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# Longevity of leafy spurge seeds in the soil following various control programs

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# Longevity of Leafy Spurge Seeds in the Soil Following Various Control Programs

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Highlight: Although picloram provided adequate control of leafy spurge (*Euphorbia esula* L.) for a minimum of 3 years, from 3,500 to 11,000 viable seeds remained in the soil, providing a source for rapid reestablishment of the infestation. Continuous sheep grazing for 8 years prevented annual seed set and reduced the size of the soil seed bank from > 3,500 to 15 seeds/m<sup>2</sup>, greatly reducing the chance of reestablishment from seed. Combining the data from the various treatments indicated that the average annual loss from the soil seed bank is 13% of the original population. This means that even though an initial application of picloram kills most of the vegetative portion of the plant, a repeat treatment is necessary to greatly reduce the number of seeds in the soil seed bank to prevent reestablishment by seed.

Leafy spurge (Euphorbia esula L.) is an introduced weed from Europe which was reported in Massachusetts as early as 1827 (Gussow et al. 1942). It is now found in most of Canada and in the United States south to Pennsylvania, Illinois, and Nebraska (Fernald 1950). Numerous reports have described leafy spurge as a weed of cropland, pastures, and rights-of-ways (Anderson 1956: Mitich 1969: Selleck et al. 1962). Anderson (1956) estimated that 3,640 ha of land were infested with leafy spurge in western Canada. This estimate may be conservative because Selleck et al. (1962) reported more than 3,960 ha in one-third of the settled portion of Saskatchewan. In 1962, Selleck et al. (1962) speculated that there were 14,160 to 16,190 ha of land infested with leafy spurge in Canada. No recent estimates of the acreage infested in Saskatchewan are available. However, in neighbouring North Dakota, the plant was reported to cover 202,340 ha in 1969 and to be spreading at a rate of 3,360 ha per year (Mitich 1969).

The rapid spread and the difficulty of controlling this weed are due in part to its methods of reproduction. Leafy spurge can reproduce and spread vegetatively from roots as well as by seeds. The effectiveness of the root system for vegetative reproduction is well documented by Selleck et al. (1962). However, the importance of seeds in plant establishment has not been adequately investigated. The seeds are scattered by a special mechanism. The seed capsules break open with an explosive force often propelling the seeds up to 4.6 m from the parent plant, thus effectively dispersing them (Bakke 1936). Each flowering shoot normally produces from 10 to 50 fruits and each fruit usually contains 3 seeds, but larger plants will produce hundreds of seeds per plant (Bakke 1936).

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Many biological and chemical control methods have been used on leafy spurge. Smothering leafy spurge patches with straw has been attempted but proved ineffective because new shoots appeared around the edge of the piles (Batho 1938; Muenscher 1930). The straw piles must be left in place for 3 to 4 vears to kill the roots below the center of the pile ((Batho 1938). Close grazing of leafy spurge with sheep has been recommended and used as a control measure for over 35 years (Batho 1938; Bibbey 1952; Helgeson and Thompson 1939; Muenscher 1930; and Wood 1945). In order to achieve control with sheep, the pasture must be continually grazed during the growing season. Chemical control methods have had some degree of success. Repeat applications of 2,4-dichlorophenoxy acetic acid (2,4-D) in the spring and fall for 3 or 4 years have given satisfactory weed control (Helgeson and Jansen 1959). More recently, a single application of 4-amino-3,5,6-trichloropicolinic acid (picloram) has given satisfactory weed control for 3 to 5 years, but the population rapidly recovered (Bowes and Molberg 1975).

All these control methods were designed to deplete carbohydrate reserves in the leafy spurge root system by preventing shoots from appearing above ground for at least 3 years. It is not known if the shoots appearing after these short-term control methods originated from perennial roots or seeds. If seedlings are important in the recovery of the population following control methods, the survival characteristics of leafy spurge seeds in the soil become important. However, none of the studies mentioned above have taken into account the amount of viable seeds in the soil either before, during, and after the various treatments. The following experiment was designed to estimate the amount of viable seeds remaining in the soil following the prevention of seed set with chemicals and sheep for 1 to 8 years.

#### **Materials and Methods**

Seeds were recovered from the soil at three separate locations that were chosen to represent a range of time since the initiation of control procedures. Two locations received a herbicide treatment and the other location was grazed by sheep. At the Moose Jaw location ( $50^{\circ}22'N$ and  $105^{\circ}46'W$ ) the potassium salt of picloram was applied at the recommended rate of 2.2 kg/ha on June 5, 1975. Two years previous, July 1973, picloram had been applied at the same rate on leafy spurge in an area 22 km east of Regina, Sask. ( $50^{\circ}25'N$  and  $104^{\circ}22'W$ ) at the Jameson location. At both locations, the degree of control was satisfactory. In the fall of 1967, ten dense patches of leafy spurge had been permanently marked in a pasture area near Mortlach, Sask. ( $50^{\circ}26'N$  and  $105^{\circ}59'W$ ). The area was not grazed prior to 1967 but was continuously grazed with sheep from the spring of 1968 until the termination of the experiment. The number of leafy spurge shoots/m<sup>2</sup> was recorded once or twice a year under four wire exclosures, each 1.2

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 $\times$  1.2 m, which were randomly placed near the permanent markers. The exclosures were moved once a year at the time of the earliest counts but were not moved after the spring of 1974.

Ten soil samples,  $20 \times 20$  cm and 2.5 cm in depth, were obtained on October 30, 1975, from each of the sheep grazed pasture, picloram treated areas at Jameson and Moose Jaw, and adjacent untreated areas. On October 29, 1976, ten additional samples were obtained from the picloram treated and from the adjacent untreated area near Regina. Leafy spurge seeds were separated from the dry sandy soil of the Regina and Mortlach locations with a series of sieves. The Moose Jaw soil had a higher clay and organic matter content so it was necessary to wash the soil through the sieve. Only whole seeds were hand-picked from the remaining debris at all locations. When the embryo dies and decays, the seed coat splits apart. Any seeds of this type were obviously nonviable and were not selected. From the date of collection until the commencement of germination tests in February, all samples were stored at  $-12^{\circ}$ C.

Percent germination was determined by placing 50 seeds in  $100 \times 20$ -mm glass petri dishes on 2 layers of No. 1 Whatman filter paper moistened with 5 ml of water. Three replicates of 50 seeds were randomly selected from each of the 10 soil samples per location. When there were fewer than 150 seeds per soil sample, all seeds were used for germination (Mortlach location only). Environmental conditions for the alternating, cool + alternating, and hot + alternating temperature portion of the germination sequence are presented in Table 1. Optimum temperature for germination and the sequence of cold and hot conditions used to stimulate the germination of dormant seed were determined in previous experiments (unpublished data). The germination test was conducted in a germinator with a programmable light and temperature sequence. During the hot phase (40°C) of the sequence, the seeds were kept dry in small envelopes in a temperature controlled oven. In all other cases, water was added when necessary to the petri dishes. The number of seeds with radicals longer than 2 mm were recorded twice a week during the alternating (30 day/10 night) part of the sequence. After the hot + alternating phase, the seeds were examined and sorted into two groups. Those with a well-formed white embryo were classed as firm seeds and assumed to be viable. The remainder of the seeds were hollow or had a brown shrivelled embryo and were considered nonviable. This method of determining viable and nonviable seeds was confirmed on a random sample using the tetrazolium test (Grabe 1970).

Table 1. Light and temperature sequences used in germination tests.

	Duration (wk)	Photoperiod		Tempera		
Temperature sequence		(h light	nr) dark	day (8 hr)	night (16 hr)	Moisture level
Alternating	4	8	16	$30 \pm 1$	$10 \pm 1$	wet
Cool +	4	0	24	2 - 5	2 - 5	wet
Alternating	2	8	16	$30 \pm 1$	$10 \pm 1$	wet
Hot +	4	0	24	$40 \pm 2$	$40 \pm 2$	dry
Alternating	2	8	16	$30 \pm 1$	10 ± 1	wet

Results of the germination test are presented on a percentage basis. Total germination is the summation of the alternating, cool + alternating, and hot + alternating portions of the temperature sequence. Germination data were subjected to angular transformation prior to analysis of variance and Student-Newman-Keul multiple range test (Zar 1974).

#### Results

The density of leafy spurge remained high following 3 years of continuous sheep grazing (Fig. 1). The following year there was a drastic reduction in the shoot density which remained low during the duration of the experiment. From the fourth year until the end of the experiment, the few remaining shoots were observed to be perennial. The exclosures were not moved after



Fig. 1. Shoot density of leafy spurge with continuous sheep grazing (shoots/m<sup>2</sup> with standard deviations).

1974 and the population of leafy spurge started to reestablish from roots in 1976.

The large seeds of leafy spurge were easily recognized and separated from the debris after the sieving and cleaning process. This method yielded a large number of seeds representing the seed reserve in the soil (Table 2). The size of these seed banks varied considerably from location to location. Two to three times as many seeds were recovered in 1975 than in 1976 from untreated plots at the Jameson location. On the other hand, the numbers of seeds recovered in 1976 from Jameson and Moose Jaw untreated locations were similar. The herbicide treatment with picloram prevented seed set for 1 year at the Moose Jaw location and 3 and 4 years at the Jameson location. This elimination of seed input into the soil was reflected by fewer seeds recovered in the picloram treated than in the untreated areas. However, only during 1975 at the Jameson location was the difference statistically significant. The lowest number of seeds were found on the Mortlach location where sheep had grazed continuously for 8 years.

Only a portion of the recovered seed population germinated when placed in a suitable environment (Table 2). The greatest germination response occurred during the alternating temperature sequence for all treatments. A cool + alternating temperaure sequence increased the germination total but the hot + alternating temperature sequence had no effect. Seed recovered during 1976 was not subjected to a hot temperature sequence. There was considerable variation in the percentage of seed that germinated on the untreated sites. The seeds recovered during 1976 at Jameson had a much higher percentage germination than did the 1975 seed sample. During the alternating portion of the temperature sequence, the number of seeds that germinated in picloram treated areas was less than the untreated but this was only statistically different for seeds recovered during 1975 at Jameson. A similar relationship was found during the cool + alternating portion of the temperature sequence. Less than 1% of the seeds germinated at Mortlach during all parts of the temperature sequence.

The seeds that did not germinate were either firm or nonviable. Most of the firm seeds were viable as confirmed by the tetrazolium test performed on nongerminating seeds collected during 1975 from the 1975 picloram treated location (Table 3).

	Treatment	Sample date	Treatment date	Number of seeds/m <sup>2</sup>	Germination of leafy spurge seed (%) <sup>2</sup>					Seeds <sup>3</sup>	
Location					Temperature sequence (°C)				- Firm	capable of Nonviable germination	
					Alternating	Cool	Hot	Total	seeds	seeds /m <sup>2</sup>	/m <sup>2</sup>
Moose Jaw	Untreated	1975		7043 b	56 d	5 b	0.4 ab	61 d	8 b	31 c	4860
	Picloram	1975	1975	6073 b	53 d	5 b	0.2 ab	58 d	15 e	27 bc	4433
Jameson	Untreated	1975		17823 d	29 c	16 c	0.8 ab	46 c	39 d	15 ab	15150
	Picloram	1975	1973	14053 c	12 b	6 b	0.6 ab	19 Б	62 e	19 abc	11383
Jameson	Untreated	1976		6128b	66 d	20 c		85 e	4 b	11 a	5454
	Picloram	1976	1973	4515b	57 d	15 e		73 e	5 b	23 bc	3522
Mortlach	Grazed	1975	1967	1133 a	0.2 a	0.2 a	0 a	0.4 a	0.9 a	99 d	15

<sup>1</sup> Means followed by the same letter are not different at the 0.5 probability level.

<sup>2</sup> Alternating =  $30/10^{\circ}$ C for 4 weeks; Cool =  $2-5^{\circ}$ C for 4 weeks followed by  $30/10^{\circ}$ C for 2 weeks; Hot =  $40^{\circ}$ C for 4 weeks followed by  $30/10^{\circ}$ C for 2 weeks <sup>3</sup> (Percentage of seeds germinating + percentage firm seed) × number of seeds recovered/m<sup>2</sup>.

In the untreated areas, the percentage of seeds classed as firm varied considerably among the four locations (Table 2). The seeds recovered from picloram treated plots at Moose Jaw, 1975, and Jameson, 1975, had a significantly larger percentage of firm seeds than the untreated. Compared to the other locations, the percentage of firm seeds following continuous grazing with sheep for 8 years was very low. A comparison of picloram treated and untreated areas indicated that the proportion of nonviable seeds in the samples was similar on three of the four locations, the exception being Jameson, 1976. The proportion of nonviable seeds in the sample might be expected to be similar on the two treatments since only whole seeds were selected.

After 8 years of continuous grazing at Mortlach, the number of seeds capable of germination was only  $15/m^2$  while at the other locations over 3,500 were capable of germination if subjected to a suitable environment (Table 2). Preventing seed set for 1 to 4 years with picloram reduced the number of seeds capable of germination in the soil seed bank.

## Discussion

To achieve long-term control of leafy spurge, the shoots coming from perennial rootstocks and the soil seed bank must be drastically reduced. Three or more years of continual sheep grazing were necessary to greatly reduce the shoot density of leafy spurge. However, 5 to 10 shoots/m<sup>2</sup> were still growing from perennial rootstocks after 8 years (Fig. 1). These few shoots did not increase in number until the second year after the pressure from continuous sheep grazing was removed. It is not known how many years are necessary for the leafy spurge density to reach the pre-1968 level. The control at the Moose Jaw site averaged 2 shoots/m<sup>2</sup> during 1976 (unpublished data), which was similar to that reported for the Jameson site (Bowes and Molberg 1975). Therefore, during the first 3 to 4 years of a leafy spurge control program, picloram was as effective as sheep grazing in reducing the density of leafy spurge.

Regardless of the degree of control of shoots obtained from herbicide application, there are many leafy spurge seeds in the

Table 3. Percentage germination and percentage firm and nonviable seeds of leafy spurge in the soil seed bank 3 years after treatment with picloram (Jameson 1975 location).<sup>1</sup>

Germinating seeds	$37 \pm 4^*$	
Firm seeds	$48 \pm 4$	
Nonviable seeds	$15 \pm 3$	

<sup>1</sup> Mean plus standard deviation based on ten samples.

soil at the end of the first 3 to 4 years of a control program (Table 2). The ability of these seeds to germinate varies with the site from which they were collected (Table 2 and Selleck et al. 1962). Regardless of the variability between locations, the most important factor was the length of time seed set was prevented. Most seeds collected from the soil following the elimination of seed set for 8 years were either hollow or contained a shrivelled embryo. Our results indicated that ample seeds were available for reestablishment of leafy spurge following a single application of picloram (Table 2). However, if the initial application of picloram is followed by a repeat treatment 3 to 5 years later, then the amount of seed remaining in the soil should be similar to the area grazed with sheep.

To determine the rate of change in the numbers of seeds in the soil bank, a seed depletion curve was constructed using data from various locations (Fig. 2). The number of seeds capable of germination on treated areas was expressed as a percentage of the untreated plots. It was assumed that the rate of depletion in the soil was similar on all sites. An arithmetic regression equation was the best fit, which meant the average annual loss from the soil seed bank was linear and was in the amount of 13%. This figure was less than the annual depletion rate of 20% found by Roberts (1969) for natural populations of seeds in uncultivated soils in England.

The trend to a lower percentage germination and higher percentage of firm seeds on picloram treated areas (Table 2)



Fig. 2. Total number of seeds capable of germination on picloram treated areas expressed as a percentage of the untreated areas.

suggested that the chemical may have been toxic to a part of the embryo vital for germination, that the chemical may have induced dormancy in the seed, or that the germination characteristics of the seed changed with age. Laboratory investigations revealed that the germination of leafy spurge seeds in 0, 63, 125, 250, 500, and 1,000 ppb of picloram was 70, 73, 62, 75, and 68%, respectively, which was statistically nonsignificant at the 5% probability level. Picloram did not inhibit the germination process through chemical injury or by inducing dormancy. Picloram has been found by others to inhibit the germination of very susceptible species such as soybeans (*Glycine max* (L.) Merr. var Lee) and to a lesser extent safflower (*Carthamus*) tinctorius L.), whereas radish (Raphanus sativus L.) and grass species were unaffected (Chang and Foy 1971; Scifres and Halifax 1972). It is not surprising that picloram did not inhibit germination since only very susceptible species are affected. Tetrazolium tests on the ungerminated seeds collected during 1975 from the 1973 picloram treated area revealed that most of the seeds were viable (Table 3). This suggests that seeds remaining in the soil bank for several years germinate under a different set of environmental conditions. The specific requirements which promote germination of these dormant seeds are unknown.

The conclusions drawn from the experiment are that there are many viable seeds present for reestablishment of leafy spurge following satisfactory control for at least 3 years with picloram. Continuous sheep grazing gave satisfactory long-term control of leafy spurge because the vegetative portion and the number of viable seeds in the soil were greatly reduced. For equivalent weed control with picloram, it is necessary that repeat applications of picloram prevent seed set for at least 8 years.

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