

EFFECT OF HERBICIDES ON GROWTH AND NITROGEN FIXATION POTENTIAL
(ARA) OF FIELD PEA AND LENTIL

J.J. Germida¹, A.E. Slinkard², L.M. Nelson³ and R.B. McKercher¹,
¹Saskatchewan Institute of Pedology, ²Crop Development
Center and ³Plant Biotechnology Institute

This study assessed the effects of the herbicides trifluralin (Treflan), metribuzin (Sencor), MCPB (Tropotox) and sethoxydim (Poast) on the growth and symbiotic nitrogen fixation of the field pea and lentil (in separate experiments). Sites were established in the Dark Brown and Black soil zones at Sutherland and Laird, respectively. Legume seeds were inoculated with a commercial preparation of rhizobia. The herbicides were tested at the recommended field rate and twice field rate in a split-plot design with herbicides as the main effect and rate as the split effect. The parameters measured were legume growth and response to herbicide, plant dry matter and grain yield, nodule number, and nitrogen fixing potential (ARA).

In general, there was no significant effect of herbicides on the growth and yield of field pea at the Sutherland site, whereas MCPB caused a significant reduction in growth and yield of lentil. The average grain yield ranged from 1782 to 2325 kg/ha for field pea and 456 to 1703 kg/ha for lentil. All plants at this site, including controls, were poorly nodulated and several herbicides (Sencor, MCPB, Poast) significantly reduced nitrogen fixation potential (ARA) of both field pea and lentil at certain sampling times. Similar results occurred at the Laird site. The average grain yield ranged from 1053 to 2363 kg/ha for field pea and 48 to 1915 kg/ha for lentil. MCPB significantly reduced yield of both crops, whereas Treflan and Poast at two times field rate increased lentil grain yield, probably as a result of better control of volunteer wheat. The herbicides reduced nitrogen fixation potential (ARA) in a similar fashion to that observed for Sutherland plants even though the number of nodules was about ten-fold greater.

Based on one year of results, MCPB adversely affected the growth, yield, and nitrogen fixation potential (ARA) of lentil, and under certain conditions that of field pea. Additional studies are required to fully document herbicide effects on nitrogen fixation of grain legumes.

INTRODUCTION

A variety of herbicides are used in Saskatchewan to protect legume crops from weed competition. These herbicides not only control weed growth but, may also affect the Rhizobium-legume symbiosis or the population of rhizobia in the soil. The effect of herbicides on these

non-target systems may lead to a decrease in nitrogen fixation and also in soil fertility.

Little information is available on the effects of herbicides on the nodulation and nitrogen fixation of legumes grown in western Canada. Studies by Germida et al (1983; 1984) demonstrated that the soil incorporated herbicide, trifluralin, inhibits the growth of rhizobia in pure culture, and causes a severe reduction in nodulation and nitrogen fixation of pea plants grown in a growth chamber. However, the effect of trifluralin on pea plants was not as severe under field conditions. Rennie and Dubetz (1984) found that five different herbicides including trifluralin, metribuzin, and diclofop had no effect on seed yield or nitrogen fixation of soybean grown in southern Alberta. Investigation by other workers (Brock, 1972; Harvey and Gritton, 1977; Bollich et al 1985) have shown that trifluralin and other herbicides can cause a reduction in nodulation and nitrogen fixation of legumes such as soybean, clover, and pea. These results, however, are not necessarily applicable to western Canada as the soils, climatic conditions, and crop varieties were not the same as in Canada.

This study evaluated the effects of four herbicides on the growth, nodulation and nitrogen-fixing potential (ARA) of field pea and lentil under Saskatchewan field conditions. The following report summarizes the first year field study results.

MATERIALS AND METHODS

Herbicide experiments were conducted with lentil (Lens culinaris cv. Eston) and field pea (Pisum sativum cv. Trapper) grown

in a Dark Brown Chernozem and a Black Chernozem at Sutherland and Laird, Saskatchewan, respectively. Both soils contained low levels of $\text{NO}_3\text{-N}$ in the 0-24 cm layer (Table 1). The experiments were set-up in a randomized split-plot design with herbicides as the main effect and rates as the split effect with four replicates.

Table 1. Fertility analysis of Sutherland and Laird field soils.[†]

Site	Depth (cm)	Text.	pH	Cond. (mS/cm)	$\text{NO}_3\text{-N}$ -----	P mg/kg	K -----	$\text{SO}_4\text{-S}$ -----
Sutherland	0-6	l	7.4	0.4	5.1	27	>450	4.7
	6-12	l	7.8	0.4	7.0	ND	ND [‡]	5.2
	12-24	cl	8.1	0.8	22.4	ND	ND	>1.2
Laird	0-6	l	6.8	0.4	4.7	8	268	3.4
	6-12	l	7.1	0.3	2.5	ND	ND	3.1
	12-24	cl	7.7	0.6	2.5	ND	ND	7.0

[†] Soil analysis data from Saskatchewan Soil Testing Laboratory.

[‡] ND, not determined.

Herbicides

The herbicides tested were 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- (ethoxyimino) butyl]-5-[2-(ethylthio) propyl]-3-hydroxy-2-cyclohexen -1-one). Herbicides were tested at the recommended field rate and twice the field rate. Trifluralin (Treflan) was soil incorporated at 1.73 L/ha (0.94 kg AI/ha) and 3.46 L/ha (1.89 kg AI/ha); metribuzin (Sencor) at 0.42 L/ha (0.21 kg AI/ha) and 0.85 L/ha (0.42 kg AI/ha), MCPB (Troptox) at 3.46 L/ha (1.29 kg AI/ha) and 6.91 L/ha (2.59 kg AI/ha), and sethoxydim (Poast) at 1.35 L/ha (0.25 kg AI/ha)

and 2.70 L/ha (0.50 kg AI/ha) were applied post-emergence according to manufacturer's recommendations.

Inoculation

Seeds were inoculated (200 g inoculum/25 kg seed) with a seed-applied, peat-based rhizobial inoculant appropriate for pea, vetch and lentil : "C" culture (Nitragin Co., Milwaukee, WI) using skim milk powder as sticker.

Plant Growth

Pisum sativum cv. Trapper seeds (129 kg/ha) or Lens culinaris cv. Eston seeds (38 kg/ha) were sown at a depth of 2" (using a Rem Plot Seeder) in 8-row plots (rows 15 cm apart). Phosphorus and starter nitrogen (as 11-55-0) was sidebanded at 49 kg/ha. Plots were checked for emergence and herbicides applied at the 2-3 leaf stage (ca. 10 to 14 days post-emergence).

Plots were examined at various times intervals and observations made on plant growth, response to herbicides, nodulation and nitrogen fixation potential (ARA). Plant dry matter yield was determined at 3 or 4 times intervals (ca. 4, 6, 8 and 10 weeks post emergence). Two sets of three plants were taken from the inner rows of one-half of the plot. The roots were excised and the loose soil removed. The roots were placed (3 per jar) into a 909 mL gas-tight mason jar sealed with a screw-cap lid fitted with a flanged rubber septum (455 mL jars were used for 5-6 week old plants). Air (40 mL) was removed and replaced with 60 mL of acetylene (one half these amounts for the 455 mL jars). The jars were incubated at 23-26 C for 15 min and then duplicate 10 mL gas subsamples were taken using

evacuated vacutainers. The acetylene reduction assay (ARA) (Postgate 1972) was performed to indicate the dinitrogen fixing activity. For this assay, ethylene was measured 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 size were evaluated for each root system.

The shoot material was dried at 70 C for 72-96 h and weighed.

At harvest, yield was determined by sampling 3 meters of the 3 inside rows (ca. 1.6 m²) from the bottom half (undisturbed during the growing season) of each plot. This material was dried, threshed, and then straw and grain yield determined.

Statistical Analysis

Data were analyzed statistically by analysis of variance. The least significant difference (LSD) was used to test significant differences among treatment means. The acetylene reduction data were logarithmically transformed prior to analysis to homogenize the variances.

RESULTS

General observations:

There were several differences between the Sutherland and Laird field sites that must be noted. First, soil conditions at the Sutherland site were considerably drier than at the Laird site. The Laird site was also subjected to hail during the growing season. Second, the Laird site was plagued with a severe problem of volunteer wheat. Third, there was a notable difference between nodulation of

legumes at both sites (see below) and this might be related to soil moisture. Finally, the Laird site was only partially sampled at the second time interval and this information is not included in the results.

Herbicide effects: visual observations on plant growth

The soil incorporated herbicide Treflan had no effect on seed emergence of field pea or lentil at either field site. Emergence in the Treflan field rate plots and twice field rate plots ranged from 92 to 105% and 80 to 120% of the control plots, respectively. These values were typical for the other plots that were eventually treated with post-emergence herbicides.

Application of Sencor or Poast caused some leaf burning of both field pea and lentil plants, but the plants recovered and no long lasting visual effects were noted. MCPB caused wilting of pea plants which lasted for approximately one week. The effect of MCPB on lentil plants was more severe as plants were burned and killed; partially damaged plants never fully recovered. Treflan had no visual effect on growth of either legume.

Field pea at Sutherland

There was no significant effect of herbicides on the growth, nodulation and nitrogen fixation potential (ARA) of field pea at the Sutherland site (Table 2). There was no significant effect of herbicide rate on the grain yield, although overall yield from the Sencor plots (control and treated plots) was significantly greater than from other plots (Table 2; Fig. 1a). The average grain yield ranged from 1780 to 2277 kg/ha (Fig. 1 a). The average straw yield ranged from

Table 2. Summary of statistical significance from analysis of variance for plant shoot dry weight, nodulation, acetylene reduction (ARA) and grain yield of field pea grown at Sutherland (site A) and Laird (site B).

Site	Parameter	Source	df	Sampling time				
				T1	T2	T3	Harvest	
A	Dry wt	Herbicide	3	NS	NS	NS		
		Rate	2	NS	NS	NS		
		H x R	6	NS	NS	NS		
	Nodule no.	Herbicide	3	NS	NS	NS		
		Rate	2	NS	NS	NS		
		H x R	6	NS	NS	NS		
	ARA	Herbicide	3	NS	*	NS		
		Rate	2	NS	NS	NS		
		H x R	6	NS	NS	NS		
	Grain yield	Herbicide	3				*	
		Rate	2				NS	
		H x R	6				NS	
	B	Dry wt	Herbicide	3		NS	NS	
			Rate	2		NS	NS	
			H x R	6		NS	NS	
Nodule no.		Herbicide	3		NS	NS		
		Rate	2		NS	NS		
		H x R	6		NS	NS		
ARA		Herbicide	3		*	**		
		Rate	2		*	NS		
		H x R	6		NS	NS		
Grain yield		Herbicide	3				**	
		Rate	2				NS	
		H x R	6				NS	

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

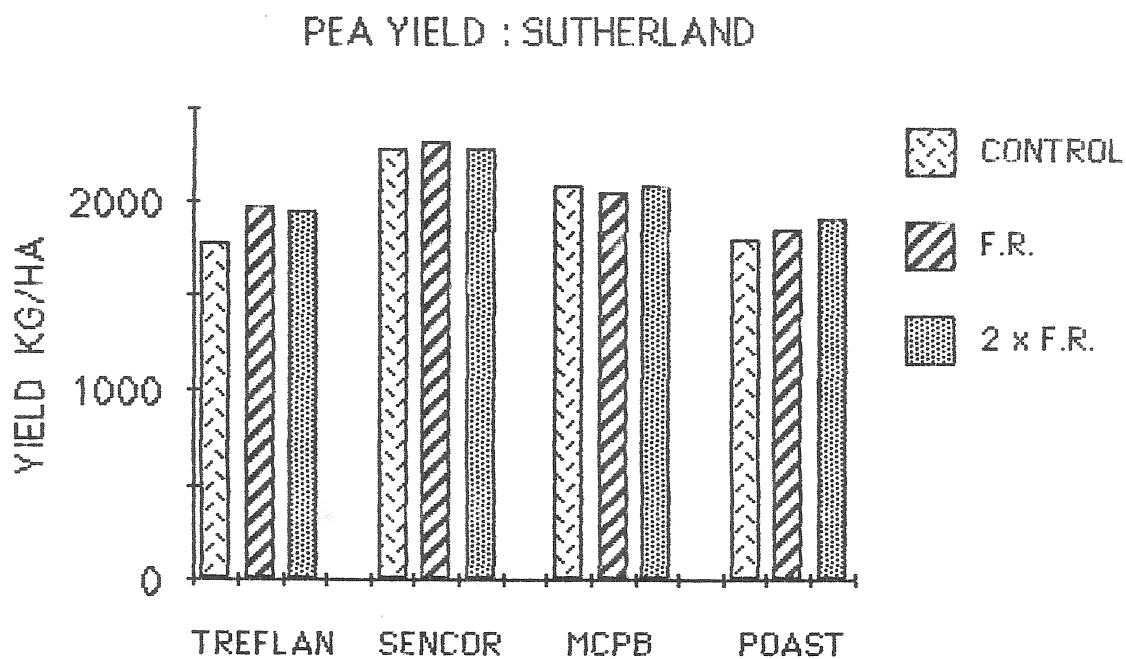
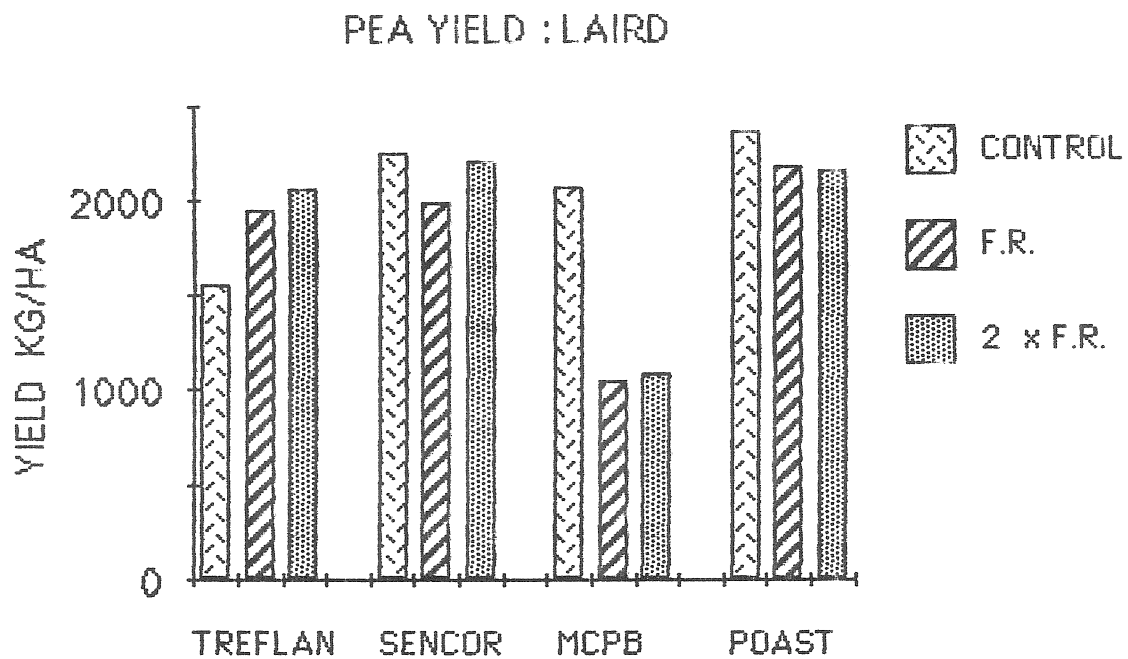


Figure 1. Effect of herbicides on the grain yield of field pea grown at Sutherland (A) and Laird (B), Saskatchewan.

1692 to 2214 kg/ha. In most cases the straw yield represented approximately 50% of the total yield and closely paralleled the response curves for grain yield. All plants at this site, including controls, were poorly nodulated (i.e., ca. 5-10 nodules per root system) and this might be the consequence of the dry soil conditions. The ARA activity of plants at the first, second and third sampling times ranged from 327 - 768, 117 - 930, and 15 - 373 n moles C₂H₄ hr⁻¹ plant⁻¹, respectively.

Field pea at Laird

There was no significant effect of herbicides on the growth and nodulation of field pea at Laird, although there was a significant effect on ARA activity and yield (Table 2). The average grain yield for the pea crop ranged from 1053 to 2363 (Fig. 1 b). There was a significant reduction in yield due to MCPB at both tested rates. Straw yield ranged from 863 to 2635 kg/ha and exhibited a response trend similar to grain yield. Pea plants at this site were well nodulated having approximately 60 to 100 nodules per root system. Adequate soil moisture and slightly lower soil NO₃-N levels compared with the Sutherland site might account for the difference in nodulation at both sites. There was a significant effect of some herbicides (e.g., MCPB) on the ARA activity (Table 2). For example, at the second sampling interval (T2) the ARA activity ranged from a low of 476 (MCPB, twice field rate) to 1822 (Treflan, twice field rate) whereas the average control ARA was 1183 n moles C₂H₄ hr⁻¹ plant⁻¹. A similar trend was noted for the third sampling interval. It should be noted that in some case there was an increase (e.g.,

Treflan) or decrease (e.g, Sencor) in ARA activity throughout the growing season but the effect was not significant (see Fig. 2). These effects on ARA activity appear related to plant growth, i.e., the better the growth the greater the ARA activity. Thus, herbicides that controlled the volunteer wheat stimulated ARA activity.

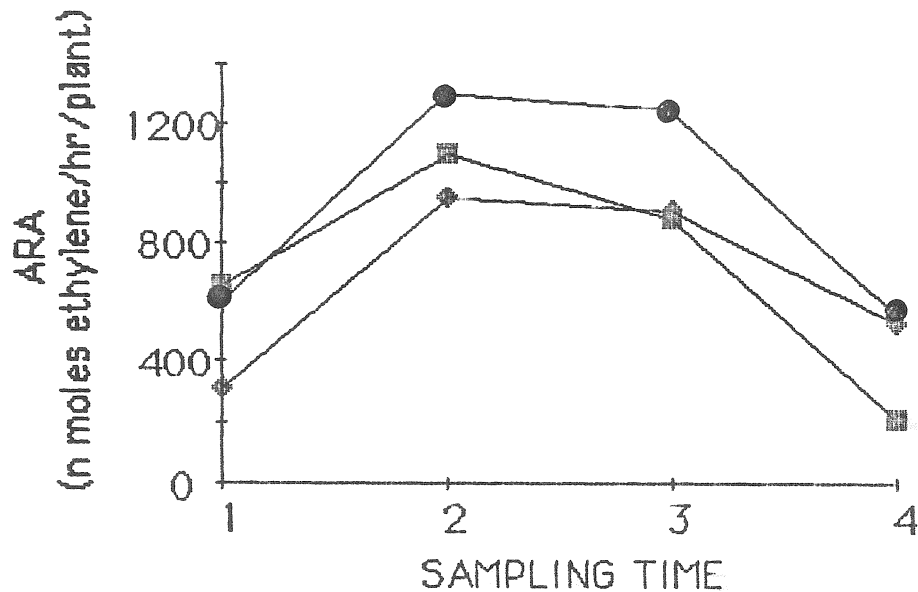


Figure 2. Effect of metribuzin (Sencor) on the ARA activity of field pea grown at Laird, Saskatchewan; ● , control; ■ , field rate; ◆ , twice field rate.

Lentil at Sutherland

There was a significant effect of herbicides on the growth and ARA activity, but not nodulation of lentil grown at the Sutherland site (Table 3). The average grain yield ranged from 456 to 1703 kg/ha (Fig. 1 c). MCPB affected yield at both tested rates. This was due to the inhibitory effect of MCPB on lentil growth. The average straw yield ranged from 910 to 2000 kg/ha and the response trend was similar to that for grain yield. As was the case with pea plants grown at

Table 3. Summary of statistical significance from analysis of variance for plant shoot dry weight, nodulation, acetylene reduction (ARA) and grain yield of lentil grown at Sutherland (site A) and Laird (site B).

Site	Parameter	Source	df	Sampling Time				
				T1	T2	T3	Harvest	
A	Dry wt	Herbicide	3	**	*	**		
		Rate	2	**	NS	NS		
		H x R	6	**	*	**		
	Nodule no.	Herbicide	3	NS	NS	NS		
		Rate	2	NS	NS	NS		
		H x R	6	NS	NS	NS		
	ARA	Herbicide	3	**	NS			
		Rate	2	**	*	NS		
		H x R	6	*	*	**		
	Grain yield	Herbicide	3				**	
		Rate	2				*	
		H x R	6				**	
	B	Dry wt	Herbicide	3		**	**	
			Rate	2		**	NS	
			H x R	6		**	**	
Nodule no.		Herbicide	3		**	*		
		Rate	2		NS	NS		
		H x R	6		NS	**		
ARA		Herbicide	3		**	**		
		Rate	2		**	NS		
		H x R	6		**	NS		
Grain yield		Herbicide	3				**	
		Rate	2				NS	
		H x R	6				**	

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

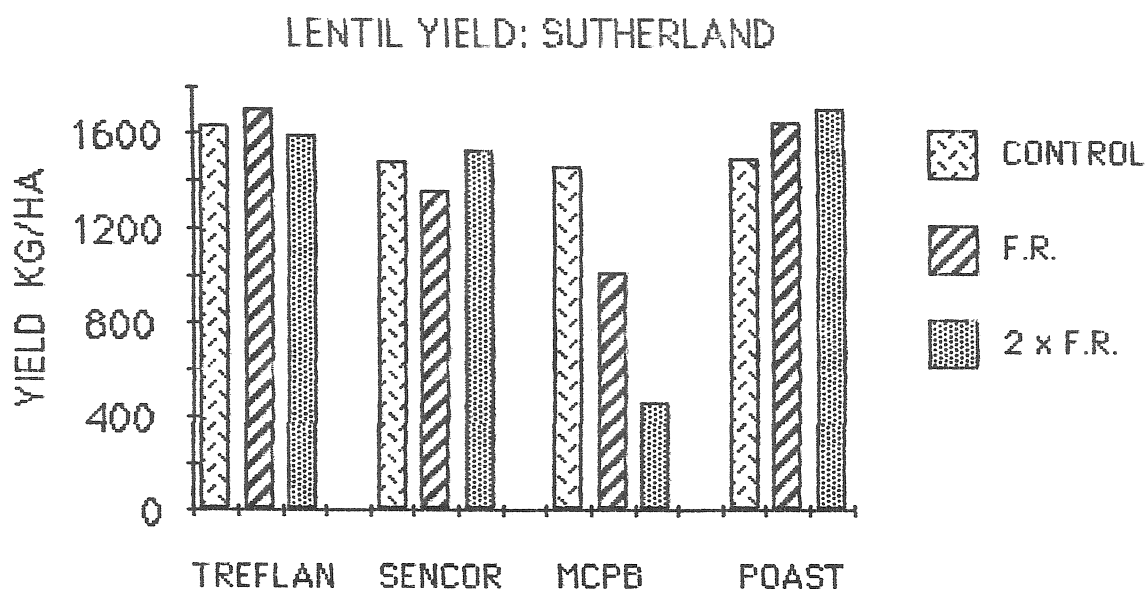
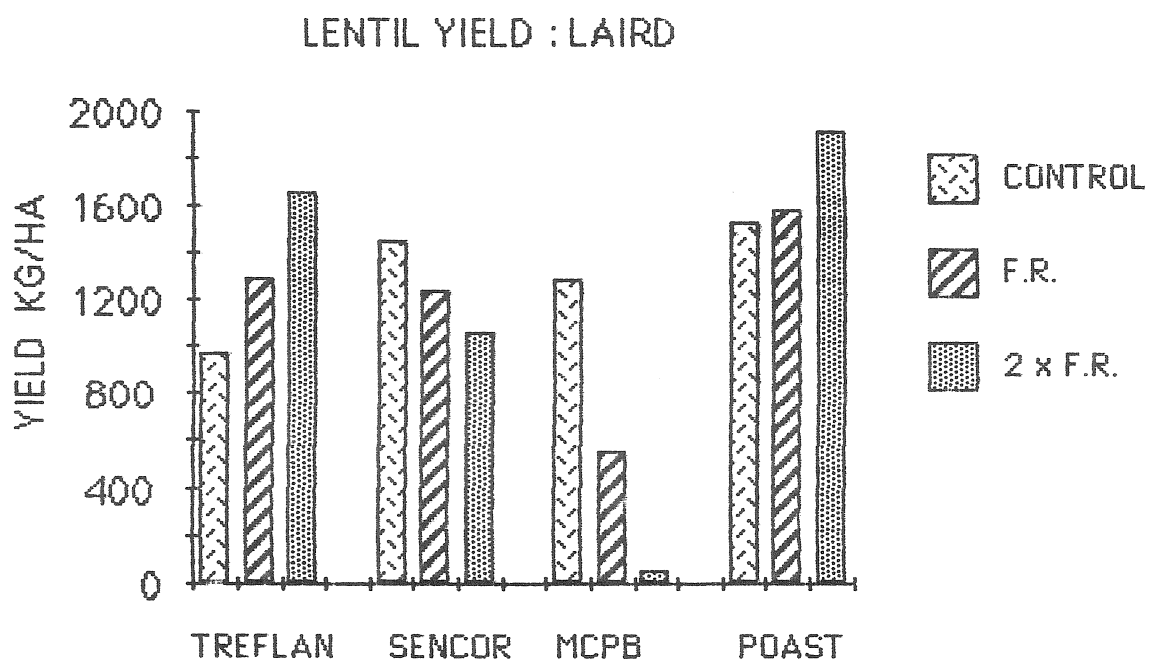


Figure 1. Effect of herbicides on the grain yield of lentil grown at Sutherland (C) and Laird (D), Saskatchewan.

this site, all lentil plants were poorly nodulated (i.e., 2 to 8 nodules per root system). A significant reduction in ARA activity was noted at various time intervals due to MCPB, and was most probably related to poor plant growth. The ARA activity at the three sampling times ranged from 15 - 207, 7 - 128 , and 1-57 n moles C_2H_4 hr⁻¹ plant⁻¹, respectively. Note that the ARA activity of lentil was substantially less than that of field pea at all sampling intervals.

Lentil at Laird

There was a significant effect of herbicides on the growth, nodulation, and nitrogen fixation potential of lentil (Table 3). The average grain yield ranged from 48 to 1915 kg/ha (Fig. 1 d). There was a significant reduction in yield due to MCPB at both tested rates. However, the herbicides Treflan and Poast tended to increase yields at this site, most likely due to better control of volunteer wheat. These yield increases were not significant (Fig. 1 d). The average straw yield at this site ranged from 134 to 2155 kg/ha and the response trend was similar to that for grain yield. The lentil plants at this site were well nodulated (i.e., 30 to 70 nodules per root system), but MCPB caused a significant reduction in nodulation. The ARA activity at the second and third sampling times ranged from 20 - 441 and 9-612 n moles C_2H_4 hr⁻¹ plant⁻¹, respectively. There was a significant reduction in the ARA activity due to MCPB, whereas Treflan and Poast increased ARA activity. As described previously, the effects on ARA activity most likely reflected plant growth. Lentils at this site had lower N_2 fixing activity (ARA) than field pea.

DISCUSSION

The present study investigated the effects of four herbicides on the growth, nodulation and nitrogen fixation potential of field pea and lentil grown under Saskatchewan field conditions. Two of these herbicides, Treflan and Sencor are registered for use with these legumes whereas the other two MCPB and Poast are registered for use on pea only. In general, Treflan, Sencor and Poast were safe to use on these pulse crops at recommended field rates. MCPB, however, caused a significant reduction in the growth and yield of lentil and thus adversely affected nitrogen fixation potential (ARA). Under certain conditions (i.e., such as those found at Laird site) this herbicide caused a reduction in yield of field pea. Our results agree with those of Rennie and Dubetz (1984) who found that Treflan (trifluralin) and Sencor (metribuzin) had no effect on yield and nitrogen fixation of legumes (i.e., soybean) grown in southern Alberta.

We found that some herbicides such as Treflan or Sencor could affect the nitrogen fixation potential (ARA) of pulse crops under certain conditions. These effects were either stimulatory or inhibitory in nature and were not easy to predict. Usually, these effects were not significant, but the trends were clear-cut. Factors such as drought (Sutherland), hail damage and the severe volunteer wheat problem at the Laird site may have been involved. Additional studies are needed to better document these effects. In all cases it appeared that herbicide effects on ARA activity were the consequence of herbicide effects on plant growth. Thus, herbicides such as MCPB that severely limited plant growth likewise had the greatest effect on ARA

activity. Studies by other workers indicated that Teflan and Sencor had relatively little effect on nitrogen fixation (ARA) of soybean grown in a greenhouse, but adverse effects were noted on coarse-texture soils low in organic matter (Bollich et al., 1985). Our previous study (Germida et al., 1984) indicated that the adverse effects of Treflan on ARA of pea plants grown under greenhouse conditions are difficult to demonstrate under field conditions. Our present findings support this observation.

This research program has made a good start. One year's field data were collected and interesting trends were observed. Data from an additional year are required for the two representative soil types tested and the effect of these herbicides on pea and lentil grown on other soil types needs assessment. Based on the first year results, it is obvious that the herbicide MCPB adversely affects growth and yield of lentil, and under certain conditions that of field pea. The effects of herbicides on nitrogen fixation (ARA) were less clear and additional studies are needed to clarify preliminary observations.

ACKNOWLEDGEMENTS

This study was supported by a grant from the Western Grains Research Foundation. The technical assistance of C. Lindsay is appreciated.

REFERENCES

- Bollich, P.K., E.P. Dunigan and A.W.M. Jadi. 1985. Effects of seven herbicides on N_2 (C_2H_2) fixation by soybeans. *Weed Science* 33:427-430.
- Brock, J.L. 1972. Effect of the herbicides trifluralin and carbethamide on nodulation and growth of legume seedlings. *Weed Res.* 12:150-154.
- Germida, J.J., B.M. Olson and R.B. McKercher. 1983. Soil microorganisms and the herbicide trifluralin. pp. 223-236. In Proceedings Saskatchewan Soils and Crops Workshop, February, 1983, Saskatoon, Sask.
- Germida, J.J., B.M. Olson and R.B. McKercher. 1984. Effect of trifluralin on growth, nodulation, and nitrogen fixation of peas. pp. 177-182. In Proceedings Saskatchewan Soils and Crops Workshop, February, 1984, Saskatoon, Sask.
- Harvey, R.G. and E.T. Gritton. 1977. Susceptibility of pea cultivars to trifluralin. *Weed Science* 25: 23-26.
- Postgate, J.R. 1972. The acetylene reduction test for nitrogen fixation. pp. 343-356. In *Methods of Microbiology* 6B. J.R. Norris and D.W. Ribbons (eds). Academic Press, London.
- Rennie, R.J. and S. Dubetz. 1984. Effect of fungicides and herbicides on nodulation and N_2 fixation in soybean fields lacking indigenous Rhizobium japonicum. *Agronomy J.* 76: 451-454.