Published January 25, 2017

Early Application of Harvest Aid Herbicides Adversely Impacts Lentil

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ABSTRACT

Applying harvest aid herbicides can dry down lentil (*Lens culinaris* Medik.) crops evenly and quickly, and can help control late-emerging weeds. However, improper application timing may reduce yield and quality, and leave unacceptable herbicide residues in seed, which can cause commercial issues when marketing lentil. The objective of this research was to determine the response of lentil to various application timings of glyphosate, saflufenacil, and the combination of these two herbicides. A field experiment consisting of a randomized complete block design was run at Saskatoon and Scott, SK, Canada in 2012, 2013, and 2014 to address the objective. Application of harvest aid herbicides before 30% seed moisture content reduced seed yield and thousand seed weight up to 25 and 8%, respectively. Moreover, application timings before 30% seed moisture resulted in lentil seed samples exceeding residue levels of 2.0 and 0.03 mg kg^{-1} for glyphosate and saflufenacil, respectively. Adding saflufenacil to glyphosate did not reduce glyphosate residue in lentil seed compared to glyphosate applied alone. However, this tank mixture significantly reduced seed residues of saflufenacil and improved crop desiccation compared with either glyphosate or saflufenacil applied alone. Our data lead us to conclude that a tank mix of saflufenacil+glyphosate should be recommended for crop desiccation and pre-harvest weed control in lentil over using either product alone. In addition, it is critical to ensure applications of glyphosate or saflufenacil are not made prior to 30% seed moisture in lentil crops.

Core Ideas

- Improper application timing of harvest aids may reduce lentil seed yield and quality, and leave unacceptable herbicide residues in seed.
- Application of harvest aids before 30% seed moisture content reduced lentil seed yield and thousand seed weight.
- These application timings resulted in lentil seed samples exceeding residue levels of 2 and 0.03 mg kg^{-1} for glyphosate and saflufenacil, respectively.

Published in Agron. J. 109:239–248 (2017)
doi:10.2134/agronj2016.07.0419
Received 19 July 2016
Accepted 1 Nov. 2016

Lentil is one of the most important and widely grown pulse crops in western Canada, with production totals of approximately 2.0 million tonnes in 2015 (Agriculture and Agri-Food Canada, 2016). Nevertheless, harvesting lentil remains a challenge because the indeterminate growth habit of lentil, combined with variability in field conditions, can result in non-uniform maturity (Saxena, 2009) that can decrease seed quality and slow harvesting operations (Saskatchewan Pulse Growers, 2011; Alberta Pulse Growers, 2013). Growers frequently desiccate lentil crops at physiological maturity (Zhang et al., 2016) to improve lentil dry down, lower seed moisture for storage, and control late-emerging weeds to reduce weed seed return to the seedbank. Collectively, this allows for early harvesting and enhances lentil harvest efficiency (Ali et al., 2009; Saskatchewan Pulse Growers, 2011; Alberta Pulse Growers, 2013).

Herbicides registered in Canada as lentil desiccants include diquat [6,7-dihydrodipyrido[1,2-a:2',1'-c]pyrazinediium dibromide], glyphosate [N(phosphonomethyl)glycine], saflufenacil [N-{2-chloro-4-fluoro-5-[1,2,3,6-tetrahydro-3-methyl-2,6-dioxo-4-(trifluoromethyl)pyrimidin-1-yl] benzoyl}-N-isopropyl-N-methylsulfamide], and glufosinate [(RS)-2-amino-4-(hydroxy(methyl)phosphonoyl)butanoic acid] (Saskatchewan Ministry of Agriculture, 2016). These herbicides are applied late in the growing season, and improper timing can result in reduced seed yield, seed weight, and quality, as well as unacceptable herbicide residues in the seed (Cessna et al., 1994, 2000; Bennett and Shaw, 2000: Cessna et al., 2002; Boudreaux and Griffin, 2011). Unacceptable levels of seed residue cause trade issues if residue levels exceed the maximum residue limits (MRL) for importing countries.

Glyphosate is commonly used as a harvest aid in Canadian pulse and cereal crops. It provides perennial grass and broadleaf weed control, and can reduce the time between physiological maturity and harvest (Cessna et al., 2000, 2002; Zhang et al., 2016). The recommended application timing is typically when the crop is at or below 30% seed moisture content (Saskatchewan Ministry of Agriculture, 2016). However, if

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Abbreviations: AUDPC, area under the desiccation progress curve; DAA, days after application; GR, glyphosate residue; MRL, maximum residue limit; SMC, seed moisture content; SR, saflufenacil residue; TSW, thousand seed-weight.

glyphosate is applied to crops that have not reached physiological maturity, the herbicide may be translocated to developing seeds, resulting in residue levels exceeding MRLs (Cessna et al., 1994, 2000; 2002). In 2011, the European Union rejected shipments of Canadian lentils due to glyphosate seed residues over 0.1 mg kg^{-1} (Pratt, 2011). Maximum residue limits vary by crop, herbicide, and foreign market requirements (Bryant Christie Inc., 2015); therefore, it is critical to ensure residues are below acceptable levels to ensure market acceptance.

Saflufenacil, a protoporphyrinogen IX oxidase inhibitor with rapid crop dry-down, has recently been introduced to the market and is newly registered as a desiccant in lentil (Grossmann et al., 2011; Zhang et al., 2016). Safluflenacil has both contact and systemic activity via limited translocation through the phloem and xylem, and could translocate to sucrose sinks such as seeds. Therefore, saflufenacil residues may be a concern for growers when applied as a harvest aid at early crop growth stages. Similar to glyphosate, major importing countries also have set MRLs for saflufenacil (Bryant Christie Inc., 2015).

Saflufenacil provides more rapid weed control than glyphosate, but does not provide adequate control of perennial weeds in lentil fields (Baylis, 2000). Ideally, growers should apply both products if they are seeking rapid crop dry-down and perennial weed control. Zhang et al. (2016) reported that saflufenacil tank-mixed with glyphosate and applied at 30% seed moisture content enhanced crop desiccation compared to the untreated control and either herbicide applied alone; however, the benefit was not consistent across environments. Knezevic et al. (2009) reported similar results indicating that a saflufenacil + glyphosate tank-mix improved weed control for several species. In contrast, Ashigh and Hall (2010) showed that the activity of glyphosate was reduced in plants when combined with saflufenacil due to saflufenacil's rapid contact activity, which accelerated cell death and decreased glyphosate translocation to meristematic tissues. In addition, saflufenacil translocation was reduced in glyphosate-susceptible plants by adding glyphosate.

The European Union's rejection of Canadian lentil shipments in 2011 had a significant negative impact on the Canadian pulse industry, and it raised questions about effective management of residues in lentil. It is now imperative that producers follow good management practices to ensure that residue limits are not exceeded. Data on the effect of application timing of glyphosate and saflufenacil on lentil desiccation, seed yield, seed weight, and seed residues is limited. Consequently, research was undertaken to determine the effect of glyphosate, saflufenacil, and glyphosate + safluenacil tank-mix application timing on these parameters.

MATERIALS AND METHODS

Experiment Site and Design

A field trial was conducted at Saskatoon (52.13° N, 106.64° W) and Scott, (52.1° N, 106.3° W), SK, Canada, from 2012 to 2014. However, the trial at Scott in 2012 was lost due to hail damage. The soil at both sites is a Dark Brown Chernozem (Typic Boroll). Soil texture at Saskatoon ranged from clay to sandy loam with a pH of 7.5 to 7.9 and an organic matter content of 2.4 to 4.5%. Soil at Scott is a silty loam with a pH of 5.3 to 6.8 and an organic matter content of 2.4 to 2.6%.

Plots were set up in a randomized complete block design with four replications per treatment. Two experimental factors were used in the study: herbicide treatment (glyphosate, saflufenacil, and the tank mixture of glyphosate plus saflufenacil) and application timings (60, 50, 40, 30, and 20% seed moisture content). An unsprayed control also was included in the study. Individual plot sizes were 2 m wide by 6 m long and 2 m wide by 5 m long at Saskatoon and Scott, respectively.

Experimental Procedure

The cultivar used in the trial was CDC Maxim, an imidazolinone-resistant variety that is the most commonly grown cultivar in western Canada. Prior to planting, seeds were treated with Apron Maxx RTA [0.73% fludioxonil (4-(2,2-difluoro-1,3-benzodioxol-4-yl)-1H-pyrrole-3-carbonitrile); 1.10% metalaxyl-M and S-isomer [methyl 2-(N-(2-methoxyacetyl)-2,6-dimethylanilino)propanoate)] at a rate of 325 mL per 100 kg seed. Seeds were also inoculated (2.76 mL kg^{-1}) with Liquid Nodulator (BASF, Research Triangle Park, Durham, NC) containing *Rhizobium leguminosarum* biovar *viceae* in 2012, or with Tag Team (Monsanto BioAg, St. Louis, MO) Granular (2.8 kg ha^{-1}) containing *Rhizobium leguminosarum* and *Penicillium bilaii* in 2013 and 2014. Lentil was direct-seeded into chem-fallow plots at a depth of 3 cm. Seeding was performed with a small plot drill equipped with single shoot hoe openers on 22 cm row spacing. Planting dates at Saskatoon were 17, 12, and 14 May in 2012, 2013, and 2014, respectively; the Scott site was planted on 21 and 12 May in 2013 and 2014, respectively. The plant density targeted was 130 lentil plants m^{-2}, with seeding rates adjusted for germination test results. Plots were rolled at both sites immediately following planting. Fertilizer was not applied as soil test recommendations did not call for the addition of nutrients.

At Saskatoon, ethalfluralin [N-ethyl-N-(2-methylprop-2-enyl)-2,6-dinitro-4-(trifluoromethyl)aniline] was applied at a rate of 1400 g a.i. ha^{-1} each fall prior to plot establishment the following spring. Glyphosate (675 g a.e. ha^{-1}) was applied prior to crop emergence, while post-emergence weed control was achieved with a tank mix of imazamox plus imazethapyr [5-ethyl-2-(4-methyl-5-oxo-4-propan-2-yl-1H-imidazol-2-yl)pyridine-3-carboxylic acid; 30 g a.i. ha^{-1}] applied between the five- to six-node stage. Any weeds not controlled by herbicides were removed by hand. At the early flowering stage, prothioconazole {2-[2-(1-chlorocyclopropyl)-3-(2-chlorophenyl)-2-hydroxypropyl]-1H-1,2,4-triazole-3-thione} was applied (166 g a.i. ha^{-1}) to control ascochyta blight (*Ascochyta fabae* f.sp. *lentis*), with a second application of chlorothalonil (2,4,5,6-tetrachlorobenzene-1,3-dicarbonitrile) applied (1500 g ha^{-1}) at the early pod stage. At Scott, imazethapyr was applied (13 g a.i. ha^{-1}) for weed control each fall prior to plot establishment. Pre-emergence weed control was achieved with glyphosate (900 g a.e. ha^{-1}) applied immediately after planting, while an in-crop application of quizalofop (ethyl (2R)-2-[4-(6-chloroquinoxalin-2-yl)oxyphenoxy]propanoate; 420 g a.i. ha^{-1}) was made at the four-node-stage of crop development. Preventative disease control was achieved with boscalid applied {2-chloro-N-[2-(4-chlorophenyl)phenyl]pyridine-3-carboxamide; 294 g a.i. ha^{-1}} at early flowering.

Harvest aid herbicides were foliar-applied as follows: glyphosate at 900 g a.e. ha^{-1}, saflufenacil at 50 g a.i ha^{-1}, and glyphosate at 900 g a.e. ha^{-1} plus saflufenacil at 36 g a.i. ha^{-1} (Table 1). All herbicide rates were based on label recommendations (Saskatchewan Ministry of Agriculture, 2016). Merge (BASF, Research Triangle Park, Durham, NC) adjuvant (50% surfactant and 50% petroleum hydrocarbons solvent) was added to treatments containing saflufenacil at a rate of 1 or 0.5 L ha^{-1} when applied alone or with glyphosate, respectively (Saskatchewan Ministry of Agriculture, 2016). Application timings and application dates are shown in Tables 1 and 2. Products were applied based on seed moisture content in 10% seed moisture decrements (60, 50, 40, 30, and 20%) to facilitate regression analysis. Herbicides were applied with a CO_2–pressurized backpack sprayer (110-015 AirMix nozzle at 45 cm spacing) at Saskatoon in 2012 and 2013 and with an air-pressurized tractor mounted sprayer equipped with shielding (110-015 AirMix nozzles at 45 cm spacing) at Saskatoon in 2014. At Scott, a CO_2–pressurized bicycle sprayer (110-003 AirMix nozzles, at 25 cm) was used. Sprayer speed and application pressure were calibrated to deliver 200 L ha^{-1} of spray carrier volume.

Prior to the application of harvest aid herbicide treatments, a random subsample of plants (10 plants per plot) was excised from border plots and bulked to create a composite seed sample on which seed moisture content could be determined for each application timing. Each composite seed sample was weighed (fresh weight), placed in paper bags and dried in an oven at 80°C for 24 h to determine dry weight. Seed moisture content (SMC) of each sample was calculated by the following equation:

$$\mathrm{SMC} = \frac{(M_f) - (M_d)}{M_f} \times 100\% \quad [1]$$

where M_f is fresh weight of the composite seed samples, and M_d is the dry weight of the composite seed samples.

DATA COLLECTION

Desiccation (plot visual color change) was rated 7, 14, and 21 d after each harvest aid herbicide application (DAA) based on a visual scale of 0 to 100%, where 0 indicates no visible symptoms and 100 indicates complete plant mortality. The three visual ratings were used to determine desiccation progress over time, which is calculated by the area under the desiccation progress curve (AUDPC):

$$\mathrm{AUDPC} = \left(\frac{D_1 + D_2}{2}\right)(t_2 - t_1) + \left(\frac{D_2 + D_3}{2}\right)(t_3 - t_2) \quad [2]$$

where D_1, D_2, and D_3 represent observed desiccation ratings at each evaluation day; t_1, t_2, and t_3 represent the number of the days after each herbicide application (Jeger and Viljanen-Rollinson, 2001; Simko and Piepho, 2012). The AUDPC equation converted the three desiccation ratings and crop moisture contents into a single relative value for the purpose of reporting; the greater the calculated AUDPC value, the further desiccation had progressed between ratings (McNaughton et al., 2015).

Lentil plots were harvested with a small plot combine at both sites. The harvested sample was weighed, cleaned with a dockage tester, and weighed again to determine clean seed yield adjusted to 13% seed moisture content. Thousand seed weight (TSW) was determined by counting and weighing 250 seeds and multiplying by a factor of four.

Glyphosate residues at Saskatoon (2012 and 2013) and Scott (2013) were assessed on three samples of each treatment containing glyphosate and an untreated control as per Zhang et al. (2016). Each 250-g sample was collected at 7 DAA from border rows, cleaned, placed into plastic bags and kept in a freezer at –20°C until all samples were collected. Collecting glyphosate residues 7 DAA may have biased the samples toward

Table 1. Herbicide treatments, rates, and application timings (percent seed moisture content) for each herbicide treatment evaluated at Saskatoon and Scott, SK, from 2012 to 2014.

Common name	Trade name	Rate	Manufacturer	Application timing
		g a.e. ha^{-1}/g a.i. ha^{-1}		%
Glyphosate	Roundup WeatherMax	900	Monsanto Canada, Winnipeg, MB	60
				50
				40
				30
				20
Saflufenacil†	Heat	50	BASF Canada Inc., Mississauga, ON	60
				50
				40
				30
				20
Glyphosate+Saflufenacil‡		900 + 36		60
				50
				40
				30
				20

† Merge at 1 L ha^{-1} was added to saflufenacil.

‡ Merge at 0.5 L ha^{-1} was added in the tank mixture of saflufenacil+glyphosate.

Table 2. Dates of application timings and environmental conditions (temperature at time of application and relative humidity) for each herbicide treatment in timing trials at Saskatoon and Scott, SK, from 2012 to 2014.

Site	Year	Application timing	Application date	Temperature	Relative humidity
		%		°C	%
Saskatoon	2012	60	17 Aug.	26.0	43.1
		50	20 Aug.	29.0	33.0
		40	28 Aug.	27.0	43.0
		30	30 Aug.	16.0	55.0
		20	6 Sept.	20.0	49.0
	2013	60	9 Aug.	20.1	56.1
		50	14 Aug.	20.3	69.0
		40	16 Aug.	27.0	64.1
		30	19 Aug.	30.1	30.5
		20	23 Aug.	19.5	63.0
	2014	60	12 Aug.	30.0	29.0
		50	15 Aug.	24.0	66.7
		40	19 Aug.	29.0	51.0
		30	27 Aug.	30.0	35.5
		20	5 Sept.	15.0	58.6
Scott	2012	na†	na	na	na
	2013	60	20 Aug.	13.4	73.9
		50	23 Aug.	17.0	50.1
		40	29 Aug.	19.6	74.5
		30	3 Sept.	12.2	83.8
		20	12 Sept.	10.7	61.8
	2014	60	12 Aug.	19.1	70.8
		50	15 Aug.	22.5	73.8
		40	19 Aug.	20.4	69.3
		30	22 Aug.	13.8	46.9
		20	27 Aug.	21.0	49.3

† na: no applicable data recorded due to hail damage.

Table 3. *P* values derived from analysis of variance of area under desiccation progress curve (AUDPC), lentil seed yield, thousand seed weight (TSW), glyphosate residue (GR), and saflufenacil residue (SR) as influenced by herbicide (H), and application timing (T) at Saskatoon and Scott, SK, from 2012 to 2014.

Source	AUDPC	Yield	TSW	GR	SR
			P value		
Site-year (SY)	0.094	0.111	0.104	0.251	0.438
Timing (T)	0.806	0.083	0.256	0.018*	0.000***
Herbicide (H)	0.045*	0.725	0.705	0.240	0.067
T × H	0.003***	0.003**	0.045*	0.367	0.079
SY × T	0.005**	0.030*	0.046*	0.051	0.110
SY × H	0.068	0.040*	0.388	0.244	0.150
SY × T × H	0.422	0.202	0.055	0.219	0.023*

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

Table 4. *P* values derived from analysis of variance of fixed effects (herbicide and application timing) for the area under desiccation progress curve (AUDPC) at Saskatoon and Scott, SK, from 2012 to 2014.

	AUDPC				
	P value				
Source	Saskatoon 2012	Saskatoon 2013	Saskatoon 2014	Scott 2013	Scott 2014
Herbicide (H)	<0.001***	0.247	0.003**	0.279	0.368
Timing (T)	<0.001***	0.001***	0.016*	<0.001***	0.077
H × T	0.010**	0.393	0.188	0.145	0.328

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
*** Significant at the 0.001 probability level.

higher residue levels as growers typically harvest lentils around 12 to 14 DAA, but we wanted to ensure our values represented the upper bound of those likely to occur under field conditions. Samples were sent to ALS Laboratories in Edmonton, AB, Canada. Using a standardized process provided by ALS Laboratories, high performance liquid chromatography (HPLC) using column switching and post-column derivatization with fluorescence detection was employed to determine glyphosate residues (Zhang, 2015).

Saflufenacil residue data was collected for both Saskatoon (2012, 2013, and 2014) and Scott (2013 and 2014) locations. Cleaned seed samples (75 g) were collected at 21 DAA (just prior to harvest), dried at 14°C in a paper bag, and then kept in a freezer at –20°C until processed. Because growers often harvest prior to 21 DAA, saflufenacil residues presented here may be lower than those expected under commercial production. Liquid chromatography-mass spectrometry (LC-MS) was used to determine the saflufenacil residues as per Mueller et al. (2014). Saflufenacil concentrations were determined by comparison to standards of known concentration responses. Saflufenacil recoveries were >97% based on fortified untreated samples, so concentrations were not corrected for percent recovery (data not shown). The lower limit of detection of this procedure was 5.6×10^{-4} mg kg^{-1} of lentil seeds; all saflufenacil-treated samples had detectable saflufenacil residues.

STATISTICAL ANALYSIS

Residuals were tested for normality and homogeneity of variances with PROC UNIVARIATE and Levene's test, respectively (SAS Institute, 2014). Where residuals did not conform to the assumptions of ANOVA, heterogeneous variance structures were modeled with mixed models. All data were analyzed using the MIXED Procedure in SAS 9.3 (SAS Institute, 2014). Herbicide treatments and application timings were considered fixed effects in the model, while site-year (environmental effects), replication (nested within site-year), and the interaction between fixed and environmental effects initially were treated as random effects.

The significance of random effects and their interactions with fixed effects was assessed with the COVTEST option in PROC MIXED (SAS Institute, 2014). Meanwhile, scatterplots of variables were observed to determine whether data could be combined for analysis. Where data could not be combined, data were treated as fixed effects and analyzed within site-years. Single degree of freedom contrasts were used to make specific preplanned comparisons of interest. Where ANOVA indicated a significant effect of application timing, data were subjected to linear and quadratic regression analysis using PROC REG (SAS Institute, 2014).

RESULTS AND DISCUSSION

Lentil Desiccation

The interaction of site-year × application timing was significant for desiccation progress (AUDPC) and thus, data were analyzed within site-years (Table 3). The herbicide × application timing interaction did not affect crop desiccation except at Saskatoon in 2012, so desiccation data were combined across three herbicide treatments for the other 4 site-years (Table 4). Lentil desiccation (AUDPC) decreased linearly with later application timings at Saskatoon in 2013 only (Fig. 1). There was no relationship between AUDPC and application timing at any of the other site-years. The Saskatoon 2013 data exhibited a similar pattern to that reported by McNaughton et al. (2015), where dry bean (*Phaseolus vulgaris* L.) desiccation was consistently reduced at higher seed moisture contents. On the other hand, since there was no relationship between application timing and AUDPC at most of our locations, it indicates that either lentil desiccation is less responsive to application timing than dry bean, or that the effect of application timing varies with environmental conditions.

Across five application timings, orthogonal contrasts showed that saflufenacil alone, or mixed with glyphosate, resulted in faster desiccation than glyphosate applied alone at Saskatoon 2012 and Saskatoon 2014, respectively (Table 5). There was no difference between treatments at the remaining 3 site-years. These results are consistent with Zhang et al. (2016) wherein saflufenacil + glyphosate resulted in better desiccation than either herbicide applied alone in some environments, but the response was not consistent across all environments tested. Soltani et al. (2013) and McNaughton et al. (2015) also reported that the addition of saflufenacil to glyphosate increased dry bean desiccation compared to each herbicide applied alone, which corroborates our results.

Generally, crop desiccation varied between site-years, likely due to variables such as temperature and relative humidity (Table 2). Interestingly, site-years that exhibited no variation in patterns with delayed application timings tended to have lower temperature at later application timings (Table 2). This probably slowed crop desiccation rate compared to earlier application timings, as reported by Wilson and Smith (2002). Similar observations by Moyer et al. (1996) showed higher temperature and reduced rainfall contributed to more rapid alfalfa (*Medicago sativa* L.) desiccation.

Table 5. Orthogonal contrasts for area under desiccation progress curve (AUDPC) of each herbicide treatment at various application timings (percent seed moisture content). Each value is the estimate of the difference between means at Saskatoon and Scott, SK, from 2012 to 2014.

	AUDPC				
Herbicide compared	Saskatoon 2012	Saskatoon 2013	Saskatoon 2014	Scott 2013	Scott 2014
Glyphosate vs. saflufenacil	–144***	6	–30	13	–19
Glyphosate vs. glyphosate+saflufenacil	–190***	–51	–95***	–20	–40
Saflufenacil vs. glyphosate+saflufenacil	–46	–57	–66*	–34	–22

* Significant at the 0.05 probability level.

*** Significant at the 0.001 probability level.

Lentil Seed Yield

The effects of site-year × timing ($P = 0.0161$), and site-year × herbicide ($P = 0.0382$) were statistically significant for yield data (Table 3), but scatterplots of yield data (not shown) showed consistent patterns across site-years. Moreover, the interactions of site-year × application timing and site-year × herbicide occupied relatively small proportions of the total sum of squares (5 and 9%, respectively) and showed little influence on model performance. Based on this, seed yield data were pooled across all site-years.

An interaction between herbicide treatment and application timing necessitated an analysis within herbicide treatments (Table 3). Glyphosate alone did not affect lentil yield, regardless of application timing (Fig. 2). Similar effects were observed across all application timings for saflufenacil applied alone, with the exception of 60% seed moisture content, where yield decreased by 22% compared to the untreated control (untreated control yield = 3358 kg ha^{-1}, Fig. 2). Lentil yield also decreased at earlier application timings (i.e., 60% seed moisture content) when glyphosate was tank mixed with saflufenacil (Fig. 2). In fact, lentil yield was 25% greater when

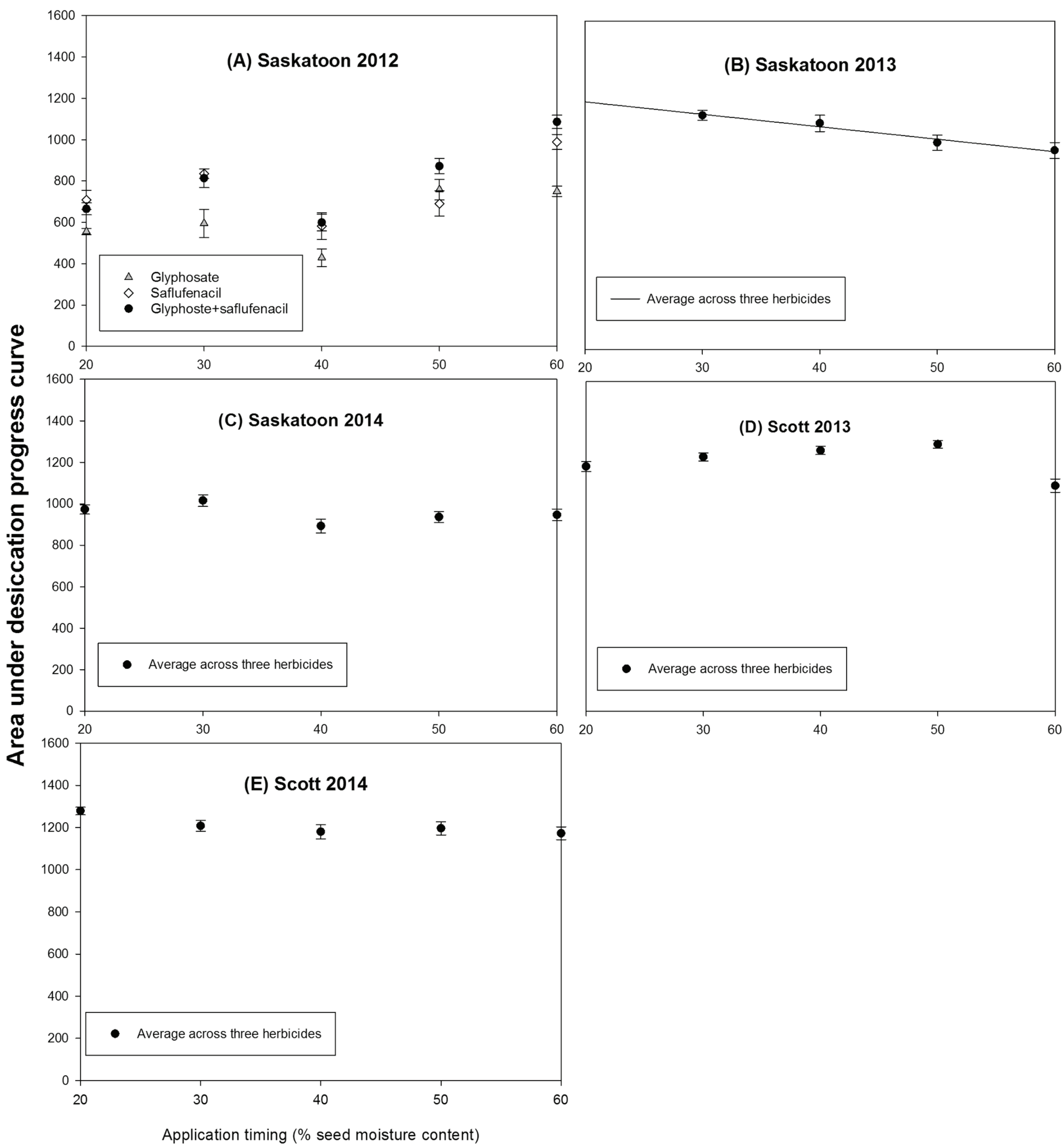

Fig. 1. The relationship between area under the desiccation progress curve (AUDPC) and application timing at (A) Saskatoon 2012, (B) Saskatoon 2013, (C) Saskatoon 2014, (D) Scott 2013, and (E) Scott 2014. Regression equation across three herbicide treatments at (B) Saskatoon 2013: $Y = -6.06x + 1327.70$, $R^2 = 0.967$, $P = 0.0167$. No relationship was observed for herbicides at any other site-year. Error bars represent one standard error of the mean.

the tank mixture of saflufenacil + glyphosate was applied at 20% seed moisture compared with 60% seed moisture content (Fig. 2). Saflufenacil and glyphosate did not reduce yield at any of the application timings when compared to the untreated control (Fig. 2). Contrasts showed no differences between the untreated control and the average of the three herbicides across application timings, indicating desiccants did not reduce seed yield when compared to the untreated control (Table 6).

Our results showed that lentil seed yield was generally reduced by an early application of saflufenacil (Fig. 2). However, seed yield was not adversely impacted by herbicide treatments when applications were made at or below 50% moisture content (Fig. 2). At early application timings lentil pods are unlikely to have reached physiological maturity. Similar results have been reported in other legume crops. Boudreaux and Griffin (2011) reported sizeable yield reductions in soybean when harvest aid herbicides were applied at 50 to 60% seed moisture, but applications at moisture contents lower than 40% seed moisture did not have adverse effects on seed yield. McNaughton et al. (2015) found that glyphosate or saflufenacil applied alone or in a tank mix increased dry yield as applications were progressively delayed toward lower seed moisture contents. Results from our study indicate that 50% seed moisture content is earliest that applications could safely be made to lentil without compromising yield.

Thousand Seed Weight

The interactions between site-year, herbicide treatment, and application timing with respect to TSW were not significant so data were combined across site-years (Table 3). Due to an interaction between herbicide treatment and application timing, TSW data were analyzed within individual herbicide treatments (Table 3). No statistical relationship between TSW and application timing was detected when glyphosate was applied alone, but quadratic responses were observed for both saflufenacil and the tank mixture of glyphosate + saflufenacil (Fig. 3). The TSW

Table 6. Orthogonal contrasts of yield, thousand seed weight (TSW), glyphosate residue (GR), and saflufenacil residue (SR) for specific herbicide treatments of interest at Saskatoon and Scott, SK, from 2012 to 2014. Each value is the estimate of the difference between means for each treatment.

Treatments compared	Yield	TSW	GR	SR
	Kg ha^{-1}	g	mg kg^{-1}	
Control vs. glyphosate	–66.6	1.9*	na†	na
Control vs. saflufenacil	10.9	2.1**	na	na
Control vs. glyphosate+saflufenacil	–207.3	2.1**	na	na
Glyphosate vs. saflufenacil	77.5	0.3	na	na
Glyphosate vs. glyphosate+saflufenacil	–140.7	0.2	1.1	na
Saflufenacil vs. glyphosate+saflufenacil	–218.2	0.0	na	0.0085*

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† na: no applicable data recorded due to absence of glyphosate or saflufenacil in treatments.

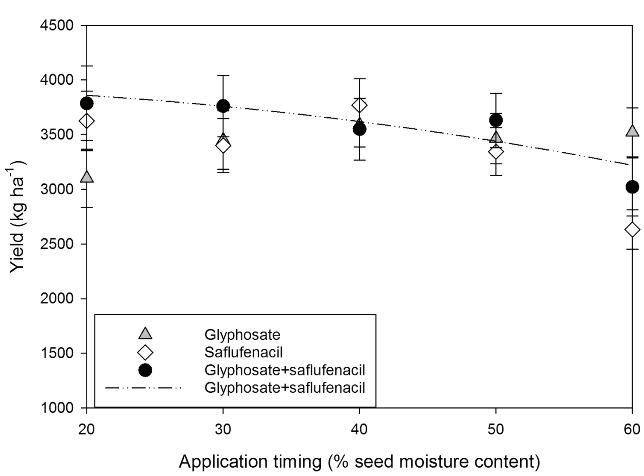

Fig. 2. The relationship between seed yield and application timing across 5 site-years in Saskatchewan. Regression equation for the tank mixture of glyphosate+saflufenacil: $Y = -0.2x^2 + 3940.4$, $R^2 = 0.7935$, $P = 0.0426$. No relationship was observed for glyphosate or saflufenacil applied alone. Points represent glyphosate (closed squares); points represent saflufenacil (open diamond); points represent glyphosate + salfufenacil (dash + dot dot + closed circle). Control yield was 3358.0 ± 252.0 kg ha^{-1}. Error bars represent one standard error of the mean.

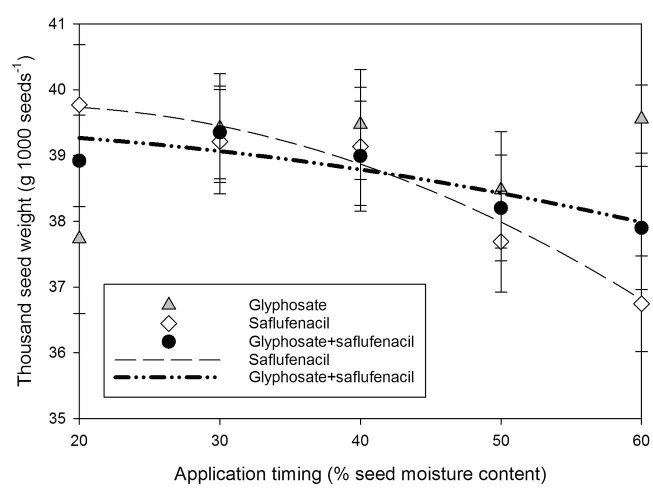

Fig. 3. The relationship between thousand seed weight and application timing across 5 site-years in Saskatchewan. Regression equation for saflufenacil: $Y = -0.0015x^2 + 0.0469x + 39.3940$, $R^2 = 0.9680$, $P = 0.032$; regression equation for the tank mixture of glyphosate+saflufenacil: $Y = -0.0004x^2 + 39.7278$, $R^2 = 0.7969$, $P = 0.0415$. No relationship was observed between TSW and glyphosate applied alone. Points represent glyphosate (closed squares); points represent saflufenacil (open diamond); points represent saflufenacil + glyphosate (closed circle). Control TSW was 40.6 ± 0.8 g. Error bars represent one standard error of the mean.

increased from 36.8 to 39.8 g when saflufenacil was applied at 60 and 20% seed moisture content, respectively (Fig. 3). However, compared to the untreated control (TSW = 40.6 g), there was no reduction in TSW with saflufenacil application unless it was applied at 60% seed moisture content (Fig. 3). The tank mixture treatment of glyphosate+saflufenacil exhibited a similar curvilinear relationship with seed moisture content wherein TSW decreased as moisture content increased to a minimum of 37.7 g at 60% seed moisture, although this did not differ statistically from the untreated control (Fig. 3).

Although there was no relationship between application timing and seed moisture content when glyphosate was applied alone, contrasts indicate that the overall TSW mean from glyphosate application was lower than the untreated control (Table 6). This agrees with Ratnayake and Shaw (1992), who reported that glyphosate did not affect soybean TSW if applied between the R5 (beginning seed development) and R8 (full seed maturity) growth stages in soybean. Saflufenacil, on the other hand, produced a decrease in TSW when applications were made beyond 50% seed moisture (Fig. 3), which is in agreement with findings by McNaughton et al. (2015) in dry bean. Bennett and Shaw (2000) and Boudreaux and Griffin (2011) both reported substantial reductions in soybean seed weight when desiccants were applied prior to 40% seed moisture content. The differences we observed between glyphosate and saflufenacil probably result from the slow action of glyphosate at early growth stages, which permitted more time for seed growth prior to the arresting of seed development. Alternatively, it is possible that saflufenacil rapidly limited lentil growth, which resulted in less time for seed development and lower seed weights. Although the impact of glyphosate application timing was not obvious in this study, saflufenacil treatments displayed adverse effects on TSW at 60% seed moisture content. Consequently, growers must avoid early application of these desiccants, but applying the tank mixture treatment is an alternative, as it did not have adverse effects on TSW.

Herbicide Residues

There was no interaction between herbicide treatment and application timing ($P = 0.3670$) and thus, data were pooled across herbicide treatments (Table 3). Glyphosate residue generally increased as treatments were made at progressively more immature growth stages (higher moisture contents) (Fig. 4). For example, glyphosate seed residues increased from 0.7 at 20% seed moisture to 6.2 mg kg^{-1} at 60%, which represents an approximate ninefold increase (Fig. 4). Contrasts showed that adding saflufenacil to glyphosate did not influence glyphosate residues (Table 6).

The accumulation of glyphosate residue in lentil seed is crucial for lentil exporters because importing countries may reject lentil shipments if the glyphosate residue exceeds the MRL (Pratt, 2011). In the current study, average glyphosate residues did not exceed 2.0 mg kg^{-1} at the 30% application timing, nor did they exceed 4.0 mg kg^{-1} at 40% application timing (Fig. 4). These values are not above the new EU MRL of 10 mg kg^{-1}, which was established in 2012. However, our results show that average glyphosate residues do exceed the Canadian and Japanese limits of 4.0 and 2.0 mg kg^{-1}, respectively, and could exceed international CODEX levels of 5.0 mg kg^{-1} (Bryant Christie Inc., 2015). It is therefore critical that growers do not apply glyphosate as a harvest aid when seed moisture content is above 30%. Our results also show that applications made prior to 40% seed moisture content consistently produced higher glyphosate residues. At early seed developmental stages, seeds are major sucrose sinks and glyphosate will translocate to those developing seeds. As the crop matures, the demand for sucrose from these sinks declines and less glyphosate is translocated to the developing seeds, resulting in reduced glyphosate residues (Zhang et al., 2016).

Although there was a site-year × timing × herbicide interaction for saflufenacil residue data, further examination of the data and residuals indicated that saflufenacil residue responded consistently to treatments across all site-years; therefore, the data were pooled. There was no herbicide treatment × application timing interaction for saflufenacil residues so data were combined across herbicide treatments (Table 3). Saflufenacil residues consistently decreased as application timing was progressively delayed (Fig. 4B). For example, saflufenacil residues in seed harvested 21 DAA decreased approximately 85% as application timing was delayed from 60 to 20% seed moisture content. Contrasts showed that tank-mixing saflufenacil and glyphosate decreased saflufenacil residues further compared to

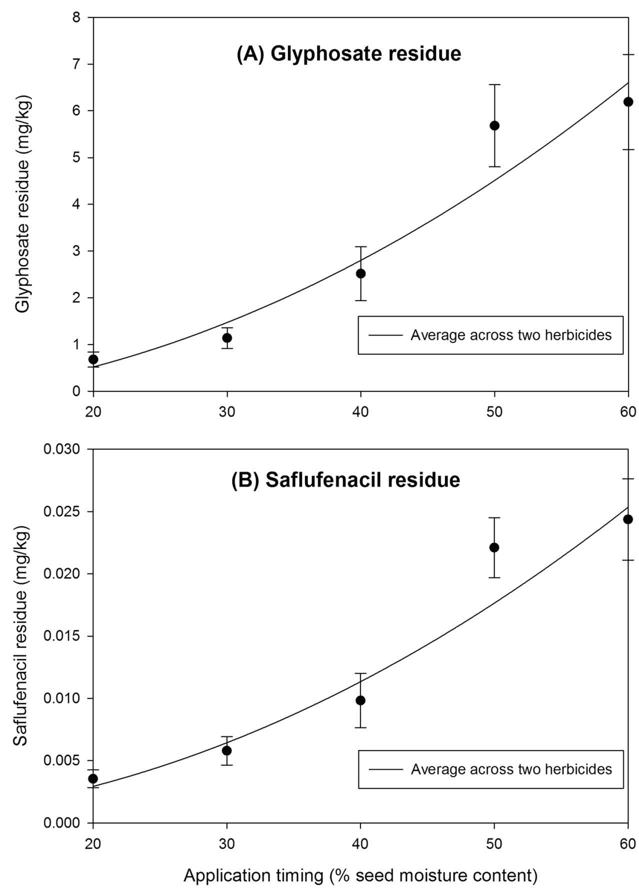

Fig. 4. The relationship between herbicide residue and application timing averaged across two herbicide treatments at 3 site-years in Saskatchewan. (A) Regression equation of glyphosate residue across two herbicides: $Y = 0.0019x^2 - 0.2377$, $R^2 = 0.9339$, $P = 0.0074$. (B) Regression equation of saflufenacil residue across two herbicides: $Y = 0.0000072x^2 + 0.0001433$, $R^2 = 0.9372$, $P = 0.0068$. Error bars represent one standard error of the mean.

saflufenacil applied alone (Table 6). This is not surprising given that as per label recommendations (Saskatchewan Ministry of Agriculture, 2016), a 65% rate of saflufenacil was used in the tank mixture (Table 1).

An interesting finding was a lack of differences in glyphosate residues if glyphosate was applied alone compared to the tank mix treatment of saflufenacil + glyphosate. Other research has also found that the addition of saflufenacil to glyphosate did not reduce seed residues, with an exception of application at 50% crop maturity in dry bean (McNaughton et al., 2015). Zhang et al. (2016) reported that saflufenacil did not consistently reduce glyphosate residues when applied at 30% seed moisture content; however, tank mixes with other contact herbicides such as diquat and glufosinate generally resulted in lower glyphosate residues. Our results contrast with Ashigh and Hall (2010), who reported that glyphosate activity in plants was limited by adding saflufenacil, which can destroy plant phloem quickly. The contrasting results may be due to lower sensitivity of pulse crops to saflufenacil (Zhang et al., 2016) compared with the brassicaceous species used in Ashigh and Hall (2010), or they may be due to differences in application timing as applications were made at the three- to four-leaf stages in that study.

Saflufenacil can be translocated in xylem and phloem (Ashigh and Hall, 2010; Soltani et al., 2010) and therefore, its residue is detectable in seeds. The lowest current acceptable MRL for saflufenacil residue in lentil seed is 0.03 mg kg^{-1} set by the European Union (Bryant Christie Inc., 2015). In our study, saflufenacil residues generally increased with earlier application timing (Fig. 4B). Saflufenacil applied alone at 60 and 50% seed moisture resulted in unacceptable seed residue levels, exceeding 0.03 mg kg^{-1} in some cases. However, we found that saflufenacil residues were lower in the glyphosate tank-mix treatment, and did not exceed 0.03 mg kg^{-1}, regardless of application timing. This can be partially attributed to the lower rate of saflufenacil in the tank mixture (36 g a.i. ha^{-1}) compared with saflufenacil applied alone (50 g a.i. ha^{-1}). It is also possible that reduced saflufenacil residues may result from reduced saflufenacil translocation with the tank mixture, as glyphosate has been shown to limit the translocation of saflufenacil in glyphosate-susceptible canola (Ashigh and Hall, 2010). Alternatively, saflufenacil residues in dry bean did not change with the addition of glyphosate compared with the application of saflufenacil alone (McNaughton et al., 2015). Based on these contrasting results, the interaction between saflufenacil and glyphosate in lentil needs further investigation.

CONCLUSIONS

Application of desiccants below 30% seed moisture content, when lentil was close to physiological maturity, did not impact seed yield or TSW, and did not result in lentil seed samples that exceeded residue levels of 2 and 0.03 mg kg^{-1} for glyphosate (sampled 7 DAA) and saflufenacil (sampled 21 DAA), respectively. Although glyphosate residue levels were substantially lower in the tank mixture, adding saflufenacil to glyphosate did not reduce glyphosate residue in lentil seeds compared to glyphosate applied alone. It did, however, improve crop desiccation and reduce seed residues of saflufenacil compared with either glyphosate or saflufenacil applied alone. This tank mixture should also improve weed control over using either herbicide alone and offers two distinct modes of action, which is important to delay the evolution of herbicide resistance. Therefore, we recommend a tank mix of saflufenacil + glyphosate for crop desiccation and pre-harvest weed control in lentil over using either product alone. Regardless of the product chosen, our results with respect to seed residue levels show it is imperative to ensure applications of glyphosate or saflufenacil are not made prior to the 30% seed moisture stage. Further, consideration must be given to accurately determining 30% seed moisture content across fields with variable topography containing low areas, swales, and north aspects, which can dry down at much different rates than other areas of the field.

ACKNOWLEGEMENTS

We would like to acknowledge all funding agencies including the China Scholarship Council, Pulse Canada, and Canadian Agricultural Adaptation Program. We also would like to thank Gerald Stuber, Ken Sapsford, Gerein Aaron, Ryan Regush, Cindy Gampe, Eric Tozzi, Cory Jacob, Matthew Lawless, Ethan Bertholet, and Jessica Weber for providing technical assistance.

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