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Productivity of wheat (*Triticum aestivum*) as affected by continuous use of new low dose herbicides for management of littleseed canarygrass (*Phalaris minor*)

R. S. DHAWAN*, S. S. PUNIA*, S. SINGH, D.YADAV AND R. K. MALIK*

CCS HAU Regional Research Station, Uchani, Karnal 132 001

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ABSTRACT

A field experiment was conducted at villages Uchana and Sagga near Karnal, Haryana during *rabi* of 1998-99 through 2006-07 to study the effect of continuous and rotational use of fenoxaprop, sulfosulfuron and clodinafop on control of *Phalaris minor* and productivity of wheat. Application of all the 3 herbicides resulted in 35-50% increase in wheat yield over weedy plots during first year of application. With continued application of these herbicides, the increase in yield remained 23-27% in sulfosulfuron- sprayed plots, 12-23% in fenoxaprop-sprayed plots and 17-35% in clodinafop- sprayed plots in 2005-06 and 2006-07. A similar decline was observed in plots that received rotational treatment of herbicides. Herbicide sprays resulted in a decline in density of *P. minor* till 2003-04 after which it increased till 2006-07. In 2006-07 the density of *P. minor* in some of the herbicide sprayed plots was equivalent to that in the weedy plots. Bioassay test showed increased insensitivity of *P. minor* to the herbicides. Mortality declined from 85-100% in 2001-02 to 10 % with fenoxaprop and 30-40% with sulfosulfuron in 2006-07. With clodinafop, the decline was relatively lesser. It was upto75% only. GR_{50} values of surviving populations showed 10-fold increase than the initial value in sulfosulfuron, 8-fold increase in fenoxaprop and 3-4-fold increase in clodinafop indicating the evolution of cross resistance to these herbicides in this weed.

***Key words*:** Clodinafop-propargyl, Cross-resistance, Fenoxaprop-p-ethyl, Non-linear regression, *Phalaris minor*, Sulfosulfuron, Wheat

Rice (*Oryza sativa* L.) – wheat (*Triticum aestivum* L. emend. Flori & Paol.) is an important cropping system in India comprising 10 m ha. One of the major thrusts to maintaining yield potential in cropping systems is to check the yield losses owing to weeds, besides checking the losses caused by insect pests and diseases. In India since 1970s, isoproturon - a phenylurea herbicide and an inhibitor of PS II in photosynthesis provided effective control of grassy weeds especially *P. minor* in wheat maintaining yields. Its continuous use has resulted in the evolution of resistance in *P. minor* to this herbicide in Haryana and Punjab (Malik and Singh, 1995; Yaduraju *et al.*, 1995; Walia *et al.*, 1997). The resistance affected area ranged between 0.8 and 1.0 m ha in north-western India which is on the increase. Since weed populations resistant to one category of herbicides are known to be managed by herbicides with alternate mode of action, three alternate herbicides, *viz.*, fenoxaprop-p-ethyl, clodinafop –propargyl and sulfosulfuron were recommended for management of herbicide- resistant populations of *P. minor* in 1997-98. While fenoxaprop and clodinafop are inhibitors of acetyl coenzyme –a carboxylase (ACCase), the first enzyme in fatty acid synthesis pathway, sulfosulfuron is an inhibitor of acetolactate /acetohydroxyacid synthase (ALS/AHAS) the first enzyme involved in synthesis of branched chain amino acids leucine, isoleucine and valine. Cross resistance and multiple resistance are known to evolve in herbicide resistant populations after continuous use of new herbicides in many weeds (Heap, 1997). In this investigation, the efficacy of these herbicides and the shifts in density of *P. minor* and in wheat yield were monitored in a long-term trial. An assessment of the change in sensitivity of *P. minor* populations to these herbicides was planned to be studied by shift in GR_{50} values (Herbicide dose at which 50% reduction of growth takes place) of different herbicides. Linear and logarithmic modes of regression on dose response curves have been carried out by a number of workers to make assessments regarding GR_{50} values (Finney, 1979; Streibig, 1988). Some of the workers proposed a better fit by non-linear modes of regression on dose response curves (Brian and Cousens, 1989; Seefeldt *et al.*, 1995). A comparison of polynomial mode of regression with linear and logarithmic modes of regression on dose response curves was also made during this investigation to assess the best mode of regression for estimating GR_{50} values.

Correspondence address: * *Department of Agronomy, CCS HAU, Hisar, Haryana 125 004* (E-mail: rupadhawan@hotmail.com)

MATERIAL AND METHODS

A permanent herbicidal trial was laid at farmers' fields at 2 sites in villages Uchana and Sagga around Karnal(29°43'N, 76°58'E at 215.2 m above mean sea-level) in 1998. Wheat 'PBW 343' was sown in November each year following conventional tillage practices. In July each year, rice was transplanted in the same plots after puddling of the soil. *Phalaris minor* population was reported to be resistant to isoproturon in the area. The soil was clay loam in texture, alkaline in reaction (pH 8.6 at Uchana and pH 8.2 at Sagga), low in organic carbon (0.30 % at Uchana and 0.33% at Sagga), medium in available phosphorus (9 kg/ha at Uchana and 14 kg/ha at Sagga) and high in potassium (250 kg/ha). Recommended dose of fertilizers (120-60-60 kg N-P-K/ha) was uniformly applied to all the treatments. Herbicide treatments consisted of sulfosulfuron (25 g/ha), fenoxaprop (120 g/ha) and clodinafop (60 g/ha), a rotational treatment and a weedy check. A single plot of 15 m × 66 m was kept for each treatment at both the locations. The same herbicide was sprayed in the same plot continuously for investigation. Weedy plots were maintained as such over the years. The sequence of herbicides in rotational treatment has been clodinafop for 1 year fb sulfosulfuron in the second year fb fenoxaprop in the third year and the treatments were repeated in the same order. All the herbicides were applied by knapsack sprayer using flat fan nozzle delivering 300 litre/ha volume at 2-3 leaf stage 30-35 days after sowing. Observations for *P. minor* density /m^2 were recorded at 4 different locations within a plot 30 days after spray of herbicide. Each plot was divided into 4 sub-plots for the purpose of creating replication for recording yield data at the time of harvest. The data was analyzed statistically by employing a three factor analysis of variance in a randomized block design.

Seeds of *P.minor* were collected from different plots sprayed continuously with different herbicides at Uchana and Sagga in April each year for bioassay test. Seeds from unsprayed plots were also collected to serve as controls. The bioassay test was conducted in pots in a screen house in November from 2,000 onwards each year. Seeds were sown in sandy loam soil in earthen pots (9" dia). 20 plants were maintained in 3 replicates for each biotype. Fenoxaprop (0 to180 g/ha), clodinafop (0 to 75 g/ha) and sulfosulfuron (0 to 60 g/ha) were sprayed with a knapsack sprayer. The pots were kept in an area of 10m^2 and the herbicide was sprayed at the doses mentioned above when the plants attained 2-3 leaf stage approximately 30-35 days after sowing. Observations were recorded 30 days after spray on population mortality and on biomass accumulation. Fresh weight of 5 plants/treatment was recorded 30 days after spray. Data on percentage mortality at herbicidal doses was analyzed by one factor analysis of variance following completely randomized design.

For plotting dose response curves, Microsoft Excel computer programme was used. The doses of herbicides were plotted on X-axis. The biomass accumulated as percentage increase or decrease over control was plotted on Y-axis. Trend lines for linear, logarithmic and polynomial regression (second order) were drawn for each data set. An estimate regarding the degree of fit was made from values for co-efficient of determination (R^2). Since it was observed that the polynomial mode of regression provided the best fit, only these regression lines were used for plotting GR_{50} values. GR_{50} value was regarded as the herbicide dose at which 50 % reduction in fresh biomass accumulation was observed as compared to untreated controls.

RESULTS AND DISCUSSION

Weed flora

Phalaris minor (Littleseed canarygrass) constituted about 49% of the total weed flora at the start of the investigation. The other predominant weeds were 9% *Rumex maritimus* (Golden dock). 7% *Chenopodium album* (Lamb's quarters), 5% *Chenopodium murale* (Nettleleaf goose foot), 4% *Coronopus didymus* (Swine cress), 6% *Anagallis arvensis* (Pimpernel), 3.7% *Melilotus indica* (Yellow sweet clover), 4.0% *Malwa parviflora* (Small whorl mallow), 2.5% *Medicago denticulata* (Bur clover) and 2% *Spergula arvensis* (Corn spurry). Weeds like *Polypogon monospliensis* (Rabbit foot's grass), *Poa annua*(Annual meadow grass), *Avena ludoviciana* (Wild oat), *Circium arvensis* (Canada thistle), *Vicia sativa* (Vetch), *Fumaria parviflora* (Fineleaf fumitory) and *Convolvulus arvensis* (Field bindweed) constituted rest of the weed flora. The weed spectrum, however, changed with herbicide sprays over the years and there was a predominance of broad leaf weeds when *P.minor* was controlled by the new herbicides. A shift in the density of *P.minor*, the major weed of the fields was monitored over the years.

Shift in P.minor density

In 1999-2000 the density of *Phalaris minor* was 460-580 plants/m^2 at the two sites. The density declined significantly (95-98%) after the use of all the three herbicides irrespective of the site (Table 1). The decline continued till 2003-04 in sulfosulfuron and fenoxaprop and till 2004-05 in clodinafop and rotational treatment after which it started to increase. The increase in sulfosulfuron sprayed and fenoxaprop sprayed plots was significantly more in 2005-06 than the previous years. A similar increase in the density of this weed under rotational treatment of herbicides and clodinafop sprayed plots was observed in 2006-07 over the previous years. The values were higher than the mean values in fenoxaprop sprayed plots at both the sites in 2005-06 and in 2006-07 and also in clodinafop sprayed plots at Uchana in 2006-07 at both the sites. An initial decline in weed density up to 2003-04 in sulfosulfuron and fenoxaprop sprayed plots and up to 2005-06 in clodinafop sprayed plots and in plots receiving rotational treatment of herbicides is indicative of the good efficacy of these herbicides against the weed. A continuous decline in the density of this weed in weedy plots was also observed till 2003-04 and was 60% of the mean density over the years. This could be due to two factors: *(i)* lesser recharge of the weed seed bank from the same and adjoining fields and *(ii)* lesser percentage of the weed seed contaminating the crop seed after effective management of the weed by the new herbicides. The fact that the weed seed reservoir in a particular field comprises of seeds shed by the weeds in the same plot as also seeds carried to the same plot from adjoining fields by wind, irrigation or farm equipments during tillage operations or with crop seed has been largely emphasized (Yaduraju, 1999). An increase in density of the weed after 5-6 years of continuous use of herbicides is an indication of selection of individuals cross resistant to these herbicides and their multiplication. The fact that the weed density in clodinafop sprayed plots started to increase later as compared to fenoxaprop sprayed plots perhaps indicates that the herbicide pressure by the two ACCase inhibitors selects for different mutants amongst the resistant individuals. The increase in weed density even in plots receiving rotational treatment of herbicides is indicative of the fact that the rotations are less effective probably because the mode of evolution of resis-

Table 1. Year wise data on density of *P. minor* (no./m^2) as affected by herbicide sprays continuously in the same plot every year.

Treatments	Sites	1999-00	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	Mean (Herbicide)
Sulfosulfuron	Uchana	32	10	8	4	9	22	38	70	
Sulfosulfuron	Sagga	16	12	6	4	14	16	36	57	22
Fenoxaprop	Uchana	55	13	18	14	14	26	70	154	
Fenoxaprop	Sagga	21	11	20	10	14	28	58	109	39.6
Clodinafop	Uchana	18	8	4	10	11	8	26	122	
Clodinafop	Sagga	16	12	6	8	10	8	16	37	19.8
Rotational	Uchana	30	13	0	3	6	4	22	102	
Rotational	Sagga	28	12	0	2	9	6	20	81	21
Weedy	Uchana	576	435	388	260	88	118	142	194	
Weedy	Sagga	460	448	372	228	96	153	122	131	263
Mean (Year)		125.2	96.9	82.2	54.3	27.1	38.8	54.9	105.5	
Mean (Site)	Uchana	78.6								
	Sagga	67.6								
CD (P=0.05)										
Herbicide	3.9	Site	2.5	Year	5.0					
Herbicide × site	5.6	Herbicide×year	11.2	Site×year	7.4	Herbicide × site × year			15.9	

Table 2. Year wise data on grain yield of wheat (t/ha) as affected by herbicide sprays of continuously in the same plot every year

Treatment	Site	1999-00	2000-01	2001-02	2002-03	2003-04	2004-05	2005-06	2006-07	Mean (Herbicide)
Sulfosulfuron	Uchana	6.1 (49)	5.3 (28)	5.0 (38)	5.4 (31)	5.2 (30)	4.2 (26)	4.3 (25)	3.7 (27)	
Sulfosulfuron	Sagga	6.1 (45)	5.6 (35)	5.5 (36)	5.8 (31)	5.4 (2.7)	4.0 (25)	4.2 (26)	3.8 (23)	4.9
Fenoxaprop	Uchana	6.0 (48)	5.3 (28)	4.9 (38)	5.4 (31)	5.1 (29)	4.1 (24)	4.2 (23)	3.5 (22)	
Fenoxaprop	Sagga	6.0 (45)	5.2 (30)	5.5 (36)	5.8 (31)	5.4 (2.7)	4.0 (25)	3.8 (18)	3.3 (12)	4.0
Clodinafop	Uchana	6.0 (48)	5.2 (26)	5.0 (38)	5.5 (32)	5.2 (30)	4.2 (26)	3.9 (17)	4.1 (34)	
Clodinafop	Sagga	6.0 (45)	5.6(35)	5.5 (36)	5.8 (31)	5.5 (29)	4.1 (26)	4.2 (26)	3.9 (25)	4.9
Rotational	Uchana	5.1 (39)	5.2 (26)	5.1 (39)	5.5 (32)	5.2 (30)	4.2 (26)	4.3 (25)	3.8 (28)	
Rotational	Sagga	5.2 (36)	5.6 (35)	5.6 (37)	5.9 (32)	5.5 (29)	4.1 (26)	4.2 (26)	3.8 (23)	4.8
Weedy	Uchana	3.1	3.8	3.7	3.6	3.1	3.1	3.2	2.7	
Weedy	Sagga	3.3	3.6	3.5	4.0	3.9	3.0	3.1	2.9	3.3
Mean (Year)		5.2	5.0	4.8	5.2	4.9	3.9	3.9	3.5	
Mean (Site)	Uchana	4.5								
	Sagga	4.6								
CD(P=0.05)										
Herbicide	0.1	Site	0.07	Year	NS					
Herbicide×site	0.1	Herbicide×year	0.30	Site × year	0.2	Herbicide × site × year		NS		

tance in this weed is metabolic detoxification of herbicides. These are more effective when the mode of evolution of cross resistance is target site of the herbicides.

Wheat yield

The grain yield in herbicide treated plots in 1999-2000 was 35-50% higher (5-6 t/ha) as compared to weedy plots (3 t/ha). This yield, however, declined over the years. It remained only 12-28% higher in 2006-07 except for plots sprayed with clodinafop at Uchana where it was still 35 % higher (Table 2). The yield in fenoxaprop sprayed plots at Sagga was lesser than the mean value. While interaction effect of herbicide and year on wheat yield was significant, while that of herbicide x site x year was not significant. This indicated that both herbicide application and the number of years for which it was used formed important factors for the reduction in yield while site was not an important factor. The decline in yield could possibly be due to the facts that *(i)* there may be an increased competition provided by the broad leaved weeds that start to emerge and provide competition after the management of *P. minor* and *(ii)* selection of *P. minor* populations insensitive to the herbicides used and competition provided by these. While an increased competition of a broad leaved weed *Rumex spinosus* has been reported after the management of *P. minor* (Sidhu *et al.*, 2000), the sensitivity of the *P. minor* populations over the years to the different herbicides was assessed by the bioassay test.

Bioassay test

Percentage mortality as well as biomass accumulated provided indications regarding sensitivity of weed over the years. Percentage mortality in the bioassay test at the herbicidal doses was 85% with sulfosulfuron and 100 % with fenoxaprop and clodinafop in 2001-02 (Table 3). This indicated that a majority of *P.minor* population at this stage was susceptible to the new herbicides. In 2006-07, the mortality level declined to 30-40% in samples from plots sprayed with sulfosulfuron and 10% in samples from plots sprayed with fenoxaprop. Mortality level remained higher up to 76-96% in samples from plots sprayed with clodinafop till this time.

GR_{50} values computed from data on biomass accumulation indicated that the value for sulfosulfuron which was 8g/ha in 2001-02 increased to 50-55g/ha in 2006-07. The GR_{50} value for fenoxaprop from unsprayed plots increased from 60g/ha in 2001-02 to 180-210g/ha in 2006-07. GR_{50} values for clodinafop which was 18 g/ha in 2001-02 in samples from unsprayed plots also increased to 50 to 60g/ha in samples from sprayed plots. Over the years samples from unsprayed plots also showed an increase in GR_{50} values although it was 40 to 50% lesser as compared to samples from sprayed plots (Table 4). The GR_{50} values before the start of the investigation against *P. minor* population were 5, 22 and 14g/ha for sulfosulfuron, fenoxaprop and clodinafop respectively (Yadav and Malik, 2005). A rise in GR_{50} values for all the 3 herbicides is indicative of the selection of individuals insensitive to the herbicidal doses. Resistance factor varied with the herbicide. A 10-fold increase in GR_{50} values against sulfosulfuron, 8-fold increase against fenoxaprop and 3-4-fold increase against clodinafop as compared to the initial values was observed. This is an indication of evolution of cross- resistance to both the category of the herbicides in the weed. A multiplication of these individuals resulted in build up in density of these individuals, creating competition and affecting yield. An increase in GR_{50} values for all the herbicides even on samples from unsprayed plots is perhaps an indication of mixing of the resistant individuals with the susceptible ones in these plots.

It, could, therefore, be inferred that a continuous use of herbicides resulted in decline in wheat yields. These provided control of the target weed *Phalaris minor* for about 4-5 years only. After this selection of individuals cross-resistant to both category of herbicides took place. Non-target weeds replace the target weed and provide competition during the period of effective management of the target weed by the herbicides. Therefore, herbicide mixtures targeting the major weed flora may be preferred to be

Table 3. Percent mortality of *Phalaris minor* seedlings 30 days after spray at herbicidal doses in bioassay test.

Year	Uchana			Sagga		
	Sulfosulfuron (25g/ha)	Fenoxaprop (120g/ha)	Clodinafop (60g/ha)	Sulfosulfuron (25g/ha)	Fenoxaprop (120g/ha)	Clodinafop (60g/ha)
2001-02	85	100	100	-	-	-
2002-03	85	90	100	-	-	-
2003-04	10	65	83	30	90	94
2004-05	7.5	33	60	7.7	52	99
2005-06	24	25	70	23	15	75
2006-07	40	11	76	30	10	96
SEm±	2.1	2.7	1.8	1.9	3.8	1.5
CD (P=0.05)	6.4	8.1	5.4	6.1	12.1	4.6

Table 4. GR_{50} values of *P.minor* samples in bioassay tests as affected by a continuous application of herbicides

Year	Polynomial Regression equation	GR_{50}	R^2
HAU Research Farm (Unsprayed plots)			
Sulfosulfuron			
2001-02	$Y=142-55.x+6.1x^2$	8	0.90
2003-03	$Y=160-56x + 6.5x^2$	28	0.87
2004-05	$Y=-150-64x +6.6x^2$	15	0.95
2005-06	$Y=155-63x+7.11x^2$	16	0.97
2006-07	$Y=50+66x-15.6x^2$	37	0.90
Fenoxaprop			
2001-02	$Y= 127-1.6x-3.4x^2$	60	0.75
2003-04	$Y=143-57x+7.4x^2$	75	0.85
2005-06	$Y=132+16.3x-8.5x^2$	110	0.47
2006-07	$Y= 119+47x-15.4x^2$	100	0.50
Clodinafop			
2001-02	$Y=153-23x-0.3x^2$	18	0.70
2003-04	$Y= 119-33.9x+3.1x^2$	35	0.74
2005-06	$Y= 146-58.7+6.2x^2$	18	0.95
2006-07	$Y= 50+60x-15.6x^2$	37	0.90
Uchana			
Sulfosulfuron			
2001-02	$Y=154 -67.1x +8.0x^2$	8	0.95
2003-04	$Y= 147-53.4x +6.9x^2$	30	0.82
2004-05	$Y=115-29.6x+1.9x^2$	23	0.68
2005-06	$Y= 119-10x+1.17x^2$	55	0.92
2006-07	$Y= 29+98.4x-21.3x^2$	45	0.91
Fenoxaprop			
2001-02	$Y=118-5.9x-0.2x^2$	65	0.77
2003-04	$Y= 101+3.8x-24.5x^2$	175	0.94
2005-06	$Y= 47+78.4x-14.7x^2$	155	0.72
2006-07	$Y= 67+63.6x-12.9x^2$	180	0.57
Clodinafop			
2001-02	$Y= 96+32.9x-79x^2$	60	0.60
2003-04	$Y= 104-13.2x -0.04x^2$	52	0.85
2005-06	$Y= 102 -4.89x-1.3x^2$	60	0.78
2006-07	$Y= 29 +98x-21.3x^2$	45	0.91
Sagga			
Sulfosulfuron			
2003-04	$Y= 135-32.7x+2.8x^2$	47	0.83
2004-05	$Y= 98-3.2x-2.2x^2$	47	0.93
2005-06	$Y= 155-53x +5.2x^2$	30	0.92
2006-07	$Y=23+142x-27x^2$	50	0.95
Fenoxaprop			
2003-04	$Y= 144 -40x+4.2x^2$	120	0.66
2005-06	$Y= 29+115x -22x^2$	135	0.71
2006-07	$Y= -26 +154x -24x^2$	210	0.91
Clodinafop			
2003-04	$Y= 84 +17.9x+4.9x^2$	60	0.86
2005-06	$Y=129 -31x +1.9x^2$	32	0.84
2006-07	$Y=23 +142x -27x^2$	50	0.95

used rather than application of single herbicides on a long-term basis. A rotation of the herbicides helps delay the selection but may not do so for a long time. The presence of cross-resistant population in unsprayed plots provides indications regarding the spread of these populations to an area which has never been exposed to the herbicide. Strict measures would be required to be undertaken to check the spread of cross resistant populations from an area where these evolved. Biochemical and molecular approaches gain relevance for identification of the resistant individuals. Molecular markers could also be helpful in enforcing quarantine measures.

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