



## FINAL PROJECT REPORT

### Canola Agronomic Research Program (CARP)

The Final Project Report should fully describe the work completed for the project and note the personnel involved. It should also note any deviations from the original plan and any corrective steps that were taken during the course of the project. A complete statement of expenses should be included.

**Project Title:** Reliable and Effective use of Managed Bees for Canola Pollination

#### Research Team Information

<b>Lead Researchers:</b>		
<b>Name</b>	<b>Institution</b>	<b>Expertise Added</b>
Shelley Hoover	Alberta Agriculture and Forestry	Experience managing and assessment of honey bee hives, familiarity with leafcutter bee production and management, physical proximity to field sites near Lethbridge, experience in bee behavior and nutrition
<b>Research Team Members</b>		
<b>Name</b>	<b>Institution</b>	<b>Expertise Added</b>
Ralph Cartar	University of Calgary	Expert in bee behavior and foraging patterns
<b>Name</b>	<b>Institution</b>	<b>Expertise Added</b>
Stephen Pernal	AAFC	Physical proximity to field sites in Peace, expertise in canola pollen nutrition, expertise in honey bee pathogen identification and treatment, honey bee management
<b>Name</b>	<b>Institution</b>	<b>Expertise Added</b>
Andony Melathopoulos	University of Oregon	Experience in pollination requirements of other crops, assessing pollination deficit, honey bee management

**Project Start Date:** April 1 2014      **Project Completion Date:** March 31 2017

**CARP Project Number:** 2014-1

**Instructions:** This Final Project Report shall be completed and submitted on or about March 31<sup>st</sup> of the project completion year. The Lead Researcher of the project in question shall complete and submit the report on behalf of his/her complete research team.

This Report is a means by which to provide a final summary on the status and activities of the project. Details may be general in nature unless major issues or changes arise (e.g., change of scientists, significant change or delay of activities) including impacts on budgets. Please note that financial reports of major impact on budgets.

The following template is provided to assist you in completing this task. Please forward the completed document electronically to your appropriate CCC contact.

**1. Forecasted Date of Completion:**

All fieldwork is complete for this project, and approximately 85% of the plant samples have been processed. We also have a small number of mounted flower stigmas remaining that we need to count the pollen on. We expect sample analysis to be completed by the end of the summer 2017. We are currently analyzing the data, and preparing final reports and papers for publication.

**2. Status of Activity: (please check one)**

Ahead of Schedule  On Schedule  Behind Schedule  Completed

**Comment:** We are slightly behind schedule – we hoped to have all the plant material processed by the end of March, but it will likely take a couple more months.

**3. Completed actions, deliverables and results; any major issues or variance between planned and actual activities.**

**No major variance between planned and actual activities**

**Presentations:**

Waytes R (2017) Pollination of hybrid canola – pollinator efficacy and movement. Alfalfa Seed Commission Meeting, Brooks.

Hoover S (2017) Apiculture Research Program Update. Southern Alberta Beekeepers, Fort MacLeod.

Ovinge L and Hoover S. (2017) Singles versus doubles in Canola Pollination. Southern Alberta Beekeepers, Fort MacLeod.

Waytes Riley, Cartar R, Hoover S (2017) How pollinators contribute to hybrid canola pollination. Southern Alberta Beekeepers, Fort MacLeod.

Waytes R, Cartar R, and Hoover S. (2017) Competitors facilitate pollinator movement in hybrid seed canola. Oral presentation at: 51<sup>st</sup> Annual Prairie University Biology Symposium. University of Saskatchewan, Saskatoon, SK.

Hoover S (2017) Lethbridge Research Centre Apiculture Research Program. Calgary Beekeepers Club, Calgary.

Waytes R, Cartar, R, and Hoover, S (2016) Are bees ideal and free? The role of competitor density and floral rewards in patch selection of managed pollinators in a gynodioecious crop system. Entomological Society of Alberta, Calgary

Robinson S, Cartar R, Pernal S, and Hoover S (2016) The spatial distribution of central place foraging pollinators in mass-flowering crops. Entomological Society of Alberta, Calgary

Robinson S, Cartar R, Pernal S, Hoover S (2016) The Spatial distribution of central place foraging pollinators in a landscape of mass-flowering crops. British Ecological Society, Liverpool.

Hoover S, and Ovinge L (2017) Honey Production, pollen collection, and the provisioning of pollination services in canola. North American Beekeeping Convention / American Bee Research Conference, Galveston.

Hoover S (2016) Pollen collection, honey production, and the provision of pollination services. Alberta Beekeepers Commission AGM, Edmonton

Hoover S (2016) Bees in Canada. Organic Farm Tour, Lethbridge

Hoover S (2016) Research Update. Southern Alberta Beekeepers Meeting, Cayley

Melathopoulos A (2016) Bang for your Buzz: Commodity Canola Pollination. Southern Alberta Beekeepers Meeting, Cayley

Ovinge L (2016) Singles versus doubles in canola seed pollination. Southern Alberta Beekeepers Meeting, Cayley

Hoover S Bee Research. "Science-O-Rama, Canola Council, Edmonton April 6 2016

Waytes R (2016) Pollinator Efficacy and choice in hybrid seed canola. Prairie University Biology Symposium, Lethbridge.

Cartar R (2016) Return of the native? Wild bees in agro-ecosystems. FarmTech meeting, Edmonton.

Melathopoulos A, and Hoover S, (2015) The challenges of leafcutter bee pollination of hybrid canola. Alberta Alfalfa Seed Commission. Brooks.

Vandervalk L, Hoover S (2015) Canola Pollination – Cost of Pollination. Alberta Beekeepers AGM, Edmonton (Oral)

Hoover S (2015) Canola Pollination – Bee behaviour and crop traits. Alberta Beekeepers AGM, Edmonton (Oral)

Robinson S, Cartar R, Hoover S, Pernal S (2015) Pollination, insect visitation, and nectar availability in commercial canola (*Brassica napus* L.). Entomological Society of Alberta, Jasper.

Waytes R, Cartar R, Hoover S (2015) Bee pollination effectiveness in hybrid seed canola measured with an interview bouquet. Entomological Society Alberta, Jasper

Vandervalk L, Hoover S (2015) Honeybee pollination of hybrid seed canola in southern Alberta. Société d' Entomologie du Québec and the Entomological Society of Canada, Montreal.

Hoover S, and Vandervalk L (2015) Singles versus Doubles for pollination in seed canola. Beaverlodge field day, Beaverlodge.

Hoover S (2015) Canola Pollination. CAPA / CHC AGM, New Brunswick Beekeepers meeting, Moncton.

Hoover S (2015) Health of honey bees in Canada. Public Pollinator Forum, CAPA / CHC AGM, New Brunswick Beekeepers meeting, Moncton.

Hoover S, and Vandervalk L (2014) Use of singles versus doubles in canola pollination. Alberta Beekeepers Commission AGM, Edmonton

Robinson S, Cartar, RV, Hoover S, Pernal S (2014) Determinants of pollination, insect visitation, and nectar availability in commercial canola (*Brassica napus* L.). Ent Soc Alberta, Lethbridge.

Hoover SER, and Wolf-Viega P. (2014) Healthy Bees: Healthy Agriculture. Growing the North. Grande Prairie.

**Completed Theses:**

Waytes, R (2017) Pollinator Movement and Pollen Transfer in Hybrid Seed Canola. MSc Thesis, University of Calgary.

#### 4. Significant Progress/Accomplishments

##### Progress to end of March 2017

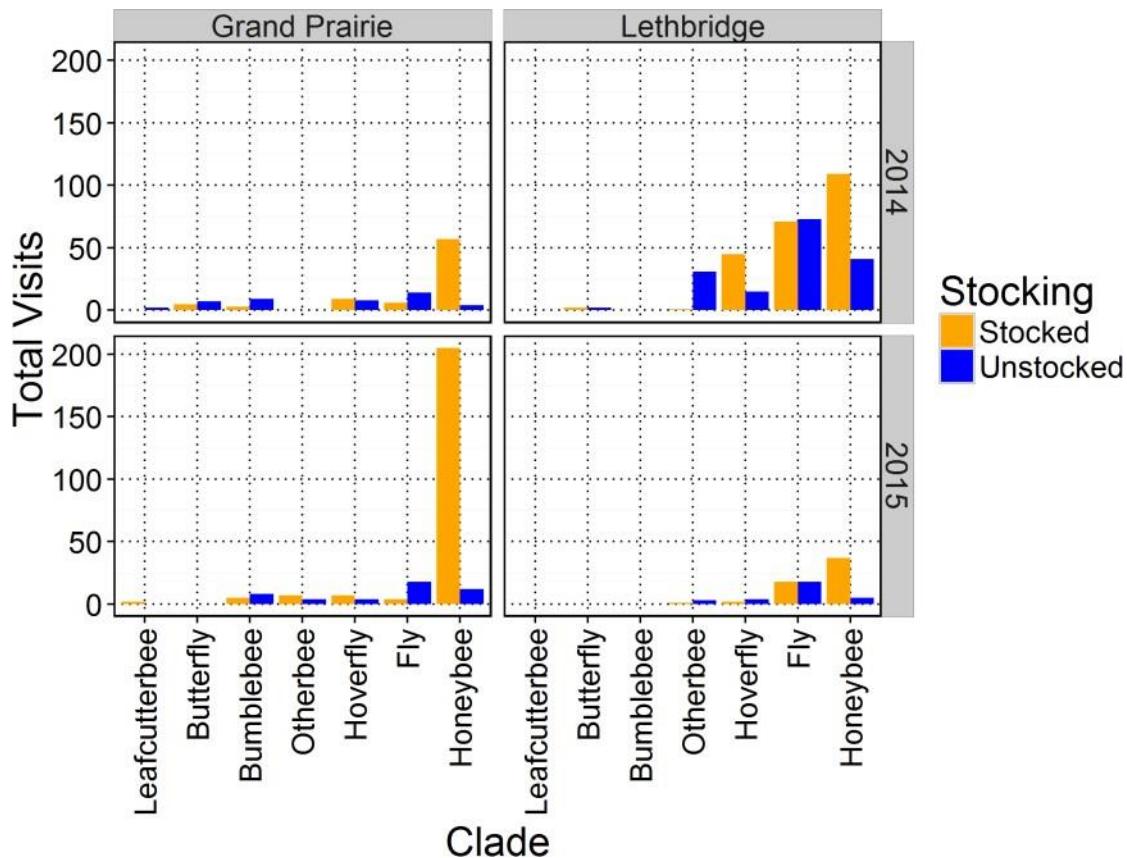
###### Survey 1. Canola yields versus bee abundance:

###### (a) Commodity canola fields

This component of the project asked the questions: what pollinators are present in commodity fields? How important are pollinators for canola yield in these fields?

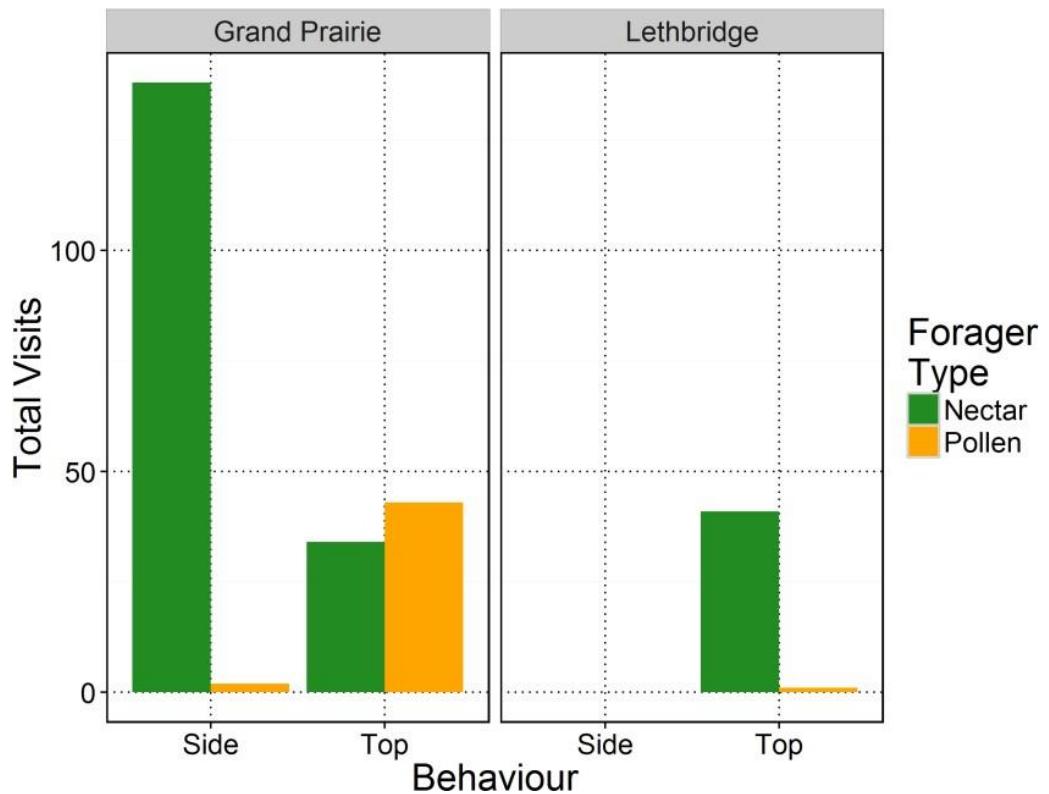
Completed to date: 2015 was the second year of surveys, in which pollinator diversity and abundance were surveyed at 4 distances from the field edge in commodity canola fields (5, 20, 100, and 400 m into the field center), and related to pollen counts on floral stigmas, and seed yield. In each of 2014 and 2015, we conducted the survey at two sites in Alberta (Lethbridge, Peace), with about 30 fields per year. We also measured traits of plants from which seeds were obtained, to relate yield to plant floral success and branching. The pollinator observations are complete, and all plants have been harvested and processed.

We found that in fields stocked with honey bee hives, honey bees were the dominant flower visitor, followed by large flies (Muscidae, Anthomyiidae, Calliphoridae) and hoverflies (Syrphidae), and wild bees (Figure 1). In unstocked fields, large flies were the dominant flower visitor. Visitation rates were lower overall in 2015 than in 2014 in Lethbridge, even in stocked fields. While honey bees dominated in both the north and south, there were also distinct differences in the pollinator communities at our two sites (Lethbridge and Grande Prairie); flies were more common in the south, and bumble bees more common in the north.



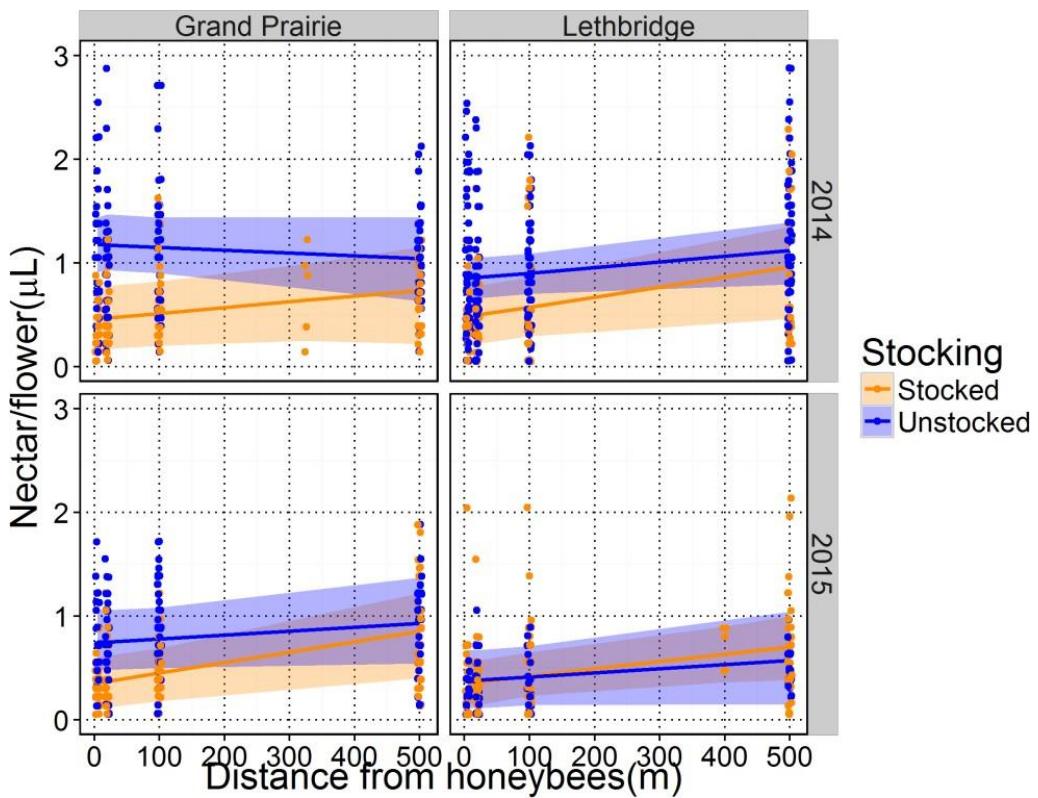
**Figure 1. Number of flower visitors for each site in 2014 and 2015 by taxonomic group. Gold bars represent fields stocked with managed honey bees, blue bars are unstocked fields.**

In 2015, we observed flower visitation behaviour in the visiting honeybees, and found that most of the flower visitation by honey bees was to collect nectar. A greater proportion of bees in the Grand Prairie than Lethbridge region engaged in nectar-robbing behaviour by side-working the flowers (Figure 2). This side-working behavior does not contribute substantially to pollination.



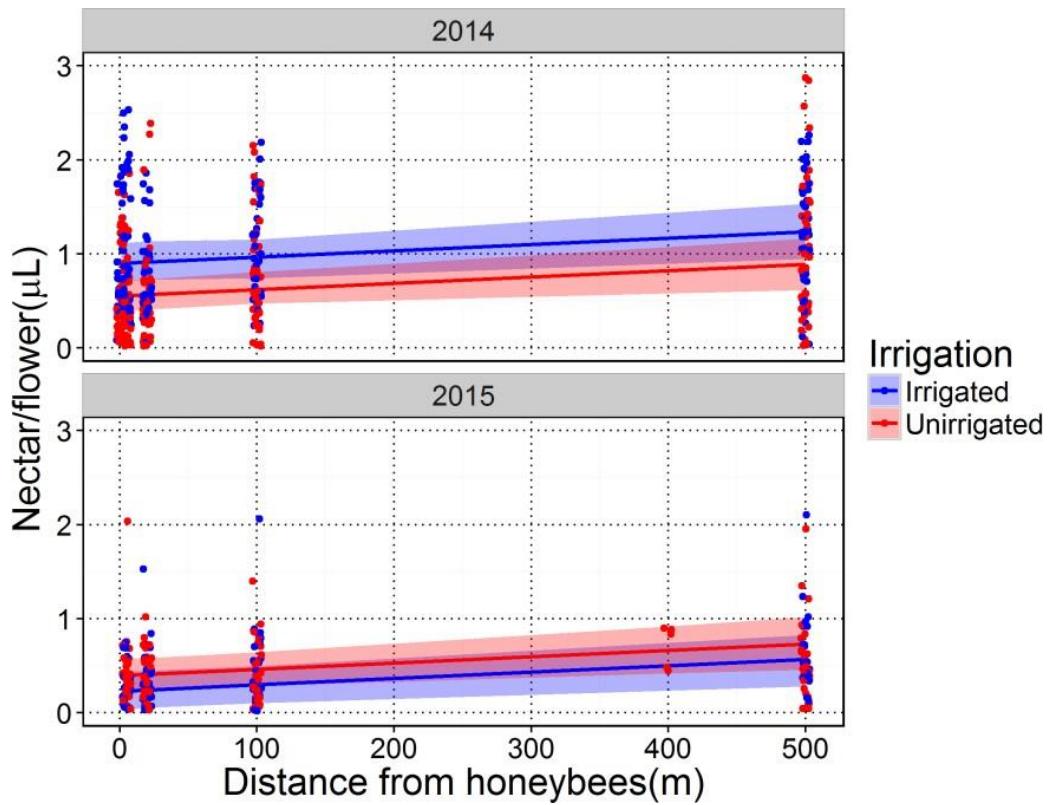
**Figure 2. Behaviour of honey bees visiting commodity canola flowers; bees either side-worked the flower to access nectar (side), or accessed the flower from the front (top) thus coming in contact with pollen as well as accessing the nectaries. Gold bars represent honey bees with pollen on their corbiculae, green bars represent nectar foragers.**

The standing crop of nectar in the flowers generally increased with distance away from the field edge in commodity fields, but this pattern differed between years, between stocked and unstocked fields, and between the Lethbridge and Grand Prairie regions (Figure 3). In unstocked fields, there was no decline with distance in Grand Prairie, and a marginal distance effect in Lethbridge ( $p=0.08$ ). Standing crop decreased with distance from the honey bee hives at the edge of the field in stocked fields in both regions, and in Grand Prairie there was significantly less nectar available in the field in 2015 than 2014.

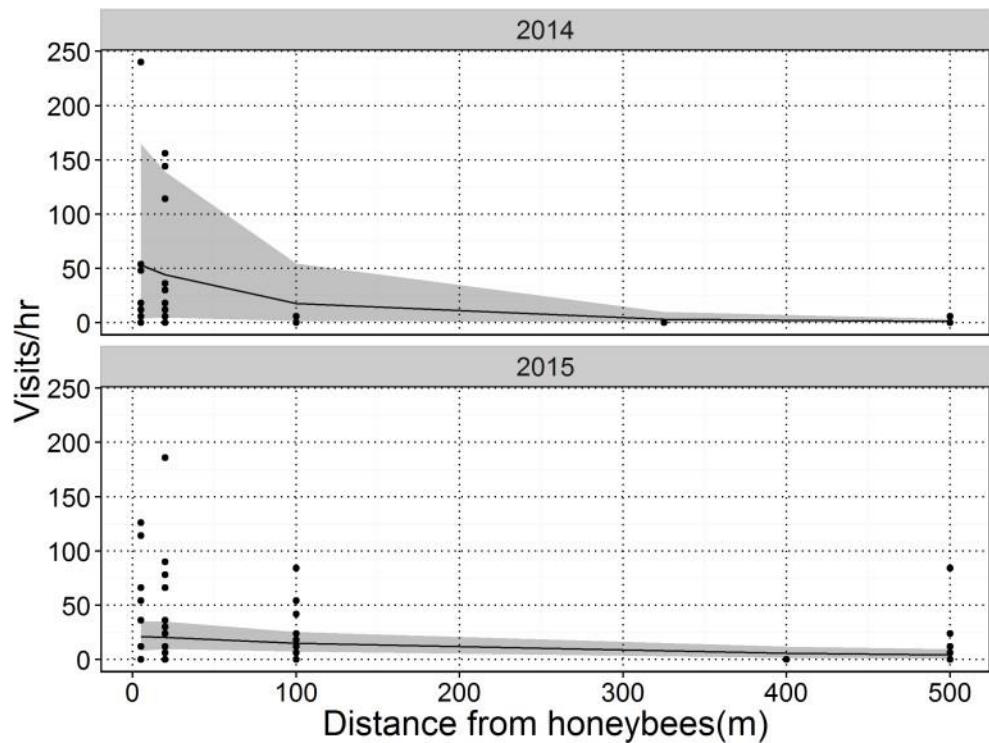


**Figure 3. Standing crop of nectar versus distance into the field for either unstocked (blue) fields, or fields stocked (gold) with honey bee hives. Stocked fields showed a consistent pattern of increase in nectar availability with increasing distance from the bee hives.**

In the Lethbridge region, nectar standing crop was higher in irrigated commodity canola fields, but only in 2014 (Figure 4). There was less overall standing nectar crop in 2015 in Lethbridge. Decreased standing crop at the edge of the field is likely the result of depletion by foraging insects, as there was no significant trend between soil moisture and nectar standing crop in 2015. Honeybee visitation to plots in the fields declined rapidly with distance into the field in both 2014 and 2015 (figure 5).

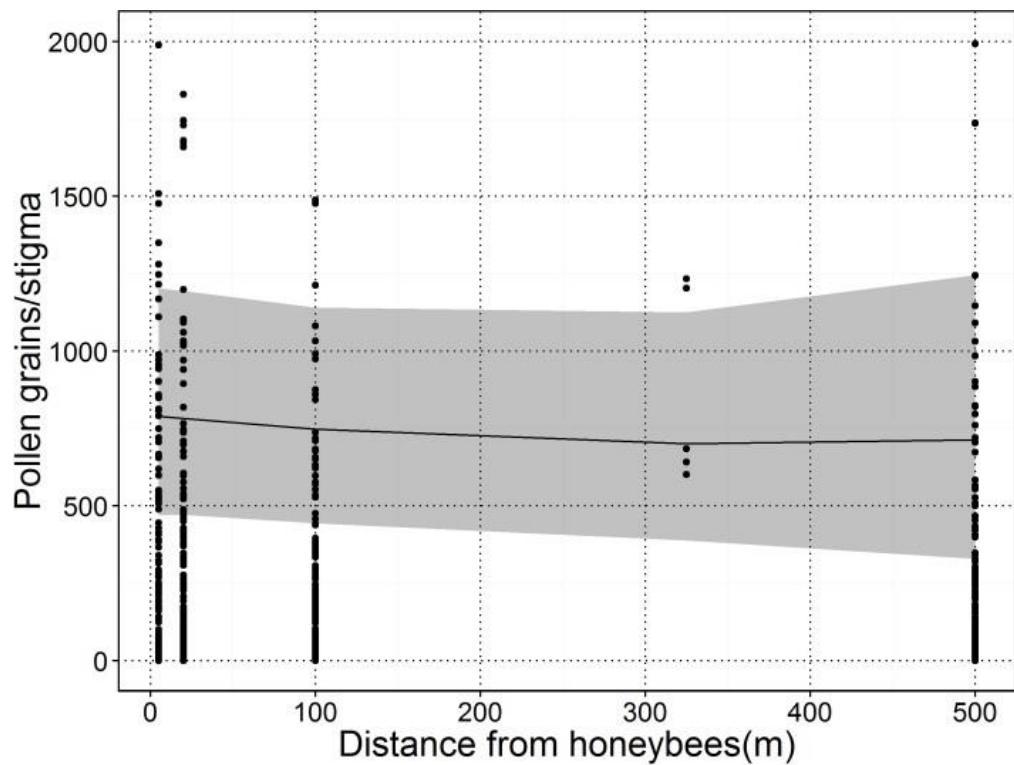


**Figure 4.** Standing crop of nectar in irrigated (blue) and unirrigated (red) commodity canola fields. There was greater nectar availability in 2014.



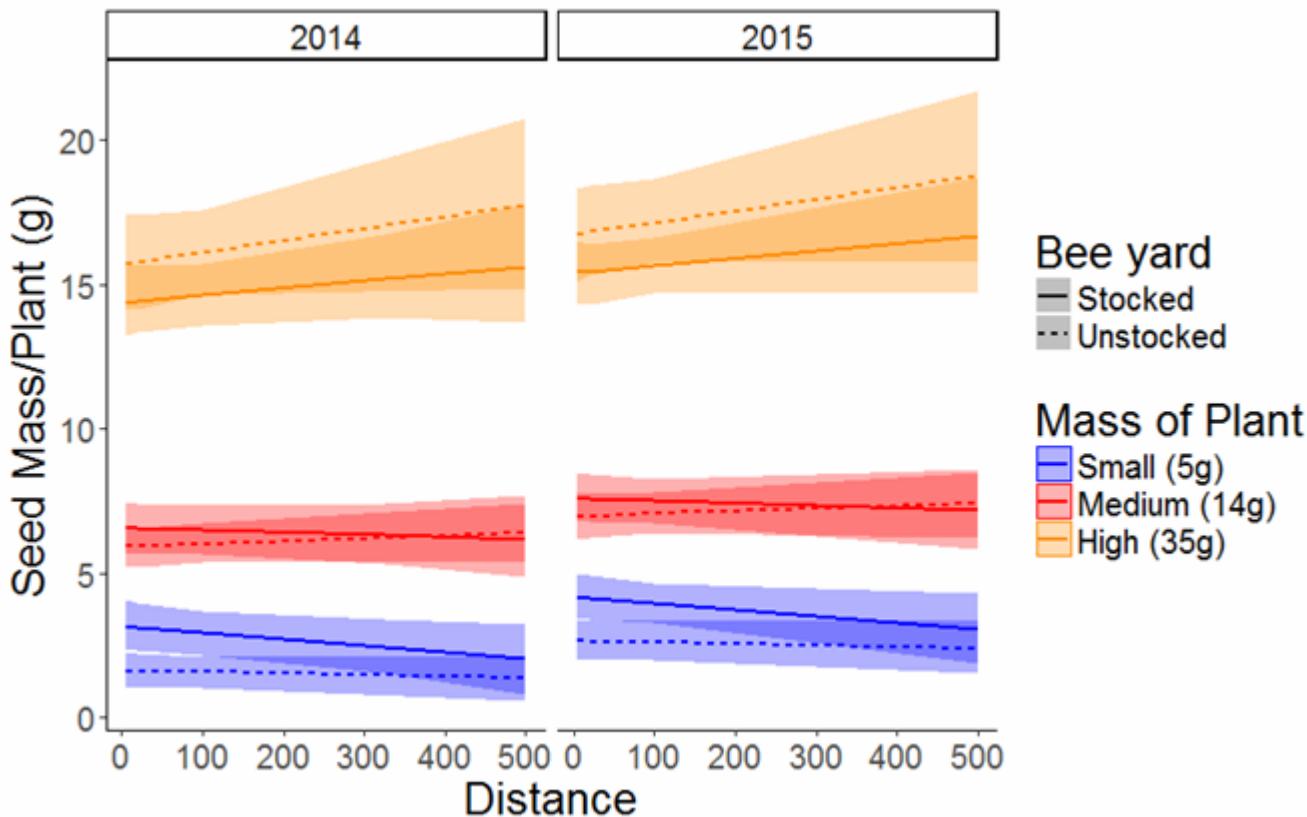
**Figure 5.** Honey bee visits to experimental flower plots at different distances into commodity canola fields. Bee abundance declined rapidly with distance.

However, while bee abundance declined with distance (figure 5), pollen deposition on stigmas did not similarly decline ( $p=0.1$ ) in 2014, meaning that bee pollination accounts for only a fraction of the total pollen deposited on stigmas in these fields (Figure 6).



**Figure 6. Pollen deposition on stigmas with increasing distance into the field, and away from honey bee hives. While bee abundance declined with distance into the fields, pollen deposition did not.**

Finally, we found that seed yield per plant was strongly correlated with size of plant (larger plants produced more seeds), but that proximity to honey bee hives was important for yield in smaller plants (Figure 7). This suggests that smaller canola plants may benefit from additional pollination, but that the effect is negligible for larger plants.



**Figure 7.** Seed yield per plant with increasing distance into the field, for a variety of plant sizes (5-35g), under stocked (honeybee hives present) and unstocked conditions, for both 2014 and 2015. Plants in 2015 produced significantly more seed, and there was a decline in seed yield with distance from honey bees, but only for smaller plants.

Remaining work: field/plot-level yield data processing is in progress, stigma pollen counts for 2015 samples is in progress.

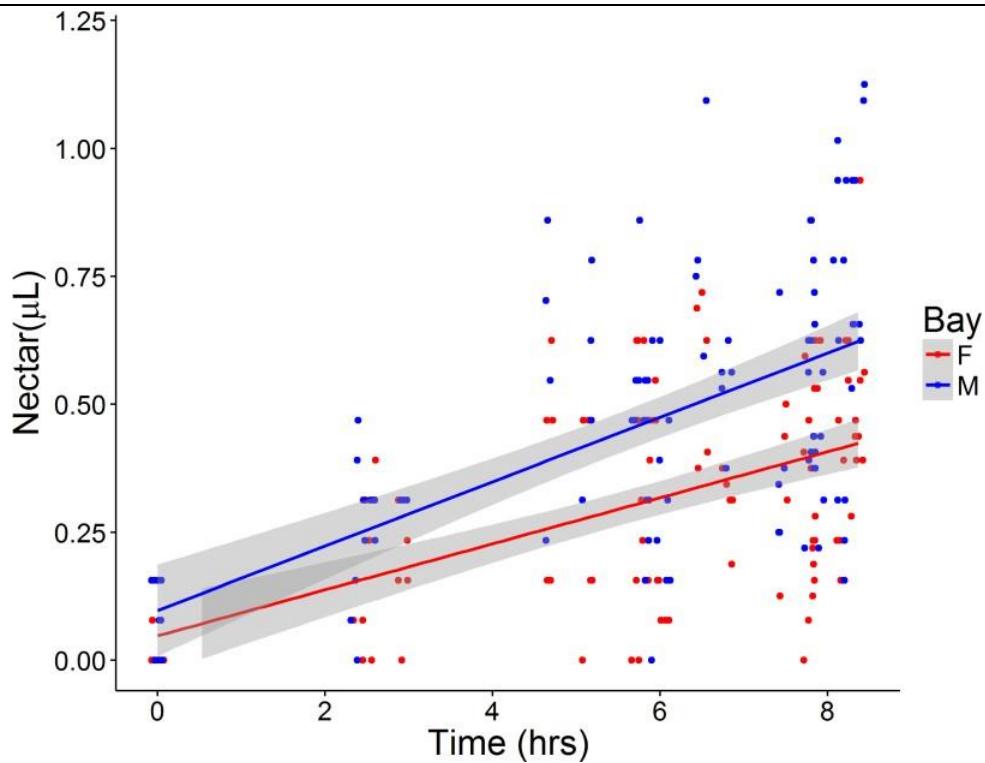
#### **Survey 1. Canola yields versus bee abundance.**

##### **(b) Seed canola fields**

2015 was the first year of field work for this aspect of the project, where pollinator densities were surveyed at 4 distances from the field edge closest to honey bee colonies (5, 20, 100, and 400 m into the field). In 2015 and 2016, we also measured the densities of pollinators at the edge and center of female “bays” (i.e., those flowers responsible for producing the seed), and the flower-visiting behaviour (pollen collection vs. nectar collection vs. nectar robbing) of pollinators in the 2 bay types (male and female).

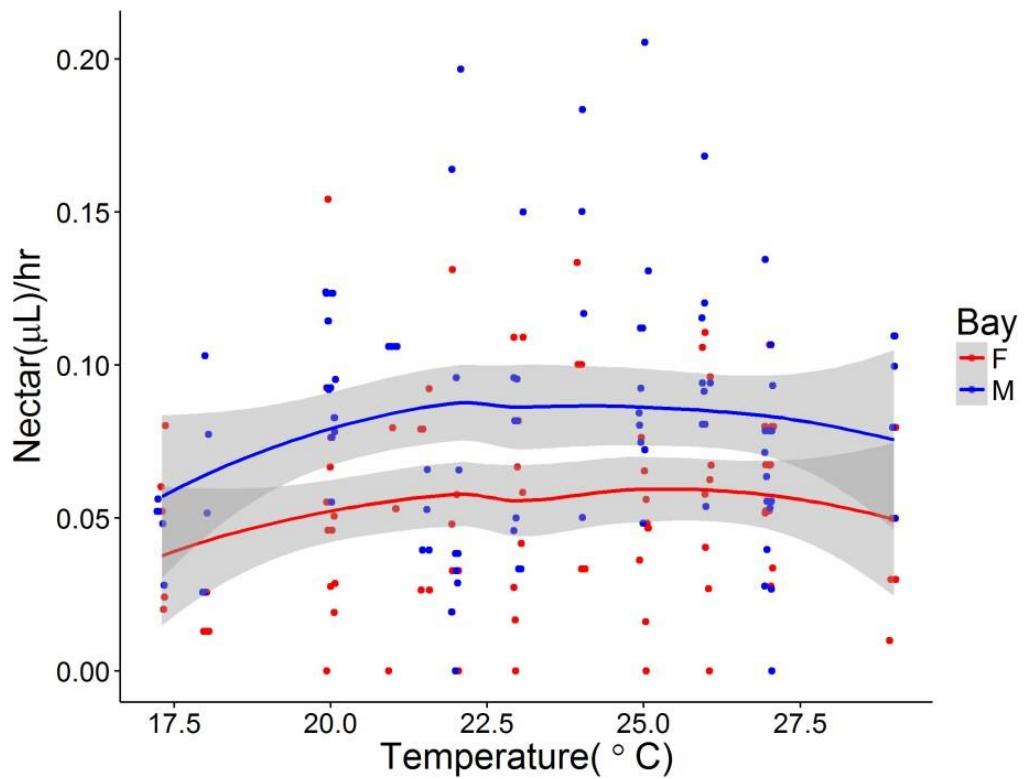
Completed: We surveyed hybrid canola seed production fields in 2015 and 2016, plants from 2015 have been harvested and processed, and observational data have been analysed.

There were negligible amounts of nectar in the standing crop in the open flowers in seed fields both in 2015 and 2016, only a small fraction of what is available in commodity fields. However, using netted flowers, we were able to gauge the nectar production rate over time. We found that male-sterile (‘female’) plants produce about 0.04uL/hr, whereas male-fertile (‘male’) plants produce about 0.07uL/hr (figure 8) in the varieties examined.



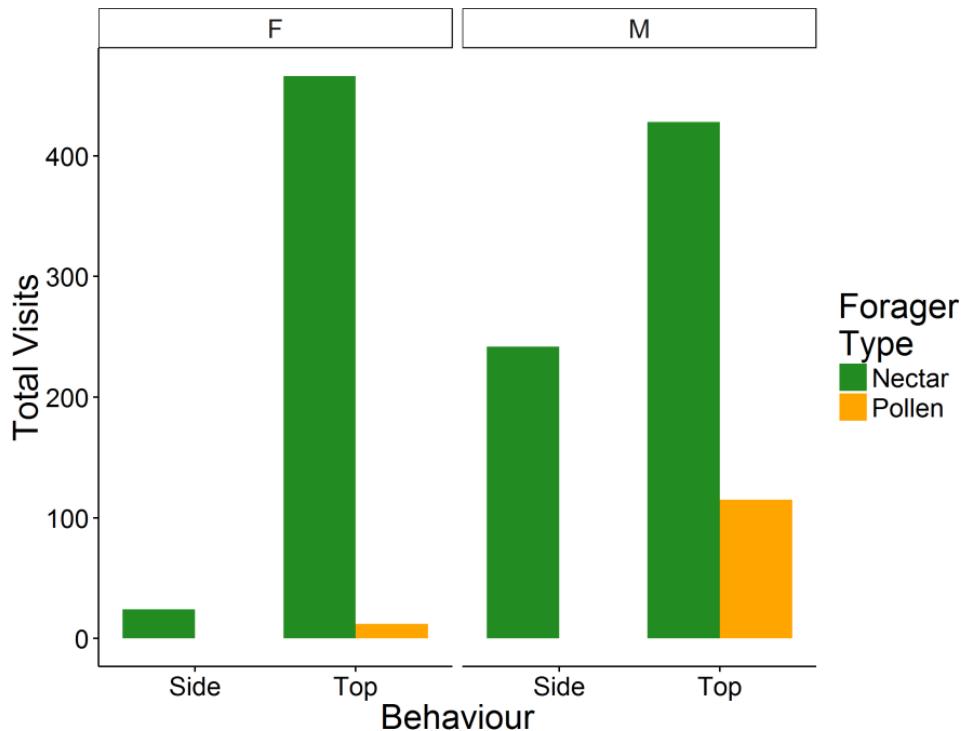
**Figure 8. Nectar production over time in netted flowers inaccessible to bees. 'Male' plants produced more nectar than 'female' plants per hour.**

Both male and female plants experienced peak nectar production during days where the temperature was about 21-27°C (Figure 9).



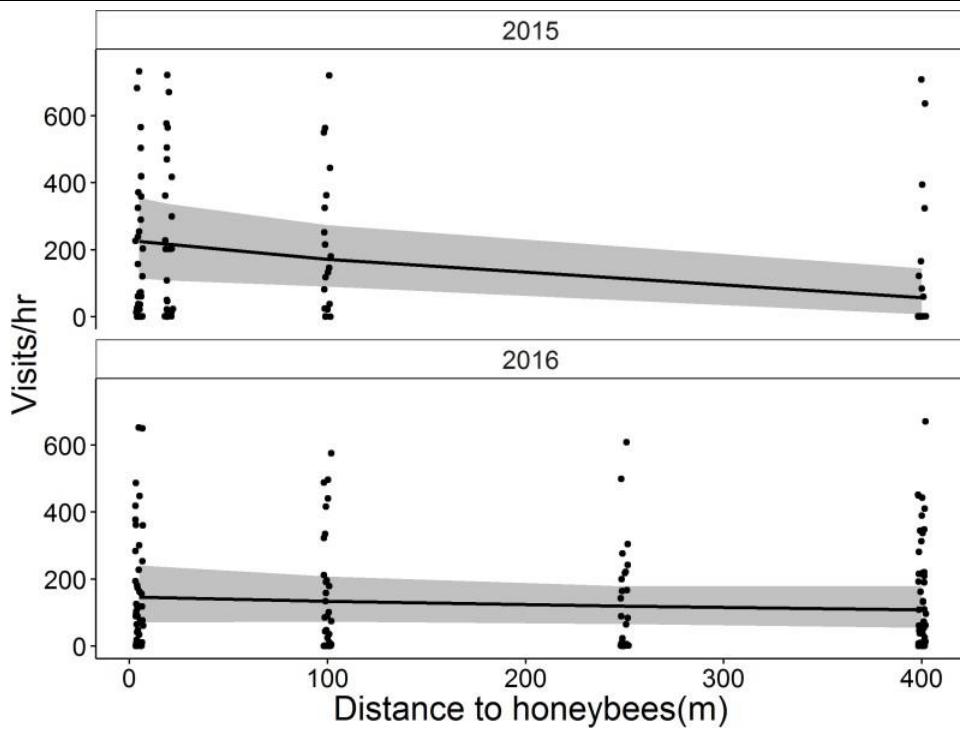
**Figure 9. Nectar production per hour in male (blue) and female (red) plants, at different temperatures.**

In 2015, we found that the majority of honey bees in seed fields were nectar foragers (no pollen loads observed), and that approximately equal numbers of foragers were observed/ m<sup>2</sup> in male and female bays (Figure 10). Pollen foragers were observed primarily in the male bay, which makes sense, given that only the male plants produce pollen. Side-working nectar foragers were mainly observed in the male bay.



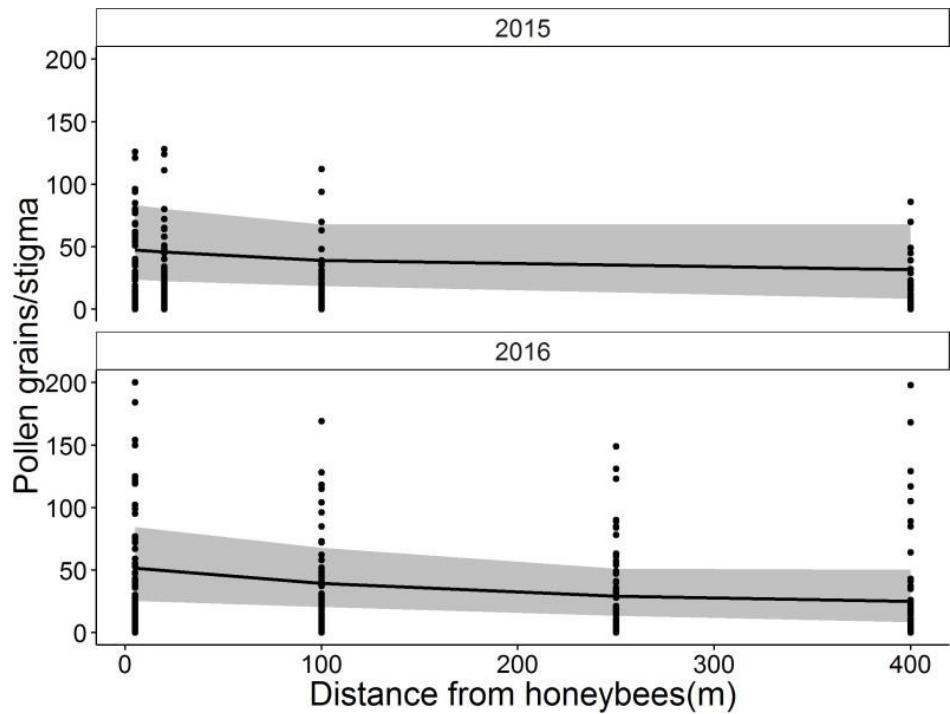
**Figure 10.** Total visits of honey bees per plot in seed canola fields for both pollen (gold) and nectar (green) foragers in both male (M) and female (F) bays; foragers either accessed the flower from the top (thus coming in contact with pollen) or side-worked them (nectar robbing).

Honeybee visitation declined with distance from field edge / hives in 2015 but not 2016. We found no difference in visitation of honey bees between male and female bays.



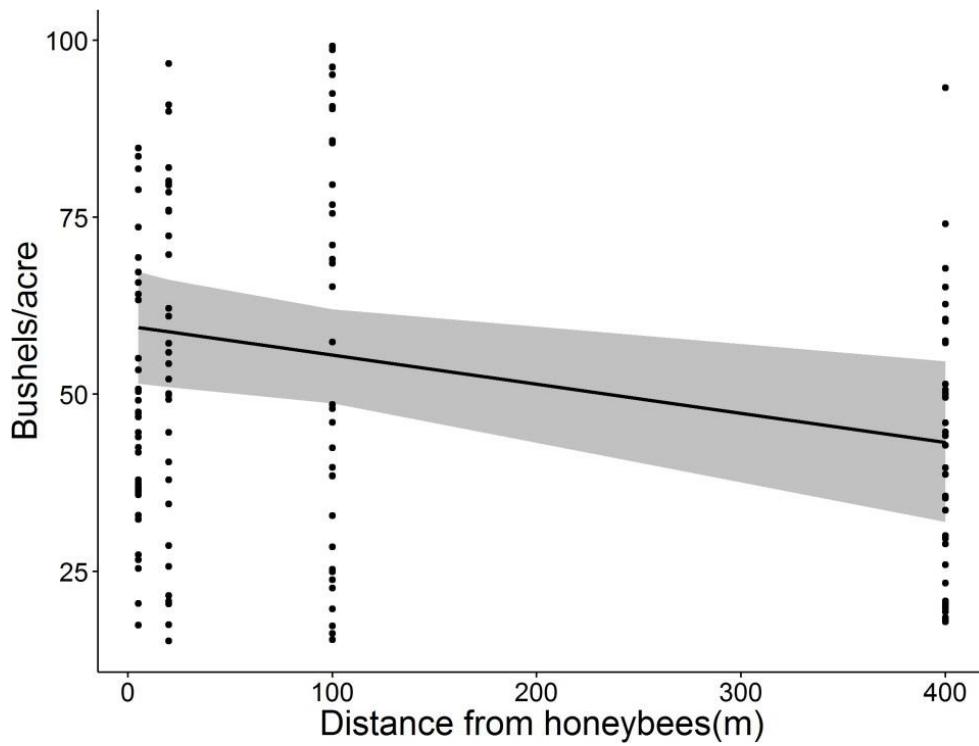
**Figure 11. Honey bee visits per plot with increasing distance into the field (and away from hives).**

In contrast to our findings from commodity canola, there was a decline in pollen deposition with distance into the seed production fields in both 2015 and 2016 (figure 12). This indicates that honey bees likely play a larger role in pollen deposition in the seed fields than the commodity fields, and is present despite the activity of leafcutter bees in all our study fields.



**Figure 12. Pollen deposition on stigmas with increasing distance into seed fields / away from hives.**

We also observed a decline in seed yield with distance from honey bee hives in 2015, indicating that there may be a pollination deficit at greater distances from the honey bee hives (figure 13).



**Figure 13. Seed yield at increasing distances from honey bee hives in seed production fields in 2015.**

Remaining: Analyses of the above factors for leafcutting bees to be completed summer 2017, and data processing, finish processing remaining plants from 2016 (March -May 2017).

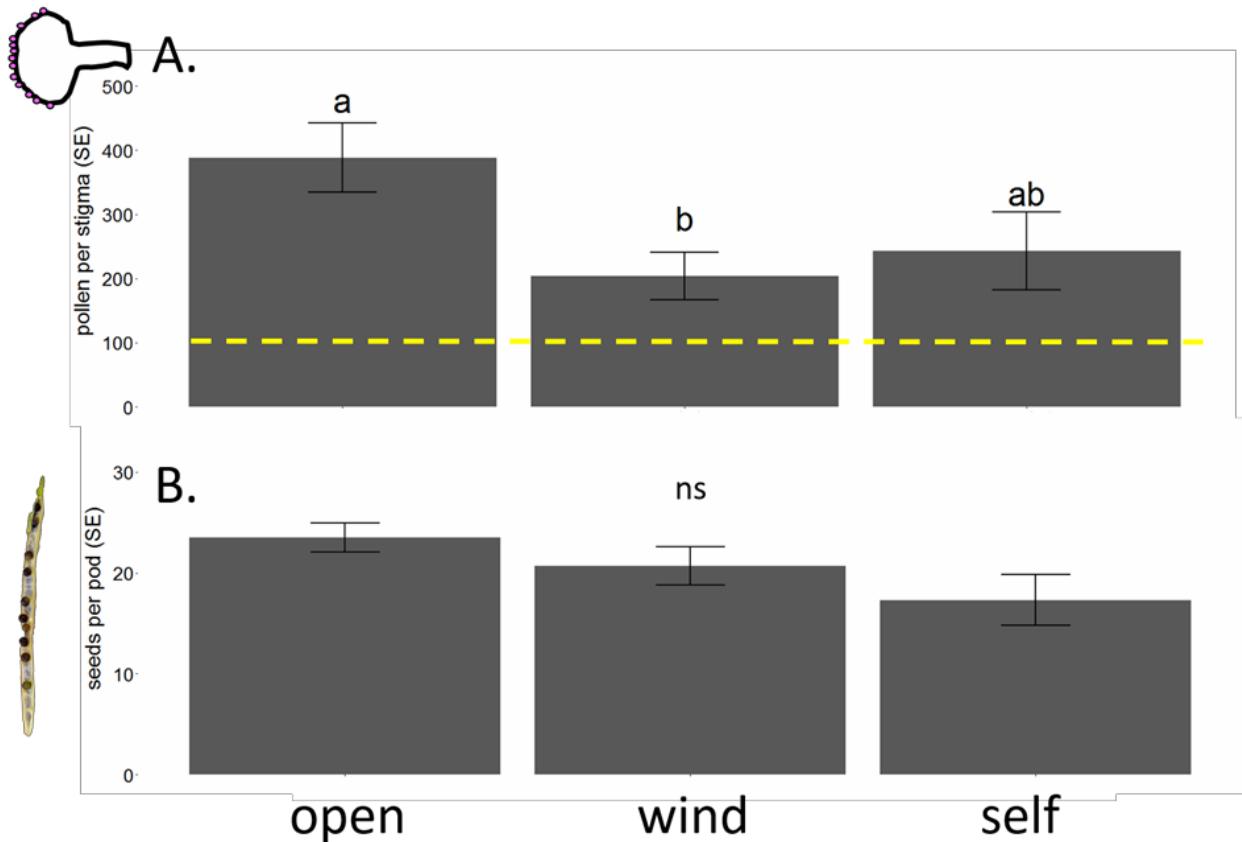
### Experiment 1: Canola Yield versus bee abundance

In 2016 we repeated our experiment from 2015 to determine if the yield of commodity canola increases with honey bee pollination or whether most of the pollination is accomplished either by wind- or self-pollination (adding 6 fields in 2016 to the 3 fields used in 2015). As in 2015, we separated these effects by comparing yield in plots that were subjected to one of the following three treatments:

- 1) **open**-pollination (uncaged plot)
- 2) **wind**-pollination (plants placed in a coarse mesh cage during bloom to exclude pollinators but not wind)
- 3) **self**-pollination (fine mesh cage to exclude both pollinators and the wind)

Given the importance of wind-pollination in 2015, we tried to measure the extent to which wind increased the amount of airborne pollen by periodically sampling the airspace above the plants in the three different treated plots in 2016. The measurement and analysis of airborne pollen will be completed in April 2017. Also, given that our plots in 2015 had inconsistent visitation from honey bees during bloom we expanded our study to include an additional 14 commercial fields throughout Lethbridge County with and without commercial apiaries adjacent to the plots. These additional fields also spanned a wider gradient of soil moisture than in 2015, with half the fields under irrigation and half dryland. Unlike the 3 fields from 2015 and 6 fields from 2016, which included three treated plots, these additional 14 fields focused on the effect of open vs. wind pollination (i.e., 2 plots per field). Pod counts and plant measurements from the 2016 field trials have been completed and we anticipate having yield measurements finished by January 2017.

Finally, our analysis of stigmas collected from 2015 provides a clue as to why yields fail to increase under conditions of open-pollination compared to wind pollination. Preliminary analysis suggests that seed set did not continue to increase beyond 100 grains of pollen per stigma. Consequently, even though flowers in open-pollinated plots had significantly more pollen per stigma compared to wind-pollinated plots (figure 14), flowers in both plots exceeded the threshold of 100 pollen grains, translating into a lack of difference in seed set. We have completed counting our stigma samples from 2016 and will begin analysis of these data in December 2016 to confirm our findings from 2015.



**Figure 14. Measures of (A) pollen deposition on the stigmas and (B) resulting seeds per pod from canola flowers located in one of three treated plots: open-pollination (uncaged plot), 2) wind-pollination (plants placed in a coarse mesh cage during bloom to exclude pollinators but not wind) and 3) self-pollination (fine mesh cage to exclude both pollinators and the wind). The hashed yellow line indicates the threshold level of pollen required to fully set the ovules of a canola flower (from 2015 data). Means followed by the same lower-cased letter indicate no significant differences (Tukey-Kramer HSD,  $\alpha=0.05$ ).**

## Experiment 2: Pollinator Efficacy

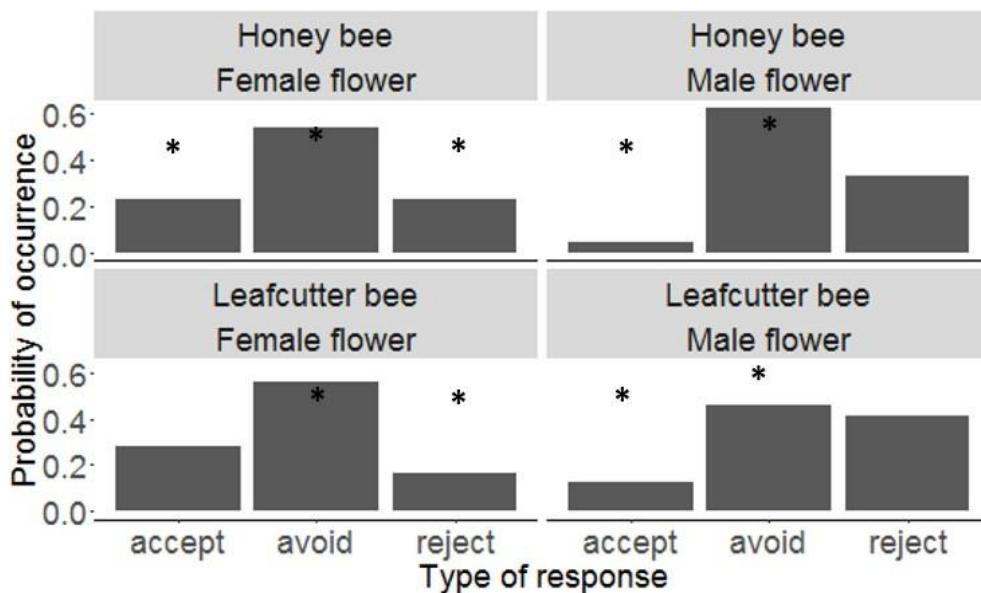
Pollinator efficacy and response were tested in 21 hybrid canola fields in 2015, and 18 fields in 2016. Pollinators were offered a virgin female inflorescence (flower) using an interview bouquet method and allowed to visit the flower. Pollinator responses to the inflorescence were recorded via video, and visitation to a flower resulted in collection of both the pollinator and flower stigma for processing. Managed pollinators as well as wild bees and hoverflies (when present) were included.

After the end of the field season, videos were analyzed to establish pollinator identity and behaviour, the type of flower the pollinator was visiting before being offered the bouquet (male or female), and the amount of time pollinators spent on the flower. Pollinator behaviour was separated into three separate categories: avoid, reject, or accept. The 'avoid' category included all pollinators that did not visit the flower but also showed no

indication of seeing the flower; this is the broadest category, and contains pollinators that potentially refused to visit the flower (but not in a way that was obvious to the video reviewer), did not see the flower, or were scared away by the interview bouquet apparatus. The 'reject' category implied that there was a visual indication that the pollinator saw and potentially inspected the flower, but flew away before touching the flower's stigma. The last category, 'accept,' contains all pollinators that contacted the reproductive parts of the flower. Once a pollinator accepted a flower, it was collected and the stigma of the flower was preserved on a slide.

Collected stigmas were examined under a microscope to count pollen deposition. Collected pollinators were sonicated in ethanol to remove any pollen stuck to their body, and the amount of pollen released into the solution was counted with the use of a hemocytometer.

In 2015, only two types of pollinators (honey bees and alfalfa leafcutter bees) were numerous enough to be included in the pollinator response analysis. An interaction between the flower of origin and pollinator type ( $\chi^2=6.99$ ,  $df=2$ ,  $p=0.03$ ), as well as Julian date ( $\chi^2=9.76$ ,  $df=2$ ,  $p=0.001$ ), significantly influenced pollinator choice (Figure 15).



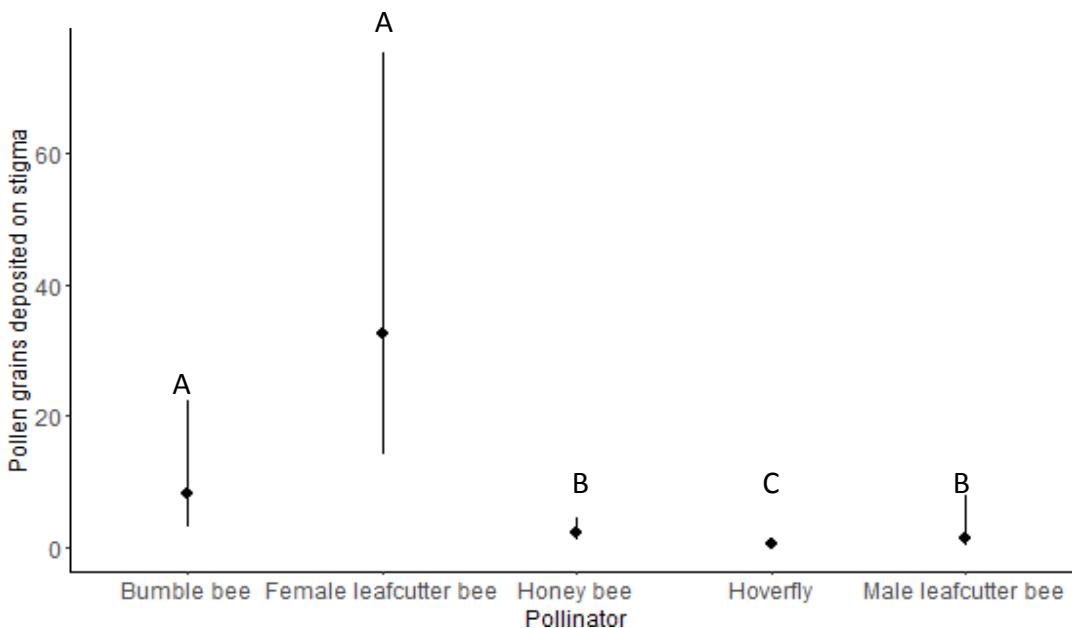
**Figure 15. Proportion of actions taken by alfalfa leafcutter bees (ALCB;  $n=196$ ) and honey bees (HB;  $n=226$ ) in response to female inflorescences, separated by flower of origin (male or female). Response actions include accept, avoid, and reject. There is a significant interaction between pollinator type and flower of origin ( $p=0.003$ ). Asterisks denote behaviours that significantly deviate from the expected 1:1:1 ratio.**

To parse out the interaction between pollinator type and flower of origin, we used post hoc analyses to compare the likelihood of a pollinator exhibiting a response against the chance that responses occurred randomly (in which case actions would occur at a 1:1:1 ratio, where each was just as likely to occur as the other). Both leafcutter and honey bees foraging on female and male plants avoided inflorescences more than would be expected ( $p>0.001$  in each case), which is likely an artifact of how pollinators interact with the interview bouquet apparatus. While foraging on female flowers, honey bees were less likely to accept or reject a female inflorescence than expected ( $p=0.01$  for both cases). When foraging on male flowers, honey bees were less likely to accept a female inflorescence ( $p>0.001$ ), but 'reject' was not different from expected proportions ( $p=0.86$ ), suggesting that honey bees are more likely to reject a female inflorescence while foraging on male flowers but not female flowers.

While foraging on female flowers, leafcutter bees accepted female inflorescences with the expected frequency ( $p=0.12$ ), and were less likely to reject them ( $p>0.001$ ). While foraging on male flowers, leafcutter bees accepted female inflorescences less than the expected 1/3 frequency ( $p>0.001$ ) and rejected them with the expected frequency ( $p=0.06$ ). Leafcutter bees therefore were less likely to accept female inflorescences while foraging on male flowers than while foraging on female, and more likely to reject them. The results of this analysis suggest that both leafcutter and honey bees exhibit floral constancy, with honey bees being more likely to reject a female inflorescence when foraging on male flowers (as opposed to when foraging on female flowers), and leafcutter bees are more likely to accept and less likely to reject a female inflorescence when foraging on female flowers (as opposed to when foraging on male flowers).

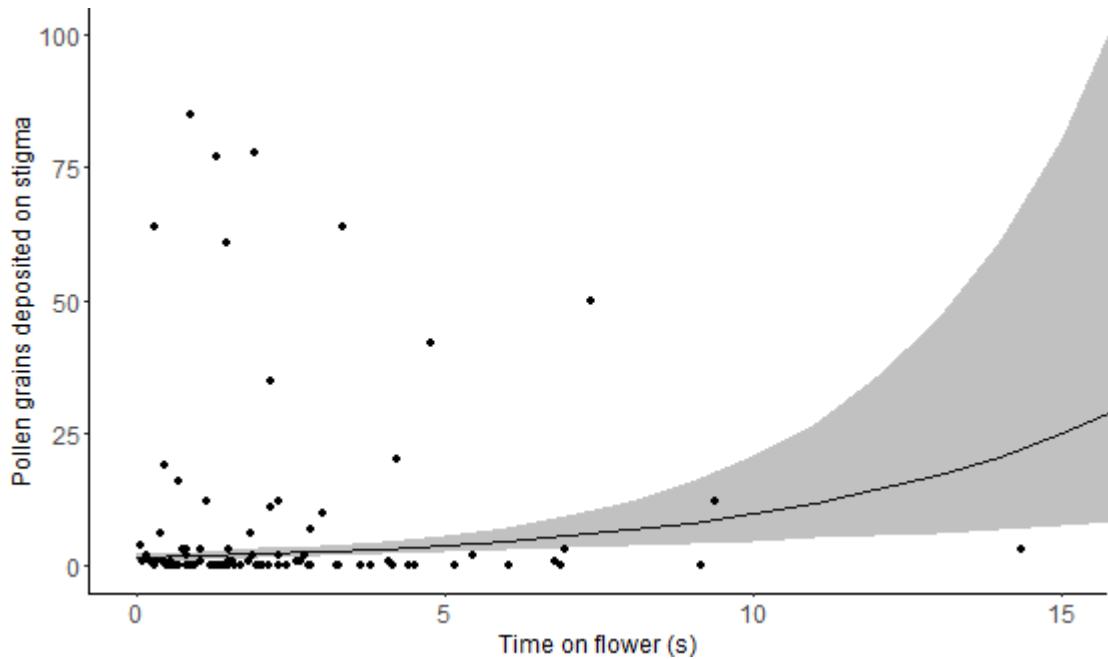
We compared the effectiveness of pollen deposition among honey bees, male and female alfalfa leafcutter bees, bumble bees, and hoverflies. We expected bees would be better than flies at pollen deposition, and that larger, hairier pollinators would pick up more pollen and therefore deposit more. The amount of pollen a pollinator had on its body, the type of flower that a pollinator was originally foraging on (male or female), and the time a pollinator spent visiting a flower were also considered as factors that could influence pollen deposition. Pollinators spending longer times handling flowers or that had more pollen on their bodies were expected to be more effective at depositing pollen. Pollinators travelling from male plants were expected to deposit more pollen than those travelling from female plants. Pollinator efficacy was analyzed using a GLM model with a negative binomial distribution to correct for overdispersion.

The type of pollinator ( $\chi^2=62.26$ ,  $df=4$ ,  $p<0.001$ ), the amount of pollen a pollinator had on its body ( $\chi^2=4.40$ ,  $df=1$ ,  $p=0.04$ ), the flower it was originally foraging on ( $\chi^2=39.58$ ,  $df=1$ ,  $p<0.001$ ), and the time it spent on a flower ( $\chi^2=18.93$ ,  $df=1$ ,  $p<0.001$ ) all significantly affected the amount of pollen deposited on a stigma. A post hoc Tukey test showed that female alfalfa leafcutter bees deposited significantly more pollen on stigmas than male alfalfa leafcutter bees ( $p=0.001$ ), honey bees ( $p<0.001$ ) and hoverflies ( $p<0.001$ ) (Figure 16). There was no significant difference in pollen deposition between bumble bees and female alfalfa leafcutter bees ( $p=0.10$ ) or honey bees ( $p=0.13$ ).

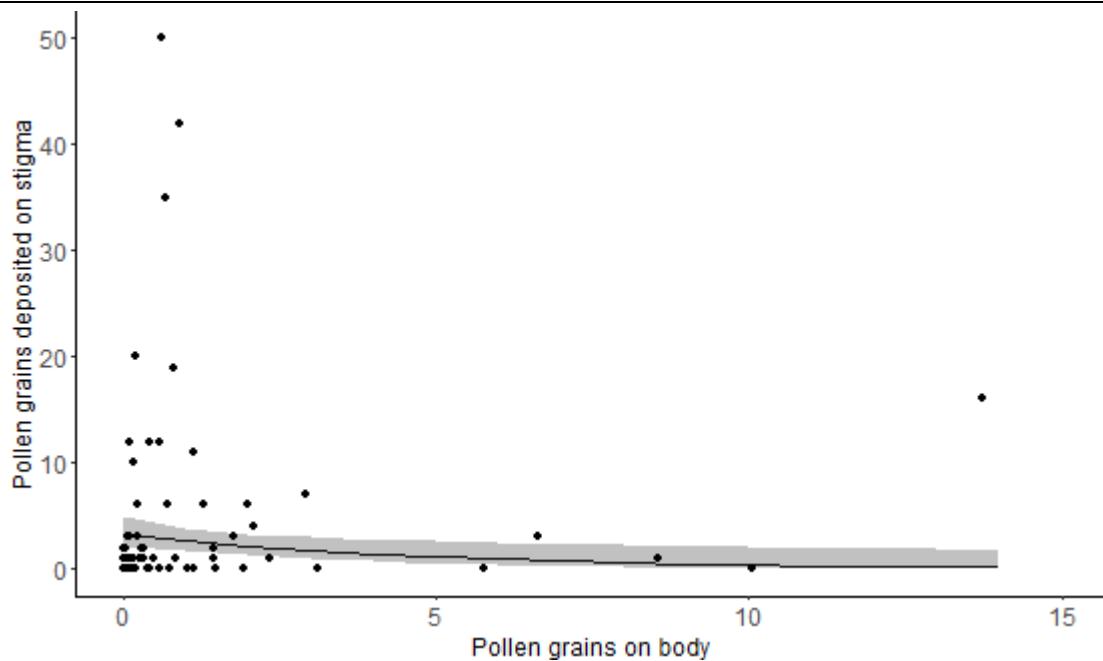


**Figure 16. The effect of pollinator taxa on the amount of pollen grains deposited on stigmas. Pollinator taxa included bumble bee ( $n=13$ ), female leafcutter bee ( $n=20$ ), honey bee ( $n=43$ ), hoverfly ( $n=21$ ), and male leafcutter bee ( $n=6$ ). Points represent means and lines represent the 95% CI. Pollen deposition was averaged between flower of origin; time spent on flower and pollen on body were held constant. Letters indicate significant differences ( $p<0.05$ ).**

Pollinators travelling directly from male flowers deposited on average  $32.2 \pm 10.1$  pollen grains onto stigmas, compared to the  $1.7 \pm 1.2$  grains deposited by pollinators travelling from female flowers. Pollinators with longer floral visits deposited more pollen grains (Figure 17). The relationship between pollen on body and pollen deposited was negative (Figure 18), contrary to expectations.



**Figure 17.** Relationship between the amount of time spent on a flower and pollen grains deposited, with predicted trend line plotted against observed (non-adjusted) points. For trend line, variables pollinator type (5 levels), flower of origin (2 levels), and pollen on body were held constant. Shaded area represent the 95% CI.



**Figure 18. Relationship between average pollen grains on body (per  $10^{-4}$  mL) and pollen grains deposited on stigma, with predicted trend line plotted against observed (non-adjusted) points. For trend line, variables pollinator type (5 levels), flower of origin (2 levels), and time spent on flower were held constant. Shaded area represent the 95% CI.**

The placement of pollen on a pollinator's body, rather than pollinator size, may be more indicative of how effective a pollinator will be at depositing pollen on canola flowers. Female leafcutter bees, who hold their pollen on hairs located ventrally on their abdomen (scopae), were the best at depositing pollen (although they did overlap with bumble bees). They were more effective at pollen deposition than honey bees and even male leafcutter bees (who have much less substantial abdominal hair). While bumble bees were similarly effective as female leafcutter bees at depositing pollen, their limited presence in hybrid canola fields suggests that they cannot contribute greatly to hybrid canola pollination.

The more time a pollinator spent on a flower, the greater the amount of pollen was deposited. Unexpectedly, the more grains of pollen that were on a pollinator's body, the less were deposited on a stigma. Pollinators with high amounts of pollen on their bodies in this system may have been specifically foraging for pollen (especially in the case of honey bees and bumble bees). Pollen foragers may groom more rigorously than nectar foragers and pack their pollen where it might be inaccessible for pollen deposition.

Pollinators seem to exhibit floral constancy to morph in hybrid canola, preferring to visit female or male flowers (rather than visiting both indiscriminately). However, to achieve pollination success, it is important for pollinators to switch between morphs, as pollinators moving from a male to a female inflorescence deposited more pollen than those moving from a female to another female inflorescence.

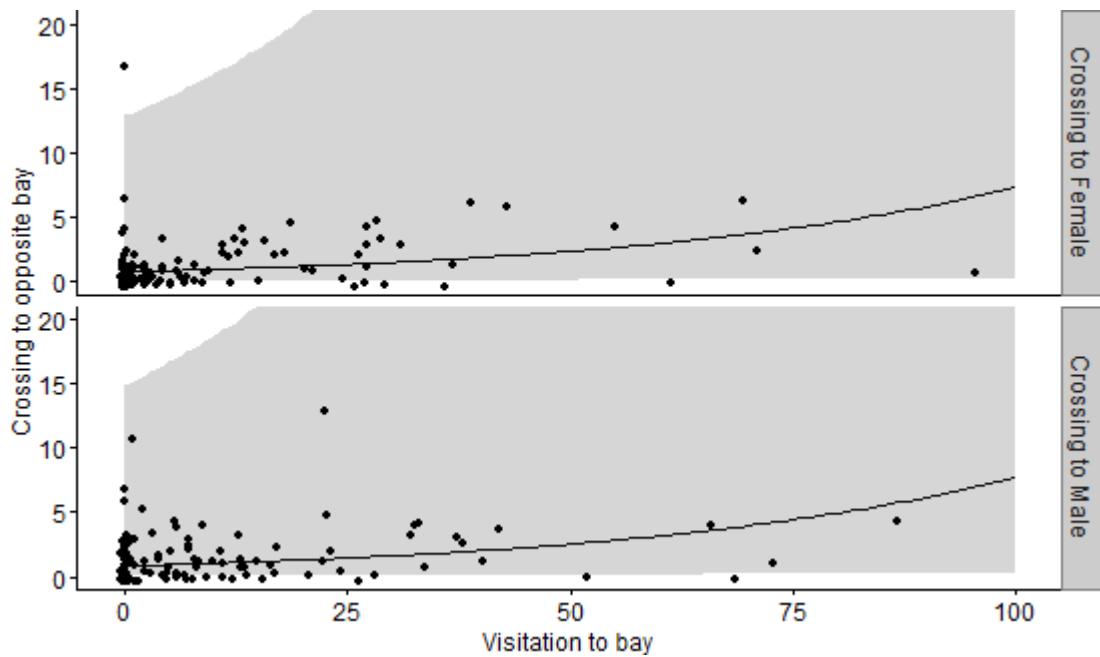
### Experiment 3: Bee Behaviour

In 2015 we examined different factors that could influence pollinator visitation to male and female hybrid canola bays, and how this affected pollinator movement between the bays. At each field site we established plots at distances near to and away from sources of pollinators (honey bee hives, leafcutter bee shelters, and wild bee habitat) to measure pollinator visitation. We then used this pollinator visitation data to look at pollinator movement between the bays using two different methods, transect line crossing and individual pollinator follows. Transect line crossing involved measuring the directionality and amount of pollinator movement between the male and female bay to see how competitor densities and floral rewards motivated

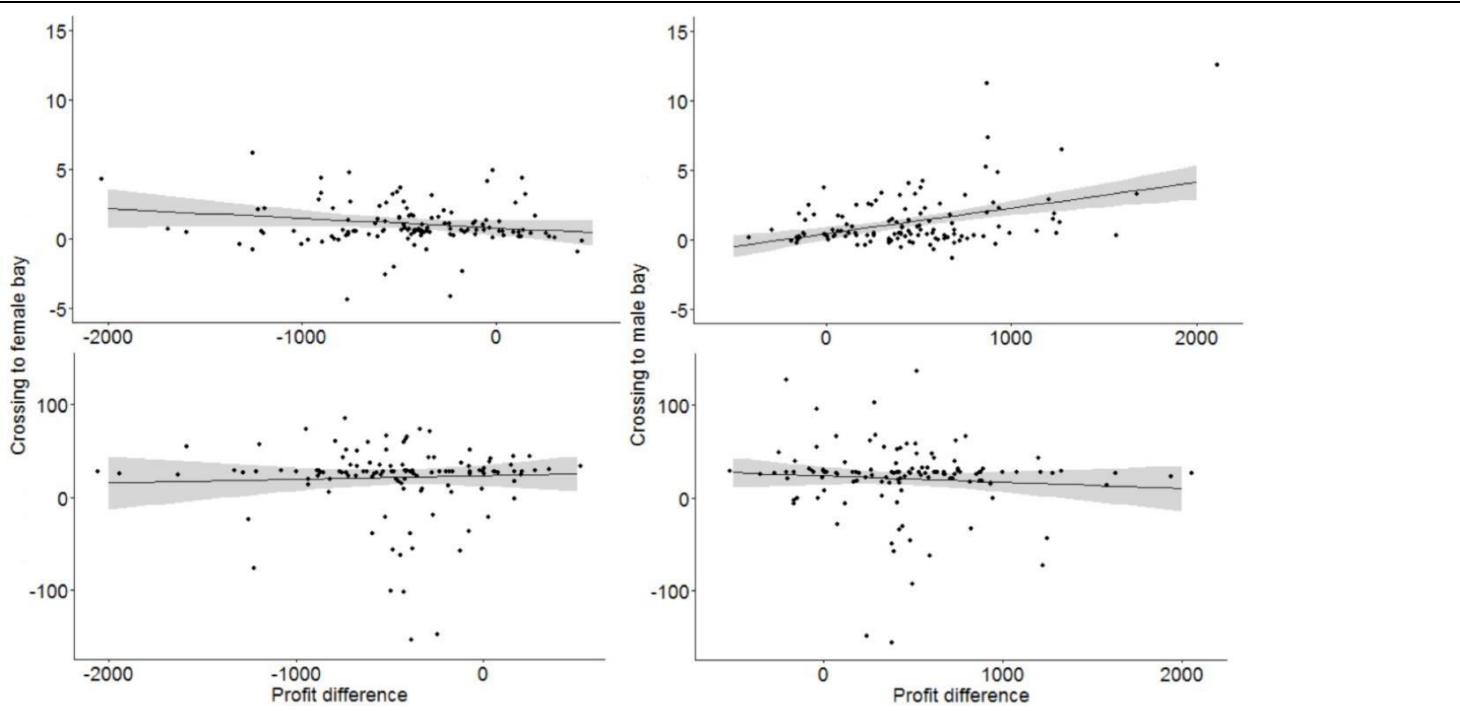
bay crossing. Individual travels involved following individual foraging alfalfa leafcutter bees and honey bees (both pollen and nectar foragers) for a minute on the male bay to measure what factors increased the likelihood of them switching to the female bay.

The main motivator that was considered for whether pollinators crossed between bays was competition, represented by pollinator visitation to the plot and diversity of pollinators (taxon richness). Pollinator visitation was broken down into two categories, conspecific visitation by managed pollinators and heterospecific visitation (all bees other than the focal species). Because non-managed pollinators were not identified to species, diversity was represented instead by the number of rarefied taxa (including honey bees, leafcutter bees, native bees, syrphid flies, calyptate muscoids, and Lepidopterans). Resource availability, represented by the energetic production of each morph of flower multiplied by the floral density of the bay (profit; J/hr\*m<sup>2</sup>), was also included as a motivator for movement between bays.

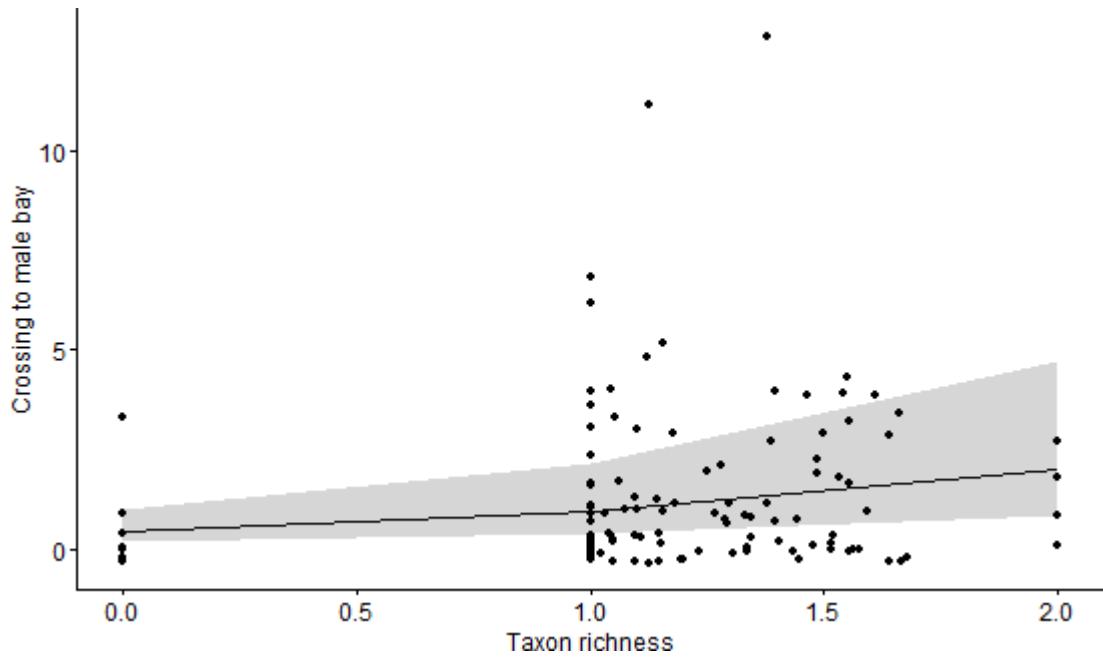
For the transect line crossing measurements, an increase of honey bee visitation to the male bay prompted crossing to the female bay ( $df=6, \Delta AIC=1.49$ ) (Figure 19).  $\Delta$  profit also influenced the likelihood of honey bees to cross to the female bay, although honey bees were unexpectedly less likely to cross to the female bay as its profit increased relative to the male bay (Figure 19). Profit and honey bee visitation were likewise influential in causing honey bees to cross to the male bay ( $df=7, \Delta AICc=1.16$ ), although in this case increases in both honey bee visitation to the female bay (Figure 18) and in  $\Delta$  profit (Figure 20) increased the likelihood of honey bee crossing to the male bay. Along with profit and honey bee visitation, an increase in taxon richness in the male bay ( $df=8, \Delta AICc=0$ ) increased honey bee crossing to the female bay (Figure 21).



**Figure 19. Relationship between honey bee visitation to the male or female bay (X) and crossing to the female (top) or male (bottom) bay (Y), with predicted trend line plotted against observed (non-adjusted) points (n=121).  $\Delta$  profit, temperature, and Julian day were held constant for trend line. Shaded area represents the 95% CI.**



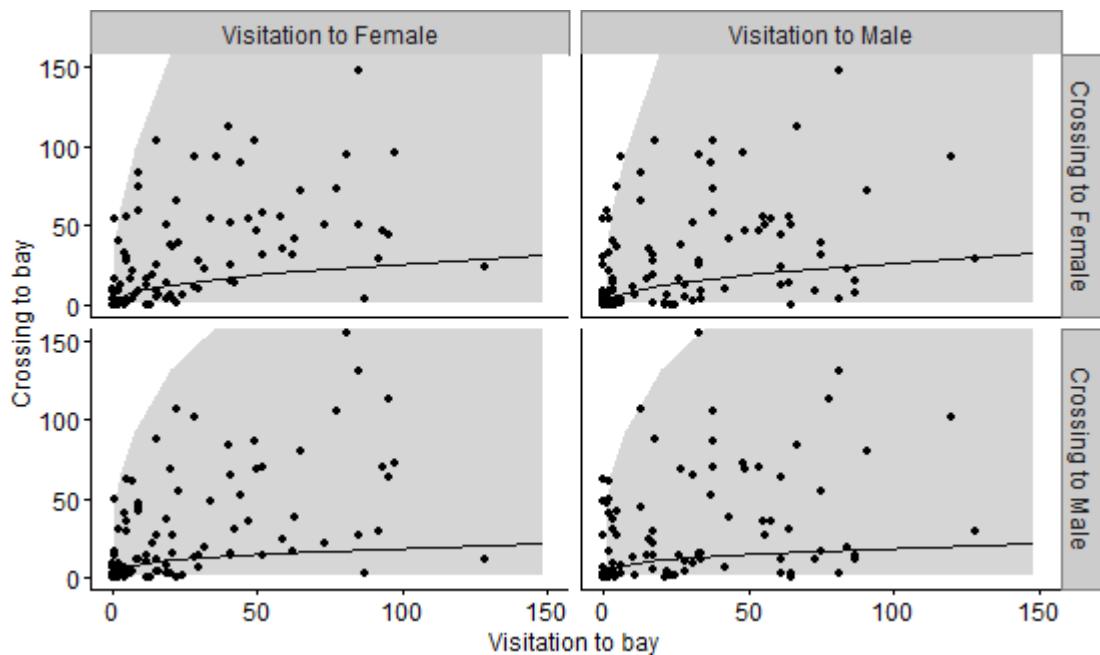
**Figure 20.** Partial regression plots of honey bees (top;  $n=121$ ) and leafcutter bees (bottom;  $n=122$ ) crossing between bays as influenced by  $\Delta$  profit between the bays, adjusting for the effects of visitation, temperature, and Julian day. The figures on the left represent female profit minus male profit, while the figures on the right represent male profit minus female profit. Shaded area shows the 95% CI.



**Figure 21.** Frequency of honey bees crossing to the male bay as influenced by rarefied taxon richness ( $n=121$ ), with predicted trend line plotted against observed (non-adjusted) points. For trend line, variables  $\Delta$  profit, visitation, temperature, and Julian day were held constant. Shaded area represents the 95% CI.

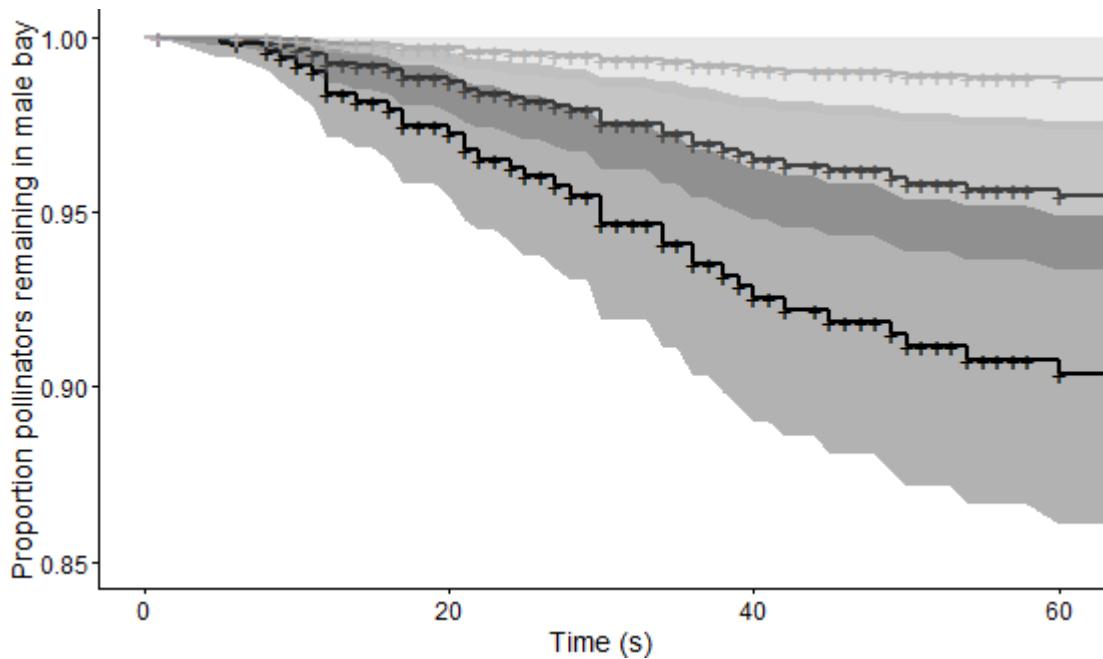
Increased visitation by alfalfa leafcutter bees to both the male and female bay motivated leafcutter bees to cross to the female bay ( $df=7, \Delta AICc=1.5$ ) (Figure 22), as did an increase in  $\Delta$  female profit ( $df=8, \Delta AICc=0$ ) (Figure 20). Similarly, an increase in visitation by leafcutter bees to both the male and female bays motivated leafcutter bees to cross to the male bay ( $df=7, \Delta AICc=1.42$ ) (Figure 22), although an increase in profit in the male bay relative to the female bay resulted in less leafcutter bees crossing to the male bay ( $df=8, \Delta AICc=0$ ) (Figure 20).

Leafcutter bees respond to increasing visitation to an area by moving away from it (and therefore crossing to a different bay), but also seem to be attracted to areas with increased visitation. This could be due to conspecific cuing, where foragers use the presence of conspecifics to assess resource availability in an area. It is also possible that the presence of male leafcutter bees prompted the unexpected attraction of leafcutter bees to higher densities of conspecifics, since female leafcutter bees represent mating opportunities for male bees. In 2016 leafcutter bee sex ratio surveys were completed in 15 hybrid seed canola fields to see if a male-biased sex ratio was more likely with higher densities of leafcutter bees. This could support the idea that the movement of leafcutter bees towards higher densities of leafcutter bees was driven by males seeking females to mate with. The results of the surveys are currently being analyzed.



**Figure 22.** A partial residual plot of alfalfa leafcutter bees crossing to the female (top) and male (bottom) bays, as influenced by conspecific visitation to the female (left) and male (right) bay, with all other variables held constant. Shaded area represents the 95% CI.

For individuals moving from foraging on male to female inflorescences, diversity (rarefied taxon richness) had a positive effect on the tendency of pollinators to switch inflorescence type over time. Pollinator type also affected the tendency to switch (with honey bees switching less than leafcutter bees ( $p<0.01$ ), and pollen foragers switching less than nectar foragers ( $p=0.03$ )) ( $df=5, \Delta AIC=0$ ). An increase of the number of pollinator taxon meant that a bee would more likely switch to the female bay. Leafcutter bees were the most willing to switch to the male bay, while honey bees (especially pollen foragers, which was to be expected) were less likely to move from the male bay (Figure 23).



**Figure 23. Propensity of leafcutter bees (black line), honey bee nectar foragers (grey line), and honey bee nectar foragers (light grey line) to remain in the male bay for 60 s (n=728). Slopes are Cox model coefficients (see Table 3.4). Shading around lines represents the 95% CI.**

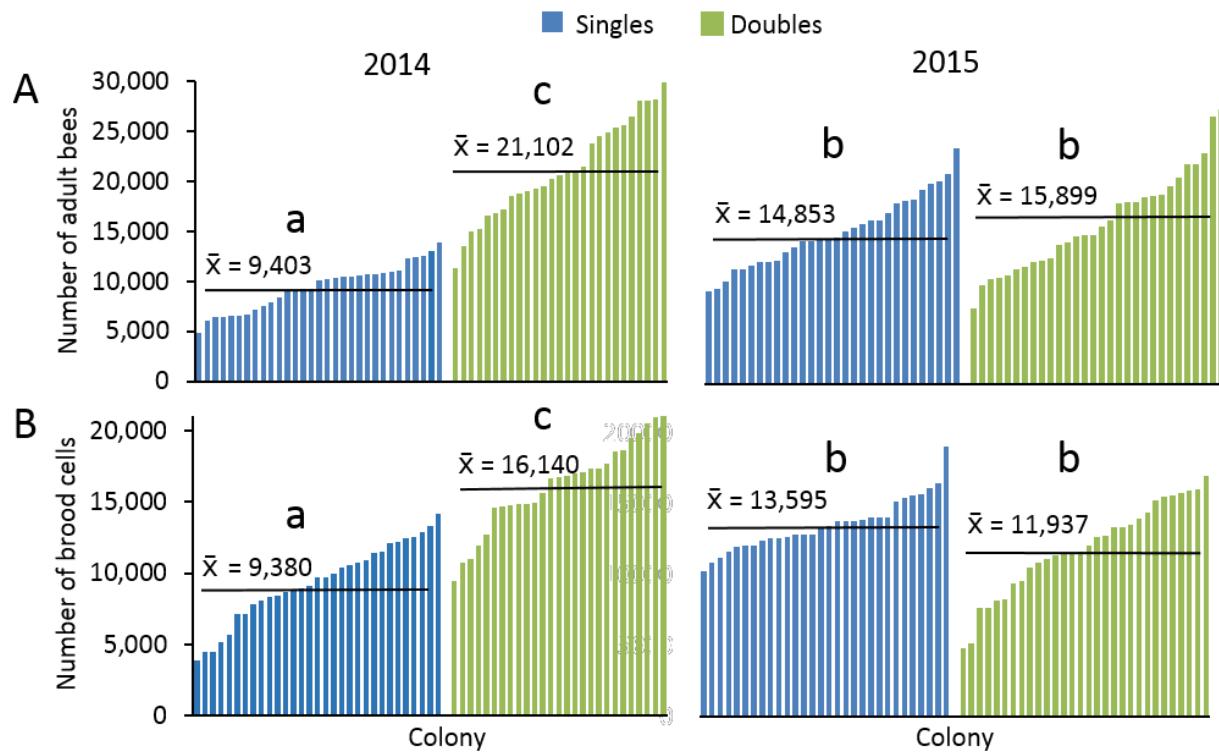
The results show that while managed pollinators seem to respond most immediately to the visitation of conspecific pollinators, the diversity of pollinators might also be important in motivating movement between the bays. Wild pollinators, which may not contribute directly to pollination due to a low presence, may still contribute to hybrid canola pollination by inducing more movement between the male and female flowers.

Neither honey bees nor leafcutter bees were negatively influenced by pollinator density in the opposite bay, suggesting that bees may respond to resource to consumption rather than more directly to the presence of competitors. The discrepancy of the effects of  $\Delta$  profit (in which some cases motivated pollinator crossing, and in others made crossing less likely) suggests possible interactions between pollinator visitation and floral profit.

#### Experiment 4. Hive grade versus efficacy

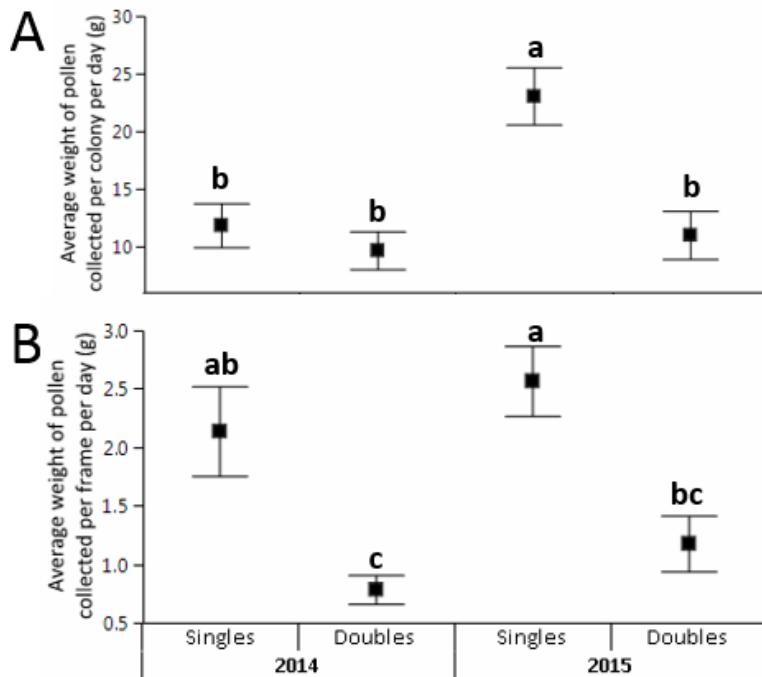
In 2014 and 2015 we compared the performance in hybrid canola pollination of two units of hives currently managed by beekeepers: singles (one brood chamber) and doubles (two brood chambers). Currently all colonies rented for hybrid canola pollination must be doubles, although singles are also kept by many beekeepers in Alberta, including many who pollinate with doubles. Between these two groups we compared the number of bees, the number of brood cells, the average weight of pollen collected, the number of nectar and pollen foragers per 10 min, load weights of nectar and pollen, and the honey production.

We found that the number of bees and number of brood cells in each colony was highly variable within each grouping of singles and doubles and also between years (figure 24). In 2014 the singles had much smaller bee and brood populations than the doubles. However in 2015, the two groups were statistically similar. As the hybrid canola seed production companies rent colonies and then pay based on the number of frames of bees in each colony, several of our measurements were compared between the singles and doubles on a colony level and a per frame basis (a frame was assumed to be 1600 bees).



**Figure 24. Number of adult bees (A) and number of brood cells (B) in the singles (blue) and doubles (green) in 2014 and 2015. Each bar represents an individual colony (sorted from smallest to largest), and the lines represent the population mean ( $\bar{x}$ ) of each grouping. Different letters above the groupings indicate significant differences (within and between years) according to Factorial ANOVAs with Tukey LS Means separation ( $P < 0.005$ ).**

We found that the average weight of pollen collected per day (averaged across 3-4 collections) varied significantly between the singles and doubles at the colony level (figure 25A), with the singles collecting statistically similar amounts (2014) or even greater amounts (2015) than the doubles. This difference was even more pronounced at the frame level (figure 25B), where the singles collected 1 or 2 times as much pollen per day as the doubles.

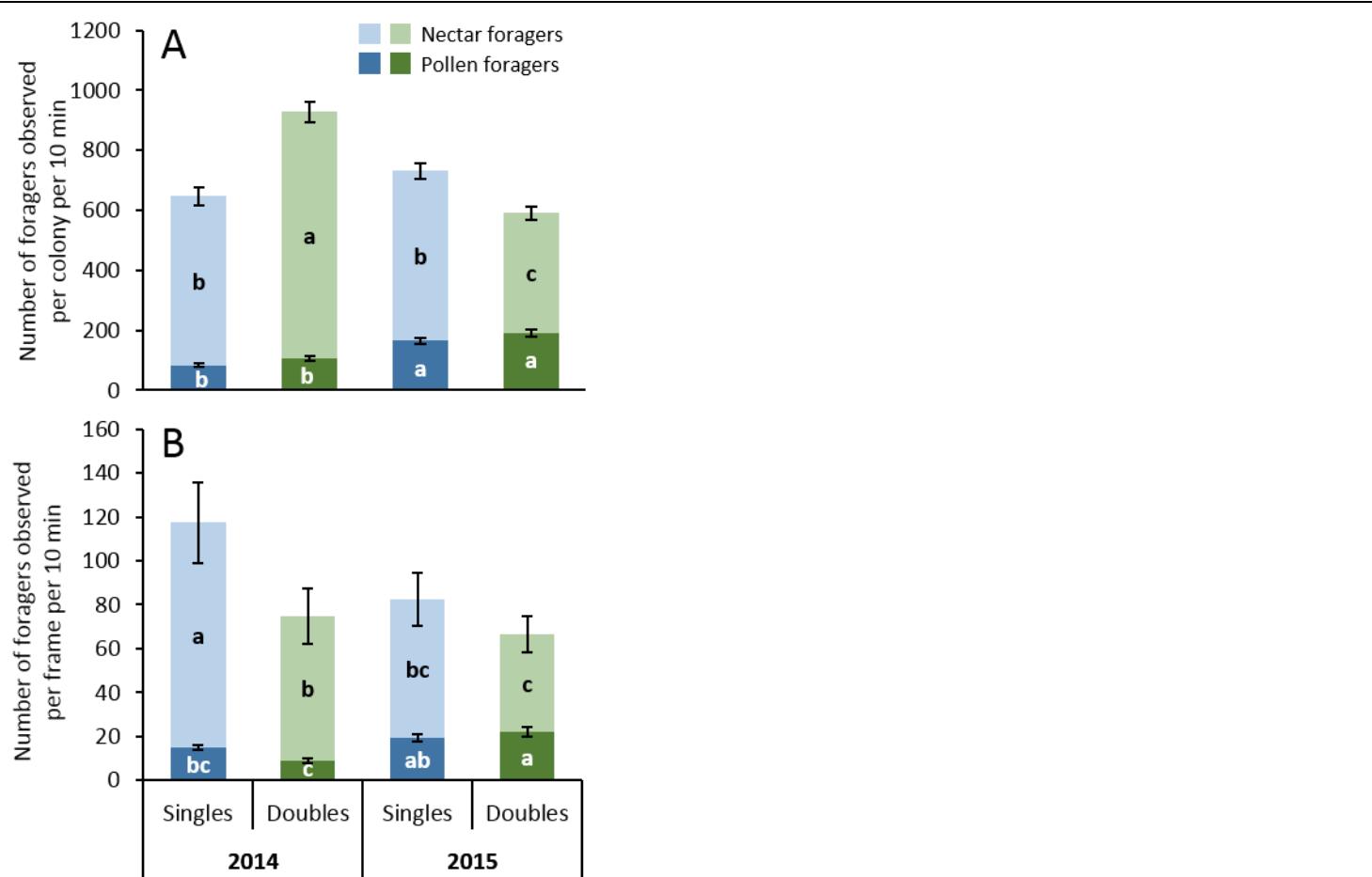


**Figure 25. Average weight of pollen collected per day  $\pm$  SE at the colony level (A) and at the frame level (B) in the singles and doubles in 2014 and 2015. Different letters above the groupings indicate significant differences (within and between years) according to Factorial ANOVAs with Tukey LS Means separation ( $P < 0.005$ ).**

The number of pollen foragers per 10 min at the colony level was statistically similar between the singles and doubles, while the number of nectar foragers was greater in the doubles in 2014 and in the singles in 2015 (Figure 26A). At the frame level (26B), the number of pollen foragers and nectar foragers per 10 min tended to be statistically similar, although there were significantly more nectar foragers in the singles in 2014.

The load weights of nectar and pollen of individual bees were weighed, and found not to vary significantly between the singles and doubles or between years.

In 2014 the honey production at the colony level in the doubles was twice that of the singles, which was significant ( $T=-6.72$ ,  $df=48.9$ ,  $P<0.0001$ ). At the frame level, the singles and doubles did not vary significantly ( $T=0.38$ ,  $df=34.3$ ,  $P=0.7053$ ). The honey production data for 2015 was unfortunately not taken by the participating beekeeper.



**Figure 26. Mean  $\pm$  SE number of nectar and pollen foragers observed in 10 min at the colony level (A) and the frame level (B) in the singles (blue) and doubles (green) in 2014 and 2015. Different letters above the groupings indicate significant differences (within and between years) according to factorial ANOVAs with Tukey LS Means separation ( $P < 0.005$ ).**

To summarize both years, singles were statistically as efficient as or even more efficient than doubles on a per-frame basis in terms of pollen collection, nectar and pollen foragers, and honey production. Furthermore, although the singles were statistically less populous colonies in 2014, in 2015 they were statistically similar to the doubles, suggesting that there are singles that are the same size as pollination grade doubles. Therefore it is likely that singles could be included in hybrid canola pollination alongside doubles, as long as a similar stocking rate of number of frames of bees per acre is met.

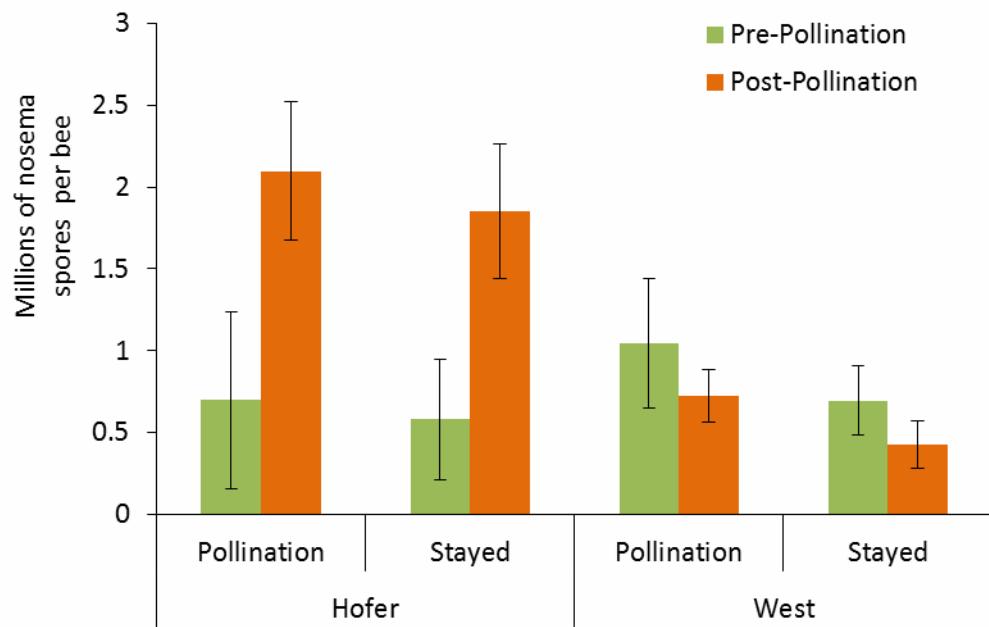
### **Survey 2. Bee health. (a) Honey Bees**

#### **Effects of Hybrid Canola Pollination on Colony Health and Productivity**

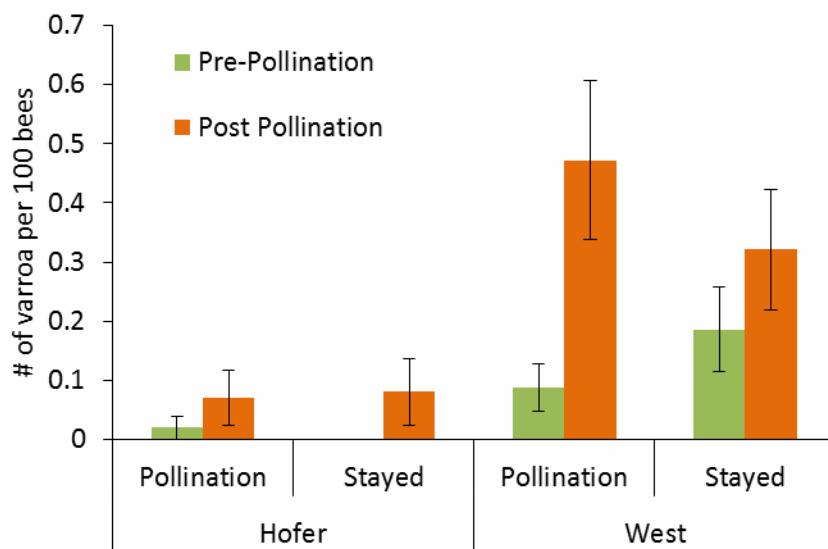
It is assumed that moving honey bee colonies from their original locations to hybrid seed canola pollination comes at a loss in terms of colony production and health due to the stresses placed on the colonies. The colonies undergo a thirty-fold increase in stocking rate as they move from about 40 colonies per 1280 acres (.03 colonies per acre) to about 160 colonies per 160 acres (1+ hive per acre). Given that forage resources are limited in high stocking rates, it follows that the colonies be less productive as they would have access to less nectar and pollen resources. Additionally, as colonies are in close proximity, and other beekeepers' colonies may also be nearby, it is likely that there would be effects on colony health as bees transmit diseases as they drift between colonies. Finally, the stress and disorientation associated with transport may result in decreased health due loss in population, foraging effort, or loss of queens.

To answer whether moving honey bee colonies into canola pollination has negative effects on colony health and production, in 2015 we assessed two apiaries of colonies (40 in each) for health and population, then moved half of each (20 colonies) to a canola pollination field. During this time the honey and pollen production was evaluated both in pollination and in the colonies that remained in their original yards. Furthermore, the health and population of the colonies that pollinated and those that remained were assessed upon the return of the pollinating colonies to the original yards.

The colony health indicators we used were *Nosema* infection, *Varroa* infestation, and queen loss. *Nosema* infection did not vary significantly between the hives that went to pollination and the hives that stayed, rather it varied between the two source apiaries we observed (figure 27).



**Figure 27.** *Nosema* infection (an indicator of colony health, mean spore count  $\pm$  SE) as determined pre-pollination (green) and post-pollination (orange) compared between the colonies that went to pollination, and those that stayed, for each of the yards Hofer and West.

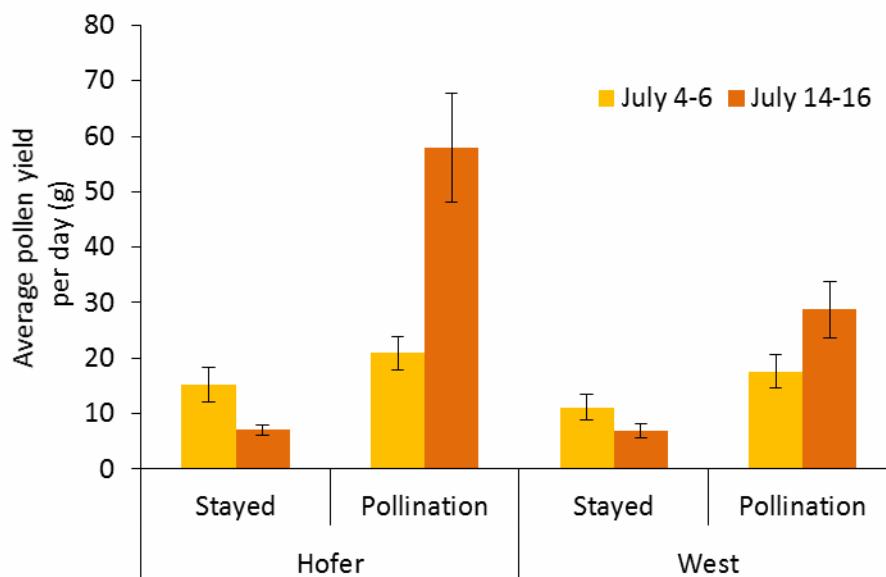


**Figure 28. *Varroa* infection (an indicator of colony health, *Varroa* / 100 bees  $\pm$  SE) as determined pre-pollination (green) and post-pollination (orange) compared between the colonies that went to pollination, and those that stayed, for each of the yards Hofer and West.**

*Varroa* infestation (an indicator of colony health) as determined pre-pollination (green) and post-pollination (orange) compared between the colonies that went to pollination, and those that stayed, for each of the yards Hofer and West. As with *Nosema*, *Varroa* levels were significantly different between source apiaries, but not colonies that went to pollination fields or did not (figure 28). Similarly, there were no significant effects on queen loss (Table 1).

**Table 1. Number of colonies that were queenless after the pollination period compared between the colonies that went to pollination (yellow) and those that stayed (green), for each of the yards Hofer and West.**

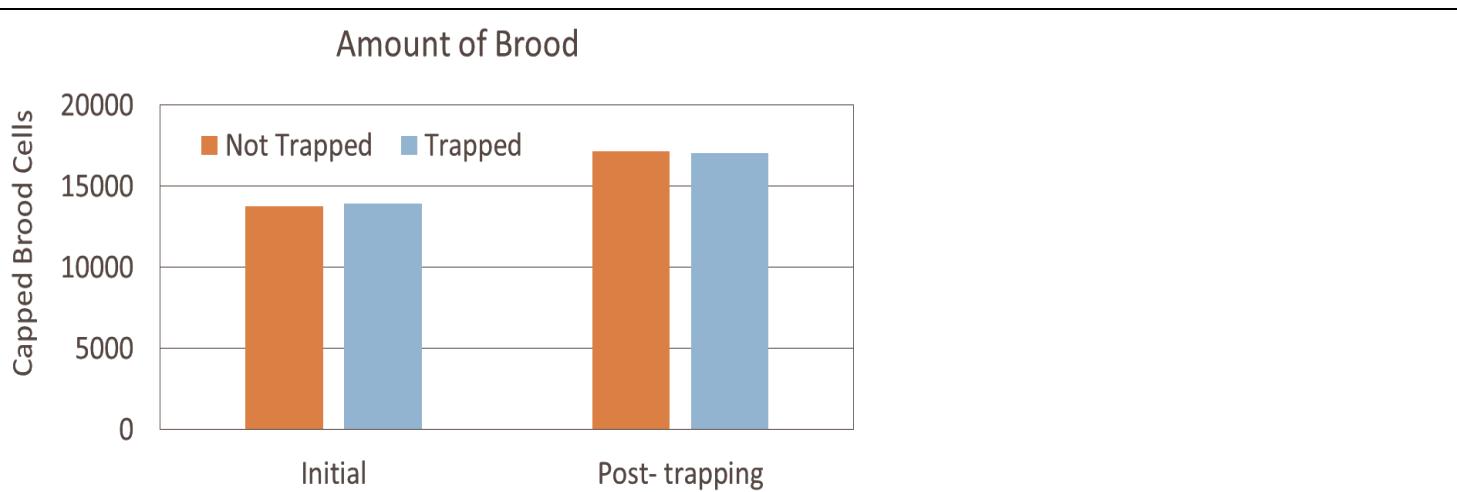
Hofer		West	
Stayed	Pollination	Stayed	Pollination
2 queenless	3 queenless	3 queenless	1 queenless



**Figure 29. Average  $\pm$  SE pollen yield per day (an indicator of colony productivity) as determined July 4-6 2015 (yellow) and July 14-16 2015 (orange) compared between the colonies that went to pollination, and those that stayed, for each of the yards Hofer and West.**

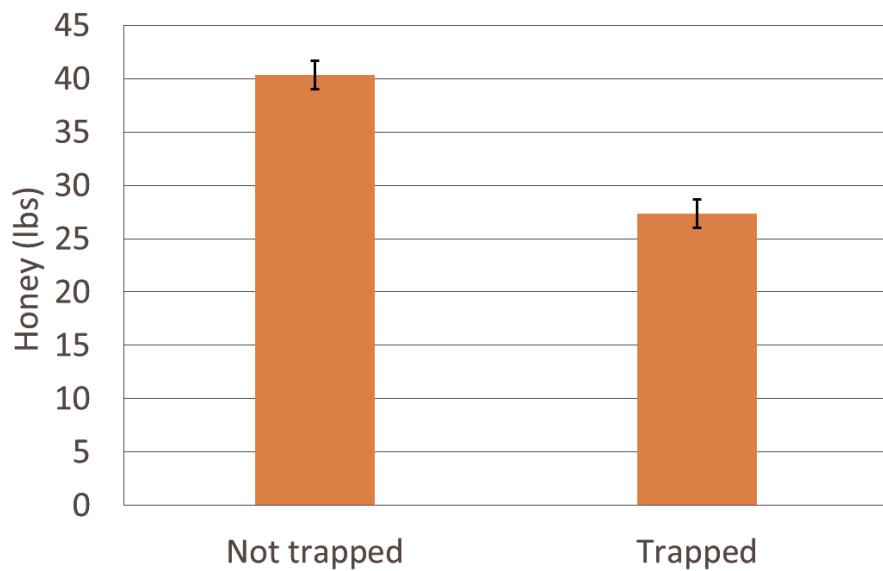
Colonies that were used for canola pollination produced significantly more pollen than those that stayed in their 'home' apiary (Figure 29). This led to further research on pollen production in 2016.

We identified 60 queenright double brood chamber colonies in each of three hybrid canola seed production fields (total n=120 colonies). The total amount of sealed brood in each colony was assessed by taking a photo of each side of each frame containing brood, and subsequent analysis with HoneyBee Complete (version 4.2) software. The brood population for all colonies was assessed when the colonies were first brought into pollination (4-7 July 2016), then again shortly before they were removed from the field (25-28 July 2016). Between these two assessment periods, we placed pollen traps on 20 double brood chamber hives per field (total n=60 trapped colonies, figure 4), dispersed amongst the untrapped hives. We collected the pollen twice weekly throughout the time the colonies were on the field. We then dried, cleaned, and weighed all the pollen collected. We also recorded the honey production for each of the 120 colonies over the same time period.



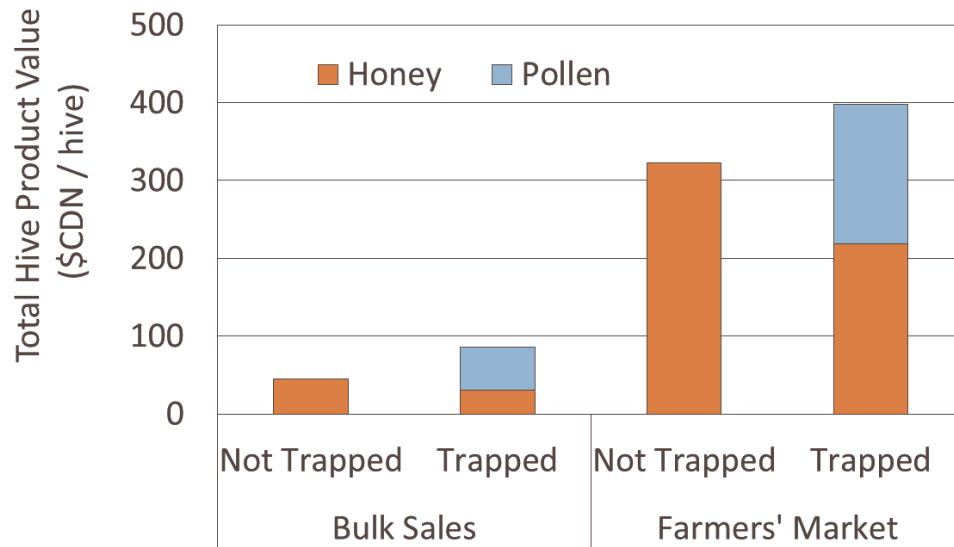
**Figure 30. Amount of brood in colonies with (blue bars) and without (orange bars) pollen traps. There was no effect of collecting pollen on brood production.**

Despite previous reports of negative impacts on brood production of prolonged pollen trapping, short-term trapping during an abundant pollen flow had no impact on brood production (figure 30). In contrast, honey production was negatively impacted by the addition of pollen traps beneath the colonies, or by the collection of the pollen (figure 31). Honey production decreased from a mean of 40.3 lbs among untrapped colonies to only 27.4 lbs from colonies fitted with pollen traps.



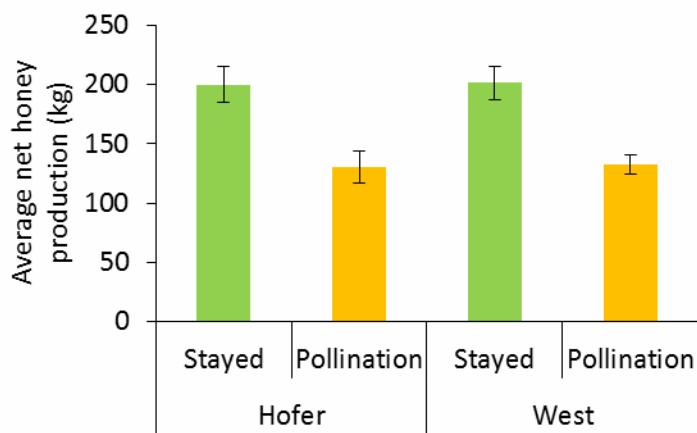
**Figure 31. Honey production (mea per colony  $\pm$  SE) in trapped (right) and untrapped (left) colonies. Trapped colonies had a pollen trap on their entrance while in canola pollination.**

However, the per hive profit was larger from pollen-trapped colonies (figure 32) than untrapped colonies because of the high value of pollen relative to honey, and the large amounts of pollen collected.



**Figure 32. The total value of hive products was greater for colonies that had pollen traps (pollen + honey) compared to colonies with no trap (honey production only), regardless of whether the products were sold at current bulk or farmers' market prices.**

Pollen collection can fit into a management paradigm focused on pollination service delivery, increasing the per hive profit without negative effects on pollination services or colony health.

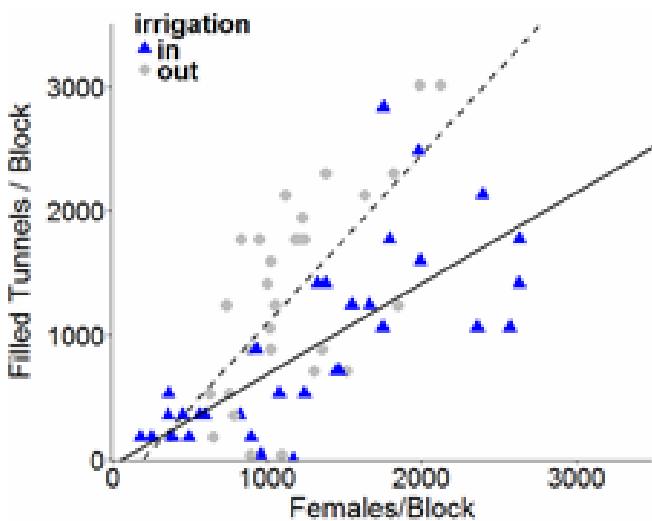


**Figure 33.** Average  $\pm$  SE net honey production (an indicator of colony productivity) as compared between the colonies that went to pollination (yellow), and those that stayed (green), for each of the yards Hofer and West.

Finally, honey production was significantly less from colonies that were used to pollinated hybrid canola seed fields (figure 33) than colonies that stayed in their home apiary, however the magnitude of this effect will vary with the location of the 'home' apiary or honey production site, and among years.

#### Survey 2b. Alfalfa leafcutter bee health

We conducted a preliminary survey of the reproductive success of alfalfa leafcutter bees reared on hybrid canola seed versus alfalfa seed fields. The survey entailed randomly selecting 10 bee shelters within each of four commercial hybrid canola and alfalfa seed fields that were pollinated by three different commercial leafcutter bee producers. Cocoon returns from each of the fields is pending analysis using a multivariate model to predict the total returns on cells as a product of the number of nesting females, their field-level density, the crop (either canola or alfalfa) and all two-way interactions. One preliminary result, however, is that shelters placed immediately outside the irrigation drip line in canola have a higher overall number of cocoons produced per shelter than shelters placed nearby, but under the pivot (figure 34).



**Figure 34.** Leafcutter reproductive success (measured in terms of the number of tunnels filled with cocoons) among shelters located outside the irrigation drip-line in canola fields compared to outside the drip-line.

**5. Research and Action Plans/Next Steps**

We have stigma and canola plant samples that remain to be analyzed. We expect to finish these by the end of the summer. We will finish analyzing the data from each survey and experiment in 2017, and expect to share results on presentations, industry newsletters, and scientific publications in 2017 and 2018.

**6. Budget impacts in the event major issues or variance between planned and actual is noted:**

A financial statement will follow after AF has time to finish accruals for the fiscal year ending March 31 2017.

**Please forward an electronic copy of this completed document to:**

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