Final Report

ADF20120028 – Emergence timing and management of cleavers in Saskatchewan canola crops

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October, 2015

1. Project title and ADF file number.

ADF20120028 – Emergence timing and management of cleavers in Saskatchewan Canola Crops.

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4. Abstract/ Summary: *This must include project objectives, results, and conclusions for use in publications and in the Ministry database. Maximum of 300 words in lay language.*

Galium species (cleavers) have been identified as the most competitive common broad-leaved weed in winter cereals (Wilson and Wright 1987). An increased presence of two species, *G. aparine* and *G. spurium*, has been observed in western Canada (Leeson et al. 2005), but these species are difficult to differentiate. Moreover, these species are difficult to control in many crops, such as canola. Proper identification and improved control can lead to better management practices for cleavers in canola. In this study, experiments were completed to aid growers in managing cleavers by characterizing the emergence and genetic characteristics of cleavers populations in Western Canada. In addition, we determined the response of cleavers to new herbicides such as quinclorac and clomazone. Finally, we assessed the response of cleavers populations to glufosinate-ammonium, glyphosate, and quinclorac to assess whether differences among

populations existed.

The variation between *G. aparine* and *G. spurium* at position 230 within the 5.8S was used to create a molecular marker that determined all cleavers samples we obtained from across western Canada *G. spurium* species. Vegetative morphological traits were not significantly different between the Canadian populations, with the exception of start and end of flowering period and emergence timing. Emergence timing was significantly different between years in the spring and fall, but populations did not separate by geographical location. These differences suggest growers will have to consider the emergence timing of local populations when controlling cleavers.

All populations within each herbicide system responded similarly to glufosinate-ammonium, imazapyr+imazamox, and quinclorac, despite being from different locations in western Canada. This is quite favorable for growers since the field plots showed that clomazone and quinclorac significantly reduced cleaver biomass, cleaver seed contamination, and improved cleavers control in canola crops. Therefore, registration of these herbicides will significantly improve cleavers control in western Canadian canola crops.

5. Introduction: *Brief project background and rationale.*

Galium species (cleavers) are a member of the *Rubiaceae* or Madder family that has flourished in a variety of crops. Three species, *Galium aparine* L.*, Galium spurium* L.*,* and *Galium boreale* L. are present on the Canadian prairies. An increased presence of two species, *G. aparine* and *G. spurium,* has been recorded in field surveys. Research in Canada has referred to cleavers populations as *G. spurium* or *G. aparine* and European researchers identify their cleavers as predominately *G. aparine,* even though molecular technology has not been used to identify *Galium* species. Currently, chromosome counts are currently the most effective way to tell the species apart from one another, but this process is very tedious and time consuming.

The increasing frequency of cleavers in western Canada makes it vital to understand why they are successful and how to better control them. Cleavers have been identified as the most competitive common broad-leaved weed in winter cereals (Wilson and Wright 1987). Malik and Vanden Born (1988) established that a cleavers density of 100 plants/m^2 caused yield reduction in canola between 4 and 28% depending on the emergence date of the weed relative to the crop. At high infestation levels, *G. spurium* emerging with a rapeseed crop, or one week after the crop, resulted in a seed contamination level of 31 and 72 seeds$\cdot g^{-1}$ of rapeseed, respectively (Malik and Vanden Born 1988). In addition to competing with the crop for light, water, and nutrients, *Galium* species have weak, climbing stems, which can cause crop lodging and harvesting problems (Defelice, 2002). Cleavers are described as a highly prolific seed producer with an average of 300 and 400 seeds from a single *G. spurium* plant and with a maximum production of 3500 seeds when grown under ideal conditions (Malik and Vanden Born 1988).

The population dynamics of cleavers in canola is extremely important to crop quality, and emergence timing may play a large role in cleavers management. Even at relatively low infestation levels, canola oil quality can be dramatically reduced by the presence of cleavers seeds (Malik and Vanden Born, 1987). The seeds of

both cleavers and canola are very similar in size and shape, making them difficult to separate (Canola Council, 2014). Currently, mechanical removal of cleavers from canola is not possible. According to the Canadian Grain Commission, cleavers found in canola can downgrade samples, as samples must contain less than 1.0% other seeds that are conspicuous and not readily separable from canola (Canola Council, 2014).

The substantial increase in cleavers distribution and frequency may be partially attributed to the increase in canola acres. However, with the advent of herbicide resistant canola varieties, it was believed that cleavers would no longer be a significant weed species on the Canadian prairies. Nevertheless, a population of Group 2 resistant biotypes found in central Alberta was identified as also cross-resistant to quinclorac, an auxin-type Group 4 herbicide (Hall, et al. 1998). Despite wide spread screening in subsequent years, no others cases of quinclorac resistance have been found (Beckie, 2011). This is not surprising given that resistance in other auxinic herbicide-resistant species has not become widespread, regardless of the genetic inheritance mechanism between *Galium* species (Saskatchewan Ministry of Agriculture, 2015. A study published by Froud-Williams and Ferris-Kaan, (1991) found that there is significant intraspecific genetic variation within *Galium* species with respect to the responses to mecoprop. Another major problem for growers is that cleavers resistant to Group two herbicides have also been spreading across western Canada, especially in Saskatchewan (Beckie et al., 2012). Although these cases of resistance have been reported in *Galium spurium*, it is not know whether cleavers populations of are mixed species across the prairies, which could affect the spread of resistance.

Advancements in biotechnology can potentially increase the efficiency in species/biotype identification and aid in proper management. The increased use of molecular biology in weed science, such as markers in the ITS region, have helped in proper species identification, detecting weedy traits, and monitoring the effects of selection pressures on weed populations. *G. aparine* and *G. spurium* are nearly impossible to visually distinguish, as many of their morphological characteristics very similar. For example, *G. aparine* has whitish flowers with a diameter of 2 mm, seeds normally 2.8-4 mm long, and linear leaves that are oblanceolate and up to 50 mm long and 5 mm in width. *G. spurium* has greenish-yellow flowers 1-1.5 mm in diameter, fruits 1.5-2.8 mm long, and narrow leaves that are linear to lanceolate in shape (Moore 1975). Such small visual differences would suggest that these species are unlikely to be differentiated by producers or agronomists, which is problematic when recommending effective and efficient control options. Hence, there is a need to determine a reliable method of detection and also, to determine which species exist in western Canada.

6. Methodology: *Include approaches, experimental design, methodology, materials, sites, etc.*

1) Assessing the efficacy of several novel herbicides on cleavers

Field experiments were conducted in 2013 and 2014 at two locations, the Scott Research Farm (Scott) (52^{O} 07’ N, 106^{O} 43’W) and the Saskatchewan Pulse Growers research site (SPG) near Saskatoon (52^{O} 36’ N, 108^{O} 83’ W). An additional site was added at Rosthern (52^{O} 67’ N, 106^{O} 38’ W) in 2014. The Scott site is located in on Dark Brown Chernozemic soil with a pH of 5.9 and 4.0% organic matter, the SPG site was

located on Moist Dark Brown Chernozemic soil with a pH of 7.0 and 3.5% organic matter, while the Rosthern site was located on Black Chernozemic soil with a pH 7.9 and 4.6% organic matter. Each canola herbicide system was set up as its own trial. Treatments within each trial were eight different herbicide combinations, with border plots surrounding the trial. The experimental layout was a randomized complete block design with four replicates. Each replicate consisted of eight treatments with a 2 x 6 m plot size.

Experimental treatment and establishment

Table 1. Field trial Experiment, treatment order for field efficacy research

	Clearfield System
1	Control (untreated check)
2	HT Standard - ARES herbicide (9 g ai/ha imazapyr + 20 g ai/ha imazamox + Merge (0.5 v/v)
3	Quinclorac alone (100 g ai/ha) + Merge Adjuvant (0.5v/v)
4	Clomazone Alone (120 g ai/ha)
5	Clomazone (120 g ai/ha) FB quinclorac (100 g ai/ha) + Merge Adjuvant (0.5 v/v)
6	Ares herbicide (as above) FB quinclorac (50 g ai/ha) + Merge Adjuvant (0.5 v/v)
7	Clomazone (120 g ai/ha) FB ARES (as above)
8	Clomazone (120 g ai/ha) FB ARES (as above) + quinclorac (50 g ai/ha) + Merge Adjuvant (0.5 v/v)

	Liberty-Link System
1	Control (untreated check)
2	HT Standard- Liberty herbicide (500 g ai/ha)
3	Quinclorac alone (100 g ai/ha) + Merge Adjuvant (0.5v/v)
4	Clomazone Alone (120 g ai/ha)
5	Clomazone (120 g ai/ha) FB quinclorac (100 g ai/ha) + Merge Adjuvant (0.5 v/v)
6	Glufosinate herbicide (as above) FB quinclorac (50 g ai/ha) + Merge Adjuvant (0.5 v/v)
7	Clomazone (120 g ai/ha) FB glufosinate (as above)
8	Clomazone (120 g ai/ha) FB Glufosinate (as above) + quinclorac (50 g ai/ha) + Merge Adjuvant (0.5 v/v)

Roundup-Ready System	
1	Control (untreated check)
2	HT Standard- glyphosate herbicide (450 g ae/ha)
3	Quinclorac alone (100 g ai/ha) + Merge Adjuvant (0.5v/v)
4	Clomazone Alone (120 g ai/ha)
5	Clomazone (120 g ai/ha) FB quinclorac (100 g ai/ha) + Merge Adjuvant (0.5 v/v)
6	Glyphosate herbicide (as above) FB quinclorac (50 g ai/ha) + Merge Adjuvant (0.5 v/v)
7	Clomazone (120 g ai/ha) FB glyphosate (as above)
8	Clomazone (120 g ai/ha) FB glyphosate (as above) + quinclorac (50 g ai/ha) + Merge Adjuvant (0.5 v/v)

*FB = Followed by

Eight herbicide treatments were used in this experiment to evaluate their efficacy on cleavers. The herbicide standard for each canola system was used alone and with the addition of quinclorac and/or clomazone (Table 1). At all sites canola varieties (L130, 73-75, and 45H73), resistant to their respective herbicide system, were seeded into cereal stubble. In 2013 clomazone treatments were applied May 22nd at SPG and May 28th at Scott, SK with canola seeding occurring two days after (clomazone was applied pre-plant). In 2014, clomazone treatments were applied May 26th at SPG, May 29th at Scott, and June 2nd at Rosthern, SK. Seeding occurred on May 29th at SPG, May 31st at Scott, and June 4th at Rosthern. Canola was seeded at 150 seeds per m^{-2} in 25 cm rows and at a depth of 2 cm. The seeding rate was adjusted for each canola variety based on 1000 kernel weight and an assumed 40% mortality rate. At both the SPG site (2013 & 2014) and Rosthern (2014), cleavers were broadcast and then lightly harrowed. At Scott (2013 & 2014), cleavers were broadcast mid row at the time of seeding. Cleavers seed was broadcast at 350 seed per m^{-2} to target a plant stand of 75-100 plants per m^{-2}. Nitrogen and phosphorous fertilizers were applied at the time of seeding at each location based on spring soil test recommendations. Pre-emergence weed control was achieved by applying glyphosate at 950 g a. i. ha^{-1}. Target timing for herbicide applications was the 2-4 whorl stage. In-crop herbicide treatments at SPG (2013 & 2014) and Scott (2013) were applied when the crop was at the 4-leaf stage. Quinclorac treatments were applied either alone or as a tank-mixture with herbicide standards for their respective herbicide-resistant canola system. Data collection occurred as outlined in Table 2. Data collected are shown in Table 2.

Table 2. Field trial Experiment data collection

Measurement	Details
Crop Count	2-3 weeks after emergence, count number of plants in 2, 1m rows per plot

Weed Count	2-3 weeks after emergence, count number of plants in 3, 0.25 m^2 quadrates per plot
Crop/cleavers injury Rating	Rate pre-seed treatment plots prior to in crop herbicide and others @ 7-10, 14-21, > 28 days after herbicide application on CWSS scale.
Crop/weed biomass	Cut all plants (at canola pod fill) at soil surface in 2 x 0.5m^2 quadrats in each 2 x 6 m plot. Separate cleavers from canola and place in separate cloth or paper bags, dry until moisture dissipated from plants, and weigh (record the dry weight).
Plant Height	During the podding stage, measure the height of 5 individual canola plants
Crop yield	Seed Yield, % moisture at harvest. Keep all of the samples - dry to below 10% and to uniformity. Cleaver seed will be cleaned from 200g samples and weighed to estimate the number of seeds.
Thousand seed weight	Count 250 seeds of each sample, multiply by factor of 4 to achieve TSW

2) Assessing the response of cleavers populations to various herbicides

To determine the response of cleavers populations to herbicides, growth chamber experiments were carried out at the phytotron facility at the University of Saskatchewan in November of 2013 and again in March of 2014. Dose response experiments were performed in a controlled environment with a separate experiment for each herbicide (glufosinate-ammonium, imazamox+imazapyr, quinclorac) (Table 3). All treatments were replicated three times. Dose response experiments were conducted on three different *Galium* populations, with seed obtained from Lacombe, AB, Vegreville, AB, and Saskatoon. Prior to conducting the experiment, *Galium* populations were screened for group 2 resistance.

Experiments were conducted in 1-L plastic pots, using commercial potting mixture, which were watered and fertilized to maintain optimum growth. Several seeds were planted in each pot, and pots were thinned to five individuals per pot at the two-whorl stage. All pots were randomized weekly. Herbicides were applied at the three-whorl stage, and data collection occured as listed in Table 4.

Table 3. Dose Response Experiment treatments. All treatments conducted at the 3-whorl stage.

Glufosinate-ammonium (g ai/ha)	Ares (imazapyr + imazamox) (g ai/ha)	Quinclorac (+merge adjuvant) (g ai/ha)
0	0/0	0
19	0.563/1.125	2.5
37	1.125/2.5	5
75	2.25/5	10
125	4.5/10	40
250	9/20	80
500	18/40	160
1000		

Table 4. Dose Response Experiment Data Collection

Measurement	Details
Weed Injury	Rate all pots @ 7-10, 14 days after herbicide application on CWSS scale.
Weed biomass	In both dose response trials, aboveground biomass was harvested 21 days after herbicide application, oven dried, weighed and expressed as a % of the untreated control.

3) Genetic, morphological, and molecular characterization of *Galium* species in Western Canada

Sequencing of the Internal Transcribed Spacer Region

Cleavers samples were collected from nine locations across Western Canada and two locations from Europe (only one reported below). Land locations are in Table 5.

Table 5. Canadian cleavers populations to determine species complex

Name	Land Location	Name	Land Location
*Lacombe	52.4851, -113.6393	*Melfort - Heavin	52.8710, -104.6117
*Vegreville	53.5200, -112.0781	*Melfort- Trawin	52.8697, -104.5103
*Carrot River – Clancy	53.2794, -103.5847	Yorkton	51.6370, -102.4298
*Saskatoon – SPG	52.0637, -106.4440	Ontario	N/A
Moosomin	49.9964, -101.8942	Manitoba	N/A

Five plants of each reference population (known *Galium spurium* and *aparine*) and ten plants of each Canadian population for molecular analyses were grown in a controlled growth chamber at the University of Saskatchewan in 11 cm diameter pots filled with a soil-less mix. Seeds were germinated in 24 hour darkness at 10°C for 8 hours and 15°C for 16 hours. After germination, plants were established in a 16 hour photoperiod at 18°C/12°C day/night. Plant material was harvested at the two-whorl stage 4-6 weeks after planting and stored on ice before DNA extraction. DNA extraction was done using the CTAB procedure modified by the Cytology and Molecular Genomics lab at the University of Saskatchewan. The ITS region was amplified in polymerase chain reaction using ITS1 and ITS4 primers. The amplified region was ligated into Invitrogen TOPO-3 vectors and cloned into E. coli bacteria. A Qiagen plasmid prep kit was used to remove the cloned ITS region from three bacteria colonies of each plant to be sent for sequencing. Sequencing was done at the Plant Biotechnology Institute at the University of Saskatchewan.

Emergence Timing and Morphological Characteristics

Initial germination tests were conducted on each sample by lining eight petri dishes with two layers of filter paper. Fifty seeds of each sample were placed in their corresponding petri dish and the filter paper was wetted with water before being placed in the dark. Supplemental water was added when necessary and germinated seeds were counted after fourteen days. Seeding rates were then adjusted for germination rate and each individual sample (plot) was blended with sand and broadcast onto fallow at a rate of 400 seeds per m^2. Microplots (1 m x 2 m) were then lightly raked (individually) to cover the seeds with soil. Seeds were planted in early May and early September to determine whether populations were spring or fall emerging, respectively. Soil preparation included soil samples to determine nutrient requirements, tillage to eliminate residue, and a pre-burn herbicide application of glyphosate (900 g ae ha^{-1}) before seeding to control emerged weeds. Group 1 herbicides were used to control grassy weeds as needed, while broadleaf weeds were hand weeded throughout the season.

Several morphological characteristics were collected on each plot throughout the growing season, including leaf area. Leaf area was collected from five randomly selected plants by removing all of the leaf material and

running it through a leaf area meter. Biomass measurements were also collected from these same plants by cutting plants at the soil surface, drying them, and then determining the sample dry weight.

The experimental design was a randomized complete block design containing 4 replications at two locations (Kernen, SK and Goodale Research Farms, SK). The experiment was repeated (2013-2014), though only a single site and year are necessary for the common garden approach. We chose a common garden approach (growing several populations of diverse geographic origin) because it minimizes environmental variability when testing for genetic causes underlying variability between populations. A total of 64 microplots were present at each location. Newly emerged cleavers plants within three randomly placed 0.15 m^2 quadrats were enumerated daily and marked with a rubber band to ensure they were not counted twice. Emergence data from each population was collected daily and the data were fit to nonlinear curves that best described the sigmoidal shape of the emergence curve using the *drc* package of R.

7. **Research accomplishments**: *(Describe progress towards meeting objectives. Please use revised objectives if Ministry-approved revisions have been made to original objectives.)*

Objectives	**Progress**
1) To assess various herbicides with regard to their efficacy on cleavers in canola. This should provide growers with new herbicide options to manage cleavers in canola crops.	Objective was met. Trials conducted over two years and at three sites consistently showed that tank-mixing quinclorac with any of the herbicide standards improve cleavers control in canola. Applying clomazone prior to seeding (pre-plant) canola followed by an in-crop application of a herbicide standard also provided acceptable control of cleavers. It is expected that both of these products will be widely utilized in canola once registered.
2) To determine the response of cleavers species and/or populations to glufosinate-ammonium, glyphosate, and quinclorac herbicides.	Objective was met. In a series of greenhouse dose-response trials, populations appeared to have minor differences in their parameter estimates. Small differences in the ED_{50} (dose to 50% injury) were evident across populations for all herbicides tested, as were minor differences in the slopes. Further analyses are required to confirm these responses.

3) To characterize the differences in morphological characteristics among cleavers populations in western Canda	Objective was met. A molecular marker was identified (and confirmed to be accurate) that can be used to differentiat between Galium species. Using this marker, it was determined that all sampled populations from across western Canada were *Galium spurium*, or false cleavers. Populations generally exhibited little variation for morphological traits, but exhibited some differences in emergence timing.

add additional lines as required

8. Discussion: *Provide discussion necessary to the full understanding of the results. Where applicable, results should be discussed in the context of existing knowledge and relevant literature. Detail any major concerns or project setbacks.*

1) Assessing the efficacy of several novel herbicides on cleavers

There was a significant treatment effect on cleaver biomass ($P < 0.05$) in the imidazolinone-resistant trial (Table 6), indicating that there are differences between imazamox+imazapyr (Ares), quinclorac, clomazone and various combinations of these herbicides. All herbicide treatments significantly decreased cleavers biomass compared to the unsprayed check (Figure 1). Imazamox+imazapyr, clomazone, and quinclorac applied alone reduced cleavers biomass by 66, 42, and 80% respectively (Figure 1). The additive herbicide treatments of clomazone+ imazamox+imazapyr, clomazone+quinclorac, imazamox+imazapyr +quinclorac and clomazone+ imazamox+imazapyr +quinclorac reduced cleavers biomass by 52, 83, 95 and 99% respectively (Figure 1). With the exception of the combination of clomazone+ imazamox+imazapyr, the additive treatments all significantly ($P < 0.05$) decreased cleavers biomass compared to the single herbicide treatments (Figure 1). The efficacy of clomazone on cleavers that we observed was comparable to research conducted in Norway (Dæhli et al. 2011); clomazone is registered on cleavers throughout most of western Europe (FMC, 2014). These results are also similar to those reported by Sapsford et al. (2015), which found that clomazone applied as a sole active ingredient did not achieve control of cleavers. However, when used in conjunction with an in-crop herbicide, cleavers control significantly increased. Quinclorac effectively controlled cleaver biomass, which is similar to the results of Grossmann et al. (2001).

Table 6. ANOVA of field trial for weed biomass, plant height, crop yield and thousand seed weight as affected by herbicide treatment combined across locations and years.

Herbicide System	Weed biomass	Plant height	Crop Yield	Thousand Seed Weight
Imidazolinone-resistant	0.0049**	0.0812	0.0246*	0.131
Glyphosate-resistant	0.0061**	0.0754	0.0385*	0.0698
Glufosinate-resistant	0.012**	0.3761	0.0396*	0.0984

*,**,*** denote significant at the 0.05, 0.01 and 0.001 † denotes significant at the 0.1 level.

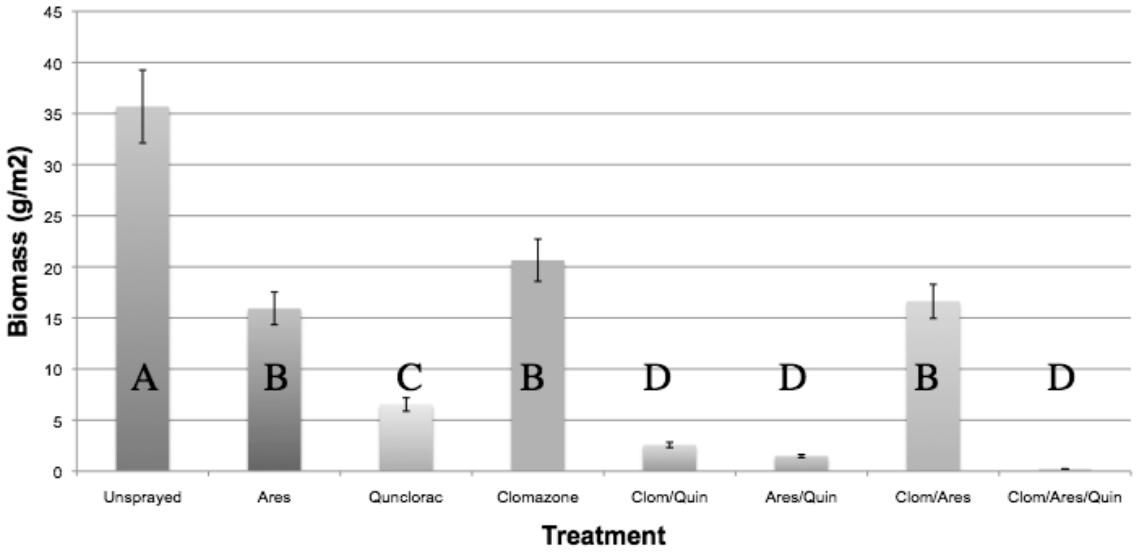

Figure 1. Cleavers biomass in imidazolinone tolerant canola as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

In the glyphosate-resistant system, all herbicide treatments significantly reduced cleavers biomass compared to the unsprayed check (Table 6, Figure 2). Clomazone alone reduced cleaver biomass by 43%, while glyphosate and quinclorac treatments showed an 80% reduction. Combination treatments either by tank mixing or sequential application significantly improved cleavers control. Clomazone+quinclorac reduced cleavers biomass by 91%, whereas glyphosate+quinclorac reduced cleavers biomass by 96% and clomazone+glyphosate almost eliminated cleavers from the plots (99% reduction in biomass).

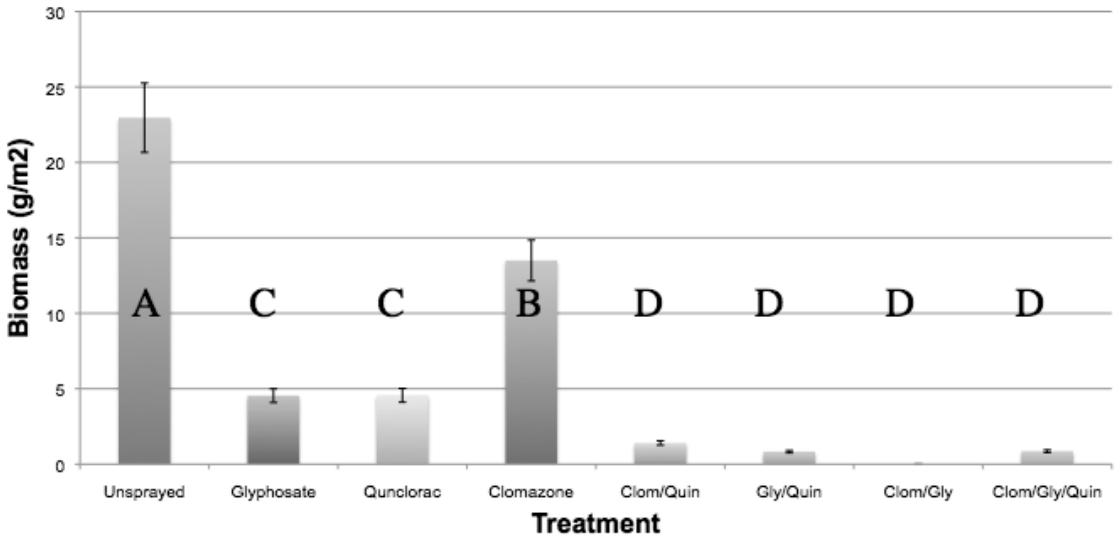

Figure 2. Cleavers biomass in glyphosate-resistant canola as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

Glufosinate alone did not control cleavers, and only provided a 40% reduction in cleavers biomass (Figure 3). Clomazone applied alone was considerably better, exhibiting a 62% reduction in biomass, while quinclorac provide excellent control and a 94% reduction in biomass. The addition of clomazone, glufosinate or both to quinclorac did not significantly improve control over quinclorac alone (Table 6). Relative to the unsprayed control, clomazone applied prior to glufosinate did substantially decrease cleavers, however (59%).

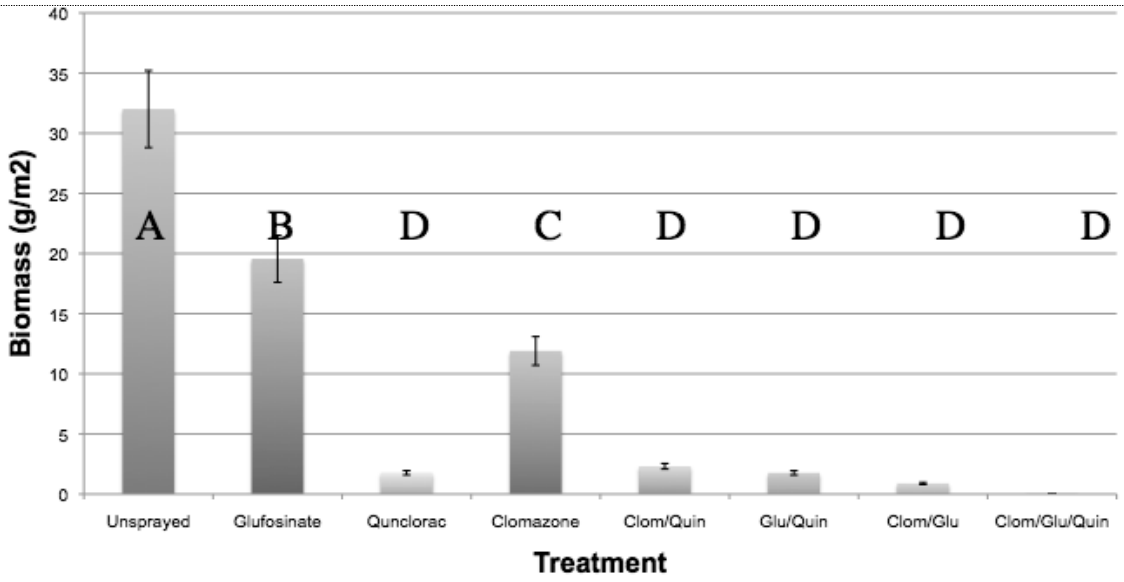

Figure 3. Cleavers biomass in glufosinate-resistant canola as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

Plant Height

There was no significant herbicide effect on plant height ($P > 0.05$) (Table 6), indicating that there was no interaction between herbicide treatments and plant height at any site, year, or herbicide system. Much research has documented the relationship between plant height and competition as it relates to light (Singh et al. 2000, Xiao et al. 2006, Massings et al. 2003). It is well established that many species, in an attempt to outcompete their neighbours for light, will modify their growth habit and grow taller (Schmitt et al., 1995; Dudley and Schmit, 1996; Huber and Wiggermann, 1997; Anten and Hirose, 1998; Huber et al. 1998). *Galium* species do not fall into this category primarily because they are known to be shade tolerant, reducing the need for direct sunlight (Defelice, 2002). In addition, cleavers exhibit a semi-self-supporting, scrambling-ascending growth habit, which forces them to partially rely on nearby plant species to vertically extend themselves. Both the growth habit and light preference of cleavers may explain the lack of significance between crop height and herbicide treatments.

Crop Yield and seed weight

In all sites, years, and herbicide systems, crop yield was affected by herbicide treatment ($P < 0.05$) (Table 6,7,8). In the glufosinate system yield was significantly increased from the unsprayed check in all treatments except when clomazone was applied alone (Figure 4). Glufosinate when applied alone improved yield by 30% over the unsprayed control. The addition of quinclorac, clomazone and clomazone/quinclorac to glufosinate also significantly improved yield by 45, 24, and 53%, respectively, compared to glufosinate applied alone (Figure 4). Collectively this shows that the addition of these tank-mixtures provides excellent

crop safety and increased canola yield under competition from cleavers. Clomazone applied alone did not increase yield over glufosinate applied alone, which is consistent with visual efficacy ratings that showed a steady decline in clomazone performance (cleavers control) as the season went on.

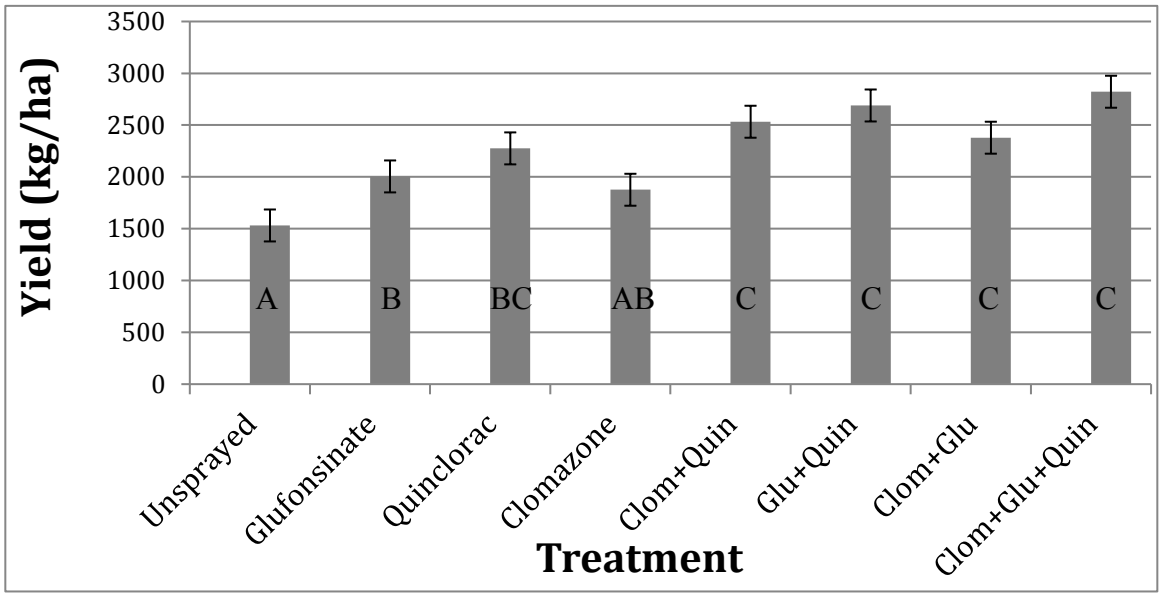

Figure 4. Canola yield in glufosinate-resistant canola as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

In the glyphosate-resistant system, all treatments (with the exception of clomazone alone) significantly improved yield compared to the unsprayed check (Figure 5). Glyphosate and quinclorac applied alone improved yield by 36 and 29%, respectively. Applying clomazone prior to a tank-mix of glyphosate and quinclorac further improved yields by an additional 13 and 15%, respectively. Tank mixing glyphosate and quinclorac significantly improved canola yield compared each herbicide applied alone. Moreover, this tank-mix exhibited 46% greater yield than the unsprayed check, and 10% better yield than glyphosate alone. The combination of clomazone (pre-emergence), quinclorac and glyphosate (tank-mix) was the highest yielding treatment, exhibiting a 54% yield increase compared to the unsprayed check, and an 18% better yield than glyphosate.

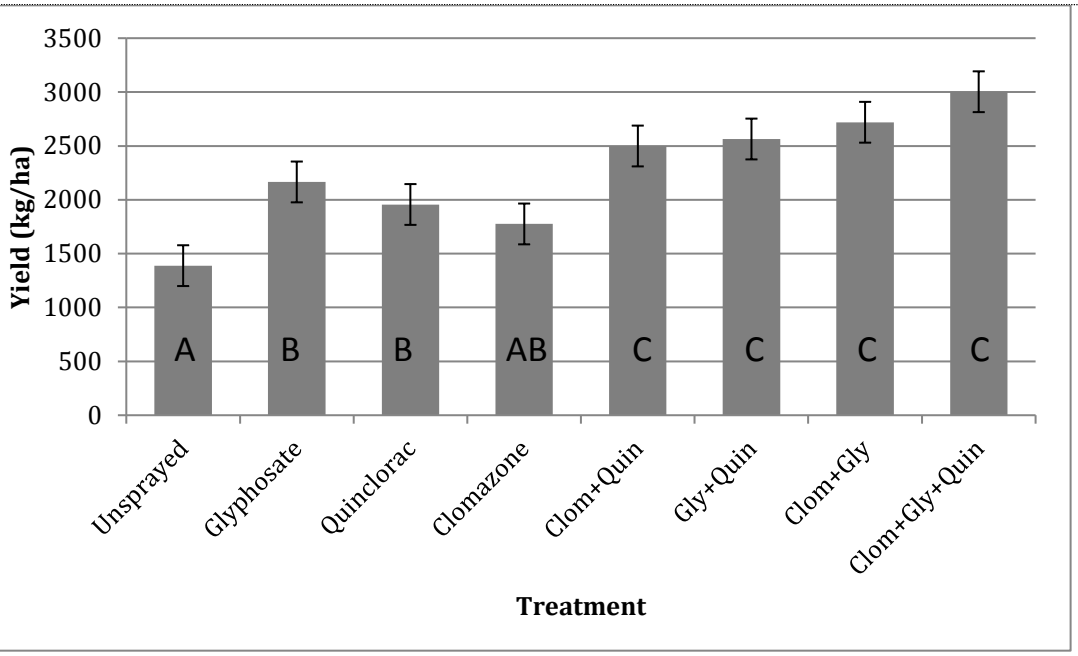

Figure 5. Canola yield in glyphosate-resistant canola as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

All herbicide treatments significantly improved yield when compared to the unsprayed check in the imidazolinone-resistant system (Figure 6). Imazamox+imazapyr and clomazone applied alone both increased yield by 18% over the unsprayed control, whereas quinclorac applied alone improved yield by 36%. The herbicide combination treatments were not significantly different from each other, but all exhibited higher yields than any of the individual components applied alone. A pre-emergence application of clomazone combined with a tank-mixture of quinclorac and imazamox+imazapyr improved yields by an additional 25 and 26% compared with the individual products applied alone. This was also the highest yielding treatment, improving canola yield by 51% over the unsprayed check and 33% over the herbicide standard (Imazamox+imazapyr).

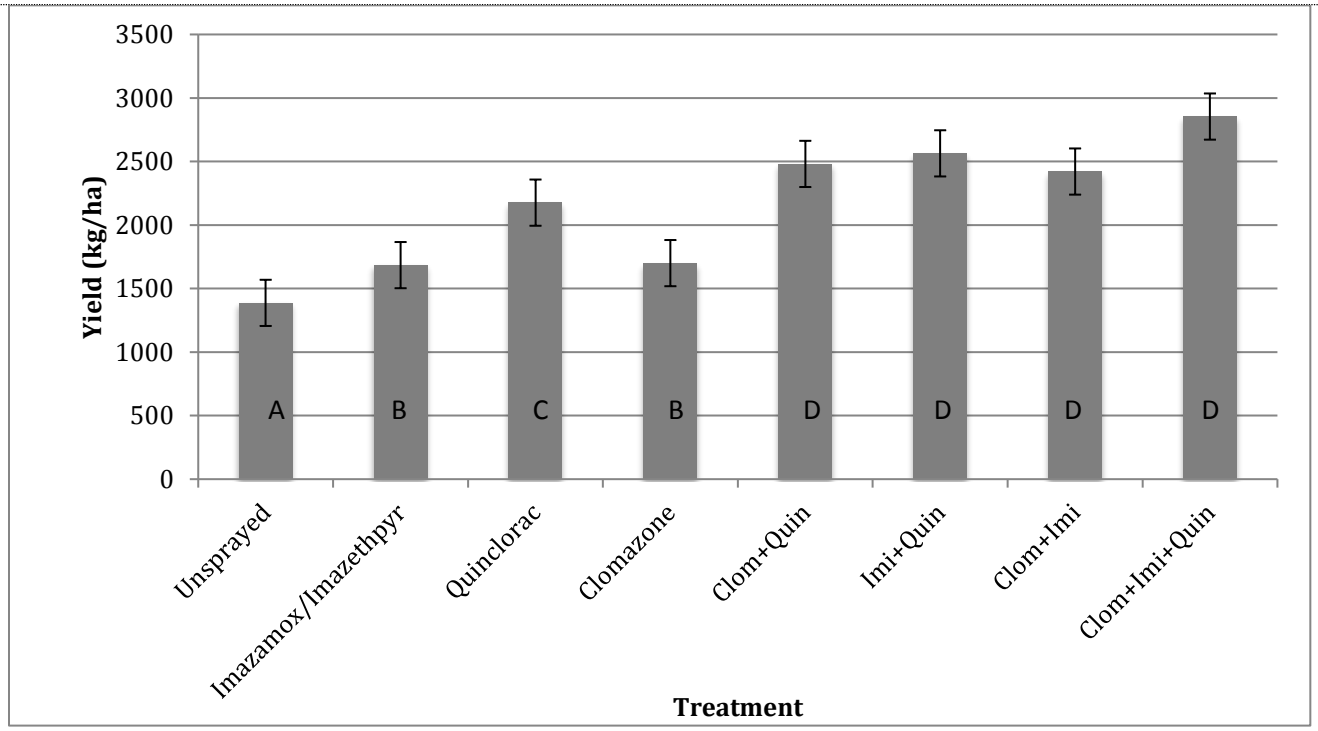

Figure 6. Canola yield in imidazolinone-resistant canola as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

Thousand seed weight was not significant affected ($P > 0.05$), by any of the treatments for any site, year or treatment (Table 6,7,8). During the vegetative stage canola has a low canopy height and consequently, weeds that grew faster and taller were allowed to effectively outcompete the crop and affect yield and yield components. Cleavers species are characterized by weak stems and hooked bristles, which cause a climbing growth habit (Defelice, 2002). Cleavers require other taller plants in which to attach themselves to in order to vertically grow. In addition *gallium* species are not known for rapid growth (Defelice, 2002). The lack of canopy competition during the vegetative stage of the crop may be why herbicide treatment affected overall crop yield but not thousand seed weight. Research conducted by Yaghoobi and Siyami (2008) found that thousand seed weight was not affected by weed competition, which may have contributed to the results observed in this study.

Cleavers Contamination

Cleaver contamination of harvested canola seed differed significantly between herbicide treatments among all systems ($P < 0.05$) (Table 6). Generally, trends were similar to those observed for cleavers biomass, and were inversely related to canola yield. In the glyphosate-resistant system, the application of a single in-crop herbicide such as glyphosate or quinclorac (alone) reduced cleavers contamination by 72% and 64% (Figure 7), respectively. Clomazone, on the other hand, reduced the contamination rate by 28%. The combination of clomazone followed by quinclorac as well as clomazone followed by glyphosate was not significantly different from either the quinclorac or glyphosate applied alone. As with cleavers biomass, the greatest in cleavers contamination was achieved by either tank mixing glyphosate and quinclorac (94%) or pre-seed

clomazone followed by glyphosate plus quinclorac (97%).

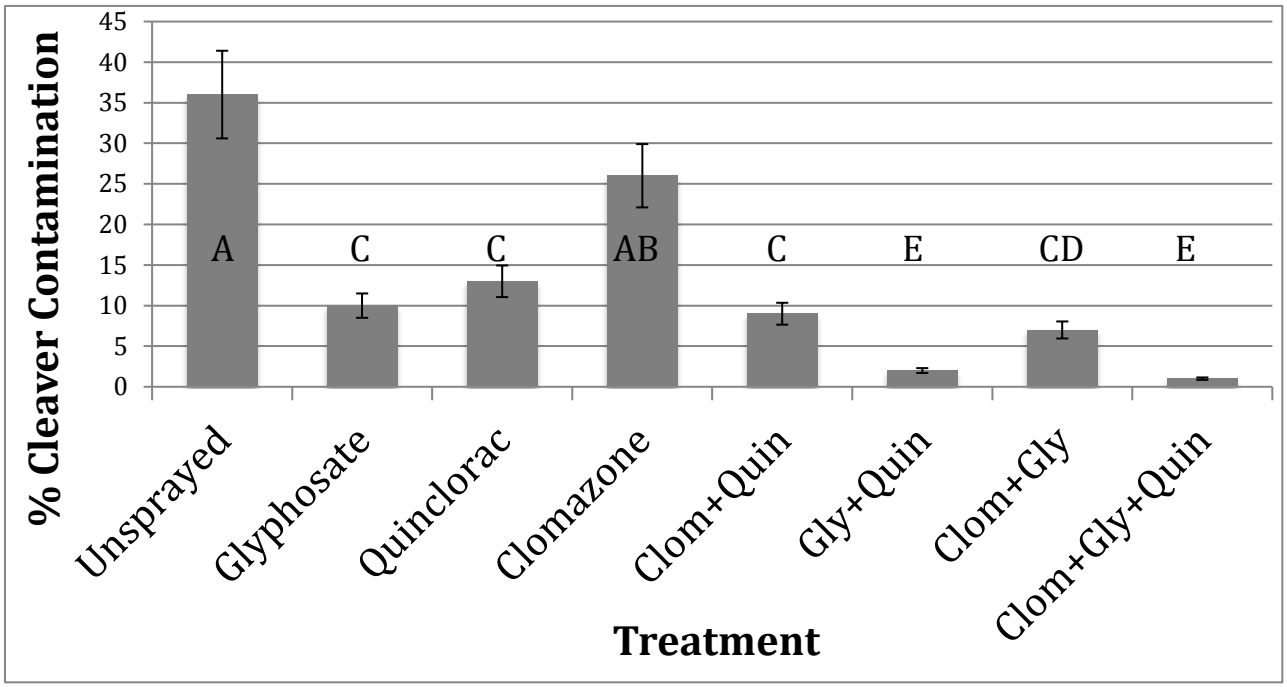

Figure 7. Cleavers contamination in canola seed in a glyphosate-resistant system as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

In the glufosinate-resistant system, all herbicide treatments significantly reduced cleavers contamination relative to the unsprayed check. Glufosinate and clomazone applied alone resulted in reductions in cleavers contamination of 36 and 20%, respectively (Figure 8). A pre-emergence application of clomazone followed by glufosinate improved reduced cleavers contamination by 64% relative to the unsprayed check. Quinclorac applied alone produced a 71% reduction in cleavers contamination, while the application of clomazone prior to quinclorac produced a 79% reduction in cleavers contamination. The greatest reduction in cleavers contamination was achieved by either tank-mixing quinclorac with glufosinate (82%) or applying clomazone pre-emergence, followed by a quinclorac and glufosinate tank mixture (89%).

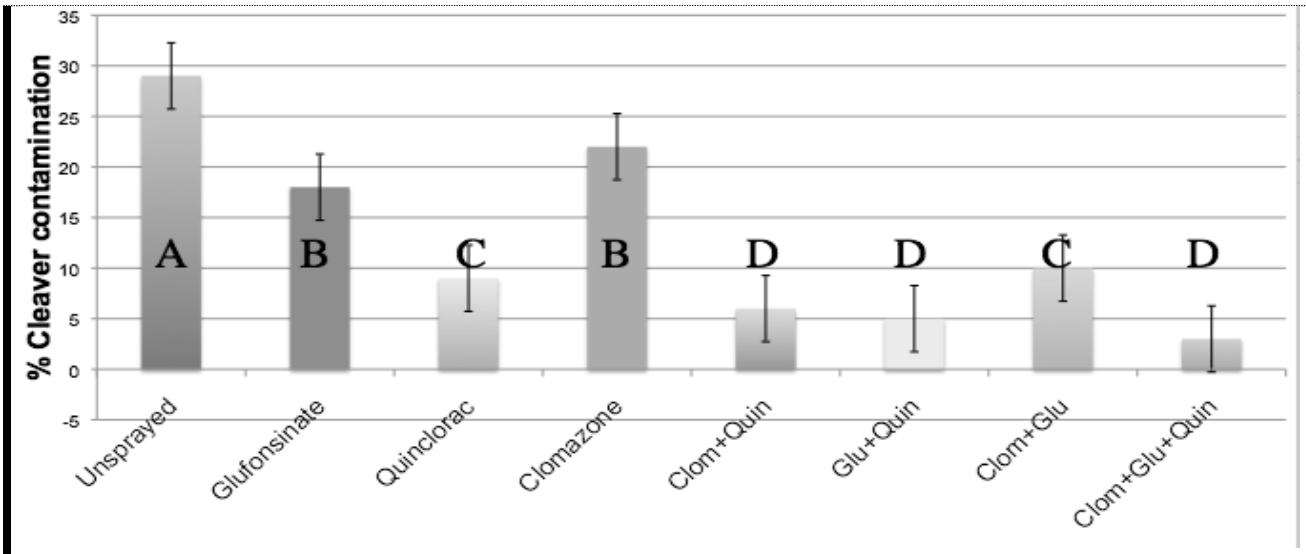

Figure 8. Cleavers contamination in canola seed in a glufosinate-resistant system as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

In the imidazolinone-resistant system, all herbicide treatments, with the exception of clomazone applied alone, significantly reduced cleavers contamination (Figure 9). Imazamox+imazapyr only reduced contamination by 38%, which can be partially attributed to the presence of group 2 resistant biotypes in the cleaver populations. Quinclorac, clomazone+quinclorac, and imazamox+imazapyr +quinclorac significantly reduced cleavers contamination by 68%, 78% and 81%, respectively. All three treatments significantly reduced contamination compared to imazamox+imazapyr applied alone. Clomazone followed by an in-crop application of Imazamox+imazapyr +quinclorac exhibited the greatest reduction in cleaver contamination (88%).

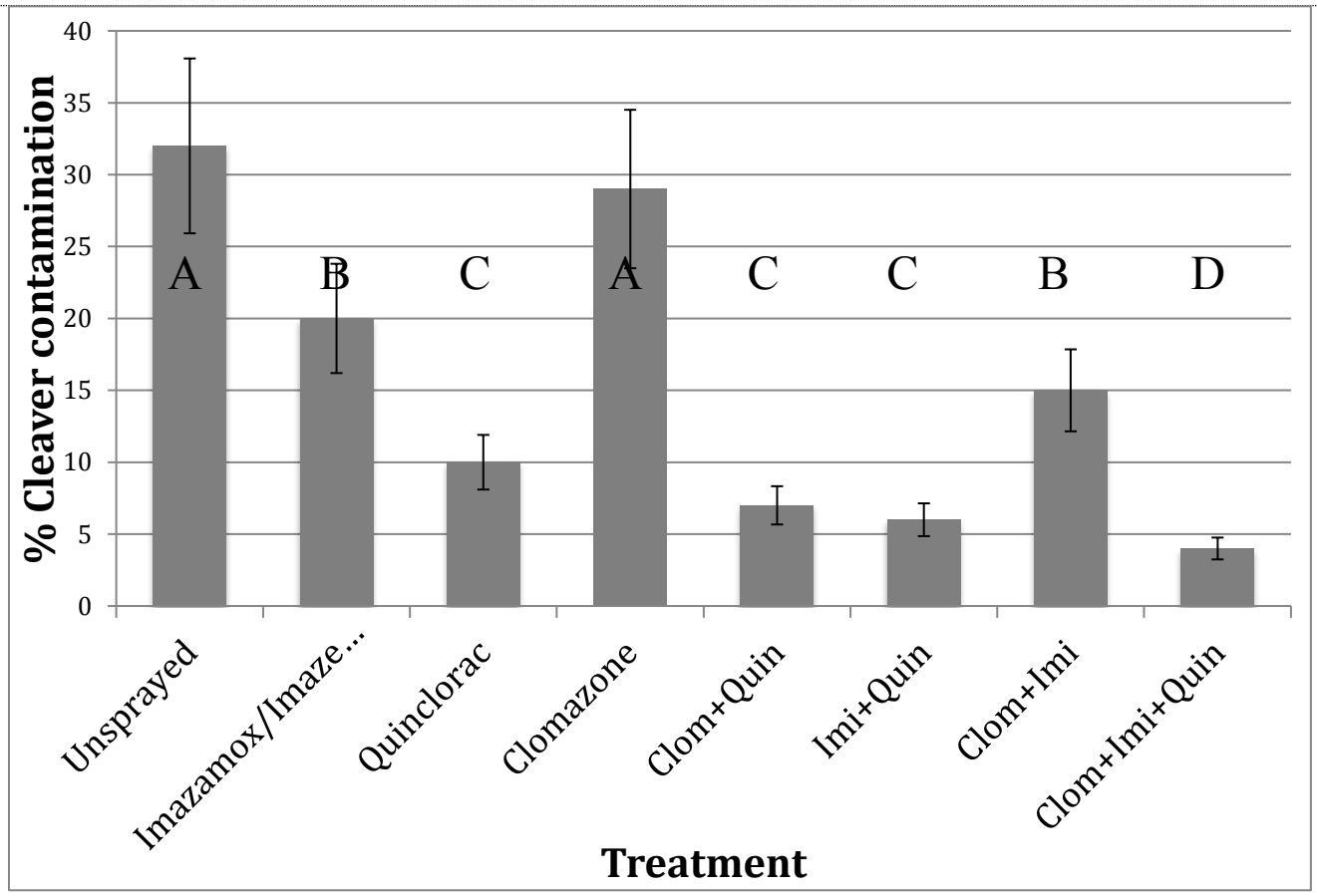

Figure 9. Cleavers contamination in canola seed in an imidazolinone-resistant system as affected by herbicide treatment across five site-years. Error bars represent the standard error. Comparisons made between herbicide treatments with the same letter indicate no significant difference at Tukey 0.05.

The aforementioned results show that clomazone, while largely ineffective when applied as the sole active ingredient did substantially reduce cleaver biomass and seed contamination while improving canola yield when used in conjunction with an in-crop herbicide standard such as glyphosate, glufosinate or imazamox+imazapyr. As a soil applied herbicide, clomazone exhibited the greatest control of cleavers early in the growing season, which is consistent with work done by (Shimi, et al. 2007). Group 13 herbicides, such as clomazone, injure cleavers by reducing growth and bleaching affected individuals (Ferhatoglu and Barrett, 2005). Current in-crop herbicide such as glyphosate, glufosinate and imazamox+imazapyr are registered for control of cleavers, but only at early growth stages 2 whorl, 1-4 whorl and 15 cm, respectively (Saskatchewan Ministry of Agriculture, 2015). The significant growth reduction caused by clomazone allowed the in-crop herbicides to more effectively control cleavers species.

Quinclorac, on the other hand, provided effective control of cleavers when applied as a sole ingredient, and did not cause any crop injury. Similar results were reported by Grossmann et al. (2001) as cleavers are known to be sensitive to auxin herbicides. Other work by Sheltrup and Grossmann (1995) and Ali (1998) has also shown that quinclorac readily controls cleavers. The addition of quinclorac to the in-crop herbicide standards also significantly increased efficacy on cleavers. In the imidazolinone-resistant canola, quinclorac controlled group 2 resistant biotypes. Likewise, glufosinate, which only controls cleavers when they are

extremely small (two whorl), also benefited from the addition of quinclorac. Glufosinate is known to be highly susceptible to environmental conditions and the addition of quinclorac improves cleavers control in a variety of environmental conditions. Glyphosate is presently the most efficacious in-crop herbicide in regard to cleaver control, but improved efficacy with the addition of quinclorac was observed in this study. The addition of quinclorac improved control of larger individuals, which may be close to or exceeding the registered stage for control by glyphosate. Both glyphosate and glufosinate are registered for two in-crop applications per year and due to the small stage at which cleavers need to be sprayed for effective control, growers often spray extremely early, missing other weed flushes and forcing a second application. The use of either clomazone or quinclorac in addition to the in-crop herbicide standard may reduce the need for a second in-crop herbicide application.

2) Assessing the response of cleavers populations to various herbicides

There were no significant differences in shoot biomass between any of the Galium populations (Lacombe, AB, Vegreville, AB, and Saskatchewan Pulse Growers (Figure 9,10,11). Due to the lack of significant differences between populations, a common curve could be fit to all three Galium populations in each herbicide treatment. This suggests that populations did not differ in response to the various herbicides. Moreover, the ED50 parameter for imidazolinones, quinclorac, and glufosinate populations were very similar, although slight differences in the parameter estimates were noted between populations within each system. While the ED50s for quinclorac were similar, the ED50s for SPG, Lacombe, Vegreville populations were 0.31, 1.79, and 0.55, respectively in response to imazamox+imazapyr. These results suggest that as little as 18% of highest dose to reduce cleaver biomass in the Lacombe population was needed to reduce the biomass by the same levels in the SPG population. It is important to note these populations were screened for resistance prior to conducting this research and thus, resistance is not the underlying factor contributing to this variance. Similarly, the ED50s for SPG, Lacombe, Vegreville populations were 1.27, 1.58 and 1.76, respectively, in response to glufosinate. These results suggest that 72% of highest dose to reduce cleaver biomass in the Vegreville population was needed to reduce the biomass by the same levels in the SPG population.

Despite the fact that a common curve could be fit across populations, these data suggest that the populations may be responding differently to herbicides, with the greatest variance exhibited to glufosinate. Inherent genetic differences may have influences the response of each population to each herbicide, but this needs further testing. The ability to combine populations within each herbicide system is likely due to the similarity of the other parameter estimates between populations (data not shown). Further study is needed to determine why such large differences between the ED50s of each population and increasing the number of replications and run may assist in that.

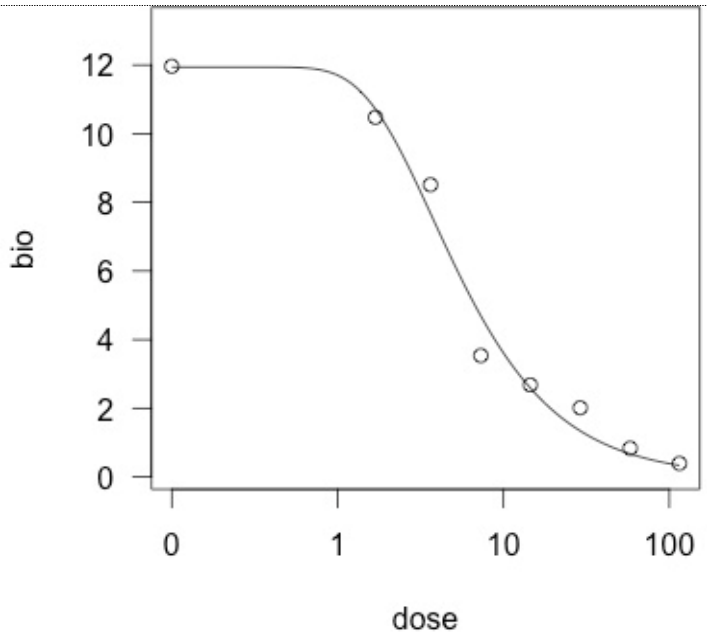

Figure 9. Relationship between imazapyr + imazamox and the shoot dry weight (g) of cleaver populations from western Canada in 2013 and 2014. Bio = shoot biomass of cleavers.

Table 9. Parameter estimates for the Combined relationship between Ares® herbicide (imazapyr + imazamox) and the shoot dry weight (g) of cleaver populations from western Canada in 2013 and 2014. ED50 represents the dose needed to reduce shoot dry weight by 50%.

Intercept	Estimate	Std. Error	t-value	p-value
Slope	1.94	0.31	6.35	0.00001
Lower limit	1.23	2.42	0.51	0.61
Upper limit	108.49	6.61	16.40	0.00001
ED50	1.13	0.12	9.27	0.00001

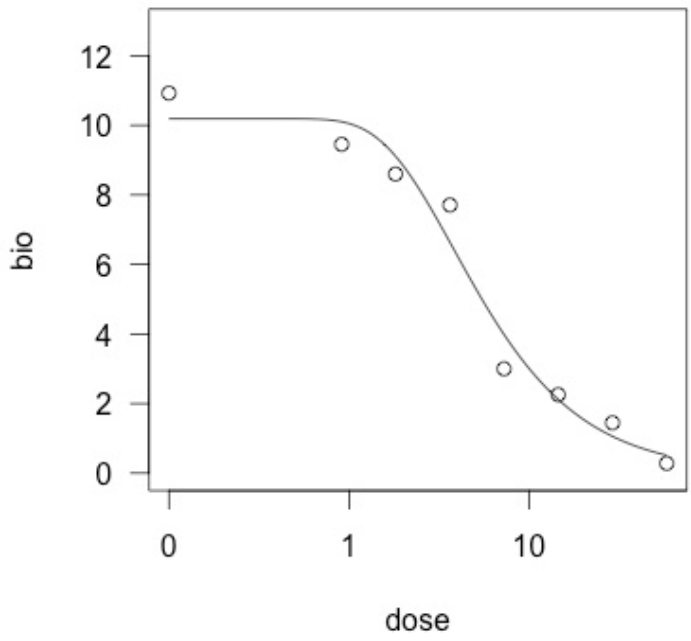

Figure 10. Relationship between quinclorac and the shoot dry weight (g) of cleaver populations from western Canada in 2013 and 2014. Bio = shoot biomass of cleavers.

Table 10. Parameter estimates for the Combined relationship between quinclorac herbicide and the shoot dry weight (g) of cleaver populations from western Canada in 2013 and 2014. ED50 represents the dose needed to reduce shoot dry weight by 50%.

Intercept	Estimate	Std. Error	t-value	p-value
Slope	2.14	0.41	5.25	0.00001
Lower limit	0.98	3.01	0.32	0.74
Upper limit	106.04	7.56	14.01	0.00001
ED50	1.14	0.14	8.11	0.00001

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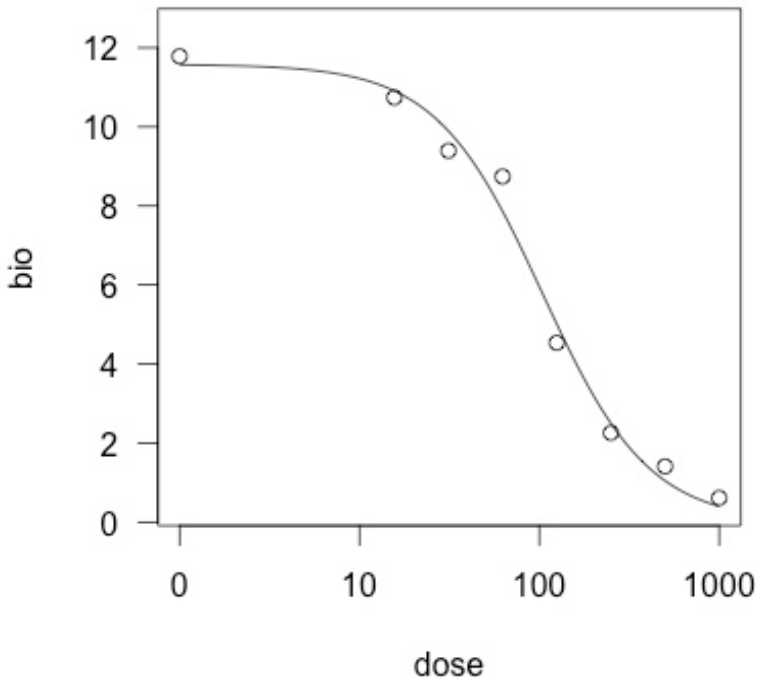

Figure 11. Relationship between glufosinate and the shoot dry weight (g) of cleaver populations from western Canada in 2013 and 2014. Bio = shoot biomass of cleavers.

Table 11. Parameter estimates for the Combined relationship between Liberty® herbicide (glufosinate-ammonium) and the shoot dry weight (g) of cleaver populations from western Canada in 2013 and 2014. ED50 represents the dose needed to reduce shoot dry weight by 50%.

Intercept	Estimate	Std. Error	t-value	p-value
Slope	1.58	0.16	9.57	0.00001
Lower limit	-21.90	20.56	1.06	0.28
Upper limit	1143.17	50.24	22.75	0.00001
ED50	1.44	0.09	14.52	0.00001

3a) Genetic, morphological, and molecular characterization of *Galium* species in Western Canada

The ITS1-5.8S-ITS2 complex of the three *Galium* species was 691- 741 bp in length, suggesting that there was variation in the ITS complex that could be used to differentiate species (Figures 11, 12). While the 5.8S gene was 138 bp for all species, the ITS1 region was 210 bp in both *G. aparine* and *G. spurium*, but 221 bp

in *G. boreale.* Similarly, the ITS2 region was 391-393 bp in *G. spurium* and *G. aparine*, but was much shorter, (332 bp) in *G. boreale*.

Sequencing of samples from the Canadian populations resulted in various ITS lengths from 740 -744 bp long. Very little variation occurred within ITS1 and ITS2 among the populations, which suggests that they are highly related. No differences in the 5.8S gene were found between any of the populations, and all exhibited species identity consistent with *G. spurium*. Additionally, the Canadian populations matched *G. spurium* at locations of variation between *G. aparine* and *G. spurium* in the ITS1 and ITS2 regions, which further confirms that the cleavers populations we sampled from across western Canada are predominantly comprised of *G. spurium.* The final consensus sequences of the three species and various collected Canadian populations were separated in a dendrogram, shown in Figure 13, which shows that populations are highly-related.

The variation in the 5.8S gene was used for the TaqMan assay to separate *G. aparine* from *G. spurium.* A SNP within the rDNA was chosen because these genes are highly conserved across taxa and very useful for phylogenetic relationships (Baldwin et al. 1995). The first spot of variation at position 230 was used for TaqMan to differentiate *G. aparine* and *G. spurium*. The first SNP was used after variation in second SNP at position 312 was found using BLAST. To confirm that the Taqman assay would select for individual species, the assay was run on previously sequenced DNA samples of the reference populations and Canadian populations. The assay ran successfully and additional plant samples from the *Galium* populations were collected for analysis. All samples were shown to be *G. spurium*, further confirming that this species is the predominant species in cleavers populations from across western Canada. In addition, we detected only *G. spurium* in these populations, and our analyses failed to detect *G. aparine* in any of the populations. This suggests that the populations we sampled are not a mixture of species, but rather, are comprised of *G. spurium*.

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Spurium GTAACCAATACGACTGTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGC
Aparine GTAACCAGTACGACTGTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGC
Boreale GTAACCAATACGACTCTCGGCAACGGATATCTAGGCTCTCGCATCGATGAAGAACGTAGC

Spurium AAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAA
Aparine AAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAA
Boreale AAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAATCATCGAGTTTTTGAACGCAA

Spurium GTTGCGCCCAAAGCCACT
Aparine GTTGCGCCCGAAGCCACT
Boreale GTTGCGCCCGAAGCCATT
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Figure 12. The 5.8S Gene of the three *Galium* species that shows SNPs to be used to differentiate the species via TaqMan Assay screening.

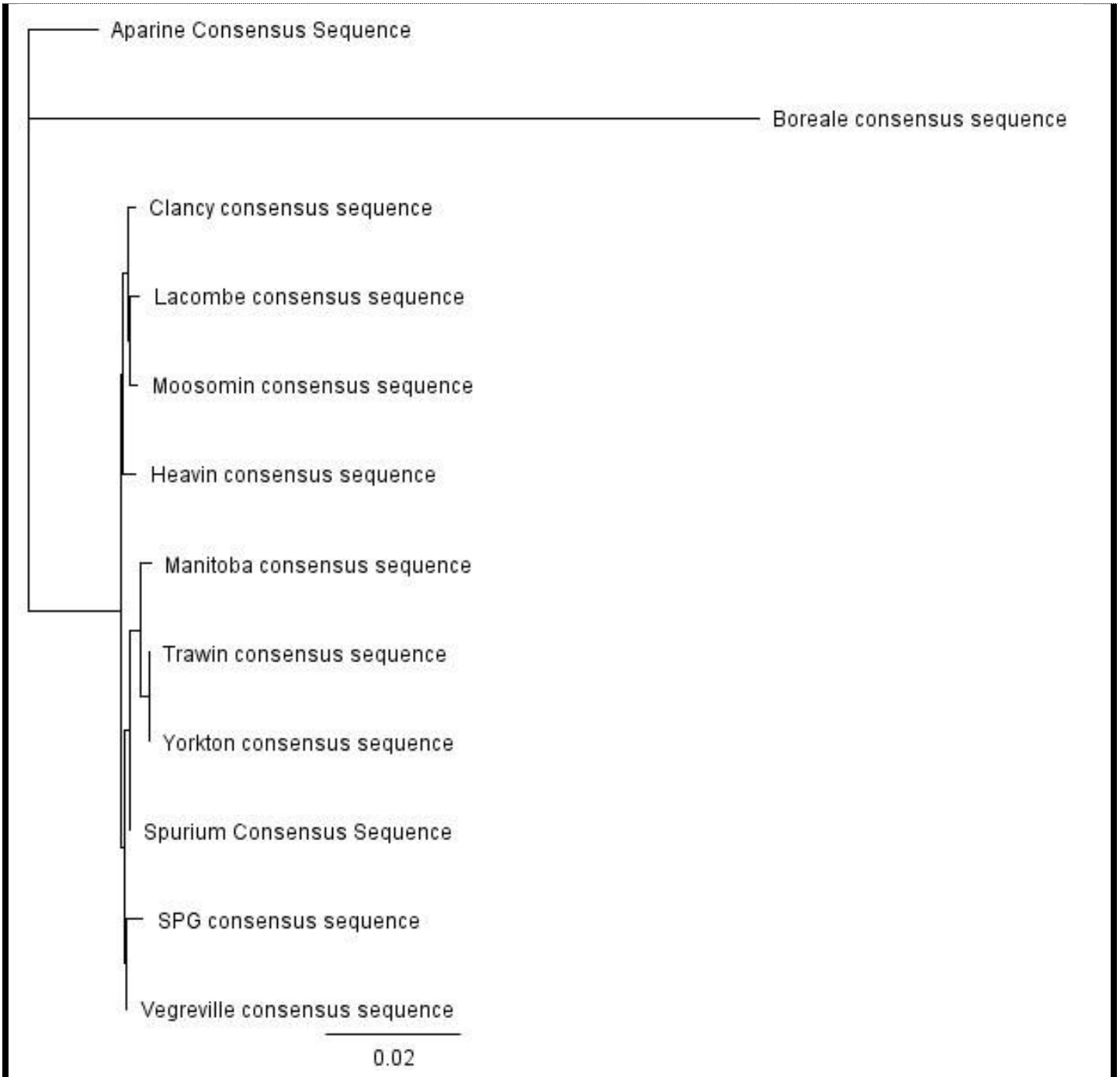

Figure 13. Dendrogram created using genetic variability between known (reference) populations and sampled cleavers populations (from western Canada) detected by Geneious 6.8.1. The reference populations separate out from each other, while the Canadian populations all correspond with *G. spurium*.

White et al. (1990) initially suggested that the ITS complex could be used to differentiate and characterize closely related species or populations of the same species, and this appears to be acceptable in the case of distinguishing *Galium* species. Furthermore, variation in the 5.8S gene is unusual within species or closely related species, which makes it an ideal candidate target site for molecular markers (Hübner et al. 2003). Because previous molecular work on *G. aparine* employed the ITS complex to divide populations into two groups (Hübner et al. 2003), their analyses together with our research suggests that the ITS is an acceptable DNA region which can be used successfully to differentiate species.

3b) Emergence timing of cleaver populations

Spring emergence started on June 3rd at 223 growing degree-days (GDD) and June 1st after 214 GDD in spring of 2013 and 2014, respectively (Figure 14, Table 12). Emergence was complete after 23 days in 2013 at 574 GDD and 33 days in 2014 after 617 GDD. Spring median emergence time (MD50) was significantly different between all populations, with the exception of Heavin and SPG. The population with the lowest MD50, and therefore the most rapid germination, was Clancy. In 2014, the slope parameter differed only between Vegreville and Trawin. Median emergence was significantly lower for Heavin, and Trawin, suggesting they exhibited an earlier time to 50% emergence. The upper limit in 2014 was only significantly different between Trawin and all the other populations (Table 12). There was generally no regional similarity between the Saskatchewan or Alberta populations that proved cleavers populations in close proximity to each other had similar emergence patterns. However, populations from the Melfort area (Heavin and Trawin) consistently exhibited lower median emergence timings than populations from Alberta (Lacombe, Vegreville).

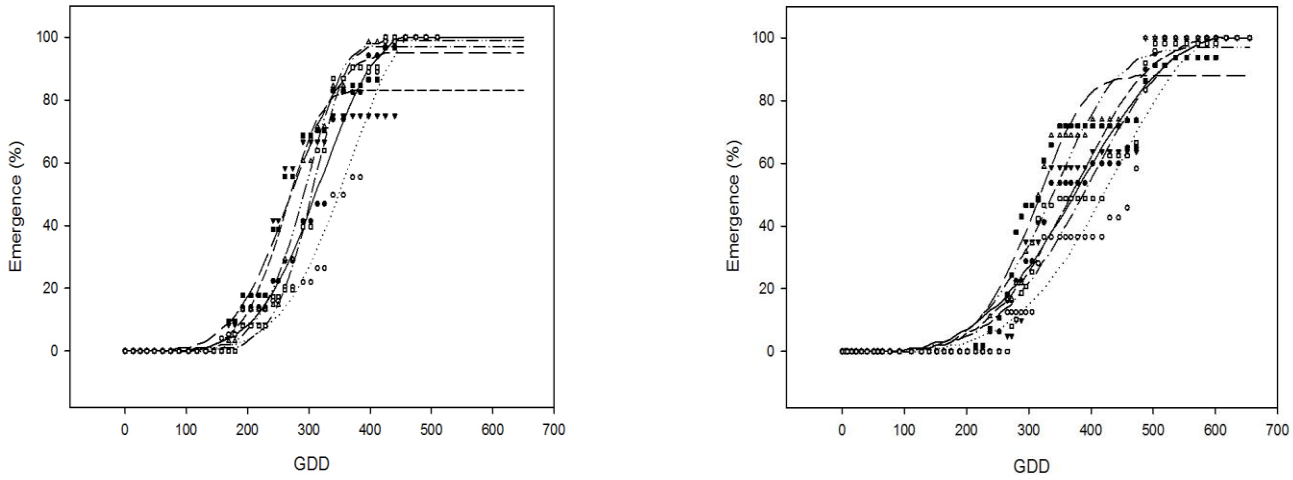

Figure 14. Emergence timing of cleavers populations at Goodale in the spring of 2013 (left) and 2014 (right), respectively. Closed circles represent Vegreville, open circles represent Lacombe, closed squares represent Trawin, open squares represent SPG, closed triangles represent Clancy, open triangles represent Heavin. Data were fit using nonlinear regression with the drc package of R.

Table 12. Parameter estimates and standard errors of spring emergence data in 2013 and 2014.

	2013			2014		
Population	Slope	MD50	Upper Limit	Slope	MD50	Upper Limit
Vegreville	4.68 (0.334)	338.29 (5.59)	1.02 (0.020)	3.60 (0.260)	417.54 (11.508)	1.03 (0.038)
Lacombe	5.24 (0.455)	380.07 (6.808)	1.06 (0.040)	4.32 (0.351)	467.58 (11.701)	1.07 (0.047)
SPG	7.25 (0.721)	318.99 (3.7)	0.97 (0.019)	4.04 (0.291)	425.89 (10.063)	1.02 (0.035)
Heavin	6.13 (0.538)	308.58 (4.081)	0.99 (0.020)	4.30 (0.401)	363.80 (8.832)	0.97 (0.027)
Trawin	4.26 (0.364)	290.58 (5.982)	0.95 (0.023)	5.15 (1.013)	328.81 (11.137)	0.88 (0.029)
Clancy	5.75 (0.818)	273.85 (6.137)	0.83 (0.020)	4.10 (0.293)	403.24 (9.102)	1.00 (0.031)

Fall emergence of cleavers population was highly variable between years; therefore data were not combined (Figure 15, Table 13). In 2013, fall emergence started on September 6th after 307 GDD (after planting) and ended on October 26th at 702 GDD. Fall emergence in 2014 started on August 26th at 201 GDD and finished on September 19th with 448 GDD. The slope parameter in 2013 differed significantly between Vegreville and Heavin only; all other populations were not significantly different from each other (Table 13). With regard to median emergence timing, the populations separated into three groupings. Heavin exhibited a significantly higher median emergence time than all other populations, indicating that it had the slowest emergence of all populations. Although the Lacombe, Clancy, and SPG populations did not differ from each other, these populations exhibited significantly longer median emergence times than Trawin and Vegreville. Similarly in 2014, median emergence time differed between all populations and ranged from 250 to 328 growing degree days.

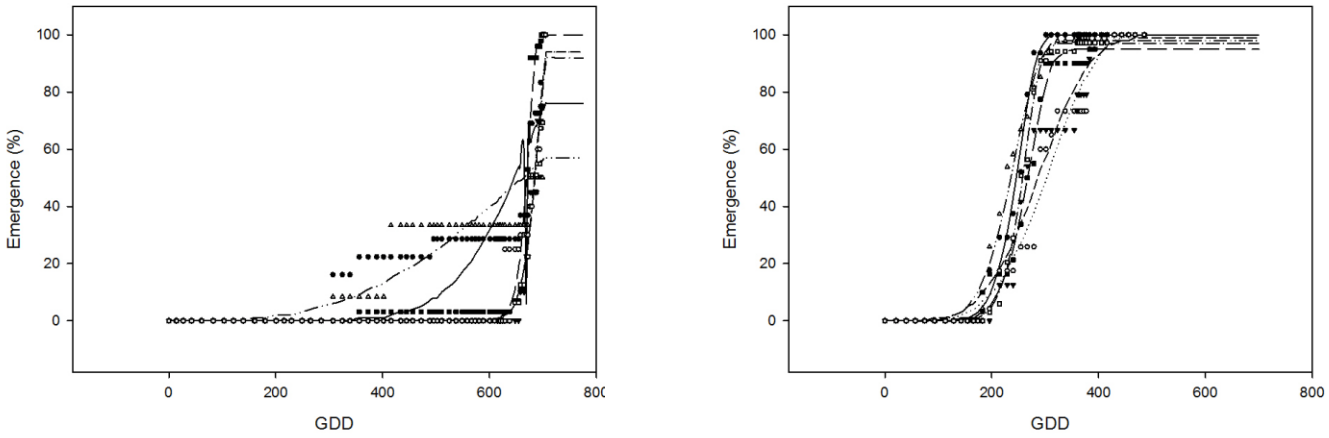

Figure 15. Emergence timing of cleavers populations at Goodale in the fall of 2013 (left) and 2014 (right), respectively. Closed circles represent Vegreville, open circles represent Lacombe, closed squares represent Trawin, open squares represent SPG, closed triangles represent Clancy, open triangles represent Heavin. Data were fit using nonlinear regression with the drc package of R.

Table 13. Parameter estimates and standard errors of fall emergence data in 2013 and 2014. Only a two-parameter model was used to fit the data in 2015.

	2013		2014		
Population	Slope	MD50	Slope	MD50	Upper Limit
Vegreville	8.20 (2.078)	676.20 (4.955)	8.64 (0.798)	256.55 (2.166)	1.00 (0.012)
Lacombe	42.65 (6.925)	690.20 (1.345)	4.55 (0.274)	328.44 (5.358)	1.01 (0.028)
SPG	41.66 (4.369)	691.56 (1.297)	10.23 (1.016)	265.91 (2.198)	0.97 (0.012)
Heavin	3.17 (0.379)	743.32 (17.630)	6.13 (0.462)	248.83 (2.997)	0.98 (0.012)
Trawin	53.63 (7.642)	674.37 (1.373)	8.29 (0.838)	276.66 (2.440)	0.95 (0.012)
Clancy	43.20 (4.298)	690.42 (1.283)	4.27 (0.254)	312.31 (5.775)	0.99 (0.027)

Royo et al. (2010) modelled the emergence of three different Galium species and found emergence was related to the temperature and moisture during the emergence period. Seedlings in that study started to emerge after 250-300 GDD and took 400-500 GDD to reach 50% emergence (Royo et al. 2010). In our study, emergence in the spring of 2014 was longer and more gradual than in 2013. This variability in emergence between years was likely the result of greater moisture in May and June of 2014. *G. spurium* is more sensitive to soil water potential than *G. aparine* (Royo et al. 2010), and time to 50% emergence of some populations responded substantially different between years. These differences may be driven by variability in base temperature or water potential required for germination among the populations, and further investigation is required in this area.

Final emergence percentages were analyzed separately for each year similar to the emergence curves. Final emergence among spring-sown treatments was significantly different between populations in both 2013 ($P=0.02$) and 2014 ($P<0.001$) (Figure 16). In 2013, the Clancy population exhibited a final emergence that was 5 to 8 times lower than the other populations. In 2014 all populations exhibited lower emergence percentages than the SPG population. This is not surprising since the SPG population was a locally collected and likely locally adapted population. Clancy and Vegreville both exhibited lower emergence than the Trawin population (approximately 2.5-fold lower) (Figure 16).

Differences final emergence percentages in the fall sowing were not significant in both years (data not shown), although fall emergence was lower than emergence in the spring. Although emergence may be lower, fall emerging cleavers that overwinter are very competitive. Malik and Vanden Born (1987b) found *G. spurium* emergence could occur throughout the growing season and those emerging in July could potentially overwinter until spring. In Spain, late season emergence of *G. aparine* and *G. spurium* was also noted and plants could overwinter when conditions were favourable (Royo et al. 2010).

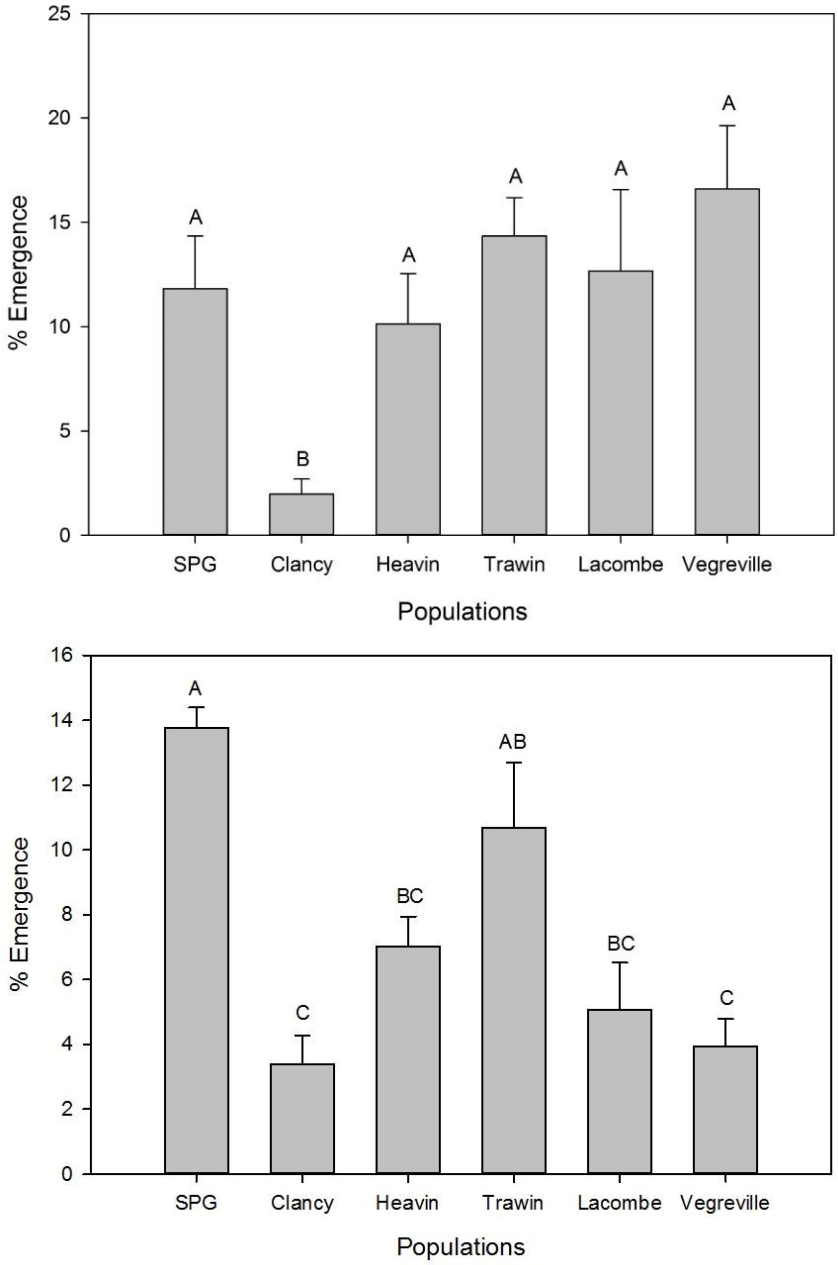

Figure 16. Final emergence percentage of populations in spring of 2013 (top) and 2014 (bottom). Error bars represent standard error and letters indicate significant differences at P<0.05.

3c) Morphological Trait Differences

Morphological data were analyzed using analysis of variance, which showed that there was a significant year by population effect for height and consequently, data were analyzed within years (Table 14). This showed th regardless of year, cleavers plant height did not differ significantly among populations (P=0.4630) or 2014 (P=0.2549). Days to commencement (P=0.0281) and termination of flowering (P=0.0492) were the only trait found differ significantly between populations. The Clancy population started flowering approximately 4 day before SPG, Trawin, and Vegreville. On the other hand, the Heavin population took 5 days longer to finish flowering than Vegreville, and thus exhibited a longer flowering period than the other populations. No patter of similarity link flowering period to emergence period.

All other traits did not exhibit statistically significant differences between populations. The lack of significan between morphological traits of cleavers populations is, perhaps, not surprising given that this project found that all populations are of the same species. Nevertheless, it is interesting that different biotypes were not evident among the populations sampled, despite the fact that they originated from wide areas of western Canada. This is beneficial as it suggests traits ranges are common across the Prairies and thus, individual populations are unlikely to exhibit greater variation in competitive traits than are others.

Table 14. Analysis of variance results (P-values) for leaf area index (LAI), leaf weight (LW), total weight (TW), base branch number (Branch), height, start of flowering (FLW5), mid-flowering (FLW50), end of flowering (FLW95), thousand seed weight (TSW), and fecundity (FED) of cleavers grown in field plots

Source	LAI	LW	TW	Branch	Height	FLW5	FLW50	FLW95	TSW	FED
Population (Pop)	0.7831	0.7865	0.6894	0.4220	N/A	0.0281*	0.2743	0.0492*	0.2913	0.078
Year	0.4644	0.4650	0.4598	0.5406	0.6326	0.4797	0.2426	0.4813	0.2777	0.251
Rep (Year)	0.1953	0.1482	0.1475	0.8659	0.3363	0.1419	0.1903	0.5669	0.4610	0.328
Year*Pop	0.1521	0.2792	0.3431	0.9868	0.0054**	0.8700	0.1249	0.2135	0.2760	0.422

*, **, *** denotes significance at the 0.05, 0.01, 0.001 probability levels, respectively.

9. Conclusions and Recommendations: *Highlight significant conclusions based on the previous sections, with emphasis on the project objectives specified above. Provide recommendations for the application and adoption of the project.*

1) Taken together, the results of the first study revealed that the addition of clomazone and quinclorac significantly improves cleavers control in canola production. Use of clomazone and quinclorac, in conjunction with existing herbicides such as glyphosate, glufosinate, and imazamox+imazapyr reduced cleaver biomass, cleaver seed contamination and increased canola yield. Consequently, these products can and should be registered for use in western Canada. To our knowledge, both of these products have been or will soon be registered for use with canola.

In addition to the reductions in cleavers biomass and seed contamination as well as the associated canola yield increases observed in this study, adding clomazone and quinclorac to production practices in western Canada will have long-term benefits. Spring applied clomazone reduces the size and stage of cleavers found in-crop, and it is known that lower population numbers reduce the risk of developing herbicide resistance. Quinclorac, which can be mixed with any of the in-crop herbicides, also lowers the risk of further developing herbicide resistance by adding a different mode of action to in-crop applications. Cleaver resistance to group two herbicides is already widespread throughout Alberta and Saskatchewan, and cleavers rank second among weeds likely to develop glyphosate resistance, in the black soil zone, (Hugh Beckie, 2010).

2) The lack of any significant, substantial differences between cleaver populations in response to the various herbicides suggests that cleavers populations can be expected to respond similar to new and existing products. Although some differences in various parameters were noted, all populations were well-controlled with the herbicides. Thus, there appears to be no genetic basis for differential herbicide responses in populations of Galium spurium across western Canada. Further testing will be necessary to further analyze the parameter estimates for variability and stability across populations. However, the lack of substantial variation is of benefit to growers as similar responses to herbicides can be expected, regardless of whether growers choose quinclorac, imazamox+imazapyr, or glufosinate. This also suggests that it is unlikely that differential responses among populations herbicides is the underlying mechanism behind the expansion of cleavers, although the spread of group 2 resistant cleavers has clearly played a role in that regard.

3) Results from the molecular analyses showed that cleavers populations in western Canada are dominated by *G. spurium*. Due to the habitat preferences of each species, it was not unexpected that *G. spurium* was the most common, but the absence of *G. aparine* was unexpected. Although no *G. aparine* was found in the collected samples, it does not mean there is none present in fields across the prairies. Fields with established shelter belts or fields surrounded by natural forested habitat may be hosts for *G. aparine*. Extensive sampling of field and bush cleavers populations would need to be done to confirm the *Galium* spp. complex in western Canada is primarily *G. spurium*, although *G. boreale* is known to reside in field margins and wooded habitats. Additional sequencing of populations could also further examine if variation between ITS regions is

in some part correlated to competitive characteristics.

Historically, cleavers have been classified as Galium species in the weed surveys, due to the difficulty in determining which species are present. The current study has developed a molecular marker that will allow for samples to be identified to the species level. Because our study included eight populations, it would ideal to have multiple samples from across the Prairies on which to run the markers, thus providing an accurate map of cleavers species distributions across western Canada.

Emergence timing of cleavers varied between years in both the spring and fall. In each year, some populations exhibited variation in emergence timing, although the differences between populations generally did not correspond with geographical location. The differences owing to variation among populations suggests that growers will have to pay close attention to emergence timing in their fields in order to ensure they do not miss the window for control, and this window may vary with location and year. More importantly, our data demonstrate that substantial portions of cleavers populations emerge in the fall and may potentially overwinter into spring, making post-harvest weed control even more important. Cleavers that overwinter are very difficult to control in spring and given the proportion of emergence reported in this research, fall control of cleavers should be a priority. A sustainable approach to long-term cleavers management will thus employ a strategy to effectively target cleavers in the fall. However, understanding which traits contribute to the aggressiveness of cleavers will also help inform this strategy and produce better control strategies, although it is clear from this work that vegetative morphological traits do not exhibit variation across cleavers populations.

10. Success stories/ practical implications for producers or industry: *Identify new innovations and /or technologies developed through this project; and elaborate on how they might impact the producers /industry.*

In addition, there are a number of practical implications that are the result of this project:

1. Use of clomazone and quinclorac in conjunction with existing herbicides will provide excellent control of cleavers in canola crops. For the first time, growers will be able to manage cleavers in canola crops via the use of herbicides
2. We have created a new molecular marker to distinguish between cleavers species. This can be used to differentiate between cleavers species in future projects, such as the weed survey conducted by Agriculture Canada.
3. The cleavers species of prevalence across the west is *Galium spurium*, and populations appear homogenous for this species. This is also the species that possesses resistance to group 2 herbicides.
4. Morphological traits and emergence timing are relatively homogenous between populations from across western Canada. Thus, a common approach can be taken to targeting this weed without having to manage different biotypes with different approaches in different locales.
5. Some differences exist between populations for emergence characteristics, and growers will need to pay close attention to emergence timing of this weed to ensure the small window for control is not missed. Moreover, a significant proportion of cleavers populations emerged in the fall and thus, management in the fall is key to the sustainable long-term management of cleavers in western Canada.

A significant success story is that this research contributed to the training of two MSc. students, which is a key outcome of any academic grant. Both students intend to contribute to the SK agricultural economy, and one is already working for the canola industry (Canola Council of Canada).

11. Patents/ IP generated/ commercialized products: *List any products developed from this research.*

None to report

12. List technology transfer activities: *Include presentations to conferences, producer groups or articles published in science journals or other magazines.*

Conference and Extension Talks

- DeRoo, A. and C.J. Willenborg. 2014. Emergence timing and morphological characteristics of *Galium* species in western Canada. Soils and Crops Annual Meeting. Saskatoon, SK. Mar 11-12, 2014. *Graduate Student Oral presentation and abstract.*
- Epp, I. and C.J. Willenborg. 2014. Evaluating the response of gallium species and populations to herbicides. Soils and Crops Annual Meeting. Saskatoon, SK. Mar 11-12, 2014. *Graduate Student Oral presentation and abstract.*
- Epp, I. and C.J. Willenborg. 2015. Response of Galium species to herbicides. Soils and Crops Annual Meeting. Saskatoon, SK. Mar 16, 2015. *Graduate Student Oral presentation and abstract.*
- DeRoo, A. and C.J. Willenborg. 2015. Molecular discrimination of Catchweed Bedstraw (Galium aparine L.) and False Cleavers (Galium spurium L.). Soils and Crops Annual Meeting. Saskatoon, SK. Mar 16, 2015. *Graduate Student Oral presentation and abstract.*
- DeRoo, A. and C.J. Willenborg. 2015. Emergence timing and morphological characteristics of *Galium* species in western Canada. Soils and Crops Annual Meeting. Saskatoon, SK. Mar 16, 2015. *Graduate Student poster.*
- Epp, I. and C.J. Willenborg. 2015. Response of Galium species to herbicides. Canadian Weed Science Society Meeting. Montreal, QC. Nov 17-20, 2015. *Graduate Student Oral presentation and abstract.*
- DeRoo, A. and C.J. Willenborg. 2015. Molecular discrimination of Catchweed Bedstraw (Galium aparine L.) and False Cleavers (Galium spurium L.). Canadian Weed Science Society Meeting. Montreal, QC. Nov 17-20, 2015. *Graduate Student Oral presentation and abstract.*
- Willenborg, C.J., A. DeRoo, I. Epp, K. Sapsford, and E.N. Johnson. 2015. Untangling the sticky issue of cleavers. Top Notch Farming Conference. Melfort, SK. Feb. 26, 2015. *Invited Presentation*.

Interviews and Press

- Featured in Canola Digest Magazine (November 2015) – Management of cleavers in canola; page 57.
- Featured in Crops Guide (December, 2013) – Stopping Cleavers – Knowing the enemy could lead to better control; pages 26-27.
- Feature interview for "Canola Connection" airing on CJWW week of April 2, 2014. Topic was short canola rotations and weed management.

13. List any industry contributions or support received.

We received approximately $90,000 in industry support for this research over a three-year period.

14. Is there a need to conduct follow up research? *Detail any further research, development and/or communication needs arising from this project.*

There is always a need to continue research. This research began to shed light on some of the differences that exist between populations for emergence characteristics. While this may explain some of the differences observed in the field, we still cannot account for whether temperature and moisture are driving the increase in cleavers across western Canada, nor do we know how these populations respond to differences in moisture and temperature. In that regard, we are proposing to do more research on the interactive response to temperature and moisture stress of *Galium spurium* and *Galium aparine*. We will also test the populations used in the current study for their response to these abiotic stresses. Finally, the base temperature for germination is not known for either *Galium spurium* and *Galium aparine*, and we will attempt to determine this in future work. This knowledge would provide better predictive modeling of the emergence timing of these species.

15. Acknowledgements. *Include actions taken to acknowledge support by the Ministry of Agriculture and the Canada-Saskatchewan Growing Forward 2 bilateral agreement.*

All presentations and extension activities have listed an acknowledgement of the funding contributors.

All publications, peer-reviewed or otherwise, will also contain an acknowledgement of funding sources.

16. Appendices: *Include any additional materials supporting the previous sections, e.g. detailed data tables, maps, graphs, specifications, literature cited*

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