FINAL REPORT

Assessing the influence of base germination temperature and chemical desiccants on the recruitment biology of cleavers (*Galium* species) (SCDC CARP 2015-1).

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Non-Confidential Abstract/Summary

Galium aparine L. (cleavers or catchweed bedstraw) and *Galium spurium* L. (false cleavers) are problematic weed species in canola. They can reduce crop yield, impair harvest operations, and reduce crop processing efficiency. The development of herbicide resistance in these species, as well as the potential for evolved glyphosate resistance, means that knowledge of their biology and the development of alternative management strategies is critical. The objective of this study was twofold: to determine the differences in base germination temperatures of *Galium* spp. populations from different locations in western Canada, and to investigate options for reducing cleavers seed return in canola crops. The first experiment was conducted at Agriculture and Agri-Food Canada in Saskatoon, SK. Seven *Galium spurium* populations and one *Galium aparine* population were subjected to germination studies on a thermogradient plate that included a range of temperatures (1-10° C) set at 1°C increments. Using this experimental protocol, we were able to determine the base temperature for germination, which was a consistent 2° C for all *Galium spurium* populations and 4° C for the *Galium aparine* population. The second experiment was a field study conducted at two sites (Saskatoon, Scott) in 2016 and 2017. This experiment evaluated the effect of pre-harvest herbicides on cleavers contamination in canola crops, as well as cleavers seed viability and vigour. Seed vigour was evaluated using an electrolyte leakage test. Herbicides evaluated included saflufenacil, diquat, glufosinate, saflufenacil plus glyphosate, diquat plus glyphosate, and glufosinate plus glyphosate. Glufosinate + glyphosate and saflufenacil + glyphosate application resulted in significantly lower cleavers contamination. A number of the herbicides reduced seed viability and increased electrolyte leakage in cleavers seeds, indicating potentially lower seed vigour. The establishment of the base temperature for cleavers germination indicates that western Canadian populations of *Galium spurium* are able to germinate both very early in the spring and late into the fall. Pre-harvest herbicides show potential to manage cleavers seed production and reduce competition with canola by increasing seed mortality, and by reducing seed viability and vigour.

Introduction

Galium species (cleavers) have been identified as one of the most competitive common broad-leaved weeds in major crops within North America (Wright and Wilson 1987). An increased presence of two species, *G. aparine* (cleavers or catchweed bedstraw) and *G. spurium* (false cleavers) has been recorded in field surveys of Saskatchewan, where they are now ranked 7th and 6th in abundance in all annual crops and canola, respectively (Leeson 2016). Research done in our lab identified that *G. spurium* was the predominate species of samples collected in western Canada (De Roo 2016). Cleavers reduce yield, increase crop lodging, interfere with harvest operations, and reduce canola grades through seed contamination. Malik and Vanden Born (1987a) established that a cleavers density of 100 plants m^{-2} resulted in canola yield reductions of 4 to 28%, depending on the emergence date of the weed relative to the crop. Seed contamination is due to the seeds of both cleavers and canola being very similar in size and shape, making them difficult to separate (Canola Council of Canada 2014). In addition, about 20% of cleavers surveyed in Saskatchewan have evolved resistance to ALS-inhibitor herbicides and cleavers are considered a high-risk weed for evolved glyphosate resistance (Beckie 2010; Beckie et al. 2013).

Herbicide applications are the major method of weed control in western Canada and spray timing is critical to ensure adequate control of weeds such as cleavers. Emergence timing studies and advances in emergence modelling are providing better information of when producers should implement control methods. The timing of weed emergence makes a significant contribution to the potential success of weedy species. Annual weeds are often more susceptible to herbicides at early growth stages; hence, understanding their germination and emergence patterns is important for management (Kusdk and Streibig 2003).

The main conditions influencing weed seed emergence are soil temperature, water potential, light quality, and air quality (Forcella et al. 1992); however, the effect of temperature on weed emergence has been well-documented and thermal time (growing degree days) can be used directly as a predictor of plant emergence (Angus et al. 1981). Currently, the literature has placed the base germination temperature of cleavers species between 2-20^{o}C, which is not accurate enough to be useful for producers or weed scientists attempting to accurately model emergence timing (Malik and Vanden Born 1988). Some studies have focused on the optimum

temperature for germination and have found ranges of 12-15^{o}C, 10-20^{o}C, and 0.5-12^{o}C, depending on population (Malik and Vanden Born 1988). These studies used *G. aparine* from European locations, however. Unfortunately, the base temperature for *G. spurium* has never been investigated, despite being the predominant *Galuim* species in western Canada. Moreover, De Roo (2016) reported that *G. spurium* populations collected from different locations in the Canadian Prairies varied in their emergence timing and rate of emergence. However, a base germination temperature for these populations could not be determined based on study design. Mechanistic determination of a different base germination temperature for these cleavers populations would allow for a better model to predict emergence, which is essential for properly timed weed management.

With an increase in the amount of canola grown each year and a move towards more direct harvesting techniques, the need for chemical desiccation methods has also increased. Research has been conducted on chemical desiccants in canola in the United States, no such research has been very limited in Canada. Even fewer studies have investigated the impact of desiccants on weed seed production and viability. In Australia, the practice of "crop-topping", applying a non-selective pre-harvest herbicide to reduce annual ryegrass (*Lolium rigidum* Gaud.) seed production, is a common practice. Steadman et al. (2006) reported that glyphosate or a combination of paraquat and diquat applied pre- and post-anthesis, respectively, resulted in up to a 90% reduction in ryegrass seed production. In addition, subsequent germination and seedling vigour of ryegrass seed was reduced. More recently, Bertholet and Willenborg (unpublished data) found that pre-harvest application of glufosinate and diquat were effective in reducing kochia (*Kochia scoparia* L. Schrad.) and mustard (*Brassica juncea* L. Czern.) seed production in lentil (*Lens culinaris* Med.). Seedling emergence and vigor of the treated seed was reduced in their study as well. In addition, it has been demonstrated that cleavers retains most of its seed until canola, peas, and wheat reach maturity (Burton et al. 2016; Tidemann et al. 2017). Therefore, there is a high potential for pre-harvest herbicides to influence fitness, although there have not been any studies on canola desiccation and its effect on cleavers seed production and viability. The goal of this research project was to improve cleavers management in canola crops, specifically with regard to seed and seedbank management. Thus, the specific objectives of this research were twofold: 1) to determine if differences in base germination temperature exist

between *Galium* populations from across western Canada, and 2) to investigate herbicide options that may reduce viable seed return from cleavers in canola production.

1. Thermogradient Plate Experiment

Materials and Methods

Experimental Procedure. The germination response of seeds from several different *Galium* populations to temperature was investigated using a thermogradient plate (TGP) located at Agriculture and Agri-Food Canada in Saskatoon, SK Canada. The TGP provides over 200 individually-controlled cells varying in temperature. The temperature of each cell is controlled with a thermoelectric pump, and each cell can deliver temperatures accurate within 0.1°C (Tozzi et al. 2014). The methodology used for the TGP experiment was similar to Tozzi et al. (2014). Populations of *Galium spurium* from Carrot River, Melfort, Saskatoon, Canora, SK, and Vegreville and Lacombe, AB, along with a single *Galium aparine* population from Saskatoon, were used in the experiment. One-hundred seeds from each population were counted and placed in small paper envelopes and stored at -4°C (De Roo 2016). Any seed dormancy induced by storage was broken using methods developed from previous experiments at the University of Saskatchewan. Namely, the seed was placed in a freezer (-18^{o}C) for 1 month, then a refrigerator (2^{o}C) for 2 weeks, then seed was scratched on abrasive sandpaper to scarify the testa.

At the initiation of the experiment, 25 *Galium* seeds were placed in a petri dish lined with filter paper, which was then dampened using de-ionised water and placed in a TGP cell. Filter paper was kept moist throughout the experiment. Seeds were then subjected to 10 constant temperatures ranging from 1-10°C and at 1.0°C increments. Temperature treatments were arranged in a completely randomized design. Because the range of germination temperatures is quite wide for *Galium*, this experiment focused on identifying the lowest threshold for germination in these populations. Germination times were recorded daily for 21 days to determine the lowest temperature at which seeds germinated. Seeds were deemed to have germinated when the radicle broke through the seed coat and was at least 1 mm in length. For each *Galium* spp. population, there were two replicates of each treatment and the experiment was repeated.

Statistical analysis. Data were subjected to ANOVA using a mixed model procedure that accounted for the number of trial runs (JMP software V13, SAS Institute Inc., Cary, NC). The assumptions of ANOVA were met (normal error distribution, homogeneity of error variance, and independence of residuals). Data from each run was combined for non-linear regression analysis. The DRC package in R-software was used to fit a nonlinear three-parameter Gompertz sigmodial model to the data representing the relationship between temperature and germination. The germination response model was:

$$yt=aebect$$

where y is percentage germination, t is temperature, a the asymptote, b the rate of change in germination and c the inflection point. Models were fitted for each population separately and parameters were compared by ANOVA. A statistical comparison of the inflection point of the modelled germination curve for each population with the average inflection point for all populations was conducted. Means for parameter values were separated using estimate statements at $P<0.05$.

Results

The germination characteristics of all populations, with the exception of *G. aparine*, responded similarly to varying temperatures (Figure 1). The model provided a good fit of the data for each population with coefficients of determination (R^2) ranging from 0.92 to 0.97 (Table 1). Few statistical differences were observed between parameter estimates, which were often similar among many of the populations. The inflection point can be used as a representation of when 50% of the total germination (T_{50}) has occurred and in this case, that would mean the *G. aparine* population required a temperature of 8.34^{o}C to reach 50% germination. In contrast, the T_{50} for all other populations were similar at about 6.5°C, on average. *G. aparine* (8.34°C) exhibited a significantly different T_{50} than the Clancy (6.04°C), Canora (6.12°C), Heavin (6.13°C), and Trawin (5.77°C) populations (Table 1). *G. spurium* (3.78%) also had a significantly faster germination rate than both the SPG (1.82%) and Trawin (2.05%) populations. However, there were no significant differences in germination rates among the other cleavers populations. Furthermore, there were no significant differences between the upper germination asymptote for any of the populations (Table 1).

The base germination temperature was 2°C for all populations except *G. aparine*, which had a base germination temperature of 4°C (Table 1). This suggests that all *G. spurium* populations examined in this study exhibited similar base temperatures; thus, they have the potential to germinate earlier in the spring and later into the autumn months.

Table 1. Base germination (°C) and inflection point (°C for 50% Germination) of each logistic three-parameter model on each population of *Galium spurium* and *Galium aparine* from across western Canada in 2016. Standard errors are in parentheses. *a* represents the asymptote, *b* represents the rate of germination, R^2 = coefficient of determination. Letters indicate a significant difference between populations at p=0.05.

Population	Base Germination (°C)	Parameters					Inflection point (°C for 50% Germination)			R^2
		a		*b*						
Canora	2.0	117.90	(13.34)	2.03	(0.44)	AB	6.12	(0.32)	B	0.96
Clancy	2.0	111.52	(10.32)	1.77	(0.36)	AB	6.04	(0.24)	B	0.96
Heavin	2.0	118.89	(17.18)	2.40	(0.57)	AB	6.13	(0.45)	B	0.96
Lacombe	2.0	144.79	(43.60)	3.14	(1.15)	AB	6.72	(1.13)	AB	0.92
Saskatoon-*aparine*	2.0	168.15	(56.45)	2.91	(0.91)	AB	8.34	(1.08)	A	0.96
Saskatoon-*spurium*	4.0	164.49	(42.58)	3.78	(0.96)	A	7.45	(1.10)	AB	0.97
SPG	2.0	113.29	(9.72)	1.82	(0.33)	B	6.07	(0.23)	B	0.97
Trawin	2.0	114.06	(12.17)	2.05	(0.44)	B	5.77	(0.31)	B	0.96
Vegreville	2.0	129.15	(21.25)	2.64	(0.66)	AB	6.16	(0.55)	B	0.95

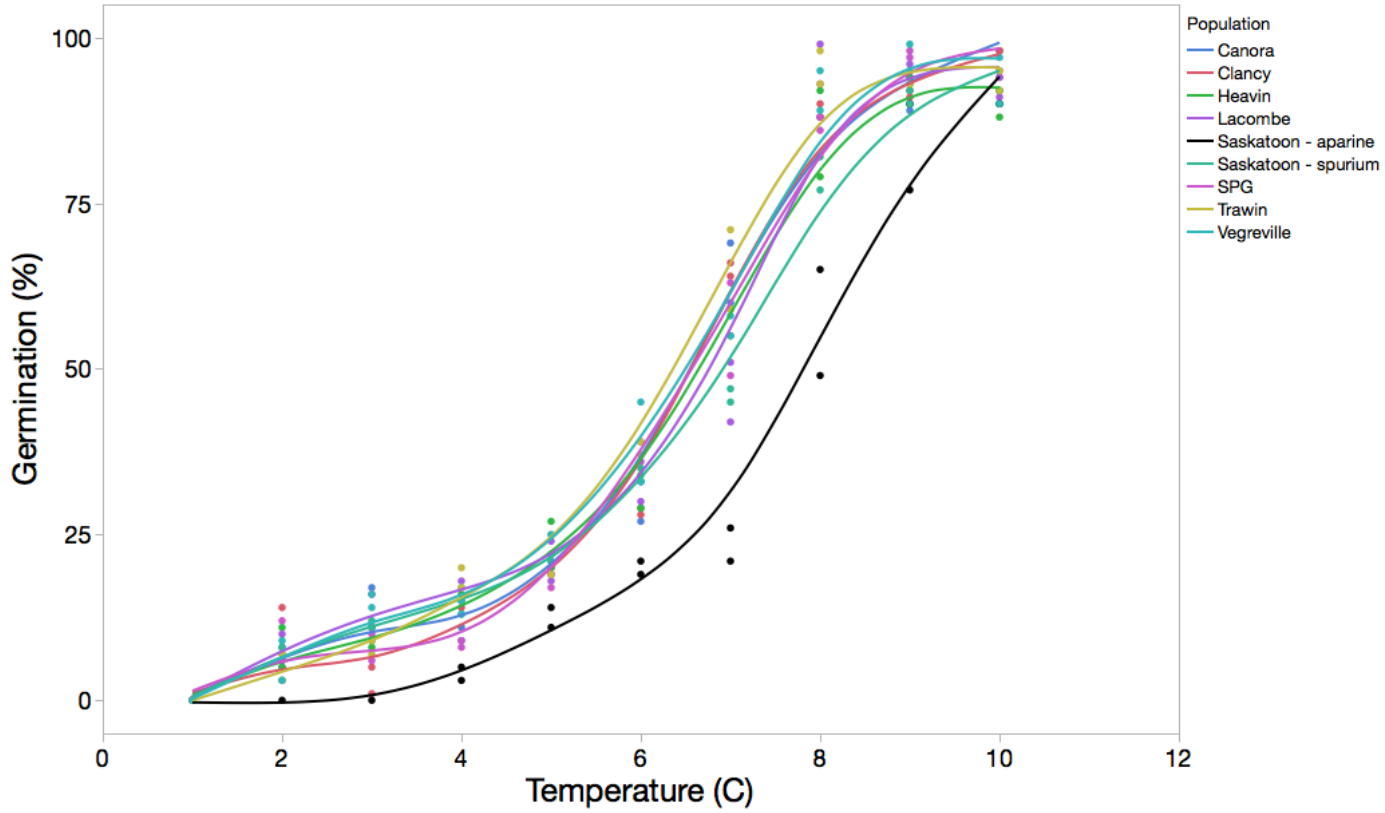

Figure 1: Logistic three-parameter model describing the effect of temperature on percent germination of eight *Galium spurium* populations and one *Galium aparine* population from different locations on the Canadian Prairies. Data points represent the means of two trial runs.

2. Field Experiment

Materials and Methods

Experimental Procedure. The experiment was conducted at the Kernen Research Farm at Saskatoon, SK and at the Western Applied Research Corporation located in Scott, SK in 2016 and 2017. The Kernen Research Farm is located on an Orthic Dark Brown Chernozem soil, and the Western Applied Research Corporation is located on a Dark Brown Chernozem. A detailed summary of soil characteristics can be found in Table 2. Imidazolinone-resistant canola (Clearfield® canola *cv.* 45H75) was seeded at a target density of 75 seeds m^{-2} in 2 x 6 m plots. Imidazolinone-resistant canola was used because group 2 resistant cleavers are prevalent in western Canada and additional options are needed to improve control of these herbicide resistant populations. Cleavers were seeded just prior to canola to achieve a target density of 50 plants m^{-2} (350 seeds m^{-2}). Plots were seeded on chemical fallow in Saskatoon and cereal stubble at Scott. Fertilizer was applied based on soil test recommendations. The experiment was arranged as a randomized complete block with seven pre-harvest treatments and four replications. A treatment summary can be found in Table 3. Prior to seeding at Scott, all plots were treated with glyphosate (675 g ae ha^{-1}) + bromoxynil (280 g ai ha^{-1}). Likewise at Saskatoon, all plots were treated with glyphosate (450g ae ha^{-1}) prior to seeding. Plots were seeded using an R-Tech plot seeder equipped with mid-row banders and hoe openers on a 25 cm row spacing at Scott, and at Saskatoon the trial was seeded with a plot drill, with hoe openers on a 23 cm row spacing.

Table 2. Soil classification and soil descriptions for 2 years and two site locations: Kernen Crop Research Farm (Saskatoon, SK) and Western Applied Research Corporation (Scott, SK)

		Soil Description				
		pH	OM[a]	Sand	Silt	Clay
Site	Soil Type		(%)	(%)	(%)	(%)
Kernen: 2016	Black Chernozem	7.2	4.4	20	30	50
Scott: 2016	Dark Brown Chernozem	6.0	4.5	31	47	23
Kernen: 2017	Black Chernozem	7.2	4.4	20	30	50
Scott: 2017	Dark Brown Chernozem	6.0	4.5	31	47	23

Table 3. Herbicide common name, herbicide group, herbicide concentration, herbicide rate, surfactant/adjuvant used, and adjuvant rate for the cleaver pre-harvest desiccation in canola

at Saskatoon and Scott in 2016 and 2017.

Trt. #	Herbicide common name	Herbicide group	Conc. g/l or g/kg	Rate g a.i. ha^{-1}	Surfactant/ Adjuvant	Adjuvant rate (%v/v)
1	Saflufenacil	14	342	50	Merge	0.5
2	Diquat	22	240	420	None	-
3	Glufosinate	10	150	600	None	-
4	Glyphosate	9	540	900	None	-
5	Saflufenacil +	14	342	35	Merge	0.5
	Glyphosate	9	540	900		
6	Diquat +	22	240	420	Agral 90	0.1
	Glyphosate	9	540	900		
7	Glufosinate +	10	150	600	None	-
	Glyphosate	9	540	900		

Pre-harvest applications were made when 60-75% of the canola plants had changed color from green to brown. Pre-harvest herbicides were applied at Kernen using a tractor-mounted sprayer equipped with TurboTee Jet Airmix 10015 nozzles calibrated to deliver a volume of 100 L ha^{-1} at 275 kPa. A bicycle sprayer equipped with TurboTee Jet AirMix 110015 nozzles calibrated to deliver a volume of 100 L ha^{-1} at 275 kPa was used to apply treatments at Scott. Both canola and cleavers were harvested from the entire plot using a small plot combine. Cleavers seed were separated manually from canola seed to calculate cleavers seed contamination and canola seed yield. In 2017, cleavers plants lodged and laid on the ground below the combine cutterbar at both locations, so they could not be picked up with the combine. Therefore, we were unable to calculate cleavers contamination percentage in 2017. Cleavers plants were hand-picked from the plots after harvest in 2017 and seed was hand-harvested for viability and vigour testing. Specific dates of field operations can be found in Table 4. Cleavers control ratings were initially to be recorded, but low levels of cleavers establishment made data collection difficult and inaccurate.

Table 4. Field operation for controlling cleavers in canola at the Kernen Crop Research Farm (Saskatoon, SK) and the Western Applied Research Corporation (Scott, SK)

Site Year	Seeding	Spraying	Harvest

Kernen	2016	May 18	August 20	September 13
	2017	May 18	August 28	September 15
Scott	2016	May 13	n.a.	September 1
	2017	May 16	August 18, 22 & 25	September 11

Cleavers seed from mature plants was tested for vigour and viability with an electrical conductivity and tetrazolium test, respectively. The tetrazolium test allows us to evaluate seed viability in a matter of hours rather than over the course of several days (de Carvalho et al. 2013). A solution of 2, 3, 5 - triphenyl tetrazolium chloride was applied to the seeds (Borza et al. 2007). Once absorbed, the tetrazolium undergoes an enzymatic reduction in living cells which results in these cells becoming stained red, which is an indicator of viability (Patil and Dadlani 2014). Cleavers seeds were imbibed with water, then prepared for colouration with a longitudinal cross section of the embryo, followed by treatment with a 1.0% (water to volume) tetrazolium solution (de Carvalho et al. 2013). The tetrazolium test is used frequently for numerous crop species for quality control (de Carvalho et al. 2013).

The electrical conductivity (EC) test measures electrolyte leakage from the intact seed. Electroconductivity tests are quick and relatively simple to conduct (Milošević et al. 2010). Higher EC values (i.e. higher electrolyte leakage) have been correlated with lower seed vigour in a number of seed crops. The electrical conductivity test followed the procedure outlined in Ghassemi-Golezani and Hosseinzadeh-Mahootchy (2009). Two replicates of 50 seeds from each plot were weighed (SW1 and SW2). Seeds of each replicate were immersed in 250 ml deionized water in a container at 20°C for 24 hours. The seed-steep water was then gently decanted and electrical conductivity (EC) of seed leachates were measured using an EC meter (EC1 and EC2). The following equation was applied to calculate conductivity per gram seed weight for each sample (Powell et al., 1984).

$$EC\mu Scm^{-}\ g^{-} = EC\ SW + EC\ SW$$

Statistical analysis. Linear mixed models were constructed using the MIXED procedure of SAS 9.4 (SAS Inst. 2016, Cary, NC), with pre-panned contrasts used to make specific comparisons of interest. Residuals were initially tested for normality with the UNIVARIATE procedure, while

homogeneity of error variance was confirmed using Shapiro Wilk's test in SAS (SAS Inst. 2016). All variables were analyzed using PROC GLIMMIX with a Guassian distribution because the residual data was normally distributed. Fixed effects in the model were herbicide treatments while site, replication (nested within site) and their interactions with fixed effects were treated as random effects. These random effects and their interactions with herbicide treatments (fixed effect) were assessed with a COVTEST to determine if site-years could be combined for analysis (SAS Inst. 2016). Because the aim of this research was to assess if pre-harvest herbicides could reduce cleavers contamination and viability relative to the untreated check, a DUNNETT'S test was used to compare treatment means to the untreated check. The calculated minimum significant difference (MSD) was used to determine if the mean of the treatment significantly differed from the untreated check.

Results

Environmental conditions. In 2016 and 2017 the mean temperatures of all sites were similar to the long-term average at each respective site, with Kernen experiencing slightly warmer conditions in 2016 and 2017 compared to the 10 year average (Table 5). May to September precipitation was variable between the different site years. Total precipitation at Kernen between May to September was 14% and 23% lower than the long term average in 2016 and 2017, respectively. Scott 2016 had excess precipitation (+36% of normal), with 85% of rainfall occurring in May, July and August. 2017 was substantially drier than the long term average at Scott, with the months of June (-44%), July (-69%), and September (-48%) having the greatest deficit.

The COVTEST (test of covariance) indicated no significant site year x treatment interactions and therefore, all site years of data were combined for analysis (data not shown). P-value results from the ANOVA are presented in Table 6. Treatment had a significant effect on cleavers contamination, electroconductivity, and viability. There was no effect of the desiccation treatments on canola yield.

Table 5. Mean monthly temperature (°C) and precipitation data (mm) at the Kernen Crop Research Farm (Saskatoon, SK), and Western Applied Research Corporation (Scott, SK) in 2016 and 2017.

Site		May	June	July	August	September	Average/ Total
		----------Temperature (°C) ----------					
Kernen	2016	13.4	17.4	18.4	17.1	12.1	15.7
	2017	11.6	16.0	19.5	17.8	13.1	15.6
	Long term	**10.4**	**15.5**	**18.5**	**17.3**	**12.9**	**14.9**
Scott	2016	12.4	15.8	17.8	16.2	10.9	14.6
	2017	11.5	15.1	18.3	16.6	11.5	14.6
	Long term	**10.8**	**15.3**	**17.1**	**16.5**	**10.4**	**14.0**
		----------Precipitation (mm) ----------					
Kernen	2016	45.0	51.0	80.5	66.0	19.8	232.3
	2017	56.0	43.6	32.4	30.0	46.4	208.4
	Long term	**44.3**	**73.9**	**68.8**	**52.8**	**29.7**	**269.5**
Scott	2016	64.8	20.8	88.1	98.2	22.2	294.1
	2017	69.0	34.3	22.4	53.0	18.9	197.6
	Long term	**36.3**	**61.8**	**72.1**	**45.7**	**36.0**	**215.9**

Table 6. Analysis of variance results (F-values) for measured variables as affected by pre-harvest herbicides. Data was combined over 4 site years from Kernen Research Farm (Saskatoon, SK) and WARC (Scott, SK) in 2016 and 2017

	Treatment			
Variable	ndf	ddf	F-value	
Canola yield	7	1	1.21	
Cleavers contamination	7	56	8.08	***
Electro conductivity	7	119	37.62	***
Viability	7	8	26.56	***

*** indicates significance of P<0.001.

The field study showed that percentage of cleavers found in the harvested sample for all treatments was < 2.0% (Figure 2). Glufosinate + glyphosate (1.1%) and saflufenacil + glyphosate (0.77%) treatments resulted in 32% and 50% lower cleavers contamination than the control,

respectively. The only treatment to reduce cleavers contamination below 1.0% was saflufenacil + glyphosate (0.77%). All other treatments did not show a significant difference when compared to the untreated check. It is important to note that no sample had greater than 2.0% cleavers contamination, meaning that these samples can all be expected to grade a #2 canola or better.

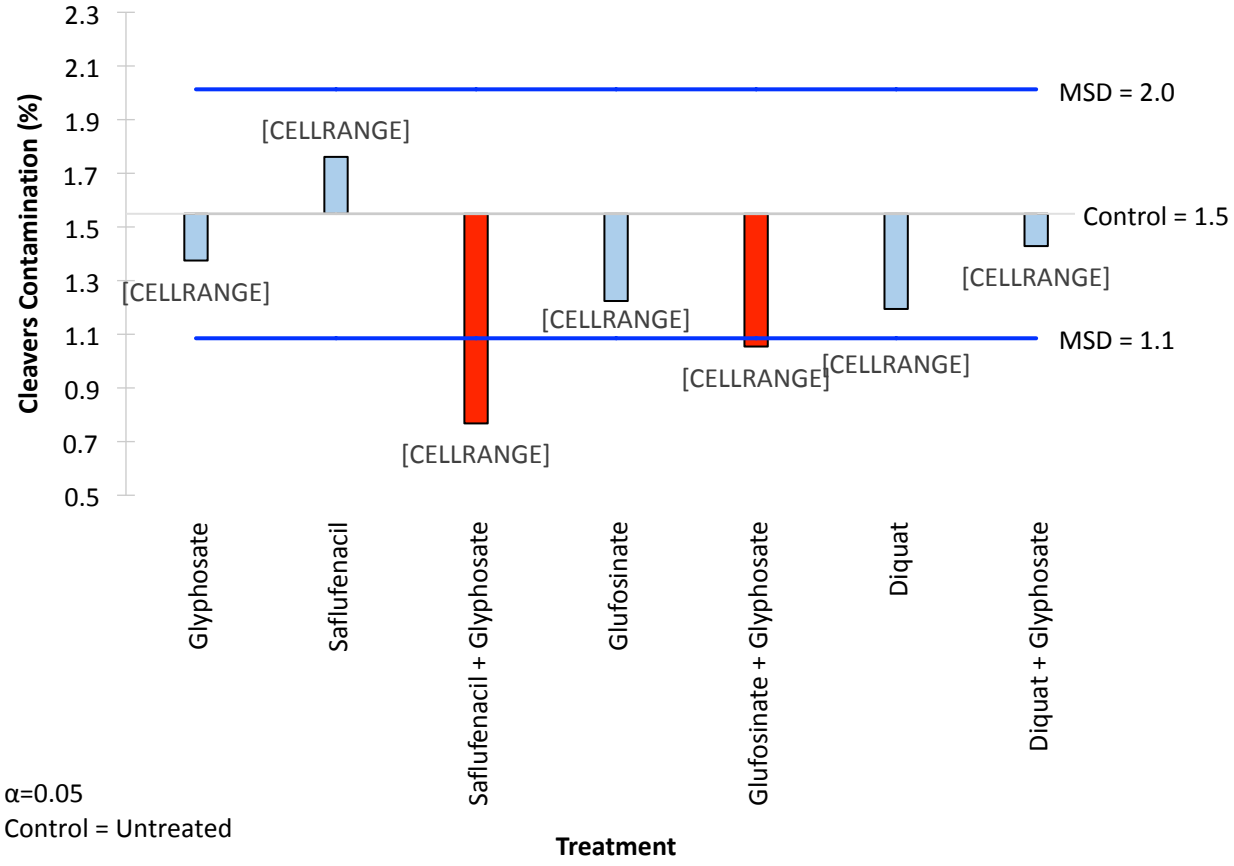

Figure 2. Dunnett's means comparison with untreated plots for cleavers contamination (%) in 2016. The X-axis represents the mean of the untreated check (1.5%). Blue lines represent the minimum significant difference (MSD) between the untreated check and all treatments. Red bars indicate a significant difference between the treatment and the untreated check. Letters above/below bars indicate a significant difference between treatments.

The electrical conductivity of the seed leachate collected from the seed soaking process was often higher with the herbicide treatments compared to the untreated check (Figure 3). Glyphosate applied alone, saflufenacil alone, and the combination of glyphosate + saflufenacil

increased cleavers seed electroconductivity by 92%, 88%, and 67% respectively compared with the check. Treatments of glufosinate + glyphosate and diquat applied alone did not produce significantly greater conductivity values compared to the untreated check. The addition of diquat to glyphosate did, however, produce a 3.3 $\mu Scm^{-1}g^{-1}$ increase in electrolyte leakage. Glufosinate applied alone also significantly increased seed electrolyte leakage by 2.6 $\mu Scm^{-1}g^{-1}$.

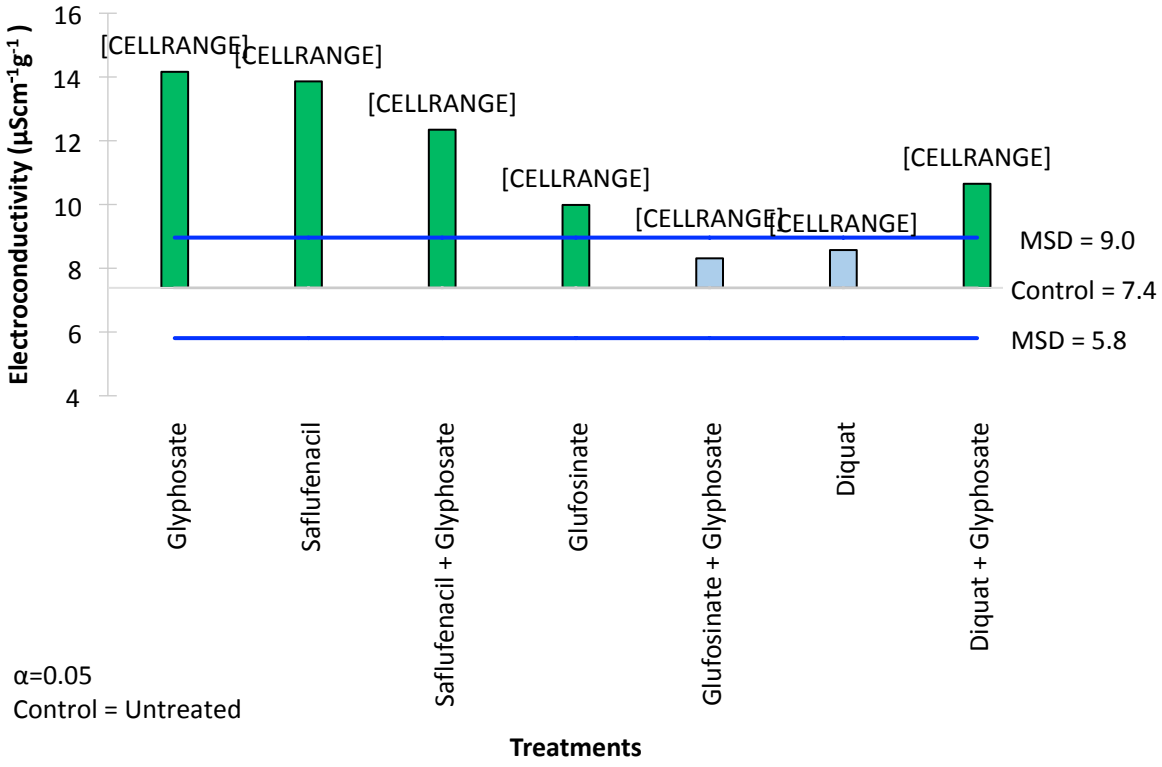

Figure 3. Dunnett's means comparison with untreated seed for cleavers electro conductivity $\mu Scm^{-1}g^{-1}$ in 2016 and 2017. The X-axis represents the mean of the untreated check (7.4 $\mu Scm^{-1}g^{-1}$). Blue lines represent the minimum significant difference (MSD) between the untreated check and all treatments. Green bars indicate a significant difference between the treatment and the untreated check. Letters above/below bars indicate a significant difference between treatments.

All pre-harvest herbicides significantly lowered cleavers seed viability compared to the untreated check (Figure 4). Treatments containing glyphosate and saflufenacil reduced cleavers seed viability by 49% and 44% respectively, and by 42% when both were applied together. Tank mixing diquat + glyphosate did not significantly reduce seed viability compared to diquat applied

alone (Figure 4). Both treatments resulted in 63% and 68% of cleavers seed being viable. The combination of glufosinate + glyphosate did not did not significantly reduce seed viability of cleavers more than when glufosinate was applied alone. Both treatments resulted in 69% viable cleaver seed. However, all treatments significantly reduced cleavers seed viability significantly more than the untreated check, where 89% of cleaver seeds were viable.

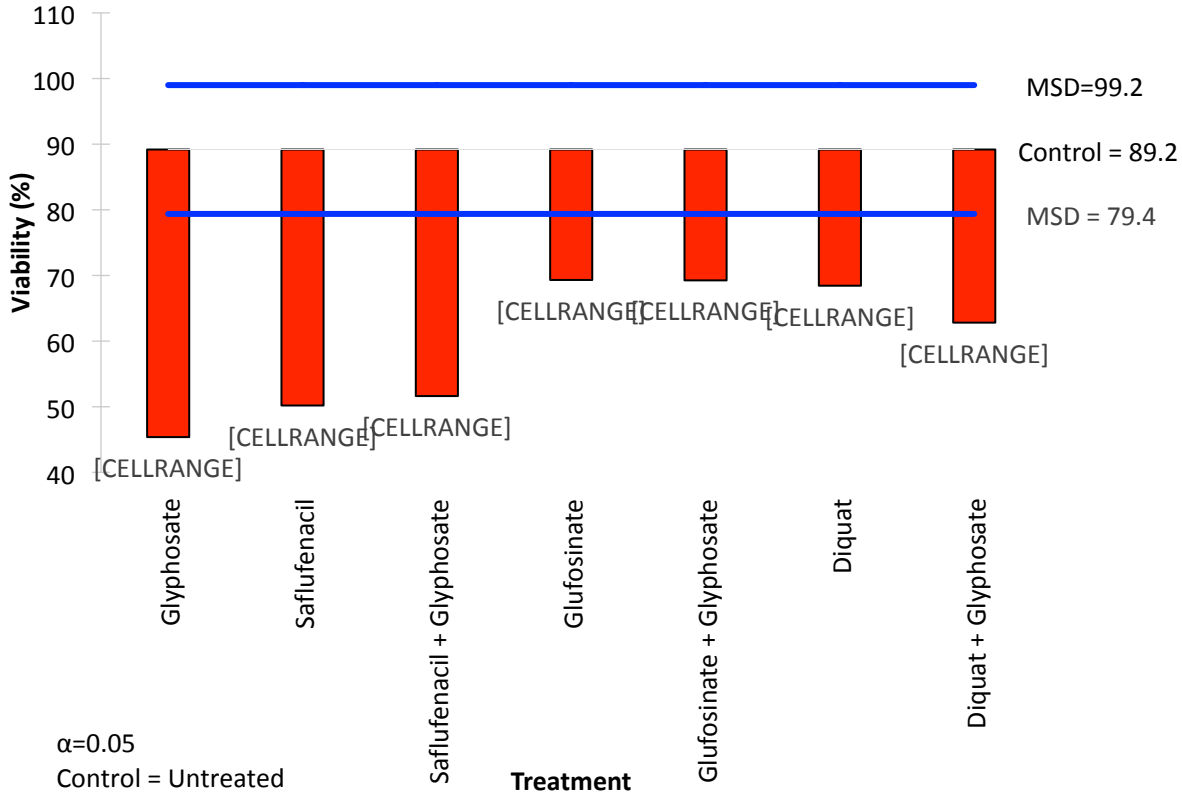

Figure 4. Dunnett's means comparison with untreated seed (89.2%) for cleavers seed viability (%) in 2016 and 2017. The X-axis represents the mean of the untreated check (89.2%). Blue lines represent the minimum significant difference (MSD) between the untreated check and all treatments. Red bars indicate a significant difference between the treatment and the untreated check. Letters above/below bars indicate a significant difference between treatments.

Discussion

Determination of the base germination temperature for western Canadian cleavers populations fills a knowledge gap that was not known prior to this project. The base temperature of 2° C determined in this study is lower and more precise than those previously reported in the

literature. Malik and Vanden Born (1988) reported that the base temperature for cleavers germination was between 2 and 20 °C, but they determined this base temperature using European accessions of *G. aparine*. In contrast, we used western Canadian accessions confirmed to be *G. spurium.* The lower base temperatures of the western Canadian accessions may be due to evolutionary adaption to the local climate. Tozzi et al. (2014) reported that seed of *Conyza canadensis* (*Conyza canadensis* (L.) Cronq. var. *canadensis*) sourced from Canada had lower base temperature requirements than seed sourced from Europe and the Middle East.

The consistency of the base germination temperature between the western Canadian accessions from diverse geographic locations indicates that the temperature required for germination is relatively constant between populations. This is not surprising as De Roo (2016) found these populations to have a high level of genetic relatedness. Using soil temperature data from the Kernen Research Farm (1994-2017), the average date at which the 5 cm soil depth reaches 2°C is April 8, indicating that cleavers germination could occur early in the growing season. Temperatures at the soil surface would likely reach that temperature threshold prior to this and so germination of seed dispersed on the soil surface could occur even earlier. The mean soil temperature at 5 cm does not drop below 2° C until November 1, indicating that germination could extend well into the fall, as has been observed years prior. Steinmaus et al. (2000) reported that winter annual weeds generally had lower base temperatures for germination than spring emerging annual weeds. The low base temperature for germination in the Canadian *Galium* accessions may help to explain why this species can act as a spring annual or winter annual weed in our environment. The ability to behave as a winter or summer annual could have arose in cleavers due to the consistent selection pressure imposed by freezing winter temperatures (Tozzi et al. 2013). As well, it would be more advantageous in a Canadian climate to have the ability to germinate at very low temperatures (Tozzi et al. 2013). Because G. *spurium* is largely an introduced, shade intolerant species and G. *aparine* is believed to be native to the woodlands of North America (Malik and Vanden Born 1988), the selection pressure to germinate earlier in the growing season could have been greater for *G. spurium* than for *G. aparine*. By germinating early, *G. spurium* could hypothetically avoid any early season competition for light and space. This evolutionary response may explain why all accessions of *G. spurium* that were evaluated had a base temperature of 2°C lower than *G. aparine*.

Combined across years, the field study showed that there was a detectable reduction in cleavers contamination from glufosinate + glyphosate and saflufenacil + glyphosate applications, although the number of sites was limited. Both of these pre-harvest herbicide mixtures reduced cleavers contamination from 1.5% (untreated check) to 1.1% and 0.7% contamination, respectively. This effect could be due to the additivity between ESPS inhibitors and other modes of action, which could impede the normal development of cleavers seeds. These results are similar to unpublished studies by Bertholet and Willenborg, which found that pre-harvest herbicides reduced kochia and mustard seed production and viability in lentil crops. Cleavers are considered conspicuous admixture in canola crops because it is inseparable from the cleaned canola sample (Canadian Grains Commission 2017). Therefore, when the weight of this inseparable material exceeds 2.0% of the net sample weight, the sample grade is lowered from a #1 to a #2 (Canadian Grains Commission 2009). While treatments with glufosinate + glyphosate did significantly reduce the percentage of cleavers found in the harvested sample, it was not enough for the sample to be classified as #1 canola.

While early emerging individuals may produce more seeds then late emerging individuals, the viability of these seeds is not impacted by competition from the crop (Bagavathiannan and Norsworthy 2012). The integrity of cell membranes ultimately determines the vigour and viability of seeds (Milošević et al. 2010). Therefore, implementing production practices that can impact both the amount of weed seeds produced and the viability of these seeds is imperative to ensure effective, long term weed control. Using pre-harvest herbicides is a production practice that can accomplish both of these goals. In addition to reducing contamination of the harvested sample, a number of pre-harvest herbicides included in the current study also reduced cleavers seed viability and increased electrolyte leakage, which indicates reduced vigor of the seeds. By subjecting cleavers seeds to an electrolyte leakage test, we are able to predict if the pre-harvest herbicides in our study are having an impact on seed viability (Milošević et al. 2010). Five of the seven treatments we examined increased electrolyte leakage from cleavers seeds, and resulted in reduced seed viability. Therefore, applying these herbicides pre-harvest significantly impacted the viability of cleavers seeds, which could reduce overall plant fitness and contributions of viable seed to the weed seed bank. However an important consideration that was not examined in this project is the amount of pesticide residue in the harvested canola sample. Saflufenacil, glyphosate, glufosinate, and diquat have maximum residue limits (MRLs) of 0.5, 20, 3, and 1 ppm respectively (Health Canada 2012). Furthermore

the pre-harvest interval varies for these herbicides, which presents a potential residue issue for the harvested grain. Specifically canola can be harvested 3 days after saflufenacil has been applied, and 7 – 10 days after glyphosate or diquat has been sprayed (Canola Council of Canada 2018). However glufosinate has a minimum pre-harvest interval of 60 days. While we did not measure MRLs in this experiment, treatments were applied 7-14 days before harvest. Therefore we can anticipate a residue issue from those treatments containing glufosinate.

As western Canadian producers begin to grow more shatter-resistant canola varieties, they are looking to desiccate rather than swath there canola crops. With this change there is an opportunity to impact the seed rain of weeds that escaped early season control (i.e. escapees) or emerged late in the growing season. Davis (2006) stated that weed seedbanks are the primary source of persistent weed infestations in the field, and the sole means for annual weeds to increase their population. Moreover, escapees are the largest contributor to weed seedbanks, and it is possible for a few individual plants to replenish the weed seed bank (Bagavathiannan and Norsworthy 2012). For instance, common lambsquarters (*Chenopodium album* L.) that emerge late in the growing season can still produce upwards of 500 seeds m^{-2}, which could replenish the soil seedbank for the upcoming growing season (Scursoni et al. 2007). Therefore expanding weed management practices beyond controlling only seedlings, to also account for weed seedbank management, is crucial to optimize weed management in the future (Davis 2006). Cleavers management may benefit from such an approach as it retains a high percentage of its seed until crop harvest, making it more susceptible to pre-harvest herbicides (Burton et al. 2016; Tidemann et al. 2017). There is an opportunity to help mitigate cleavers seed rain to the seedbank and thus, future cleavers populations by targeting cleavers seed development with pre-harvest herbicides in canola crops.

Cleavers seed can remain dormant in the soil for approximately two years, and viability of that seed lasts for 2 – 3 years (Malik and Vanden Born, 1988). Hence, cleavers is not believed to be as persistent as other weedy species such as wild mustard. Therefore, improved cleavers control through diligent seed bank management should be possible. Davis (2006) noted that weed management systems targeting annual weeds can be optimized if weed seedbank management measures were integrated with effective seedling management. Moreover, decreasing seedbank contributions was more effective at reducing the weed population than controlling only the aboveground growth of weeds with low soil persistence and moderate seed

production. Further reductions to the seedbank can be achieved by reducing the viability of weed seeds. Thus, reducing cleavers seed rain and/or viability could improve the long term control of cleavers could by reducing the number of individuals germinating in the fall or spring. Proactive weed seedbank management is a critical component to prevent the evolution and dissemination of herbicide resistant genes in arable weeds (Bagavathiannan and Norsworthy 2012). By diligently managing the weed seedbank, producers can prevent the development of herbicide resistance, which may have arose due to mutation (Bagavathiannan and Norsworthy 2012). It is possible that *Galium* spp. will be the next weed to evolve resistance to glyphosate (Beckie 2010) and therefore, robust management practices that can be effective over the long term are necessary.

The soil seedbank is the only way an annual weed can remain persistent year after year (Davis 2006). Cleavers are an annual weed, with a wide germination window, and the ability to produce prolific amounts of seed. Managing that seed rain later in the growing season is imperative if we are to delay the onset and spread of herbicide resistant cleavers in Canada. Research has shown that by managing both the weed seedling and weed seedbank, more efficacious, long-term control can be achieved. This research has identified that cleavers have the ability to germinate from April to November, and that the viability and the amount of contamination from cleavers at harvest can be reduced by using pre-harvest herbicides. Using pre-harvest herbicides not only increases harvesting efficiency (Bagavathiannan and Norsworthy 2012), but it has a positive impact on weed seedbank management as well. While other research has explored the impact of pre-harvest herbicides on weed seedbank survival, this is an aspect of cleavers management we have yet to explore. Moreover, this study examined the effect of pre-harvest herbicides on seed vigour; however, the effect of these treatments on the growth and development of subsequent cleavers seedlings has not yet been evaluated. Understanding the impact of pre-harvest herbicides on successive cleavers populations will help to establish a more well-rounded management strategy for producers.

Conclusion

The base temperature for germination of all the western Canadian accessions of *G. spurium* was 2° C, indicating that germination can occur very early and continue very late in the growing season. This information, combined with previous information collected by this lab on

emergence patterns, fills an information gap on the germination and emergence patterns of this species. Because of the low temperatures at which cleavers can germinate, producers would be well advised to scout fields late into the fall and early in the spring after the snow has melted to ensure identification of early emerging or overwintering cleavers populations. Our results may also help to explain the ability of cleavers to act as a facultative winter annual as opposed to an obligate winter annual.

Moving forward with this research, we plan to refine the base temperature of each cleavers population using specific regression models for the purposes of publication. As well, there appears to be potential for the use of pre-harvest herbicides to reduce cleaver seed production, as well as to reduce viability and vigour. Future research should focus on determining the best timing for reducing cleavers seed development and viability by applying herbicide to a pure stand of cleavers at various stages of development. A growing degree-day model could be developed, which could also provide some indication as what crops would be most suitable for optimum control of cleavers seed. With the increasing incidence of cleavers across the prairies coupled with the increasing acres of glyphosate and glufosinate-resistant canola, an opportunity exists to improve cleavers management by targeting the seedbank. .

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