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Running title: Effect of pethoxamid on dry bean

Sensitivity of adzuki, kidney, small red Mexican and white beans to pethoxamid

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Abstract: Pethoxamid has not been evaluated for use in dry bean in Ontario. Seven field experiments were established at three Ontario locations to evaluate the effect of pethoxamid applied preplant incorporated (PPI) and preemergence (PRE) at 1200 and 2400 g ai ha⁻¹ in dry bean. At 2 weeks after emergence (WAE), pethoxamid caused as much as 19, 4, 4 and 6% injury when applied at 1200 g ai ha⁻¹ and 32, 6, 9 and 10% injury when applied at 2400 g ai ha⁻¹ in adzuki, kidney, small red Mexican and white bean, respectively. There was no difference in plant stand of dry beans with pethoxamid PPI and PRE at 1200 or 2400 g ai ha⁻¹. Pethoxamid applied PPI reduced shoot dry weight 15% compared to when applied PRE. Pethoxamid applied at 2400 g ai ha⁻¹ also reduced shoot dry weight 12% compared to when applied at 1200 g ai ha⁻¹. There

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Abbreviations: PP, preplant; PPI, preplant incorporated; POST, postemergence; PRE, preemergence; WAE, weeks after emergence

was no difference in seed yield of adzuki, kidney, small red Mexican and white bean with pethoxamid applied PPI or PRE at 1200 or 2400 g ai ha⁻¹. Based on this study, pethoxamid applied PPI or PRE at the proposed rate of 1200 g ai ha⁻¹ has the potential to cause significant injury in adzuki bean. Pethoxamid applied PPI or PRE at the proposed rate of 1200 g ai ha⁻¹ caused less injury in kidney, small red Mexican and white bean with no effect on seed yield.

Key Words: Bean, biomass, herbicide injury, plant stand, weeds, seed yield, *Phaseolus vulgaris*, *Vigna angularis*

Introduction

Dry edible bean (*Phaseolus vulgaris* L.) is an important field crop grown in southwestern Ontario. During 2015, dry bean was grown in nearly 53,000 hectares with total seed yield of 115,000 tonnes valued at approximately \$93 million (Kulasekera 2015). Market types of dry beans grown in Ontario include small seeded white (navy), black, and adzuki [*Vigna angularis* (Willd.) Ohwi & Ohashi] bean; medium seeded otebo, pinto and small red Mexican bean; and large seeded cranberry and kidney bean. Dry beans are not as vigorous as other crops such as soybean and their seed yield and quality can be significantly reduced as a result of weed interference, especially during the early growth from the second trifoliate to the first flower stage (Soltani et al. 2014; Woolley et al. 1993).

Herbicides available for weed control in dry beans are fewer than those available for other legume crops such as soybean in Ontario (OMAFRA 2016). Currently, EPTC, dimethamid-P,

pendimethalin, S-metolachlor and trifluralin are registered as soil-applied herbicides for annual grass control and imazethapyr and halosulfuron are the only soil-applied herbicides registered for broadleaf weed control in Ontario dry beans (OMAFRA 2016). To adequately control problematic weeds in dry bean fields in Ontario, new herbicide options need to be identified. New herbicide options will support weed management strategies in dry beans.

Pethoxamid [2-chloro-N-(2-ethoxyethyl)-N-(2-methyl-1-phenylprop-1-enyl) acetamide] is a new selective herbicide being developed by FMC for use in corn, soybean, cotton, rice and sunflower in North America (Hunt et al. 2015). It is a new active ingredient in the chloroacetamide family which provides primarily annual grass control (*Digitaria*, *Echinochloa*, *Panicum*, *Setaria*) but also has some activity on small seeded broadleaf weeds including *Amaranthus* (Palmer amaranth and waterhemp), *Chenopodium* and *Solanum* species (Hunt et al. 2015). Pethoxamid provides partial control of common ragweed and waterhemp, two species with glyphosate-resistant biotypes in Ontario. Pethoxamid, is a Group 15 herbicide that inhibits very long chain fatty acid elongase (VLCFAE) enzymes (Hunt et al. 2015). It can be applied preplant (PP), preplant incorporated (PPI), preemergence (PRE) or postemergence (POST) (Hunt et al. 2015). Pethoxamid is being developed as a single product, as well as in preformulated mixtures with broadleaf herbicides (Hunt et al. 2015). Pethoxamid has the potential to provide broad spectrum control of common annual grasses and small seeded broadleaf weeds in Ontario especially susceptible glyphosate resistant species. Tolerance of commonly grown dry beans such as adzuki, kidney, small red Mexican and white beans to pethoxamid is not known.

The objective of this study was to determine the tolerance of adzuki, kidney, small red Mexican and white beans to pethoxamid applied preplant incorporated or preemergence at 1200 or 2400 g ai ha⁻¹, representing the proposed registered rate (1X rate) and twice that rate (2X rate) to simulate a spray overlap in the field.

Materials and Methods

Study establishment

Seven field experiments were conducted during 2015 and 2016 in major dry bean growing areas in Ontario, Canada. Two trials (2015 and 2016) were established at Agriculture and Agri-Food Canada Research Centre, Harrow, ON. Another two trials (2015 and 2016) were established at University of Guelph, Ridgetown Campus, Ridgetown, ON. Three trials (2015a, 2015b and 2016) were established at University of Guelph, Huron Research Station, Exeter, ON. The soil type at Harrow was a Fox sandy loam soil, at Ridgetown was a Brookston loam soil and at Exeter was a Brookston clay loam soil.

Field trials were established as a three-way factorial in a completely randomized block design with four replications. Factor 1 was four market classes of dry bean, factor 2 was pethoxamid doses at 0, 1200 and 2400 g a.i. ha⁻¹, and factor 3 was PPI and PRE application timings. Each plot consisted of four rows of dry bean [adzuki ('Erimo'), kidney ('Red Hawk'), small red Mexican ('Merlot') and white ('T9905')] spaced 0.75 m apart in rows that were 8 m long at Harrow and Ridgetown and 10 m long at Exeter. Beans were planted 5 cm deep in late May to early June of each year at a rate of 200,000 seeds ha⁻¹.

A backpack sprayer (CO₂-pressurized) calibrated to deliver 200 L ha⁻¹ at 240 kPa was used to apply herbicide treatments using a boom that was 2.5 m long with six ULD120-02 nozzles spaced 0.5 m apart. The PPI treatments were applied to the soil surface one day before planting and immediately incorporated into the soil to a depth of 5 cm. The PRE treatments were applied to the soil surface one day after planting. To eliminate the confounding effect of weed interference all plots were kept free of weeds as needed by cultivation and hand hoeing.

Data collection

At 1, 2, 4 and 8 weeks after emergence (WAE), visible dry bean injury was evaluated visually using a scale of 0 to 100% (0 = no visible plant injury and 100 = complete plant death). At 4 WAE, dry bean height of ten randomly selected plants per plot was measured from the soil surface to the highest growing point. At 6 WAE, shoot dry weight of dry bean was determined by hand harvesting a 1 m row (from the two center rows) of adzuki, kidney, small red Mexican and white bean. Harvested plants were placed in an oven for at least 48 hours at 60 °C and then dry weight was recorded. Yields were measured when approximately 90% of bean pods for each market class turned to golden yellow using a small plot combine. Seed moisture content was adjusted to 13% moisture content for adzuki bean and 18% moisture content for kidney, small red Mexican and white bean.

Statistical analysis

The PROC MIXED procedure in SAS statistical software version 9.3 (SAS Institute Inc., Cary, NC) was used to conduct a 3-way analysis of variance on data collected. The fixed main effects were dry bean type, application timing and herbicide dose and the random effects were environment (year*location), and blocks nested within environment. Data for location and years were pooled and averaged when there was no significant interaction between environment and treatment. Residual plots and the Shapiro-Wilk normality test was used to make sure that the residuals were random, homogeneous, with a normal distribution about a mean of zero. To meet the assumption of normality, dry bean injury data were arcsine square root transformed and dry bean stand, shoot dry weight and height data were square root transformed. Transformation for seed yield was not required. Data shown in Tables have been back-transformed. Means for treatments were separated at the 5% level of significance using Tukey's multiple means comparison test.

Results and Discussion

Analysis of variance indicated that environment by bean type by application timing by pethoxamid rate interactions were not significant for the parameters evaluated; therefore the 7 environments (data sets) were analyzed together (Tables 1 -3). There was a significant bean type by treatment interaction for injury at 2 and 4 WAE and dry weight (Table 1). There was also a significant crop injury by application timing interaction at 1, 2 and 4 WAE. All other interactions were not significant at $p < 0.05$ (Table 1).

Crop injury

Injury symptoms with pethoxamid in adzuki, kidney, small red Mexican and white bean included wrinkling of leaf tissue, leaf yellowing, leaf necrosis, and stunted growth. Crop injury was greater with pethoxamid applied at 2400 g ai ha⁻¹ compared to 1200 g ai ha⁻¹ in adzuki bean but was not significantly different among other market classes of dry bean evaluated (Table 3). At 2 WAE, pethoxamid caused 19, 4, 4 and 6% injury when applied at 1200 g ai ha⁻¹ and 32, 6, 9 and 10% injury when applied at 2400 g ai ha⁻¹ in adzuki, kidney, Small Red Mexican and white bean, respectively (Table 3). At 4 WAE, pethoxamid caused 12, 4, 3 and 4% injury when applied at 1200 g ai ha⁻¹ and 22, 5, 6 and 6% injury when applied at 2400 g ai ha⁻¹ in adzuki, kidney, Small Red Mexican and white beans, respectively (Table 3).

There was greater injury when pethoxamid was applied PPI than PRE at 1, 2, 4 and 8 WAE (Table 2). At 1 WAE, there was 40% injury with pethoxamid applied PPI and 20% injury when applied PRE in adzuki bean (Table 3). However, injury was not significantly different with application timing among kidney, small red Mexican and white beans ranging from 2 to 10% (Table 3). Similar injury ratings persisted at 2 and 4 WAE. Pethoxamid caused 32, 7, 6 and 9% injury when applied PPI and 19, 3, 7 and 7% injury when applied PRE at 2 WAE and caused 24, 7, 5 and 6% injury when applied PPI and 10, 2, 4 and 3% injury when applied PRE at 4 WAE in adzuki, kidney, small red Mexican and white beans, respectively (Table 3).

Injury seen in adzuki bean with pethoxamid is similar to other Group 15 herbicides such as dimethenamid-P that have been shown to cause as much as 38% injury in adzuki bean (Soltani et al. 2005). Dimethenamid-P applied PPI at 693 g ha⁻¹ has been shown to cause no significant

visual injury in pinto and small red Mexican beans at 1, 2 and 4 WAE; however, at 1386 g ha⁻¹ it has been shown to cause as much as 7% visual injury (Soltani et al. 2008). Other Group 15 herbicides such as S-metolachlor caused no significant visual injury in pinto and small red Mexican beans and cranberry, kidney and white bean (Sikkema et al. 2004; Soltani et al. 2003, 2004). Minimal injury have been found with PRE applications of S-metolachlor for pinto (Arnold et al. 1993), kidney (Sikkema et al. 2004), cranberry, black and white beans (Soltani et al. 2003, 2004).

Plant stand and shoot dry weight

There was no difference in plant stand (density) of adzuki, kidney, small red Mexican, and white bean with pethoxamid applied PPI and PRE at 1200 or 2400 g ai ha⁻¹ (Table 2). There was also no difference between application timings (PPI vs PRE). However, pethoxamid applied PPI reduced shoot dry weight 15% compared to when applied PRE (Table 2). Pethoxamid applied at 2400 g ai ha⁻¹ reduced shoot dry weight 12% compared to when applied at 1200 g ai ha⁻¹ (Table 2). Pethoxamid applied at 2400 g ai ha⁻¹ reduced shoot dry weight of small red Mexican 20% and white bean 25% but had no significant effect on other market classes of dry beans evaluated compared to the untreated control (Table 3).

Other Group 15 herbicides such as S-metolachlor have been shown to reduce shoot dry weight of black bean 6% but had no adverse effect on shoot dry weight of cranberry, kidney and white beans (Sikkema et al. 2004; Soltani et al. 2003, 2004). Minimal biomass reductions have

been reported with S-metolachlor in pinto (Arnold et al. 1993), kidney (Sikkema et al. 2004), cranberry, black and white beans (Soltani et al. 2003, 2004).

Plant height

Plant height varied between market classes of dry bean with small red Mexican having the greatest height followed by kidney, white and then adzuki bean (Table 2). Pethoxamid applied PPI reduced height 4% compared to when applied PRE; however, there were no difference in bean heights between pethoxamid application rates (1200 vs 2400 g ai ha⁻¹) (Table 2). Similarly, other Group 15 herbicides such as dimethenamid-P and S-metolachlor applied PPI have been shown to cause no adverse effect on plant height of pinto and small red Mexican beans (Sikkema et al. 2006; Soltani et al. 2008). Other studies have also shown no adverse effect of S-metolachlor applied PRE on height of pinto (Arnold et al. 1993), kidney (Sikkema et al. 2004), cranberry, black and white beans (Soltani et al. 2003, 2004).

Seed yield

Seed yield varied among dry bean market classes as expected with white bean producing the greatest seed yield followed by small red Mexican, kidney and then adzuki bean (Tables 1 and 2). There was no differences in seed yield of adzuki, kidney, small red Mexican, and white bean with pethoxamid applied PPI and PRE at 1200 or 2400 g ai ha⁻¹ (Table 2). There was also no difference between application timings (PPI vs PRE).

In other studies, other Group 15 herbicides such as dimethenamid-P applied PPI caused a

38% seed yield reduction in adzuki bean (Soltani et al. 2005). However, dimethenamid-P applied PPI at 693 g ha⁻¹ caused no adverse effect on maturity and seed yield of pinto and small red Mexican beans (Soltani et al. 2008). Dimethenamid-P applied PPI caused no adverse effect on maturity and yield of otebo bean (Sikkema et al. 2006). Another Group 15 herbicide, S-metolachlor applied PPI at 1600 and 3200 g ha⁻¹ caused no adverse effect on maturity and yield of pinto and small red Mexican beans (Soltani et al. 2008). No seed yield reductions were also found with PRE applications of S-metolachlor in pinto (Arnold et al. 1993), kidney (Sikkema et al. 2004), cranberry, black and white bean (Soltani et al. 2003, 2004). Alachlor, another Group 15 herbicide has been shown to cause no adverse effect on seed yield of various market classes of dry beans (Urwin et al. 1996).

Based on this study, pethoxamid applied PPI or PRE at the proposed rate of 1200 g ai ha⁻¹ has the potential to cause greater than 10% in adzuki bean although final seed yield was not affected. Injury was generally greater when applied PPI compared to when it was applied PRE. Pethoxamid applied PPI or PRE at the proposed rate of 1200 g ai ha⁻¹ causes less than 10% injury in kidney, small red Mexican and white beans with no effect on final seed yield. The biologically effective rate of pethoxamid to control troublesome weeds in dry bean needs to be determined if lower rates of pethoxamid can be used to reduce injury in dry beans.

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Table 1. Analysis of variance (ANOVA) table for percent injury, density, dry weight, height and yield of dry bean treated with pethoxamid applied PPI and PRE at 7 environments (averaged) in Ontario, Canada during 2015 and 2016.

Source	Injury				Density	Dry Weight	Height	Yield
	1 WAE	2 WAE	4 WAE	8 WAE				
Type	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Treatment	0.0003	<0.0001	0.0004	0.10	0.24	0.0002	0.36	0.17
Timing	0.0002	0.0013	<0.0001	0.03	0.28	0.0002	0.0003	0.10
Type*treatment	0.25	0.02	0.04	0.95	0.91	0.0006	0.16	0.79
Treatment*timing	0.29	0.18	0.05	0.77	0.70	0.08	0.57	0.93
Type*timing	<0.0001	0.002	0.003	0.99	0.70	0.06	0.35	0.91
Type*treatment*timing	0.70	0.79	0.20	0.98	0.89	0.37	0.43	0.89

^a Abbreviations: WAE, week after crop emergence; Treatment, herbicide treatment; Type, dry bean market class; Timing, preplant incorporated or preemergence.

Table 2. Significance of main effects for percent injury, density, dry weight, height and yield of dry bean treated with PPI and PRE applications of pethoxamid at 7 environments (averaged) in Ontario, Canada during 2015 and 2016.

Main Effects	Injury				Density	Dry Weight	Height	Yield
	1 WAE	2 WAE	4 WAE	8 WAE				
	%				no. m ⁻²	g m ⁻²	cm	t ha ⁻¹
<u>Dry Bean Type</u>	**	**	**	**	**	**	**	**
Adzuki	30	25	17	9	14	8.3	32.3	1.5
Kidney	4	5	5	3	14	34.6	49.7	1.7
Small Red Mexican	7	7	4	2	16	36.6	54.7	2.7
White	9	8	5	3	17	24.9	47.4	2.9
SE	2	1	1	1	0.4	2.5	1	0.1
<u>Application Timing</u>	*	*	**	*	NS	*	*	NS
Preplant incorporated	16	13	10	6	14	24.0	45.0	2.2
Preemergence	10	9	5	3	16	28.1	47.0	2.3
SE	1	1	0.8	0.8	0.3	1.8	0.7	0.06
<u>Herbicide (g ai ha⁻¹)</u>	*	**	*	NS	NS	*	NS	NS
Pethoxamid (1200)	10	8	6	3	15	27.8	46.8	2.3
Pethoxamid (2400)	16	14	10	6	15	24.4	45.2	2.2
SE	1	1	0.7	0.6	0.3	1.7	0.6	0.05

^a Abbreviations: WAE, week after crop emergence; NS, not significant at P=0.05 level; PPI, preplant incorporated; PRE, preemergence.

^b Significance at P < 0.05 and P < 0.01 levels denoted by * and **, respectively.

Table 3. Percent injury and shoot dry weight (g m^{-2}) of four dry bean types treated with PPI and PRE applications of pethoxamid at 7 environments (averaged) in Ontario, Canada during 2015 and 2016. Means followed by the same letter within an interaction grouping are not significantly different according to a Tukey's multiple means comparison test at a Type I error rate of 0.05.

	Dry Bean type			
	Adzuki	Kidney	Small Red Mexican	White
<i>Injury 2 WAE (%)</i>				
Untreated	0d	0d	0d	0d
Pethoxamid (1200 g ai ha ⁻¹)	19b	4cd	4cd	6c
Pethoxamid (2400 g ai ha ⁻¹)	32a	6c	9c	10c
<i>Injury 4 WAE (%)</i>				
Untreated	0c	0c	0c	0c
Pethoxamid (1200 g ai ha ⁻¹)	12b	4c	3c	4c
Pethoxamid (2400 g ai ha ⁻¹)	22a	5bc	6bc	6bc
<i>Dry weight (g m^{-2})</i>				
Untreated	10.8c	39.0a	41.6a	29.7ab
Pethoxamid (1200 g ai ha ⁻¹)	9.0bc	34.9ab	39.8a	27.5ab
Pethoxamid (2400 g ai ha ⁻¹)	7.6bc	34.4ab	33.3ab	22.3bc
<i>Injury 1 WAE (%)</i>				
Preplant incorporated	40a	6c	7c	10c
Preemergence	20b	2c	8c	8c
<i>Injury 2 WAE (%)</i>				
Preplant incorporated	32a	7c	6c	9c
Preemergence	19b	3c	7c	7c
<i>Injury 4 WAE (%)</i>				
Preplant incorporated	24a	7bc	5bc	6bc
Preemergence	10b	2c	4bc	3bc

^a Abbreviations: WAE, week after crop emergence; PPI, preplant incorporated; PRE, preemergence.