Growth and Nitrogen Fixation of Field Pea and Lentil as Influenced by Herbicides: A Three Year Field Study

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A three year field study was conducted in the Dark Brown and Black soil zones of Saskatchewan to assess the effects of trifluralin (Treflan), metribuzin (Sencor), MCPB (Tropotox) and sethoxydim (Poast) on growth and symbiotic nitrogen fixation of field pea (*Pisum sativum* cv. Trapper) and lentil (*Lens culinaris* cv. Eston). Herbicides were applied at Saskatchewan recommended field rates and twice the recommended field rates. Treflan, Sencor and Poast did not adversely affect plant growth or nitrogen fixation potential of field pea and lentil when applied at recommended field rates. Similarily, application of MCPB at the recommended rate did not adversely affect field pea under normal growing conditions. In some instances, herbicide application at the recommended rate was observed to have a stimulatory effect on growth and nitrogen fixation. In contrast, at application rates in excess of those currently recommended in Saskatchewan, or when plants were severely stressed by unfavorable growing conditions such as poor soil moisture, plant growth, yield, nodulation, and nitrogen fixation were adversely effected by herbicides. These effects were not consistant nor were they predictable. MCPB is not registered for use in lentil and, when applied in this crop, often resulted in reduced nitrogen fixation and seed yield. In all cases, data suggest that reductions in the nitrogen fixation potential were due to indirect effects of herbicide application on plant growth rather than to direct effects on nodulation and nitrogenase activity.

INTRODUCTION

Several herbicides are currently registered for use, or are reportedly in use, for weed control in legume crops such as field pea and lentil. These herbicides may not only control weed growth but may also affect the legume-*Rhizobium* symbiosis either directly, through inhibition of nodulation and nitrogenase activity, or indirectly, through deleterious effects on root growth and plant development. As a consequence of the effect of herbicide application on non-target systems, symbiotic nitrogen fixation may be reduced with subsequent adverse effects on the nitrogen nutrition of the legume crop.

Little information is available regarding the effects of herbicide application on legume-*Rhizobium* symbiosis and, of that available, little is applicable to the western Canadian agro-climate. Studies by Germida et al. (1983; 1984) demonstrated that the soil incorporated herbicide trifluralin inhibits growth of rhizobia in pure culture and causes a severe reduction in nodulation and nitrogen fixation in pea plants under growth chamber conditions. However, under field conditions, the effects of trifluralin on growth and nodulation were not as severe. Dunigan et al. (1972) reported that, although high rates of trifluralin had an adverse effect on the nodulation process, no detrimental effects were observed when trifluralin was applied at recommended rates. Similarily, Rennie and Dubetz (1984) reported that, when applied at recommended rates, herbicides including trifluralin, metribuzin and diclofop had no effect on seed yield and nitrogen fixation of soybean grown in southern Alberta. Bebb et al. (1985) examined the effects of several herbicides including sethoxydim on legume growth and nodulation and concluded that

effects of herbicides applied at recommended rates were generally small; although, sethoxydim significantly reduced nodulation when applied at high rates. In contrast, studies by other workers (Brock, 1972; Harvey and Gritton, 1977; Bollich et al, 1985) have indicated that herbicides (including trifluralin) applied at rates equivalent to those presently recommended can cause a reduction in nodulation and nitrogen fixation of legumes such as soybean, clover and pea.

This study was initiated to evaluate the effects of four herbicides on the growth, seed yield, nodulation and nitrogen fixing potential (ARA), of field pea and lentil grown under Saskatchewan conditions.

MATERIALS AND METHODS

Herbicide field experiments were conducted during 1985, 1986 and 1987 with field pea and lentil on soils representative of the Dark Brown and Black Chernozemic soils of Saskatchewan. Some physical and chemical characteristics of the soils used in these experiments are reported in Table 1.

Table 1. Some physical and chemical characteristics of soils used in field experiments†.

Site	Depth (cm)	Texture	pH	Conductivity (mS cm^{-1})	NO_3-N (mg kg^{-1})	P (mg kg^{-1})
1985						
Sutherland	0-20	l	7.4	0.4	5.1	27
	20-40	l	7.8	0.4	7.1	ND††
	40-60	cl	8.1	0.8	22.4	ND
Laird	0-20	l	6.8	0.4	4.7	8
	20-40	l	7.1	0.3	2.5	ND
	40-60	cl	7.7	0.6	2.5	ND
1986						
Sutherland	0-20	l	7.6	0.5	5.5	13
	20-40	cl	7.8	0.6	5.3	7
Bellevue	0-20	l	7.8	0.4	21.1	21
	20-40	l	8.1	0.4	10.1	10
1987						
Aberdeen II	0-20	cl	7.5	0.4	4.0	13
	20-40	c	7.8	0.5	2.2	7
Aberdeen III	0-20	cl	7.8	0.4	4.4	14
	20-40	c	7.9	0.4	3.4	8
Bellevue	0-20	l	7.4	0.3	6.9	>60
	20-40	l	7.8	0.3	8.2	>60

† Soil analysis from Saskatchewan Soil Testing Laboratory.
†† Not determined.

Trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-*p*-toluidine), metribuzin ([4-amino-6-*tert*-butyl-3-(methylthio)-*as*-triazin-5(4H)-one], MCPB, and sethoxydim {2-[1-(ethoximino)butyl]-5-[2-(ethylthio)propyl]-3-hydroxy-2-cyclohexen-1-one} were applied at both the recommended field rates for Saskatchewan and at twice the recommended field rates. Trifluralin (Treflan) was soil incorporated prior to seeding at rates of 1.73 L ha^{-1} (0.94 kg a.i. ha^{-1}) and 3.46 L ha^{-1} (1.89 kg a.i. ha^{-1}). Metribuzin (Sencor), applied at 0.42 L ha^{-1} (0.21 kg a.i. ha^{-1}) and 0.85 L ha^{-1} (0.42 kg a.i. ha^{-1}), MCPB (Tropotox) applied at 3.46 L ha^{-1} (1.29 kg a.i. ha^{-1}) and 6.91 L ha^{-1} (2.59 kg a.i. ha^{-1}) , and sethoxydim (Poast) applied at 1.35 L ha^{-1} (0.25 kg a.i. ha^{-1}) and 2.70 L ha^{-1} (0.50 kg a.i. ha^{-1}) were all applied post emergence, according to manufacturers' recommendations. MCPB is not registered in Saskatchewan for use in lentil and was applied to this crop based on the rates recommended for use in field pea.

At each field location in 1985 and 1986, and at Bellevue in 1987, separate experiments for peas and lentils were conducted using a split plot design with herbicides as main plots and rates of application as subplots. Lentils were the only test crop grown in 1987 at Aberdeen II and Aberdeen III. Treflan and Sencor were applied at Aberdeen II and Sencor and MCPB were applied at Aberdeen III using a split plot design.

Pea and lentil were inoculated with an appropriate commercial "C" culture (Nitragin Co., Milwaukee, WI) seed-applied, peat-based rhizobial inoculant at a rate of 200 g 25 kg^{-1} seed immediately prior to seeding. Seeding was accomplished using a Rem Plot Seeder at seeding rates of 129 kg ha^{-1} and 38 kg ha^{-1} for pea and lentil, respectively. Subplots were six meters long and contained eight rows at 15 centimeter spacings. Phosphorus and nitrogen, both supplied as 11-55-0, were sidebanded at rates of 27 kg P_2O_5 ha^{-1} and 5 kg N ha^{-1}.

The experiments were monitored throughout the growing season and, at various time intervals, plants were sampled to evaluate dry matter accumulation and nodulation. At these intervals nitrogen fixation potential was evaluated using the acetylene reduction assay (ARA) (Postgate, 1972). Two samples of three plants each were harvested from the inner rows of one-half of each subplot and aerial dry matter was determined. The plant roots were exised and loose soil was removed. Three roots from each harvested sample were then placed in a gas-tight mason jar sealed with a screw cap and fitted with a flanged rubber septum. At the early sampling dates, 455 ml jars were employed whereas at the later sampling dates 909 ml jars were used. When the larger jars were used, 40 milliliters of gas were removed and replaced with 60 milliliters of acetylene. Quantities of gas removed and acetylene reinjected for smaller sampling jars were 20 milliliters and 30 milliliters, respectively. Jars were incubated at 23-26° C for 15 minutes. Following incubation, duplicate ten milliliter samples were withdrawn from the incubation jars and stored in evacuated vacutainers for future analysis. Samples were analyzed for ethylene using a Hewlett Packard 5890A gas chromatograph fitted with a stainless steel (1/8" O.D.) Poropak R (80/100 mesh) column. The oven temperature was 45° C and the detector temperature was 50° C. Nodule numbers and relative size were evaluated.

At maturity, plants were harvested from a one to two square meter area from within the undisturbed half of each subplot. Aerial portions were dried and threshed and both seed and straw yield were determined.

Analysis of variance was performed on all data. Least significant differences were determined and used to test differences among, and within, treatment means. Data obtained from acetylene reduction were logarithmically transformed prior to statistical analysis to homogenize the variance.

RESULTS AND DISCUSSION

Emergence of field pea and lentil was generally unaffected by preplant incorporation of Treflan . Post emergence application of Sencor and Poast caused some leaf burning of both field pea and lentil, although plants recovered from initial damage. Application of MCPB to field pea typically resulted in temporary wilting and some yellowing. Application of MCPB to lentil plants caused considerable damage from which the crop never fully recovered. Severity of crop damage was dependant on prevailing climatic conditions and tended to be worse when plants were already suffering moisture stress.

In 1985, herbicide application did not significantly affect the growth and yield of field pea at Sutherland. In contrast, growth and yield of lentil was significantly reduced by the application of MCPB. Nitrogen fixation potential of both field pea and lentil was significantly reduced at various sampling periods throughout the growing season by the application of Sencor, MCPB and Poast, particularly when applied at rates in excess of those recommended. In some cases application of Treflan resulted in increased ARA activity. At Laird, MCPB significantly reduced yield of both crops whereas application of Treflan and Poast at twice the recommended field rate resulted in lentil seed yield increases. Yield increases were likely due to control of volunteer wheat by the grassy weed herbicides. It was concluded that herbicide effects on ARA activity were the consequence of herbicide effects on plant growth. Thus, stimulation of plant growth as a result of better weed control enhanced fixation potential. A more detailed discussion of the 1985 field results is found elsewhere (Germida et al., 1986).

At Sutherland in 1986, application of herbicides to pea plants significantly affected dry matter accumulation whereas nodule numbers and ARA activity were significantly affected only by rate of application (Table 2). Dry matter yields tended to be highest on Treflan mainplots and lowest on MCPB mainplots. Although not statistically significant, data suggest that nodule numbers were reduced by all herbicides, particularily when applied at twice the recommended field rates. Increasing rates of herbicide application tended to decrease ARA activity (Table 3). These reductions in ARA activity were statistically significant at the second and third sampling period.

Seed yield of field pea was significantly affected by herbicide application at Sutherland (Table 2). Although there were no statistically significant effects of herbicide rate on grain yield, overall seed yield from the Treflan mainplot (control and treatments) was significantly greater than from other herbicide treatments (Fig 1). MCPB applied at the recommended field rate limited seed yield to a greater extent than when applied at twice the recommended field rate. This observaton was not consistant with results obtained in either 1985 or 1987 where both rates of application gave a similar response. Seed yields ranged from 584 kg ha^{-1} (MCPB,recommended field rate), to 2191 kg ha^{-1} (untreated control).

Table 2. Summary of statistical significance from analysis of variance for dry matter accumulation, nodulation, acetylene reduction (ARA) and grain yield of field pea grown at Sutherland and Bellevue, 1986.

Parameter	Source	df	*Sampling Time* T1	T2	T3	T4	Harvest
Sutherland							
Dry matter	Herbicide	3	**	NS	NS	**	
	Rate	2	NS	NS	NS	NS	
	H x R	6	NS	NS	NS	NS	
Nodule #	Herbicide	3	NS	NS	NS	NS	
	Rate	2	NS	NS	NS	*	
	H x R	6	NS	NS	NS	NS	
ARA	Herbicide	3	NS	NS	NS	NS	
	Rate	2	NS	**	*	NS	
	H x R	6	NS	NS	NS	NS	
Grain Yield	Herbicide	3					*
	Rate	2					NS
	H x R	6					NS
Bellevue							
Dry matter	Herbicide	3	NS	NS	NS	*	
	Rate	2	NS	NS	NS	NS	
	H x R	6	NS	NS	NS	NS	
Nodule #	Herbicide	3	NS	NS	NS	NS	
	Rate	2	NS	NS	NS	NS	
	H x R	6	NS	NS	NS	NS	
ARA	Herbicide	3	*	NS	NS	NS	
	Rate	2	NS	NS	NS	NS	
	H x R	6	NS	NS	NS	NS	
Grain Yield	Herbicide	3					NS
	Rate	2					NS
	H x R	6					*

*,**, Significant at 0.05 and 0.01 level, respectively.

At Bellevue in 1986, high levels of soil NO_3-N encouraged healthy growth of field pea and may have reduced both plant dependence on symbiotically fixed nitrogen and response to herbicide application. Statistical analysis indicated that few significant responses were obtained at this site (Table 2). Although not statistically significant, data suggest that MCPB may have limited dry matter accumulation at the first three sampling periods. A statistically significant reduction in dry matter yield at the fourth sampling period was due to the application of MCPB. The nitrogen fixation potential of peas at Bellevue was significantly affected by herbicide application only at the first sampling period. It is interesting to note that, with the exception of Poast, herbicide application generally resulted in increased ARA activity at this harvest (Table 4). Seed yields of field pea at Bellevue were not significantly affected by herbicide or rate of application although a significant herbicide by rate interaction was observed. Seed yields ranged from 2098 kg ha^{-1} (Sencor, twice recommended rate) to 3189 kg ha^{-1} (Poast, twice recommended rate) (Fig. 1).

Table 3. Effect of herbicide application on nitrogen fixation potential (ARA) of field pea at Sutherland, 1986.

Herbicide	Application Rate (x field rate)	ARA at Sampling Period (n moles C_2H_4 $plant^{-1}$ hr^{-1})			
		1	2	3	4
Treflan	0	691	1910	2495	15816
	1	604	2140	1180	20061
	2	624	1135	770	7324
Sencor	0	1479	3548	2420	9802
	1	1019	2293	1507	11988
	2	745	957	1701	13425
MCPB	0	1010	3783	7085	15290
	1	694	979	1441	10064
	2	513	996	1020	9909
Poast	0	1052	4688	1896	13138
	1	679	2765	706	6390
	2	1345	1260	766	10191

Table 4. Effect of herbicide application on nitrogen fixation potential (ARA) of field pea at Bellevue, 1986.

Herbicide	Application Rate (x field rate)	ARA at Sampling Period (n moles C_2H_4 $plant^{-1}$ hr^{-1})			
		1	2	3	4
Treflan	0	231	320	2310	3496
	1	388	264	1189	3019
	2	967	422	2815	4864
Sencor	0	366	349	3350	3785
	1	779	378	5218	5648
	2	898	374	2765	5262
MCPB	0	1696	1496	5739	4830
	1	1218	1082	7431	7379
	2	2180	875	7691	5736
Poast	0	1501	289	3404	3386
	1	1052	608	4306	7421
	2	357	359	4148	3879

In 1986 at Sutherland, herbicide application significantly affected dry matter accumulation, nodule numbers , and ARA activity of lentils at various sampling periods (Table 5). Dry matter accumulation was significantly affected by herbicide application at each sampling period although the effects of herbicide rate were statistically significant only at the second and third sampling period. MCPB tended to have the greatest effect on dry matter accumulation, resulting in significantly lower dry matter yields at each sampling period. With the exception of Treflan and Sencor at the first and third sampling periods, application of herbicides generally reduced ARA activity as compared to control treatments (Table 6). Furthermore, the highest level of herbicide application generally exaggerated the reduction in nitrogen fixation potential.

Table 5. Summary of statistical significance from analysis of variance for dry matter accumulation, nodulation, acetylene reduction (ARA) and grain yield of lentil grown at Sutherland and Bellevue, 1986.

			Sampling Time				
Parameter	Source	df	T1	T2	T3	T4	Harvest
Sutherland							
Dry matter	Herbicide	3	*	**	*	*	
	Rate	2	NS	**	**	NS	
	H x R	6	NS	NS	NS	NS	
Nodule #	Herbicide	3	*	**	NS	NS	
	Rate	2	*	NS	NS	NS	
	H x R	6	NS	*	NS	NS	
ARA	Herbicide	3	NS	NS	**	NS	
	Rate	2	NS	**	*	NS	
	H x R	6	NS	NS	NS	NS	
Grain Yield	Herbicide	3					NS
	Rate	2					NS
	H x R	6					NS
Bellevue							
Dry matter	Herbicide	3	NS	NS	NS	NS	
	Rate	2	NS	NS	NS	NS	
	H x R	6	NS	NS	**	NS	
Nodule #	Herbicide	3	*	**	NS	NS	
	Rate	2	NS	NS	NS	NS	
	H x R	6	NS	*	NS	NS	
ARA	Herbicide	3	NS	NS	NS	NS	
	Rate	2	NS	**	NS	NS	
	H x R	6	NS	NS	NS	NS	
Grain Yield	Herbicide	3					NS
	Rate	2					NS
	H x R	6					NS

*,**, Significant at 0.05 and 0.01 level, respectively.

Seed yields of lentil grown at Sutherland in 1986 were not significantly affected by application of herbicide despite earlier indications that herbicide application reduced nitrogen fixation potential and dry matter accumulation (Table 5, Fig. 2). Although not statistically significant, data suggest that seed yields were reduced by the application of MCPB and Poast, particularily at rates equivalent to twice field rate.

Table 6. Effect of herbicide application on nitrogen fixation potential (ARA) of lentil at Sutherland, 1986.

Herbicide	Application Rate (x field rate)	ARA at Sampling Period (n moles C_2H_4 $plant^{-1}$ hr^{-1})			
		1	2	3	4
Treflan	0	114	318	74	1588
	1	128	322	71	1473
	2	139	280	92	1098
Sencor	0	87	452	91	1218
	1	117	388	138	986
	2	124	210	91	625
MCPB	0	165	618	75	648
	1	128	389	56	579
	2	130	230	47	296
Poast	0	111	462	177	1183
	1	116	177	80	872
	2	97	282	92	821

As was previously indicated, fewer responses to herbicide application were obtained at Bellevue as compared to Sutherland (Table 5). Dry matter accumulation of lentil plants grown at Bellevue in 1986 was not significantly affected by either herbicide application or rate of application although a significant herbicide by rate interaction was noted at the third sampling period. Our results indicate a strong trend for reduced dry matter yields at both the field rate and twice the field rate of MCPB. Nodule numbers were not significantly affected by rate of herbicide application, although overall numbers of nodules on plants grown within the Sencor main plot (control and treatment) were significantly higher than all other herbicides at the first and second sampling period. Nitrogen fixation potential of lentils at Bellevue was significantly affected by rate of herbicide application only at the second sampling period. At this time, significant reductions in ARA activity were associated with increasing rates of herbicide application (Table 7). In particular, Sencor and Treflan application reduced ARA activity at both the field rate and twice the recommended field rate. MCPB and Poast applied at the field rate led to increased ARA activity at the second sampling time whereas application of herbicides at the highest rate limited nitrogen fixation potential. As was the observation at Sutherland in 1986, seed yield of lentil was not significantly affected by herbicide application at Bellevue (Table 5). However, data suggest that application of MCPB and Treflan led to reduced seed yields, particularily when herbicides were applied at the highest rate (Fig. 2).

Table 7. Effect of herbicide application on nitrogen fixation potential (ARA) of lentil at Bellevue, 1986.

Herbicide	Application Rate (x field rate)	ARA at Sampling Period (n moles C_2H_4 $plant^{-1}$ hr^{-1})			
		1	2	3	4
Treflan	0	108	584	1659	800
	1	200	516	1464	889
	2	84	310	908	809
Sencor	0	388	366	1609	1301
	1	298	251	752	889
	2	222	144	1064	966
MCPB	0	63	235	702	766
	1	214	330	852	1175
	2	171	180	742	945
Poast	0	119	246	871	1069
	1	147	546	1289	968
	2	131	115	775	820

Preliminary analysis of the 1987 field results indicate that there were no significant effects of either Treflan or Sencor on seed yield of lentil at Aberdeen II. Lentil seed yields at this site were generally poor, ranging from 670 kg ha^{-1} to 876 kg ha^{-1} and this likely reflects lack of soil moisture and poor growing conditions (Fig.3). Similarily, lentil seed yields at Aberdeen III were poor and ranged from 662 kg ha^{-1} to 809 kg ha^{-1}(Fig. 3). Sencor and MCPB did not appear to affect seed yields.

Growing conditions at Bellevue in 1987 were more favourable. Pea yields ranged from 1034 kg $ha^{-1}$to 1509 kg ha^{-1} (Fig. 4) and were not significantly affected by the application of herbicide. Lentil seed yields at Bellevue ranged from 120 kg ha^{-1} to 1709 kg ha^{-1} (Fig. 4). There was a highly significant effect of herbicide and herbicide rate on seed yield of lentil at Bellevue. MCPB treatments caused the greatest yield reductions, followed by Treflan.

SUMMARY

The results of this three year study suggest that, under normal growing conditions, Treflan, Sencor and Poast pose little threat to the growth, yield, nodulation and effective nitrogen fixation of field pea and lentil when applied at the Saskatchwan recommended rates. Similarily, MCPB can be safely used in field pea at the recommended rate. These results are in keeping with those of other workers (Dunigan et. 1972; Rennie and Dubetz 1984; Bebb et al. 1985) who reported that herbicides could be safely used in a variety of legumes when applied at the recommended rate. However, at application rates in excess of those presently recommended, or if plants are unduly stressed by unfavorable growing conditions, growth, yield, nodulation and nitrogen fixation may all be adversely affected by herbicide application. MCPB is not recommended for use in lentil and was observed to significantly reduce plant growth, yield and nitrogen fixation potential of lentil. Reduction of yield and /or nitrogen fixation is obviously undesirable and, thus, use of this herbicide

for weed control in lentil should be avoided. With few exceptions, results indicated that nitrogen fixation potential was affected through the indirect effects of herbicide application on plant growth rather than through the direct effects on nitrogenase activity. Thus, application of herbicides which strictly limited plant growth had the greatest effect on ARA activity.

ACKNOWLEDGEMENTS

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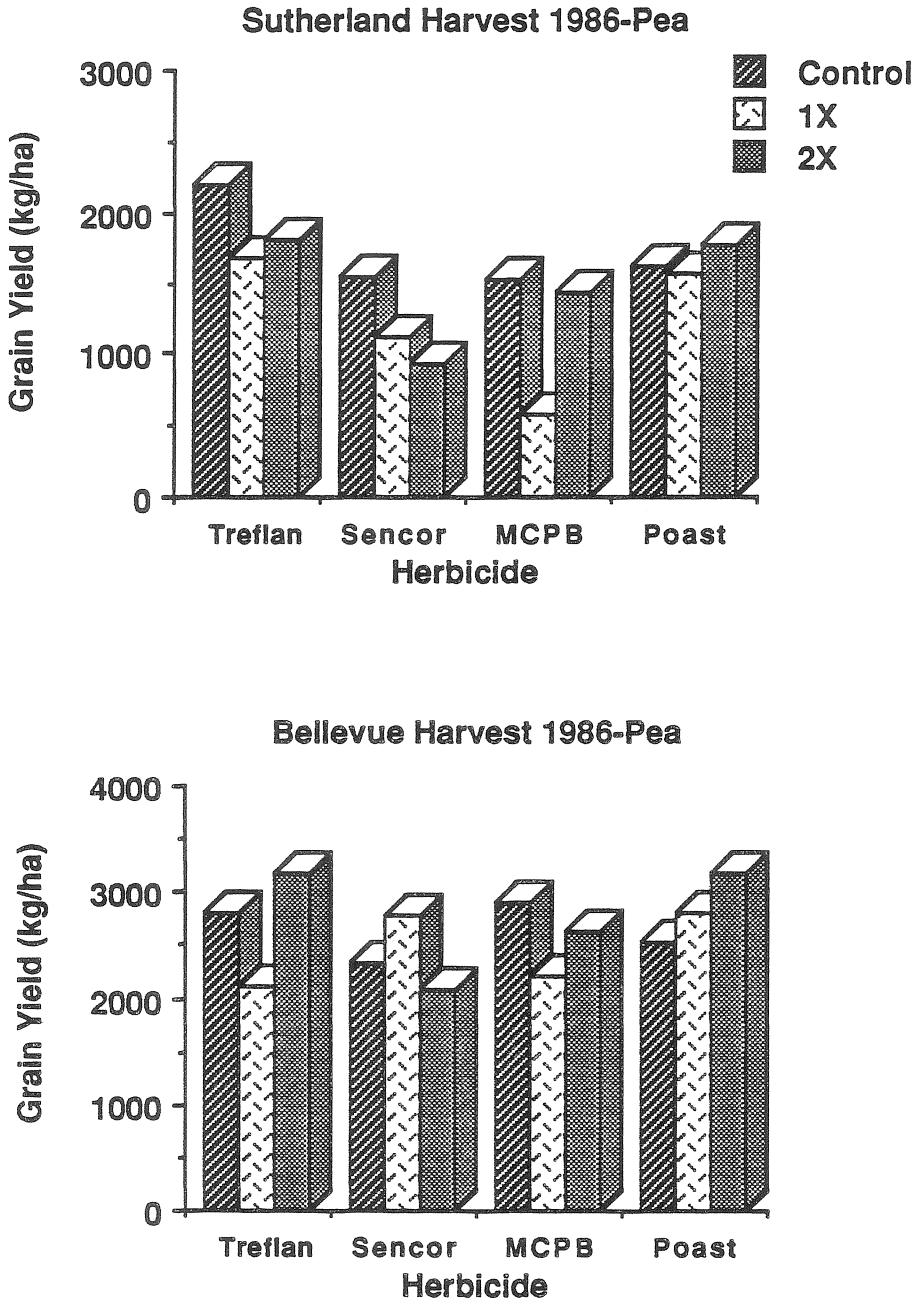

Figure 1. Effect of herbicides on the grain yield of pea, 1986.

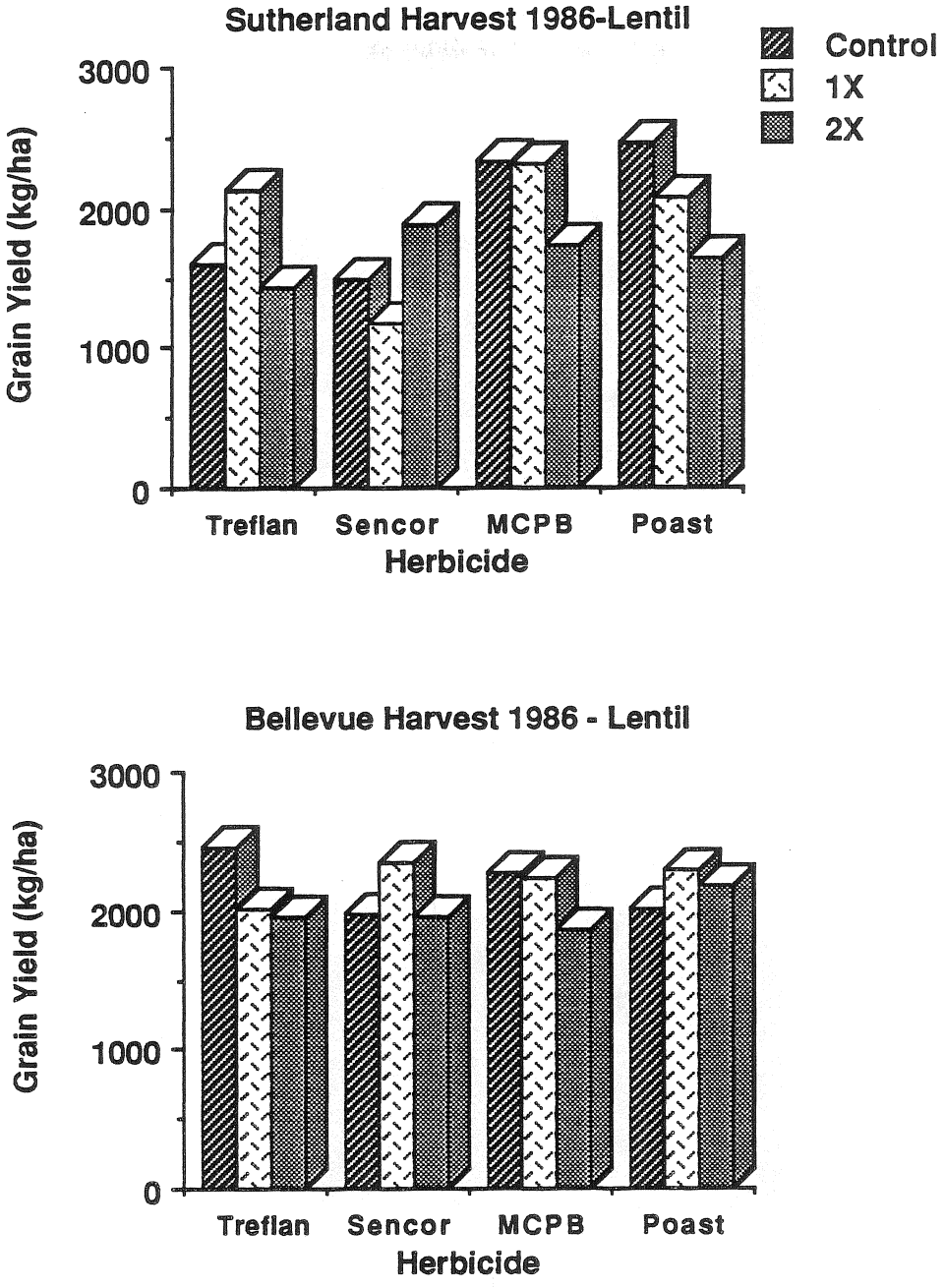

Figure 2. Effect of herbicides on the grain yield of lentil, 1986.

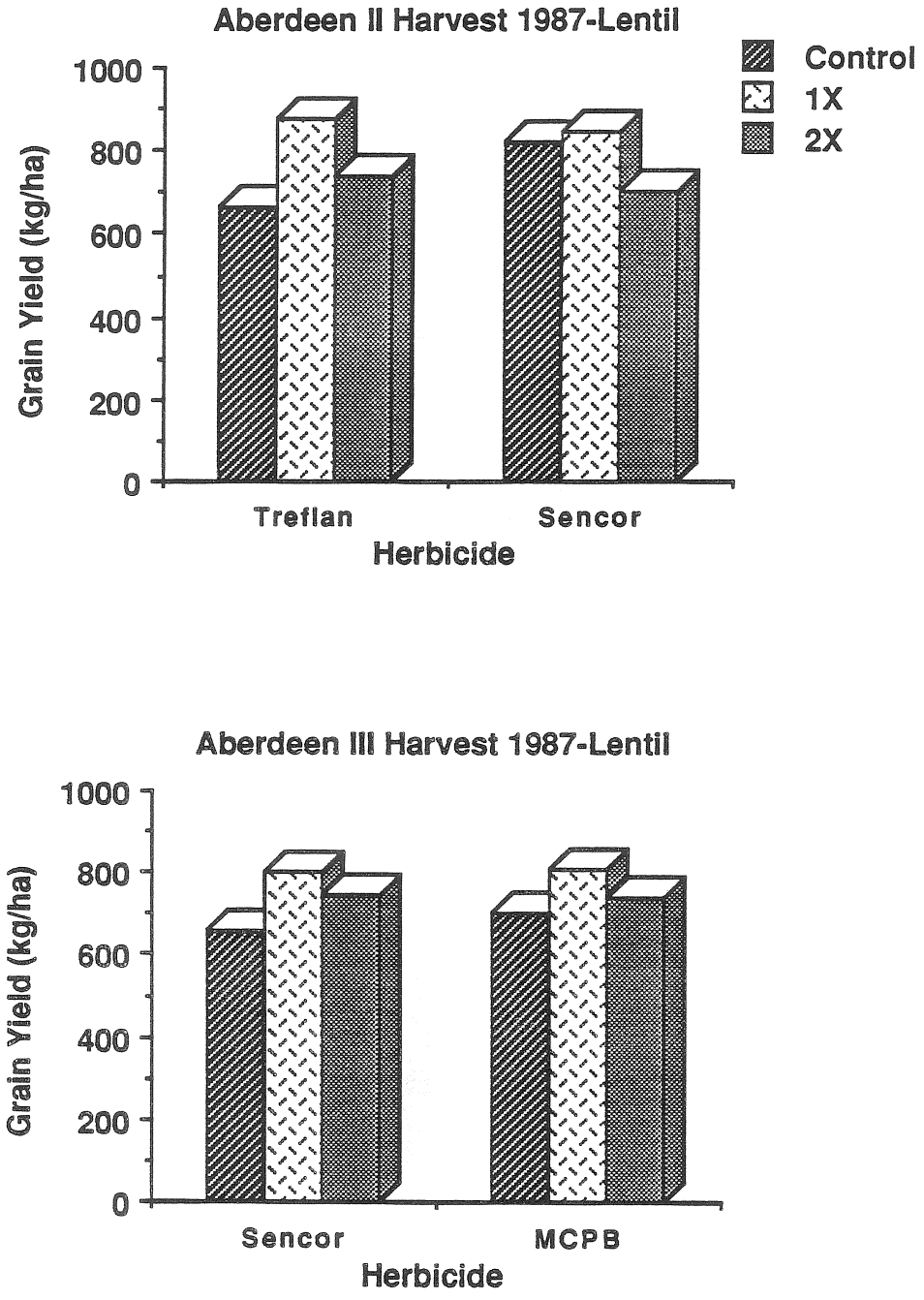

Figure 3. Effect of herbicides on the grain yield of lentil, 1987.

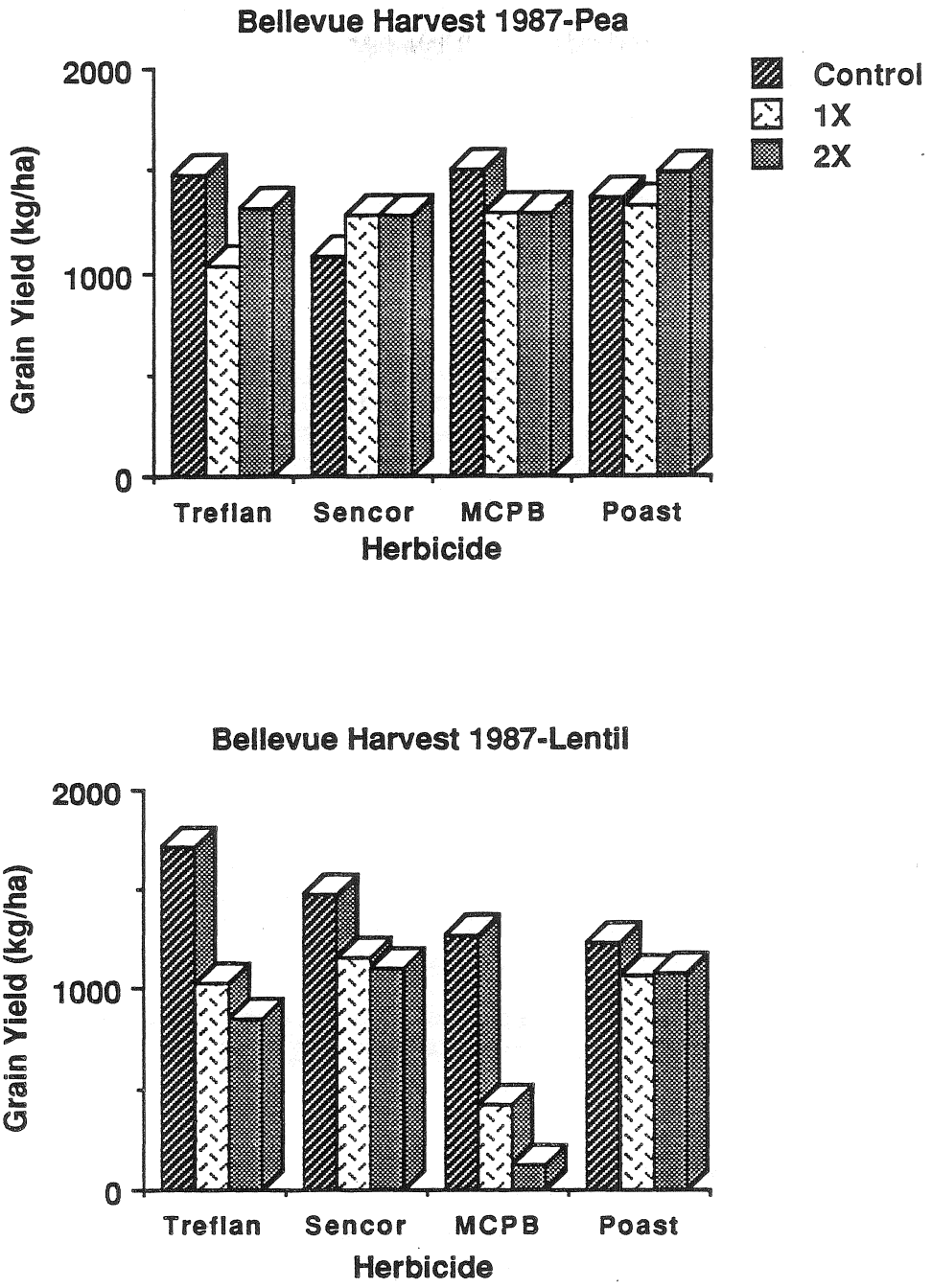

Figure 4. Effect of herbicides on the grain yield of pea and lentil, 1987.