SESSION 3C POSTER

NEW CONCEPTS AND NOVEL METHODS

Proceedings 1982 British Crop Protection Conference - Weeds

A TECHNIQUE USING RHIZOME NODES OF AGROPYRON REPENS TO ASSESS HERBICIDE ACTIVITY. SOME RESULTS USING GLYPHOSATE AND FLUAZIFOP-BUTYL

D. Coupland and D.F. Wyatt
ARC Weed Research Organization, Begbroke Hill, Yarnton, Oxford, OX5 1PF

Summary. Techniques are described using single-node rhizome fragments of Agropyron repens to assess herbicide activity. Details of plant preparation and herbicide treatment are given. Some preliminary results using glyphosate and fluazifop-butyl are also presented. Unsprouted rhizome nodes were affected more than the larger, sprouted ones and, for fluazifop-butyl, sterile nodes were affected more than non-sterile ones. The techniques may be useful for the specific screening of herbicides and other compounds for perennial species such as A. repens and are ideally suited for mode of action studies.

INTRODUCTION

Agropyron repens (common couch) is regarded as a serious weed in many temperate regions of the world because of its ability to propagate swiftly by underground rhizomes. Buds at the nodes on these rhizomes have the potential to develop into new plants and thus spread the weed vegetatively. These nodes are the main 'target areas' for translocated herbicides such as glyphosate and fluazifop-butyl (Plowman et al., 1980; Claus and Behrens, 1976). The evaluation of such herbicides for the control of A. repens is often made difficult because of the time required to assess long-term effects on whole plants (Turner and Cussans, 1981). It is also reported, for example, that regeneration can occur from these underground parts up to 18 months after the apparent death of the foliage (Fryer and Makepeace, 1977). A quick, and preferably simple method for assessing herbicide effects directly on rhizome nodes and buds is obviously of value.

The present work describes a system using both sterile and non-sterile rhizome nodes and presents some preliminary results using glyphosate and fluazifop-butyl.

METHODS AND MATERIALS

Plant material. Uniform single-node rhizome fragments were obtained from clonal stock material. Apices, immature nodes (up to 3 nodes from the apex), and sprouted nodes were discarded. All roots and scale leaves were removed to expose the bud.

Surface sterilization of nodes. Nodes were washed clean in tap water, rinsed in absolute alcohol, then placed in 50% sodium hypochlorite solution (approximately 7% available chlorine) containing 0.01% Agral surfactant. The nodes were removed after 20 minutes and rinsed in sterile distilled water (4 x 100 mls). The mercuric chloride/hydroxylamine hydrochloride method of Barber (1967) was also tried. Although this method gave the same visual reduction in contamination, it produced plants that were less uniform than the hypochlorite method and was therefore not used.

Agar preparation. Agar medium was prepared containing Murashige and Skoog nutrients (obtained from Flow Laboratories Ltd.). Each 100 ml of agar mixture contained 99 ml nutrient solution (made by dissolving 4.71 g of the commercial mixture in 1 litre sterile distilled water) plus 1 ml of kinetin stock solution (10 mg kinetin dissolved in 5 drops 1 M NaOH and made up to 500 ml). This mixture was then adjusted to pH 5.6 using dilute NaOH, and 0.4 g agar added ("Lab M" No. 2 agar obtained from

London Analytical and Bacteriological Media Ltd.). After autoclaving and allowing to cool, but before the mixture had set, the antibiotics nystatin (10 µl of a suspension of 1 million USP units in 2 ml absolute alcohol) and gentamycin (20 µl of a stock solution containing 1 g gentamycin sulphate in 10 ml sterile distilled water) were added. Three ml of this nutrient agar mixture was pipetted into each cell of a sterile 25 cell repli dish.

Preparation of herbicide-containing agar. Commercial formulations of glyphosate and fluazifop-butyl were diluted with sterile distilled water to give 3.33 mg a.i./10 ml. Different amounts (1 ml, 0.1 ml and 0.01 ml) of these solutions when made up to 100 ml with the nutrient agar mixture gave the required quantities of active ingredient per repli dish cell. The herbicide stock solutions were added to the agar medium after autoclaving and the addition of antibiotics but before the mixture had set.

Treatment of sterile nodes. After sterilizing, the bud together with a small amount of rhizome tissue on either side (2-3 mm) was cut from each fragment and placed singly in repli dish cells. The dishes were then sealed with Parafilm and placed in a controlled environment room set at 16/10°C (day/night) and 90 W/m^2 (14 h daylength). After 3 weeks, when there was good shoot and some root growth, the nodes were transferred to new dishes containing fresh agar and the herbicides. The nodes were planted just beneath the surface of the agar with bud and shoot tissues above it. These dishes were sealed and kept in the same conditions as above. Nodes were assessed after 2 weeks (see below). Sterile, unsprouted nodes were planted directly in herbicide-containing agar as above and left for 3 weeks before assessment. 'Control' nodes were planted in nutrient agar with no herbicide.

To determine if nodes grown under sterile conditions would produce normal, healthy plants, some untreated nodes were kept in nutrient agar for 4 weeks, then carefully removed and planted in Levington compost in trays. These were kept moist and placed inside plastic bags to maintain a humid atmosphere.

In all the above procedures, all sterile operations were carried out in laminar-flow cabinets using sterile equipment.

Treatment of non-sterile nodes. Single-node rhizome fragments were cut 2 cm long and treated either immediately with herbicide or grown on moist filter paper (conditions as above) until the shoots were 3-4 cm long and there was some root growth. Plastic tubes (1 cm) were placed securely on the cut ends of the rhizomes, and herbicide solutions added to these tubes. The herbicide concentrates were diluted with sterile distilled water to give 0.1, 1.0 and 10.0 µg a.i./40 µl; for each treatment 20 µl was dispensed to both tubes on each node fragment. The nodes were placed in small trays on moist filter paper and put into plastic bags to maintain a humid atmosphere, then placed in the same controlled environment conditions as above. The nodes were watered through the plastic tubes after the herbicide solutions had been taken up. 'Control' nodes were treated with distilled water only. Nodes were assessed after 2 weeks (sprouted) or 3 weeks (unsprouted).

Assessments. At the end of the 2 or 3 week period, quantitative measurements of shoot fresh weight and chlorophyll content were made. Root growth was variable and accordingly was not considered as a reliable measure of herbicide effect. Shoot growth and chlorophyll content have both been shown to be affected by glyphosate (Cole, 1979; Kitchen et al., 1981) and fluazifop-butyl (Plowman et al., 1980). Chlorophyll was determined after extraction in 80% acetone according to the method of Arnon (1949).

Experimental design and statistics. Each sterile treatment was replicated 5 times. The three doses, controls and a row of blank cells (to check for dish or agar contamination) were conveniently accommodated in the 5 x 5 repli dishes. Non-sterile treatments were replicated 6 or 8 times (see Tables). Each experiment was repeated twice. Results were subjected to analyses of variance where possible and treatment means were compared using S.E. values.

RESULTS

Technique. Both the sterile and non-sterile methods were relatively quick and simple to perform. The rhizome nodes used for the tests were easy to prepare and to treat, and results were obtained within 2 to 3 weeks. The methods are best suited to water soluble compounds, or to compounds that form stable emulsions in water. It would be difficult to use other types of compounds, such as wettable powders, for example. Volatile compounds could be used with modifications in the procedure to more effectively separate the treatments.

Node growth. Experiments, using surface-sterilized rhizome material, showed that node tissues could be grown successfully in sterile nutrient agar but that microbial contamination did occur occasionally despite the use of the antibiotic and anti-fungal agents. The main contaminant was tentatively identified as Sporobolomyces sp., a yeast-like fungus (M.P. Greaves, personal communication). Whenever such contamination occurred, the cultures were discarded. In this way, the effects on the plant tissues could be attributed to the herbicides rather than any contamination. The non-sterile tissues were undoubtedly contaminated, but the absence of any additional nutrients kept microbial growth to a visual minimum within the 3-4 week experimental period.

Sterile nodes grown on in soil developed into normal, healthy plants with well-formed roots and developing rhizome systems.

Herbicide effects. In general, the unsprouted nodes were affected more than the sprouted nodes. Because of the high phytotoxicity to the unsprouted sterile nodes, a significant difference between the two herbicides could not be demonstrated (Table 1). With non-sterile, unsprouted nodes, glyphosate, at the two lower doses, reduced shoot fresh weight significantly more than fluazifop-butyl (Table 2). With sprouted nodes, there were differences between sterile and non-sterile tissues. Non-sterile nodes were affected by glyphosate to approximately the same extent as sterile ones but, with fluazifop-butyl, non-sterile ones were affected much less than sterile ones (shoot weight, Tables 3 and 4). Chlorophyll data showed different effects. With glyphosate, non-sterile nodes were affected more than sterile nodes, but with fluazifop-butyl there was no consistent trend in dose response.

DISCUSSION

A wide dose range was used in these experiments with glyphsoate and fluazifop-butyl in order to test the methods rather than to obtain specific dose response data. The results showed that, in general, immature unsprouted nodes were affected more than sprouted nodes. There were probably several reasons for this effect. Unsprouted nodes were smaller and the bud tissues were not developing to any extent. Sprouted nodes were larger, with developing root and shoot tissues. These differences in size could have resulted in an effective dilution of the herbicides in the larger tissues. Furthermore, the shoot tissues were chlorophyllous and therefore the sprouted nodes were physiologically distinct from the unsprouted nodes. From a technique point of view, this difference between sprouted and unsprouted nodes means that care must be taken to obtain uniform plant material.

Differences were also observed between sterile and non-sterile sprouted nodes. There could be several reasons for these differences. First, the sterile and non-sterile nodes were treated in different ways. Sterile nodes could absorb herbicide only by diffusion through the cut ends of the node and any other surface in contact with the agar. Also, the herbicides were dissolved throughout the agar so the nodes were not directly treated with all of the herbicide. With the non-sterile nodes, all of the herbicide solution was absorbed by the nodes.

Second, there were differences in herbicide concentration between the treatment solutions (non-sterile method) and the agar mixtures (sterile method). However, this was probably insignificant in these studies because more phytotoxicity was observed with the sterile technique in which lower concentrations of herbicide were

used.

Third, the sterile and non-sterile nodes were of different size. The larger non-sterile material could have "inactivated" more herbicide through adsorption, endogenous enzymes and enzymes released during sample preparation. In the sterile work there was much less internode tissue present and any enzymes released during sample preparation were more likely to be washed out during the sterilizing and rinsing steps.

And finally, the differences in herbicide effect with the sterile and non-sterile nodes could be attributed to the presence of surface micro-organisms, especially in or around the cut ends of the node fragments.

In conclusion, the methods described allow comparisons of the relative phyto-toxicities of chemicals presented in the vicinity of the site of action to be made without the complications and limitations often associated with uptake and translocation. These methods may be of use in the specific screening of herbicides and PGR compounds and may help supplement the existing, more time-consuming tests for perennial species such as A. repens. However, interpretation or comparison of data to whole plant studies may be initially difficult. Additionally, the use of small pieces of plant tissue that are easily surface sterilized makes this technique ideally suited to PGR and mode of action studies.

REFERENCES

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in Beta vulgaris. Plant Physiology, 24, 1-15.

Barber, A. (1967). The effect of micro-organisms on the adsorption of inorganic nutrients by intact plants. I. Apparatus and culture technique. Journal of Experimental Botany, 18, 163-169.

Claus, J.S.; Behrens, R. (1976). Glyphosate translocation and quackgrass rhizome bud kill. Weed Science, 24, 149-152.

Cole, D.J. (1979). Studies into the mode of action of glyphosate. Ph. D. Thesis, University of Bath, pp 130.

Fryer, J.D.: Makepeace, R.J. (Eds.). (1977). In: Weed Control Handbook, Vol. I, Principles. Blackwell Scientific Publications, London, p 247.

Kitchen, L.M.; Witt, W.W.; Rieck, C.E. (1981). Inhibition of chlorophyll accumulation by glyphosate. Weed Science, 29, 513-516.

Plowman, R.E.; Stonebridge, W.C.; Hawtree, J.N. (1980). Fluazifop-butyl - a new selective herbicide for the control of annual and perennial grass weeds. Proceedings 1980 British Crop Protection Conference - Weeds, 1, 29-37.

Turner, D.J.; Cussans, G.W. (1981). Techniques for the assessment of perennial grass weeds. Proceedings Association of Applied Biologists Conference on grass weeds in cereals in the United Kingdom, 109-114.

Table 1

Effects of glyphosate and fluazifop-butyl applied to sterile, unsprouted rhizome nodes

Herbicide Dose applied (μg)	Glyphosate	Fluazifop-butyl	Glyphosate	Fluazifop-butyl
	Shoot fresh wt (mg)		Chlorophyll (mg/g fr. wt.)	
0	29.5 (±7.24)		0.36 (±0.075)	
0.1	14.8 (±4.21)	12.5 (±5.38)	0.29 (±0.069)	0.26 (±0.091)
1.0	*7.6 (±5.48)	0	*0.10 (±0.067)	0
10.0	**9.7 (±3.54)	0	**0.17 (±0.049)	0

NB. Because of the many zero values in this set of data, no overall SE of means can be presented. Individual S.E. of means are given in parenthesis (DF=9).

* 2 out of 10 replicate nodes viable.
** 6 out of 10 replicate nodes viable.

Table 2

Effects of glyphosate and fluazifop-butyl applied to non-sterile, unsprouted rhizome nodes

Herbicide Dose applied (μg)	Glyphosate	Fluazifop-butyl	Glyphosate	Fluazifop-butyl
	Shoot fresh wt (mg)		Chlorophyll (mg/g fr.wt.)	
0	19.3		0.23	
0.1	11.5	19.5	0.20	0.26
1.0	0	7.4	0	0.36
10.0	0	0	0	0
± SE (DF = 31)	2.58		0.054	

Table 3

Effects of glyphosate and fluazifop-butyl applied to sterile, sprouted rhizome nodes

Herbicide Dose applied (μg)	Glyphosate	Fluazifop-butyl	Glyphosate	Fluazifop-butyl
	Shoot fresh wt (mg)		Chlorophyll (mg/g fr. wt.)	
0	200		0.49	
0.1	127	122	0.35	0.25
1.0	91	38	0.19	0.14
10.0	41	36	0.09	0.05
± SE (DF = 69)	27.0		0.059	

Table 4

Effects of glyphosate and fluazifop-butyl applied to non-sterile, sprouted rhizome nodes

Herbicide Dose applied (μg)	Glyphosate	Fluazifop-butyl	Glyphosate	Fluazifop-butyl
	Shoot fresh wt (mg)		Chlorophyll (mg/g fr. wt.)	
0	395		0.87 (1.92)*	
0.1	298	297	0.22 (1.34)	0.17 (1.20)
1.0	148	207	0.03 (0.46)	0.24 (1.37)
10.0	105	238	0.03 (0.46)	0.19 (1.27)
± SE (DF = 41)	26.2		0.026 (0.056)	

* Values in parentheses are log transformations for statistical comparisons (log variate = $\log_{10}$ (variate x 100)).

Proceedings 1982 British Crop Protection Conference - Weeds

THE INVOLVEMENT OF STOMATA IN BENTAZONE ACTION IN CHENOPODIUM ALBUM, L.

P.J. Dunleavy, A.H. Cobb, K.E. Pallett and L.G. Davies
Department of Life Sciences, Trent Polytechnic, Burton Street, Nottingham. NG1 4BU

Summary. Leaf diffusive resistance porometry has shown that in a controlled environment stomatal movement in C. album follows a circadian rhythm with pre-dawn opening, pre-dusk closure and partial midday closure. Application of bentazone caused stomatal closure and disruption of this rhythm, particularly when applied during the mid-morning period when the stomata were open. This response was less apparent following midday applications and the corresponding herbicidal efficacy was also reduced. Penetration of ^{14}C-bentazone into C. album leaves was found to be relatively slow but also influenced by the time of day of application. However, the addition of the surfactant Actipron to the formulation enhanced penetration regardless of application time. Further data has shown that bentazone exerts rapid and significant reductions in both intact leaf photosynthesis and transpiration, and it is suggested that stomatal closure is of importance in the complete mode of action of bentazone in this species. Herbicide, fat hen, porometry, penetration, transpiration, photosynthesis.

INTRODUCTION

The susceptibility of weeds to bentazone is influenced by both the prevailing environmental conditions (Davies et al., 1979) and the time of day of application (Doran and Andersen, 1976). Stomatal movement is also under tight environmental and physiological control (Jarvis and Mansfield, 1981), and in C. album bentazone has been shown to significantly influence stomatal movement in epidermal peels (Taylor et al, 1980).

This paper examines the effect of time of day of bentazone application in relation to stomatal movement and herbicide penetration in intact leaves of C. album, and also monitors leaf photosynthesis and transpiration in the presence or absence of the herbicide.

METHODS AND MATERIALS

(1) Plant growth conditions: In all experiments the youngest fully expanded leaf was used from 4-6 week old C. album plants grown in a 1:1 mix of J. Arthur Bowers potting compost and John Innes No. 2 compost in Fisons 600G3 growth cabinets. A 16h photoperiod was provided by Atlas Grolux and Warmwhite fluorescent tubes at a photon flux density of 200$\mu E.m^{-2}.s^{-1}$ (P.A.R.) and temperatures maintained at 20°C (day) and 12°C (night).

(2) Measurement of leaf diffusive resistance: Adaxial leaf diffusive resistance (LDR) was determined in the growth cabinet using a Crump Automatic Diffusive Resistance Porometer, which functions by measuring the time taken for the humidity in the sensor cup surrounding the leaf to rise between two pre-set levels. The porometer was calibrated using perforated plates before each set of readings was taken. Up to 20 readings of different leaves could be obtained in a 30 min period, although no single plant was measured more than twice in one day to avoid leaf damage. Thus, for example, 96 plants were monitored over a 10d period to obtain the data in Fig. 1. For herbicide studies, 20 plants were sprayed with field-rate bentazone (31 in 280l water. ha^{-1}; formulation BAS 3517H, containing 48% (w/v) a.i.bentazone) at either 0930-0945h or 1215-1230h using a Binks-Bullows gravity-fed sprayer at 1 bar pressure

whilst a further 20 plants were left untreated to act as controls. LDR was monitored at 0900-1030h and 1200 - 1330h for 6d before and 5d after when chlorotic symptoms began to develop.

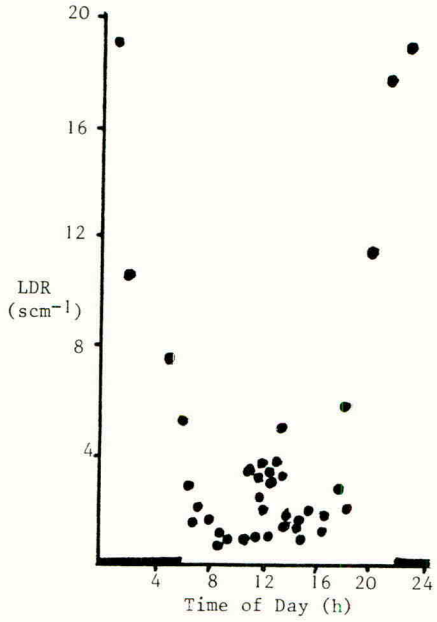
(3) ^{14}C-bentazone uptake by C. album leaves: Penetration of ^{14}C-bentazone into the youngest fully expanded leaf of known LDR was determined according to Pallett and Caseley (1981) at either 0930 or 2030h. Five 0.5µl drops of field-rate bentazone containing 2.5 x 10^5 dpm total activity, were applied to each of 4 leaves using a Burkard microsyringe. After 0, 24, 48, 72 and 168h in the growth cabinet the treated leaves were excised and washed in 10cm^3 0.5% (v/v) Actipron, and the radioactivity in the washings determined by liquid scintillation counting.

(4) Measurement of C. album photosynthesis and transpiration: C. album leaf photosynthesis was continuously measured for 5h after application of field-rate bentazone at 0930h using an Infra-Red Gas Analysis (IRGA) circuit, as described by Dunleavy (unpubl.). Light intensity was maintained at 750µE.m^{-2}.s^{-1}, temperature at 20°C and relative humidity at 60 ± 10% during measurement. Photosynthesis was calculated by determining CO_2 depletion rates according to Gaastra (1959). Transpiration was measured in a similar fashion by monitoring increase in r.h. using a Vaisala HMP 13ST sensor.

RESULTS

Fig. 1

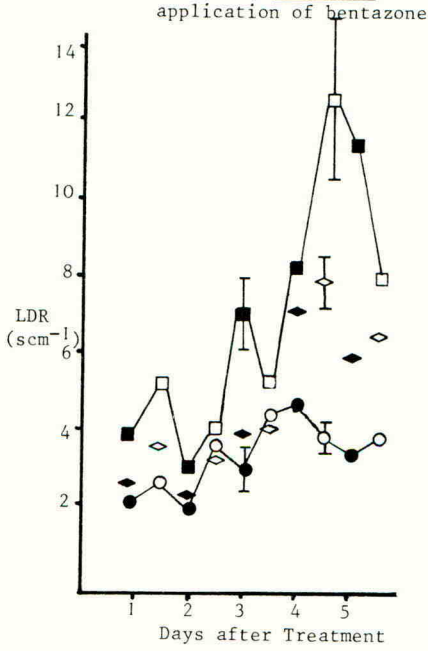
Relationship between C. album LDR and time of day



Each point represents the mean of upto 20 measurements of different leaves taken within a 30 minute period.

Fig. 2

Changes in C. album LDR after application of bentazone



Closed symbols measured 0900-1030h; open symbols measured 1200-1330h; ●—○, control; ◆—◇, sprayed 1230h; ■—□, sprayed 0930h. Bars represent S.E. values, n = 15.

The relationship between LDR and time of day in C. album leaves (Fig. 1) infers a circadian rhythm of stomatal movement in this species when grown under the conditions described, with pre-dawn opening, pre-dusk closure, and a tendency to close around midday. Minimum LDR, and hence maximum stomatal opening, is observed both before and after the midday closure period. Fig. 2 shows the response of C. album LDR to bentazone application. Although control plants show midday closure throughout the period of observation, plants sprayed with field-rate bentazone at 0930h show departures from this rhythm in two major respects. Firstly, treated plants show higher values than the controls, and secondly, the rhythm in LDR is interrupted by bentazone application. Thus, plants treated at 0930h show a marked increase in LDR one day after treatment and after day 2 the expected midday closure is lost, culminating in further stomatal closure by day 6. However, plants sprayed during the midday closure period (1230h) show a more gradual increase in LDR, and only begin to deviate from control values 4 days after treatment. Furthermore, fresh and dry weight determinations show that more damage is shown in plants sprayed at 0930h than at 1230h (Table 1).

Table 1

Fresh and dry weights of plants before and 7 days after treatment with field-rate bentazone or water at either 0930 or 1230h

Time of day of Treatment	Days after treatment 0		7	
	Fresh Weight	Dry Weight	Fresh Weight	Dry Weight
Control	1.16 ± 0.5	0.116 ± 0.050	3.83 ± 1.3	0.415 ± 0.160
0930h	-	-	0.98 ± 0.37	0.154 ± 0.030
1230h	-	-	3.07 ± 0.50	0.303 ± 0.100

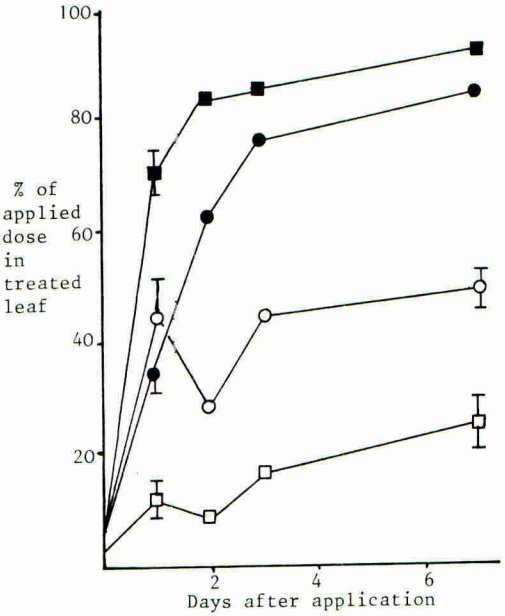
(Weights in g; values expressed as means ± S.D. where n = 8)

Fig. 3 shows that the penetration of ^{14}C-bentazone into intact C. album leaves is approximately doubled if the herbicide is applied at 0930h when mean LDR is 1.67s.cm^{-1} and stomata open, than if applied at 2030h when mean LDR is 15.6s.cm^{-1} and the stomata effectively closed. However, the addition of 0.5% (v/v) Actipron to the formulation ensured a more rapid penetration and reversed the time of day response.

Fig. 4 clearly illustrates the time-course of inhibition of both whole leaf photosynthesis and transpiration by field-rate bentazone. Total inhibition of photosynthesis occurred after 4h of measurement and no subsequent recovery was observed, whereas the inhibition of transpiration took only 2.5h.

Fig. 3

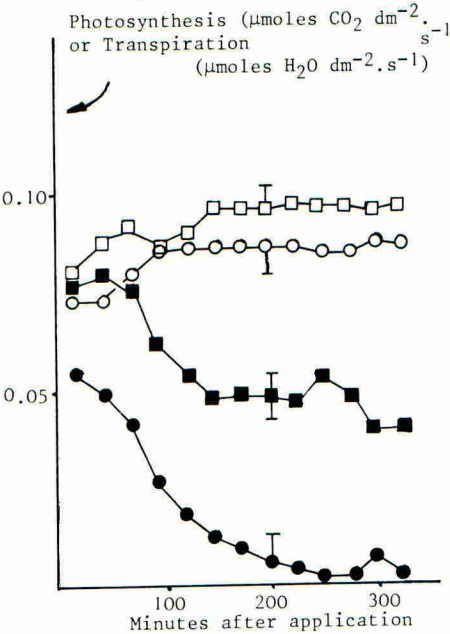
Penetration of ^{14}C-bentazone into C. album leaves in the presence or absence of 0.5% (v/v) Actipron



Open circles, bentazone, applied 0930h; closed circles, bentazone & actipron 0930h; Open squares, bentazone, 2030h; closed squares, bentazone & actipron, 2030h. Bars represent S.E. values.

Fig. 4

Effect of bentazone on C. album leaf Photosynthesis and Transpiration



Open circles, control leaf photosynthesis; closed circles, treated leaf photosynthesis; open squares, control leaf transpiration; closed squares, treated leaf transpiration. Bars represent S.E. values.

DISCUSSION

The data presented above clearly shows that when C. album is grown in a controlled environment, leaf adaxial stomata show a pronounced circadian rhythm of movement, and that LDR is altered by bentazone application. Some supporting data has been obtained with field-grown C. album, although rapid changes in leaf external environment make porometer readings more variable and prone to error (Dunleavy, (unpubl.). However, this study, and a previous one using abaxial epidermal peels (Taylor et al., 1980), clearly show that stomatal movement in this species is affected by bentazone.

The observation that ^{14}C-bentazone penetrates C. album leaves more readily following 0930h application than at 2030h presents further supporting evidence for stomatal involvement in bentazone uptake. However, more than 30% of the applied dose could be washed off the treated leaf 7 days after application, indicating that bentazone uptake is a slow process in this species (c/f < 10% after 24h in the case of rapid ^{14}C-difenzoquat plus Agral uptake in wheat, Pallett and Caseley, 1981). Nonetheless, uptake was considerably improved in the presence of 0.5% (v/v) Actipron.

Previous studies in this laboratory have shown that C. album leaf surfaces are well endowed with water-repellent epicuticular wax platelets but that the guard cells

and antechambers of the stomatal complexes appear virtually wax free (Taylor et al., 1981). Direct bentazone passage through the stomatal pore is considered unlikely since actual pore dimensions are very small (1-2μm), although the presence of Actipron may facilitate this by reducing the surface tension of the herbicide formulation (Schönherr and Bukovac, 1972). It is considered more likely, however, that the important role of Actipron in this case is to provide better coverage of the leaf surface and thus expose a greater proportion of the epidermis to the herbicide. Indeed, the clear evidence that bentazone causes an inhibition of transpiration more rapidly than of photosynthesis (Fig. 4) supports the earlier data in Fig. 2, and suggests that the initial effect of bentazone application may be to close stomata thus limiting the CO_2 supply for photosynthesis. Later cumulative penetration of the herbicide may then inhibit photosynthesis at the chloroplast level by blocking electron transport, and eventually plant death.

ACKNOWLEDGEMENTS

We acknowledge the gifts of pure, formulated and radioactive forms of bentazone from BASF UK and Germany and B.P. Oil Ltd. for Actipron.

REFERENCES

Davies, L.G.; Cobb, A.H.; Taylor, F.E. (1979). The susceptibility of Chenopodium album to bentazone under different environmental conditions. Proceedings of the European Weed Research Society Symposium, 97-104.

Doran, D.L.; Andersen, R.N. (1976). The effectiveness of bentazone applied at various times of day. Weed Science, 24, 567-570.

Gaastra, P. (1959). Photosynthesis of crop plants as influenced by light, carbon dioxide, temperature and stomatal diffusion resistance. Mededelingen Van De Landbouwhogeschool Te Wageningen, Nederland, 59, 1-68.

Jarvis, P.G.; Mansfield, T.A. (1981). Stomatal Physiology. Society for Experimental Biology. Seminar Series; 8, Cambridge University Press.

Pallett, K.E.; Caseley, J.C. (1980). Differential DNA synthesis in difenzoquat tolerant and susceptible U.K. spring wheat cultivars. Pesticide Biochemistry and Physiology, 14, 144-152.

Schönherr, J.; Bukovac, M.J. (1972). Penetration of stomata by liquids - dependence on surface tension, wettability and stomatal morphology. Plant Physiology, 49, 813-819.

Taylor, F.E.; Cobb, A.H. and Davies, L.G. (1980). The effect of bentazone on stomatal behaviour in Chenopodium album, L. New Phytologist, 85, 369-376.

Taylor, F.E.; Davies, L.G.; Cobb, A.H. (1981). An analysis of the epicuticular wax of Chenopodium album leaves in relation to environmental change, leaf wettability and the penetration of the herbicide bentazone. Annals of Applied Biology, 98, 471-478.

Proceedings 1982 British Crop Protection Conference - Weeds

SELECTION FOR ASULAM TOLERANCE IN BARLEY

S.C. Giffard, H.A. Collin and P.D. Putwain
Botany Department, Liverpool University, L69 3BX

R.H. Hewett
May and Baker Ltd., Ongar Research Station,
Ongar, Essex, CM5 0HW

Summary An alternative approach to the development of new selective herbicides to cope with problem grass weeds in cereal crops is to improve the selectivity of appropriate existing herbicides. Ultimately plant breeding methods may be used to obtain more tolerant cereal varieties. Selection for tolerance to asulam in barley was carried out by post-emergence screening of intact plants and by screening excised embryos of barley grown on agar culture with asulam incorporated into the medium. The excised embryo technique was used to test the asulam tolerance of progeny derived from a single cycle of intact plant selection within the variety Midas. The majority of selected families showed enhanced tolerance to asulam in comparison with unselected families. Excised embryos, folic acid.

INTRODUCTION

One of the potential problems with intensive cereal cropping is the build up of grass weeds such as wild oat (Avena fatua L.), couch (Agropyron repens L.) and bromes (Bromus sterilis L., B. tectorum L.). Although many current herbicides will give satisfactory control of one or two of these species, very few will control the full spectrum of grass weeds. For example, difenzoquat, benzoylprop-ethyl and diclofop-methyl will effectively control A. fatua but are relatively ineffective against Bromus spp. In addition a lack of herbicide selectivity may sometimes blunt the effectiveness of even commonly used herbicides. For example, Smith and Finch (1978) reported that crop damage and therefore potential yield losses occurred with the use of barban, difenzoquat and diclofop-methyl to control A. fatua in spring barley.

An obvious solution to the problems is the development of new selective herbicides. However the rising costs of research and development are limiting factors. The number of compounds screened per marketed product has increased considerably (Green, Hartley and West, 1979). In addition the costs of satisfying stringent registration regulations including obtaining toxicological data are reducing the potential for new successful herbicides.

An alternative approach, first suggested by Pfeiffer and Zeller, (1954), would be to utilise the natural variability exhibited by crops in response to herbicides and to use plant breeding methods to obtain cereal crop plants which are tolerant to existing herbicides which are known to be efficient in controlling a broad spectrum of grass weeds e.g. asulam. Such an approach can be successful, for example, the recently developed strains of paraquat-tolerant perennial rye-grass (Lolium perenne L.) (Faulkner, 1978, 1982) and strains of oilseed rape (Brassica napus L.) which are resistant to atrazine and simazine (Souza Machado, 1982). The aim of the work described here was to investigate the possibility of selecting for herbicide tolerance in barley. Asulam was selected as an appropriate

herbicide since it is effective against a broad spectrum of grass and broadleaved weeds and it is an environmentally safe chemical. Work by Flack (1982) showed some intraspecific variation for asulam tolerance in barley indicating that it may be possible to ultimately breed tolerant varieties of this crop.

METHODS AND MATERIALS

Screening for tolerance

Resistance is only used to describe a strain which survives a dose (equivalent to a normal agricultural application rate) which is completely lethal to a normal susceptible strain or variety. We define tolerance to describe anything less than resistance, i.e. implying differential susceptibility between varieties, strains, lines and families or between individuals within a population.

Screening for asulam tolerance was carried out on intact plants and also using a tissue culture system. The method of selecting for asulam tolerance in intact plants was to apply a post-emergence spray onto large numbers of barley seedlings at the two leaf stage of development. The concentration of asulam had been previously determined by applying a range of concentrations to a control variety, Midas, and calculating a LC95 value using probit transformation of percent mortality values. The surviving plants were allowed to set seed and the progeny were used for further selection and to test the degree of inheritance of tolerance. Survival was the main basis for selection of parent plants, although vigour, degree of visible phytotoxic symptoms and spatial isolation of survivors were other selection criteria which were used.

Tissue culture techniques provide an alternative to intact plant screening. Selection may be based on cell rather than whole plant differences. This method of selection has produced several biochemical mutants (Widholm, 1978) and herbicide tolerant lines (Chalef and Parsons, 1978; Merrick and Collin, 1981). The main advantage of cell selection is that very substantial numbers of potential individuals may be screened relatively quickly in a relatively small controlled-environment. However a major disadvantage of cell selection is that regenerated plants may not show the herbicide tolerance characteristics of the selected cell lines and in addition valuable agronomic characters may have been lost.

Problems such as these can be overcome by using partially differentiated systems such as tissue culture embryoids (Merrick & Collin, 1980) or by using excised embryos (Bright et al., 1979). The embryoids and embryos can be obtained in large numbers and unlike undifferentiated cells can be easily grown into adult plants.

Barley seed embryos were used as the partially differentiated tissue culture system. Each embryo was excised asceptically, then 10 embryos were transferred to 50ml nutrient agar medium in each of 7 x 8cm screw topped specimen jars. The embryos were maintained at 25°C under fluorescent lights (500 mWatts cm^{-2}) on a 12 hour diurnal cycle. In certain experiments asulam and other additives such as folic acid and 4 amino benzoic acid were included in the medium. The effects on percent mortality and other growth parameters were assessed after 21 days. The survivors were transferred to John Innes No. 2 compost and grown on in a greenhouse. Many of the survivors developed normally and produced fertile seed.

RESULTS

Tolerance to asulam in embryos and intact plants

Selection of excised embryos for tolerance to asulam provides an alternative

selection method to post-emergence screening of intact plants. However it is necessary to demonstrate that both sources of material show the same pattern of response to increasing concentrations of asulam and that the mechanism of action of the herbicide was the same in embryos and intact plants.

Figure 1 shows a comparison of the percent mortality of excised embryos and intact plants in the presence of asulam or after spraying with asulam, respectively, plotted against log-concentration. The regression coefficients for excised embryos and intact plants were very similar. Response to asulam in both types of material was apparently very similar although all the embryos exposed to 5mg/l were dead within 25 days whereas intact plants sprayed with 4500 mg/l asulam required six weeks for mortality of all treated individuals.

Asulam exerts its inhibition of growth in the intact plant by blocking folic acid synthesis (Veerasekaran et al., 1981). In our investigation, the mechanism of action of asulam on the excised embryos was also shown to involve folic acid synthesis (Figure 2) since the inhibition, measured as percentage green seedlings, leaf number and shoot length, was overcome by the presence of either a precursor of folic acid, 4 amino benzoic acid (4 ABA) or folic acid itself. The reversal of the asulam inhibition in excised embryos indicates that asulam exerts its action in a similar way in both the excised embryo and intact plants. This would suggest that the two approaches are comparable, and that excised embryos can be used in a selection procedure for asulam resistance.

Screening excised embryos for asulam tolerance

The technique of screening excised embryos was first attempted using a modern barley variety, Midas, which was already known to be less susceptible to asulam than many other modern and older varieties - 1500 excised embryos were grown in nutrient agar in the presence of 5mg/l asulam. A small proportion of individuals showed a similar amount of leaf greening and short elongation as the control embryos which had not been exposed to asulam. After 21 days, approximately 80 individuals were arbitarily classed as 'tolerant' on the basis that their growth was at least 75% of control growth. Figure 3 shows the mean performance of 'tolerant', susceptible and control embryos. The 'tolerant' individuals were removed from the agar and were grown on to maturity in John Innes No. 2 potting compost. Seed was collected for subsequent progeny testing for asulam tolerance.

Screening intact plants and subsequent progeny tests

An excised embryo screening was used to test progeny derived from a previous intact plant screening of a large number of individuals of the variety Midas. Selected families of Midas were obtained from predominantly self-pollinated survivors of the intact plant screening. Excised embryos were taken from seeds belonging to several selected families and were compared with excised embryos from unselected families of Midas. The embryos were plated onto nutrient agar containing 5 mg/l asulam or with no asulam (controls). Growth parameters were measured after 21 days. The response of embryos exposed to asulam was expressed as a percentage of the control values for each family (Figure 4). It is clear that several of the selected families show enhanced tolerance to asulam although expression of tolerance depends on which growth parameter is measured.

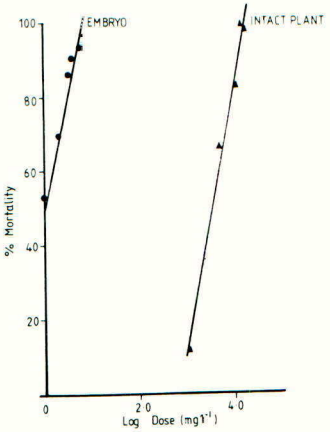


Fig. 1

A comparison of percent mortality of excised embryos and intact plants treated with a range of doses of asulam

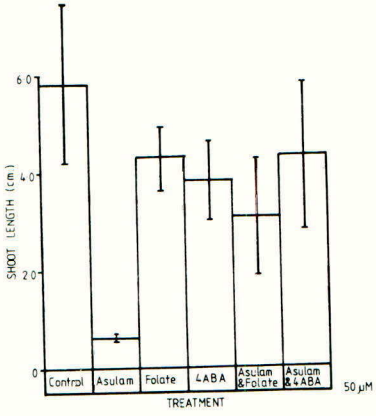


Fig. 2

Reversal of asulam induced inhibition of shoot growth by folate and 4 amino benzoic acid

DISCUSSION

Using excised embryos for screening for tolerance to asulam appears to be a useful new technique. Progeny tests may be carried out relatively quickly and with more precise environmental control than can normally be obtained using intact plant screening methods. It is possible that the technique could be adopted by plant breeders to test varietal susceptibility to existing herbicides or new products or to test potential new cereal varieties which may be in late stages of development or about to enter national list trials.

Fig. 3

A comparison of tolerant and susceptible excised embryos of barley growing in the presence of 5 mg/l asulam

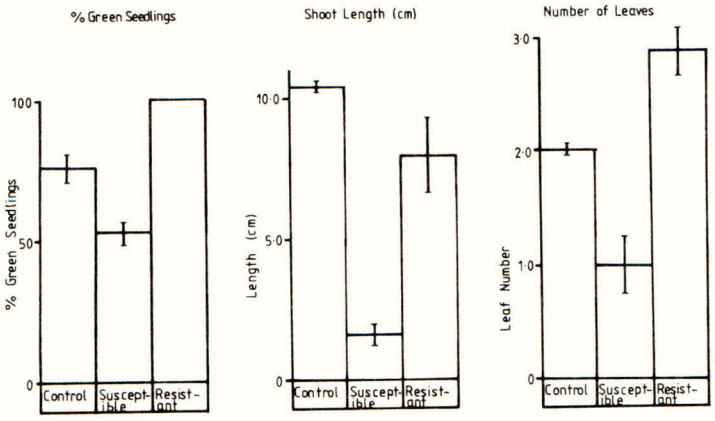
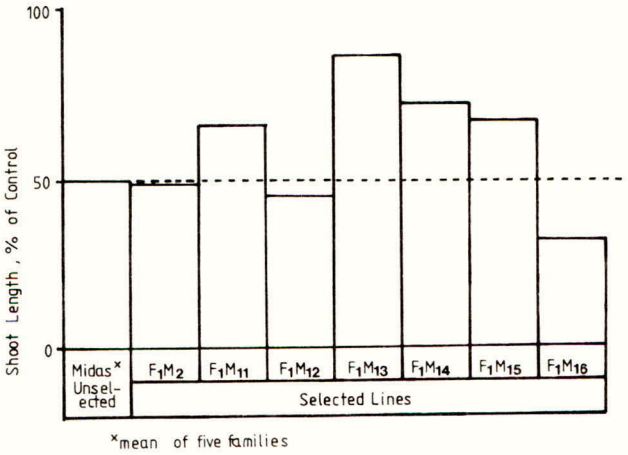


Fig. 4

Growth of barley single plant progeny (excised embryos in 5 mg/l asulam) after one cycle of selection by asulam



Screening of excised embryos may be used as a primary screening technique for asulam tolerance since the response to a range of concentrations of asulam, of intact plants and embryos, is similar. The technique probably has potential for development as a routine primary screening method for assessment of herbicidal activity.

The progeny tests of selected families of Midas indicate that there is variability in tolerance to asulam within barley varieties which can be selected for in a single cycle of selection. Recurrent selection may lead to considerable improvements in tolerance. Some barley varieties are already sufficiently tolerant to asulam to enable this herbicide to be used experimentally for control of Bromus sterilis (Pollard, 1981) without undue damage to the crop.

REFERENCES

Bright, S.W.J., Norbury, P.B. and Miflin, B.J. (1979). Isolation of a recessive barley mutant resistant to S- (2-aminoethyl) L-cysteine. Theoretical and Applied Genetics, 55, 1-4.

Chalef, R.S. and Parsons, M.F. (1978). Direct selection in vitro for herbicide resistant mutants of Nicotiana tabacum. Proceedings of the National Academy of Sciences, U.S.A., 75, 5104-5107.

Faulkner, J.S. (1978). The use of paraquat for controlling weeds in seedling swards of paraquat resistant Lolium perenne L. Proceedings 1978 British Crop Protection Conference - Weeds, 1, 341-348.

Faulkner, J.S. (1982). Breeding herbicide-tolerant crop cultivars by conventional methods. In: Herbicide Resistance in Plants, H.M. LeBaron and J. Gressel (eds.), John Wiley & Sons, New York, pp. 235-256.

Flack, J.L.E. (1982). The selection for asulam resistance in crop plants. Ph.D. thesis, University of Liverpool.

Green, M.B., Hartley, G.S. and West, T.F. (1979). Chemicals for Crop Protection and Pest Control. Pergamon, Oxford.

Merrick, M.M.A. and Collin, H.A. (1981). Selection for asulam resistance in tissue cultures of celery. Plant Science Letters, 20, 291-296.

Pfeiffer, R.K. and Zeller, A. (1954). Practical aspects of weed control by plant growth regulating substances in cereals. Proceedings of the 2nd British Weed Control Conference, 70-73.

Pollard, F. (1981). Personal communication.

Smith, J. and Finch, R.J. (1978). Chemical control of Avena fatua in spring barley. Proceedings 1978 British Crop Protection Conference - Weeds, 841-849.

Souza Machado, V. (1982). Inheritance and breeding potential of triazine tolerance and resistance in plants. In: Herbicide Resistance in Plants, H.M. LeBaron and J. Gressel (Eds.), John Wiley & Sons, New York, pp. 257-273.

Widholm, J.M. (1977). Selection and characterization of biochemical mutants. In: Plant tissue culture and its biotechnological applications, pp. 112-22. Bary, W., Reinhard, E., Zenk, M.H. (Eds.), Stringer, Berlin.

Veerasekaran, P., Kirkwood, R.C., and Parnell, E.W. (1981). Studies of the mechanism of action of asulam in plants. Part 2: Effect of asulam on the biosynthesis of folic acid. Pesticide Science, 12, 330-338.

Proceedings 1982 British Crop Protection Conference - Weeds

THE POST-EMERGENCE CONTROL OF A. FATUA, A. MYOSUROIDES AND SOME BROAD-LEAVED WEEDS IN WINTER CEREALS WITH CHLORSULFURON AND AC 222293

A. W. Lovegrove, P. J. W. Lutman and M. E. Thornton
ARC Weed Research Organization, Begbroke Hill, Yarnton, Oxford OX5 1PF

Summary. In four field experiments chlorsulfuron (0.01-0.02 Kg a.i./ha) improved the performance of isoproturon (1.2-2.5 kg a.i./ha), methabenzthiazuron (1.6-3.2 kg a.i./ha) and metoxuron (2.25-4.5 kg a.i./ha) against Alopecurus myosuroides. Mecoprop (1.1-2.2 kg a.i./ha) and isoproturon (1.2 kg a.i./ha) were also more effective against Veronica persica and Veronica hederifolia when mixed with chlorsulfuron. Reasonable control of Avena fatua was achieved with AC 222293 (0.5-1.0 kg a.i./ha) when applied in the autumn or as a sequence in the autumn and spring, but it had little effect on the other three species.

INTRODUCTION

Although herbicides are available for the majority of weed problems in winter cereals there is still a requirement for reliable control of mixed infestations of A. fatua and A. myosuroides, and for grass weed herbicides with a wider broad-leaved weed spectrum. Chlorsulfuron in worldwide field trials (Palm et al., 1980) and greenhouse experiments (Richardson et al., 1980) achieved good control of many broad-leaved weeds and grass weeds at doses of 0.04-0.28 kg a.i./ha. However, as some crop species including sugar beet and oilseed rape are susceptible to small soil residues (Ray, 1982) only doses of 0.01-0.03 kg a.i./ha can be used commercially. At these low doses activity on some weed species is reduced and so it may be necessary to mix this herbicide with others to improve overall performance. Experiments at WRO (Richardson et al., 1981) and by Cyanamid of Great Britain, suggested that another novel herbicide AC 222293 (Kirkland & Shafer, 1982) might have the potential for controlling both A. fatua and A. myosuroides, particularly if additional wetter was used. The field trials described were started in 1980 to study the potential for increasing the control of A. myosuroides and broad-leaved weeds by mixing chlorsulfuron with selected herbicides, and to study the performance of AC 222293 with or without wetters against A. myosuroides and A. fatua.

METHODS AND MATERIALS

The four experiments described were on commercially grown winter cereal crops with naturally occurring populations of broad-leaved and grass weeds. All trials were of a randomized block design, replicated three times, each plot 2 m x 6 m. All applications were made with a modified Oxford Precision Sprayer fitted with a 2 m boom delivering 250 l/ha through four matched TeeJets at 210 kPa pressure.

1980 Hatherop

The performance of AC 222293 alone, and chlorsulfuron alone and in mixture with isoproturon, methabenzthiazuron or metoxuron was studied against A. myosuroides. The experiment was on a sandy clay soil and the winter wheat (cv. Mardler) was drilled on 19.10.80. Treatments were applied on 26.11.80 and A. myosuroides heads were counted in 10 x 0.1 m^2 quadrats for each plot on 8.6.81.

1980 Dean

This experiment investigated the activity of AC 222293 (50% w.p.) alone and of chlorsulfuron (20% d.f.) alone and in mixture with a range of herbicides used for broad-leaved weed control against V. persica. Details of herbicides, formulations and doses are given in Table 3. The soil was a Cotswold brash and the winter wheat (cv. Mardler) was sown on 27.10.80. All treatments were applied on 3.12.80. The biomass of V. persica was visually assessed on 23.6.81 on a scale of 0-10 (10 = as control, 0 = none found) scores of 0-2 were considered commercially acceptable.

Table 1

Stages of growth of crops and cereals and weeds at spraying (Zadoks* scale used for grass weeds and cereals)

Site	Hatherop	Dean	Alcester	Milton	
Year	1980	1980	1981	1981	
				autumn	spring
Crop	13	12-13	14 21	14-15	31
A. myosuroides	11-14		11-16		
A. fatua				11-15	11-14, well tillered
V. persica		0-4 leaves			
V. hederifolia			0-4 leaves		

* Zadoks, Chang & Konzak (1974) Weed Research, 14, 415-422

1981 Alcester

The performance of AC 222293 (50% w.p.) alone, chlorsulfuron alone and in mixture with isoproturon or methabenzthiazuron, together with a formulated product of chlorsulfuron and methabenzthiazuron was studied against A. myosuroides and V. hederifolia. The crop winter barley (cv. Sonja) was drilled in early October into a sandy loam soil and all applications were made on 1.12.81. V. hederifolia plants were counted in 10 x 0.25 m^2 quadrats/plot on 25.3.82 and A. myosuroides heads counted in ten 0.1 m^2/plot on 9.6.82.

1981 Milton

In this experiment on a sandy clay loam soil the control of A. fatua from treatments of difenzoquat and from AC 222293 alone with or without three rates of Agral 90 and in mixtures with chlorsulfuron (75% d.f.) was investigated. The winter barley (cv. Igri) was drilled on 25.9.81 and applications were made either singly in the autumn (2.12.81) or spring (16.4.82) or as a sequence at both dates. When the spring applications were made two flushes of A. fatua were present, those germinating in the autumn which were well tillered and those germinating in the spring which had no tillers (Table 1). A. fatua panicles were counted and graded in 4 x 1 m^2 quadrats for each plot on 16.6.82 and 5.7.82. The number of spikelets m^2 was calculated. Fixed quadrats 0.25 m^2 were placed on the control plots and all A. fatua within them marked with coloured wire rings when treatments were applied (2.12.81 and 16.4.82). The rings on these fixed quadrats were relocated on 16.6.82 when the plants were counted and spikelet numbers for each plant estimated.

RESULTS

A. myosuroides

Chlorsulfuron. At Hatherop chlorsulfuron when applied alone at 0.01, 0.02 and 0.03 kg/ha achieved 51, 68 and 78% control of A. myosuroides heads (Table 2). Metoxuron and methabenzthiazuron alone, particularly the latter, gave unsatisfactory control leaving over 300 heads/m^2. Isoproturon alone at 1.2 kg/ha was slightly better but only the 2.4 kg/ha dose approached commercially acceptable levels of control reducing the number of heads to 87/m^2. The addition of chlorsulfuron to all three herbicides significantly improved the level of A. myosuroides control, the higher dose having a greater effect than the lower one. When mixed with the lower doses of methabenzthiazuron and metoxuron the control was similar to that achieved by chlorsulfuron alone. The best control (97%) resulted from the mixture of 2.4 kg/ha isoproturon with 0.02 kg/ha chlorsulfuron.

The results of Alcester the following year were generally similar (Table 2); methabenzthiazuron alone achieving no more than 40% control and isoproturon between 45-75% (Table 1). The best single treatment (isoproturon 2.5 kg/ha) still left over 100 heads/m^2. Chlorsulfuron alone at 0.02 kg/ha reduced the number of heads to 197/m^2, a 64% reduction. The addition of the higher rates of methabenzthiazuron to chlorsulfuron appeared to improve the latters performance but the differences were not statistically significant. The performance of the formulated product was similar to that of the tank mixtures. Mixtures of chlorsulfuron with isoproturon improved the level of control leaving only 77 heads/m^2 following 1.87 kg/ha and 39 following 2.5 kg/ha.

Table 2

The control of A. myosuroides (heads/m^2) at two sites by chlorsulfuron alone and in mixtures with isoproturon, methabenzthiazuron and metoxuron

Herbicide	Dose (kg a.i./ha)	Hatherop Chlorsulfuron (kg a.i./ha) (20% d.f.)x				Alcester (75% d.f.)	
		0	0.01	0.02	0.03	0	0.02
-		513	255	167	114	549	197
Metoxuron (50% s.c.)	2.25	334	158	132			
	4.50	342	101	67			
Isoproturon (50% s.c.)	1.25+	276	155	50		293	169
	1.87					300	77
	2.50+	87	25	13		123	39
Methabenzthiazuron (70% w.p.)	1.60	489	249	130		344	162
	2.40					489	173
	3.20	479	125	82		362	115
*Methabenzthiazuron + chlorsulfuron (70% w.p.)	2.40					94*	
ESE of mean (n = 3)			37.6			41.8	

\+ at Hatherop 1.2 and 2.4
* Formulated product Glean C
x d.f. = dry flowable

In the trial at Hatherop both doses of AC 222293 failed to reduce A. myosuroides by more than 50%. Its activity was similar in the second year at Alcester.

Veronica spp.

V. persica. Chlorsulfuron alone at 0.01, 0.02 and 0.03 kg/ha achieved 50-60% control at Dean (Table 3). Methabenzthiazuron, ioxynil + bromoxynil + mecoprop and ioxynil + bromoxynil applied alone all achieved commercially acceptable control, consequently the addition of chlorsulfuron had little effect. Mecoprop at 2.2 and 1.1 kg/ha and isoproturon 1.2 kg/ha alone did not fully control this weed. The performance of isoproturon was not enhanced by adding chlorsulfuron but all mixtures of chlorsulfuron with mecoprop improved the level of control. Best results followed the mixture of 2.2 kg/ha mecoprop with 0.02 kg/ha chlorsulfuron. AC 222293 at both doses had no effect.

V. hederifolia. At Alcester all doses of methabenzthiazuron alone achieved 90-98% control, therefore no enhancement was noticed from mixing with chlorsulfuron. This also applied to the formulated product. In contrast isoproturon had no effect. Chlorsulfuron alone and all the mixtures with isoproturon reduced the number of plants by approximately 50%. AC 222293 had little effect on V. hederifolia at all doses

Table 3

The control of Veronica spp. with chlorsulfuron alone and in mixtures with a range of herbicides for broad-leaved weed control

Herbicide	Formulation	Dose kg a.i./ha	Chlorsulfuron (kg a.i./ha) (20% d.f.) 0	0.01	0.02	0.03	(75% d.f.) 0	0.02
			V. persica+				V. hederifolia*	
-	-	-	10	5.3	3.7	3.7	103	48
Methabenzthiazuron	70% w.p.	1.6	1.8	0.7	1.8		11	9
		2.4					6	7
		3.2					1	2
Methabenzthiazuron! + chlorsulfuron	70% w.p.	2.4					7!	
Isoproturon	50% s.c.	1.25	4.0	4.7	3.3		94	53
		1.87					126	41
		2.5					83	57
Ioxynil+bromoxynil + mecoprop	49.5% e.c.	1.24	0.7	0.7	1.7			
Mecoprop	60% a.c.	1.1	4.3	2.8	2.3			
		2.2	4.5	3.0	1.3			
Ioxynil+bromoxynil	40% a.c.	0.4	1.8	0.7	0.0			
		0.8	1.0	0.7	1.0			

! Formulated product (Glean C)
+ V. persica Dean 1980 (10 = as control 0 = none found)
* V. hederifolia Alcester 1981: plant counts m^2, ESE of mean (n=3) = 10.6

A. fatua

Fixed quadrats. On 2.12.81 approximately 47 plants/m^2 were present in thequadrats. A further 53/m^2 emerged subsequently, but before 16.4.82. However 40% of the autumn emerging seedlings died during the winter. Despite their fewer numbers the surviving plants were larger and produced 82% of the total spikelets.

AC 222293. At 0.5, 0.75 and 1.0 kg/ha with 0.25% Agral 90 this herbicide significantly reduced the numbers of A. fatua spikelets when applied in the autum and as a sequence in the autumn and spring (Table 4) . Although the levels varied slightly between the two assessments the overall trends were similar. It appeared that the highest dose gave the best control but there was no statistically significant difference between the doses. The same treatments applied in the spring, only significantly reduced A. fatua spikelets at the 1.0 kg/ha dose. The level of control appeared much better at the first assessment than at the second. Difenzoquat at 1.0 kg/ha achieved almost 100% control of A.fatua spikelets when applied in the spring and as a sequence in the autumn and spring. The performance in the autumn alone was less good, achieving around 70% control. The addition of several concentrations of Agral 90 to AC 222293 at 0.75 kg/ha had no significant effect on activity when applied in the autumn. Its effect on the spring applications was less clear as the two assessments differed considerably. At the first assessment AC 222293 without Agral failed to reduce significantly the number of A. fatua spikelets. The addition of Agral 90 at all concentrations significantly increased the degree of control. At the second assessment all treatments failed to reduce the number of A. fatua spikelets, although the performance of AC 222293 tended to be better at the higher rates of Agral 90. The addition of chlorsulfuron to AC 222293 had no significant effect.

Table 4

The reduction in A. fatua spikelets/m^2 achieved by AC 222293 and difenzoquat

Herbicide	Dose kg ai /ha	% Agral	Autumn (1)	Autumn (2)	Spring (1)	Spring (2)	Autumn +Spring (1)	Autumn +Spring (2)	Transformed log 10 (x+1.0) Autumn (1)	Autumn (2)	Spring (1)	Spring (2)	Autumn +Spring (1)	Autumn +Spring (2)
AC 222293 (50% w.p.)	0.5	0.25%	305	203	365	756	111	147	2.36	2.10	2.53	2.85	1.78	2.08
	0.75	0.25%	144	107	254	788	17	53	2.15	2.01	2.32	2.85	1.13	1.67
	1.0	0.25%	164	86	51	396	18	79	2.15	1.86	1.70	2.54	1.27	1.79
Difenzoquat (63% w.p.)	1.0	0.5%	289	374	8	21	4	24	2.41	2.47	0.62	1.16	0.55	1.01
	ESE of mean (n = 3)								0.18	0.18				
AC 222293 (50% w.p.)	0.75	0	217	257	730	962			2.34	2.39	2.83	2.96		
		0.1	178	132	229	798			2.19	2.09	2.31	2.86		
		0.25	144	107	254	788			2.15	2.01	2.32	2.85		
		0.5	242	285	99	579			2.36	2.44	1.95	2.75		
AC 222293 + Chlor-sulfuron	0.75 0.02	0.25	285	342	134	590			2.40	2.47	2.01	2.69		
Control	mean		950	1364					2.96	3.11				
	ESE of mean (n = 3)								0.17	0.14				

(1) Assessment 16.6.82 (2) Assessment 5.7.82

DISCUSSION

At two sites, Dean 1980 and Alcester 1981, chlorsulfuron alone achieved useful control of V. persica and V. hederifolia. The performance of mecoprop was enhanced in mixtures with chlorsulfuron, but the other broad-leaved weed herbicides alone adequately controlled V. persica. Methabenzthiazuron controlled both species alone, therefore no enhancement was noticed with mixtures. As isoproturon alone failed to control both species the performance of the isoproturon/chlorsulfuron mixture depended entirely on the activity of the chlorsulfuron. Stellaria media was also assessed at Dean but this was controlled by all herbicides alone apart from AC 222293 which also failed to control Veronica spp.

A. myosuroides was assessed at two sites, Hatherop 1980 and Alcester 1981. Both sites had a history of poor control of this weed and applied alone the urea herbicides failed to achieve commercially acceptable control of A. myosuroides, although isoproturon approached it. Chlorsulfuron improved the performance of all herbicides in mixtures. Mixtures of chlorsulfuron with methabenzthiazuron or metoxuron achieving useful control of A. myosuroides when the full doses were mixed with 0.02 kg a.i./ha chlorsulfuron. However the results were not as good as exspected from the results of Palm et al. (1980). At both sites the best control was achieved from the full dose isoproturon with 0.01 or 0.02 kg a.i./ha chlorsulfuron. Mixtures of the recommended dose of methabenzthiazuron with 0.01 and 0.02 kg a.i./ha chlorsulfuron at Hatherop resulted in crop scorch following applications but by harvest no effects were noticeable.

AC 222293 achieved good control of A. fatua at all doses when applied in the autumn or as a sequence in the autumn and spring but its performance was poorer than that of the spring application of difenzoquat. Addition of various concentrations of Agral had no significant effect on activity. The trial was assessed twice due to the A. fatua plants reaching maturity at different times. At the first assessment many of the panicles appeared small, the larger panicles had not fully expanded due to herbicide treatment, whereas at the later assessment all the larger panicles had fully expanded and many of the smaller panicles had shed their spikelets and fallen into the crop. It is clear that the control of A. fatua from spring treatments of AC 222293 was much poorer as judged at the second assessment compared with the first. Suggesting that AC 222293 had damaged A. fatua plants, thus delaying development of the panicles. This effect was not apparent when AC 222293 was applied in the autumn or as a sequence in the autumn and spring.

REFERENCES

Kirkland, K.; Shafer, N. E. (1982). AC222293 a new post-emergance herbicide for cereals: field studies. Proc. 1982 British Crop Protection Conference - Weeds, (in press)

Palm, H. L., Riggleman, J. D.; Allison, D. A. (1980). Worldwide review of the new herbicide DPX 4189. Proc. 1980 British Crop Protection Conference - Weeds, 1, 1-6.

Richardson, W. G.; West, T. M.; Parker, C. (1980). The activity and post-emergence selectivity of some recently developed herbicides. Weed Research Organization Technical Report No. 61.

Richardson, W. G.; West, T. M.; Parker, C. (1981) The activity and post-emergence selectivity of some recently developed herbicides. Weed Research Organization Technical Report No.63.

Ray, T. B. (1982) The mode of action of chlorsulfuron - a new herbicide for cereals. Pesticide Biochem and Physiol., 17, 10-17.

R-33865: A NOVEL CONCEPT FOR EXTENDED WEED CONTROL BY THIOLCARBAMATE HERBICIDES

B. Miaullis
Stauffer Chemical Company, Mountain View Research Center, California, USA

G.J. Nohynek, F. Pereiro
Stauffer Chemical S.A., Geneva, Switzerland

Summary: In soils, thiolcarbamate herbicides are readily degraded via microbial action. R-33865 appears to inhibit certain microbial enzymes involved in the breakdown of certain thiolcarbamates. Thus addition of R-33865 to thiolcarbamates such as EPTC (ERADICANE) and Molinate (ORDRAM) results in delayed soil degradation and extended soil persistence. This effect enhances herbicidal activity of both compounds against late germinating annual grasses, perennial grasses, cyperaceae and several broadleaf weeds. R-33865 shows desirable toxicological and environmental properties: it is of low acute and subacute toxicity, readily degradable and does not leave any detectable residues in crops or soils.

INTRODUCTION

Thiolcarbamate herbicides such as EPTC, vernolate, molinate are readily degraded in treated soils by microbial activity and hydrolysis (Gray, 1981). Although their persistence is sufficient to insure commercial weed control, suppression of some late germinating weeds such as some Digitaria, Echinochloa, Panicum and Setaria spp. could require enhanced persistence under certain conditions. R-33865, a "Herbicide Extender" represents a new concept for extending soil persistence and biological activity of many thiolcarbamates. R-33865 has recently been registered in the US as an inert ingredient of the herbicide ERADICANE EXTRA (EPTC + R-25788 + R-33865) (Federal Register, 1981). This paper describes some physical, chemical and biochemical properties of R-33865 and includes results of field trials with EPTC/R-33865 in maize, and molinate/R-33865 in rice.

PHYSICAL/CHEMICAL PROPERTIES

Chemically, R-33865 is a thiophosphorus ester displaying the following properties:

Table 1: Physical and chemical properties of R-33865

Chemical Name: O,O-Diethyl-O-phenyl phosphorothioate

Structure:

Water Solubility: 22 ppm at 25°C

Physical state: liquid

BIOLOGICAL PROPERTIES

R-33865 has been described by Fukuto and Metcalf as early as 1956 as the least toxic compound in a series of organophosphorus esters (Fukuto and Metcalf, 1956). R-33865 displays no significant insecticidal activity (Stauffer, 1980).

Table 2

R-33865 : Absence of Insectididal Activity

Topical LD_{50} values of R-33865, permethrin and sulprofos (micrograms/insect)

	Blatella germanica	Heliothis virescens	Trichoplusia ni
R-33865	0.2	0.2	0.05
Permethrin	0.005	0.0008	0.0003
Sulprofos	0.01	0.01	0.03

The cholinergic activity of R-33865 is low, the oxon analogue being 1000 times less active than paraoxon (Fukuto and Metcalf, 1956). Although inactive as an insecticide, R-33865 has been reported to inhibit paraoxon metabolism in resistant houseflies (Oppenoorth et al, 1971).

TOXICOLOGICAL AND RESIDUAL PROPERTIES

R-33865 is of moderate acute toxicity. Its addition to thiolcarbamate formulations at the standard rate of 120 g/l does not alter the acute toxicity or hazard classification of the formulation:

Table 3

Acute toxicity of R-33865, EPTC and Combined Formulations

Compound	Acute oral LD_{50}, male rat (mg/kg)
R-33865, technical	740
EPTC, technical	1640
EPTC, 720 g/l EC (EPTAM 6E)	1590
EPTC/R-33865, 720/120g/l EC (EPTAM EXTRA)	1326

R-33865 has been subjected to a rigorous battery of toxicological safety evaluations. No major adverse effects were noted in these studies. R-33865 was found to be readily metabolized in soils, plants and mammals. Crop residue studies with thiolcarbamates + R-33865 failed to detect measurable residues of either compound at a detection limit of 0.05 ppm.

MODE OF ACTION

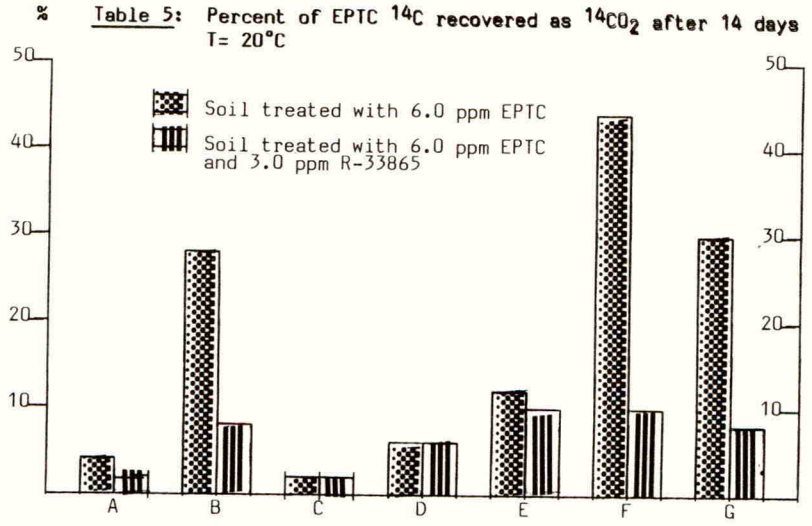
Bioassays indicated that R-33865 delays soil degradation of EPTC. As soil fungi play a major role in soil degradation of EPTC (Miaullis, 1980), in-vitro soil degradation assays were conducted with EPTC/R-33865 in sterilized soil and in soil inoculated with a fungal isolate of Rhizopus spp. The following typical degradation patterns were observed:

Table 4

EPTC-degradation in Keeton Sandy Loam Soil, inoculated with a Rhizopus species isolate, in presence and absence of R-33865

Medium	R-33865 (ppm)	EPTC, initial (ppm)	EPTC after 72 h (ppm)
Sterilized soil	--	6.0	4.07
Sterilized soil	--	6.0	4.32
Inoculated soil	--	6.0	2.76
Inoculated soil	--	6.0	1.93
Inoculated soil	1.0	6.0	4.01
Inoculated soil	1.0	6.0	4.02

Further investigations measured evolution of $^{14}CO_2$ from 7 soils treated with carbonyl labelled ^{14}C-EPTC in presence and absence of R-33865:



A= California clay loam
B= California clay loam, EPTC pretreated
C= Mississippi sill loam
D= Missouri,type unknown
E= Missouri Clay loam
F= North Dakota loam
G= Minnesota loam

The beforementioned studies have indicated that R-33865 may act by interfering with microbial breakdown of EPTC. However, R-33865 lacks bacterial and fungistatic properties and does not inhibit growth and activity of soil microorganisms (Miaullis, 1980). Recent data have suggested that R-33865 may influence microbial degradation patterns of EPTC: EPTC is be broken down via several pathways, the major route being oxidation to the herbicidal sulfoxide and sulfone (Casida et al, 1975; Horwarth and Pulay, 1980). Other degradation routes lead via dehydroxylation to deactivation of EPTC. It has been postulated that R-33865 may block these secondary breakdown pathways without affecting the herbicidal activation (Miaullis, 1982). Thus, R-33865 would display a unique activity, i.e. selective inhibition of undesirable metabolic deactivation pathways without affecting metabolic activation.

FIELD RESULTS

MATERIALS AND METHODS

42 field trials were carried out in maize in Western Europe during 1980/81 with combinations of EPTC/crop safener, in presence and absence of R-33865, formulated as emulsifiable concentrates containing 720 g/l EPTC and 720 + 120 g/l EPTC + R-33865. Use rates were 3.6, 4.32 and 5.72 kg EPTC/ha. All three rates were used for control of annual grass weeds, the two high rates for perennial weed control. Trials were designed as randomized complete blocks with 3-4 replicates, plot size generally being 4 x 5 m.

All EPTC-containing formulations were applied pre-plant, followed within 30 minutes by soil incorporation to a depth of 6-10 cm. Alachlor, at 3.0 kg/ha, applied pre-emergence surface, was included as a reference standard. Weed control ratings were conducted at 4-6 weeks (initial control), and at 8-10 weeks (residual control) after application. Counts are expressed as percentage weed control over untreated.

In paddy rice, 14 trials were conducted in France, Italy, Portugal and Spain using a 7.5% granular formulation of molinate and a granular containing 7.5% molinate + 1.25% R-33865. Both were applied post-flood, post-emergence, at 1-2 leaf stage of the rice plants, in rates corresponding to 4.125 kg molinate/ha. Weed control ratings were carried out as described before.

RESULTS

Annual grass weeds in Maize: 4-6 weeks after application, EPTC/safener + R-33865 showed significantly improved control of Digitaria sanguinalis at all rates and of Panicum dichotomiflorum at 3.6 and 4.32 kg a.i/ha respectively. All treatments resulted in satisfactory initial control of Echinochloa crus-galli, Setaria spp. and Sorghum halepense growing from seeds.

After 8 - 10 weeks after application, EPTC/safener + R-33865 showed increased activity when compared to EPTC/Safener alone, significantly improving control of D. sanguinalis, S. virides and of P. dichotomiflorum (Table 6)

Perennial grass weeds in maize: Perennial species occurred in a number of trials and assessments were made at 8-10 weeks after application. EPTC/safener + R-33865 at both rates showed improved activity over EPTC/Safener alone, resulting in enhanced control of Agropyron repens, Cynodon dactylon and S. halepense from rhizomes, by 24-31, 21-25 and 16-35 % res-pectively. All treatments resulted in excellent control of Cyperus esculentus and C. rotundus (Table 7)

Grass weeds in rice: Molinate + R-33865 showed significaltly enhanced suppression of Echinochloa spp. when compared to molinate alone. Molinate + R-33865 controlled late germinating seedlings of E. crus-galli up to 6 weeks after application, improving performance by 20% over molinate alone. Cyperus difformis, a weed difficult to control by molinate, was commercially controlled by molinate + R-33865 (Table 8)

Table 6

Percent Control of Annual Grass Weeds in Maize 4-6 & 8-10 Weeks After Application

Species	No. of Trials	Weeks after application	EPTC/Safener+R-33865 (kg ai/ha) 3.6	4.32	5.76	EPTC/Safener (kg ai/ha) 3.6	4.32	5.76	Alachlor kg ai/ha 3.0
D. sanguinalis	7	4 - 6	94	99	99	85	92	90	86
"	11	8 - 10	81	97	100	56	87	90	70
E. crus-galli	5	4 - 6	-	94	99	-	92	99	-
"	11	8 - 10	99	95	99	94	94	96	89
P. dichotomiflorum	5	4 - 6	95	97	-	58	63	-	94
"	5	8 - 10	90	87	-	59	58	-	86
S. viridis	5	4 - 6	84	-	-	83	-	-	87
"	5	8 - 10	78	-	-	65	-	-	81
S. verticillata	3	8 - 10	100	-	-	96	-	-	78
"									

Table 7

Percent Control of Perennial Weeds in Maize 8-10 weeks after application

Species	No. of Trials	EPTC/Safener+R-33865 (kg ai/ha) 4.32	5.76	EPTC/Safener (kg ai/ha) 4.32	5.76
A. repens	4	74	78	40	54
C. dactylon	2	92	94	57	73
C. rotundus	2	94	-	93	-
C. esculentus	4	95	98	97	97
S. halepense	4	81	97	62	62

Table 8

Percent Control of Weeds in Rice

Species	No. of Trials	Weeks after application	Molinate 4.125 kg ai/ha	Molinate + R-33865 4.125 kg ai/ha
E. crus-galli	14	2 - 4	90	91
	9	4 - 6	69	90
C. difformis	3	4 - 6	14	80

DISCUSSION

In vitro experiments have indicated that R-33865 may extend the persistence of EPTC in soils. Field trials with combinations of R-33865 with EPTC/safener confirmed the observations of the in-vitro studies. Addition of R-33865 to EPTC/safener formulations resulted in prolonged and enhanced herbicidal activity when compared to the EPTC/safener alone. This became particularly evident in control of late germinating annual grass species, such as D. sanguinalis and P. dichotomiflorum and improved suppression of perennial grass weeds such as S. halepense, C. dactylon and A. repens.

Enhanced residual weed control by R-33865 was seen in both organic and mineral soils. In addition, there are indications that depth and quality of incorporation may be less critical for formulations containing R-33865. The results of EPTC/safener + R-33865 field trials could be confirmed in experiments with molinate/R-33865 combinations. Molinate /R-33865 showed superior efficacy in suppression of late germinating Echinochloa spp. and C. difformis when compared to molinate alone. In all in-vitro experiments and field trials on R-33865 it became evident that the unique properties of R-33865 represent an excellent tool for further improvement of thiolcarbamates, an existing class of successful herbicides.

REFERENCES

Casida, J.E.; Kimmel, E.C.; May, M.; Okhawa, H.; Rodebush, J.E.; Gray, R.A.; Tseng, C.K.; Tilles, H. (1975). Thiocarbamate Sulfoxide Herbicides. Environm. Qual. Safety, Vol. III, 675-679, Georg Thieme Verlag, Stuttgart, W-Germany.

Federal Register (Sept. 25, 1981) Vol. 46, 186, p. 47224, U.S.A.

Fukuto, T.R.; Metcalf, R.I.; (1956). Structure and Insecticidal Activity of Some Diethyl Substituted Phenyl Phosphates. J. Agric. Food Chem. Vol. 4, 11, 930-935.

Gray, R.A.(1971). Behavior, Persistence and Degradation of Carbamate and Thiocarbamate Herbicides in the Environment. Proc. Calif. Weed Control Conf., 128-141.

Horvath, L.; Pulay, A.(1980). Metabolism of EPTC in Germinating Corn: Sulfone as the True Carbamoylating Agent. Pestic. Biochem. Physiol. 14, 265-270.

Miaullis, B.(1980), Stauffer Chemical Co., Mt. View Research Center. Unpublished Report.

Miaullis, B.(1982), Stauffer Chemical Co, Mt. View Research Center. Unpublished Report.

Oppenoorth, F.J.; Voerman, S.; Welling, W.; Houx, N.W.H.; Wouters van den Oudenweyer, J.(1971). Synergism of Insecticidal Action by Inhibition of Microsomal Oxidation with Phosphorothionates, Nature New Biology 233, October, 187-188.

Stauffer Chemical Co. (1980), Anonymous, Mt. View Research Center. Unpublished Report.

TRANSLOCATION OF ^{14}C-LABELLED FOSAMINE AMMONIUM AT DIFFERENT STAGES OF THE DEVELOPMENT OF *PTERIDIUM AQUILINUM* AND *EQUISETUM ARVENSE*

F. MÜLLER
Institute of Phytomedicine, Section Phytopharmacology, University of Hohenheim, D-7000 Stuttgart-70, Federal Republic of Germany

Summary. The distribution of ^{14}C-fosamine ammonium in *Pteridium aquilinum* (L.) Kuhn and *Equisetum arvense* was investigated during the seasonal development.

In early stages of *P. aquilinum*, when the fronds are still partially uncurled respectively not yet completely developed, the main distribution goes acropetally into the growing parts. The export into the rhizomes is rather low. After full development of the fronds, large amounts are transported into the subterranean parts, first into the horizontal rhizomes and later into the still growing rhizome buds. This distribution pattern is an important assumption for the good action of fosamine ammonium in late summer and early autumn.

In *E. arvense*, fosamine ammonium is translocated in young shoots mainly into growing parts with extremely little downward transport. After conclusion of shoot growing in summer and early autumn, the transport into the rhizomes increased a little. Field experiments will have to confirm whether the amounts of active ingredient will be effective against this weed. *Pteridium aquilinum, Equisetum arvense,* fosamine ammonium, translocation, stage of development.

INTRODUCTION

The plant growth regulator fosamine ammonium is being used in forestry for reducing growth of numerous broad-leaved woody plants when plantations of young conifers are cleared (VON ZITZEWITZ 1976, ROEDIGER 1977). Besides, it has a special herbicidal effect so that also a control of perennial weeds is possible. In this case, not a mild growth regulating, but a true herbicidal, weed-killing effect is wanted.

Herbicides against perennial weeds must effect roots and rhizomes. A chemical "mowing" of the shoots does not suffice as then the roots and rhizomes will grow out again. Pre-requisite for a lasting effect is the transport of the active ingredient into the subterranean parts which is only possible for substances with a good phloem mobility. But also these can be translocated into the roots and rhizomes during times with an intensive transport of assimilates. The phases of deposition of reserve carbohydrates are determined by the seasonal phases of development of the plants. Only by treatments during these periods, a reasonable effect can be expected (For additional details see MÜLLER 1976).

The control of *Pteridium aquilinum* in forests and on pasture-land is necessary in many areas. The widely spread species takes away space and displaces in forests many plants and prevents natural growth of forest trees. On pasture-land, it presents a competetive factor for pasture plants, interferes with grazing cattle and, in addition, is poisonous for cattle. Since a long time already, intensive efforts for

controlling this weed are taking place. Mechanical methods like mowing, crushing and digging out were applied in the past. They proved to be ineffective. Chemicals in form of total and soil herbicides were also tried, in recent times the new phloem-mobile leaf herbicides. Here especially the compounds asulam and glyphosate brought some improvements, however, without longer lasting effects and with strong impairment of the other vegetation (WILLIAMS 1980). Fosamine ammonium could offer very favourable possibilities in the control of bracken as the herbaceous plants on the ground are affected only little and the conifers are not disturbed. Experiments in practice have confirmed the good suitability of formulated fosamine for controlling *P. aquilinum*.

Another weed hard to control is *Equisetum arvense*. Phenoxy compounds are failing in the control as apparently too little active ingredient reaches the subterranean parts (MÜLLER 1970, 1976). Recently, here also glyphosate and asulam offer certain possibilities for control (COUPLAND and PEABODY 1981). Whether fosamine ammonium is suited for control, must first be determined by practical experiments. An increase of effectiveness of this compound could be reached by its application during the period of the best herbicide transport into the rhizomes. For determination of this optimum point of time, the experiments described here will be carried out. As, on principle, the same periodicity must apply to all phloem-mobile substances, the translocation experiments with fosamine ammonium could yield valuable information whether the low deposition rate observed with the phenoxy compounds would also occur in case of fosamine ammonium. This would mean that also with other substances, which are translocated into the roots after application to the shoots, *E. arvense* could not be controlled.

MATERIALS AND METHODS

Plant material: Plants of *Pteridium aquilinum* (L.) Kuhn obtained from a forest near Marxzell/Black Forest were cultivated in late summer in large Mitscherlich containers (30 cm Ø, 30 cm high). Under normal weather conditions, they stood in the open part of a wirehouse at the Institute of Phytomedicine in Hohenheim.

Plants of *Equisetum arvense* L. grew under slightly shady conditions near the institute.

Radioactive substance: Fosamine ammonium (Ammonium-ethyl-carbamoyl-phosphonate(^{14}C-carbonyl)) was supplied by Du Pont de Nemours (Wilmington, Del., USA) (Spec. activity: 9.53 µCi/mg; 1 µCi = 0.10493 mg).

Application: The application of 1.0 µCi ^{14}C-fosamine ammonium was carried out together with 0,1 ml of 1 % aqueous Krenite solution by placing small drops on certain areas of the plants. Rings made of lanolin prevented by runoff the solution.

Harvest and processing: After exposure to up to 12 days, the plants were dug out, divided into shoot and rhizome and dried at 85°C in an oven with air circulation. The plant parts to be used for autoradiography were pressed. The rhizome systems were cut into sections of 5 - 10 cm length after preparation of schematic drawings and then dried at 85°C. After determination of the dry weights, the rhizome sections were pulverized in a micro mill (Retsch, Haan/Rheinl.) or directly combusted if the pieces were small.

Autoradiography: The dried, pressed fronds and shoots were fixed on paper sheets with transparent adhesive tape. For autoradiography, x-ray film (AGFA-GEVAERT Blue base) was used. Exposure time: 14 days.

Determination of radioactivity: The quantitative determination of radioactivity in the plant samples was carried out after combustion in a sample oxidizer TRICARB 306 and measuring with a liquid scintillation spectrometer 3380 (Packard Instrument, Downers Growe, Ill., USA). At least two plants were used per treatment. The radioactivity in the rhizome systems agreed within a range of deviation of ± 5 % if the individual shape of the plants is considered.

Graphic representation: The radioactivity measured in each rhizome section was represented as a rectangle, the long sides corresponding with the length of the section. By joining together the individual retangles, a schematic survey of the radioactivity in the rhizome system was obtained.

Extraction of radioactive substances: Fresh material was extracted with 80 % methanol. The strongly concentrated methanol extract was shaken out with chloroform after addition of a small amount of water. The chloroform fraction was dried over water-free sodium sulphate concentrated to dryness and the residue taken up in a mixture of chloroform/methanol/water (4:3:1). The water phase was also concentrated.

Thin-layer chromatography: The chloroform and water extracts were chromatographed on silica gel plates 60 F_{254}, 0.25 mm (Merck, Darmstadt) (Developing mixtures: ethyl acetate/methanol/water (4:1:1); methanol/acetic acid (9:1)), radioactive zones were determined by scanning the thin-layer plates with the Radiochromatograph II (Berthold, Wildbad).

RESULTS

Distribution in *Pteridium aquilinum*

The distribution of fosamine ammonium in *P. aquilinum* plants after treatment at different stages of development with ^{14}C-substance is represented in the following with special emphasis on the degree of transport into the rhizome.

After treatment of plants, the fronds of which were just emerging from the ground and the pinnae of which were still rolled in (Stage 1), the radioactive substance was present in the whole frond without a special direction of distribution. At this time, only very little substance was translocated into the rhizomes (Fig. 1). In dead basal stumps of last year's fronds and in buds and rhizome tips no deposition could be detected.

But after somewhat later treatment at advancing development of the fronds, when the middle pinnae have not yet unfolded (Stage 2), the substance is translocated towards the top. At this phase of development relatively much fosamine ammonium moves into the adjacent parts of the rhizome, especially into buds located near the treated shoot and also into more distant parts of rhizomes with vertical buds.

When the middle pinnae have just unfolded (Stage 3), the substance is translocated mostly into the growing pinnae and into the tops of fronds. In stage 3, more ^{14}C-fosamine ammonium as before is moving into subterranean parts, especially into the rhizome parts near the treated frond and into near vertical buds which soon will emerge as young shoots. The mode of distribution allows the conclusion that a particular frond supplies certain parts of the rhizome with carbohydrates.

After treatment at a stage when all pinnae of the frond are unfolded (Stage 4), almost no ^{14}C-fosamine ammonium is translocated from the treated basal side pinnae into the higher inserted side pinnae as these apparently produce their own carbohydrates necessary for their growth. The export of assimilates and, thus, the transport of the active ingredient from the fronds takes place almost exclusively downwards into the

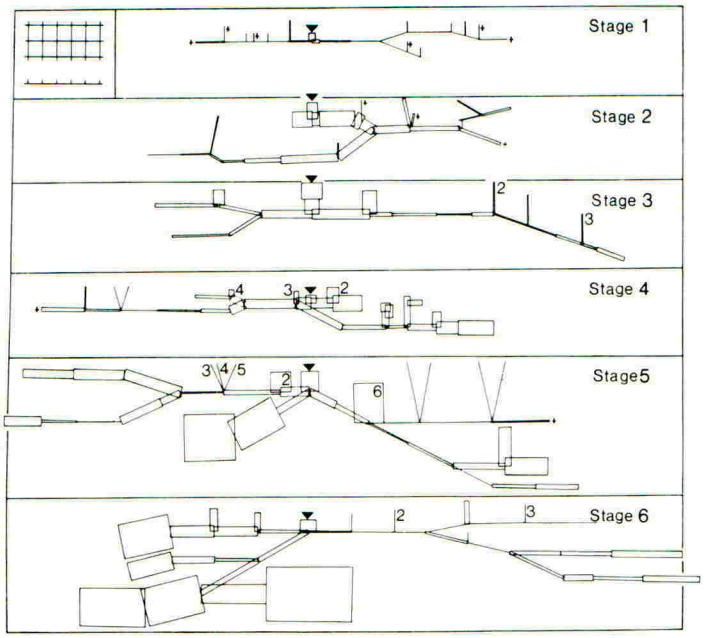


Fig. 1: Distribution of ^{14}C-fosamine ammonium in the rhizome systems of *P. aquilinum* at different stages of development (application 1 uCi/ plant; duration of action 12 days; scale: 1 square = 2 000 dpm, 1 unite of length = 2 cm; ▼ = treated frond)

rhizomes concentrating in the at this time very intensively growing regions of the horizontal rhizome, in all buds and in the tips of the rhizomes. Into certain parts of the rhizomes, however, only little substance is transported as these obviously are supplied with carbohydrates from other fronds.

In the middle of September, the fronds are fully developed (Stage 5), the basal pinnae show sometimes already a weak yellowing. Treatment with ^{14}C-fosamine ammonium at this time leads to a picture similar to that of stage 4. A large amount of substance moved into the rhizome system - especially after 6 and 12 days of exposure - resulting in heavy accumulations in some still growing rhizome tips and terminal buds. To some extent ^{14}C-activity can be found in young fronds, this can only mean a secondary transport of the substance from the rhizome system.

Investigations in the middle of October show the relationships during the last phases of development of *P. aquilinum* (Stage 6). The leaves are still fully turgescent, but show slight yellowing. From the point of application, no substance moves anymore acropetally into the tips of the fronds. The transport is exclusively directed towards the basal plant parts. There occurs no deposition in the horizontal rhizome, but only a heavy translocation into the horizontal buds which apparently are still growing at this point of time. Some radioactive material can still be found in the growing vertical buds.

Carbohydrate contents in the rhizomes

The carbohydrate contents (mainly glucose and starch) and its changes during the seasonal development of the plants give indications on phases with especially intensive export of sugars and later deposi-

tion in the storage organs. In order to obtain predicatory results, the carbohydrate contents in the different parts of the rhizome must be analyzed separately as mixed very much obscure the relationships.

The amount of glucose and hydrolyzable carbohydrates is lowest in the horizontal rhizomes at the beginning of the vegetation period when the fronds appear at the soil surface (Stage 1). During the year it increases more and more due to new deposition (Stage 4 - 6) (Tab. 1). There exist no differences between parts of rhizomes which contain much ^{14}C-fosamine ammonium and those containing only little. In the terminal buds of horizontal rhizomes, a relatively high amount is found after completion of the frond development. Because then the surplus of the assimilation is fully translocated into the subterranean parts, firstly for storage and secondary for extension of the rhizome system. The same applies to the terminal buds of the vertical rhizomes. At first they contain only little, but later a high amount of carbohydrates. In the basal parts of treated shoots, also a high contents of carbohydrates is found after complete development of the fronds.

Tab. 1: Mean values of the carbohydrates (soluble sugars + hydrolysable carbohydrates) in the rhizomes of *P. aquilinum* in different stages of development of the fronds (mg sugar in 100 mg dry weight of plant materials).

Stage	Horizontal-rhizomes[1]			Horizontal-rhizome end buds	Vertical-rhizome end buds	Basalpart of applicated fronds
	group 1	group 2	group 3			
0[2]	7.10	-	-	-	-	-
1	5.04	-	-	-	-	-
2	5.34	5.10	4.87	-	4.29	-
3	5.14	9.72	3.43	-	4.31	-
4	9.65	12.72	11.38	15.05	12.09	14.26
5	10.85	9.42	13.11	12.84	13.54	16.09
6	15.27	14.46	12.52	12.18	12.42	20.65

[1]) The three groups contain small, medium and high amounts of radioactivity, respectively.

[2]) Stage of development 0 are plants in spring before appearance of the fronds at the soil surface.

Metabolism of fosamine ammonium

The radioactive material extracted from the rhizomes could be separated by thin-layer chromatography into three different substances. One of these substances behaves like fosamine ammonium, the second agrees in its behaviour with carbamoyl-phosphonic acid, whereas the third one is unknown. The values show that after 12 days of exposure 10 % of the radioactivity are not extractable from the rhizome material with 80 % methanol, but that between 65 and 75 % of the ^{14}C-substances have the same chromatographic behaviour like fosamine ammonium.

Distribution in *Equisetum arvense*

In order to analyse the direction and intensity of distribution of the compound in the shoot and to get information about the degree of transportion into the rhizomes, *E. arvense* was treated at the third and fourth node with ^{14}C-fosamine ammonium. Tab. 2 shows the total activity in each of two nodes with the frond whorls appertaining to them.

Following application shortly after the shoots appeared at the soil surface (Height of shoots: 4 - 6 cm) (Stage 1), almost the entire radioactivity is confined to the area of application, only little moves into the direction of the shoot tip. In the rhizomes practically no radioactivity can be detected.

If the treatment is performed somewhat later (Shoot height 10 - 15 cm; lower side branches 3 cm) (Stage 2), fosamine ammonium is translocated more into the tips of the plants, into side branches and into the crowns of the side branches.

Treatment of about 25 cm high fronds (Low side branches 7 cm, middle side branches 5 cm) (Stage 3) shows that the substance is mainly moving away from the place of application in acropetal direction. Only little goes into the rhizomes.

Also after treatment of plants with a height of 25 - 30 cm in summer (Stage 4), the transport of fosamine ammonium is directed towards the shoot tip. Somewhat more than before is deposited in the rhizomes.

Is ^{14}C-fosamine ammonium applied to fully grown fronds in summer (Stage 5), almost no transport occurs anymore in direction shoot tip. However, there takes place a certain export into the rhizome system.

The same relationships exist after treatment in early autumn (Stage 6). Here the transport in basal direction is especially pronounced. Again rather much substance moves into the rhizome system.

Tab. 2: Radioactivity in the shoots (whorls and shoot axis, side branches, resp.) and uppermost sections of rhizomes of *E. arvense* plants treated with ^{14}C-fosamine ammonium at different stages of development (Application at the whorls 3 - 4; duration of action 12 days; radioactivity in dpm; application 0.5 µCi/plant).

Part of plants		Stage 2	Stage 3	Stage 5	Stage 6
whorls	13-14	3 239	173	65	227
	11-12	2 013 5 844	516 5 634	274 1 029	680 848
	9-10	2 671 7 938	920 6 446	672 1 342	248 1 201
	7- 8	2 981 23 144	1 398 8 154	997 3 158	682 2 162
	5- 6	4 098 23 146	2 560 10 144	1 865 7 128	4 196 4 409
	3- 4 (appl.)	892 522 45 490	959.669 54.870	886 094 76 598	961 395 87 612
	1- 2	66 170	23 049	31 938	33 580
rhizomes (depth in cm)	0- 5	1 022	1 233	1 653	8 525
	5-10	480	644	838	1 743
	10-15	215	238	588	1 682
	15-20	68	691	463	882

DISCUSSION

Fosamine ammonium is distributed in the phloem (MULLER 1979, 1981). An effect against perennial weeds can be expected only in developing phases when an extensive phloem transport takes place from the leaves to the subterranean parts (For details refer to MULLER =976).

Also in *P. aquilinum* and in *E. arvense*, a pronounced transport of active ingredient into the rhizomes occurs which had been published for amitrole (VOLGER 1969), for asulam and glyphosate (MARTIN 1976, WILLIAMS 1980). The carbohydrate content in the rhizomes is relatively low at the beginning of vegetation. It decreases somewhat when the fronds start to develop. It increases considerably only when the almost completely developed fronds assimilate strongly. This is at the end of September according to WILLIAMS and FOLEY (1976). The deposition of sugars occurs at first in the growing buds, then in the storage organs and finally in the horizontal, growing ends of rhizomes. The accumulation of ^{14}C-fosamine ammonium in the different parts of the rhizomes confirms this. It is not surprising that the best control with fosamine ammonium can be achieved in early autumn, when much active ingredient moves into the rhizomes with the flow of carbohydrates.

In the case of *E. arvense*, the situation is very similar. Practically no substance is translocated from the growing fonds in a basal direction. However later, after growth of the fronds is completed, some transport into the rhizomes occurs. The intensity is not as strong as that in *P. aquilinum*. Similar relationships were found for phenoxy compounds with *E. arvense* (MULLER 1971) or *E.palustre* (KOHLER and MULLER 1969). Whether the low amount of fosamine ammonium in the rhizomes suffices for successful control can only be found out by practical experiments.

ACKNOWLEDGEMENTS

The author wishes to thank the E.I. Du Pont de Nemours Company, Wilmington, Del., USA, for providing ^{14}C-fosamine ammonium.

REFERENCES

COUPLAND, D.; PEABODY, D.V. (1981): Absorption, translocation and exudation of glyphosate, fosamine, and amitrole in field horsetail *Equisetum arvense*. Weed Sci., 29, 556-560.

HAMILTON, S.; CANNY, M.J. (1961): The transport of carbohydrates in Australian bracken. Aust. J. biol. Sci., 13, 479-485.

KOHLER, I.; MULLER, F. (197¼): Untersuchungen zur Translokation von ^{14}C-markierten Herbiziden in *Equisetum palustre* L. Z. PflKrankh., 77, 429-437.

MARTIN, F. (1976): Control of bracken. Botanical J. of the Linnean Soc. 73, 241-246.

MULLER, F. (1970): Untersuchungen zum Transport von ^{14}C-markiertem MCPA in verschiedenen Entwicklungsstadien von *Equisetum arvense* L. Z. PflKrankh., S. -H. V. 103-114.

MULLER, F. (1976): Translokation von ^{14}C-markiertem MCPA in verschiedenen Entwicklungsstadien mehrjahriger Unkrauter. Acta Phytomedica, Heft 4, Verlag Paul Parey, Berlin u. Hamburg.

MULLER, F. (1979): Behaviour of ^{14}C-Krenite in young forest plants and braken. IX. Intern. Congr. of Plant Protection, Washington, Sect. VII, 501.

MULLER, F. (1981): Untersuchungen zum Verhalten von Fosamine in jungen Forstpflanzen. Mitt. Biol. BundAnst. Ld. - u- Forstw., Berlin-Dahlem, Heft 203, 264-265.

ROE, J.H. (1965): The determination of sugar in blood and spinal fluid with the anthrone reagent. J. biol. Chem., 212, 335.

ROEDIGER, K. -J. (1977): Krenite ermoglicht Kulturpflege nach MaB und Kulturvorbereitung. Erfahrungen aus funfjahrigen Versuchen. Allg. Forstz., 32, 859-862.

VOLGER, C. (1969): Der Alderfarn (*Pteridium aquilinum* (L.) Kuhn) and seine Bekampfung mit Aminotriazol. Schr.Reihe Forstl. Fak. Univ. Gottingen, Heft 41.

WILLIAMS, G.H. (1980): Bracken control; a Review of Progress, 1974 - 1979. The West of Scotland Agricultural College, Research and Development Publication Nr. 12, 55 pp.

WILLIAMS, G.H.; FOLEY, A. (1976): Seasonal variations in the carbohydrate content of bracken. Botanical J. of the Linnean Soc., 73, 87-93.

ZITZEWITZ, VON, H. (1976): Mit Krenite zur waldgerechten Kulturpflege. Allg. Forstz., 31, 701-704.

Proceedings 1982 British Crop Protection Conference - Weeds

THE USE OF CHLORSULFURON FOR THE CONTROL OF *RUMEX OBTUSIFOLIUS* IN GRASSLAND

A.K. Oswald, T.M. West and W.G. Richardson

ARC Weed Research Organization, Begbroke Hill, Yarnton, Oxford, OX5 1PF

Summary. Chlorsulfuron at 0.02-0.2 kg ha^{-1} a.i. gave excellent control of established *Rumex obtusifolius* grown in pots, and doses of 0.015-0.06 kg ha^{-1} a.i. applied in August were equally effective on established plants growing in a grass sward.

Maximum effects were reached 9 weeks after spraying and there was no re-growth during the following spring, 9 months after treatment. A slight depression in grass herbage was recorded but this was transitory.

This herbicide looks promising for use in grassland and further work with lower doses and on other grassland weeds is suggested.

INTRODUCTION

Although herbicides are available for controlling *R. obtusifolius* in grassland (Fryer and Makepeace, 1978), improved treatments are continually being sought.

The results of an initial screening experiment carried out at the Weed Research Organization indicated good control of seedling *R. obtusifolius* by chlorsulfuron (Richardson *et al.*, 1980).

The object of this present study was to test the effectiveness of this herbicide on more mature plants grown in pots and on established plants present in a grass sward.

METHODS AND MATERIALS

1. Glasshouse experiments

Three pot experiments were started in September 1979, September 1980 and November 1981. Seeds of *R. obtusifolius* were sown 0.5 cm deep in 9 cm pots filled with a mixture of soil, peat and sand in the ratio 4:1:1 with added fertilizer and insecticide. Plants grown in 1979 were kept in a cold frame during the winter, before planting outside, but in subsequent years the plants were kept in the glasshouse before transferring to 23 cm diameter pots and placing outside in March-April. In addition to normal rainfall each pot was connected to a drip feed through which the plants could be irrigated before and after treatment.

Treatments

Chlorsulfuron was applied at 0.1 and 0.2 kg ha^{-1} a.i. on 22 May 1979, at 0.04 and 0.08 kg ha^{-1} a.i. on 29 May 1980 and at 0.02 and 0.04 kg ha^{-1} a.i. on 28 May 1981. On each occasion an untreated control was included for comparison. At spraying, plants in all three years had from 40 to 50 leaves which were 30 to 40 cm long. In 1980 and 1981 some plants were already flowering at the time of spraying. All treatments were applied in 185-200 l/ha aqueous spray solution containing Agral 90 surfactant at 0.5% (1979/80) and 0.25% (1981). Pressure was 210 kPa using a Teejet No. 8001 fitted to the laboratory pot sprayer. After treatment pots were laid outside in a randomised block design of three replicates.

Assessments

All foliage was cut to soil level on 6 July 1979 (45 days after spraying), 15 July 1980 (47 days), and 6 July 1981 (39 days), using secateurs and then weighed fresh. The defoliated plants were allowed to regenerate before harvesting again on 2 October 1979, 6 October 1980 and 1 September 1981.

2. Field experiments

The 6-year-old sward contained perennial ryegrass and white clover with some Poa trivialis and Holcus lanatus. Rumex obtusifolius plants, raised from seed sown in the glasshouse, had been transplanted on 26 October 1978 into plots measuring 1 x 1 m laid out in a randomised block design of 4 replicates. The sward was cut to a height of 3 cm on three occasions during 1979 and 1980 and on 15 May and 14 July 1981. Annual fertilizer applications prior to and including 1980 averaged 190 kg ha^{-1} N, 70 kg ha^{-1} P_2O_5 and 120 kg ha^{-1} K_2O with a final application of 150 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5 and 80 kg ha^{-1} K_2O on 2 April 1981.

Treatments

Chlorsulfuron was applied on 24 August 1981 at 0, 0.015, 0.03 and 0.06 kg ha^{-1} a.i. A standard treatment of asulam at 1.12 kg ha^{-1} a.e. was applied. All treatments were applied in 300 l/ha aqueous spray solution at 210 kPa pressure with Teejets No. 8002 fitted to a square metre sprayer.

The conditions at spraying were: temperature 23°C, rh 78%, cloud cover 50%. Rumex obtusifolius plants were 26 cm high, with 20% flowering. Grass was 22 cm high and all foliage was dry. Ground cover of R. obtusifolius ranged from 54% to 70%.

All plots were cut to a height of 8 cm on 23 October 1981 using an autoscythe. A total of 180 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5 and 80 kg ha^{-1} K_2O was applied on 29 March 1982.

Assessments

A quadrat measuring 1 x 1 m was divided to give 100, 10 cm squares and this was used to measure the ground cover of R. obtusifolius on each plot by recording the number of squares where weed foliage was present. Assessments were made immediately before spraying on 26 August 1981 and repeated on 24 September and 24 October 1981, and 11 May 1982.

Scores were used to measure the effects on green R. obtusifolius and grass herbage on 4, 14 and 24 September and 4, 14 and 24 October 1981. A score from 0 (no green herbage visible) to 9 (equal to unsprayed herbage) was made by two people independently and a mean of both scores was recorded.

RESULTS

1. Glasshouse experiments

Rumex obtusifolius fresh weight, measured 45 days after treatment, was reduced by 80-90% by chlorsulfuron at 0.10 and 0.20 kg ha^{-1} a.i. in 1979 and at 0.04 and 0.08 kg ha^{-1} a.i. in 1980 (Table 1). Although the dose of 0.04 kg ha^{-1} a.i. applied in 1981 was less effective initially, giving a reduction of 55%. there were no signs of plant regrowth following treatment in this or any of the other years.

2. Field experiment

Ground cover of R. obtusifolius was not reduced by any of the treatments 30 days after spraying (Table 2). However, significant reductions were recorded after 60 days with all treatments being equally effective. None of the plants treated with chlorsulfuron had recovered when assessed during the following spring, as indicated

by the negligible area of ground covered by the weed compared with the untreated herbage or that treated with the standard application of asulam. The ground cover recorded on plots where asulam had been sprayed indicated that regrowth had taken place since the previous assessment.

Table 1

The effects of chlorsulfuron on the fresh weight (g/pot) of Rumex obtusifolius grown outdoors in pots

Date of treatment:	22 May 1979		29 May 1980		28 May 1981	
	Assessed		Assessed		Assessed	
Dose of chlorsulfuron (kg ha^{-1} a.i.)	6 July 1979	20 Oct. 1979	15 July 1980	6 Oct. 1980	6 July 1981	1 Sept. 1981
0	547	197	474	69	355	220
0.02	-	-	-	-	131	0
0.04	-	-	83	0	160	0
0.08	-	-	101	0	-	-
0.10	58	0	-	-	-	-
0.20	93	0	-	-	-	-
S.E.	±38.0		±27.0		±39.0	

- indicates that this dose was not applied in this experiment

Table 2

The frequency of Rumex obtusifolius in a grass sward at different times after treatment with chlorsulfuron and asulam on 24 August 1981 (presence in 100 x 10 cm squares/plot)

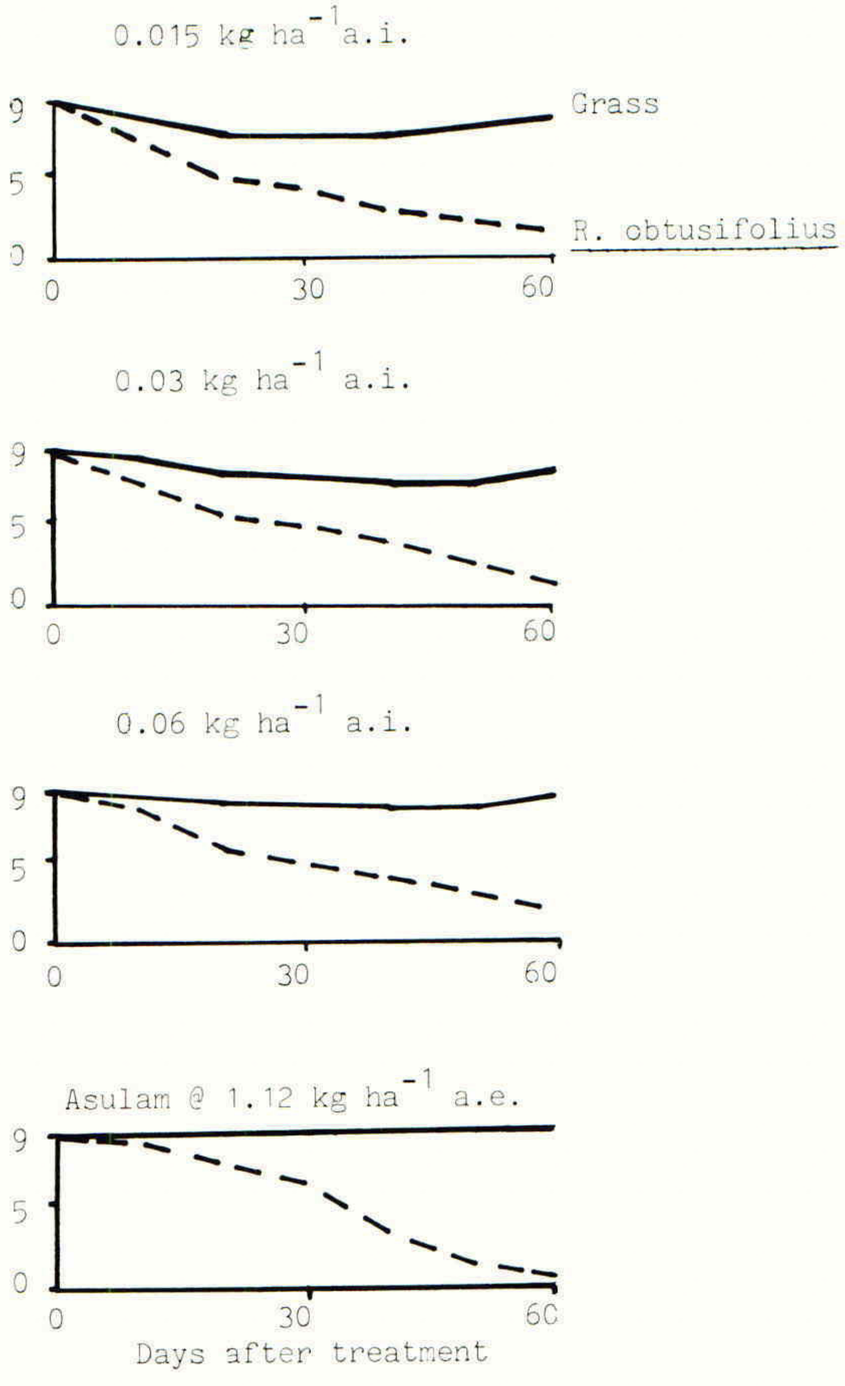
	Treatment dose (kg ha^{-1} a.i.)	24 September 1981	24 October 1981	11 May 1982
Chlorsulfuron	0.015	47	6	2
"	0.03	59	6	2
"	0.06	67	14	2
Asulam	1.12	58	5	25
Untreated control	0	72	49	78
S.E.		±17.8	±8.0	±6.9

The visible effects of all doses of chlorsulfuron on green R. obtusifolius herbage were similar to those of the standard treatment (Fig. 1). Reductions occurred 10 days after treatment with discolouration and plant death increasing to a 90% level after 60 days when the final assessment was made prior to senescence.

Chlorsulfuron caused only a slight ($< 20\%$) check in grass growth (Fig. 1). This effect was reached 20 days after treatment with the low dose and 50 days after the higher doses. However recovery started after 50 days and this was complete by the following spring.

Fig. 1

The effects on green herbage following application of chlorsulfuron on 24 August 1981 to a grass sward containing Rumex obtusifolius



DISCUSSION

The results of this study indicate the potential of chlorsulfuron for the control of R. obtusifolius in grassland. The most promising aspect was the lack of regrowth after treatment, with no recovery even 9 months after spraying the well-established plants in the field. This suggests an advantage compared with currently available selective herbicides following which regrowth usually occurs (Frame and Harkess, 1972; Oswald and Elliott, 1970; Oswald and Haggar, 1976).

There was a slight depression in grass growth which lasted for 6-7 weeks before recovery took place. This is similar to effects recorded elsewhere in perennial ryegrass with this herbicide (McAteer and Courtney, 1981) and to the effects of other herbicides used for control of R. obtusifolius in grassland, particularly where species other than perennial ryegrass are present (Martin, 1970; Soper, 1970).

The white clover present on the experiment area was not evenly distributed and no assessment was made of its response to treatment. However, the visual impression was that although clover was unaffected by asulam, damage had been caused by all doses of chlorsulfuron.

The flat dose response indicated in the pot and field experiments suggests that effective weed control might be achieved by even lower doses than those used, especially where R. obtusifolius seedlings are present. The effect on grass, although only transitory, would also be reduced by use of lower doses.

Thus, further evaluation of this promising herbicide is warranted, concentrating on the effectiveness of doses below 0.015 kg ha^{-1} a.i. for controlling R. obtusifolius.

ACKNOWLEDGEMENTS

The authors thank R.H.Webster and R.M.Porteous for growth of plant material and P.G.Smith and G.P.White for assistance in the field and glasshouse.

REFERENCES

Frame, J.; Harkess, R.D. (1972). Herbicides for the control of docks (Rumex spp.) in grassland. Proceedings 11th British Weed Control Conference, 323-330.

Fryer, J.D.; Makepeace, R.J. (1978). Weed Control Handbook, Vol. II Recommendations. Oxford, Blackwell Scientific Publications, 242-244.

Martin, G.S. (1970). The effect of asulam applied as for dock control (Rumex spp.) on the production of the grass sward. Proceedings 10th British Weed Control Conference, 476-480.

McAteer, S.; Courtney, A.D. (1981). Evaluation of DPX 4189 for selective weed control in grassland. Annals of Applied Biology Supplement, Tests of Agrochemicals and Cultivars, 97, (2), 48-49.

Oswald, A.K.; Elliott, J.G. (1970). The toxicity of three herbicides to the docks (Rumex spp.) and grasses growing in a mainly ryegrass pasture. Proceedings 10th British Weed Control Conference, 481-487.

Oswald, A.K.; Haggar, R.J. (1976). The effect of asulam on two Lolium perenne swards containing Rumex obtusifolius. Weed Research, 16, 7-10.

Richardson, W.G.; West, T.M.; Parker, C. (1980). The activity and post-emergence selectivity of some recently developed herbicides: R 40244, DPX 4189, acifluorfen, ARD 34/02(NP55) and PP 009. Technical Report Agricultural Research Council Weed Research Organization, 1980, 61, pp 57.

Soper, D. (1970). The tolerance of pasture grasses to asulam. Proceedings 10th British Weed Control Conference, 465-475.

R-40244, A NEW HERBICIDE FOR WEED CONTROL IN POTATOES, SUNFLOWERS AND WINTER WHEAT

F. Pereiro, J.C. Ballaux
Stauffer Chemical S.A. (Geneva)

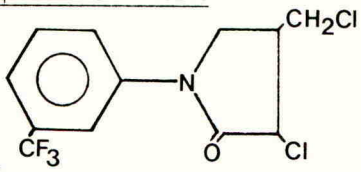
J.M. Béraud
Stauffer Chemical S.A.R.L. (Paris)

Summary : Field work during 1979-1981 has demonstrated the potential of R-40244 (proposed common name : fluorochloridone) as a pre-emergence herbicide for use in carrots, potatoes, sunflowers, winter wheat, cotton, tree and bush fruits and non-crop situations. Data from over 100 European field trials in sunflowers and potatoes indicate that rates of 0.5-1.0 kg a.i./ha, applied as soon as possible after sowing or planting, will provide excellent control of a wide range of broad-leaved weeds. The species controlled include a number of problem weeds such as Amaranthus spp., Chenopodium album, Galium aparine, Polygonum spp., Sinapis arvensis and Solanum nigrum. Mixtures with appropriate grass herbicides have also been evaluated. R-40244 used in winter wheat as a pre-emergence treatment at reduced rates combined with existing products improves control of major weeds such as Galium aparine, Veronica hederaefolia, and Viola arvensis.

INTRODUCTION

R-40244 (1-(m-trifluoromethylphenyl)-2-chloro-4 chloromethyl-2-pyrrolidone) is a novel herbicide discovered by Stauffer Chemical Company. Physical and chemical properties are summarized in the following table :

Table 1 : Physical / chemical properties of R-40244

Chemical Structure	: 
Molecular weight	: 312.12
Isomers (technical)	: 30% cis / 70% trans
Purity of technical material	: > 90%
Physical State (technical)	: Beige powder
Water solubility at 20°C	: Cis isomer : 27 ppm Trans isomer : 12 ppm Technical : 28 ppm
Partition Coefficient Octanol / Water	: 2.2×10^3 (trans isomer)

R-40244 presents favorable acute toxicity values as shown in the following table.

Table 2 : Acute Toxicity of Technical R-40244

Acute oral LD_{50}, male rat	:	4000 mg/kg
Acute oral LD_{50}, female rat	:	3650 mg/kg
Acute dermal LD_{50}, rabbit	:	> 5000 mg/kg
Skin irritation, rabbit	:	Mild irritant
Eye irritation, rabbit	:	>0.121 mg/lt/4hr

Mutagenicity evaluations using the Ame's Salmonella assay and the mouse lymphoma forward mutation assay, observed no mutagenic activity both in presence and absence of a liver microsome activation system.

Further investigations on metabolism and degradation of R-40244 in plants and animals indicate that the compound is readily degraded. In rats, 90% of a single oral dose of ^{14}C-labelled R-40244 is excreted within 48 hours after administration.

Residue evaluations in winter wheat, sunflowers and potatoes have been conducted in France, U.K., Netherlands and the USA during 1978 - 1981. No detectable residues were observed in any of these investigations at the lowest sensitivity limit of the analytical residue method, i.e. 0.01 - 0.02 ppm.

R-40244 shows a high degree of biological activity (Richardson et al. 1979) and is a powerful inhibitor of carotenoid synthesis (Devlin et al. 1979 and 1980, Sandmann and Böger 1981). It is selective in a wide range of crops including carrots, cotton, potatoes, sunflowers, winter wheat and tree and bush crops. Although primarily a pre-emergence herbicide, R-40244 also shows useful post-emergence activity in a number of established crops.

Data presented in this paper summarize the results from 43 sunflower trials, 60 potato trials and 48 wheat trials conducted during 1979-81 in Austria, France, Italy, Spain, United Kingdom, West Germany and Yugoslavia.

METHODS AND MATERIALS

In all countries trials were of a randomized block design with each treatment replicated 3-4 times. Plot sizes were generally 20 m^2. All treatments were applied with a small plot precision sprayer in 300-400 l/ha of water at a pressure of 2.3 - 2.8 bar. Commercially available products were used as standard treatments, including linuron, trifluralin and terbutryne in sunflowers, linuron and metribuzin in potatoes, chlortoluron and nitrofen + neburon in wheat.

Weed control assessments were made by counting the number of each weed species present in (2-4) x 0.25 m^2 quadrats per plot. These counts are expressed as percentage control over untreated check.

RESULTS

A. Sunflowers

Crop tolerance was good when R-40244 was applied pre-emergence immediately after drilling at rates of 0.5 - 0.75 kg a.i./ha. However, some temporary leaf bleaching was recorded at 1.0 - 1.5 kg a.i./ha in compacted or water-logged soils. This level of tolerance was maintained when R-40244 was combined with trifluralin at 0.75 kg a.i./ha or linuron at 0.5 kg a.i./ha.

At application rates of 0.5 - 0.75 kg a.i./ha, R-40244 was more effective than the commercial standard terbutryne against Sinapis arvensis, Raphanus raphanistrum, Amaranthus spp., Anagallis arvensis and Galium aparine. (Table 3). Acceptable control of Fumaria officinalis was achieved only when R-40244 was applied in tank-mixture with 0.5 kg a.i./ha of linuron. The addition of 0.75 kg a.i./ha. trifluralin improved the activity of R-40244 against Polygonum convolvulus (Table 5). Apart from Digitaria sanquinalis and Setaria spp., R-40244 shows only moderate activity against grass weeds. However control of these weeds is greatly improved by the addition of trifluralin, either as a tank-mixture with R-40244 or when the products are used sequentially (Table 5). In general R-40244 shows a wider spectrum of activity and longer residual control when applied to the soil surface.

B. Potatoes

R-40244 shows good selectivity on all varieties of potatoes when applied prior to 7-10 days of emergence. In a number of trials, however, when applied pre-emergence R-40244 caused some yellowing or bleaching of the merging shoots. These symptoms were particularly apparent in trials where the compound was sprayed within a few days prior to, or after, plant emergence. This effect was transient.

Weed control is satisfactory at rates between 0.5 and 0.75 kg a.i./ha. R-40244 alone controls most common economic broad-leaved weeds in potatoes including Galium aparine, Solanum nigrum, Amaranthus retroflexus, Chenopodium album, Polygonum spp., Stellaria media and all cruciferous weeds (Table 3). The use of rates between 0.75 and 1.0 kg a.i./ha provides excellent control of heavy infestations of Galium aparine (99%) and Solanum nigrum (97%).

At high levels of infestation some weeds appear less susceptible to R-40244, but control of these species can be improved by the addition of a second herbicide. A tank-mixture of R-40244 + linuron at 0.5 + 0.5 kg a.i./ha showed good activity against Matricaria chamomilla, Mercurialis annua and Alopecurus myosuroides (Table 5).

C. Winter Wheat

Several mixtures including R-40244 applied pre-emergence within 2 days after drilling show good selectivity on winter wheat and excellent efficacy on the usual weed spectrum of the crop.

The addition of 0.25 kg a.i./ha to chlortoluron, metoxuron and neburon (all 3 at 2.0 kg a.i./ha) provided improved control of Galium aparine, Veronica hederaefolia and Viola arvensis over commercial standards used. Level of control of other major weeds such as Alopecurus myosuroides, Matricaria chamomilla and Polygonum aviculare was similar to standards (Table 4). Other common weeds such as Stellaria media, Papaver spp. and Lolium spp. are well controlled with all combinations.

DISCUSSION

The new compound R-40244 shows considerable potential for broad-leaved weed control in a number of arable crops. It may be used either alone or in combination with other herbicides, depending on the spectrum of weeds to be controlled. The recommended rates are between 0.5 - 0.75 kg a.i./ha. In sunflowers R-40244 can be applied on its own or in tank-mixture with trifluralin as a single spray or as a sequential treatment. Advantages of R-40244 over existing materials include a wider spectrum of activity and a higher level of weed control, particularly against Amaranthus retroflexus, Galium aparine, Raphanus raphanistrum and Sinapis arvensis.

In order to avoid temporary leaf bleaching in potatoes, experience has shown that R-40244 should be applied pre-emergence soon after planting or immediately after the last ridging operation. However no application should be made within 7-10 days of crop emergence. When sprayed alone at rates of 0.5 - 1.0 kg ai/ha R-40244 will control a wide range of troublesome weeds in potatoes including Chenopodium album, Senecio vulgaris, Galium aparine and Solanum nigrum. At the low rate of 0.5 kg. ai/ha. the spectrum and level of weed control may be improved by mixture with linuron.

The addition of 0.25 kg a.i./ha R-40244 to substituted ureas used in winter wheat improves control of most common broad-leaved weeds and shows an interesting efficacy on Galium aparine and Veronica hederaefolia in different European situations.

REFERENCES

Devlin, R.M.; Kisiel, M.J.; Kostusiak, A.S. (1979). Influence of R-40244 on pigment content of wheat and corn. Weed Research, 19, 59-61.

Devlin, R.M.; Kisiel, M.J.; Kostusiak, A.S. (1980). Enhancement of Giberellic Acid Sensitivity in Corn (Zea mays) by fluridone and R-40244. Weed Science, 28, 11-12.

Richardson, W.G.; West, T.M.; Parker, C. (1979). The activity and pre-emergence selectivity of some recently developed herbicides: R-40244, AC-206784, pendimethalin, butralin, acifluorfen and FMC 39821. Weed Research Organization Technical Report, 57, 1-15.

Sandmann, G.; Böger, P. (1981). Mode of action of herbicidal bleaching. American Chemical Society Symposium Series 1981, 1-18.

*****

Table 3 : Average percentage control of broadleaved weeds in sunflowers and potatoes. Europe 1979-81.

	Sunflowers			Potatoes			
(kg a.i./ha)	R-40244		Terbutryne	R-40244		Metribuzin	No. of
Weed Species	0.5	0.75	2.0	0.5	0.75	0.7	Trials
Amaranthus retroflexus	85	89	83	89	98	99	15
Anagallis arvensis	99	99	91	98	99	98	13
Capsella bursa-pastoris	96	100	96	100	100	100	8
Chenopodium album	86	93	90	91	95	95	37
Fumaria officinalis	67	73	78	90	94	96	14
Galium aparine	94	97	50	86	89	33	13
Matricaria chamomilla	99	99	96	69	68	98	14
Mercurialis annua	51	60	69	87	89	98	7
Polygonum aviculare	95	97	94	91	94	90	20
Polygonum convolvulus	68	79	72	84	92	85	17
Polygonum persicaria	-	-	-	79	89	90	8
Raphanus raphanistrum	99	99	82	100	100	100	9
Senecio vulgaris	-	-	-	85	97	99	8
Sinapis arvensis	98	99	81	98	99	99	10
Solanum nigrum	-	-	-	81	88	89	8
Urtica urens	-	-	-	89	97	87	8

All treatments pre-emergence.

Table 4 : Weed sensitivity in winter wheat. Summary of 48 trials. Europe 1979-81.

Weed Species (kg a.i./ha)	R-40244 + Chlorotoluron 0.25 + 2	R-40244 + Metoxuron 0.25 + 2	R-40244 + Neburon 0.25 +2	Chlorotoluron 2.5	Nitrofen +neburon 1 + 2
Alopecurus myosuroides	S	-	MS	MS	MS
Galium aparine	MS	MR	MR	R	R
Matricaria chamomilla	S	S	S	S	MS
Polygonum aviculare	S	S	S	S	-
Veronica hederaefolia	MS	MS	MS	R	MS
Viola arvensis	S	-	MS	MR	MS
Basically developed in :	F.R. Germany	Spain	France		

All treatments pre-emergence.
S : ⩾90% MS : 75 - 89% MR : 60 - 74% R : <60%

*****

Table 5 : Average percentage weed control with R-40244 combinations in sunflowers and potatoes. Europe 1979-81.

Weed Species (kg a.i./ha)	R-40244 0.5	R-40244 0.75	R-40244 Combinations	No. of Trials
A) Sunflowers : Incorporated prior to drilling.			R-40244 + trifluralin, 0.75 + 0.75	
Fumaria officinalis	67	73	100	4
Polygonum convolvulus	68	79	97	6
Alopecurus myosuroides	63	70	100	3
B) Sunflowers : Incorporated prior to drilling.			R-40244 + trifluralin, 0.5 + 0.75	
Avena fatua	71	76	85	3
Echinochloa crus-galli	85	86	100	2
C) Potatoes : Pre-emergence spray.			R-40244 + linuron, 0.5 + 0.5	
Galium aparine	86	89	95	9
Matricaria chamomilla	69	68	95	7
Mercurialis annua	87	89	97	3
Solanum nigrum	81	88	94	8
Alopecurus myosuroides	82	82	91	3

Proceedings 1982 British Crop Protection Conference - Weeds

GLYPHOSATE (N-(PHOSPHONOMETHYL) GLYCINE) AS A PRE-HARVEST RETTING AGENT IN FLAX (Linum ussitatissimum)

A. D. Courtney
Field Botany Research Division, Department of Agriculture, Newforge Lane, Belfast BT9 5PX, N. Ireland

E. Robinson
Lambeg Industrial Research Association, Lambeg, Co. Antrim

Summary Glyphosate at a range of rates 0.7 - 2.8 kg a.e. ha^{-1}, in a pot experiment, produced a satisfactory degree of desiccation and fibre separation (retting) in the standing crop. This was however associated with a loss of seed and a decline in straw d.m. In plot experiments glyphosate activity in the plant, recorded as moisture loss, was virtually complete in 20 days from application, and appeared to be influenced little by rate of application, variety or use of surfactant. The processing and ease of fibre separation from the treated straw was improved by use of the herbicide; 1.4 kg a.e. ha^{-1} appeared to be an adequate rate of glyphosate and 2.8 kg a.e. ha^{-1} or too long an interval after spraying tended to reduce the proportion of long fibre.

INTRODUCTION

In the period 1968-1970 studies in France (Association Generale de Producteurs de Lin 1968) and in N. Ireland (NI Agriculture Trust 1968) had established that the bipyridylum quaternary salts paraquat and diquat applied as a pre-harvest desiccant induced separation of the flax fibre bundles in a manner similar in many respects to that observed in the dew or dam retting processes (Green 1969). Desiccation in the field however was uneven particularly at the base of the stem, a fact also most recently confirmed for linseed by Gubbels & Kenaschuk (1981). The development of glyphosate (N-(phosphonomethyl) glycine) by Monsanto Ltd., (Baird et al, 1971) as a phloem-mobile chemical (Sprankle et al, 1975) presented a fresh opportunity to examine the pre-harvest effects of a highly translocated herbicide on desiccation and fibre separation of the standing crop.

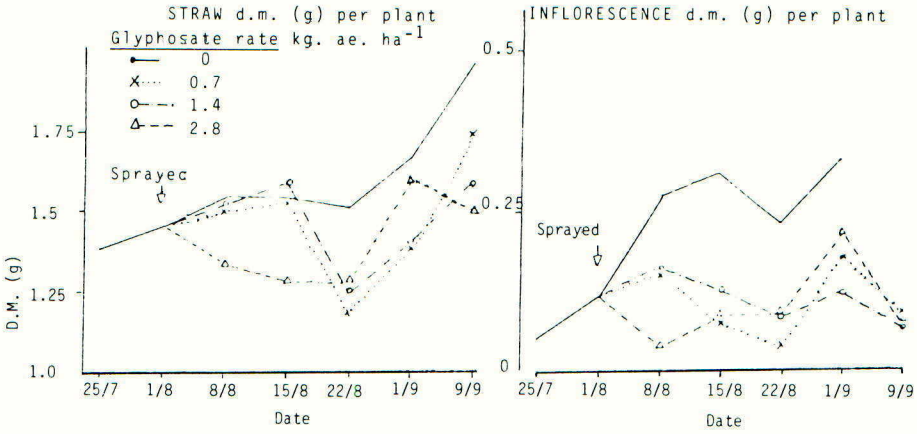
Preliminary studies were made in 1979 using glyphosate at 2.1 kg a.e. ha^{-1}. The data presented represent the experiments conducted in 1980 to confirm the pre-harvest 'retting' activity of glyphosate and to obtain information on appropriate rates of glyphosate and other factors influencing the activity of glyphosate in the standing flax crop.

METHODS AND MATERIALS

Experiment 1 (cv. Eva) was conducted in pots at Newforge, to investigate the influence of glyphosate on the subsequent development of the crop. The flax was sown (20 seeds per pot) on 25 April in 250 mm diameter plastic pots containing a 1:1 mixture by volume of compost and grit. The pots were placed outdoors and watered as required. The eight herbicide treatments comprised glyphosate at rates equivalent to 0, 0.7, 1.4 and 2.8 kg a.e. ha^{-1} with or without the additives ammonium sulphate at 4 kg ha^{-1} plus a fatty amine ethoxylate surfactant (Ethylan TF, Diamond Shamrock Agrochemicals Ltd.) at a final concentration of 5 g l^{-1}. The treatments were applied with a small plot sprayer at a spray pressure 2.0 bars in a volume equivalent to 225 l ha^{-1} just after the first flowers opened on 25 July or on 1 or 8

CROP DRY MATTER DEVELOPMENT

Fig. 1



CROP DESICCATION

Fig. 2(a)

Expt. 2. Effect of Glyphosate (cv. Hera)

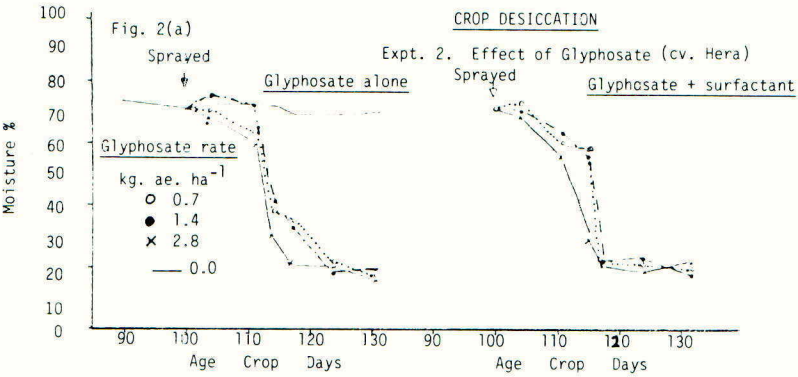
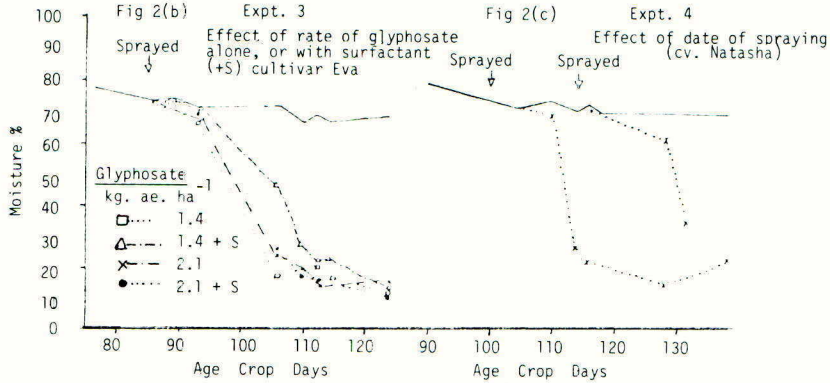


Fig 2(b) Expt. 3

Effect of rate of glyphosate alone, or with surfactant (+S) cultivar Eva

Fig 2(c) Expt. 4

Effect of date of spraying (cv. Natasha)



August 1980. Two replicates of each herbicide treatment were applied at each spray date. Individual plant samples were harvested at weekly intervals after spraying and the remaining plants recorded at the final harvest on 9 September.

Experiments 2, 3 and 4 were conducted at the Lambeg Industrial Research Association (L.I.R.A.). The flax seed was broadcast on April 23 at a rate equivalent to 90 kg ha^{-1}. Soil analysis had indicated high levels of potash and phosphate and only nitrogen was applied to the seed bed as ammonium sulphate at a rate of 25 kg ha^{-1}. Three cultivars, Hera, Eva and Natasha were sown in individual blocks. Each cultivar block was used to investigate a different aspect of glyphosate 'retting' activity.

Experiment 2, (cv. Hera). Glyphosate was applied at rates of 0, 0.7, 1.4, and 2.8 kg a.e. ha^{-1} alone or with a surfactant, Ethylan TT15, at a final concentration of 5 g l^{-1}. Individual unreplicated plots, approximately 3 m x 12 m, were sprayed with a small plot sprayer at a pressure of 2.0 bars in volume of 450 l ha^{-1}, 2 weeks after the time of mid-flowering (1 August).

Experiment 3, (cv. Eva). This flax was sprayed with glyphosate at 0, 1.4, 2.1 kg a.e. ha^{-1} alone or with the spray additives as in Expt. 1, 11 days after the mid-point of flowering (24 July). Two periods of retting after application were investigated, half the plot being pulled after 28 days and half after 35 days.

Experiment 4 (cv. Natasha). A single rate of glyphosate 2.1 kg a.e. ha^{-1} was applied on four dates, 0, 2, 3 or 4 weeks after the mid-point of flowering (5 August) to investigate the time of glyphosate application in relation to flowering.

Crop Assessments

In the trials at Lambeg, samples were taken at 2 - 3 day intervals and the stem cross sections prepared to determine fibre bundle and stem integrity. It was on the basis of this assessment that harvest dates were determined. In addition prior to harvest straw moisture contents was recorded in Expts. 1, 3 and 4.

At harvest the flax straw samples of about 1.0 kg were subjected to a process of mechanical fibre separation on the L.I.R.A. flax analyser. The main straw parameters recorded were:- (a) total fibre % as a proportion of straw d.m. (b) long fibre % as a proportion of straw d.m. (c) of total fibre and (d) Separation Index - a qualitative assessment of the ease of fibre extraction.

RESULTS

Crop Development

In Expt. 1 the activity of the glyphosate on the development of the flax was recorded according to rate and date of application, (Table 1). The earliest date of application (25 July) which was approximately coincident with the mid-point of flowering resulted in total seed loss and an excessive degree of desiccation and loss of straw d.m., 6 weeks after spraying. The spraying one week later (1 August) still resulted in nearly total seed loss and severe reduction in straw d.m. although desiccation was reduced. Glyphosate application two weeks after the mid-point of flowering, harvested four weeks later came nearest to reconciling a satisfactory degree of desiccation with minimal loss of seed and straw d.m., although these were still quite large.

The progressive effect of the glyphosate on individual plant d.m. for the 1 August spray date is illustrated in Fig. 1. The yield of both straw and seed d.m. continued to increase throughout the experiment to its conclusion, 9 September. Straw d.m. showed an immediate decline with the highest rate of glyphosate and a more gradual response at the other rates, reaching a minimum 3 weeks after application. Some recovery of straw d.m. occurred after this date. Capsule d.m.

showed a similar trend of decline and recovery, followed however by a further irreversible decline as capsule shedding occurred.

Table 1 Desiccation and % loss of capsule and straw d.m. according to rate and date of glyphosate application. Harvested 9.9.80

Application Date	Glyphosate kg ha^{-1}	Desiccation Score	d.m. % loss Seed	Straw
	Control 0	1	(0.16 g.) 0	(1.7 g.) 0
1. 25.7.80	0.7	8.0	100	40
	1.4	10.0	100	39
	2.8	10.0	100	47
2. 1.8.80	0.7	3.0	99	26
	1.4	3.5	98	40
	2.8	5.0	100	48
3. 8.8.80	0.7	3.0	83	27
	1.4	4.5	98	31
	2.8	5.0	88	32

Crop desiccation 1-10, 1 natural senescence stems yellow ochre
10 extreme desiccation, stems grey brown
Scores of 4-7 represent acceptable desiccation and tissue breakdown.

The detailed microscopic examination of selected treatments on tissue integrity in this experiment was also recorded and constitutes a separate publication (Fraser et al, 1982).

Crop Desiccation

In these experiments the application of glyphosate to the standing flax crop resulted in a sequence of visual effects on the crop. Yellowing of the leaves and later of the stem occurred 7-10 days after treatment followed by a progressive browning of the stem and loss or withering of the leaves along the full length of the plant. Integrity of the stem tissues was lost progressively after about 4 weeks, resulting in the desired separation of the fibre bundles in preparation for the extraction of the fibre.

The course of moisture loss in the crop gave a guide to the progressive activity of the glyphosate. In relation to rate of glyphosate (Fig. 2a, cv. Hera) the 2.8 kg a.e. ha^{-1} rate alone or with surfactant gave the most rapid desiccation and the moisture content dropped to 20% in 20 days from spraying, the lower rates (0.7, 1.4 kg a.e. ha^{-1}) taking about 7 days longer to attain the same degree of desiccation. The addition of surfactant in this instance or with the other cultivar Eva (Fig. 2b) at 1.4 or 2.1 kg a.e. ha^{-1} also did not appear to influence desiccation.

Fig. 2c illustrates for the later maturing cultivar Natasha that desiccant activity occurred equally well for each of the times of application, in relation to the mid point of flowering.

Fibre Extraction Table 2

Variety did not significantly influence fibre extraction and the surfactant materials used in conjunction with certain of the glyphosate treatments, although they appeared to have a slight but not significant effect on the ease of retting,

Table 2.

Influence of rate, variety and surfactant on process of flax straw

	Factors													
	Variety				Glyphosate Rate (kg/ha)						Surfactant			
	(a)	(b)	(c)	Sig	0	0.7	1.4	2.1	2.8	Sig	-	+	Sig
Total fibre %, d.m.	30.0	30.6	29.8	NS	31.7	30.9	32.1	31.9	23.9	NS	29.6	30.6	NS
(S.E.M.)	(1.4)	(2.0)	(2.8)		(2.2)	(3.7)	(2.2)	(1.6)	(3.7)		(1.5)	(2.0)	
Long fibre % d.m.	12.8	14.4	12.0	NS	15.4	16.0	13.9	12.9	7.3	**	14.0	12.2	NS
(S.E.M)	(0.7)	(1.0)	(1.3)		(1.1)	(1.8)	(1.1)	(0.8)	(0.8)		(1.3)	(1.7)	
% Long fibre	42.4	46.3	41.9	NS	49.5	52.4	43.8	41.5	30.6	***	46.7	40.4	**
(S.E.M.)	(1.2)	(1.7)	(2.4)		(1.9)	(3.2)	(1.9)	(1.4)	(3.2)		(14.0)	(12.2)	
Separation Index†	8.0	6.6	7.2	NS	5.9	6.3	7.0	8.0	9.2	*	6.7	7.8	NS
(S.E.M.)	(0.4)	(0.5)	(0.8)		(0.6)	(1.0)	(0.6)	(0.5)	(1.0)		(0.4)	(0.6)	

Varieties:- (a) Hera (b) Eva (c) Natasha

† 0 = nil 10 = extreme ease of fibre separation

tended to reduce the proportion of long fibre ($P<.01$). The rate of glyphosate had a significant effect on the majority of fibre parameters recorded. Total fibre yield remained virtually constant up to a rate of 2.1 kg a.e. ha^{-1}. At the highest rate (2.8 kg a.e. ha^{-1}) both total fibre and the proportion of long fibre declined. Fibre extraction was improved ($P<0.05$) by the use of increased rates of herbicide.

DISCUSSION

It was evident that glyphosate, could be used to give a uniform 'retting' along the full length of the stem in the standing flax crop but can result in a decline in fibre yield and proportion of long fibres if excess desiccation occurs. The problems only partially resolved are the loss of seed and or effects on seed maturation. Timing of application is likely to be critical in this respect and also in maintaining maximum straw and fibre yields. The 'retting' process itself appears to be a balance of chemical and microbial activity.

The use of glyphosate is also being examined in France by Institute Technique du Lin (I.T.L.) and in Belgium by Onderzoek en Voorlichtingscentrum voor Land en Tuinbouw. The predominant retting method used in both countries is dew retting and glyphosate application is being studied principally as an aid to dew retting. The homogeneous drying of the standing crop observed after spraying with this herbicide is a valuable aid in obtaining good retting of the crop after pulling and laying. A field trial in France in 1980, conducted for L.I.R.A. in collaboration with I.T.L. showed nearly identical long fibre percentages in the dew retted (16.9%) and glyphosate treated (17.9%) areas of the crop. (Sultana, C. pers. comm.).

Field trials evaluating commercial scale use of the procedure in N. Ireland and in Scotland are producing encouraging results. In N. Ireland flax fibre imports for the linen industry are valued at some £5 x 10^6 p.a. (N. Ireland Department of Commerce (1981)) and this coupled with a long standing tradition for growing the flax crop would make it an important agricultural development.

REFERENCES

Association Generale de Producteurs de Lin (1968) Compte Rendu 1968.

Baird, D.D.; Upchurch, W.B.; Homesley, W.B.; Franz, J.E. (1971) Introduction of a new broadspectrum post emergence herbicide class with utility for herbaceous perennial weed control. Proceedings North Central Weed Control Conference. 26, 64-68.

Fraser, T.W.; Courtney, A.D.; Harvey, B.M.R. (1982) Pre-harvest retting of flax: a light microscope study of the effects of glyphosate treatment on the maturation of stem tissues. Annals of Applied Biology. In Press.

Green, H.W. (1969) A fresh look at flax, Lambeg Research Review 1968/69 pp 4-5.

Gubbels, G.H.; Kenaschuk, E.O. (1981) Desiccation of flax with diquat. Canadian Journal of Plant Science 61, 575-581.

Northern Ireland Agriculture Trust (1968) Flax Investigations in 1968.

Northern Ireland Department of Commerce (1981) Statistics Branch Belfast. U.K. imports of flax Jan-Dec 1980.

Sprankle, P.; Meggitt, W.F.; Penner, D. (1975) Absorption action and translocation of glyphosate. Weed Science 23, 235-240.

Sultana, C. (Personal Communication) Institute Technique du Lin. 5, Rue Cardinale Mercier, Paris.