ADF Progress Report

1. Project title, ADF file number and reporting period:

Integrated Management Approach to Optimize Red Clover Seed Production in Saskatchewan (20170007).

Reporting period: February 2019

2. **Prepared by:**

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3. **Activities during the reporting period:**

The project is progressing, generally, as planned.
Activities during the reporting period include:

- A M.Sc. student has been recruited for this project (Daniel Malamura)
- Experimental plots have been planted in both Clavet and Melfort, SK
- Data and sample collection have been completed for the first research year
- Data analysis is ongoing, as is pollinator identification
- The first manuscript is currently in preparation

4. Project activities undertaken during this reporting period:

Methodology

This research was conducted during the field season of 2018 using the red clover (*Trifolium pratense*) cultivar "Altaswede". The experiments took place at five locations: Clavet, SK (Livestock and Forage Centre of Excellence), Melfort (AAFC Research Farm), and three commercial fields were included north and south of Carrot river. The commercial fields are an addition from the original research plan. All the fields on conventional/commercial land grew red clover of the same cultivar "Altaswede" intended for seed production. The distance between commercial fields was at least 2 km. Locations in Clavet and Melfort were used for seeding rate and nitrogen fixation trials. The layout of the experiments at Clavet and Melfort was a split-plot arrangement on Randomized Complete Block Design (RCBD) with four replications while Latin Square design with four replications was used at conventional sites. Six seeding rates of red clover (0.5, 2.5, 4.5, 6.5, 8.5, 10.5 kg/ha) were seeded at Clavet and Melfort sites by May 27th and June 15th respectively. Red clover seeds were inoculated with commercial inoculant that

contains *rhizobium leguminosarum* biovar *trifolii* according to the manufacturer's recommendation, which is 189 g (6.67 oz) per 22.7 kg (50 lb) of seed. Also, crested wheatgrass (*Agropyron cristatum*) strips were seeded as a non-N fixing check. In the commercial (grower) sites, three different weevil control strategies were applied: (i) Untreated control, (ii) Decis (*Deltamethrin*) and (iii) Exirel (*Cyantraniliprole*) at conventional sites. The insecticides were applied according to a producer's instructions using Co2 backpack sprayer. The original proposal was to use a combination of Exirel and BeelteGone! However, the manufacturers had production issues and that material was not available. Insecticides were sprayed when a number of lesser clover leaf weevil (LCLW) larvae exceeded three individuals per five randomly chosen shots, which took place on June 15th. Additional spraying was conducted in one of the conventional sites two weeks after the first application due to remaining LCLW pressure. To ensure adequate pollination at the commerical fields, beehives including honey bees (*Apis mellifera*) were placed by growers at a distance no farther than 250 m from the experimental plots in all sites. No insecticide treatments were applied at the Clavet and Melfort sites since single cut "Altaswede" red clover starts blooming either late or following year after sowing.

Notably, ggermination of red clover in Clavet was somewhat worse than in Melfort. This can be explained by red clover sensitivity to drought and limited precipitation, which occurred more in Clavet.

Yield harvesting of each individual plot from conventional the sites was conducted at the end of September when 75 percent of the heads had turned brown to dark brown.

Measurements

1. **LCLW**

To estimate efficiency of treatments on the number of LCLW in red clover three different methods were used at all plots of all treatments. The methods included: sweep netting, field counting of LCLW larvae in a field out of 10 shots and rearing LCLW larvae collected from the treated fields in lab conditions. The last method included collecting twenty red clover shoots and rearing larvae in growing chambers with optimal conditions for LCLW development. The clover shoots were placed in two small plastic cups (diameter 6.35cm, height 12.06 cm) field with

rooting hormone solution and caged in big plastic containers (width, 34 cm; height, 11.7 cm) with small meshed nets on sides to ensure that no condensation formed. The containers were stored at 20°C and 70% relative humidity for 10 days after which larvae and emerged adult weevils were identified and counted. All conventional sites were sampled for LCLW at least 5 times during the field season including the prior (pre) to and after (post) insecticide applications. The Melfort and Clavet sites were sampled twice since no treatments were applied in the first year. Temperature (°C), precipitation (mm), wind velocity (kph) were recorded every hour at a meteorological station "Nipawin Saskatchewan weather station" situated at a distance about 15 km from the experimental sites.

2. Pollinators

To estimate the effect of insecticide treatments on the abundance and diversity of pollinators, the two most commonly used methods for sampling pollinators were used at all plots of all treatments. Blue Vane Traps (Springstar Inc.) and "bee bowls" (blue, yellow, and white cups) were filled with either a combination of water and blue Dawn dish detergent or propylene glycol (antifreeze) and deployed into the fields at fixed 1-meter distances in each plot for twenty-four hours. In addition to bee bowls and vane traps, yellow sticky cards and sweep nest sampling were also used. Yellow sticky cards were deployed one per plot. Sweep sampling was performed by taking ten sweeps swung in a 180º arc such that the net was striking the top inches of red clover growth. Each 180º arc was counted as one sweep. We surveyed commercial (grower) plots for pollinators four times during the field season including pre-spraying and post-spray sampling. In addition, growers' fields were monitored outside the experimental plots using similar methods. The Melfort and Clavet sites were sampled two or three times respectively. Sampling was performed between May 28th and August 30th. Pollinators collected from samples are being identified to the lowest possible taxonomic level (typically genus or species) using a Zeiss SteREO microscope Discovery V8.

3. Winter survival

To assess red clover winter survival in Melfort and Clavet among the six seeding rates, the number of plants in one-meter transects were counted in the last week of September as a refrence. The number of overwintered red clover plant will be counted from the same rows in both sites at

the end of spring or when weather conditions will be favourable. This will be compared to the September counts to estimate winter survival.

4. Red clover seed yield data

To assess potential seed yield of red clover under the three different insecticide treatments all plots from conventional sites were sampled in the last week of September. A systematic random sampling technique was applied. Two sub-samples were collected from each plot using a 50 cm^2 square-shaped frame. All inflorescences were hand-harvested. Additionally, samples were collected from plots in segmentas of the commerical fields being grown under the grower's management for red clover seed yield. All samples were dried in a hot air seed dryer up to 11% of moisture and processed through a seed thresher machine.

Results and discussion

The results from the field experiments in 2018 have been analysed. Preliminary data indicates that both Decis (*Deltamethrin*) and Exirel (*Cyantraniliprole*) significantly reduced the number of LCLW larvae per 20 shoots compered to the untreated control when the counts were done ten days after treatments out of shoots grown in growing chambers. In contrast, the number of LCLW larvae was not significantly reduced by any treatment when LCLW larvae counts were done in fields 24 hours after spraying. Presumably, LCLW larvae were more vulnerable to insecticide treatments when the insects digested treated plants for a period longer than than 24 hours. Also, we suggest that LCLW adults may be more vulnerable to insecticide treatments then larvae; therefore, the number of adults and subsequent larvae pressure may be reduced with time. However, to test this hypothesis, sweep net specimens need to be fully processed and analyzed. This is ongoing.

Red clover seed yield was not significantly affected by insecticide treatments. On average seed yield varied from 410 to 520 lb/acre. However, seed yield from grower managed land decreased significantly in a field where Decis (*Deltamethrin*) was sprayed twice during the field season compared to non-sprayed fields. To understand whether yield reduction was due to agronomic practices or due to a reduction in pollination/pollinators resulting from insecticides we are analyzing bee samples collected during the red clover vegetation period.

The process of identifying insects (bees and pests) collected in the 2018 filed season is ongoing. However, preliminary observations indicate that pollinators in red clover mostly consist of wild bees from the bee genera *Bombus* and *Lasioglossum*, followed by commercially managed honey bees (*Apis mellifera*). Results on bee's vulnerability to applied insecticides during the 2018 season, and the role of bee diversity in red clover pollination in 2018, will be obtained by prior to the start of the 2019 field season.

Interim conclusion

Both Decis and Exirel have the potential to control LCLW pressure. A significant effect of these chemicals was not found immediately after application; nevertheless, pest reduction was noticed on the 10^{th} day. To understand the impact of our treatments on adult LCLW, red clover pests and pollinators will require more data and samples. This process in in progress.

Even though there was a trend toward increased yield in Decis treated plots, and with the lowest yield in the untreated controls, seed yield in insecticide-treated plots was not significantly affected.

Since LCLW pressure may fluctuate in different regions, new Northern Saskatchewan fields will be used for our trials in the coming field season (summer 2019) to assess the impact of LCLW on red clover seed yield and effects of chemical treatments on pollinators, LCLW and other pests.

Seed yield from seeding rate trials (Clavet, Melfort) will be determined this year (2019). Provided there is adequate seed production in the 2020 year, Clavet and Melfort plots will be also harvested for seed yield determination. The ^{15}N isotope dilution method will be used in spring-early summer (2019) to estimate N fixation rate by red clover.

Technology Transfer Activities

Results from insect studies were presented at conferences including: The Joint Annual Meetings of the Canadian and American Entomological Society (November 2018), The Entomological Society of Saskatchewan Annual General Meeting (December 2018), Western Forum, and the Saskatchewan Advisory Council on Forage Crop Meeting (January 2019).

Identify any changes expected to industry contributions

This research is intended to directly address issues of concern to Saskatchewan Agriculture, specifically producers of forage seed. By identifying the effects of red clover seeding rate on seed yield and N fixation, it will be possible to make recommendations on optimal seeding rates for red clover seed production. These recommendations will lead to better agronomic practices and will also provide a guide for crop insurance program which is of direct economic benefit to forage seed growers. The remainder of this research will produce information that will result in the development of Integrated Pest Management strategies for both pest and beneficial insects. This research will lead to recommendations and methods for managing the lesser clover leaf weevil. Potentially a new insecticide will be registered for controlling LCLW. Conversely, managing insect pests can harm pollinators. Finally, this research will produce information leading improved in red clover and thus greater yields.

Appendices

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