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ORIGINAL ARTICLES

Physiological response of purslane weed (*Portulaca oleracea*) and two common beans (*Phaseolus vulgaris)* recombinant inbred lines to Phosphorus fertilizer and bentazon herbicide.

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ABSTRACT

Pot experiments were conducted in March 2009 and 2010 to evaluate physiological response of purslane (*Portulaca oleracea*) weed and two recombinant inbred lines (RILs) of common bean (*Phaseolus vulgaris*) RIL-115 & RIL-147 to postemergence applications of bentazon herbicide at registered rate **[**1L / Fed. (2.4 L/ha)**]** and reduced rate **[**½ L / Fed (1.2 L/ha)**]**. Bentazon was applied postemergence to all pots with or without phosphorus (P) at 27.7 kg / fed. (66kg / ha). Purslane weed control increased with higher rate of bentazon. Addition of phosphorus improved purslane control. The reduction in dry weight of purslane in RIL-115 reached 79.4 and 95.7% at harvest with the combined application of P and bentazon at low and high rates respectively. The corresponding results were 66.8 and 84.4% % in RIL-147. Control of purslane weed by bentazon alone or bentazon and phosphorus was accompanied by increases in growth and yield of the two genotypes, RIL-115 and RIL 147 of common bean. Generally, the increase in growth and yield exceeded 100% in the two genotypes as compared to the corresponding controls. The increases in bean growth and yield were concomitant with increases in both phosphorus and nitrogen contents in bean leaves as well as metabolic processes which are represented by increases in the percentage of carbohydrate and protein in the yielded seeds. The results suggested improving purslane weed control by addition of phosphorus to bentazon herbicide.

Key words: *Portulaca oleracea, Phaseolus vulgaris,* Bentazon and Phosphorus.

Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the most important food legumes for direct human consumption; it comprises 50% of the legumes consumed worldwide (Broughton *et al*., 2003; Graham *et al.*, 2003). It is high in protein, phosphorus, iron, vitamin B, fiber, and it is free of cholesterol. Beans are an attractive crop for farmers, because of its adaptability to different cropping systems and short growing cycle. Bean productivity is limited by soil fertility especially phosphorus and nitrogen **(**Kumar and Puri, 2002; Graham *et al*., 2003**).** Common bean suffers from several abiotic and biotic production constraints. The major abiotic constraints include nitrogen and phosphorus deficiency. Phosphorus (P) is an essential element for plant growth; it plays a vital role in plant nutrition **(**Nielsen *et al*., 1998)**.** A lack of this element is serious since it may prevent other nutrient from being acquired by plants (Mengel and Kirkby, 2001). Its concentration in soil solution is approximately 0.05 mg/L (Goldstein, 1994). So, the possibility of using phosphate as a fertilizer has received significant interest (Nielsen *et al*., 1998; Gniazdowska *et al*., 1999; Olivera *et al*., 2004; Wittenmayer and Merbach, 2005; Camachoa *et al*., 2008; Suleiman and Hago, 2009 and Suleiman *et al*., 2009).

Weeds are one of the most important biotic constraints in bean plants. Weeds are considered important reducing factors of bean yield. Crop yield may be reduced significantly when weeds compete with plants for light, water and minerals (Lehoczky and Reisinger, 2003). So, using herbicides is one option to control weeds effectively. Weed control in white beans is currently limited by the small number of registered herbicides, e. g., thifensulfuron, chlorimuron, bromoxynil, imazamox, fomesafen, bentazon, and cloransulam-methyl. Bentazon is a selective herbicide related to groups that control broadleaved weeds through inhibition of photosynthesis at photosystem II (its site of action is D-1 quinone-binding protein of photosynthetic electron transport) (Min and Mutsunaka, 1975). In general bentazon controlled 90% of broad leaved weeds in legumes without crop injury (Ngouajio and Daelemans, 1987; Metwally, 2002; Saad El-din, 2003; Saad El-din and El-Metwally, 2003). Soltani *et al*. (2008) found that foliar application of bentazon once or twice had no adverse effect on plant height; shoot dry weight, seed moisture content, and yield. On the other hand, postemergence applications of bentazon plus imazamox, decreased plant height, shoot dry weight, and yield by 41% (Sikkema *et al*., 2004).

Some evidences have been gathered that adding fertilizers to herbicide solution could increase its activity on controlling annual weeds (Metwally and Hassan, 2001; El–Metwally, 2002; El-Shahawy, 2007).

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Consequently, the lower dose could be effective and its damage on crop could be decreased. Moreover, lowering the dose of any herbicide is much appreciated of minimizing pollution.

This work was carried out to study the influence of using phosphorus (P) on the efficiency of the herbicide bentazon on controlling selected broad leaved weed, *Portulaca oleracea* associated with two genotypes of *Phaseolus vulgaris* L RIL-115 and RI -147 as well as the effect of both P and bentazon on their yield.

Materials and Methods

The experiments were conducted in greenhouse at the National Research Centre, Dokki, Egypt, during the two seasons 2009 and 2010. The treatments consisted of the two doses of the herbicide bentazon with or without phosphorus in addition to weed free and unweeded control in each genotype (RIL-115& RIL-147). Two Recombinant inbred lines (RILs) of common bean (*Phaseolus vulgaris*) RIL-115 & RIL-147 were introduced from Dr. Jean-Jacques Drevon, Institut National de la Recherche Agronomique, UMR1222 Ecologie Fonctionnelle & Biogéochimie des Sols, INRA-IRD-SupAgro, 2 Place Viala, 34060 Montpellier Cedex, France for possible cultivation under Egyptian agricultural condition. Common bean seeds were selected for uniformity by choosing those of equal size and with the same colour. The selected seeds were washed with distilled water, sterilized with 1% sodium hypochlorite solution for about 2min and thoroughly washed again with distilled water and left to dry at room temperature (25◦C) for about 1h. Commercial *Rhizobium leguminosarum* used to inoculate the common bean. Uniform air dried common bean seeds were sown 2 cm depth in pots which had a 30 cm diameter and 30 cm height with surface area = 0.07 m^2 contained equal amounts of sieved soil. Seeds of the two genotypes were sown on March in the two seasons 2009 and 2010 and final harvest at maturity was done on July 2009 and 2010. The pots were infested with constant weight of seeds of purslane: *Portulaca oleracea* L. (broad-leaved weed) and in such a way that all pots were infested with the same weight of the weed seeds. The experiment consisted of 16 treatments with 6 replicates for each treatment. The treatments are in complete randomized design. Daytime temperatures ranged from 17.7 to 40.5◦C with an average of 29.1± 5.4 ◦C whereas temperatures at night were 16.6± 4.1◦C, with minimum and maximum of 8.2 and 23.5◦C, respectively. Daily relative humidity averaged 50.9±9.2%, in a range between 21.0and 70.0%.

The soil had a sand loamy texture; pH (1:2.5) 7.36; EC (dS m^{-1}) 1.56; organic matter 1.42%; CaCO3 9.24 %; Total N 0.09%. Available nutrients (mg/kg soil) were as follows: P 5.16; K 201.24; Fe 7.03; Mn1.52; Zn 0.88; Cu 0.67. Total nitrogen measures the total amount of nitrogen present in the soil is 0.09 considered low based on Hazelton and Murphy (2007). So, granular ammonium sulfate (20.5% N) at the rate of 40 kg ha−1 N was added to the soil before cultivation. Critical values for K that begin to limit plant growth are around 80–200 mg/kg (Gourley,1999). The value of soil K was 201.24 mg/kg soil, so, no more K fertilizer was added. Soil P test was 5.16 (mg /kg), so, P test Interpretation is very low, hence, P recommended application (kg /ha) is 66 kg P ha^{-1} in form of superphosphate (15% P_2O_5) was added before cultivation. The N and P fertilizers were mixed thoroughly into the soil of each pot immediately before sowing.

Thinning of common bean was done after 2 weeks so that 3 homogeneous seedlings were left per pot. Common bean plants as well as infested weeds 25 days later (25 days after sowing) was sprayed with the recommended dose [1L /Fed. (2.4 L/ ha.)] and half the recommended dose [1/2L /Fed. (1.2 L/ha.) of the herbicide bentazon [48% (3-isopropyl 1 H-2, 1, 3-benzathiadiazin-4-(3 11) one, 2, 2-dioxide) with or without phosphorus which applied at rate of 27.7kg/Fed. (66kg/ha).

Soil water content was checked by weighing and daily loss of water supplemented twice (morning and afternoon). Soil water content was maintained at about 65% of field water capacity. All recommended agricultural practices were followed.

Samples of weeds as well as common bean plants were taken from three pots at flowering of bean and at harvest.

Weed sample:

The infested weeds were collected from each pot. Weed samples were taken from three pots at each stage (all weed samples in each pot were pulled up). The data on fresh and dry weight of collected weeds were recorded.

Common bean:

Data on common bean were recorded as the three plants in each pot were pulled up (three pots in each stage). Some morphological and growth characteristics of common plants were recorded at flowering for each individual plant. The recorded characteristics included plant height (cm), number of leaves/ plant, and fresh and dry weight/plants (g). The fresh materials were washed carefully several times with tap water, then with distilled

water and oven dried at 60°C for determination of dry weight (g/plant). The dried materials were ground to fine powder and stored in paper bags until used in further steps (extraction and testing).
Common bean green and dry yield were recorded for each treatment at the end of the season.

Biochemical analyses:

Determination of some chemical contents:

A-Nitrogen and phosphorus contents:

Nitrogen and phosphorus contents were determined in dried leaves of common bean according to the official and modified methods of analysis (AOAC, 1984).

B-Percentage of carbohydrate contents:

Total carbohydrates were extracted from dry finely ground yielded seeds according to Herbert *et al*. (1971) and estimated colourimetrically by the phenol-sulphoric acid method (Montogomery 1961).

C- Percentage of protein contents:

Total protein contents were extracted from dry finely ground yielded seeds according to the method of Lowery *et al*. (1951).

Statistical Analysis:

The data were statistically analyzed according to Snedecor & Cochran (1980).

Results and Discussion

Weed growth:

Inhibition in weed growth showed different significant responses between the two common bean genotypes (RILs 115 & 147) due to bentazon herbicide (BH) and Phosphorus (P) at 27.7kg/Fed. treatments as indicated by the mean values (Table 1). The herbicide at half the recommended rate (1/2L/ fed.) and at the recommended rate (1L/fed) reduced significantly the fresh and dry weight of broad leaved weed purslane with or without P in RIL 115 as well as RIL-147 in comparison to the corresponding controls. The inclusion of P with the recommended rate of BH induced maximum significant reduction at flowering and at harvest. The reduction in dry weight of purslane reached 79.4 and 95.7 % at harvest in RIL-115 with the combined application of P and BH at low and high rates respectively. The corresponding results were 66.8and 84.4% in RIL-147. In this connection, several investigators proved that addition of fertilizers especially N and P increased the efficiency of the herbicides in increasing weed control (Metwally and Hassan, 2001; El–Metwally, 2002; El-Shahawy, 2007 and El-Metwally *et al*., 2010). The reduction in weed growth in RIL-115 was higher than in RIL-147 that may be explained by the competitive ability of RIL-115.

Table 1: Effect of bentazon herbicide with or without phosphorus on both fresh and dry weight of purslane at flowering and at harvest of two common bean RILs (115 & 147). (Average of the two seasons).

Treatments (Fed.)	At flowering						At harvest					
	Fresh weight			Dry weight			Fresh weight			Dry weight		
	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean
Weed free	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000
Unweeded control	32.00	32.33	32.165	2.456	2.653	2.554	72.33	71.77	72.050	3.949	6.257	5.103
Bentazon 1/2L	10.70	13.23	11.965	0.987	1.390	1.188	21.74	21.28	21.510	2.032	3.177	2.604
Bentazon 1L	2.66	5.02	3.840	0.231	0.676	0.453	12.67	13.08	12.875	0.403	1.290	0.846
Weed free+ P27.7kg	0.00	0.00	0.000	0.000	0.000	0.000	0.00	0.00	0.000	0.000	0.000	0.000
Unweeded+ P27.7kg	28.60	28.99	28.795	2.352	1.845	2.098	59.63	64.40	62.015	3.575	5.509	4.542
Bentazon 1/2L+P27.7 kg	8.85	11.75	10.300	1.049	0.823	0.936	18.64	19.85	19.245	1.098	2.073	1.585
Bentazon 1L+P27.7kg	1.67	4.52	3.095	0.129	0.378	0.253	9.83	12.38	11.105	0.171	0.976	0.573
Mean	10.56	11.98		0.970	0.935		24.35	25.34		1.403	2.410	
LSD at 5%	A=0.27 B=0.48 AXB=0.56			A=0.009 B=0.045 AXB=0.052			A=0.68 B=1.40 AXB=1.61			A=0.008 B=0.065 AXB=0.075		

A= Genotype **B=Treatments** **AxB=Interaction**

Common bean growth and green yield:

When common bean plants exposed to foliar application of the two doses of BH in absence or the presence of P, plant height, number of leaves as well as dry weight increased significantly over control in the two genotypes (Table 2). Generally, the difference between the two genotypes was significant as indicated by the mean values. The addition of P to the herbicide treatments seemed to be more significantly effective in increasing plant height as well as dry weight in the two genotypes RIL-115 and RIL 147. The recommended dose of the herbicide increased dry weight in RIL-115 to 93.8% over unweeded control in absence of P, while on addition of P, dry weight increased to 135.8% over control. In RIL-147 dry weight increased to 194 and 141.6% with and without P respectively. The lower dose of BH with or without P increased also plant height and dry weight significantly in both RIL-115 and RIL-147 but to lesser extent than higher dose. The number of green pods is significantly higher in the plants treated with BH or P + BH in the two genotypes RIL-115 & RIL-147 in comparison to the corresponding controls (Table 2). The pattern of change in weight of green pods is similar to a great extent that of number of green pods in the two genotypes.

Table 2: Effect of bentazon herbicide with or without phosphorus on plant height, number of leaves per plant, total dry weight per plant, number of green pods per plant and weight of green pods per plant of two common bean RILs (115 & 147). (Average of the two season).

Treatments (Fed.)	Plant height			Number of leaves/plant			Dry weight (g/plant)		
	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean
Weed free	66.9	38.6	52.75	11.56	10.96	11.260	3.89	2.953	3.421
Unweeded control	42.4	29.3	35.85	10.10	7.30	8.700	1.93	1.33	1.630
Bentazon 1/2L	43.9	34.1	39.00	14.33	10.24	12.285	3.09	2.783	2.936
Bentazon 1L	48.6	36.3	42.45	15.83	11.16	13.495	3.74	3.214	3.477
Weed free+ P27.7kg	68.0	45.2	56.60	15.88	13.80	14.840	4.24	3.913	4.076
Unweeded+ P27.7kg	43.8	32.7	38.25	10.72	10.40	10.560	2.18	1.462	1.821
Bentazon 1/2L+P27.7 kg	49.7	36.8	43.25	12.91	10.76	11.835	3.36	2.923	3.141
Bentazon 1L+P27.7kg	68.8	41.5	55.15	16.33	14.36	15.345	4.55	3.776	4.163
Mean	54.012	36.812		13.45	11.12		3.372	2.794	
LSD at 5%	A=1.7 B=1.7 AXB=2.04		A=0.39 B=0.72 AXB=0.84				A=0.05 B=0.14 AXB=0.16		

Table 2 continue

Treatments (Fed.)	Number of green pod/plant			Weight of green pod/plant		
	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean
Weed free	8.9	6.70	7.800	29.60	25.46	27.530
Unweeded control	5.2	4.86	5.030	11.16	11.53	11.345
Bentazon 1/2L	6.5	5.43	5.965	14.60	12.26	13.430
Bentazon 1L	7.4	7.40	7.400	23.63	23.26	23.445
Weed free+ P27.7kg	10.0	8.30	9.150	32.30	36.74	34.520
Unweeded+ P27.7kg	5.4	5.10	5.250	13.20	17.43	15.315
Bentazon 1/2L+P27.7 kg	7.0	5.43	6.215	16.38	17.92	17.150
Bentazon 1L+P27.7kg	8.8	7.86	8.330	28.33	33.32	30.825
Mean	7.400	6.385		21.15	22.24	
LSD at 5%	A=0.29 B=0.37 AXB=0.54			A=0.18 B=0.97 AB=1.12		

Common bean dry yield:

Mean values indicated significant differences between the two genotypes RIL-115 and RIL-147 in number of seeds/pod, weight of 100 seeds as well as seed yield/plant. Both low (1/2L/Fed.) and high rate (1L/Fed.) of BH resulted in significant increases in number of dry pods/plant as well as number of seeds /pod, these increases reached maximum values in RIL-115 and RIL-147 by addition of P to the recommended rate of BH (Table 3). Similarly, weight of seeds /plant (seed yield /plant) as well as weight of 100 seeds in both genotypes show higher values when the plants treated with BH in the presence of P than in its absence. The increase in yield/ plant exceeded 100% over control by BH and P in RIL-115, while increased to 92% in RIL-147 (Table 3).

The present results demonstrate a good activity of P in increasing the efficiency of bentazon herbicide for controlling purslane weed. It is clear from the results that increasing the activity of BH was accompanied by increases in plant growth as well as yield and its components. These have been reported previousley that addition of feltilizers increased the efficiency of the herbicides on controlling weeds and consequently increased net return (El–Metwally, 2002; El-Shahawy, 2007 and El-Metwally *et al*., 2010). In general, controlling weeds in common bean and other legumes by the herbicide bentazon was accompanied by increases in growth and seed yield (Blackshaw *et al.*, 2000; Bailey *et al*., 2003; Sikkema *et al*., 2004; Wilson, 2005; Soltani *et al*., 2008; Blackshaw and Molnar, 2008). The effect of P on common bean growth and yield explained by the suggestion of Nielsen *et al*. (2001) who found that P increased dry weight (growth) of *Phaseolus vulgaris* genotypes. In addittion, Fisher *et al*. (2002) reported that the availability of P increased leaf dry weight from five-fold to ten fold in *P. vulgaris.* The authors added that stem dry weight increased in the presence of P than its absence. The importance of phosphorus may be attributed to that it is a constituent of nucleic acids, phytin and phospholipids (Gniazdowska *et al*., 1999). An adequate supply of P early in the life of a plant is important in laying down the primordia for its reproductive parts as it is considered essential for seed formation (Ghaffar, 1990). It is known that phospholipid molecules are one of the main structural components of membranes, and they have emerged as important second messengers (Munnik *et al*., 1998) to regulate plant growth and development and cellular responses to environmental change or stress [Xue *et al*., 2007]. Studies using genetic and molecular approaches reveal the important roles of phospholipid molecules and signaling in multiple processes of higher plants, including growth (Xu *et al*., 2005), pollen and vascular development (Lin *et al*., 2005), hormone effects (Yun *et al*., 2006) and cell responses to environmental stress (Williams *et al*., 2005).

Table 3: Effect of bentazon herbicide with or without phosphorus on number of dry pod, number of seeds per pod, weight of 100seeds and seed yield per plant of two common bean RILs (115&147).(Average of the two seasons).

Treatments (Fed.)	Number of dry pod			Number of seeds/pod			Weight of 100seeds (g)			Seeds yield/plant		
	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean
Weed free	7.62	8.50	8.060	4.20	4.40	4.300	20.00	17.82	18.91	7.24	6.60	6.920
Unweeded control	4.54	4.23	4.385	1.83	2.17	2.000	16.34	16.00	16.17	3.77	3.60	3.685
Bentazon 1/2L	6.12	5.16	5.640	3.43	3.20	3.315	18.50	16.86	17.68	4.17	4.20	4.185
Bentazon 1L	7.43	7.00	7.215	3.83	3.50	3.665	18.94	17.50	18.22	6.56	5.23	5.895
Weed free+ P27.7kg	9.16	10.00	9.580	4.80	4.22	4.510	20.10	18.10	19.10	8.30	6.90	7.600
Unweeded+ P27.7kg	4.66	4.83	4.745	2.53	2.53	2.530	18.00	16.04	17.02	4.33	4.20	4.265
Bentazon 1/2L+P27.7 kg	6.66	6.40	6.530	3.47	3.66	3.565	20.66	17.50	19.08	6.33	5.00	5.665
Bentazon 1L+P27.7kg	8.13	8.40	8.265	5.41	4.30	4.855	22.66	19.00	20.83	8.00	6.31	7.155
Mean	6.790	6.815		3.687	3.497		19.40	17.35		6.087	5.255	
LSD at 5%	A=ns B=0.26 AXB=0.31			A=0.06 B=o.24 AXB=0.28			A=0.24 B=0.66 AXB=0.77			A=0.17 B=0.27 AXB=0.31		

Some chemical constituents:

Nitrogen (N) and phosphorus (P) in common bean leaves:

Mean values indicated significant difference between the two genotypes RIL-115 and RIL-147 as well as between treatments. In addition, significant increases were recorded in the contents of both P and N in common bean leaves in plants treated with BH with or without P. The combined treatments of BH at the recommended rate and P surpassed its correspondence without P in the two genotypes (Table 4). Possible explanation is that obtained by Mengel and Kirkby, 2001 that a lack of P may prevent other nutrient from being acquired by plants, i.e. P may facilitate the uptake of N.

Carbohydrate and protein contents (%) in the yielded common bean:

It is important to estimate carbohydrate and protein contents in the yielded seeds for evaluating seed quality. The differences between the two genotypes were significant in both carbohydrate and protein contents. The treatments of BH at the two doses with or without P caused significant increase in carbohydrate percentage (Table 4). Carbohydrate contents in the yielded seeds get maximum benefits on the addition of the recommended rate of BH with P in the two genotypes RIL-115 and RIL-147. This result is coincided in protein contents. The increase in plant growth is accompanied by different metabolic processes that may explain increases in carbohydrate and protein contents.

Table 4: Effect of bentazon with or without phosphorus on leaf nitrogen % (leaf N%) and leaf phosphorus% (leaf P%) at flowering, and seed total Carbohydrate % and Seed Protein % at harvest of two common bean RILs (115 & 147). (Average of the two seasons).

Treatments (Fed.)	Leaf N%			Leaf P %			Seed total carbohydrate %			Seed Protein %		
	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean	Ril 115	Ril 147	Mean
Weed free	2.74	2.54	2.640	0.27	0.26	0.265	56.0	47.53	51.765	33.05	35.73	34.39
Unweeded control	1.80	2.18	1.990	0.33	0.23	0.28	40.3	36.18	38.240	18.11	17.75	17.93
Bentazon 1/2L	2.74	2.13	2.435	0.30	0.23	0.265	54.6	43.50	49.050	20.76	24.09	22.42
Bentazon 1L	2.33	2.58	2.455	0.35	0.26	0.305	60.2	47.59	53.895	25.85	29.49	27.67
Weed free+ P27.7kg	2.56	2.56	2.560	0.41	0.29	0.35	55.5	51.07	53.285	34.18	42.91	38.54
Unweeded+ P27.7kg	1.86	2.46	2.160	0.43	0.29	0.36	46.3	42.42	44.360	17.65	22.29	19.97
Bentazon 1/2L+P27.7 kg	3.62	2.13	2.875	0.42	0.27	0.345	56.7	44.52	50.610	22.58	26.05	24.31
Bentazon 1L+P27.7kg	3.87	3.00	3.435	0.53	0.37	0.45	64.8	49.73	57.265	27.77	30.81	29.29
Mean	2.690	2.447		0.380	0.275		54.30	45.31		24.99	28.66	
LSD at 5%	A=0.017 B=0.086 AXB=0.102			A=0.008 B=0.021 AXB=0.024			A=0.97 B=2.25 AXB=2.60			A=0.29 B=0.63 AXB=0.73		

Conclusion:

Addition of Phosphorus to the herbicide bentazon has a good activity on the effect of the herbicide on increasing control of purslane (broadleaved weed) and consequently increasing bean yield. This work would be useful for controlling purslane weed associated bean plants.

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