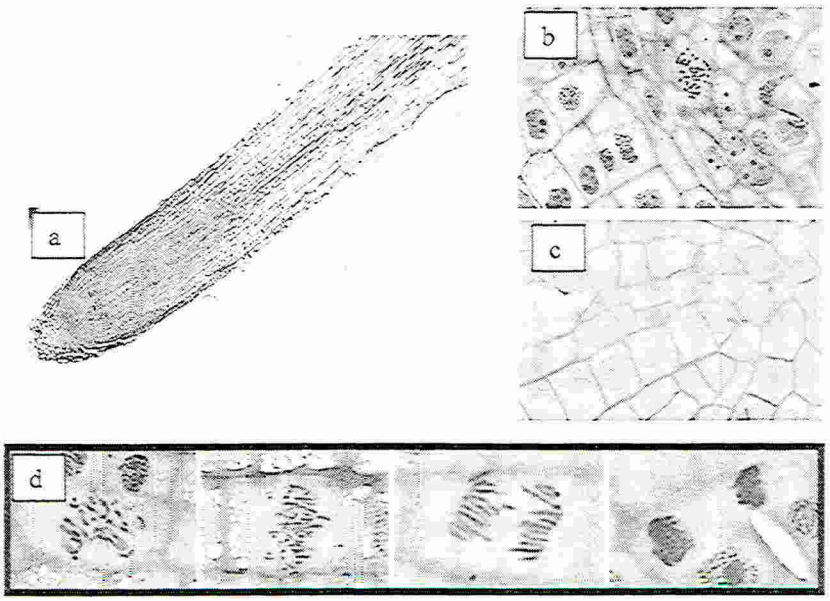

Figure 3. Longitudinal sections of the roots of untreated control plants stained with Toluidene Blue (a), (b) and (d), and Ruthenium Red (c). Stages of mitosis are clearly identifiable in the cells of control roots. Magnification (a) x10; (b), (c) and (d) x100. Encircled region in (a) indicates area examined under higher magnification in (b) and (c).

This unusual staining pattern with Toluidene Blue and Ruthenium Red was also observed when plants were treated with 18 nM cinmethylin (Figure 5c and 5d) and 100 µM dichlobenil (Figure 6c and 6d).

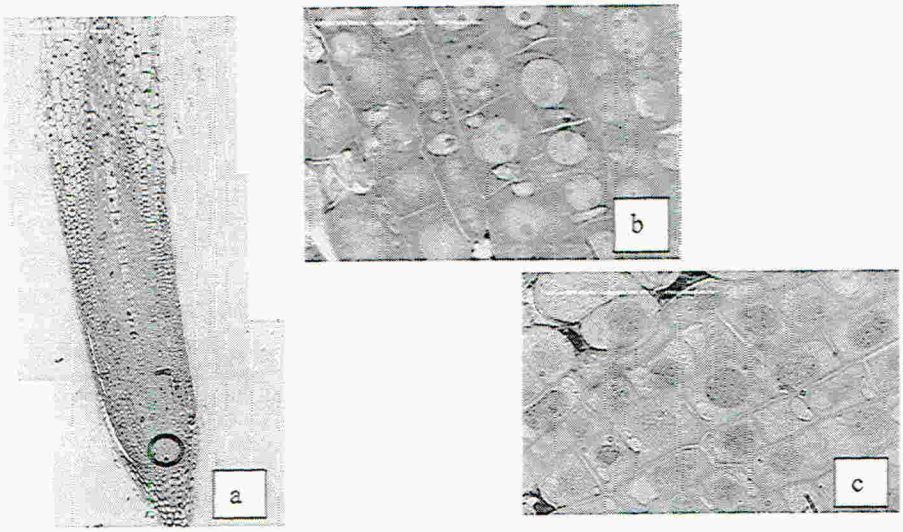

Figure 4. Longitudinal sections of the roots of wheat plants treated, via the nutrient solution, at the two-leaf stage with 24 nM oxaziclomefone for 24 h. Sections stained with Toluidene Blue (a) and (b), and Ruthenium Red (c). Encircled region in (a) indicates area examined under higher magnification in (b) and (c). Magnification (a) x10; (b) and (c) x100.

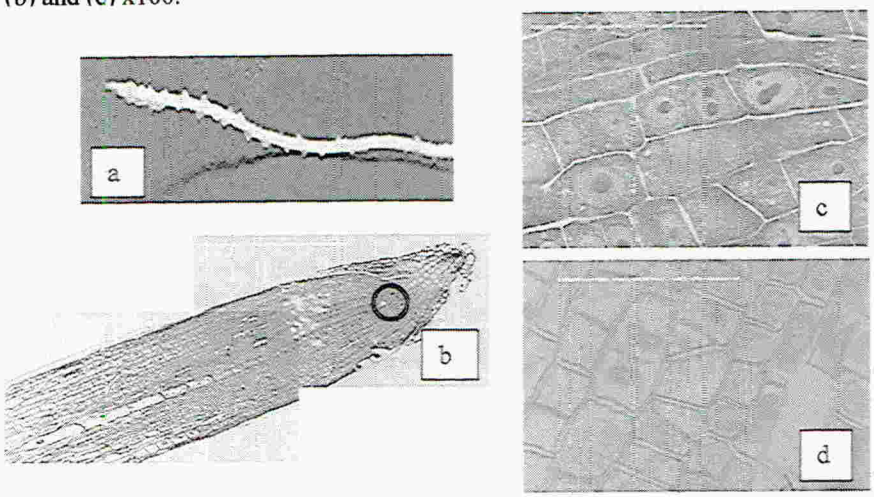

Figure 5. Effect of 18 nM cinmethylin on the roots of wheat plants treated via the nutrient solution at the two-leaf stage and grown in herbicide-amended media for either eleven days (a) or 24 h (b), (c) and (d). Longitudinal sections of the roots were stained with Toluidene Blue (b) and (c), and Ruthenium Red (d). Encircled region in (b) indicates area examined under higher magnification in (c) and (d). Magnification (b) x10; (c) and (d) x100.

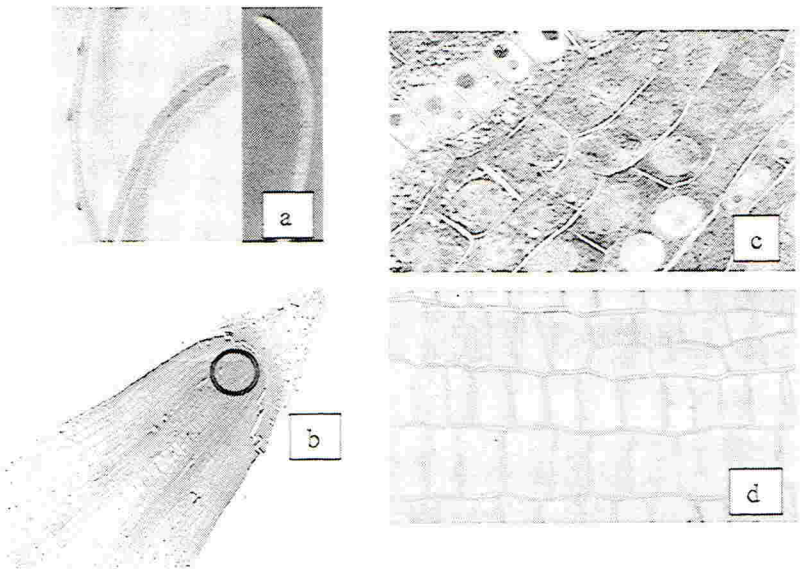

Figure 6. Effect of 100 µM dichlobenil on the roots of wheat plants treated via the nutrient solution at the two-leaf stage and grown in herbicide-amended media for either eleven days (a) or 24 h (b), (c) and (d). Longitudinal sections of the roots were stained with Toluidene Blue (b) and (c), and Ruthenium Red (d). Encircled region in (b) indicates area examined under higher magnification in (c) and (d). Magnification (b) x10; (c) and (d) x100.

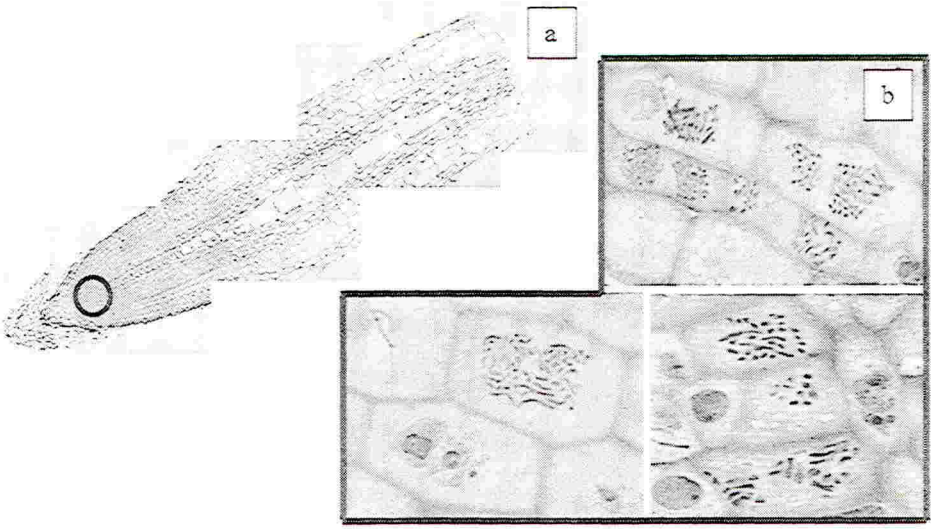

Figure 7. Effect of 10 µM oryzalin on the roots of wheat plants treated via the nutrient solution at the two-leaf stage and grown in herbicide-amended media for 24 h. Longitudinal sections of the roots were stained with Toluidene Blue. Aberrant stages of mitosis are clearly visible (b). Encircled region in (a) indicates area examined under higher magnification in (b). Magnification (a) x10; (b) x100.

As with oxaziclomefone, treatment of wheat plants with 18 nM cinmethylin via the nutrient solution caused the cessation of root and shoot growth although the plant remained alive. The protuberances that developed on the roots of cinmethylin-treated plants (Figure 5a) were similar in appearance and position to those caused by oxaziclomefone (Figure 2d). In contrast to treatment with oxaziclomefone, later stages of mitosis were occasionally seen in roots of cinmethylin-treated plants (Figure 5c and 5d).

When wheat plants were treated with 100 µM dichlobenil for eleven days, the first leaves showed signs of wilting, developing lateral roots were discoloured and the root tips were swollen (Figure 6a). In contrast to the roots of oxaziclomefone and cinmethylin-treated plants, there were no 'lateral-root-like' protuberances occurring near the root tip. Wheat plants treated with 100 µM dichlobenil (Figure 6b) and 10 µM oryzalin (Figure 7a) for 24 h showed obvious swelling of the cortical cells. Many cells in those roots treated with oryzalin showed aberrant mitotic figures as expected (Figure 7b).

DISCUSSION

These results demonstrate that some herbicide modes of action can be easily identified using histological studies. Although the swelling of root tissue caused by oryzalin and dichlobenil may appear similar, the two compounds are clearly distinguishable by the presence, as with oryzalin, or absence, as with dichlobenil, of aberrant mitotic figures. The lack of enlargement of cortical cells and aberrant mitotic figures in the roots of oxaziclomefone-treated roots further supports the biochemical studies suggesting that neither cellulose biosynthesis nor mitosis are directly affected by oxaziclomefone.

The similarities observed between the roots of oxaziclomefone and cinmethylin-treated plants did initially suggest that they may have the same mode of action. However, initial results have shown that oxaziclomefone does not inhibit asparagine synthetase as has been reported for cinmethylin (Romagni *et al.*, 2000).

An unexpected result seen in these studies is the disruption to the cell wall that has occurred in roots of those plants treated with oxaziclomefone, cinmethylin and dichlobenil. From the staining of the cell wall it appears that both the cellulose and pectin components of the cell wall are either not formed as in the control plants or that they undergo some chemical change that renders them insensitive to the stain. Further studies would be needed to determine what the cause of this is and whether is it characteristic of herbicide treatment of plants.

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POSTER SESSION 8B

HERBICIDE RESISTANT WEEDS (RISK ASSESSMENT, BASELINE SENSITIVITY AND MANAGMENT)

Session Organiser: T Godson
Pesticides Safety Directorate, York, UK

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

Herbicide resistance in *Alopecurus myosuroides* (black-grass): field testing and population plasticity

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ABSTRACT

The occurrence of herbicide resistance in grass weeds in the UK continues to rise. One proposed resistance mechanism in black-grass (*Alopecurus myosuroides*) is enhanced herbicide metabolism mediated by the enzyme glutathione *S*-transferase (GST). We have previously characterized differences in GST activity and abundance that can be correlated with herbicide resistance in black-grass. This paper presents GST abundance data from black-grass plants harvested from the field.

An ELISA-based resistance test is described and the utilization of GST abundance as a marker for herbicide resistance in this species is discussed. Preliminary observations with other grass weeds suggest GST abundance to be a useful resistance marker. Population plasticity is the individual variation in a particular trait among members of the same species. Data are presented to describe the plasticity of black-grass populations with respect to GST abundance. This is discussed in relation to the occurrence and development of herbicide resistant black-grass populations in the UK.

INTRODUCTION

Over the previous three decades grass weeds have become an increasing agricultural problem worldwide, particularly in cereal crops. The presence of such weeds results in yield losses, harvesting difficulties and poor quality product. Herbicides have been routinely relied upon to control these weeds, with varying degrees of success. Since the 1980s the presence of herbicide-resistant populations of key grass weeds (*Alopecurus myosuroides*, *Lolium rigidum*, and *Avena fatua*) have become an increasing problem. In the UK, resistance in black-grass (*A. myosuroides*) populations has now been detected in over 750 farms in 30 counties (Moss, 1997). Although resistance to a single herbicide has been reported, cross and multiple resistant populations often occur. Where this happens chemical control can be very difficult, as resistance against many herbicides may be present.

Central to any herbicide resistance management strategy is the need for quick and accurate diagnosis of resistance within weed populations. Traditional resistance testing involves growth and herbicide treatment of suspect plants under glasshouse conditions. Recently, a new generation of resistance tests have become available that are quicker and cheaper than glasshouse methods. These include the Syngenta Quick test (Boutsalis, 1999) and the Rothamsted Rapid Resistance test (Moss, 1999), which

provide results in 4-6 weeks, but are unlikely to give a diagnosis before the application of autumn-applied post-emergence herbicides. There is a need for a resistance test that will provide information to the grower prior to the application of post-emergent herbicides, allowing spray strategies to be matched to resistance profiles for the weed populations.

A resistant black-grass biotype from Peldon (Essex, UK) has been demonstrated to contain approximately double the activity of the enzyme glutathione *S*-transferase (GST) compared to susceptible biotypes. Correlation between resistance to fenoxaprop-P-ethyl and GST activity has also been demonstrated (Reade *et al.*, 1997). Purification of GST subunits from herbicide-resistant black-grass reveals a 30 kDa polypeptide that is not detected in susceptible biotypes (Reade & Cobb, 1999). Initial field studies indicate that sub-populations of black-grass surviving herbicide treatment possess higher GST abundance than untreated populations (Reade *et al.*, 1999). It therefore appears that GSTs play a role in at least some forms of herbicide resistance in black-grass. The specific nature of their role has yet to be elucidated, but it seems likely that they are involved in enhanced metabolism of herbicides to less or non-toxic metabolites. This may be accomplished by conjugating the herbicide or its metabolite to the tripeptide glutathione, although recent observations suggest that GSTs may also have glutathione peroxidase activity (Cummins *et al.*, 1999).

Population plasticity is the individual variation in a particular trait among members of the same species (Brauth *et al.*, 1991). Where the trait confers herbicide resistance to an individual the plasticity of the parent population may play a key role in the way resistance develops within the population. In target site resistance, plasticity is unlikely to be of importance, as individuals are usually either resistant or susceptible. However, in cases of enhanced metabolism, where the degree of resistance is likely to be proportional to the abundance of the metabolizing enzyme(s), plasticity within a population will have major effects on resistance development.

This paper describes field trials carried out to investigate the role of GST abundance as a marker for herbicide resistance. Data have been accrued over a two-year period, and individual plants were sampled in order that variation in GST abundance both within and between populations could be assessed.

MATERIALS AND METHODS

Field sites, herbicide treatments and plant sampling

Black-grass plants were collected from 6 different sites in the East Midlands, UK. Three field sites were used during each of the 2 years of study. All sites were treated with herbicides (see Table 1 for details), except for site 2 year 1. This site received no treatment during the year of sampling (1998/99) but had received the treatments detailed in Table 1 for the previous 6 years.

Sites 1 and 3 (year 1) were sampled once after herbicide treatment. All other sites were sampled repeatedly both before and after treatment. All above-ground biomass

was harvested and frozen on dry ice for transportation. Wherever possible, 10 plants were sampled per plot per sample date.

Table 1. Field treatments for 1998/1999 and 1999/2000.

Site \ Plot	1	2	3	4
1 (year 1)	Untreated	Isoproturon[a]	Diclofop[b]	-----
2 (year 1) *	Untreated	Isoproturon[a]	Clodinafop[c]	Fenoxaprop[d]
3 (year 1)	Untreated	Isoproturon[a]	Diclofop[b]	-----
1 (year 2)	Untreated	Isoproturon[a]	Clodinafop[c]	Fenoxaprop[d]
2 (year 2)	Untreated	Isoproturon[a]	Clodinafop[c]	Fenoxaprop[d]
3 (year 2)	Untreated	Isoproturon[a]	Clodinafop[c]	Fenoxaprop[d]

* No treatment during year of study, but stated treatments carried out during the previous six years.

[a] 2500 g a.i./ha as Auger (5 l/ha)

[b] 900 g a.i./ha as Illoxan-European (2.4 l/ha)

[c] 30 g a.i./ha as Topik (0.125 l/ha)

[d] 69 g a.i./ha as Cheetah S (1.25 l/ha)

Protein extraction and determination

Proteins were extracted and quantified as described by Milner *et al.*, (2001). GST abundance was determined by ELISA detection, as detailed in Reade & Cobb (2001).

Data handling

In order to allow comparison between sites, mean response for untreated plots was calculated and all data divided by this value. Data were subsequently grouped on a 0.2 unit scale and frequency of occurrence (0-0.2, 0.2-0.4, etc.) per trial plot was calculated. This allowed comparison between trial plots at each trial site.

RESULTS AND DISCUSSION

Field trials were performed in order to assess the suitability of GST abundance as a marker for herbicide resistance in black-grass. GST abundance in plants harvested from site 2 (year 2) is shown in Figure 1a. At sites 1 and 3 (year 1) and 1 and 3 (year 2) similar observations were made, with an absence of plants possessing low GST abundance being observed in all treated plots post-treatment. In all untreated plots these individuals were present. Sampling pre-treatment revealed no difference between plots at any sites (data not shown). Arrows in Figure 1 highlight plants possessing low GST abundance, which were found to be absent in treated plots. It is postulated that the absence of these plants possessing low GST abundance in treated plots indicates that these plants have been successfully controlled by the herbicide treatment. Those plants remaining are resistant to the herbicide used on the trial plot and possess greater GST abundance. Greater GST abundance in treated plots is not due to physiological responses of plants to herbicide treatment, as similar differences were observed in samples from site 2 (year 1). This site had not received herbicide

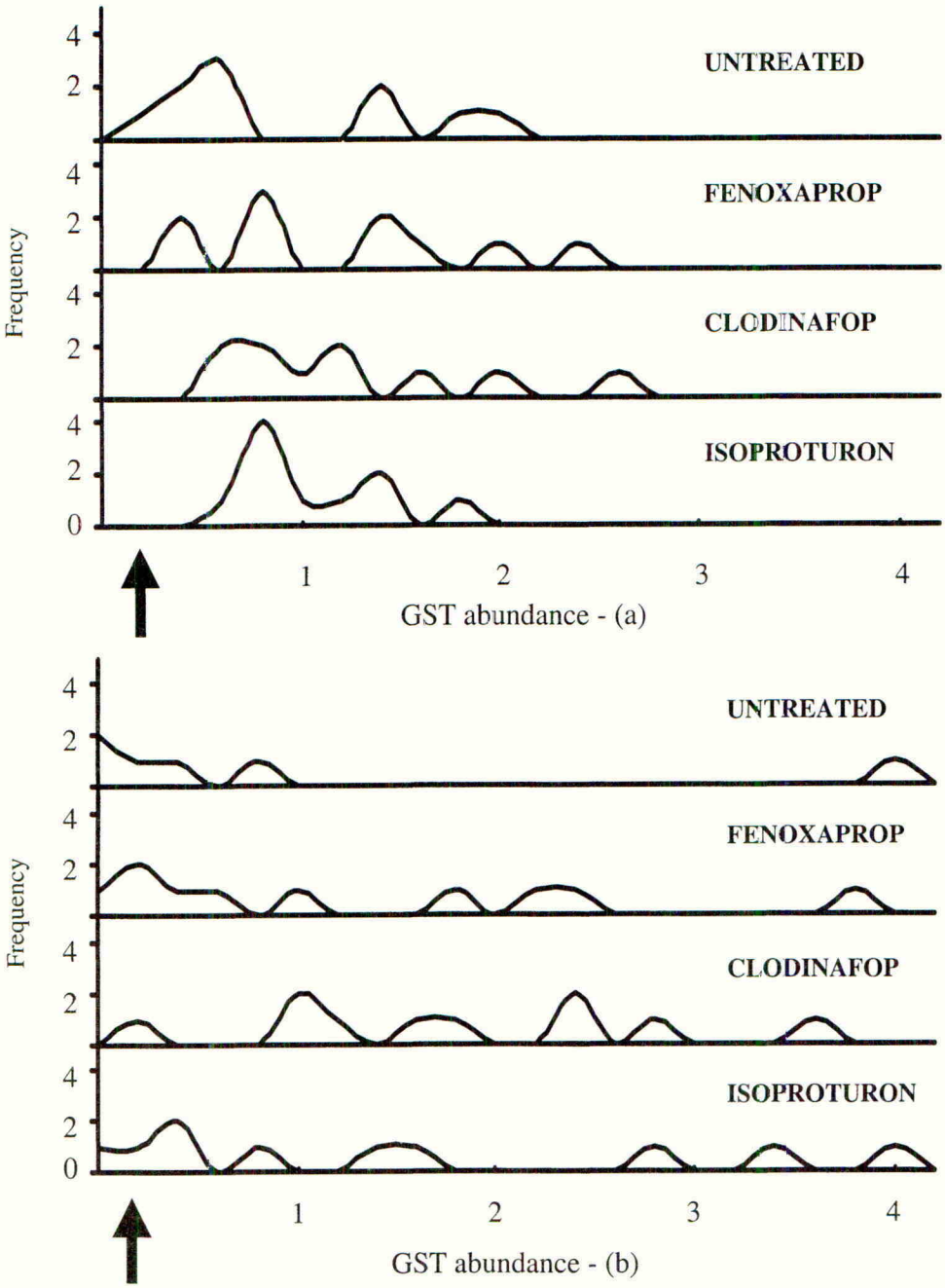

Figure1. GST abundance data for (a) site 2 year 2, where treated plots contain individuals that have survived herbicide treatment, and (b) site 2 year 1, which received no treatments during the year of study but received the indicated treatments for the previous 6 years. GST abundance is expressed as frequency of grouped responses. Arrows indicate 'low GST abundance' plants, absent from treated plots.

treatment during the year of study, but had received the indicated herbicide treatments for the previous 6 years. Data from this site are presented in Figure 1b. Plants possessing low GST abundance were present in all plots at this site. However, plots that had received repeated herbicide selection pressure contained a greater proportion of plants possessing high GST abundance.

Results from these field trials demonstrate that sub-populations of black-grass surviving herbicide treatment possess different GST abundance profiles than those of parent populations, which contain both resistant and susceptible individuals. Therefore, the number of individuals in black-grass populations possessing high GST abundance can be used to indicate the proportion of those populations that are herbicide resistant. The ELISA test takes 3 days and can be carried out on plants from GS 11, so will provide a resistance profile of a population prior to the application of post-emergence herbicides. This will allow alternative control strategies to be adopted where resistance is indicated.

Previous studies on the involvement of GSTs in herbicide resistance in black-grass have focussed on the well-characterized biotypes Herbiseed, Rothamsted and Peldon. GST activity and abundance in Peldon has repeatedly been demonstrated to be double that of susceptible biotypes (Reade & Cobb, 1999), and it was this observation that first suggested a role for this enzyme in herbicide resistance. The observations presented here demonstrate that within field populations there is a high degree of plasticity with respect to GST abundance. The putative role of GSTs in herbicide resistance is either in the conjugation of herbicides to glutathione or as a glutathione peroxidase, although general reductive/protective roles have also been suggested.

The individual variation in GST abundance among members of a population, demonstrated within all field populations studied, suggests that there may be large differences in the ability to protect from herbicide damage within each population. Susceptible individuals appear to be those that have relatively low GST abundance that are unable to carry out protection at a sufficient rate. The remaining resistant individuals, whilst having sufficient GST abundance to survive, demonstrate a range of GST abundances and hence abilities to protect from xenobiotic damage. The implication of this plasticity is that the survivors of a particular herbicide treatment may have differing abilities to survive application of a second herbicide. Such plasticity may effect the appearance and development of cross-resistant populations in the field, and implies that the use of pristine populations in glasshouse-based research might not satisfactorily explain observations made in the field.

CONCLUSION

Herbicide-susceptible black-grass plants have low GST abundance compared to resistant individuals within the same population surviving a graminicide treatment. Assessment of GST abundance within black-grass populations may therefore form the basis of a quick field test for resistance. Plasticity of black-grass populations with respect to GST abundance was observed at all sites. These results suggest that, even among sub-populations surviving herbicide treatment, there may be considerable differences in an individual's ability to protect itself from herbicide damage.

ACKNOWLEDGEMENTS

We thank Syngenta Crop Protection UK for access to their field trial sites during the period of this study and The Home-Grown Cereals Authority for funding the initial stages of this work.

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Establishing background sensitivities of a range of species from different sites to a range of herbicide treatments.

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ABSTRACT

Seed from field populations of a range of species were collected and tested for background sensitivity to herbicides. Results are illustrated using *Papaver rhoeas* and *Stellaria media*. Plants raised from these populations were sprayed with five-seven doses of metsulfuron-methyl. One population of *S. media* showed much greater tolerance than did the other populations. Aspects of seed collection, sampling and interpretation of the results are discussed.

INTRODUCTION

Variability in the sensitivity of arable weeds to herbicides is widespread (Courtney & Hill, 1988) and many factors are undoubtedly implicated. One of the key factors may well be genetic variability within the UK populations of weed species and this has never been systematically investigated. The occurrence of resistance in weeds has been summarised by Heap (2001) and 23 species listed are found in the UK. The aim of baseline sensitivity testing is to get some idea of the scale of variation in herbicide response between weed populations. There is likely to be a requirement to submit baseline sensitivity data with submissions for registration of new active substances where a risk of resistance has been identified. Consequently any subsequent changes in sensitivity of a weed to the herbicide after it is introduced commercially should be detected more reliably if a good baseline has previously been established. This will enable any cases of evolved herbicide resistance to be identified promptly and unequivocally. Moss (2001) outlined his guideline to methodologies. This paper presents results from such an investigation and discusses some of the associated practical problems.

MATERIALS AND METHODS

Seeds and plant husbandry

Seed was collected from field areas in the UK, marked out to avoid treatment, or from areas which had been missed, or from plants which had survived. Wherever possible the seed was removed from the plants in the field but in a few cases plants were pulled up and the seed removed in the laboratory. Where necessary the seed was left to air dry on a laboratory bench shaded from direct sunlight.

The seed was stored dry in paper bags, at 4°C and where possible populations were retained for subsequent testing if required. Details of the history of herbicide usage for the past 10 years were requested from each seed collection site but were rarely available in full.

Approximately 50 seeds per pot were sown directly into 9 cm diameter plastic pots containing a soil-based compost (Kettering loam 5:1 grit) and 0.5-1.0 cm depth of compost was sprinkled over the seeds. A sub-group of each population was tested with one of two herbicides (Table 1). Each herbicide treatment consisted of five to seven doses plus untreated. Each treatment was replicated either three or four times, requiring 48 or 64 pots per population. The pots were laid out on trays, in their respective populations and kept in a heated (18/12°C) and illuminated (14/10 h day/night) glasshouse. The soil was kept close to field capacity by daily use of an overhead boom watering system. Plants were thinned at the cotyledon stage of development aiming for five plants/pot.

Treatments

The full list of species collected and the two herbicides selected for application to each are listed in Table 1 but only the results for *Papaver* rhoeas and *Stellaria media* are presented for illustration (Figures 1 and 2). Herbicides were applied using a pot sprayer delivering a volume of 225 litres/ha, at a pressure of 2.0 bar, through 02 F110 nozzles, set at a height of 35 cm above target leaf. Pots were then fully randomised within replicate blocks.

Table 1. Herbicide doses used for individual weed species tested.

Weed Species	Active substance	Herbicide doses g a.i. ha^{-1}							
Avena fatua	chlorotoluron	3505	2335	1750	1165	875	440	220	0.0
	fenoxaprop-P-ethyl	82.5	55.0	41.25	27.5	20.9	10.45	4.95	0.0
Galium aparine	fluroxypyr	200.0	132.0	100.0	66.0	50.0	25.0	12.5	0.0
	mecoprop	2850.0	1881.0	1425.0	940.5	712.5	356.25	177.84	0.0
Papaver rhoeas	metsulfuron-methyl	6.0	3.0	1.5	0.75	0.375	0.0		
	chlorotoluron	2750	1375	687.5	343.75	171.85	0.0		
Stellaria media	metsulfuron-methyl	6.0	3.0	1.5	0.75	0.375	0.0		
	mecoprop	1995	997.5	498.75	249.66	119.7	0.0		
Viola arvensis	isoproturon +	250.0	125	62.5	31.25	15.625	0.0		
	diflufenican	25.0	12.5	6.25	3.125	1.5625	0.0		
	metsulfuron-methyl	3.0	1.5	0.75	0.375	0.1876	0.0		

Assessments

Three weeks after spraying, plants were cut at the soil surface and fresh weights were recorded immediately. Fresh weights were then plotted against herbicide dose for each individual species population.

Analysis of data

A logistic curve of the form y=A+C/(1+EXP(B(x-M) was fitted to each set of data, where x was the dose and y was the weight meaned over the number of replicates. Parallel curve

analysis was then carried out across the various sets of data to see if logistic curves could be fitted keeping one or more of the parameters constant. This was generally not successful and separate parameters were needed for each set of data. An additional problem was that the three replicates for each set of data often produced different shape responses for each replicate. An alternative curve, the critical exponential, of the form y=A+(B+Cx)r**x was also fitted. This fitted the data slightly better, in that it allowed for an increase in fresh weight at the lower doses which was sometimes present in the data, but it was still not possible to calculate values for the effective dose to reduce fresh weight by 50% (ED_{50})with sufficient confidence from the curves of the means, because of the variable results obtained from each individual replicate. It was therefore decided to show the overall shapes of each individual set of data in order to give a general picture of what was happening, rather than presenting statistically fitted curves.

Field uniformity

Samples of black-grass were collected from each of three replicates in a field experiment which had been sprayed annually for four seasons with clodinafop-propargyl at 30 g a.i./ha. This was tested for resistance using the Rothamsted Rapid Resistance Test and in dose response experiments similar to that described above for *S. media*.

RESULTS

There were no major differences between the response of different populations of *P. rhoeas* to metsulfuron-methyl (Figure 1).

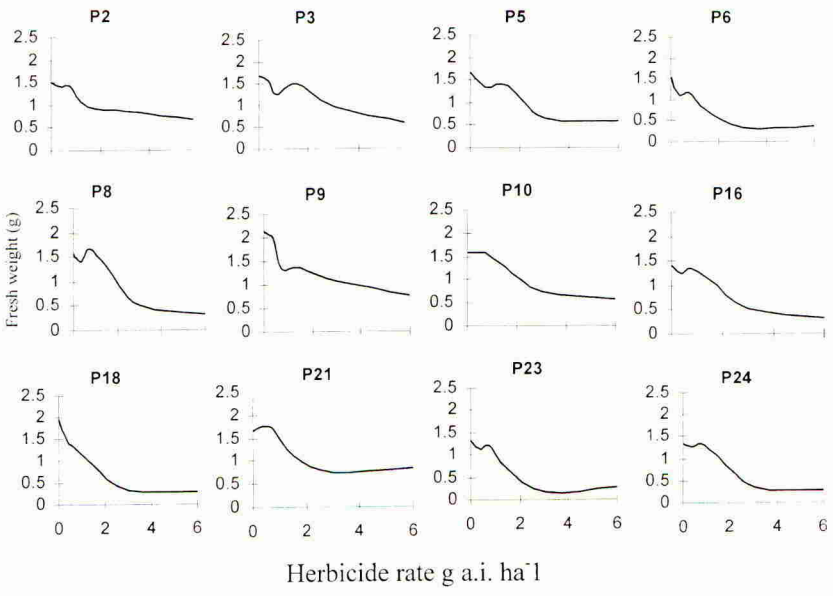

Figure 1. The response of a range of populations of *Papaver rhoeas* to increasing doses of metsulfuron-methyl (Fresh weight).

Of the *S. media* populations tested, CW30 was much more tolerant of metsulfuron-methyl than were the others (Figure 2). Differences between other populations were small.

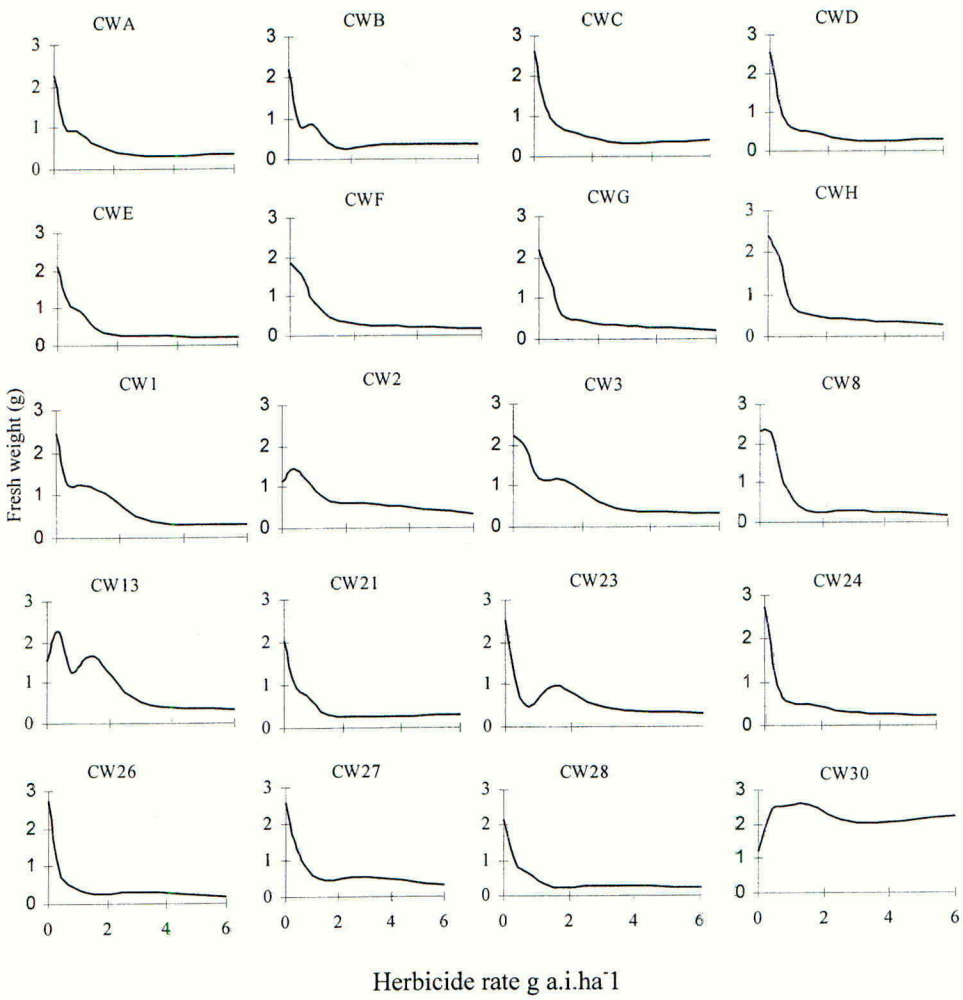

Figure 2. The response of a range of populations of *Stellaria media* to increasing doses of metsulfuron-methyl (fresh weight).

The results from the three replicate field plots (Table 2) demonstrated the importance of sampling procedures in the field and these results were confirmed for the individual replicates in the dose response experiment (not presented).

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Table 2. Resistance rating based on Rothamsted Rapid Resistance Test.

Test treatment	Field replicate		
	I	II	III
Pendimethalin	R?	S	S
Fenoxaprop	S	RR	RR
Sethoxydim	S	RR	RR

DISCUSSION

There are several aspects which have an important influence on background sensitivity testing procedures and many of these are discussed elsewhere (Moss, 2001) and supported by examples from this study.

In the series reported here the original proposal was to have a structured collection plan with two species being collected each year over a five year period. This proved difficult to implement and it may be better to identify and to collect the species as they become available and then store the seed until testing. Some species have small seeds (e.g. *V. arvensis*) and are low growing in the crop and these will tend to be more difficult to collect than species which protrude above the crop (e.g. *P. rhoeas*). Also, weed plants tend not to be very determinate with seed ripening at different times which may result in a need for more than one visit to collect seed. It can also be difficult to collect a representative number of samples for any one species. Ideally samples would be from deliberately unsprayed areas but the reality is that most will be from where herbicides have 'failed'. The example in Table 2 showed how important it is that the sample collected from the field and the final sub-sample used in the glasshouse for testing are as representative as possible of the field population. Hence it is important to collect from different parts of the field or if the weed is in patches to record this, and consider keeping them as separate samples.

Storage conditions clearly are very important both before testing samples and for the longer term. The approach used here assumes that time and storage does not influence species response to herbicides and that plants raised from freshly collected seed respond in the same way as plants raised from stored seed.

Establishing a uniform population of plants for testing would contribute to reduced variability within the test. Some species germinate and establish more reliably than others and in the ADAS resistance test (Clarke *et al.*, 1994) pre-germinated *A. myosuriodes* seed is used. This requires a lot of resource and is probably only possible where sample numbers are not large. We estimate for one black-grass sample that it could take five-seven hours to clean, pre-germinate and plant one population for testing against a range of herbicide doses. As each population of seed was obtained from an individual site it was not always possible to have adequate supplies to do more than one experiment and this was the case with the *S. media* population CW30 (Figure 2) and is not ideal. It is essential to attempt to have enough seed both for a repeat and for storage for the future. Where seed amounts are small it is important not necessarily to discard the sample as valuable information may be missed. Fewer replicates, fewer doses or only testing for one herbicide may be alternatives in this situation.

The selection of the correct herbicide dose range in glasshouse experiments is difficult particularly when there are no standards for comparison in the way that there are for *A. myosuroides* (Clarke *et al.*, 1994). One solution to this problem is clearer prior information on the dose response or more probably a greater range of doses. The practicality of having up to ten doses would have to be balanced against other factors such as the space this would take and seed availability. Where herbicide activity is strongly influenced by growing conditions at the time of the test, comparison between tests may be difficult unless there is a large range of doses.

Various curve fitting exercises were undertaken to derive ED_{50} values in this test but due to the large variability in the data it was only possible to establish significance between the response of various populations when differences were also very large e.g. *S. media* (Figure 2). The slight growth stimulation observed at low herbicide rates often occurs, particularly with sulfonylurea herbicides (Brain & Cousens, 1989) and can complicate any curve fitting exercise. Large variation within the sample of the degree seen in Table 1 would contribute to the variability and consequent precision of the analysis. Bulking field replicates for testing could mask variation.

The natural variation in weed populations in their response to herbicides needs to be quantified and future changes in sensitivity to herbicides identified which will result in field scale resistance. Small shifts in response will always be difficult to identify at an early stage.

ACKNOWLEDGEMENTS

We thank the Ministry of Agriculture, Fisheries and Food for funding this project and our many colleagues both within and outside ADAS for collecting seed samples in the field.

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Establishment of the baseline sensitivity of *Galium aparine* populations to florasulam

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ABSTRACT

In accordance with the EPPO guideline for the efficacy evaluation of plant protection products; resistance risk analysis PP 1/213(1) a method to determine the baseline sensitivity of key weed species was established. The aim of the baseline monitoring project was to understand the natural variation in herbicide response of diverse populations of *Galium aparine* to florasulam, at the time of product launch. The method entailed seed collection from representative agricultural areas throughout Northern Europe. The seed was germinated under glasshouse conditions and the subsequent plants treated with florasulam at rates ranging from 1 to 20 g ai/ha. Data was analysed using regression analysis and sensitivity indices, calculated for each country and across Europe. Thus giving an indication of the variation in *G. aparine* response to florasulam in the populations tested.

INTRODUCTION

To comply with Dow AgroSciences product stewardship strategies and the EPPO standard for the efficacy evaluation of plant protection products: resistance risk analysis PP 1/213(1) (OEPP/EPPO, 1999) the baseline sensitivity of *Galium aparine* to Boxer/Primus (50 g ai/l florasulam) was established. Florasulam is a new acetolactate synthase inhibitor (ALS) with activity against *G. aparine* and a number of other key dicotyledonous weeds in cereals. The EPPO guideline for resistance risk analysis requires the baseline sensitivity of key species to new actives to be established and monitored. The baseline sensitivity of populations of *G. aparine* to florasulam was established over a two year period to capture variations in population sensitivity. Testing over a two year period also allowed variations in data from glasshouse studies to be evaluated.

The aim of the baseline monitoring project was to understand the natural variation in herbicide response of diverse populations of *G. aparine* to florasulam, at the time of introduction of the new active substance. An accurate baseline is essential for future monitoring programs to be able to detect shifts in sensitivity, quickly and accurately. Thus allowing both an evaluation of the effectiveness of the resistance strategy in place and an opportunity to address how this may have to be altered to manage the occurrence of resistance once it has been detected.

This paper describes the methods developed and used to establish the baseline sensitivity of *G. aparine* populations collected throughout Northern Europe and the proposed method for monitoring of sensitivity.

ESTABLISHMENT OF THE BASELINE SENSITIVITY

Materials and methods

To establish the baseline sensitivity, *G. aparine* seeds from the UK, France and Germany were collected (Table 1). Representative areas of the field population were identified and marked out prior to herbicide application, headlands were considered inappropriate areas and were avoided. When seed was ripe, determined by colour of pod and ease of seed shedding, 100 g was collected and stored under cool conditions (4-7°C seed store).

One hundred grams of seed was collected to provide sufficient seed to use as a standard for resistance testing in subsequent years. In addition to the seed collected throughout Europe, two reference populations of *G. aparine*, supplied by Herbiseed UK were tested. One of these reference populations was autumn germinating and the other was spring germinating.

Table 1. Number of sites sampled in each country during the 1999 and 2000 seasons

Country of origin	1999	2000
UK	5	4
France	5	0
Germany	3	2
Hungary	1	0

The populations sampled during 1999 were predominately from agricultural areas where no ALS herbicides had been used previously for the control of *G. aparine*. Seed samples collected in 2000 were from untreated plots in florasulam trial sites, where commercial levels of control had been achieved. The samples collected in 2000 were tested in the glasshouse alongside those collected the previous year to provide two years data for the establishment of the baseline. For each collected sample, data regarding the historical herbicide usage to control *G. aparine* over the previous five years was collected using a standardised form. Variation in farmer records meant that it was not always possible to obtain the complete five year history for the site.

Seeds collected from these sites were pre-germinated in seed trays containing a peat-based soil. Plants were allowed to germinate under glasshouse conditions. When seedlings reached the cotyledon/first leaf growth stage they were transplanted into pots containing a sandy loam soil. Plants were propagated under glasshouse conditions, 14h-day length and temperatures of 12-15°C.

Florasulam was formulated as a 50 g ai/l SC and applied at rates ranging from 1 g ai/ha to 20 g ai/ha. Post-emergence applications were made when all populations were at a uniform growth stage of BBCH 12-13. Treatments were applied using an overhead track sprayer, reservoir pressure 210 kPa, 'TeeJet' SS8003, calibrated to deliver 200 l/ha. To ensure the generation of reliable dose response curves a minimum of 7 rates and 5 replicates were used.

Assessments were made when the full effects of the herbicide were evident on the reference population. Visual control was assessed as a percentage of the untreated, with 0 representing

no control and 100 representing plant death, at 14 and 21 days after application. Foliar fresh weight measurements were made 21 days after application (DAA). Plants were watered 1 hour prior to fresh weight measurement to ensure full turgor at time of assessment.

Dose response curves, using the 21 DAA visual and fresh weight data (expressed as a % of the untreated) were generated, ED 80 (Dose required to give a 80 % reduction in foliar fresh weight relative to the untreated) values (g ai/ha) were then calculated for each population using Minitab v 12.2. To demonstrate the differences in the sensitivity of the populations to florasulam a Sensitivity Index (SI) was used;

$$SI = ED\ 80\ A\ /\ ED\ 80\ B$$

Where:

A = ED 80 of most tolerant population (g ai/ha)
B = ED 80 of most sensitive population (g ai/ha)

The ratio was calculated for each country and across Europe to illustrate differences in sensitivity of *G. aparine* populations to florasulam.

RESULTS AND DISCUSSION

The ED 80 values (g ai/ha) generated using foliar fresh weight data ranged from 0.99 to 3.31 g ai/ha (Table 2) giving a sensitivity index of 3.34 across the UK (Table 2). The pattern was similar in France, with ED 80 values (g ai/ha) for foliar fresh weight data ranging from 1.52 to 5.2 g ai/ha and a sensitivity index of 3.4.

The variation in population sensitivity in Germany was very similar to that observed in the UK and France. ED 80 values for German populations ranged from 1.52 to 4.55 g ai/ha, with a sensitivity index of 2.99 based on foliar fresh weight data. Across N. Europe the ED 80 values based on foliar fresh weight ranged from 0.99 to 5.25 g ai /ha with a sensitivity index of 5.30.

Table 2. ED 80 values with 95 % confidence limits (g ai/ha) for percent visual control and foliar fresh weight 21 days after application of florasulam – Evaluated at Letcombe in 2000.

Sample number	Country of origin	ED 80 (g ai/ha) % visual control	ED 80 (g ai/ha)– foliar fresh weight
Reference – spring	UK	6.03 (4.60-7.89)	2.05 (1.44-2.91)
Reference – autumn	Germany	4.89 (3.60–6.65)	2.01 (1.53-2.64)
5328	UK	3.29 (2.50-4.31)	0.99 (0.66-1.49)
5343	UK	10.19 (7.4-13.99)	2.84 (2.22-3.66)
5344	UK	4.62 (3.42-6.24)	1.06 (0.63-1.78)
5345	UK	4.27 (3.38-5.40)	1.00 (0.67-1.51)
5347	UK	6.91 (5.47-8.72)	2.65 (2.14-3.28)
5401	UK	6.44 (4.82-8.60)	3.31 (2.50-4.37)
5402	UK	6.76 (5.24-8.71)	1.48 (1.07-2.05)
5403	UK	9.8 (7.6-12.5)	2.62 (1.94-3.53)
5398	UK	6.53 (5.40-7.89)	2.90 (2.41-3.49)
5314	France	4.87 (3.97-5.96)	1.52 (1.10-2.12)
5319	France	6.2 (4.9-7.8)	3.49 (2.78-4.38)
5290	France	9.3 (7.14-2.2)	5.2 (4.2-6.6)
5331	France	6.86 (5.52-8.53)	2.95 (2.44-3.57)
5335	France	11.35 (8.4-15.4)	2.17 (1.6-2.9)
5325	Germany	4.36 (3.08-6.17)	1.52 (0.92-2.51)
5326	Germany	7.4 (5.5-10.0)	1.98 (1.46-2.70)
5365	Germany	5.29 (4.06-6.89)	1.93 (1.45-2.59)
5388	Germany	10.2 (8.17-12.3)	4.55 (3.89-5.34)
5364	Germany	7.18 (5.58-9.25)	2.07 (1.54-2.77)
	Hungary	6.19 (4.62-8.30)	1.21 (0.68-2.14)

The variation observed in ED 80 values based on visual control data were slightly less than those recorded using foliar fresh weight data (Fig. 1) with sensitivity indices of 3.09, 2.33 and 2.33 respectively for the UK, France and Germany. The sensitivity index based on visual control data for N. Europe was 3.45. Both methods of assessment indicated the range of herbicidal sensitivity of *G. aparine* to florasulam to be narrow with a two- to four-fold difference between the most and least sensitive populations, within a country.

Frequency plots (Fig 2) illustrate that for both the UK and Germany > 80% of the populations tested had ED 80 values between 1-3 g ai/ha based on foliar fresh weight data. In France this figure was lower, with 60 % of the populations tested having ED 80 values between 1 and 3 g ai/ha.

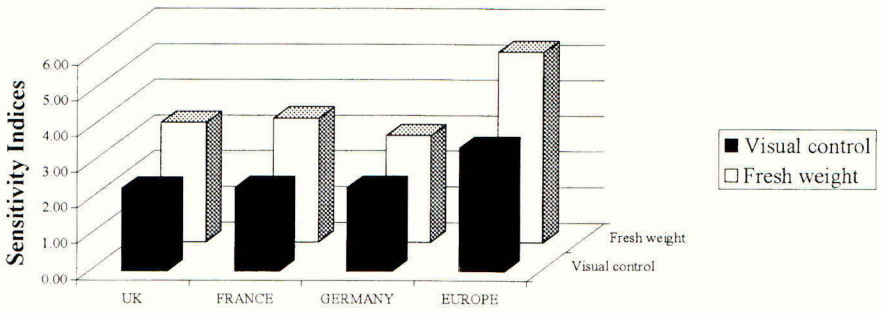
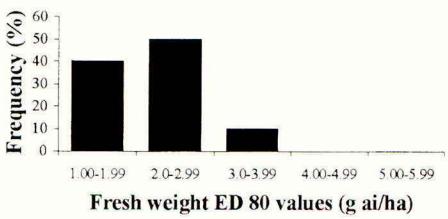
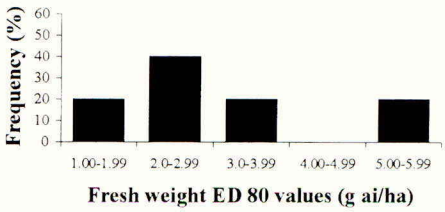


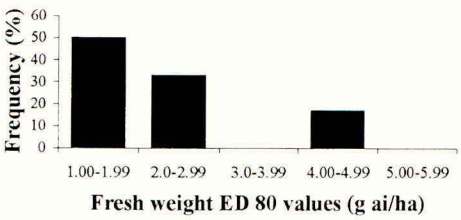
Figure 1: Sensitivity indices of *Galium. aparine* populations to florasulam from UK, France and Germany for seed collected in 1999 and 2000. SI calculated for fresh weight and visual assessments.



(A) UK



(B) France



(C) Germany

Figure 2: Frequency distribution of fresh weight ED 80 values (g ai/ha) for *Galium aparine* populations in 1999 and 2000. (A) UK, (B) France, (C) Germany.

Data from this study demonstrates that variation in herbicidal sensitivity of susceptible populations of *G. aparine* to florasulam does occur. The sensitivity of populations ranged from 2 to 3.44 fold depending on country of origin or method of assessment. This level of variation in herbicidal response is similar to that previously report by Hill and Courtney (1991) who reported a three-fold difference between the most and least sensitive population of *G. aparine* to mecoprop and fluroxypyr. Data from these studies demonstrate the need for care to be taken when interpreting small sensitivity indices generated from resistance testing or future baseline monitoring.

When diagnosing resistance, data generated in the laboratory should always be related back to activity observed in the field and the herbicide treatment history of that field. Where small changes in sensitivity, three- to four-fold, have occurred other possible reasons for herbicide failure, such as growth stage, environmental conditions and rate of use should be considered before concluding that resistance has developed. The variations observed between the populations evaluated in these studies reinforces the need for a reference standard or standards to be included in any future testing.

MONITORING OF SENSITIVITY

The objective is to continue monitoring the sensitivity of *G. aparine* populations for any shifts quickly and accurately, thus allowing the early recognition of resistance and effective management of resistance strategies with the aim of containing potential adverse effects.

The sensitivity of *G. aparine* to florasulam is currently being monitored by collecting seed from existing trial sites, demonstration plots and any commercial complaints where no clear explanation is apparent for the performance failure. This seed will be propagated under glasshouse conditions and ED 80 values generated from the dose response curves, which can then be compared to the current baseline.

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Response of a quinclorac-resistant false cleaver (*Galium spurium*) biotype to several auxinic herbicides

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ABSTRACT

Due to lack of control following treatment with an ALS herbicide, *G. spurium* seeds were collected from an Alberta field. ALS resistance was due to target-site insensitivity resulting from a point mutation in the ALS gene. This ALS-resistant biotype was also resistant to quinclorac. We are interested in characterizing quinclorac resistance in this *G. spurium* biotype, particularly the pattern of response to other auxinic herbicides. Plants were treated at the 3- to 4-whorl stage of development with $^{1}/_{4}$, 1, and 4 times the field dose of the following auxinic herbicides (1x dose in g a.i./ha): quinclorac (125.0), triclopyr (229.7), dicamba (290.4), fluroxypyr (144.1), picloram (273.8), clopyralid (306.2), and 2,4-D (568.5). Plants were harvested 14 DAT. Symptoms varied with the different herbicides and ranged from leaf hyponasty/epinasty to whole plant wilting and death. LD_{50} values for quinclorac-resistant and –susceptible biotypes were >1500 and 47 g a.i./ha, respectively. Based on calculated LD_{50} values, the resistant biotype was moderately resistance to triclopyr but not to the other auxinic herbicides tested. Cross-resistance of this *G. spurium* biotype to quinclorac and triclopyr suggests that the mechanism of resistance may be similar and related to similar chemical structure. However, differences in phytotoxic response of both biotypes suggest that each auxinic herbicide tested cause slightly different physiological responses in *G. spurium*.

INTRODUCTION

Most of the 19 species found worldwide that are resistant to auxinic herbicides (Heap, 2001), are cross-resistant to different auxinic herbicides. For example, when compared to the susceptible biotype, an auxinic-herbicide resistant wild mustard (*Sinapis arvensis*) biotype was highly resistant to picloram and dicamba, moderately resistant to 2,4-D and MCPA, but susceptible to MCPP and 2,4-DP (Penuik *et al.*, 1993). Populations of nodding thistle (*Carduus nutans*) were resistant to 2,4-D, MCPA and MCPB but susceptible to clopyralid (Harrington, 1996). In addition, a picloram-resistant yellow starthistle (*Centaurea solstitialis*) biotype was resistant to clopyralid, fluroxypyr and dicamba but susceptible to triclopyr and 2,4-D (Fuerst *et al.*, 1996). Other auxinic herbicide-resistant plants have been found that have variable response to different auxinic herbicides (Whitehead & Switzer, 1963; Bell *et al.*, 1972). In all the previous examples, auxinic herbicides were used repeatedly in the locations where the resistant biotypes were found. In contrast, the development of quinclorac resistance was not based on repetitive quinclorac use (Lopez-Martinez *et al.*, 1997; Hall *et al.*, 1998).

Quinclorac and quinmerac, members of the quinolinecarboxylic acid family of herbicides, are classified as auxinic herbicides (Grossmann, 2000). Generally, susceptible dicotyledonous

species display symptoms similar to those caused by auxinic herbicides, such as epinasty (Berghaus & Wuerzer, 1987; Grossmann, 2000). However, there is some debate whether the quinolinecarboxylic acid herbicides are 'true' auxinic herbicides because they have activity against grasses and some phytotoxic symptoms in dicotyledonous species are different from those of the benzoic acid, pyridinecarboxylic acid, and phenoxyacetic acid herbicide families. To date, there have been no reports of broad-leaved weed species with resistance to quinclorac, other than this *G. spurium* biotype (Hall *et al.*, 1998). In contrast, two quinclorac-resistant grass species, smooth crabgrass (*Digitaria ischaemum*) (Koo *et al.*, 1994) and barnyardgrass (*Echinochloa crus-galli*) (Lopez-Martinez *et al.*, 1997) have been described. To our knowledge, cross-resistance to other auxinic herbicides has not been characterized in these grass biotypes.

The objective of our research was to characterize the phytotoxic response of resistant and susceptible *G. spurium* biotypes based on susceptibility, tolerance and resistance to different auxinic herbicides. Accordingly, it may be possible to link structure-activity relationship of the herbicides to relative phytotoxicity in resistant and susceptible *G. spurium* biotypes (Figure 1).

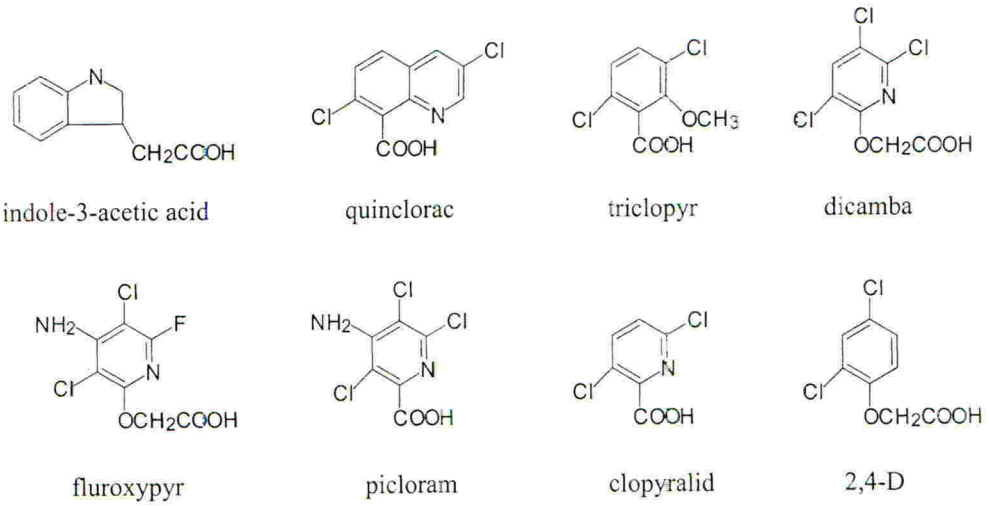

Figure 1. Molecular structures of indole-3-acetic acid, quinclorac, and the auxinic herbicides tested for cross-resistance in *Galium spurium*.

Discovery of the quinclorac-resistant *G. spurium* biotype was unusual. *G. spurium* seeds used in these experiments were collected in an Alberta field because of suspected resistance to sulfonylurea herbicides. This field was sprayed three out of six years with ALS inhibitors, but, quinclorac had never been used (Hall *et al.*, 1998). A susceptible biotype was collected in a nearby field. Greenhouse experiments confirmed that the *G. spurium* biotype was resistant to several ALS herbicides and quinclorac, but not to the auxinic herbicides fluroxypyr or MCPA/mecroprop/dicamba (Hall *et al.*, 1998). ALS resistance was attributed to target-site insensitivity based on ALS enzyme inhibition (Hall *et al.*, 1998) and a point

mutation in the ALS gene (Horsman & Devine, 2000). Our research focuses on quinclorac resistance and the possible cross-resistance to other auxinic herbicides.

MATERIALS AND METHODS

Growth of plants

G. spurium were grown, one plant per pot (600mL), in Premier Promix (Premier Horticulture Inc. Red Hill, PA), a peat moss-based potting medium. The plants were irrigated daily with water and fertilized as required, three to four times a week with 20-20-20 (N:P:K) fertilizer (20 g/litre) containing micronutrients. Plants were grown in a controlled environment growth room maintained at 24/16 ± 1°C day/night temperature with a 16-h photoperiod and an average relative humidity of 65%. The irradiance level was constant at 450 $\mu E/m^2/sec$.

Treatment and harvest of plants

G. spurium plants were sprayed at the 3- to 4-whorl stage of foliar development. The commercial formulation of each herbicide was used at $^1/_4$, 1, and 4 times the recommended field dose required for *G. spurium* control in Western Canada (Anonymous, 2000). The 1x dose in g a.i./ha and the commercial formulation for each herbicide were as follows: 125, quinclorac (Accord, BASF Corporation Canada); 229.7, triclopyr (Release, DowAgro Sciences Canada); 290.4, dicamba (Banvel, BASF Corporation Canada); 144.1, fluroxypyr (Vista, DowAgro Sciences Canada); 273.8, picloram (Tordon 22K, DowAgro Sciences Canada); 306.2, clopyralid (Stinger, DowAgro Sciences Canada); and 568.5, 2,4-D amine (Amsol 500, Rhone-Poulenc Canada Inc.). Quinclorac was sprayed with 1% v/v Merge (BASF Corporation Canada), however, no adjuvants were used with the other herbicides. To fully characterize quinclorac resistance, doses from 10.4-1500 g a.i./ha were used. All herbicides were applied with a motorized hood sprayer equipped with a flat-fan nozzle (80015E TeeJet Spraying Systems Co. Wheaton, Il) calibrated to deliver 110 litres/ha of spray solution at 250 kPa. Visual ratings of phytotoxic symptoms were determined prior to harvest 14 DAT. Shoot dry weight was determined.

Statistical analysis

All experiments were repeated twice, with at least three replications per treatment. Shoot dry weight data were expressed as a percentage of the mean of the untreated control. Statistical analysis on shoot dry weight data was performed with SAS 8.0 software (SAS Institute Inc. Cary, NC) using PROC MIXED model at the 95% confidence level. Experiments were pooled to calculate LD_{50} values using EPASTATS PROBIT 1.5 analysis. Resistance ratios were calculated using LD_{50} values by dividing the resistant biotype by the susceptible biotype.

RESULTS AND DISCUSSION

The resistant *G. spurium* biotype was resistant to quinclorac; LD_{50} values could not be calculated because there was no mortality at any of the doses tested (10.4-1500 g a.i./ha) (Figure 2a). The calculated LD_{50} for susceptible biotype was 47 g a.i./ha. At doses of 10.4 g a.i./ha and higher there was a reduction in the susceptible biotype shoot biomass compared to the untreated control (Figure 2b). Symptoms of quinclorac phytotoxicity in the susceptible biotype include leaf hyponasty, reduced leaf area, chlorosis, necrosis, and plant death.

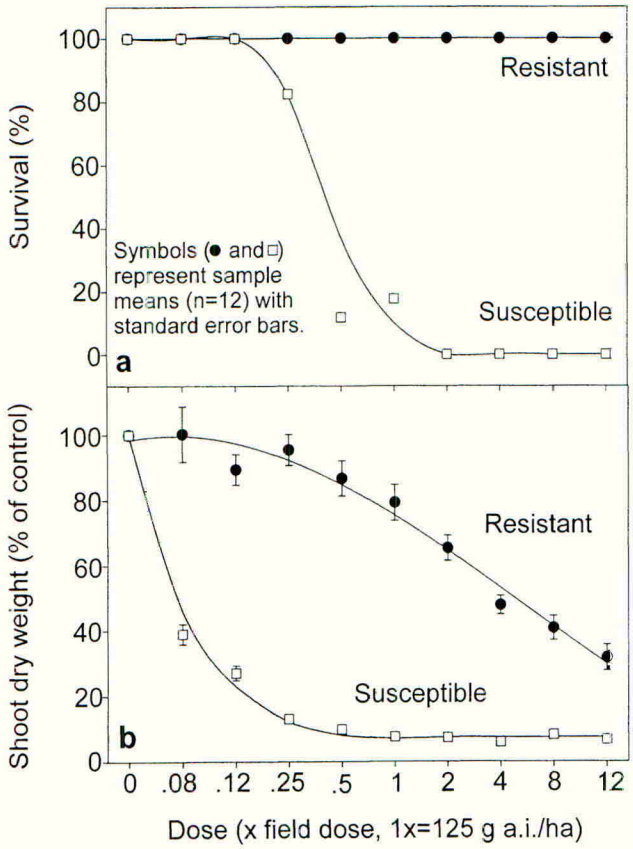

Figure 2. Response characterized by **a**) percent survival and **b**) shoot dry weight of quinclorac-resistant and -susceptible *Galium spurium* treated with quinclorac. Where no SE bars are shown, the standard error was smaller than the symbol.

Despite reduced shoot growth in resistant plants treated with 1500 g a.i./ha of quinclorac there were no phytotoxic symptoms other than minor chlorosis of some leaf tips. Phytotoxic symptoms were dependent on the auxinic herbicide used. For instance, 2,4-D-induced symptoms included shortened internodes and decreased leaf elongation 14 DAT with 2 kg/ha of 2,4-D amine. The 1x field dose of fluroxypyr caused whole plant wilting, chlorosis and necrosis. At the high dose, triclopyr reduced internode length, but leaf expansion was not inhibited in *G. spurium*. In contrast, at the 4x dose clopyralid-induced symptoms included darkened older leaves and narrow, spike-like, new leaves. Differences in phytotoxic response of both biotypes suggest that each auxinic herbicides tested caused different physiological responses in *G. spurium*.

For all herbicides, other than quinclorac, there were no differences in shoot dry weight between the resistant and susceptible biotypes (data not shown). Both *G. spurium* biotypes were tolerant to 2,4-D and clopyralid because for both biotypes the LD_{50} was at least 4x the field dose. In contrast, both biotypes were highly susceptible to picloram; the $^1/_4$x dose was lethal to both biotypes. Conversely, the resistant biotype was highly resistant to quinclorac and moderately resistance to the pyridinecarboxylic acid herbicide, triclopyr (Table 1).

Based on the lack of extensive cross-resistance to the tested auxinic herbicides, it is unlikely that previous field use of auxinic herbicides contributed to the selection of the resistant *G. spurium* biotype. Evidence in the literature indicates that quinclorac does not have exactly the same mechanism of action as 2,4-D and other auxinic herbicides, even though quinolinecarboxylic acid herbicides do have distinct auxin activity (Berghaus & Wuerzer, 1987; Sunohara & Matsumoto, 1997; Grossmann, 2000). The cross-resistance of resistant *G. spurium* to quinclorac and triclopyr suggest that the mechanism of resistance may be similar and related to the similar structure of these herbicides (Figure 1).

Table 1. LD_{50} values and resistance ratios for *Galium spurium* treated with auxinic herbicides and harvested 14 DAT.

Herbicide	LD_{50} [a]		Resistance
	Resistant	Susceptible	Ratio
Quinclorac	>12	0.38*	>31.6
Triclopyr	2.94	0.97*	≈3.0
Dicamba	0.50	<0.25	≈2
Fluroxypyr	0.56	<0.25	≈2
Picloram	<<0.25	<<0.25	≈1
Clopyralid	≈4	≈4	≈1
2,4-D	>4	>4	≈1

[a] LD_{50} values are expressed as x of field dose. *Indicates a significant difference between the LD_{50} values of the resistant and susceptible biotypes based on the 95% confidence limits.

CONCLUSIONS

The estimated cost of alternate strategies for managing herbicide-resistant weeds can be large (Beckie *et al.*, 1999). Quinclorac is a very effective herbicide for controlling *G. spurium*, and therefore provides farmers with a valuable tool for controlling this troublesome weed. The loss of quinclorac use due to resistance will have a serious economic impact on Canadian agriculture. Currently, research is being conducted to determine the mechanism of quinclorac resistance in *G. spurium*. Furthermore, the link between sulfonylurea and quinclorac resistance will be characterized.

ACKNOWLEDGEMENTS

Financial support of this research was provided, in part, by the Natural Sciences and Engineering Research Council (NSERC) of Canada. Grateful thanks are extended to Dr L M Hall for providing the *G. spurium* seed.

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Bromus diandrus population with increased tolerance to metribuzin

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ABSTRACT

A random survey of grass weeds has been done in three cereal crop areas of Spain. Glasshouse assays, conducted for metribuzin response of *Bromus diandrus* populations, detected two populations with 40% and 65% respectively of plants not damaged by a pre-emergence treatment of a 300 g a.i./ha dose of metribuzin, (susceptible population showed 2% of plants alive). The possible mechanism of metribuzin tolerance was studied in these populations by means of single plant detection of chlorophyll fluorescence in glasshouse and growth chamber assays. The data show that all three populations indicate the same pattern of chlorophyll fluorescence response. In the case of a few plants there was no inhibition of photosynthesis by the herbicide.

INTRODUCTION

Species show genetic variability in numerous characters (Allard *et al.*, 1968). In cultivated areas repeated herbicide treatments has led to a selective evolution of weeds, which in turn has led to the appearance of resistance. A previous stage (not apparent in the field) will manifest an increase of proportion of resistant plants and/or a decrease of response in a given population. This is the object of a broad study we have undertaken to determine the response of graminaceous weed populations to herbicides. There are very few references to resistance to metribuzin and to *Bromus diandrus* (Heap, 1999; Mengistu *et al.*, 2000). We have detected two populations of *B. diandrus* with a tolerance to metribuzin (Rodríguez *et al.*, 2000).

Metribuzin is an inhibitor of photosystem II (PSII) that has been used to control brome in winter cereals (Peeper, 1984). The measurement of chlorophyll fluorescence allows the study of the kinetics of translocation and/or detoxification of PSII inhibitor herbicides (Brewer *et al.*, 1979; Cadahia *et al.*, 1982; Ducruet, 1991), as well as the modification of the site of action of the herbicide (Ali & Machado, 1984; Mengistu *et al.*, 2000).

In this work we compare the behaviour of three field populations of *B. diandrus* in relation to metribuzin treatment by means of plant fresh weight inhibition, number of plants not damaged by metribuzin and by PSII inhibition.

MATERIALS AND METHODS

Three populations of *B. diandrus* out of 62 collected at random in cereal fieds in Spain were studied. Two of them, populations 59 and 104, were metribuzin-tolerant, while population 115 was sensitive to this herbicide (Rodríguez *et al.*, 2000).

Plant weight assay

Three populations of *B. diandrus* were sprayed at doses of 0 and 300 g a.i./ha of metribuzin 24 hours after sowing. Plants were grown in pots containing compost: sheep manure, sand and soil (1:1:1 by volume), using 100 seeds per pot and 6 replicates per treatment. Plants were maintained in the glasshouse under controlled conditions (12±2° C by night and 20±5° C by day) without additional illumination. Six weeks after treatment, the fresh weight of plants and the number of plants not damaged by the herbicide were measured.

Glasshouse chlorophyll fluorescence measurements assay

To study the effective quantum yield of photochemical energy conversion in photosynthesis, 500 seeds of each population were sown and treated as in the above assay. Sixteen, 19, 21, 23, 26 and 29 days after treatment (DAT) the chlorophyll fluorescence yield (yield parameter ΔF/Fm') was measured (MINI-PAM, a portable chlorophyll fluorometer (H. Walz, Germany)) in the base and in the apex of the first leaf of 50 plants chosen at random from each one of the populations.

Chamber culture chlorophyll fluorescence measurements assay

Germinated seeds, were placed in beakers filled with 175 ml of Hewitt nutrient solution. The seedlings, 25 plantlets treated and 6 control, repeated four times, were grown in a growth chamber (8 hours dark at 16±1°C , 16 hours light at 22±1°C and 160 μmol $m^{-2}s^{-1}$). At plant Growth Stage 12 the nutrient solution was replaced for 24 hours by a similar nutrient solution containing 0.2 ppm of metribuzin. Chlorophyll fluorescence was measured in the base and the apex of the second leaf of plants 24 hours after treatment (T0) and 1 (T1), 2 (T2), 4 (T4) and 7 (T7) days after treatment in each individually identified plant. The fluorescence measurements (I-O)/Fv (Ducruet *et al.*, 1984) were obtained by means of a fluorescence detector (Hansatech Ltd) and the signal was analysed by means of a computer program (Ducruet *et al.*, 1993).

RESULTS AND DISCUSSION

Plant weight assay

A survey of 62 populations of *B. diandrus* showed that 35 populations were susceptible, with less than 25% of plants surviving treatment and 16 populations being intermediate. The response in the glasshouse of three populations of *B. diandrus* to a pre-emergence treatment of metribuzin at doses of 300 g a.i./ha compared to the untreated control is shown in Table 1. Inhibition of the fresh weight of the aerial part of the plants showed that the susceptible population 115, had a plant growth inhibition of nearly 90%, while the tolerant populations showed an inhibition in weight of 43% and 29%. The data correspond with the number of plants not damaged by metribuzin in those three populations and shows that populations 59 and 104 are more tolerant to metribuzin than population 115. Since metribuzin is a PSII inhibitor two different types of assays have been carried out.

Glasshouse chlorophyll fluorescence measurements assay

Table 2 shows the effective quantum yield of photochemical energy conversion in photosynthesis, by means of chlorophyll fluorescence yield (yield parameter ΔF/Fm´)

measured in the first leaf of the plants.

Table 1. Plant response of populations 59, 104 and 115 of *B. diandrus* to metribuzin

Population	Fresh weight (% of control)	% of plants not damaged
59	57	40
104	71	65
115	13	2

Table 2. Distibution frequency fluorescence scores using the yield parameter ΔF/Fm' for the three *B. diandrus* populations. (All control plants belongs to < 40 class).

Popul.	% inhib. ΔF/Fm'	16 DAT		19 DAT		21 DAT		23 DAT		26 DAT		29 DAT	
		b	a	b	a	b	a	b	a	b	a	b	a
59	100	44	40	22	44	64	70	52	58	88	88	84	82
	80-100	38	14	42	12	30	18	40	16	12	6	12	6
	60-80	14	18	24	6	6	4	8	12	0	4	2	4
	40-60	4	28	12	28	0	8	0	14	0	2	2	8
	< 40	0	0	0	10	0	0	0	0	0	0	0	0
104	100	50	32	48	48	52	60	86	88	84	86	98	94
	80-100	32	20	20	22	38	22	14	8	14	6	2	4
	60-80	14	8	4	0	6	10	0	0	2	6	0	0
	40-60	4	40	4	2	2	6	0	4	2	6	0	0
	< 40	0	0	4	8	0	0	0	0	0	0	0	0
115	100	48	38	54	58	70	68	86	82	88	92	92	90
	80-100	40	24	26	18	26	22	14	18	10	6	4	6
	60-80	10	20	10	8	0	6	0	0	2	2	2	0
	40-60	2	18	10	14	4	4	0	0	0	0	2	4
	< 40	0	0	0	2	0	0	0	0	0	0	0	0

Eighty percent of plants show high levels of inhibition in the base of the leaf and practically 50% show a 100% inhibition. A very reduced proportion of plants has photochemical energy conversion in photosynthesis values similar to that of the controls. The inhibition of activity increases over time in view of the constant presence of the herbicide, and only a few plants show low or intermediate levels of inhibition.

Chamber culture chlorophyll fluorescence measurements assay

The treatments undertaken in hydroponic cultures in which parameter (I-O)/Fv has been measured in the apex and base of the second leaf (Figure 1), show that at T0 between 100% and 92% of the plants in the three populations are completely inhibited in the base. This inhibition becomes intermediate or low at T1 and decreases at T2. In the apex, at T0, in population 115, 55% of the plants were completely inhibited, whereas in population 59, 10% and in population 104, 30% were inhibited. At T1, distribution of plants by classes was very similar, both time-and population-wise.

Available data shows that there was a different response of plants to metribuzin in the case of the populations studied. This difference is not due to factors related to photosynthetic activity (for instance, a mutation leading to insensitivity in the site of action (Ryan, 1970; Mengistu *et al.,* 2000)). Neither have differences been observed in the responses to the measures of fluorescence that allow linking herbicide tolerance to different metribuzin absorption, translocation or metabolization mechanisms, as shown in the cases of different species (Gawronski *et al.*, 1986; 1987; Devlin *et al.*, 1987; Villarroya *et al.*, 1993). Tolerance to metribuzin has been controversial ever since it appeared, because despite the fact that its primary action mode is known (Ducruet, 1991), there are numerous contradictions in the papers on the effects of this herbicide, with inexplicable variations in the field tests. The tolerance mechanisms are diverse and there is a great intraespecific variation (Villarroya *et al.*, 1993; Al-Khatib *et al.*, 1997). Also, the genetic determinism of tolerance is polygenic in some species as in wheat (Villarroya *et al.,* 2000). Apart from which, the herbicide is very sensitive to external conditions: to the contents of the organic matter in soil, which reduces its effect, and to humidity, light and temperature, which increase it (Al-Khatib *et al.*, 1997; Janssen & Hasselt, 1994). Those of our assays that have been undertaken in glasshouses in the winter, to determine the response in weight, and in the spring, to determine fluorescence, may well have been affected by the glasshouse conditions. These external conditions may in turn have affected the plants´ response to photosynthesis-inhibiting herbicides, since they increase their effects on tolerant plants when the temperature is high (Ducruet & Lemoine, 1985; Janssen & Hasselt, 1994). However, there is always a small proportion of electron chains which are sufficiently active to guarantee the plant´s survival (Ducruet, 1991) and the activity of which may be intensified in some genotypes. In our assays, healthy four-leaf plants present levels of quasi-total fluorescence inhibition. On the basis of these results we have determined that the assays must be confirmed under strictly controlled conditions.

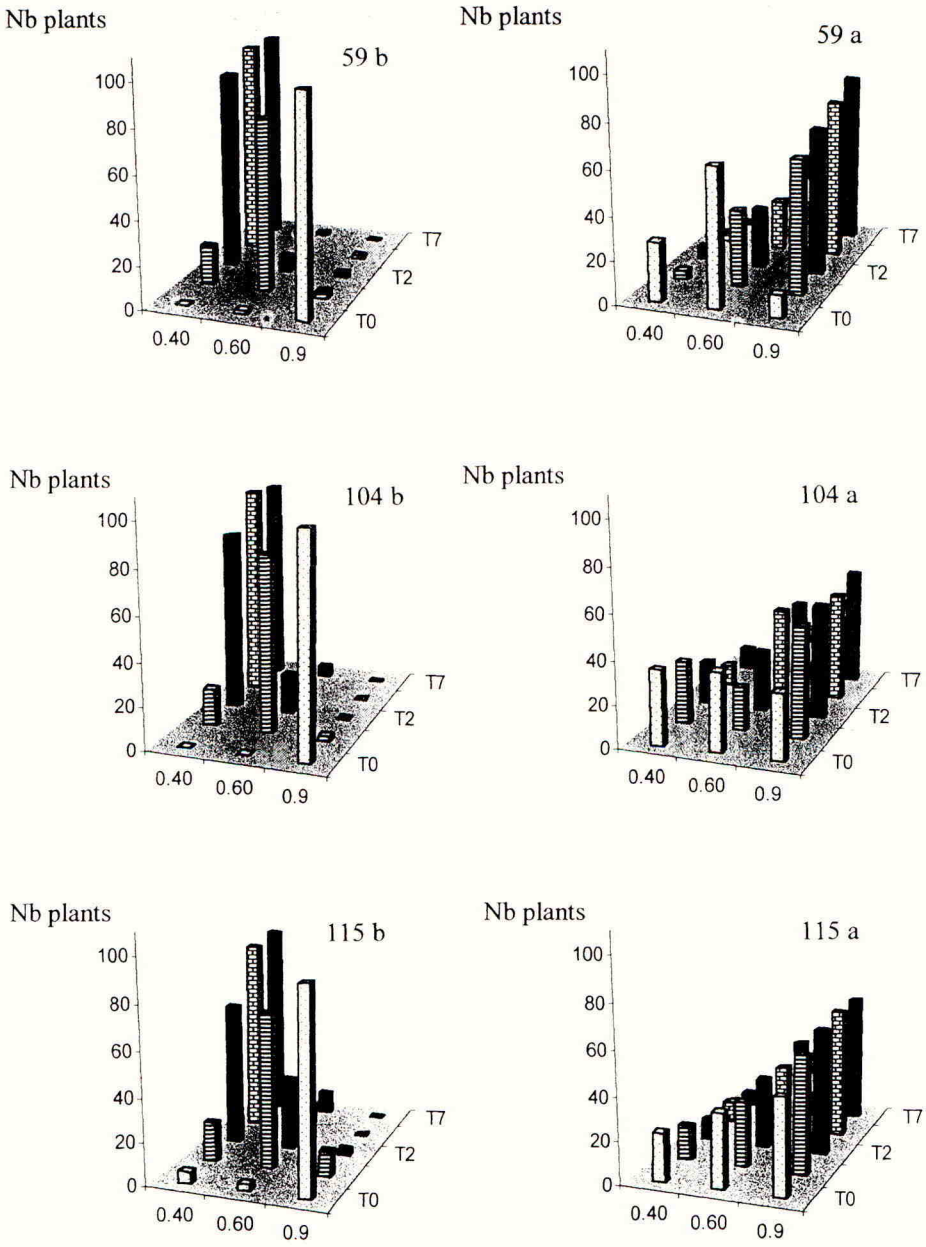

Figure 1. PSII inhibition in leaves of three populations of *B.diandrus*, after 24 hours of metribuzin treatment using the ratio (I-O)/Fv. (a) leaf apex (b) leaf base.
(Nb plants = number of plants).

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Determination of triazine resistant biotypes of *Setaria viridis*

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ABSTRACT

Since 1966 triazine herbicides (ametryn, simazine, atrazine and prometryn) have been widely used in Yugoslavia. This paper investigates the development of resistance to triazine herbicides in *Setaria viridis* from different localities. Seed of weed species that could be resistant have been collected from different localities in Vojvodina, such as Backa Palanka, Backi Maglic and Becej. Whole plant studies and Petri dish assays were performed during 1999 and 2000. Plants were treated by range of atrazine rates in controlled conditions, including also susceptible, reference population. Seeds were sown in Petri-dishes containing solutions at a range of concentrations of atrazine. Results of both tests indicate the presence of atrazine resistance in *S. viridis* from Becej locality, which has been treated by triazine herbicides for many years.

INTRODUCTION

Intensive herbicide use in developed agricultural countries of the world has resulted in a number of negative effects. First of all, crop rotation has been reduced and alternative weed control measures have been abandoned, leading to over-reliance on herbicides. This has caused the occurrence of herbicide resistance. These changes have also led to increased herbicide levels in groundwater and possible toxicity (Konstantinovic, 1999). Resistance has developed particularly widely in situations where herbicides have been used as the only weed control method. Resistance is causing increasing economic losses. (Heap, 1997).

Herbicide resistance is the naturally occurring inheritable ability of some weed biotypes within a given weed population to survive a herbicide treatment that should, under normal conditions of use, effectively control that weed population. Selection pressure is highest when weeds are controlled by pre-emergent herbicides with long persistence (Caseley *et al.*, 1991). In these cases, resistance can occur rapidly, as the susceptible weed biotypes never produce seed. By repeated use of the same herbicide or herbicides of the same action mechanism, selection will be towards elimination of susceptible biotypes and survival of resistant ones (Mallory-Smith *et al.*1990).

Cross-resistance and multiple resistance may also arise. The former describes the cases when a weed biotype is resistant to two or more herbicides as a result of one resistance mechanism, and the latter cases in which resistant plants possess two or more resistance mechanisms. (Le Baron, 1987; Budimir & Gasic, 1997). Presence of either mechanism may complicate the choice of alternative herbicides. Therefore, for the achievement of a sustainable weed control program it is necessary to rotate different herbicides, or preferably, to rotate herbicides with different modes of action.

Mechanisms of plant resistance to herbicides are as follows:
a) change to the herbicide site of action so that the target site is no longer sensitive
b) increased metabolism, whereby resistant plants can degrade herbicide into non phytotoxic metabolites faster than susceptible one, and
c) removal of the herbicide from areas in the plant cell that are susceptible, to more tolerant areas, i.e. vacuole, where it is not harmful for plant growth (Janjic,1997).

Triazine herbicides based on ametryn, simazine, atrazine and prometryn have been widely used in Yugoslavia since 1966 (Konstantinovic, 1996). Many years' use of persistent pre-emergence mixtures of atrazine and ametryn in maize have had negative consequences. These are reflected above all in change of weed flora structure, as long-term atrazine use selectively controlled annual weeds such as *Amaranthus retroflexus, Chenopodium album* and *Sinapis arvensis*, whereas it tended to favour the survival of annual and perennial weeds from the *Poaceae* (eg *Setaria* spp.) (Drazic & Konstantinovic, 1997).

There are currently 41 dicotyledon and 19 monocotyledon weed species, world-wide, that have developed resistance to triazine herbicides. High numbers of triazine resistant weeds has been identified in maize production in North America and Europe and in orchards in Europe. Nine triazine resistant weed species have been reported in the genus *Amaranthus,* five species in the genus *Polygonum* and four in the genus *Chenopodium.* The most frequently reported triazine resistant weeds have been the following: *C. album* (18 countries), *A. retroflexus* (14 countries), *Senecio vulgaris* (12) and *Solanum nigrum* (10). It has been estimated that worldwide there are over three million ha contaminated with triazine resistant weeds, which makes them the most frequent resistance problem (HRAC, 1999).

This paper reports research to establish whether poor control of *Setaria viridis* L. with triazine herbicides in Yugoslavia is due to the development of resistance.

MATERIALS AND METHODS

Studies of *S. viridis* resistance to atrazine were done in 1999 and 2000. There is little historical data on triazine herbicide use in our country and on occurrence and spread of resistant weed species (Janjic *et al.*, 1988). Consequently we have studied occurrence of resistance using whole plant studies and Petri dishes assays (Clay & Underwood, 1990).

The most important individual factor for the initial determination of resistance, is the level of non-susceptibility in the field. Consequently, we have used a method of visual assessment of atrazine efficiency to detect possible resistance. There are several factors that can indicate possibility of resistance occurrence in field, such as:
i) level of control of other susceptible species,
ii) presence of live plants alongside dead ones,
iii) past experiences, i.e. previously successful control by the same treatment,
iv) herbicide history, i.e. repetition of the same herbicide treatment, or herbicide with the same mode of action,
v) resistance occurrence in the region,
vi) harvest,
vii) cultural history, i.e. monoculture and minimum tillage (Moss,1995).

Using this method of field inspection, populations of *S. viridis* that appeared to be showing resistance were chosen from localities with long history of triazine use for its control (Table 1.). Plant material used in the trials has been collected from Becej, Backa Palanka and Backi Maglic localities. For reference, a susceptible population was used from an area that was free of herbicide treatment.

In whole plant studies, plants were grown in controlled conditions in pots from seed which was suspected to be atrazine resistant. Plants were sprayed with range of atrazine rates such as 0.75 kg a.i. ha^{-1}, 1.0 kg a.i. ha^{-1}, 1.25 kg a.i. ha^{-1}, 1.5 kg a.i. ha^{-1} and 2.0 kg a.i. ha^{-1}. Assessments have been performed visually, by recording the number of germinated plants and by measuring foliage fresh weight (Table 2). The trial was set in four replications, and assessments were done 3 – 4 weeks after treatment.

In the Petri dish assays, seed of susceptible and resistant biotypes of *S. viridis* were germinated on filter paper with the following range of atrazine concentrations: 0.75 ppm , 1.0 ppm, 1.25 ppm, 1.5 ppm and 2.0 ppm. Ten seeds per dish were spread evenly over the paper and 5ml of atrazine solution added to saturate, but not flood, the filter paper. There were three replications of each treatment. Dishes were kept at room temperature, out of direct sunlight. Germination and seedling condition were recorded at intervals up to 25 days from the start, with visual assessment of number of healthy and damaged seedlings in each dish (Table 3). Root length was also measured (Figure 1).

RESULTS AND DISCUSSION

Pot tests

It was found that atrazine at the highest rate of 2 kg a.i.ha^{-1} reduced fresh foliage weight of *S. viridis* from Becej locality by 71.4%, whereas there was 100% reduction of the herbicide free population (Table 2). Taking into consideration the fact that triazine herbicides have been used over the last 10 years at Becej locality and the results of the pot test, it seems highly likely that this population has acquired resistance. Fresh weight reduction in samples taken from the locality Backi Maglic was recorded as 88.4% at atrazine concentration of 1.0 kg ha^{-1}, and the same concentration at locality Backa Palanka caused 86.5% reduction. The susceptible standard at the atrazine concentration of 1.0 kg ha^{-1} had similar fresh foliage reduction for 82.8%, from which it can be concluded that *S. viridis* population from localities Backa Palanka and Backi Maglic are still susceptible to this herbicide, i.e. there are no signs of resistance.

Petri-dish test

No healthy plants were produced in the Petri-dishes containing 1.25ppm atrazine in the Backi Maglic, Backa Palanka and susceptible standard populations (Table 3). However there were only 38% damaged plants in the Becej population and there were still more than 50% healthy plants at 2.00 ppm. Relative hypocotyl length of *S. viridis* from locality of Becej began to drop quickly only at atrazine concentration of 1.5 ppm, whereas in the case of susceptible standard this happened at lower concentration of 1.25 ppm (Figure 1). Relative *S. viridis* hypocotyl length reduction from localities Backa Palanka and Backi Maglic was also recorded at lower atrazine rates.

The Petri dish test confirms that it is probable that the weedy population of *S. viridis* at locality Becej has acquired atrazine resistance. It also confirms previous results, which suggested that samples from two other localities are still susceptible to atrazine action.

Table 1. Details of *S. viridis* plant populations used in the studies

Species, locality	Year	Crop	Applied herbicide rates
Becej	1993	maize	Atrazine 1 kgha^{-1} Prometryn 1 kgha^{-1}
	1994	maize	Atrazine 1 kgha^{-1} Prometryn 0.5 kgha^{-1}
	1996	maize	Atrazine 1 kgha^{-1} Prometryn 0.5 kgha^{-1}
	1999	maize	Atrazine 0.6 kgha^{-1} + Prometryn 0.6 kgha^{-1}
Backi Maglic	1992	maize	Atrazine 1.5 kgha^{-1}
	1993	maize	Atrazine 1 kgha^{-1}
	1994	maize	Atrazine 1.5 kgha^{-1}
	1995	maize	Atrazine 1.5 kgha^{-1}
	1999	potato	Metribuzin 0.9 kgha^{-1}
Backa Palanka	1995	maize	Atrazine 1 kgha^{-1} Prometryn 0.5 kgha^{-1}
	1996	maize	Atrazine 0.6 kgha^{-1} + Prometryn 0.6 kgha^{-1}
	1997	maize	Atrazine 1.5 kgha^{-1}
	1998	maize	Prometryn 2 kgha^{-1}

Table 2. Effect of atrazine on number of emerged plants and foliage fresh weight

Locality	Atrazine concentrations											
	0 kg a.i./ha^{-1}		0.75 kg a.i. /ha^{-1}		1.0 kg a.i./ha^{-1}		1.25 kg a.i./ha^{-1}		1.5 kg a.i. /ha^{-1}		2.0 kg a.i./ha^{-1}	
	a	b	a	b	a	b	a	b	a	b	a	b
Becej	35	28	18.2	21	17	19	15.5	16	12.5	13	10	10
Sd	3.1	-	2.4	-	1.5	-	1.2	-	1.0	-	0.3	-
Backi Maglic	32	27	14.4	13	3.7	2	0	0	0	0	0	0
Sd	5.9	-	2.2	-	0.3	-	0	-	-	-	-	-
Backa Palanka	31	26	17.2	15	4.2	1	0	0	0	0	0	0
Sd	4.3	-	2.8	-	0.3	-	0	-	-	-	-	-
susceptible standard	32	29	15.7	18	5.5	3	0	0	0	0	0	0
Sd	3.7	-	2.8	-	0.2	-	0	-	-	-	-	-

a – mean foliage fresh weight (mg/plant) b- total number of emerged plants
Sd- standard deviation

Comparison of these results with results of whole plant studies of resistant and susceptible population showed similar reactions to different atrazine concentrations.

The intensity of herbicide use at Becej was only slightly greater than that at the other two locations so it perhaps surprising that they too did not show indications of the presence of resistance. This tends to confirm the view that resistance genes are not ubiquitous and so poor management, using the same herbicide every year does not inevitably lead to the development of resistance. Such management just increases the risk of resistance developing.

Table 3. Percentage of damaged plants 25 days after germination in petri dishes with atrazine

Locality	Atrazine concentrations											
	0 ppm		0.75 ppm		1 ppm		1.25 ppm		1.5 ppm		2 ppm	
	%	Sd	%	Sd	%	Sd	%	Sd	%	Sd	%	Sd
Becej	0	0	0	0	26.5	1.90	38.1	3.54	43.2	4.61	48.5	3.64
Backi Maglic	0	0	85.2	4.9	98.4	3.15	100	0	100	0	100	0
Backa	0	0	87.5	5.7	99.3	3.8	100	0	100	0	100	0
Palanka susceptible standard	0	0	64.1	2.46	97.8	3.08	100	0	100	0	100	0

Sd = standard deviation

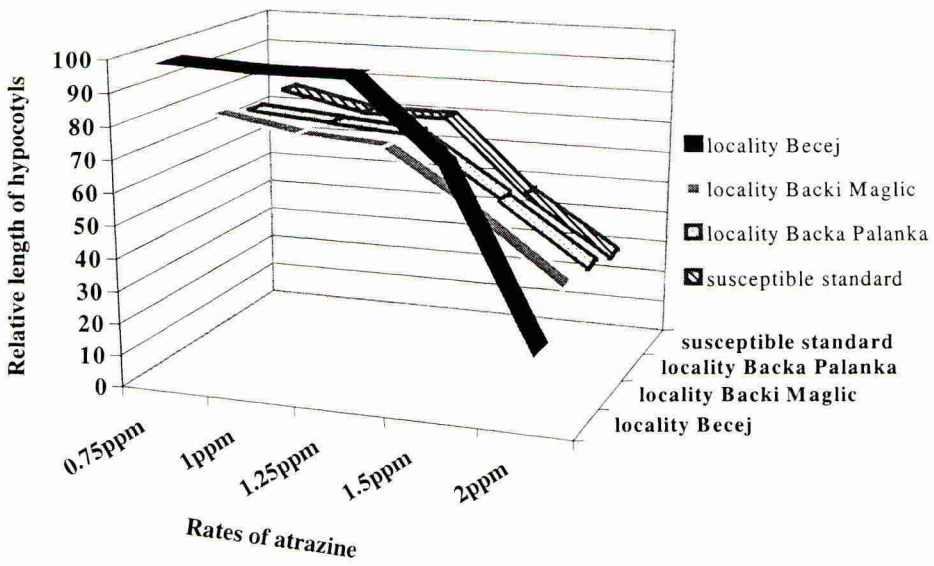

Figure 1. *Setaria viridis,* relative length of hypocotyls following treatment with atrazine (relative to length in untreated 0 ppm dishes)

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POSTER SESSION 8C

INTEGRATED CROP AND WEED MANAGEMENT IN GRAIN CROPS

Session Organiser: A D Bailey
Dow AgroSciences, Hitchin, UK

Poster Papers: 8C-1 to 8C-8

Competition between *Galium aparine* and winter wheat: optimum timing of herbicide application to minimise yield loss

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ABSTRACT

A field experiment was carried out to investigate the critical timing of competition between *Galium aparine* and winter wheat. Four herbicides (amidosulfuron, HOE 3208, fluroxypyr + metosulam and fluroxypyr) were used to remove *G. aparine* at a range of dates. All herbicide treatments gave effective control of *G. aparine* if sprayed before May, with no significant wheat yield losses compared with the weed-free control. Herbicide applications in May and June allowed large amounts of *G. aparine* biomass to develop, resulting in significant wheat yield losses compared with the weed-free control and earlier-sprayed treatments.

INTRODUCTION

In many countries, *Galium aparine* is an economically important weed that reduces crop yields, interferes with harvest and contaminates harvested grain. Although mainly germinating in the autumn, *G. aparine* can continue to emerge until May (Froud-Williams 1985) and is a late competitor putting on the bulk of its dry matter late in the season (May to July, Wilson & Wright 1987). Previous work has shown that *G. aparine* is the most competitive broad-leaved weed of winter cereals in the UK (Wilson & Wright 1990), therefore, it is vital that herbicide treatments should achieve high levels of control. Autumn herbicide applications have been found to be unreliable for the control of *G. aparine* (Lutman *et al.*, 1987); this appears to be related to temperature at the time of application (Tottman *et al.*, 1988). However, with several spring herbicides now available to control *G. aparine*, from the two leaf stage up to the booting stage of the crop (Growth Stages 12-51, Zadoks *et al.*, 1974), there is renewed interest in the selection of herbicides and their optimum timing for *G. aparine* control.

The experiment reported here investigated the critical timing of competition between *G. aparine* and winter wheat. Four herbicides to control *G. aparine* were applied at a range of dates and subsequent effects on weed biomass and wheat yields were compared.

METHODS AND MATERIALS

Experiment layout

A field experiment was established on 12 October 1998 at IACR-Long Ashton Research Station, near Bristol, UK. The experiment consisted of four replicates in a randomised block

design. Each replicate consisted of 17 plots (3m x 3m); 15 herbicide treatments (Table 1) plus a weed-free control and an untreated plot. *Galium aparine* seeds were sown by hand onto the seedbed surface and incorporated into the soil by the passage of the drill when the winter wheat (cv. Buster) was sown later the same day. The target densities for the wheat and *G. aparine* were 240 and 40 plants m^{-2}, respectively. The plots were arranged to avoid tractor wheelings and to ensure even application of fertiliser. A total of 180 kg ha^{-1} of N-fertiliser was applied in two applications, 80 kg ha^{-1}in March and 100 kg ha^{-1} in April. A standard fungicide programme was applied to all plots as necessary.

Herbicide application

The herbicides were applied at the recommended rates; Eagle (amidosulfuron) at 40 g ha^{-1}, HOE 3208 at 240 g ha^{-1}, EF 1166 (fluroxypyr + metosulam) at 1 L ha^{-1} and Starane 2 (fluroxypyr) at 1 L ha^{-1}. The herbicides were applied at a range of dates (Table 1) using a CO_2-pressurised sprayer, operating at a pressure of 210 kPa and a volume rate of 250 L ha^{-1} with a 4 m boom carried by two operators.

Table 1. Dates and growth stages when the herbicide treatments were applied

Date	Herbicide				Growth stages[+]	
	Amidosulfuron	HOE 3208	Fluroxypyr + Metosulam	Fluroxypyr	Wheat	*G. aparine*
19 March	✓	✓	✓	x	30	15 cm
08 April	✓	✓	✓	x	31	25 cm
30 April	✓	✓	x	✓	33	35 cm
20 May	✓	✓	x	✓	41	75 cm
01 June	✓	✓	x	✓	57	Flowering

[+] Zadoks *et al.* (1974) and Lutman & Tucker (1987)

Assessments

After crop and weed emergence, a 1 m^2 area in each plot was marked for later yield assessment; all wheat and *G. aparine* seedlings were counted in this area in January. The weed-free control plots were hand-weeded to remove all weed species. The plots were visually assessed at approximately one, three, six and nine weeks after treatment; the *G. aparine* plants were scored for vigour, using the score descriptions given in Table 2.

The experiment was hand-harvested in early August, the wheat and *G. aparine* in each 1 m^2 area of each plot being cut at ground level. *Galium aparine* was separated from the wheat, oven dried at 80°C and weighed. The wheat sheaf was weighed and the threshed fresh weight of grain recorded. The grain was oven dried at 100°C for 48 hours, weighed and wheat yield in t ha^{-1} at 85% dry matter was calculated.

Table 2. Score descriptions for assessing weed vigour after herbicide treatment

Score	Description
0	Completely dead
1	Moribund, but not all tissue dead
2	Alive, with some green tissue but unlikely to make much further growth
3	Very stunted but apparently still making some growth/re-growth
4	Considerable inhibition of growth
5	Readily distinguishable inhibition of growth
6	Some detectable adverse effect compared with control
7	Indistinguishable from control

Statistical analysis

Statistical analyses were performed using the Genstat 5 statistical package. Wheat yield and *G. aparine* biomass data were subjected to analysis of variance. A variance stabilising transformation ($\sqrt{x}+0.1$) was required for *G. aparine* biomass.

RESULTS

Both the crop and weed populations established well, achieving an average of 220 and 55 plants m^{-2} of wheat and *G. aparine*, respectively.

***Galium aparine* scores and biomass**

All herbicides gave effective control of *G. aparine*, especially at the first three application dates (Table 3). The mean vigour score of 3.0 on plots treated with fluroxypyr + metosulam or HOE 3208 at the early application dates showed that there was some re-growth of *G. aparine*. However, the relatively small amounts of *G. aparine* biomass produced remained at the base of the wheat canopy. Fluroxypyr + metosulam or fluroxypyr were the fastest acting treatments, with symptoms showing within a week of their application. The effects of amidosulfuron or HOE 3208 were evident at the second assessment and by the third assessment there was little difference between the treatments. By mid-May, the *G. aparine* plants had grown vigorously on the unsprayed plots. Although herbicide applications after mid-May did affect the growth of *G. aparine*, there were still large amounts of biomass remaining until harvest. Additionally, the *G. aparine* plants sprayed in June were still able to make some growth from the tips.

Galium aparine biomass was significantly reduced by all treatments compared with the untreated plots (Table 4). There was very little *G. aparine* biomass remaining at harvest on all treated plots sprayed in March or April. However, larger amounts of biomass were present in the plots sprayed in May and June. In the May application, there was significantly

less biomass on plots sprayed with fluroxypyr than with amidosulfuron, but there was no difference between the same treatments sprayed in June.

Table 3. Vigour scores for *G. aparine*

Application date	Herbicide	Assessment date									
		25/3	06/4	16/4	29/4	07/5	19/5	25/5	08/6	25/6	17/7
19 March	Amidosulfuron	7.0	3.3		0.0		0.0				
	HOE 3208	7.0	2.8		1.0		3.0				
	Flurox+Metos	6.0	3.0		2.5		3.0				
8 April	Amidosulfuron			6.0	4.3		1.8		0.0		
	HOE 3208			6.0	4.0		1.0		1.0		
	Flurox+Metos			4.0	2.0		3.0		3.0		
30 April	Amidosulfuron					5.0	4.0		1.8	1.0	
	HOE 3208					5.0	2.0		1.3	1.0	
	Fluroxypyr					4.0	2.0		1.0	0.0	
20 May	Amidosulfuron							5.8	2.1	2.1	2.5
	HOE 3208							6.0	4.0	2.0	2.1
	Fluroxypyr							4.0	1.9	2.0	2.0
1 June	Amidosulfuron								6.0	3.0	4.0
	HOE 3208								5.6	3.0	3.0
	Fluroxypyr								4.0	3.0	2.6

Table 4. Square root transformed biomass (g m^{-2}) of *G. aparine* ($\sqrt{x}$+0.1) at harvest

Application date	Herbicide				
	Untreated	Amidosulfuron	HOE 3208	Fluroxypyr + Metosulam	Fluroxypyr
	18.86				
19 March		0.27	0.72	0.89	
08 April		0.32	0.32	0.64	
30 April		3.84	3.99		1.89
20 May		12.15	10.70		9.16
01 June		16.01	17.09		16.39
SED			0.795 (d.f. = 44)		

Wheat yield

Wheat yields were not significantly different from the weed-free controls for plots treated in March and April, irrespective of herbicide (Table 5). However, yields from plots sprayed in May and June were significantly reduced compared with the weed-free control and earlier treated plots. Yield reductions were between 24 and 30% in May, with no difference between the herbicides. In June, yield reductions were between 40 and 52% and the fluroxypyr treated plots had significantly lower yields than those treated with amidosulfuron or HOE 3208. Yields from the untreated plots were 64% lower than in the weed-free controls.

Table 5. Wheat yield response to the control of *G. aparine* (t ha^{-1})

Application date	Herbicide					
	Control	Amidosulf.	HOE 3208	Fluroxypyr +Metosulam	Fluroxypyr	Untreated
	11.18					4.05
19 March		10.32	11.31	10.89		
08 April		10.49	10.63	10.91		
30 April		10.68	10.32		10.25	
20 May		7.85	7.90		8.45	
01 June		6.65	6.58		5.35	
SED			0.488 (d.f. = 47)			

DISCUSSION AND CONCLUSIONS

All herbicide treatments gave effective control of *G. aparine* if sprayed before May, with no significant wheat yield losses compared with the weed-free controls. Herbicide treatments applied in May and June resulted in large amounts of *G. aparine* biomass up to harvest and significant yield losses (24-52%) compared with the weed-free control and earlier-sprayed plots. Previous work has shown that several spring-applied herbicides give good control of *G. aparine* (D'Souza *et al.*, 1993; Bailey *et al.*, 1999). However, few other studies have reported on how the timing of *G. aparine* control reflects on crop yield losses. In this study, competition between *G. aparine* and winter wheat occurred from late-April onwards.

At each application date, there was no significant difference in wheat yield between the herbicide treatments, with the exception of the June treatment where plots sprayed with fluroxypyr had significantly lower yields than amidosulfuron or HOE 3208. It should be noted that on the final herbicide application date (1st June), the growth stage of the wheat was beyond that recommended for the application of amidosulfuron or fluroxypyr.

One reason for not spraying too early for the control of *G. aparine* is the extended period of emergence of this weed (Froud-Williams 1985). It has recently been shown that the vigour

of spring emerging *G. aparine* plants was significantly lower compared with those emerging in the autumn (Cussans & Ingle 1999), but although the spring emerging plants did not cause a significant yield loss they still had the ability to produce seeds. Control decisions need to take account of the potential increase of the population as well as the economic losses in the current crop. However, if herbicides can be applied as late as the end of April without a yield penalty, any late emerging *G. aparine* would still be controlled. In this experiment, *G. aparine* emergence was monitored but all the seedlings had emerged by January.

ACKNOWLEDGEMENTS

I would like to thank Geoff Seavers, Sarah Mitcheson and staff of the Experimental Husbandry Section for their assistance with this work and Aventis CropScience for supporting the experimental programme and supplying the herbicides. IACR receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

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Mesotrione: a new mode of action for weed control in maize

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ABSTRACT

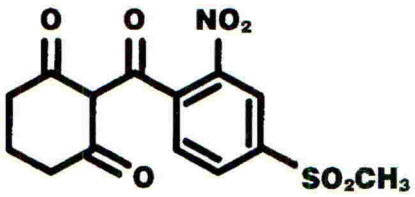
Field studies were conducted from 1997 to 2001, and included both tilled field experiments and those conducted under no-tillage conditions. The soil was a silt loam with 1.5% organic matter and pH of 6.2. Mesotrione applied postemergence controlled *Xanthium strumarium*. The compound also had activity on the grass weedy species *Brachiaria platyphylla*. Weed control was better as an early postemergent (rather than preemergent) application under these field conditions. The addition of a low rate (0.28 kg a. i./ha) of atrazine to postemergent treatments increased activity in some situations. This new mode of action would be beneficial in the management of triazine-resistant weeds, and in those areas prohibiting triazine use.

INTRODUCTION

Mesotrione (formerly ZA1296) is a new low use rate herbicide from Syngenta Crop Protection. It is chemically derived from a natural phytotoxin obtained from the Californian bottlebrush plant, *Callistemon citrinus* (Mitchell *et al.* 2001). Mesotrione has low volatility, moderate water solubility, medium soil adsorption, a short residual in soil due to microbial degradation, and a good toxicological profile (Table 1, Zeneca Technical bulletin 02-3625-001). The compound acts by competitive inhibition of the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), a component of the biochemical pathway that converts tyrosine to plastoquinone and alpha-tocopherol. Mesotrione is an extremely potent inhibitor of HPPD. Blockage of this pathway results in "bleaching symptoms" of sensitive species. It is rapidly taken up by weed species following foliar application, and is distributed within the plants by both acropetal and basipetal movement. Maize is tolerant due to its ability to metabolize the herbicide, and crop injury from mesotrione is minimal.

In plants, the tyrosine degradation pathway is crucial because homogentisate, a tyrosine degradation product, is a precursor for the biosynthesis of photosynthetic pigments, such as quinones or tocophenols (Serre *et al.* 1999). Homogentisate biosynthesis includes a decarboxylation step, a dioxygenation and a rearrangement of the pyruvate sidechain. This complex reaction is carried out by a single enzyme, the 4-hydroxyphenylpyruvate dioxygenase (HPPD), a non-heme iron dependent enzyme that is active as a homodimer in plants. Lee *et al.* (1997) reported that the triketones are potent bleaching herbicides whose structure-activity relationships and physical properties are substantially different from previous "classical" bleaching herbicides, which affect a different target enzyme (phytoene desaturase). Schulz *et al.* (1993) also reported that phytoene desaturase was not affected by SC-0051, a chemical demonstrating similar activity. Mayonado *et al.* (1989) examined the activity of SC-0051 using HPLC analysis, demonstrating a potentially novel mode of action.

Table 1. Chemical nomenclature and Properties (taken from Zeneca technical bulletin)

Chemical name (CAS)	2-[4-methylsulfonyl-2-nitrobenzoyl]-1,3-cyclohexanedione) [104206-82-8]
Chemical name (IUPAC)	2-(4-mesyl-2-nitrobenzoyl)-3-hydroxycyclohex-2-enone
common name (ISO,ANSI)	Mesotrione
Chemical family	benzoylcyclohexane-1,3-dione (triketone)
Chemical structure	
Molecular formula	C14H13O7NS
Molecular weight	339.32
Vapor pressure	4.27 * 10^{-8} mm Hg @ 20C
water solubility	2.2 g/l @ pH 4.8 @ 20C, 15 g/l @ pH 6.9 @ 20 C 22 g/l @ pH 9 @ 20 C

METHODS AND MATERIALS

Field studies (a total of 7) were conducted in 1997 through 2001 to examine the weed control and crop response of mesotrione. Corn was planted using both tilled and no-tillage production systems in several different field locations (a non-selective herbicide was used prior to planting to kill existing vegetation in no-till systems). Soils were highly fertile silt-loams with organic matter content of 1.5 - 2.0%, pH of 6.2 to 6.7, and all had good water-holding capacity. Acetochlor was applied to all plots at planting, and appropriate surfactants were included with all postemergent treatments. A pyrethroid insecticide was applied at planting to prevent stand losses from cutworms. Typical plant populations were 67,000 plants/ha. All plots were side-dressed with ammonium nitrate at 450 kg/ha when corn was 15 to 25 cm in height.

Small plot techniques were used. All herbicide applications were made using a CO_2 pressurized backpack sprayer delivering 170 L/ha. Each treatment was applied to 4 plots, each 3 m wide by 8 m in length. The herbicide treatment was applied to the center 2 meter of each plot, which allowed for an untreated border row between each plot to allow for assessment of weed populations. Weed size at the time of postemergent application was from 1 to 8 cm, and corn height was 20 to 35 cm. Field studies were conducted using a randomized complete block design, and a Fishers protected LSD was used to separate treatment means.

Table 2. *Xanthium strumarium* control in 1998

Postemergent herbicide	application dosage	application timing	*Xanthium strumarium* control (%), days after Post treatment	
	kg a.i./ ha		14	39
Mesotrione	0.20	PRE	71	44
Atrazine	2.2	PRE	88	75
Mesotrione	0.11	POST	91	64
Mesotrione+ Atrazine	0.11 + 0.28	POST	95	93
Prosulfuron + Primisulfuron	0.20 + 0.20	POST	97	94
Mesotrione	0.20	POST	93	90
Least Significant Difference			14	37

Table 3. *Brachiaria platyphylla* and *Xanthium strumarium* control with mesotrione 28 days after POST application and maize yield in 1999.

POST herbicide	dosage (Kg ai/ha)	*Brachiaria platyphylla* control (%)	*Xanthium strumarium* control (%)	maize yield (Kg/ha)
Nicosulfuron (+Atrazine PRE)	0.034	97	95	9100
Mesotrione	0.11	97	98	10500
Mesotrione	0.14	98	96	10200
Mesotrione + Atrazine	0.11 + 0.28	98	97	10400
Mesotrione + Atrazine	0.14 + 0.28	98	97	10700
No POST		0	18	7400
Weedy check		0	0	4500
LSD				3200

RESULTS

Although several studies were conducted, the data presented in each table are from a single experiment, and these results were representative of the other field studies. Environmental conditions were conducive to good herbicidal activity, including rainfall soon after application of soil-applied herbicides. Postemergent conditions were warm and moist, so both the corn and the weeds were actively growing at the time of application. At lower mesotrione dosages, the addition of a low rate of atrazine improved weed control in 1998 (Table 2). This was more evident in control >28 days after postemergent application.

In other situations, there was no need to include the atrazine, since mesotrione alone provided complete control (Table 3). Control from soil-applied mesotrione PRE treatments was substantially less than POST in all studies in all years. However, given the warm soil conditions and abundant moisture (both favoring rapid microbial breakdown), these conditions would represent the worst-case scenario for residual control of herbicides. Brachiaria platyphylla is also difficult to control and usually requires a POST treatment to achieve adequate control.

ACKNOWLEDGMENTS

This research was conducted on Tennessee Agricultural Experiment Stations, and was supported using state and federal funds (Hatch project TN0162). Thoughtful suggestions from Dan Smith and Walt Bachman added to the research.

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Integration of azolla, fish and herbicides for rice weed management

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ABSTRACT

Integrated fish farming has received considerable attention in recent years in many developing countries. Rice and fish are not only compatible but also mutually beneficial when grown together. Herbivorous fish serve the purpose of biological weed control in lowland rice. Dual culturing of azolla in rice fields besides fixing atmospheric nitrogen has the added benefit of weed suppression. Considering the multiple benefits of integrating these two component farming enterprises in lowland rice, laboratory and field experiments were conducted at the Department of Agronomy, Annamalai University, India to optimize the size of fish fingerlings and their time of release in lowland rice treated with herbicides, to study the weed control effect of azolla, fish and herbicides independently and in combination. The results of laboratory studies revealed that fish fingerlings of length 4 to 5 cm were observed to be safe with survival if released 12 days after herbicide application. From the field experiments, it was observed that azolla independently contributed for 34 per cent weed control index, fish independently contributed for 21 per cent weed control index whereas their combination contributed for 40 per cent (mean values from two seasons).

Based on these results, another field study over two consecutive seasons were conducted to compare the performance of three different herbicides in rice + fish + azolla system. Integration of azolla and fish culture with oxyfluorfen 0.25 kg/ha offered higher weed control indices (75%) and rice grain yields (6.0 t/ha). However, the histopathological studies revealed that fishes suffered tissue deformation in gills, muscle and liver.

INTRODUCTION

Integrating aquaculture into crop based farming systems can play an important role in reversing environmental degradation, improving human nutrition and increasing farmers purchasing power (Lightfoot 1991). Rice-fish culture being an age old practice in India was suggested in lowland are as where land is a scarce resource, to minimise the risk and to obtain sustained production (Ninawe 1997). Grass carp (*Ctenopharyngodon idella*) controlled weeds by effectively feeding on grasses, thereby reducing labour required for weeding and its faeces helped to fertilize the rice fields (Nie *et al.*, 1992). Azolla (*Azolla microphylla*), a free floating aquatic fern, accommodated in its upper lobes *Anabaena azolla* the blue green algae that fixes atmospheric nitrogen (Becking 1976). Azolla inoculation for biological nitrogen fixation in transplanted rice fields, also complimented smothering of weeds through rapid coverage of water surface by the

thallus (Janiya and Mood, 1984). Addition of azolla to rice-fish systems provided food for fish and fertilizer nutrient to rice (Liu Chungchu 1995). Considering the multiple benefits of integrating these two component farming enterprises in lowland rice, laboratory and field experiments were conducted to optimize the size of fish fingerlings and their time of release in lowland rice treated with herbicides, to study the weed control effect of azolla, fish and herbicides independently and in combination and to trace the histopathological impact of rice herbicides on fish.

MATERIALS AND METHODS

The data presented in this paper are obtained from the results of a series of field experiments on weed control with rice-fish farming system, over four years from 1995-1998. These experiments were conducted at the Gardenland block of Annamalai University Experimental Farm, located 11°24' N Latitude, 79°41'E Longitude at an altitude of 5.79 m above mean sea level. During the first two years, integrating component enterprises like azolla culture and fish culture, independently and in combination, with and without the use of rice herbicide butachlor 1.5 kg/ha were compared for their compatibility, synergism and weed control efficacy in transplanted rice. Whereas, the field experiments during the next two years compared the compatibility and efficacy of different weed control practices of rice like twice hand weeding, butachlor 1.5 kg/ha, oxyfluorfen 0.25 kg/ha and thiobencarb 1.5 kg/ha on rice + azolla system, independently and in combination with the fish culture in the system.

In all the experimental plots (size 8x5 m) that included fish culture as a component enterprise, trenches with the dimension of 0.5 m x 1 m were excavated along the border on one side occupying 10 per cent of rice area to serve as a permanent shelter for the fish fingerlings that moved out in to the fields as and when needed. Water management in transplanted rice is normally by impounding water upto a height of 5 cm throughout the field, upto 15 days prior to harvest. Fingerlings of grass carp of size 4-5 cm were released in the trenches, 12 days after herbicide application @ 10,000 fingerlings/ha. *Azolla* was multiplied in a separate nursery field as described by Kannaiyan (1982) and applied in the respective plots @ 500 g/m^2, one week after spraying of herbicides. The herbicides used were butachlor (Machete 50% EC), oxyfluorfen (Goal 23.5% EC) and thiobencarb (Saturn 50% EC). These herbicides at their recommended dose (butachlor 1.5 kg/ha, oxyfluorfen 0.25 kg/ha and thiobencarb 1.5 kg/ha) were sprayed pre-emergence, using 500 l/ha of spray fluid, through a knapsack sprayer fitted with flat fan nozzle maintaining a pressure of 4.2 bar. The data presented are the weed biomass recorded 60 DAT (days after treatment), weed control index computed from the data on weed biomass using the formula suggested by Mishra and Tosh (1979) and rice grain yield recorded at harvest.

The experiment to optimize the size and time of release of fish fingerlings after herbicide application was conducted in concrete tanks, filled with field soil to a height of 5 cm, and with water to height of 20 cm using 30 litres of water. All the three herbicides were sprayed at their recommended dose rates. Fish fingerlings of three different sizes; 2-3 cm, 3-4 cm and 4-5 cm were released 4, 8 and 12 days after herbicide application. Mortality and survival of fingerlings were recorded once in two days. All the field experiments were conducted in Randomised block design and the data were subjected to analysis of variance and least significant difference values were

calculated at the 5% probability level as suggested by Panse and Sukhatme (1978). Percentage values were subjected to arc-sine transformation before statistical analysis.

For the histopathological studies on fish, the fingerlings from herbicide treated and control plots were collected 15, 30 and 45 days after the release. Gill, liver, muscle and brain tissues were dissected from each of the fingerling, fixed in Bouins Zemner fixative for 6 hrs. and processed following the standard technique (Gurr 1959), for microtome. After taking sections of 6 to 8 µm thickness, they were stained in Heindenhain's iron haemotoxin and counter stained with aqueous eosin. Stained sections were mounted and observed under a microscope.

RESULTS

Size and time of releasing fish fingerlings

The results of the experiment conducted to evaluate the size and time of release of fish fingerlings in rice fields after herbicide application are presented in table 1. Among the three different sizes compared i.e. 2-3 cm, 3-4 cm and 4-5 cm and three different dates of release i.e. 4 DAT, 8 DAT and 12 DAT, fingerlings of length 4-5 cm were observed to survive better with no mortality, when released after 12 DAT, with all the three herbicides. The half life of butachlor in transplanted rice fields was observed to be 3-4 days (Kathiresan 2001). In addition to similar rapid degradation, rice herbicides like butachlor and thiobencarb were observed to be only of moderate toxicity to fishes with LC_{50} at <0.5 ppm (Ooi and Lo 1992). Larger sized fingerlings of size 4-5 cm (with comparatively better tolerance) when released leaving sufficient time for the moderately toxic herbicides to metabolize in water, withstood the negative impact of herbicides better, contributing to their survival.

Table 1. Size and time of releasing fish

Size of fingerlings	Time of release	Herbicide	Mortality rate of fish fingerlings after release (per cent)				
			2nd day	4th day	6th day	8th day	10th day
2-3 cm	4 DAT	butachlor	100	-	-	-	-
		oxyfluorfen	100	-	-	-	-
		thiobencarb	80	20	-	-	-
	8 DAT	butachlor	100	-	-	-	-
		oxyfluorfen	100	-	-	-	-
		thiobencarb	60	40	-	-	-
	12 DAT	butachlor	60	20	20	-	-
		oxyfluorfen	100	-	-	-	-
		thiobencarb	40	40	20	-	-
3-4 cm	4 DAT	butachlor	80	20	-	-	-
		oxyfluorfen	100	-	-	-	-
		thiobencarb	80	20	-	-	-
	8 DAT	butachlor	40	20	-	-	-
		oxyfluorfen	60	20	-	-	-
		thiobencarb	40	-	-	-	-
	12 DAT	butachlor	20	-	-	20	-
		oxyfluorfen	40	20	-	-	-
		thiobencarb	20	-	20	-	-
4-5 cm	4 DAT	butachlor	40	-	20	-	-
		oxyfluorfen	40	20	20	-	-
		thiobencarb	20	20	40	-	-
	8 DAT	butachlor	-	-	-	-	20
		oxyfluorfen	20	-	-	-	-
		thiobencarb	-	-	-	-	-
	12 DAT	butachlor	-	-	-	-	-
		oxyfluorfen	-	-	-	-	-
		thiobencarb	-	-	-	-	-

Complimentary weed control from component elements of the rice farming system

Results of the first two year (1995-96) experiments are furnished in table 2. The weed flora of the experimental field comprised *Echinochloa colonum*, *E. crusgalli*, *Leptochloa chinensis*, *Leersia hexandra*, *Cyperus littoralis*, *Bergia capensis*, *Eclipta alba* and *Marsilea quadrifolia*.

The integration of fish, azolla and herbicide in the cultivation of rice was superior to any other treatment. This treatment registered the lowest dry matter of weeds (100.02 kg/ha and 80.40 kg/ha during 1995 and 1996, respectively). The highest weed dry matter of 251.16 kg/ha and 230.50 kg/ha were recorded in unweeded monoculture of rice, during 1995 and 1996, respectively. Positive interaction among the component elements of rice farming has been well established. Biofertilizer azolla formed a thick mat of thallus growth over the standing water column in the field that interrupted light interception by weed seeds and seedlings at later stage of the crop. This is evident from 34 per cent of weed control index obtained independently from azolla this agrees with the reports of Janiya and Moody (1984). Azolla also supported the growth of fish, serving as food material (Liu Chungchu 1995). These fish later started feeding on the weeds in general and grasses in particular supplementing the weed control with 21 per cent weed control index recorded in the present study, the same was also observed by Nie *et al.*, (1992). Initially during establishment of these two elements, butachlor, the rice herbicide with a shorter persistence was able to control the weeds and accordingly the integration of all three resulted in the best weed control performance.

Table 2. Complimentary weed control from component elements

Treatments	Weed dry matter production (kg/ha)		Weed Control Index (%)	
	1995	1996	1995	1996
T_1 - Rice alone	251.16	230.50	-	-
T_2 - Rice + azolla	164.98	157.78	35.84 (34.30)	34.14 (31.50)
T_3 - Rice + fish	204.40	180.00	25.54 (18.60)	27.88 (21.88)
T_4 - Rice + azolla + fish	148.64	137.78	39.69 (40.79)	39.34 (40.19)
T_5 - Rice + butachlor	140.56	130.00	41.57 (44.02)	41.30 (43.57)
T_6 - Rice + azolla + butachlor	110.80	99.96	49.37 (57.57)	57.28 (60.89)
T_7 - Rice + fish + butachlor	130.50	116.40	43.87 (44.03)	45.00 (50.00)
T_8 - Rice + azolla + fish + butachlor	100.02	80.40	51.85 (61.8)	53.79 (65.11)
SE_D			2.06	1.86
CD (p=0.05)			4.14	3.75

Figures in parenthesis indicate original values

Compatibility of rice weed control measures with fish

Results of the experiments during 1997 and 1998 are presented in Table 3.

Among the herbicides compared, oxyfluorfen 0.25 kg/ha performed significantly better than butachlor and thiobencarb this is in line with the reports of Kathiresan and Gurusamy (1996). Performance of all herbicides did not show any additive or synergistic interaction or improved weed reduction or yield increment when combinedly used with fish. Though the fish were able to survive without suffering any mortality due to rice herbicides, the tissue distortion and histopathological interruption (brought out in the present study) could have caused a reduction in the feeding habit of the grass carp. This could be the reason for fish + herbicides offering only a comparable performance with herbicides alone. However, in the experiments during 1995 and 1996, fish interacted additively with herbicides. The difference in floristic composition with predominance of grasses at later years and larger degradation time of herbicides due to repeated use might have contributed for this reversing trend.

Table 3. Compatibility of rice herbicides with fish

Treatments	Weed dry matter production (kg/ha)		Grain yield (t/ha)	
	1997	1998	1997	1998
T_0 - Rice alone (unweeded control)	575.91	590.28	3.22	3.93
T_1 - Rice - twice handweeded	270.50	288.55	4.60	5.46
T_2 - Rice - unweeded + fish	460.28	485.92	2.73	3.46
T_3 - Rice - twice handweeded + fish	248.94	265.98	4.01	4.87
T_4 - Rice - butachlor 1.5 kg ha^{-1}	218.69	230.33	4.89	5.96
T_5 - Rice - oxyfluorfen 0.25 kg ha^{-1}	165.72	174.50	5.14	6.42
T_6 - Rice - thiobencarb 1.5 kg ha^{-1}	274.87	293.52	4.39	5.14
T_7 - Rice - butachlor 1.5 kg ha^{-1} + Fish	194.70	205.75	4.33	5.28
T_8 - Rice - oxyfluorfen 0.25 kg ha^{-1} + Fish	143.11	150.25	4.58	5.70
T_9 - Rice - thiobencarb 1.5 kg ha^{-1} + Fish	267.17	284.58	3.80	4.57
SE_D	13.30	14.24	0.11	0.21
CD (p=0.05)	26.74	28.62	0.23	0.41

Histopathology of fish and herbicides

Among the tissues of fish examined, gills showed a higher degree of deformation followed by muscle and liver, with respect to all the three herbicides tested. Brain was the least or unaffected tissue. The changes observed in the gill tissues were cartilagenous hyperplasia of gill rays, proliferation of lamellar epithelium, vacuolation of cytoplasm of lining epithelium and congestion of blood spaces. The changes in muscle tissue were swelling and necrosis of muscle fibres. The changes in liver tissues were congestion of sinusoids, central vein and proliferation of bile ductular epithelium. Similar tissue distortions in fishes due to herbicide treatments were reported earlier by Palarp and Ted (1985).

CONCLUSION

In rice farming system, integrating azolla and fish is observed to offer significant complimentary weed control. However, using a herbicide along with fishes is injurious from the viewpoint of fish culture enterprise. Considering the higher economic returns from rice + fish farming system, rice + fish + azolla + herbicide may prove to be a wholistic farming system approach, offering sustained weed control.

ACKNOWLEDGEMENT

We thank Dr M A M. Ramasamy, Founder Pro-Chancellor, Annamalai University for the facilities offered and Dr Lanka Lakshman Rao, RMMC, for the expertise and assistance offered in taking up histopathological studies of fishes.

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Herbicide programmes against *Alopecurus myosuroides* in the UK using MKH 6561

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ABSTRACT

MKH 6561 is a new active ingredient for control of grass weeds in winter wheat. This paper presents results from UK field trials infested with black-grass (*Alopecurus myosuroides*). MKH 6561 at 42 g a.i./ha was applied in the spring following various autumn applied standard herbicides and compared to these standards alone. Programmes with MKH 6561 in sequence gave improvements in *A. myosuroides* control over the standards alone, in terms of mean levels of control and also greater reliability of effect against all but the most resistant populations of *A. myosuroides*.

INTRODUCTION

MKH 6561 (propoxycarbazone-sodium) is a sulfonylaminocarbonyltriazolinone herbicide discovered and developed by Bayer (Feucht *et al.*, 1999). Good activity from MKH 6561 against *Alopecurus myosuroides* was demonstrated in UK field trials in the period 1993-2000. Levels of control from single applications (mean 70-80% reduction) however are typically insufficient to prevent competition with the crop and the return of weed seed to the soil.

Efficacy of even the best commercially applied herbicides against *A. myosuroides* is variable (Bolton *et al.*, 1997) and acceptable control from a single application cannot be guaranteed. Reasons for poor efficacy are not always easily explained although factors commonly implicated are, unfavourable weather, less susceptible *A. myosuroides* growth stage and *A. myosuroides* resistance to the herbicide involved.

Effective herbicidal control of *A. myosuroides* in the UK has therefore become increasingly difficult to predict. The most effective strategy and therefore common practice currently, as far as herbicidal weed control is concerned, is to use multiple applications and mixtures of different active ingredients.

The objective of this work was to examine the benefits of using a spring application of MKH 6561 as part of a programme of herbicides to control *A. myosuroides*.

MATERIALS AND METHODS

MKH 6561 was tested as a 70% WG formulation with an adjuvant mineral oil 970 g/l in all trials. The standards tested in some or all trials were clodinafop-propargyl and trifluralin 12:383 g/l EC; isoproturon 500 g/l SC; flupyrsulfuron-methyl 50% WG; flufenacet and pendimethalin 60:300 g/l EC; and UKA025, a development herbicide based on flufenacet 50% WG.

MKH 6561 was applied in the spring following autumn standards alone. Trials were conducted in commercial crops of winter wheat infested with moderate to high levels of *A. myosuroides* in the UK.

Trials were set up using a randomised block design with 2 to 3 replicates. Plot sizes were usually 36-48 m^2. Treatments were applied using knapsack sprayers pressurised by carbon dioxide, at a water volume of 200 l/ha, pressure of 2.0 bar and as a medium quality spray. Weed control was assessed as visual estimates of weed cover and quadrat counts of weed heads (minimum 5 x 0.1 m^2 per plot).

A. myosuroides seed samples from some trials were tested for herbicide sensitivity (Moss, 1995) in pot tests or by the Rothamsted Rapid Resistance Test (Moss, 2000).

RESULTS

The results demonstrate improvements in *A. myosuroides* control from herbicide sequences finishing with MKH 6561 following all autumn herbicides tested (table 1).

Table 1. % reduction in *A. myosuroides* heads from trials 1997-2000. Orthogonal data for each autumn treatment alone and in sequence with MKH 6561 42 g a.i./ha plus adjuvant oil (numbers of trials in brackets).

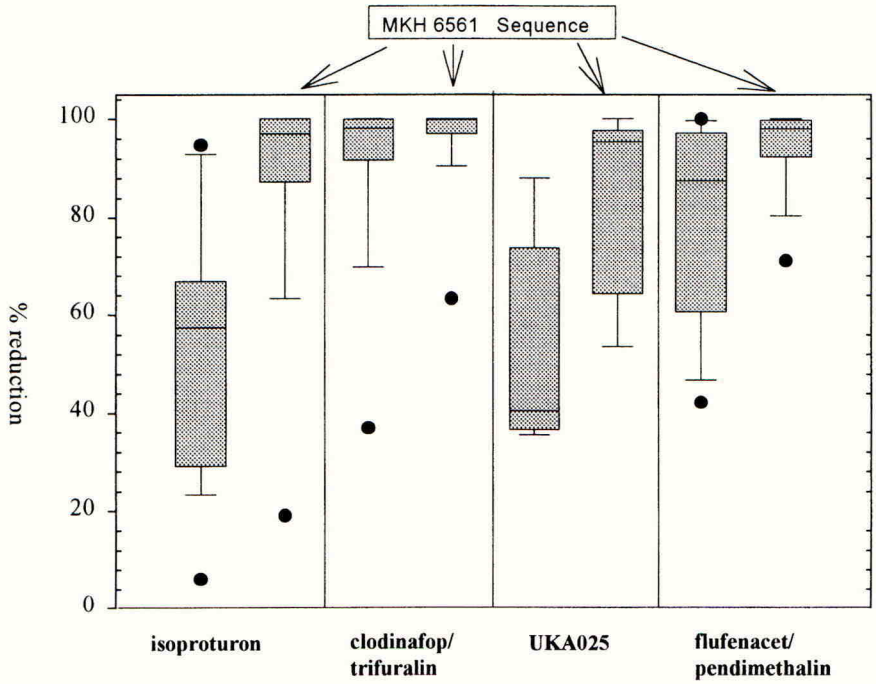
		Autumn herbicide alone		Autumn herbicide plus MKH 6561 in spring	
Autumn herbicide	Dose g ai./ha	Mean (no.) red'n	Range	Mean red'n	Range
clodinafop/ trifluralin /oil	30:958	90.7 (15)	36-100	96.2	63-100
flufenacet/ pendimethalin	240:1200	78.0 (15)	42-100	94.2	71-100
UKA025	240	54.0 (5)	36-88	83.9	59-100
isoproturon	1500-2500	54.9 (13)	6-95	83.8	19-100
flupyrsulfuron	10	83.2 (9)	50-100		
none		0.0 (25)		78.4	25-100

The variability of the data given in table 1 can be better illustrated by the use of the 'box and whisker' plots (figure 1).

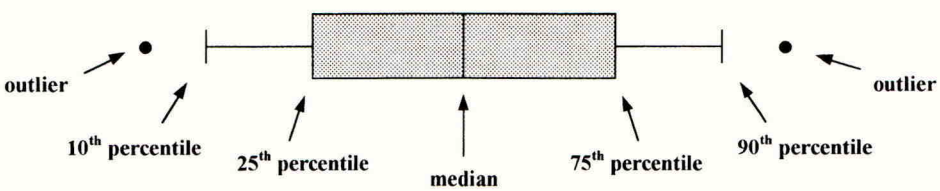
The key to the plotting of the distribution of the data is given below the plots in figure 1. The diagram shows the spread of the data by the size of the box, representing the middle 50% of results, and the whiskers, representing the middle 80% of results in the range.

The results show the wide variability of performance from the autumn herbicides and the reduction of this variability following sequential use of MKH 6561 in spring.

Figure 1. Variability of *A. myosuroides* control from trials 1998-2000 shown in box and whisker plots



Key:



In sequences with the more effective autumn herbicides, MKH 6561 boosted control levels to more than 95%, whilst those starting with weaker autumn products still gave above 80% control. Table 2 shows the numbers of trial results obtained with the different product combinations which are above the 80% or 95% control levels.

Table 2. Numbers of trials (from table 1) where treatments gave a minimum of 95% and 80% control.

Autumn herbicide	Trial no. > 95% control		Trial no. > 80% control		Total no. of trials
	Autumn herbicide alone	Autumn herbicide + MKH 6561 in spring	Autumn herbicide alone	Autumn herbicide + MKH 6561 in spring	
clodinafop/ trifluralin /oil	9	13	11	14	15
flufenacet/ pendimethalin	5	10	8	13	15
UKA025	0	3	1	3	5
isoproturon	0	7	3	11	13
none		5		13	25

At a number of sites with high populations of black-grass, the performance of the herbicide treatments was compared to the resistance status of the black-grass (table 3).

Table 3. Field performance against *A. myosuroides* and resistance status of weed samples from individual sites

	% reduction in heads				Heads	Resistance status *		
Autumn herbicide	clodinafop /trifluralin /oil	clodinafop/ trifluralin /oil	isoproturon	isoproturon	no. /m^2			
Rate g a.i./ha	30:958	30:958	(1500) or 2500	(1500) or 2500		FEN	SETH	PEND
Spring herbicide	None	MKH 6561	None	MKH 6561				
Rate g a.i./ha		42 g a.i./ha + adjuvant		42 g a.i./ha + adjuvant				
Trial number								
ER-25-98	70	95	(45)	(97)	116	RR		RR
MR-12-00	37	66	27	63	2028	RR	S	RR
MR-16-99	92	97	(25)	(68)	346	RRR	R?	R?
SM-15-99	99.7	100	(60)	(98)	804	S	S	S
NM-14-00	92	99.7	55	92	919	S	S	S
NR-11-00	100	100	91	100	97	RRR	S	R?
Mean	82	93	51	86				

Figures in brackets relate to the lower rate of isoproturon. * See text.

The resistance status was assessed on seed samples taken from untreated areas; the tests enabling the degree of resistance for three resistance types to be classified as follows:

1. Resistant – RRR
2. Partially resistant – RR
3. Marginal insensitivity – R?
4. Susceptible – S

Resistance types:

1. Fenoxyprop (FEN) – an uncharacterised mechanism that affects other 'fops'.
2. Sethoxydim (SETH) – indicates target site resistance and gives complete resistance to all 'fops' and 'dims'.
3. Pendimethalin or chlortoluron (PEND) – indicates enhanced metabolism and this can affect the performance of most herbicides including MKH 6561.

The results show that MKH 6561 gave useful additional control in sequence with clodinafop/trifluralin or isoproturon against populations with different resistance profiles although efficacy fell short of good control against some highly resistant populations.

DISCUSSION

MKH 6561 sequences have given improved reductions of *A. myosuroides* over all single applications of autumn standards, benefits in terms of higher mean levels of control, reduced variability, and were less prone to serious failures in efficacy.

Following the use of more effective autumn treatments, MKH 6561 can provide a 'mop up' of remaining weeds and consistently achieve high levels of control thus minimising seed return.

In sequences with less effective autumn treatments, MKH 6561 boosted control significantly and although final control levels were not perfect, they were sufficient to reduce competition with the crop and so protect crop yield.

Control of resistant *A. myosuroides* is increasingly difficult with the current armoury of herbicides. MKH 6561 sequences have given improvements in control against the various weed populations encountered. In cases where autumn herbicides fail, MKH 6561 sequences have still given additional reductions although total control cannot always be expected. Best control, particularly in situations of resistant weed populations, is likely to be given by combinations of applications of active ingredients from different chemical groups.

As well as efficacy against *A. myosuroides,* MKH 6561 also offers a broad spectrum of control of a range of other important arable weed species.

Susceptible grass-weed species include; *Bromus* spp., *Elymus repens*, *Arrhenatherum elatius var. bulbosum*, *Apera spica-venti* and broad-leaved species; *Brassica* spp., *Capsella bursa-pastoris*, *Sinapis arvensis* and *Thlaspi arvensis* (Feucht *et al.*, 1999).

MKH 6561 is not claimed to be a single answer to *A. myosuroides* but what it does offer UK wheat growers is a new tool for use in managed programmes to maximise control of this tenacious, yield-robbing weed.

ACKNOWLEDGEMENTS

Thanks are due to colleagues who contributed to field trials and data handling and to the growers who provided crops and weeds.

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Evaluation of a yield loss model based on wild oat and barley density and relative time of emergence

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ABSTRACT

A regression model based on wild oat (*Avena fatua* L.) and barley (*Hordeum vulgare* L.) plant density, and relative time of emergence is being used in western Canada to advise farmers on the economics of wild oat control with herbicides. Experiments were conducted in farmers' fields sown to barley in 1997, 1998 and 1999 to evaluate the reliability of the model in estimating barley losses due to wild oat. Nine fields were assessed over the three-year period. Correlation between actual and predicted barley yield loss was high. With few exceptions, the model accurately predicted whether or not a herbicide application resulted in a net profit or loss. Under certain cost and price assumptions, herbicide application was rarely economical. Seed production by unsprayed wild oat was determined from a regression model derived from data collected in five of the fields. Wild oat seed production was influenced by barley plant density, and decreased considerably as barley density increased.

INTRODUCTION

Interest in integrated approaches to weed management in western Canada is being driven by declining crop prices coupled with increased input costs, consumer concerns about the environmental and health effects of herbicides, and increasing incidences of weeds becoming resistant to herbicides. Scouting fields and assessing the nature and extent of a weed problem to determine if herbicides are necessary every year is an important component of integrated weed management (O'Donovan *et al.*, 2001). Applying the "economic threshold" concept to weeds is not an easy undertaking (Cousens 1987; Norris 1992; O'Donovan 1996a). On the other hand, applying herbicides when they are unnecessary can be a waste of time and revenue, and can lead to the selection of herbicide resistant weeds (Thill *et al.*, 1994). Information is now available in western Canada to assist in the decision-making process.

Regression models based on wild oat density were developed in the 1970's to determine the effects of wild oat on yield loss of barley and other field crops (Dew 1972). The barley model was subsequently refined to incorporate important additional factors such as the relative time of emergence of barley and wild oat (Cousens *et al.*, 1987), and barley plant density (O'Donovan *et al.*, 1999). Regression models predicting crop yield loss due to wild oat and other weeds have become important components of computerized decision support systems (Derksen et al. 1996; O'Donovan 1996b). Decision-makers using these systems need assurances that the estimates and recommendations derived from the models are reliable in practical farming situations. The objectives of this study were a) to evaluate, in farmers' fields in Alberta, the reliability of a regression model for estimating barley yield loss due to wild oat, and b) to estimate the amount of seed produced by unsprayed wild oat in the same fields.

METHODS AND MATERIALS

Model development

A regression model describing the relationship between wild oat and barley plant density, and percentage barley yield loss (O'Donovan *et al.*, 1999) was re-parameterized as a rectangular hyperbola. The re-parameterized model was:

$$yl = \frac{(0.016 \pm 0.005d)100}{1 + 0.016 \pm 0.005d + 0.018 \pm 0.008c} \quad 1)$$

where yl = percentage barley yield loss, d = wild oat plant/m^2, and c = barley plants/m^2. A third parameter from another regression model (Cousens *et al.*, 1987) was used to describe the relationship between percentage barley yield loss and relative time of emergence of barley and wild oat. The model was:

$$yl = \frac{0.503 \pm 0.099d}{e^{0.266 \pm 0.041t} + (0.503 \pm 0.099d)/49.1 \pm 3.7} \quad 2)$$

where t = relative time of emergence (days) of barley and wild oat and e is the base of natural logs. In both models, numbers are estimated regression parameters ± standard errors.

The model that was evaluated was derived from wild oat and barley density regression parameters from model 1, and the relative time of emergence regression parameter from model 2. The model was:

$$yl = \frac{1.6d}{e^{0.266t} + 0.016d + 0.018c} \quad 3)$$

Model evaluation

Over the three-year period (1997, 1998 and 1999), nine barley fields with wild oat infestations were selected in the province of Alberta in western Canada. Experiments for model evaluation were conducted on a 1-hectare area of each field. Between 20 and 25 paired quadrats (each 1-m^2) were randomly established in this area. Wild oat and crop plants were counted within each quadrat, and leaf stages of barley and wild oat determined. Prior to spraying a herbicide for wild oat control, one quadrat of each pair was covered with a plastic sheet. This was the wild oat-infested control. Where necessary, annual dicot weeds were removed by hand from the quadrats. At crop maturity each quadrat was harvested and crop seed yield determined.

Correlation analysis was used to determine if there was a significant ($p \leq 0.05$) relationship between actual and predicted barley yield losses due to wild oat. In addition, barley yield loss estimates from model 3 were used to predict if a profit or loss would result from control of wild oat with a postemergence herbicide in each of the fields. The market price of barley was assumed to be $90 Canadian/metric ton, while the herbicide and application cost was assumed to be $45 Canadian/ha. These represent approximate prices and costs for western Canada over the three years of the study.

Wild oat seed yield estimation

In five of the fields, wild oat seed was collected from the quadrats as it matured on the plants. The amount of remaining wild oat seed was determined when the quadrats were harvested. Wild oat seed yield as a function of barley and wild oat plant density was described by the model:

$$yn = \frac{d}{0.00033 \pm 0.000038\ (d - 1 + 0.265 \pm 0.061c)} \qquad 4)$$

where yn = number of wild oat seed/m^2, d = wild oat plants/m^2, c = barley plants/m^2, and b and k are estimated regression parameters. Numbers are estimated regression parameters ± standard errors.

RESULTS AND DISCUSSION

Actual vs. predicted barley yield loss and economic returns

Average wild oat densities in the nine fields varied from 8 to 57 plants/m^2 (Table 1). In most of the fields, barley plant densities varied from approximately 130 to 150 plants/m^2. In a previous study, at least 200-barley plants/m^2 was recommended for optimum wild oat management and barley yields (O'Donovan *et al.*, 1999). This suggests that farmers in Alberta may be seeding barley at sub-optimal rates. In most of the fields, barley emerged several days ahead of wild oat (Table 1). The ability of barley to emerge ahead of competitive weeds like wild oat may be largely responsible for the previously reported superior competitiveness of barley compared to other crops (Dew 1972; Dew and Keys 1976; Cousens *et al.*, 1987).

There was a highly significant correlation ($p = 0.001$, $r = 0.91$, $df = 7$) between actual and predicted barley yield losses due to wild oat. This suggests that model 3 was reasonably accurate in predicting barley yield loss due to wild oat under Alberta conditions. Yield loss was substantially underestimated in only one of the fields (1998-1, Table 1). The accuracy of the barley model in estimating yield loss caused by wild oat may be due to the fact that, in addition to wild oat density, crop density and relative time of emergence were taken into account. Both of these factors were previously shown to considerably influence the extent of crop yield loss due to wild oat (Cousens *et al.*, 1987; O'Donovan *et al.*, 1999).

In most cases, estimates on whether or not a herbicide application resulted in a net profit or loss were accurate (Table 1). Wild oat control was clearly uneconomical in seven of the nine fields. In these fields, wild oat at densities ranging from eight to 28 plants/m^2 emerged several days after barley. In a field where the average wild oat infestation was relatively high (57 plants/m^2), and wild oat emerged at the same time as the barley, a profit following herbicide application was correctly predicted (Field 1998-3, Table 1). The need to spray was underestimated in only one field, where a relatively small profit would have resulted from herbicide application (Field 1998 – 1, Table 1).

Table 1. Actual and predicted barley yield loss due to wild oat, and actual and predicted profit or loss following wild oat control with a herbicide[a]

				% barley yield loss		$ profit (+) or loss (-) following control	
Year-Field	Wild oat plants/m^2	Barley plants/m^2	Relative time of emergence[b]	Actual	Predicted[c]	Actual	Predicted[d]
1997-1	28	151	+4	1	7	-$40	-$14
1997-2	9	222	+2	0	2	-$45	-$37
1997-3	8	154	+5	0	2	-$45	-$38
1998-1	12	99	Same time	13	7	+$11	-$14
1998-2	22	208	+4	8	5	-$27	-$34
1998-3	57	129	Same time	24	22	+$41	+$34
1999-1	14	131	−4	4	4	-$25	-$25
1999-2	13	138	+4	3	4	-$28	-$22
1999-3	28	148	+5	6	7	-$28	-$25

[a]Data represent averages of 20 – 23 quadrats per field

[b]Number of days preceded by + sign indicates barley emerged before wild oat

[c]Estimates are from model 3 (see text)

[d]Assumes a barley price of $90/ metric ton and a herbicide and application cost of $45/ha.

Seed production by unsprayed wild oat

Seed production by unsprayed wild oat was determined from model 4. Wild oat and barley plant densities varied within and among the different fields, and were thus used to derive the

regression parameters. Relative time of emergence of the weeds and crops did not vary sufficiently to allow estimation of a meaningful regression parameter for this variable. Model estimates of seed production by one wild oat plant/m^2 at different barley plant densities were calculated (Table 2). Wild oat seed production was greatly influenced by barley plant density. As barley density increased, wild oat seed production decreased. An increase in barley density from 100 to 200 plants/m^2 reduced wild oat seed production by 50%.

Table 2 Estimated seed produced by one wild oat plant/m^2 at different barley plant densities

Barley plants m^{-2}	Estimated wild oat seed/m^{2a}
100	114
150	76
200	57
250	46

[a]Estimates are from model 4.

These findings are in agreement with those from small plot experiments where higher barley seeding rates reduced weed dry matter and seed production (Kirkland 1993; O'Donovan *et al.*, 1999). Seeding crops at relatively high rates would result in lower weed seed production during years when herbicides may not be applied. However, even at the highest barley plant density (250 plants/m^2), a single wild oat plant produced an estimated 46 seeds/m^2. This would be unacceptable to many producers. It should be kept in mind, however, that not all this seed would result in wild oat plants in future years. Some seed will end up in the harvested grain, succumb to predators, a tillage operation or pre-seed herbicide application, and an effective in-crop herbicide the following spring. Other seed will remain dormant in the soil for many years, its impact possibly becoming "diluted" with time. Wild oat is still one of the most ubiquitous weeds of cropland in Alberta (Thomas *et al.*, 1998) in spite of extensive herbicide application over the last 30 years, and complete elimination of wild oat seed from the soil seed bank is probably an unrealistic goal. The risk associated with seed production by uncontrolled wild oat should be weighed against the risk of selecting for herbicide resistant wild oat in future years.

ACKNOWLEDGMENTS

The excellent technical assistance of Jeff Newman, Barry McCarty, Sandi Checkel, Bob Pocock, Greg Semach and Randall Brandt is gratefully acknowledged. Western Cooperative Fertilizers Limited, and the Alberta Agricultural Research Institute provided financial support.

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GF-184 and GF-185 the flexible solution to broad leaf weed control in cereals

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ABSTRACT

GF-184 and GF-185 are novel formulations that combine the active ingredients florasulam and fluroxypyr. GF-184 provides flexible and robust control of *Galium aparine* throughout the season, allowing growers the option of either early or late applications. GF-185 can be applied later in the season for control of *G. aparine* and as it also contains florasulam the product can be applied earlier than fluroxypyr alone as the florasulam removes the variability of control observed with fluroxypyr at low temperatures. In addition to the flexibility of timing, both GF-184 and GF-185 provide a wider weed spectrum than the two actives alone.

INTRODUCTION

Galium aparine is still the most competitive weed in cereal crops (Ingle *et al.*, 1997). It has been shown that if control of *G. aparine* is greater than 98%, the seed bank can be eradicated from the field in four years (see Wilson *et al.*, 1992). In a recent survey all of the farmers stated that on their farms, numbers of *G. aparine* had not decreased in spite of their continued efforts to control them (also observed by Cussans & Ingle 1999). Current options for chemical control of *G. aparine* can be split into two separate groups; early applications *i.e.* early spring and late applications-up to flag leaf emergence. Two active ingredients which provide control of *G. aparine* are florasulam (early season applications) and fluroxypyr (late season applications).

Florasulam is an inhibitor of acetolactate synthase (ALS) and is a member of the triazolopyrimidine group of herbicides (Thompson *et al.*, 1999). Florasulam formulated as EF-1343 (tradename Boxer/Primus) is used in cereals for the control of *G. aparine* and a number of other key dicotyledonous weeds such as, *Matricaria spp, Stellaria media,* volunteer *Brassica napus* and *Papaver rhoeas*. One of the strengths of florasulam is the ability to provide weed control at low temperatures.

Fluroxypyr is a aryloxyalkanoic acid herbicide which exhibits a high degree of post-emergence activity on a range of broad leaved weeds. Since 1984, fluroxypyr formulated as EF-689 (as Starane 2 for example) has been used for the control of *G. aparine*. Due to the auxinic mode of action of the molecule, applications need to be made when the soil temperature is above 4°C. Early fieldwork indicated that to work effectively, fluroxypyr requires the soil temperature to remain 4°C for several days after application (Tottman *et al.*, 1987). This has effectively restricted applications of fluroxypyr to the end of March onwards.

GF-184 and GF-185 contain fluroxypyr and florasulam. The two products provide growers with robust and flexible control of a number of broad leaf weeds, including *G. aparine*.

This paper summarises data generated across Europe from 1999 to 2000. Control of *G. aparine* and *Matricaria chamomilla* with GF-184 and GF-185 applied early and late season were compared to control obtained with fluroxypyr and florasulam used alone.

MATERIALS AND METHODS

The trials were established in the United Kingdom, France and Germany during the spring of 1999 and 2000. All trials were carried out according to EPPO (European Plant Protection Organisation) guidelines. The trials were sprayed at various times with the applications being split into two broad categories; early season applications and late season applications. The following is a list of the products used in the trials Table 1) with the rates and timings applied in the trials (Table 2).

Table 1. Products used in trials

Product	Florasulam g ae/l	Fluroxypyr g ae/l	Formulation
GF-184	2.5	100	102.5 SE
GF-185	1.0	100	101 SE
EF-1343	50		50 SC
EF-689		200	200 EC

Table 2. Treatments used in trials

Timing	Treatment	Rate	Formulation
Early	GF-184	0.75, 1.05, 1.5, 1.8 l pr/ha	102.5 SE
Late	GF-184	0.75, 1.05, 1.5, 1.8 l pr/ha	102.5 SE
Early	GF-185	0.75, 1.05, 1.5, 1.8 l pr/ha	101 SE
Late	GF-185	0.75, 1.05, 1.5, 1.8 l pr/ha	101 SE
Early	EF-1343	75, 90, 100, 150 ml pr/ha	50 SC
Late	EF-689	0.5,0.75, 0.9, 1.0 l pr/ha	200 EC

Note: l pr/ha = litres of product per hectare
ml pr/ha = millilitres of product per hectare

RESULTS AND DISCUSSION

G. aparine control

Efficacy data for early and late control of *G. aparine* are presented (Figures 1 and 2). The accepted level of control for *G. aparine* is at least 98%. Data from thirteen trials with applications between 22nd February and 31st March are presented in Figure 1. The data show that GF-184 provided acceptable control of *G. aparine* at 1.5 and 1.8 l pr/ha (litres of product hectare^{-1}). This level of control was equivalent to EF-1343 alone at 100 and 150 ml pr/ha (ml of product hectare^{-1}). GF-185 reached 98% control at 1.8 l pr/ha but at 1.5 l pr/ha, control with GF-185 was unacceptable (92.5%).

Late season control of *G. aparine* (Figure 2) was assessed in ten trials, with applications between 28th April and 27th May. The data show that GF-184 and GF-185 gave similar levels of control at 1.05, 1.5 and 1.8 l pr/ha. 1.5 and 1.8 l pr/ha of both formulations giving commercially acceptable levels of control. EF-1343 did not achieve acceptable control levels with the late application (150 ml pr/ha only giving 93% control). EF-689, the market leader for late season *G. aparine* control required at least 0.75 l pr/ha to achieve acceptable efficacy levels.

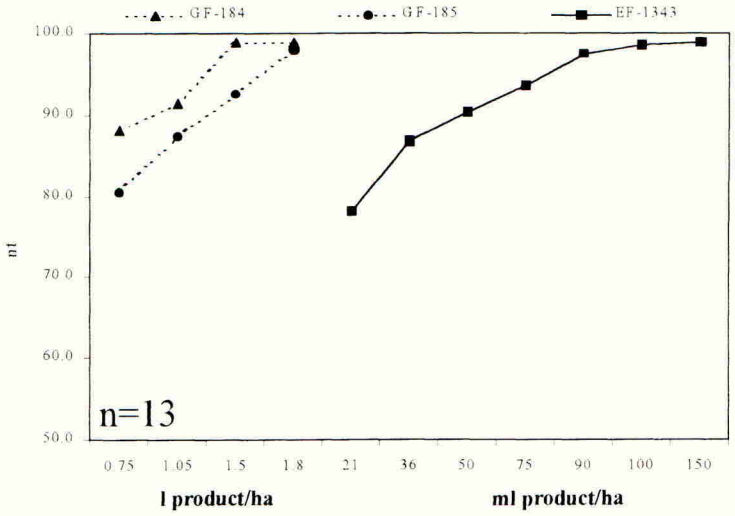

Figure 1. Early season *G. aparine* control

These trials indicate the strengths of the two formulations. GF-184 gave acceptable control of *G. aparine* with both early and late season applications. GF-185 gave some control at the early application, but acceptable control was only achieved with the late application.

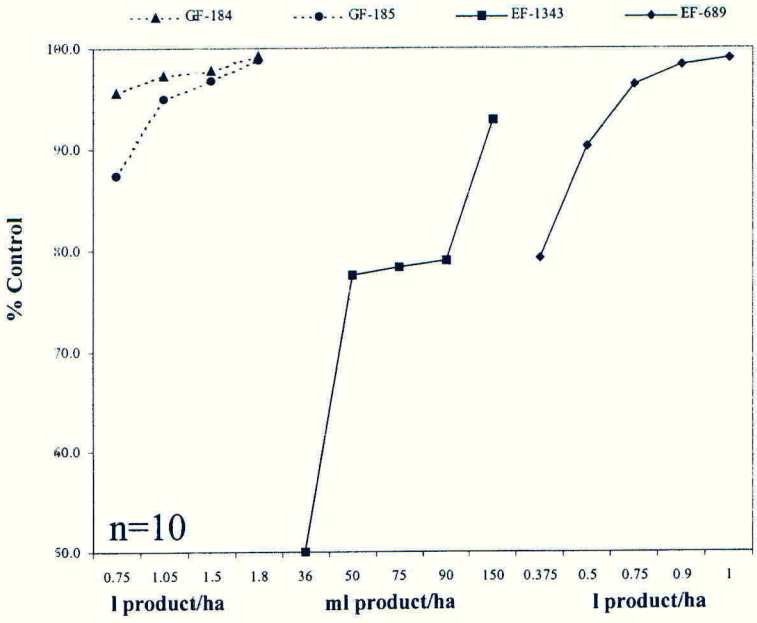

Figure 2. Late season *G. aparine* control

Mayweed control

Control of scented mayweed (*Matricaria chamomilla*) was assessed with early and late applications of GF-184 and GF-185 (Figures 3 and 4). With the early application, GF-184 achieved a very flat dose response, with all rates giving excellent control. GF-185 did not achieve acceptable control of mayweed with 0.75 or 1.05 l pr/h. On larger weeds, GF-184 gave superior control to GF-185 which only achieved acceptable levels at 1.8 l pr/ha.

Control of other weeds

Both GF-184 and GF-185 provide control of a wide range of broad leaved weeds. Amongst weeds classed as susceptible are *Polygonum* spp, *Sinapis arvensis*, *Stellaria media*, *Epilobium* spp and *Brassica napus*. The broad spectrum of weed control should help reduce growers' reliance on herbicide tank-mixes. The wide application window for the products will also result in better control levels being observed as the applications can be made during optimal weather and growing conditions.

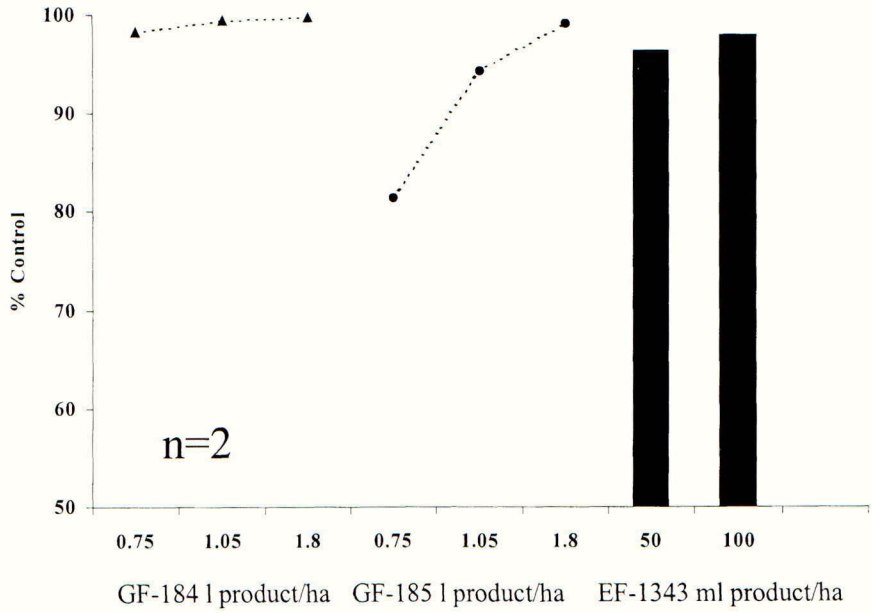

Figure 3. Early season mayweed control

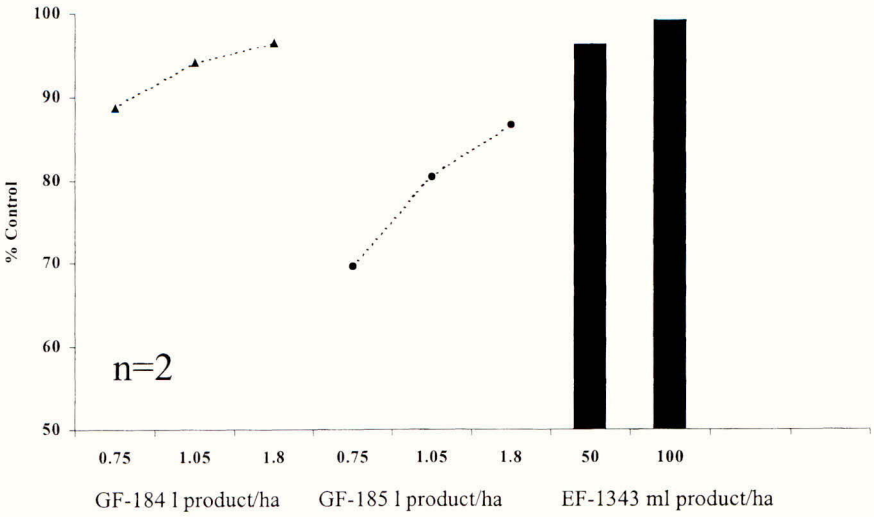

Figure 4. Late season mayweed control

RESISTANCE STRATEGY

As the two active ingredients in GF-184 and GF-185 have different modes of action, the products can be useful as part of a resistance strategy. Since the launch of fluroxypyr in 1984, there has been no reported incidence of resistance to the

molecule. Florasulam is an ALS inhibitor and resistance to other ALS inhibitors has been a concern over recent years. However, with careful use of GF-184 and GF-185, greater than 99% control of target weeds is achievable due to the flexibility of the products. This level of control is important as it helps to reduce the likelihood of resistant populations developing.

CONCLUSIONS

GF-184 and GF-185 provide robust, reliable and flexible control of a number of important broad leaved weeds, including *G. aparine*. A recent survey indicated that *G. aparine* populations are increasing[1]. This is a clear indication that control of the weed has been insufficient despite the availability of a number of products. The built in flexibility of GF-184 and GF-185 should help reduce the *G. aparine* (and other weed) populations as they provide the grower with a wide application window. For early season *G. aparine* control the 2.5 gai/l florasulam contained in GF-184 (in addition to the 100 gai/l fluroxypyr) provides reliable control under fluctuating temperatures. In addition, the product also gives excellent control of *Matricaria* spp. and can be used up to flag leaf stage of the crop. GF-185, although having the same application window as GF-184 is best suited to a later application timing. GF-185 contains 1 gai/l florasulam (plus 100 gai/l fluroxypyr), which will allow for earlier applications than with fluroxypyr alone and also giver a broader spectrum of activity.

The combination of the two actives provides an effective product which gives season long *G. aparine* control. The result of which should be a reduction in weed seed banks and in the long term a decline in *G. aparine* populations. The products should also contribute to a resistance management strategy as they contain two different modes of action.

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Sulfonylurea herbicides used in Romania for weed control in winter wheat

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ABSTRACT

Between 1997 and 2000, studies on the activity of a range of sulfonylurea herbicides used for weed control in winter wheat were carried out in the Research Institute for Plant Protection Bucharest. The efficacy and the selectivity of following herbicides were investigated: Glean 75 DF (clorsulfuron 75 %) - Du Pont de Nemours, Granstar 75 DF (tribenuron methyl 75%) Du Pont de Nemours, Grodyl 75 WG (amidosulfuron 75 %)- AgrEvo, Harmony 75 DF (thifensulfuron methyl 75%) – Du Pont de Nemours and Logran 75 WG - (triasulfuron 75%) – Novartis. These are selective systemic herbicides, absorbed mainly by the roots and moderate by the foliage and translocated throughout the plant. Plant growth is inhibited. The weed spectrum includes broadleaved weeds and some annual grasses. All treatments proved to be safe to the crop. The potential yield loss from weed competition was recouped from herbicides applications.

INTRODUCTION

The wheat crops are strongly infested with annual broadleaved weeds (Popescu *et al.*, 1994, Sarpe, 1992). Between 1995 and 2000, studies on the activity of a range of sulfonylurea herbicides used for weed control in winter wheat were carried out in the Research Institute for Plant Protection Bucharest. The efficacy and the selectivity of following herbicides were investigated in the south of Romania, an important zone to grow grain crops: clorsulfuron, tribenuron methyl, amidosulfuron, thifensulfuron methyl and triasulfuron.

The weed spectrum of these herbicides includes broadleaved weeds, resistant to 2,4-D (Bailey *et al.*, 1999; Ionescu *et al.*, 1996).

METHODS AND MATERIALS

The replicated trials were sprayed in commercial crops of winter wheat. The trials were set up as a randomised block design with 4 replications and individual plots of 100 m^2. Application was in the spring by backpack sprayers and harvesting was done using small-plot combine harvesters. These herbicides were compared with the main competitor in Romania, Icedin Super (2,4-D 29 % + dicamba 10 %).

Weed control was assessed 30 DAT and 60 DAT, as % reduction in weed bio-volume, relative to the untreated check. Weed density using random quadrat counts and crops yield were estimated.

RESULTS

Applied post-em., sulfonylurea herbicides have a high efficacy in weed control in winter wheat (Table 1). The treatments diminished weed populations (Figure 1). Clorsulfuron is the best, followed by amidosulfuron, tribenuron methyl, triasulfuron and thifensulfuron methyl. All treatments proved to be safe to the crop.

These are selective systemic herbicides, absorbed mainly by the roots and moderate by the foliage and translocated throughout the plant. The weed spectrum includes mainly annual broadleaved weeds and some annual grasses. Perennial species as *Cirsium arvense*, *Convolvulus arvensis*, *Sonchus arvensis* are resistant (Table 2). The potential yield loss from weed competition was recouped from herbicides applications (Table 3).

CONCLUSIONS

Sulfonylurea herbicides can be used for weed control of most broad-leaved weeds and some annual grasses in winter wheat. All these herbicides are registered for use in Romania.

ACKNOWLEDGEMENTS

The authors like to thank their colleagues in Du Pont de Nemours, AgrEvo and Novartis who contributed to the research, development and registration of these herbicides in Romania.

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Table 1. Efficacy of herbicides in weed control in winter wheat

Treatment	% Control of weeds							
	30 DAT				60 DAT			
	Dicotyledonous		Monocotyledonous		Dicotyledonous		Monocotyledonous	
	Annual	Perennial	Annual	Perennial	Annual	Perennial	Annual	Perennial
Clorsulfuron 15 g a.i./ha	88	12	14	0	97	33	24	0
Tribenuron methyl 15 g a.i./ha	76	8	6	0	88	28	10	0
Amidosulfuron 30 g a.i. /ha	86	14	12	0	94	34	12	0
Thifensulfuron methyl 45 g a.i. /ha	70	12	5	0	76	12	6	0
Triasulfuron 7,5 g a.i./ha	72	10	4	0	86	20	0	0
2,4-D + dicamba 290 + 100 g a.i. /ha	90	8	10	0	95	24	10	0

Table 2. Influence of sulfonylurea herbicides on weed species in winter wheat

Weed species	Clorsulfuron 15 g a.i./ha	Tribenuron methyl 15 g a.i./ha	Amido-sulfuron 30 g a.i. /ha	Thifensulfu-ron methyl 45 a.i. g /ha	Triasulfu-ron 7,5 a.i.g /ha
Annual dicotyledonous					
Amaranthus retroflexus L.	Xxx	xxx	Xxx	xxx	xxx
Anagallis arvensis	xxx	xxx	xxx	xxx	xxx
Chenopodium album L.	xxx	xxx	xxx	xxx	xxx
Brassica campestris L.	xxx	xxx	xxx	xxx	xxx
Capsella bursa pastoris (L.) Medik.	xxx	xxx	xxx	xxx	xxx
Galinsoga parviflora Cav.	xxx	xxx	xxx	xxx	xxx
Galium aparine L.	xxx	xx	xx	xx	xx
Matricaria chamomilla L.	xxx	xx	xxx	xx	xx
Polygonum aviculare L.	xx	xx	xx	xx	xx
Polygonum persicaria L.	xxx	xx	xxx	xx	xxx
Portulaca oleracea L.	xxx	xxx	xxx	xxx	xxx
Solanum nigrum L.	xx	xx	xx	x	x
Stellaria media	xxx	xxx	xxx	xxx	xxx
Thlaspi arvense L.	xxx	xxx	xxx	xxx	xx
Vicia spp.	xxx	xxx	xxx	xxx	xxx
Perennial dicotyledonous					
Cirsium arvense (L.) Scop.	x	x	x	x	x
Convolvulus arvensis L.	x	0	x	0	x
Rumex acetosella L.	x	x	x	0	x
Sonchus arvensis L.	x	x	x	0	x
Taraxacum officinale Web.	xx	x	x	x	x
Annual monocotyledonous					
Apera spica-venti L.	x	0	0	0	0
Avena fatua L.	0	0	0	0	0
Digitaria sanguinalis (L) Scop	x	0	0	0	0
Echinochloa crus-galli (L) Pal. Beav.	x	0	0	0	0
Lolium remotum Schrk.	x	0	0	0	0
Setaria spp.	x	0	0	0	0
Perennial monocotyledonous					
Cynodon dactylon (L) Pers.	0	0	0	0	0

Legend:
xxx: 85-100 % weed control
xx 50-60 % weed control
x : 10-20 % weed control
0: 0 % weed control

Table 3. Influence of treatments on the mean yields of winter wheat

Treatment	Yield	
	(kg /ha)	%
Control (untreated)	2480	100
Clorsulfuron 15 g a.i./ha	3310	133,4
Tribenuron methyl 15 g a.i./ha	3280	132,2
Amidosulfuron 30 g a.i. /ha	3300	133,0
Thifensulfuron methyl 45 g a.i. /ha	3270	131.8
Triasulfuron 7,5 g a.i. /ha	3100	125,0
2,4-D + dicamba 290 + 100 g a.i./ha	3400	137,0

DL 0,1% = 495,1

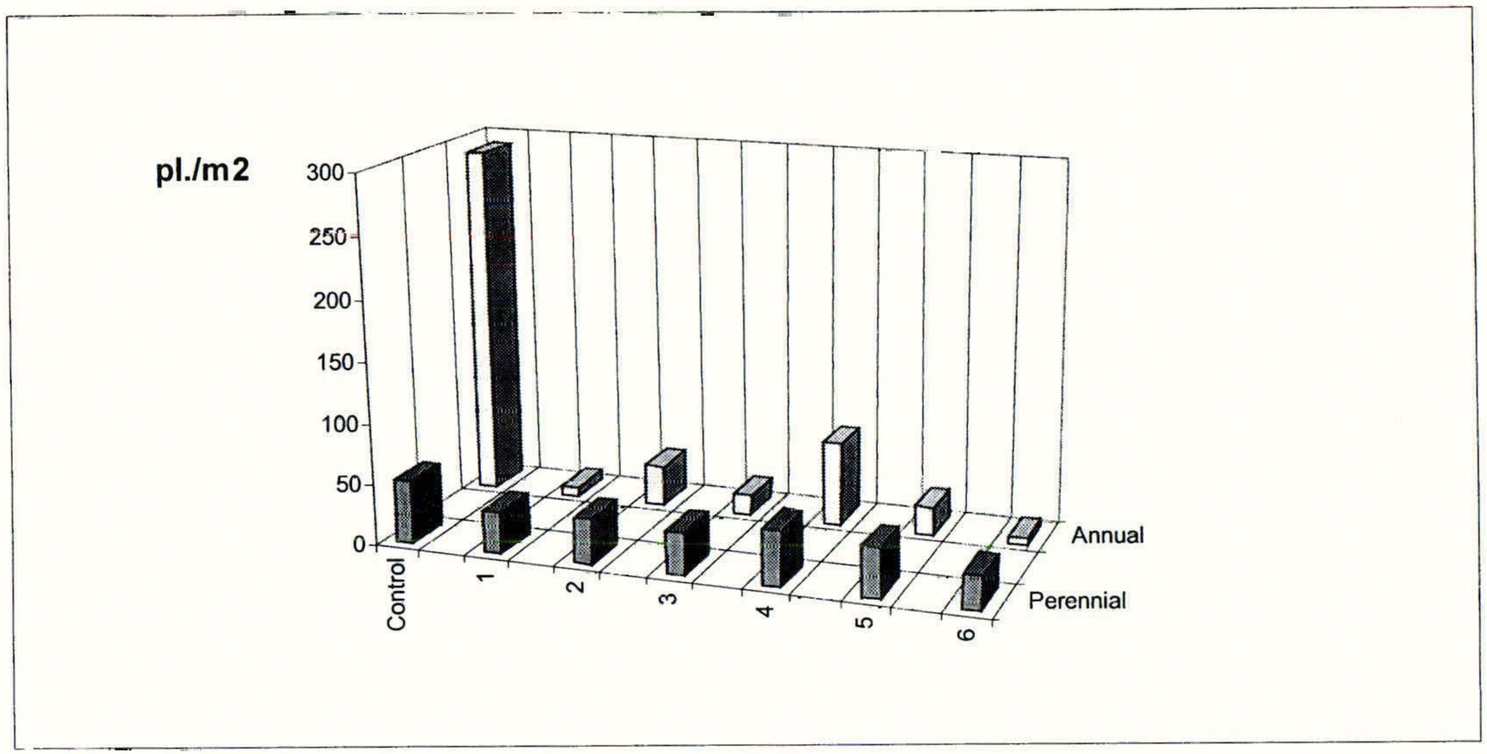

Figure 1. Sulfonylurea herbicides effect on dicotyledonous weed density (60 DAT)

Legend:
1: clorsulfuron
2: tribenuron methyl
3: amidosulfuron
4: thifensulfuron methyl
5: triasulfuron; 6: 2,4-D + dicamba

Influence of tillage and management inputs on weed growth and above ground biomass and yield of wheat varieties

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ABSTRACT

Fields experiments were made in 1999 and 2000 to investigate the effects of conventional (CT) and zero tillage (ZT), three levels of herbicides rates and three nitrogen (N) levels on weed growth and wheat production. There was a higher grain yield for ZT system compared with CT in one of the two years evaluated. Weed biomass from CT was lower than from ZT in both varieties. No differences on wheat biomass and grain yield were observed between full and reduced rate of herbicide. N fertilizer increased significantly wheat biomass and grain yield. Only N medium level had effect upon weed biomass with respect to unfertilized plots, while the highest fertilization rate lowered weed biomass. Tillage system, herbicide reduced rates and nitrogen fertilization were an effective way of limiting weed production in wheat production systems.

INTRODUCTION

Water conservation for crop usage and reduced energy inputs have contributed to the widening acceptance of conservation practices (Buhler 1995).

Nevertheless, as tillage is decreased, weed control can become a limiting factor in crop production (Buhler 1992). Changes in tillage practices can affect weed populations dynamics, which makes them dependent upon heavy use of herbicide (Buhler 1995). Numerous studies have shown the impact of reducing tillage on the population dynamics of weed species. These include increased population of perennial, summer annual grass, biennial, and winter annual species (Buhler 1995). It is important to note that the responses of population dynamics are site specific (Arshad *et al.*, 1995) and depends upon species, location and environment (Derksen *et al.*, 1993).

Fertilizer application is an important management factor in the conservation tillage systems. Conflicting results have been reported on the effect of nitrogen (N) fertilizer on the interaction of crops and weeds. Valenti & Wicks (1992) found that increasing N rates applied to winter wheat decreased annual grass weed populations and weed yields. In other study, wheat yield reductions caused by *Lolium multiflorum* were greater as N levels increased (Acciaresi *et al.*, 2000). Jørnsgård *et al.*, (1996) found differences in the biomass of individual weed species in both wheat and barley crops with N fertilizer applications.

Conservation tillage is an integral component of integrated weed management (IWM) (Swanton & Wise 1991). The design of IWM system is necessary in order to reduce environmental risk from herbicide use. Salonen, (1992) had reported that reducing the

herbicide dose by 50% decreases the control efficacy by 5-30 %. Soil tillage, herbicide, fertility and weeds are thus expected to interact strongly as to produce definitive effects on crop growth and yields. Information on the impact of several management techniques, e.g., herbicide rates, fertilizer application and different types of tillage is needed for developing a reliable IWM.

This paper is concerned with effects of two tillage systems and different management inputs of fertilizer and herbicide rates on the biomass and yield of wheat varieties and on the weed biomass.

MATERIALS AND METHODS

Field experiments were established during 1999 and 2000 at the Experimental Station of La Plata National University (34° S, 58°W, Argentina). The rainfall of the area during the study period (July-December) was 536.9 mm in 1999 and 708.1 mm in 2000 (July-December average: 528.5 mm).

The experiments were designed in a randomised complete block design with four replications, with the treatments arranged as split-split plots. The whole plot factor consisted of two tillage systems (A). This includes A1: conventional tillage (CT, ploughing-20 cm, disk-harrowing, standard sowing) and A2: zero tillage (ZT, herbicides used to control weeds and straw spread with harrows). The same tillage treatments were applied to the same whole plot each year. The subplot factor was three levels of herbicides rates (B). Three doses of metsulfuron-methyl–dicamba (0/0 (0x), 3.0/50 (0.5x) and 6.0/100 (1x) g $a.i/cm^3.ha^{-1}$, respectively) were applied at fourth leaf-unfolded stage (BBCH scale code: 14, BBCH 14) (Harrell, 1998). The sub-subplot factor consisted of three N levels (C). No N was applied in the low-N treatment areas. Urea fertilizer (46 % N, w/w) was broadcast and incorporated at BBCH 14 at rates of 50 kg and 100 kg $N.ha^{-1}.year^{-1}$ in the medium-N treatment (50 N) area and in the high-N treatment (100 N) area, respectively. Two wheat cvs (Buck Pronto (B.Pronto) and Klein Dragon (K.Dragon)) were sown at a density of 300 $pl.m^{-2}$.

The major weed species presented included *Chenopodium album*, *Viola arvensis*, *Stellaria media*, *Lamiun amplexicaule*, *Polygonum convolvulus* and *Lolium multiflorum*. Minor weed species were *Anagallis arvensis*, *Capsella bursa-pastoris* and *Spergula arvensis*. Weed population was harvested from a 0.5 m^2 area in each plot (ten samples per each sub-subplot) at BBCH 31 and their aboveground dry matter (ADM, $g.m^{-2}$) determined. Crop ADM ($g.m^{-2}$), at BBCH 31 were determined by hand harvesting samples on triplicate 0.5 by 0.5 m quadrats randomly located in each sub-subplot. Grain yield ($g.m^{-2}$) was measured on five 1m^2 quadrats on each sub-subplot.

ANOVA and LSD mean separation was made for $p \leq 0.05$. The analysis were repeated across years and tested for homogeneity of variance and normality of distribution.

RESULTS AND DISCUSSION

Soil Tillage

Tillage effects were significant ($p \leq 0.05$) for wheat and weed ADM. CT produced significantly ($p \leq 0.01$) higher wheat ADM than ZT in 2000 (table 1). The relatively drier spring of the first year could have mainly conditioned the ADM production of crop and weed at CT treatment.

There were opposite trends amongst the two evaluated years for grain yield. The tillage effects at 1999 were lower crop grain yield under CT plots with lower production in B.Pronto than in K.Dragon (table 2). There was a higher grain yield ($p \leq 0.05$) for CT than ZT plots for all the varieties tested in the second year (table 2).

Table 1. Above dry matter (ADM, $g.m^{-2}$) at BBCH 31 of wheat varieties and weeds. BP: B.Pronto. KD: K.Dragon

	Wheat				Weed			
	1999		2000		1999		2000	
	BP	KD	BP	KD	BP	KD	BP	KD
Tillage								
CT	186 [a]	137 [a]	487 [a]	383 [a]	31.5 [a]	40.4 [a]	48.5 [a]	61.1 [a]
ZT	220 [b]	245 [b]	342 [b]	286 [b]	89.8 [b]	136.3 [b]	67.3 [b]	77.9 [b]
Herbicide								
0 x	180 [a]	154 [a]	342 [a]	295 [a]	49.0 [a]	156.9 [a]	79.5 [a]	127.1 [a]
0.5 x	203 [b]	192 [b]	451 [b]	321 [b]	54.6 [b]	51.9 [b]	46.7 [b]	46.2 [b]
1 x	226 [c]	225 [c]	452 [b]	389 [b]	78.3 [c]	56.1 [b]	47.6 [b]	35.2 [b]
Fertilization								
0 N	92 [a]	81 [a]	187 [a]	166 [a]	61.9 [a]	99.2 [a]	59.0 [a]	78.1 [a]
50 N	240 [b]	208 [b]	490 [b]	424 [b]	80.0 [b]	85.2 [b]	76.2 [b]	67.1 [b]
100 N	277 [c]	206 [b]	566 [c]	420 [b]	40.5 [c]	79.8 [b]	38.6 [c]	62.8 [b]

Means in a given column followed by different letters indicate significant differences based on $LSD_{0.05}$ test.

Weed ADM varied across years. Conversely to crop biomass, the main tillage effects in all two years were a lower weed biomass production under CT in both varieties, with a lower production in 1999 than 2000. These results are in agree with Arshad *et al.*, (1995) who found a higher weed mass in ZT than in CT. In no-tillage systems, the weed's seeds remain in the upper layer and contribute immediately to the infestation. This could explain the greater biomass registered in ZT plots than CT plots in spite of the relatively drier spring of 1999. However, Buhler (1995) determined that the effect of surface residue on weed dynamics appears to be complex and controlled by interacting factors (soil type, weed species, quality and type of residue, allelopathy, environmental conditions).

Despite the higher weed ADM registered in K.Dragon, a greater grain yield has been obtained compared with B.Pronto (table 2). These results showed a varietal difference for the effect of both tillage and weed competition. K.Dragon appears as a higher competitive variety than B.Pronto. However, due the larger weed growth registered at K.Dragon plots, the long-term impact of weed seed return on seed bank dynamics must be examined.

These results are in agree with Arshad *et al.*, (1995) who found that differences in weed infestation do not always result in significant yield differences. This lack of relations between weed biomass and crop yield could be explained by the occurrence of resources complementarity (no crop-weed competence).

Table 2. Wheat grain yield (GY, $g.m^{-2}$) at BBCH 91. BP: B.Pronto. KD: K.Dragon

	1999		2000	
	BP	KD	BP	KD
Tillage				
CT	160.4 [a]	213.1 [a]	290.5 [a]	381.4 [a]
ZT	189.2 [b]	229.3 [b]	255.8 [b]	364.1 [b]
Herbicide				
0 x	162.1 [a]	207.5 [a]	226.4 [a]	356.6 [a]
0.5 x	171.1 [b]	214.6 [b]	287.6 [b]	375.1 [b]
1 x	191.4 [b]	241.4 [b]	305.5 [b]	386.6 [b]
Fertilization				
0 N	111.7 [a]	157.6 [a]	184.7 [a]	243.1 [a]
50 N	205.3 [b]	240.5 [b]	304.2 [b]	415.8 [b]
100 N	238.1 [c]	284.7 [c]	330.6 [c]	459.3 [c]

Means in a given column followed by different letters indicate significant differences based on $LSD_{0.05}$ test.

Herbicide

K.Dragon had higher competitive ability than B.Pronto when no herbicide was added ($p<0.05$). No differences were observed between the effects caused by the 1 x and 0.5 x dose in the analysed variables. Significant differences amongst these herbicide rates and 0 x were observed for weed ADM and wheat grain yield (Table 1, Table 2). Weed biomass was mostly reduced (55 % both years) by reduced herbicide rates (0.5 x). No interactions between tillage-by-herbicide were found. According to these results, herbicides influenced grain yield and weed ADM similarly irrespective of tillage treatments.

Teasdale *et al.*, (1991) revealed the risk of confounding the effect of tillage with herbicide effects and stated the need to evaluate the direct effects of tillage systems on weed populations' dynamics over several years of rotation. No significant tillage-by-herbicide interactions were presented here. These results indicate that there is no influence of tillage system on weeds despite herbicide application at reduced doses. All such data tend to indicate that reduced herbicide rate have an adequate fit with the weed flora present in the study.

Fertilization

For the two years evaluated, the N fertilizer significantly increased wheat ADM and grain yield with differences ($p \leq 0.05$) between medium and high level for grain yield. At 1999, a year with a relatively dry spring, a minor effect was obtained (Table 1, Table 2).

No significant interaction tillage-by-fertilization at every year for grain yield was obtained. A higher yield increase of wheat at no-tillage treatment (ZT) in every year (100 N: 120 % in 1999, 110 % in 2000) was registered with respect to conventional tillage (CT, 100 N: ~70 % both years).

Only at B.Pronto plots a weed ADM increase were obtained when applying moderate N rate (50 N) (table 1). However, the two fertilization levels lowered significantly weed biomass when competing with K.Dragon. Like as in tillage treatment, when nitrogen was added the cvs presented differences in competitive ability against weeds.

The results present here indicate that N optimum does not concur for wheat and weed natural populations. These results are in agree with Valenti & Wicks (1992), who found that applying N to winter wheat decreased annual grass weed populations and weed yields and with those obtained by Jørnsgård *et al.*, (1996). These authors found that above dry matter of *Chenopodium album*, *Lamiun amplexicaule*, *Stellaria media* and *Veronica spp.* cannot be improved with N application in competence with wheat and barley. Consequently, they theorized that in a low input agriculture a lower application of N could favour the increase of such species and a different proportion of them in weed natural populations. Our result are contrasting which those reached by Acciaresi *et al.*, (2000), who reported a progressively higher *Lolium multiflorum* aggressivity with increasing N rates in competence with wheat varieties and with those obtained by Cook & Clarke (1997). These authors stated that weed number increased with successive use of low herbicides and weed control was made more difficult with the continued use of low N rates.

These results suggest that variety selection may be an important component to integrate with tillage, herbicide and fertilization. Within the conditions tested here, the use of subnormal herbicide doses (50 %) and N fertilization may be useful in wheat production systems (conventional and no tillage systems) as a strategy to manage natural weed populations. More information is needed on management practices to minimize long-term effects on weed dynamics.

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POSTER SESSION 8D

DEVELOPMENTS IN HERBICIDE APPLICATION AND FORMULATION TECHNOLOGY

Session Organiser: D A Webb
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Poster Papers: 8D-1 to 8D-7

The characteristics of sprays produced by air induction nozzles

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ABSTRACT

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

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

INTRODUCTION

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

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

MATERIALS AND METHODS

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

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

Table 1. Air Induction nozzles selected

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

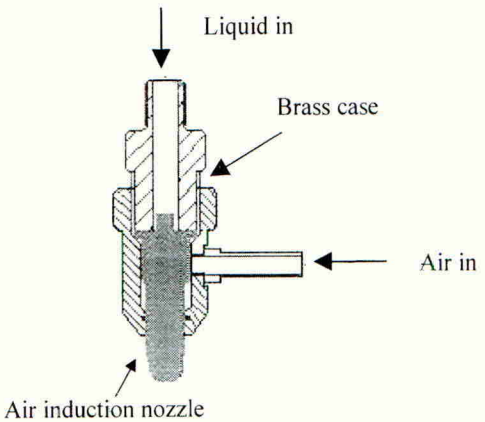

Figure 1. Arrangement for measurement of air flow into air induction nozzles

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

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

RESULTS AND DISCUSSION

Droplet size and air intake

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

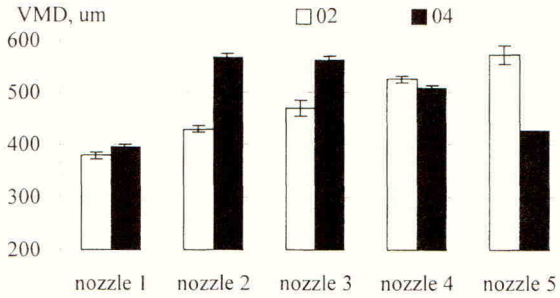

Figure 2. The effect of nozzle output on VMD

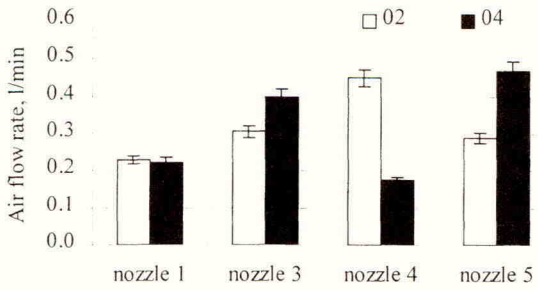

Figure 3. The effect of nozzle size on air intake

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

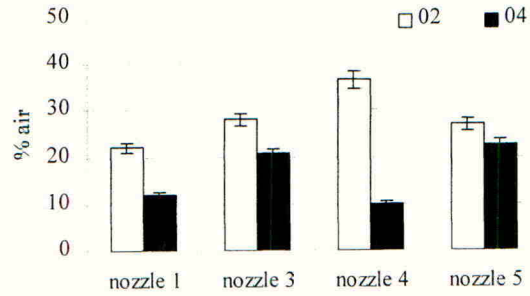

Figure 4. The effect of nozzle size on the proportion of air exiting four air induction nozzles

Droplet velocities

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

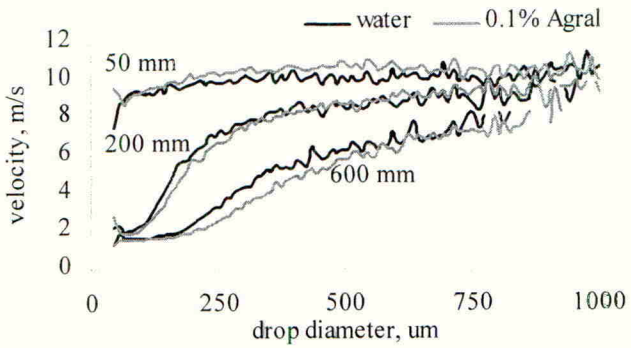

Figure 5. Variation of velocity with droplet size at three distances from AI nozzle 5

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

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

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

Spray drift

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

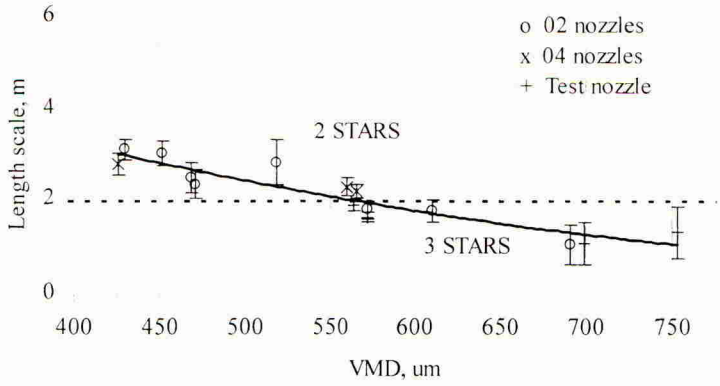

Figure 6. Relationship between VMD and drift length scale for a range of air induction nozzles at a range of pressures, compared to a standard flat fan "03" reference nozzle

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