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Full Research Project Final Report

- *This report must be a stand-alone report, i.e., must be complete in and of itself. Scientific articles or other publications cannot be substituted for the report.*
- *One electronic copy and one signed original copy are to be forwarded to the lead funding agency on or before the due date as per the investment agreement.*
- *A detailed, signed income and expenditure statement incurred during the entire funding period of the project must be submitted along with this report. Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage; and overhead (if applicable).*
- *For any questions regarding the preparation and submission of this report, please contact ACIDF*

Section A: Project overview

1. Project number: **2014C001R**
2. Project title: **Pollination Management to Maximize Canola Yield**
3. Research team leader: **Shelley Hoover**
4. Research team leader's organisation: **Alberta Agriculture and Forestry**
5. Project start date (yyyy/mm/dd): **2014/04/01**
6. Project completion date (yyyy/mm/dd):**2017/03/30**
7. Project final report date (yyyy/mm/dd):**2017/07/14**

Section B: Non-technical summary (max 1 page)

Provide a summary of the project results which could be used by the funders for communication to industry stakeholders (e.g., producers, processors, retailers, extension personnel, etc.) and/or the general public. This summary should give a brief background as to why the project was carried out, what were the principal outcomes and key messages, how these outcomes and key messages will advance the agricultural sector, how they will impact industry stakeholders and/or consumers, and what are the economic benefits for the industry.

Although the commodity canola grown in Alberta is self-compatible, recent research demonstrates that yield can be significantly increased by placing supplemental honey bee hives onto fields. However, studies demonstrating the benefits of bee pollination to the crop have been performed in areas with relatively small field sizes, and where stocks of honey bee colonies are relatively large compared to the area seeded to canola (e.g., eastern Canada or in northern Europe). Arguably, there are too few honey bee colonies and inadequate wild pollinator populations in western Canada to achieve the pollinator visitation rates needed across the ~20 million acres of canola grown in Canada. In contrast, the production of hybrid canola seed, primarily grown in the irrigation districts of Southern Alberta, is highly dependent on insect-mediated pollen transfer. To ensure effective cross-pollination, over 60,000 honey bee hives and millions of leafcutter bees are rented annually. Our project proposed to determine how pollinators might be most efficiently managed in seed and commodity canola in order to maximize both pollination and bee health. Unlike previous studies, our project focused how pollinators can be used to increase seed quality and yield, using data from a large number of fields, across multiple regions within Alberta. Furthermore, we investigated the contributions of not only managed honey and leafcutter bees, but also unpaid services contributed by wild pollinators. Our goal was to provide growers with recommendations that will allow them to increase yield through pollination management, while at the same time preserving the health of both managed and wild bees, which are highly valued by Albertans.

We found that there is a wide diversity of pollinators in canola fields in Alberta, and that the pollinator community varies among regions. Not only bees, but also flies were frequent flower visitors. In general, there is a large amount of pollen deposited to flowers in commodity fields, and yield is not pollen-limited, however there are specific contexts in which additional pollinators benefit yield and seed quality.

Hybrid seed production fields are far more dependent on the activity of honey and leafcutter bees for yield, and there are pollination management practices that will benefit yield in this crop. In particular, there are areas of the field with reduced bee abundance. Careful management of bee distribution, irrigation, and shelter placement will benefit both growers as well as beekeepers. We offer options for honey bee management that can benefit the profit of both growers and beekeepers, while maintaining high levels of bee health. In addition, pollinator diversity benefits pollen deposition in seed fields, and growers should encourage pollinator diversity on their farms. Taken together, our results indicate that pollinator diversity and abundance benefit canola yield and seed quality, and that working together, land managers and beekeepers can ensure both continued bee health as well as high canola yields.

Section C: Project details

1. Project team (max ½ page)

Describe the contribution of each member of the R&D team to the functioning of the project. Also describe any changes to the team which occurred over the course of the project.

Dr. Shelley Hoover was the lead researcher on the project, and was involved in all the experiments and surveys, both in their design and execution, as well as data analyses and written / oral dissemination of results.

Dr. Ralph Cartar was the primary academic supervisor or Melathopoulos / Waytes / Robinson at the University of Calgary, and was involved in the design and analyses of the experiments conducted by those members of the team.

Dr. Stephen Pernal supervised field work for Survey 1 in the Peace region, ensuring that the work was done as designed and providing data to Sam Robinson.

Dr. Andony Melathopoulos worked on this project as a post-doctoral fellow (2015-2016) prior to accepting his current position as faculty at Oregon State University. Dr. Melathopoulos ran the fieldwork required for experiment 1 and survey 2b, and was responsible for the subsequent data analyses and dissemination of information from these experiments.

Samuel Robinson is a PhD candidate recruited for this project, and supervised by Drs. Cartar and Hoover, at the University of Calgary. He was recruited to work on Surveys 1 and 3, which will form the basis of his PhD thesis. As such, he conducted the field work, data analyses, and writing for these aspects of the project.

Riley Waytes was an MSc candidate recruited for this project at the University of Calgary, supervised by Drs. Cartar and Hoover. Riley conducted experiments 2 and 3, which formed the basis of his MSC thesis, which he successfully defended spring 2017.

2. Background (max 1 page)

Describe the project background and include the related scientific and development work that has been completed to date by your team and/or others.

Although the commodity canola grown in Alberta is self-compatible, recent research demonstrates that yield can be significantly increased by placing supplemental honey bee hives onto fields [1-6]. However, studies demonstrating the benefits of bee pollination to the crop have been performed in areas with relatively small field sizes, and where stocks of honey bee colonies are relatively large compared to the area seeded to canola (e.g., eastern Canada or in northern Europe). Arguably, there are too few honey bee colonies and wild pollinators in western Canada to achieve the pollinator visitation rates needed to deliver the yield increases observed in these studies across the 20 million acres of canola grown in Canada. For example, to achieve the 46% increase in seed yield observed in a Quebec study[4], canola fields in western Canada would need to be stocked at a rate of 3 honey bee colonies/ha. Such a stocking rate would require a circa twenty five-fold expansion in the total number of honey bee colonies in Canada, to ~20 million colonies, all placed adjacent to canola fields during bloom.

In contrast, the production of hybrid canola seed, primarily grown in the irrigation districts of Southern Alberta, is very highly dependent on insect-mediated pollen transfer. To ensure effective cross-pollination, over 60,000 honey bee hives are rented annually (personal communication, Gertie Adair, Alberta Beekeepers Commission), at an estimated current price of $177 per colony[8] (an investment of over $10 million annually in pollination services to the hybrid canola seed industry). In 2013 it was estimated that in Canada, bees contributed over $1.1 billion to commodity canola and between $500 million - $1.83 billion in the production of the hybrid canola seed crop, suggesting that well over half of the crop pollination value generated in Canada is from canola alone[9].

Unfortunately, despite the clear economic imperative for doing so, there has not yet been resolution of either the relationship between canola production and pollinator behaviour and abundance, or the relationship between bee health and canola pollination. This situation has likely resulted in unmaximized rates of canola production, and inefficient pollinator stocking densities and management. Our project proposed to determine how pollinators might be most efficiently managed in seed and commodity canola in order to maximize both pollination and bee health. Unlike previous studies, our project focused how pollinators can be used to increase seed quality and yield, using data from a large number of fields, across multiple regions within Alberta. Furthermore, we investigated the contributions of not only managed honey and leafcutter bees, but also unpaid services contributed by wild pollinators.

3. Objectives and deliverables (max 1 page)

State what the original objective(s) and expected deliverable(s) of the project were. Also describe any modifications to the objective(s) and deliverable(s) which occurred over the course of the project.

Original objectives and deliverables:

Key Objectives:

1. Clarify the contribution of managed and native bee pollination services to commodity and seed-production canola yield and seed characteristics (Survey 1a &b, Experiment 1)
2. Quantify the impact of pollination services on bee or colony health in canola hybrid seed-production (Survey 2a &b)
3. Characterise how bee behavior affects pollination services delivered to canola crops, and determine how it can be manipulated to increase pollination efficacy and bee or colony health (Experiment 2,3 & 4)

4. Create guidelines to maximize the efficient use and maximal health of bees pollinating canola (Experiment 2,3, & 4)
5. Identify landscape-level and farm management factors that affect canola yield with respect to pollination services, and pollinator health (Experiment 3, Survey 1b, 2a & b)

Deliverables:

Canola growers and hybrid canola seed-production companies

- Identification of existing pollination deficits and factors that affect delivery of pollination service
- Recommendations for bee stocking rates in seed and commodity canola fields
- Guidelines for the concurrent management of multiple bee species to maximize canola pollination and bee health
- Determination of hive rating and management practices versus pollination effectiveness of honey bee hives
- Increased efficiency and reliability of delivery of pollination service to canola crop

Commercial honey bee and leafcutter bee operators

- Valuation of the pollination services provided to seed canola crops by leafcutter bee producers and honey bee keepers
- Quantification of the benefit of honey bees to commodity canola crop yield and value
- Quantification of the lost productivity of hives and nests in canola pollination
- Management guidelines specific to canola pollination, and documentation of hive size versus pollination efficiency
- Disease and pathogen assessment of bees in canola pollination setting

4. Research design and methodology (max 4 pages)

Describe and summarise the project design, methodology and methods of laboratory and statistical analysis that were actually used to carry out the project. Please provide sufficient detail to determine the experimental and statistical validity of the work and give reference to relevant literature where appropriate. For ease of evaluation, please structure this section according to the objectives cited above.

1. Clarify the contribution of managed and native bee pollination services to commodity and seed-production canola yield and seed characteristics (Survey 1a &b, Experiment 1)

Survey 1. Canola yields versus bee abundance:

(a) Commodity canola fields

In 2014 and 2015 we conducted surveys in which pollinator diversity and abundance were surveyed at four 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, nectar abundance, and seed yield. In both years we conducted the survey at two locations in Alberta (Lethbridge and Peace regions), with ~30 fields per year total. We also measured traits of plants from which the seeds were obtained, to relate yield to plant floral success and branching.

(b) Seed canola fields

In 2015 and 2016, pollinator densities were surveyed at four distances from the field edge closest to honey bee colonies (5, 20, 100, and 400 m into the field) in hybrid canola seed production fields near Lethbridge, AB (Appendix 2, Photo 1). We 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). We also measured characteristics of the plants in the different plots, including standing nectar crop (Appendix 2, Photo 3), nectar production over time, pollen deposition (Appendix 2, Photo 4), seed yield, % green seed, pod distribution and branching.

Experiment 1: Canola Yield versus bee abundance

We conducted two years of field trials (2015, *n* = 3 fields; and 2016, n = 21 fields) in Lethbridge County, Alberta 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 in this region (Appendix 2, Photo 2). In both years of the study, we separated these effects by comparing yield in plots treated in one of the following ways:

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

We predicted that if honey bee pollination contributed to seed yield that plants in the 'open' plots would have a larger number of pods set per plant (i.e., the number of pods/the total number of flowers produced), and/or seeds set per pod, resulting in a higher seed yield compared to plots in which bees were excluded (i.e., 'wind' and 'self' treatments).

Finally, in the three-way pollination comparison we also hand-pollinated a subset of the plants in the plots, to enable comparisons to plants that we knew had sufficient pollen deposition to ensure maximal pod set. We conducted bee observations to determine pollinator visitation rates. Upon plant maturity, plants were harvested, dried, and the number of pods and branches were recorded. In addition, we measured the seed yield, 1000 seed weight, and % green seed for all harvested plants, and compared among the pollination treatments.

- **Quantify the impact of pollination services on bee or colony health in canola hybrid seed-production (Survey 2a &b)**

Survey 2. Bee health. (a) Honey Bees

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 providing pollination services. The colonies undergo a minimum four-fold increase in stocking rate as they move from a honey production management scenario (.3 colonies per acre on stocked commodity canola) to pollination service provision (about 160 colonies per 120 acres, or 1-1.5 hive per acre). Given that forage resources are limited, it follows that the colonies are less productive as they would have access to less nectar and pollen resources per colony. Additionally, as colonies are in close proximity, and other beekeepers' colonies may also be nearby, it is believed that there can be effects on colony health as bees transmit diseases as they drift among 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, while the other 20 per apiary remained in their 'home' yard. 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. We measured rates of queen loss, as well as the occurrence of common bee pathogens and parasites such as *Varroa* and *Nosema.*

Survey 2b. Alfalfa leafcutter bee health

We conducted a 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. We also compared nest-filling rates versus the number of nesting females among shelters just inside and outside of the irrigation drip-line to examine the effects of irrigation on the reproductive success of leafcutter bees. We used an otoscope to see inside nest holes and determine the number of nesting females per shelter in the evenings when the bees had returned to the shelter. We subsequently took photos of leafcutter bee nest blocks and examined a subsample of the area of each block to determine the percent of the potential nest holes that were fully filled with cocoons to determine the rate of reproductive activity.

- **Characterise how bee behavior affects pollination services delivered to canola crops, and determine how it can be manipulated to increase pollination efficacy and bee or colony health (Experiment 2,3 & 4)**
- **Create guidelines to maximize the efficient use and maximal health of bees pollinating canola (Experiment 2,3, & 4)**

Experiment 2: Pollinator Efficacy

The efficacy of individual pollinators and their foraging decisions were tested in 21 hybrid canola seed production fields in 2015, and 18 fields in 2016. Pollinators were offered a virgin female inflorescence (flower) using an 'interview bouquet' method (Appendix 2, Video 1) and allowed to visit the flower[10]. Pollinator responses to the inflorescences were recorded via video, and visitation to a flower resulted in the collection of both the pollinator and flower stigma for further examination. Managed pollinators as well as wild bees and syrphid flies (when present) were included in the analyses.

Each video was subsequently 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 flowers. 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 the number of pollen grains deposited. 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 haemocytometer.

Experiment 3: Bee Behaviour

In 2015 we examined different factors that could influence pollinator visitation to male and female hybrid canola bays in seed production fields near Lethbridge, and how these factors influence pollinator movement between the male and female 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 examine pollinator movement between the bays using two different methods: transect crossing and individual pollinator follows. Transect 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) in the male bay to measure what factors increased the likelihood of them switching to the female bay.

The main motivator that was considered for both transect crossing and individual follow methods was competition for nectar resources, represented by pollinator visitation to the plot and diversity of pollinators (taxon richness). Pollinator visitation was broken 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, calyptrate 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^2), was also included as a motivator for movement between bays.

Experiment 4. Hive size and product diversification

Hive Size

In 2014 and 2015 we compared the performance in hybrid canola pollination of two unit sizes of hives currently managed by beekeepers: singles (one brood chamber) and doubles (two brood chambers). Recently, 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, and singles are a normal method by which beekeepers increase their hive numbers to account for winter losses. We compared the adult bee population (Appendix 2, Photo 5), the number of brood cells (Appendix 2, Photo 6), 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 for each of these two hive sizes in canola pollination.

Product diversification: Pollen production

Currently, beekeepers who pollinate canola receive financial remuneration through hive rental fees and honey production. However, these fields are managed to have an abundance of pollen available at all times during bloom. We examined whether pollen was a viable hive product to collect in this management system to offer beekeepers alternative sources of income, especially during periods of low honey prices. To assess the effects on hive health and the amount of pollen and honey produced in this system, we identified 60 queenright double brood chamber colonies in each of three hybrid canola seed production fields (total n=120 colonies) to use in our study. The total amount of sealed brood in each colony was assessed pre- and post-pollination 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), 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. We then compared the amount of brood in the trapped and untrapped colonies, as well as the potential income from honey and pollen production.

- **Identify landscape-level and farm management factors that affect canola yield with respect to pollination services, and pollinator health (Experiment 3, Survey 1b, 2a)**

Methods as described in previous sections

5. Results, discussion and conclusions (max 8 pages)

Present the project results and discuss their implications. Discuss any variance between expected targets and those achieved. Highlight the innovative, unique nature of the new knowledge generated. Describe implications of this knowledge for the advancement of agricultural science. For ease of evaluation, please structure this section according to the objectives cited above.

NB: Tables, graphs, manuscripts, etc., may be included as appendices to this report.

See APPENDIX 1 for cited tables and figures.

1. **Clarify the contribution of managed and native bee pollination services to commodity and seed-production canola yield and seed characteristics (Survey 1a &b, Experiment 1)**

What pollinators are present in commodity canola fields?

We found that in commodity canola fields stocked with honey bee hives, honey bees were the dominant flower visitor, followed by large flies (Muscidae, Anthomyiidae, Calliphoridae) and hoverflies (Syrphidae), then wild bees (Figure 1). In unstocked fields, large flies were the dominant flower visitor in both regions, followed by honey bees in Lethbridge and bumble bees and honey bees near Grande Prairie. Visitation rates were lower overall in 2015 than in 2014 in Lethbridge, even in stocked fields. There were distinct differences in the pollinator communities at our two sites (Lethbridge and Grande Prairie); flies were more abundant in the south, and bumble bees more common in the north. These results demonstrate the regional variation in pollinator abundance and diversity in canola fields in two growing regions within Alberta, as well as the abundance of non-managed bee and fly pollinators.

What are honey bees doing in commodity canola fields?

In 2015, we observed flower visitation behaviour in the honey bees, and as expected we found that in both regions, most of the flower visitation by honey bees was to collect nectar as opposed to pollen (Figure 2). Furthermore, a greater proportion of bees in the Grand Prairie than Lethbridge region engaged in nectar-robbing behaviour by ‘side-working' the flowers. This side-working behavior does not contribute substantially to pollination.

Where are honey bees in commodity canola fields, and does their distribution correlate with pollen deposition?

While honey bee abundance declined rapidly with distance into commodity canola fields (Figure 3), pollen deposition on stigmas did not similarly decline (p=0.1) (Figure 4), indicating that honey bee pollination accounts for only a fraction of the total pollen deposited on stigmas in these fields.

Is proximity to bee hives correlated with yield?

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 only in smaller plants (Figure 5). This suggests that in this agronomic context, smaller canola plants may benefit from additional pollination, but that the effect is negligible for larger plants. These results contrast previous experimental work that demonstrated large yield increases in other regions by comparing plants in pollinator-exclusion tents to open pollinated plants in small plots with high bee stocking rates[1-6]. Our results indicate that caution must be taken in extrapolating previous research to the large-scale agriculture found in Western Canada.

Given those results, what are the relative contributions of self-, wind-, and animal mediated-pollination to commodity canola yield?

Our results from Experiment 1 (comparing self, wind, and bee pollination in both 2015 and 2016 near Lethbridge AB) demonstrated no differences among our pollination treatments in yield, indicating that in this context, self-pollination alone was sufficient for maximal yield. These results are not surprising, in light of the survey results discussed above from commodity canola fields in Beaverlodge / Lethbridge. In experiment 1, we found that plots that were open to honey bee visitation had seed yields comparable to plots in which honey bees were excluded (Figure 6). Moreover, the lack of yield increase when plots were open to honey bee visitation was not the result of low honey bee visitation in 2016 (as could be the case in 2015) as over half of the fields were stocked with honey bee colonies, and visitation rates to the 'open' plots exceeded 2 flower per minute (at peak bloom). Also unexpectedly, plots that were enclosed in cages excluding most wind ('self') had comparable seed yields to plots that allowed wind to pass but excluded honey bees ('wind') (Figure 6). The unexpectedly high yield among plants with wind *and* bee exclusion may have resulted from sufficient (although highly limited) wind passing through the tightly woven fabric to agitate the plants. Another possibility is that plants in these plots experienced lower levels of pod set, but compensated by increasing 1000 seed weight. Although we observed numerically lower pod set and higher 1000 seed weight in the 'self' compared to the other treatments, this difference was not significant (Figure 6). Interestingly, while there was no difference among the pollinator treatments in overall yield, the variation in seed yield in the self-pollination only treatment was lower than in the open / wind treatments, with the maximum yield higher in the latter two treatments. In addition, the opposite trend was noted in % green seed, with self-pollinated plants having a higher maximum % green seed. This indicated that the presence of pollinators may be important for seed quality and yield, under certain circumstances or at higher stocking rates.

Our analysis of stigmas harvested from flowers in 2015 and 2016 provides another clue as to why yields fail to increase under conditions of open-pollination compared to wind- and self- pollination. We determined that seed set does not increase substantially beyond ~250 grains of pollen per stigma (Figure 7). Consequently, even though flowers in hand-pollinated plots had more pollen per stigma compared to other pollination treatments (Figure 8), flowers in all plots had median levels exceeding the 250 pollen grain threshold, translating into a lack of difference in seed set. Although we significantly increased the level of pollen deposited onto stigmas manually using hand pollination, the benefits of this additional pollination, consequently, did not translate into more seeds per pod, similar to the trend observed when bees were allowed access to flowers. This indicates that in many contexts in Western Canada, commodity canola yield is likely not pollen-limited.

Conclusions for commodity canola

Taken together, the results of Survey 1 and Experiment 1 indicate that in many agronomic contexts wind and self-pollination are sufficient for maximal seed yield in commodity canola, as sufficient pollen (~250 grains) for the maximum yield is transferred. However, flower visitation by bees does increase pollen deposition on stigmas, and in some context this may increase yield (small plants, areas with little wind). As bee abundance declines with distance into the field, this is especially important in the centre of fields, or other areas far from sources of pollinators. Targeted research to identify the agronomic contexts in which the actions of bees do increase yield would benefit growers. Furthermore, bees may benefit canola growers by promoting more even and rapid pollination, or increasing seed quality. This is an area that warrants further research.

What rewards do canola seed production fields offer bees, and how do bees behave on flowers in male versus female bays?

There were negligible amounts of nectar in the standing crop in the open flowers in seed fields in 2015 and 2016, only a small fraction of what is available to pollinators 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 9) in the varieties examined. This indicates that male and female flowers offer foraging pollinators different nectar rewards in addition to the pollen available in male flowers. Both male and female plants experienced peak nectar production during days where the temperature was about 21-27ºC indicating that weather affects the attractiveness of the crop to pollinators (Figure 10).

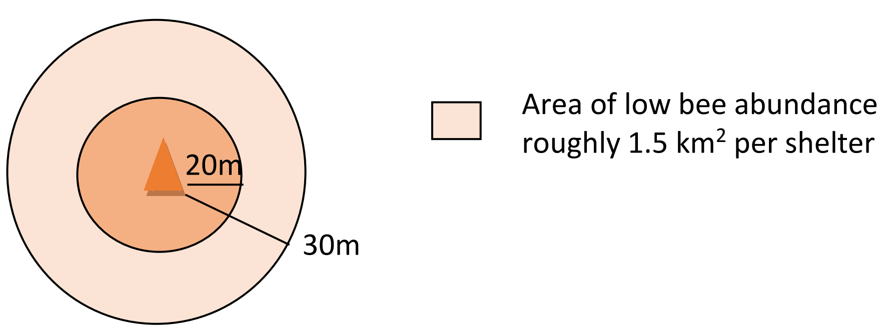
As in commodity canola fields, the majority of honey bees in seed fields were nectar foragers (no pollen loads observed), and that approximately equal numbers of foragers were observed/ m^2 in male and female bays (Figure 11). Pollen foragers were observed primarily in the male bay, which makes sense, as only the male plants produce pollen. However, pollen foragers in the female bay may have a disproportional effect on the pollination of female flowers, as they would be predicted to transfer more pollen to receptive female flowers that bees foraging for nectar. Side-working nectar foragers were also more commonly observed in the male bay.

How are bees distributed within the field, and does this are affect pollen deposition or yield?

In these heavily stocked fields, honey bee visitation declined with distance from field edge / hives in 2015 but not 2016 (Figure 12). In addition, we found no difference in visitation of honey bees between male and female bays.

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, with overall reduced pollen deposition compared to commodity canola (~50 grains) (Figure 13). This indicates that honey bees likely play a larger role in pollen deposition in the seed fields than the commodity fields, and the effect is present despite the activity of leafcutter bees across all our study fields. We also observed a decline in seed yield with distance from honey bee hives in 2015, indicating that there may be fields / years with a pollination deficit at greater distances from the honey bee hives (Figure 14).

Leafcutter bee visitation to the crop declines rapidly with distance from their shelters, reaching a base level at about 20m from the shelter (Figure 15). Honey bee visitation increases away from the leafcutter shelters, not reaching a plateauing until about 30m away; indicating that honey bees appear to feel the effects of leafcutter bees at further distances than the leafcutter bees permeate into the field. This might be due to some kind of learned association (i.e. the honey bees associate the shelters with getting attacked by female leafcutters, or a lack of forage, and avoid them), or could be caused by male leafcutters venturing further from the shelter than females and "bothering" the honey bees. This pattern results in a circular area of relatively low bee density between about 20-30m radiating out from leafcutter bee shelters. Per shelter, this represents an area of about 1.5 km^2 (see light orange area in figure below; area of circle with 30m radius =2.8 km – radius of circle with 20 m radius = 1.3 km).



Conclusions for seed canola

Similar to our findings in commodity canola, honey bee abundance decreased with distance into the fields, although not in all cases. In combination with the decrease in leafcutter bees at distances greater than about 20 m from shelters, there may be areas in seed fields that are relatively depauperate in pollinators, despite their high overall abundance in the fields. However, in contrast to the results from commodity canola, in seed canola this resulted in a decrease in pollen deposition on female flowers with increasing distance from honey bee hives. Furthermore, this translated into a decrease in yield with distance from honey bee hives. In addition, honey bees bearing pollen loads were rarely observed visiting female flowers. In general, less pollen was found on the stigmas of flowers in seed fields than in commodity fields, and this may limit yield in some parts of the field.

2. **Quantify the impact of pollination services on bee or colony health in canola hybrid seed-production (Survey 2a &b)**

What effects does providing hybrid canola pollination services have on honey bee health and productivity?

Our results from honey bee colony health surveys in 2016 showed no clear negative consequences of pollination service delivery in canola fields on honey bee health. *Nosema* infection did not vary significantly between colonies that went to pollination and colonies that did not, rather it varied between the two sources apiaries we observed (Figure 16). As with *Nosema, Varroa* levels were significantly different between source apiaries, but not colonies that went to pollination fields or did not (Figure 17). Similarly, there were no significant effects on queen loss (Table 1), at least in the short term. There was a positive effect on pollen income, however; colonies that were used for canola pollination produced significantly more pollen than those that stayed in their 'home' apiary (Figure 18). This led to further research on pollen production in seed production fields in 2016. Honey production, in contrast, was significantly less from colonies that were used to pollinated hybrid canola seed fields (Figure 19) than colonies that stayed in their home apiary. While the magnitude of this effect will vary with the location of the 'home' apiary or honey production site, and among years and canola varieties, it reinforces the economic losses incurred by beekeepers pollinating seed canola fields. The reduced honey production, increased labour costs, and increased risk must be more than compensated for by the pollination rental fees paid per colony to beekeepers.

We conducted a 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 important 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 20). This indicates that irrigation management may be used to increase the pollination activity and return rates of leafcutter bee pollinators. As this is a major investment for seed companies, the management of irrigation and nest block number and distribution is another area deserving of additional research.

3. **Characterise how bee behavior affects pollination services delivered to canola crops, and determine how it can be manipulated to increase pollination efficacy and bee or colony health (Experiment 2,3 & 4)**

Only two types of pollinators (honey bees and alfalfa leafcutter bees) were numerous enough to be included in the pollinator response analysis in 2015. The 'avoid' category of response to a proffered flower was removed due to its ambiguous nature, and the analysis was therefore restricted to whether pollinators 'accepted' (i.e. visited) or 'rejected' proffered female inflorescences (see supplementary video 1, appendix 2). Honey bees and leafcutter bees foraging on male flowers were both less likely to visit a female inflorescence than those originally foraging on female flowers (Figure 21). This result suggests that both leafcutter and honey bees exhibit floral constancy to flower type in hybrid canola, and do not commonly switch from a male to a female flower. Leafcutter bees also 'accepted' female inflorescences more than honey bees. This may be an artefact of pollinator response to the interview bouquet apparatus, suggesting that leafcutter bees are less deterred by the interview bouquet setup

We compared pollinator efficacy between honey bees, male and female alfalfa leafcutter bees, bumble bees, and syrphid flies. We found that the type of pollinator, the amount of pollen a pollinator had on its body, the flower it was originally foraging on (male or female), and the time it spent on a flower *all* significantly affected the amount of pollen deposited on a stigma. Female alfalfa leafcutter bees deposited significantly more pollen on stigmas than honey bees and syrphid flies (Figure 22), but not significantly more than male leafcutter bees. There was no significant difference in pollen deposition between bumble bees and female alfalfa leafcutter bees or honey bees. These results indicate that per bee visit, female leafcutter bees deposit more pollen than honey bees, however they may be less numerous in the field as the leafcutter bee sex ratio is male biased. We did not examine male honey bees, as they do not forage. Pollination management factors that can be used to increase the relative production of female leafcutter bees and their productivity would benefit pollination services.

Pollinators travelling directly from male flowers deposited on average 32.2 ± 10.1 pollen grains onto female stigmas, compared to the 1.7 ± 1.2 grains deposited by pollinators travelling from female flowers. This is important, as bees were previously found to be reluctant to switch from male to female flowers. Bees visiting female flowers are therefore more likely to be coming from a female flower, and depositing less pollen onto female stigmas. Crop management techniques that encourage bees to switch more readily would increase pollen deposition. Pollinators with longer floral visits also deposited more pollen grains (Figure 23). The relationship between pollen on body and pollen deposited was negative (Figure 24), contrary to expectations.

While managed female leafcutter bees and wild bumble bees may be better at depositing pollen on hybrid canola than honey bees per visit, bumble bees are relatively infrequent in hybrid canola fields. They are therefore not a dependable source of sufficient pollination. The fact that pollinators with more pollen on their bodies deposited less pollen onto stigmas may be due to the tendency of pollen foragers, especially corbiculate bees (such as honey and bumble bees), to groom and pack pollen. Grooming pollen may make it inaccessible for pollination, which means that the amount of pollen available for deposition may not be the same as the amount of pollen on the insect. Leafcutter bees, in contrast, carry pollen on their abdomen, where it is more likely to come into contact with female flowers reproductive anatomy. Finally, all female leafcutter bees collect pollen, in contrast to the specialised foraging behaviour of honey bees, which results in individuals that focus on either pollen or nectar.

For transect line crossing, an increase of honey bee visitation to the male bay prompted crossing to the female bay (df=6, ΔAIC=1.49) (Figure 25). Δ 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. Profit and honey bee visitation were likewise influential in causing honey bees to cross to the male bay (df=7, ΔAICc=1.16), although in this case increases in both honey bee visitation to the female bay (Figure 25) and in Δ profit (Figure 26) 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, ΔAICc=0) increased honey bee crossing to the female bay (Figure 27).

Increased visitation by alfalfa leafcutter bees to both the male and female bay motivated leafcutter bees to cross to the female bay (df=7, ΔAICc=1.5) (Figure 28), as did an increase in Δ female profit (df=8, ΔAICc=0) (Figure 26). 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, ΔAICc=1.42) (Figure 28), 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, ΔAICc=0) (Figure 26).

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 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.

For individual travels, diversity (rarefied taxon richness) had a positive effect on the tendency of pollinators to switch bays; 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 taxa 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 the least likely to move from the male bay (Figure 29).

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, therefore effecting pollination. The discrepancy of the effects of Δ profit (in which some cases motivated pollinator crossing, and in others made crossing less likely) suggests possible interactions between pollinator visitation and floral profit.

4. **Create guidelines to maximize the efficient use and maximal health of bees pollinating canola (Experiment 2,3, & 4)**

Colony Size

We found that the number of bees and number of brood cells in honey bee colonies rented for pollination services was highly variable within each grouping of single or double brood chamber colonies ('singles' and 'doubles') within a commercially managed pollination yard, and also between years (Figure 30). In 2014 the singles had much smaller bee and brood populations than the doubles as would be predicted. However in 2015, the two hive sizes had colony populations that were statistically indistinguishable. 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 subsequently compared between the singles and doubles on both a colony level and a per frame basis (a frame was assumed to be 1600 bees).

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 31A), with the singles collecting statistically similar amounts (2014) or even greater amounts (2015) of pollen than the doubles. This difference was even more pronounced when measured per frame of bees (Figure 31B), where the singles collected 1 or 2 times as much pollen per day as the doubles. This may have implications for pollination, as pollen foragers would visit male flowers to collect pollen. In most crops, it is pollen foraging honey bees that are the most effective pollinators, however in hybrid seed canola, it is likely bees that are willing to switch from male to female flowers that are most effective.

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 32A). At the frame level (Figure 32B), the number of pollen foragers and nectar foragers per frame 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. This indicates that there is no cost to foraging activity (and therefore likely to pollination) by managing colonies as singles.

In 2014 the honey production at the colony level in the doubles was twice that of the singles (T=-6.72, df=48.9, P<0.0001). Per frame, however, there was no difference in honey production between the two management options (T=0.38, df=34.3, P=0.7053). The honey production data for 2015 was unfortunately not recorded by the participating beekeeper.

To summarize our results across both years of the study, 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, 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. Since conducting this experiment in 2014/15, some seed production companies have contracted single brood chamber colonies, offering both the seed production and beekeeping industries management options, using the data we have provided in their management decisions.

Honey bee hive product diversification

As discussed previously, honey bee colonies used for hybrid canola seed production field pollination produced significantly more pollen than those that stayed in their 'home' apiary (Figure 18). As a result, we tested whether pollen represents a viable hive product to produce in the hybrid canola pollination management paradigm. Our results indicate that by collecting pollen in addition to honey, beekeepers may be able to increase their profit by diversifying their products, while still offering high quality pollination services. Despite previous reports of negative impacts on brood production of prolonged pollen trapping, short-term trapping during the abundant pollen flow in seed canola fields had no impact on brood production (Figure 33). In contrast, honey production was negatively impacted by the addition of pollen traps beneath the colonies, or by the collection of the pollen (Figure 34). Honey production decreased from a mean of 40.3 lbs among untrapped colonies to only 27.4 lbs from colonies fitted with pollen traps. However, the per hive profit was larger from pollen-trapped colonies (Figure 35) than untrapped colonies because of the high value of pollen relative to honey, and the large amounts of pollen collected (mean = 3.98 lbs dried pollen per colony over the 1 month pollination period (Appendix 2, Photo 7)). At current 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. Pollen trapping could in fact increase pollen collection by honey bee colonies as removing pollen may encourage an increase in pollen foraging over nectar foraging.

5. Identify landscape-level and farm management factors that affect canola yield with respect to pollination services, and pollinator health

Through the results described above, we have identified a number of management and landscape factors that can affect canola yield, grower's input costs, and bee health. In commodity canola fields, we have identified flies as a major flower visitor across Alberta, as well as regional differences in the pollinator community. We found that in general, these fields had adequate pollen deposition for high yields, however there may be specific contexts in which high honey bee stocking rates can benefit the crop. These include small plants, areas with low wind, or areas / years in which rapid, even seed set is required. The effects of bee pollination on canola seed quality and duration of flowering / seed maturity is an area deserving of further research.

In seed production fields, we have identified that there are areas in the fields that are depauperate in bee pollinators, despite the high stocking rates. These include areas 20-30 m from leafcutter bee shelters, and areas at great distances from honey bee colonies. As a result, we recommend decreasing the distance between leafcutter bee shelters, and adding multiple honey bee 'drop sites' where at all possible. Increasing the number of shelters at the same leafcutter bee stocking rate / field may not only reduce the zone of low bee density, it may also increase the proportion of offspring that are female bees (affecting pollination in subsequent years as well as interference from male bees), as well as the overall number of offspring produced[11]. We also conclude that the seed crop is very highly dependent on bee pollination compared to the commodity crop, and that overall, pollen deposition in the seed fields is much lower than the commodity crop. We have identified that (a) bees that switch from male flowers, and (b) female leafcutter bees deposit the most pollen. However, bees are reluctant to switch from male to female flowers. As such, we recommend management practices that (1) encourage a higher female : male leafcutter bee sex ratio, and (2) encourage bees to switch between the male and female bays (or rows). This could include promoting pollinator diversity overall (including flies), decreasing the width of female bays, and decreasing differences in the flowers between the male and female plants (size, nectar production). We also recommend additional research into irrigation management. As leafcutter bee shelters are located throughout the shelter, they are directly impacted by every pass of the pivot. These areas of research could benefit both leafcutter bee producers, as well as canola seed companies. Finally, we recommend additional research to determine the potential effects of climate change on canola varieties, including the male and female lines within the seed production system. Recent research has identified important effects of climate on flower attractiveness to pollinators in the hybrid carrot seed production system[12], effects that could have important implications for hybrid canola seed production in Alberta.

6. Literature cited

Provide complete reference information for all literature cited throughout the report.

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7. Benefits to the industry (max 1 page; respond to sections *a)* and *b)* separately)

a) Describe the impact of the project results on Alberta's agriculture and food industry (results achieved and potential short-term, medium-term and long-term outcomes).

Canola is an important part of Alberta's agriculture industry, generating nearly $20 billion in economic activity across Canada (Canola Council of Canada), and even incremental increases in profit for the industry can have large overall economic benefit. The results of our project provide concrete recommendations to increase seed and commodity canola yield through pollination management, as well as identifying discrete areas of canola pollination management that require further research. Some seed companies have already begun to consider alternative leafcutter bee management strategies and have contracted single brood-chamber honey bee colonies in the 2017 growing season. In addition, the project has generated interest in pollen trapping from honey beekeepers across Canada. Beekeepers in Ontario were particularly receptive to this message, as there is a demand for pollen to provision commercial bumble bee colonies (the commercial bumble bee industry then sells a portion of their colonies to greenhouses in Alberta). In the short-term, this project and the questions it generated led us to seek additional research funding from the Canola Council of Canada. We are now working with them on a research project to identify agronomic contexts in which pollination benefits the commodity crop, and varietal differences in flower attractiveness and pollinator dependency. In the long term, this project will (1) spur an on-going interest in pollination as an input to consider in canola management (2) provide guidelines that seed and commodity growers can use to increase yield and seed quality through pollination, and (3) encourage an ongoing mutually beneficial relationship between beekeepers and canola growers.

b) Quantify the potential economic impact of the project results (e.g., cost-benefit analysis, potential size of market, improvement in efficiency, etc.).

This project has provided a number of concrete recommendations for canola growers and beekeepers to increase production, increase efficiency, and provide flexibility in management and production. As commodity canola is grown across approximately 6-7 million acres in Alberta, with ~70,000 acres in seed production, the potential market for implementation of this research is rather large. Flexibility in beekeeping management (e.g. use of singles versus doubles, pollen and honey production) will assist the industry to mitigate the effects of high winter losses of honey bees and low honey prices by providing management options. The collection of pollen also opens up additional markets for beekeeping products. The identification of specific areas in seed fields with low pollinator abundance (e.g. at 20-30 m from leafcutter shelters) offers growers a discrete opportunity to increase their yield in those areas. We have also identified management areas that require refining for leafcutter bees (irrigation, shelter distance).

As the canola seed industry in Alberta is valued at approximately 1-1.5 billion dollars annually (Stephen Page, AAFC, personal communication), even a 1% increase in profit is valued at $10-15 million per annum. Our project offers not only the opportunity to increase seed and therefore profit, but also to mitigate the risk posed by high colony losses and low honey prices. Furthermore, we have identified recommendations that are of benefit to both beekeepers / producers and crop growers (e.g. decreased shelter distance to benefit both pollination as well as leafcutter bee production, pollen collection could potentially increase both pollination and beekeeper profit).

8. **Contribution to training of highly qualified personnel (max ½ page)**

Graduate Students (2):

Riley Waytes, MSC, University of Calgary (degree completed)
Samuel Robinson, PhD, University of Calgary (degree in progress)

Post-doctoral Fellows (1):

Andony Melathopoulos, Post-doctoral fellow, University of Calgary (now Faculty at Oregon State University)

Undergraduate Assistants (7):

Christian Sprinkhuysen, undergraduate assistant, University of Lethbridge
Ashley Londeau, undergraduate assistant, University of Lethbridge
Samantha Knight, undergraduate assistant, University of Calgary
Ian Johnson, University of British Columbia
Zachary Wagman, University of Ottawa
Mungunzul Amarbayan, University of Calgary
Hayley Shade, Lethbridge Collegiate Institute

Technicians (2):

Lynae Ovinge, AF
Jeff Kearns, AF

9. **Knowledge transfer/technology transfer/commercialisation (max 1 page)**

a) Scientific publications (*e.g.*, scientific journals); attach copies of any publications as an appendix to this final report

The scientific publications that will result from this project have not yet been published.

Completed Theses:

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

b) Industry-oriented publications (*e.g.*, agribusiness trade press, popular press, etc.) attach copies of any publications as an appendix to this final report

Isaacs, J. (2017) Leafcutter bees essential to western Canadian canola. Top Crop Manager. March 2017.

Barker, Bruce (2017) Understanding bee-haviour. Top Crop Manager March 27 2017

Hoover S (2017) Pollen Collection, honey production, and pollination services. Ontario Bee Journal. May 2017.

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Ovinge L, Hoover S (2016) Effective honey bee hives for pollination: *singles versus doubles* BeeScene February 2016.

c) Scientific presentations (*e.g.*, posters, talks, seminars, workshops, etc.)

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

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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.

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.

d) Industry-oriented presentations (*e.g.*, posters, talks, seminars, workshops, etc.)

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.

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

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

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

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

e) Media activities (*e.g.*, radio, television, internet, etc.)

http://www.farm.tv/watch.html/318944
http://www.producer.com/2013/08/honeybees-play-role-in-boosting-canola-yields/
http://www.producer.com/2016/04/do-more-bees-mean-more-canola-in-the-bin/
http://www.producer.com/2016/03/leafcutter-bees-operate-in-the-dark/
http://www.topcropmanager.com/other-crops/understanding-bee-haviour-20013

Section D: Project resources

1. **Statement of revenues and expenditures:**
   a) **In a separate document certified by the organisation's accountant or other senior executive officer, provide a detailed listing of all cash revenues to the project and expenditures of project cash funds.** Revenues should be identified by funder, if applicable. Expenditures should be classified into the following categories: personnel; travel; capital assets; supplies; communication, dissemination and linkage; and overhead (if applicable).
   b) **Provide a justification of project expenditures and discuss any major variance (*i.e.*, ± 10%) from the budget approved by the funder(s).**

2. **Resources:**

Provide a list of all external cash and in-kind resources which were contributed to the project.

Total resources contributed to the project		
Source	**Amount**	**Percentage of total project cost**
Funders		%
Other government sources: Cash		%
Other government sources: In-kind		%
Industry: Cash		%
Industry: In-kind		%
Total Project Cost		**100%**

External resources (additional rows may be added if necessary)		
Government sources		
Name (only approved abbreviations please)	Amount cash	Amount in-kind
Industry sources		
Name (only approved abbreviations please)	Amount cash	Amount in-kind

Section E: The next steps (max 2 pages)

Describe what further work if any needs to be done.

a) Is new research required to deal with issues and opportunities that the project raised or discovered but were not dealt with within the current project?

There were several opportunities for additional beneficial research that were identified. These included:

(a) Seed fields

(1) Effects of distance between leafcutter bee shelters on bee production and sex ratio, and the 'evenness' of the distribution of leafcutter and honey bees across the field

(2) The effect of the width of the male and female bays on pollen deposition and pollinator visitation

(3) Consequences of irrigation on leafcutter bee production and stigma receptivity

(4) Effects of pollinator diversity on pollinator behaviour

(5) Effects of climate on flower attractiveness and investment in reproduction for different varieties and male versus female plants

(6) What do honey bees cue into to avoid leafcutter bee shelters? Are they avoiding areas of depleted resources, interference from male leafcutter bees, or aggression from female bees?

(b) Commodity Fields

(1) In what contexts do honey bees benefit yield – low wind? Small plants? Low fertility soil?

(2) Does pollinator abundance reduce the time to mature seed by reducing bloom time?

(3) Does pollinator abundance improve seed quality? Under what circumstances?

(b) Is there related work that needs to be undertaken to continue advancement of the project technology or practice?

Additional work is required to continue to advance our knowledge in order to provide additional concrete information and recommendations to growers, and our group is currently undertaking some of the required research. In addition, we will continue to disseminate this research at industry meetings and in industry publications. In addition, there is one PhD thesis in progress that will include results of this project.

(c) Did the project identify any new technology or practice that needs to be developed?

We have identified beneficial management practices for beekeepers and canola growers who will refine these as required. We have been consulted by industry to provide information on our research as they endeavor to fine-tune pollinator management.

(d) What suggestions do you have that increase commercial use of results by farmers and/or companies. These may be:

1. commercial uptake.

2. **further research toward commercial use.**
3. **extension and information disbursement.**

The industry has proven very open to testing the implementation of our research results. We feel confident that if the management practices are beneficial, they will be widely adopted. We will continue to disburse extension information at grower meetings, and continue to conduct additional research to provide growers with additional information.

Section F: Research Team Signatures and Employers' Approval

The team leader and an authorised representative from his/her organisation of employment MUST sign this form.

Research team members and an authorised representative from their organisation(s) of employment MUST also sign this form.

By signing as representatives of the research team leader's employing organisation and/or the research team member's(s') employing organisation(s), the undersigned hereby acknowledge submission of the information contained in this final report to the funder(s).

Team Leader's Organisation

Team Leader	
Name: Dr. Shelley Hoover	**Title/Organisation:** Research Scientist, Alberta Agriculture and Forestry
Signature:	**Date:**
Team Leader's Employer's Approval	
Name: Dr. David Feindel	**Title/Organisation:** Section Head, Pest Surveillance, Alberta Agriculture and Forestry
Signature:	**Date:**

Research Team Members (add more lines as needed)

1. Team Member	
Name: Dr. Stephen Pernal	**Title/Organisation:** Officer In Charge, Beaverlodge Research Farm and Research Scientist, Agriculture and Agri-Food Canada
Signature:	**Date:**
Team Member's Employer's Approval	
Name: Dr. Francois Eudes	**Title/Organisation:** RTD Director, Agriculture and Agri-Food Canada
Signature:	**Date:**

2. Team Member	
Name: Dr. Ralph Cartar	**Title/Organisation:** Associate Professor Department of Biological Sciences University of Calgary
Signature:	**Date:**
Team Member's Employer's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**

Appendix 1 Figures

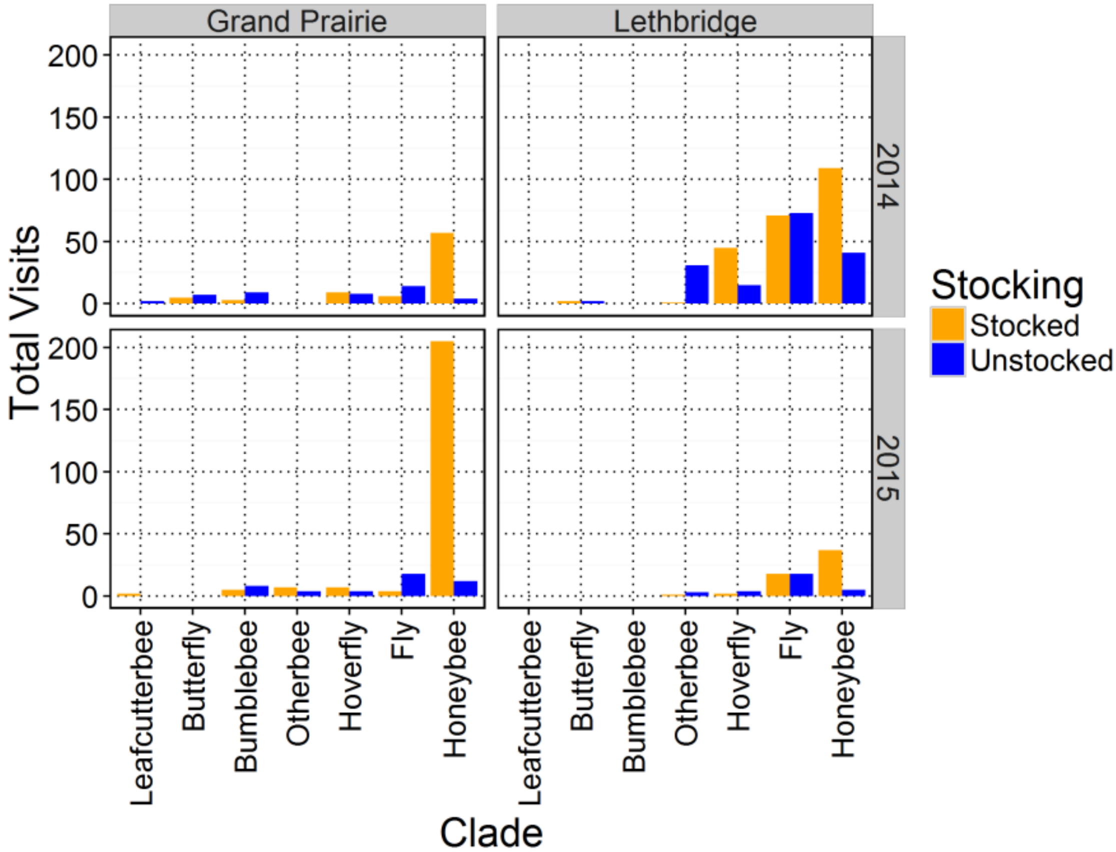

Figure 1. Number of flower visitors for each site in 2014 and 2015 by taxonomic group. Gold bars represent fields stocked with managed honey bees, blue bars are unstocked fields.

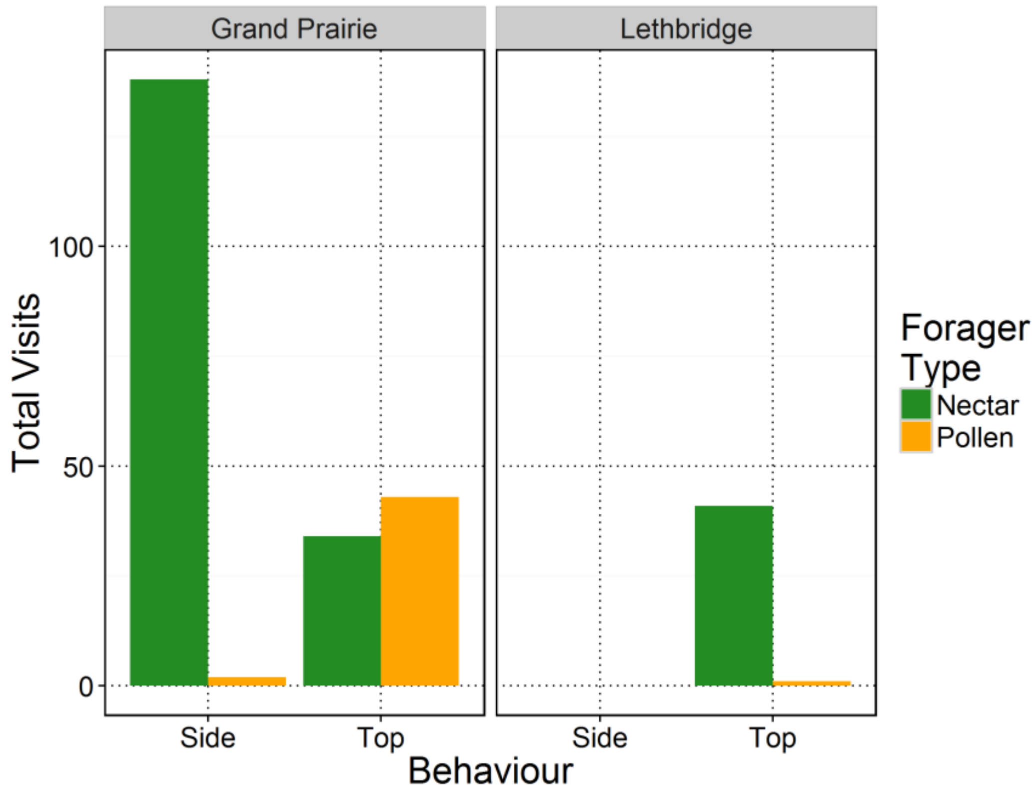

Figure 2. Behaviour of honey bees visiting commodity canola flowers; bees either side-worked the flower to access nectar (side), or accessed the flower from the front (top) thus coming in contact with pollen as well as accessing the nectaries. Gold bars represent honey bees with pollen on their corbiculae,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

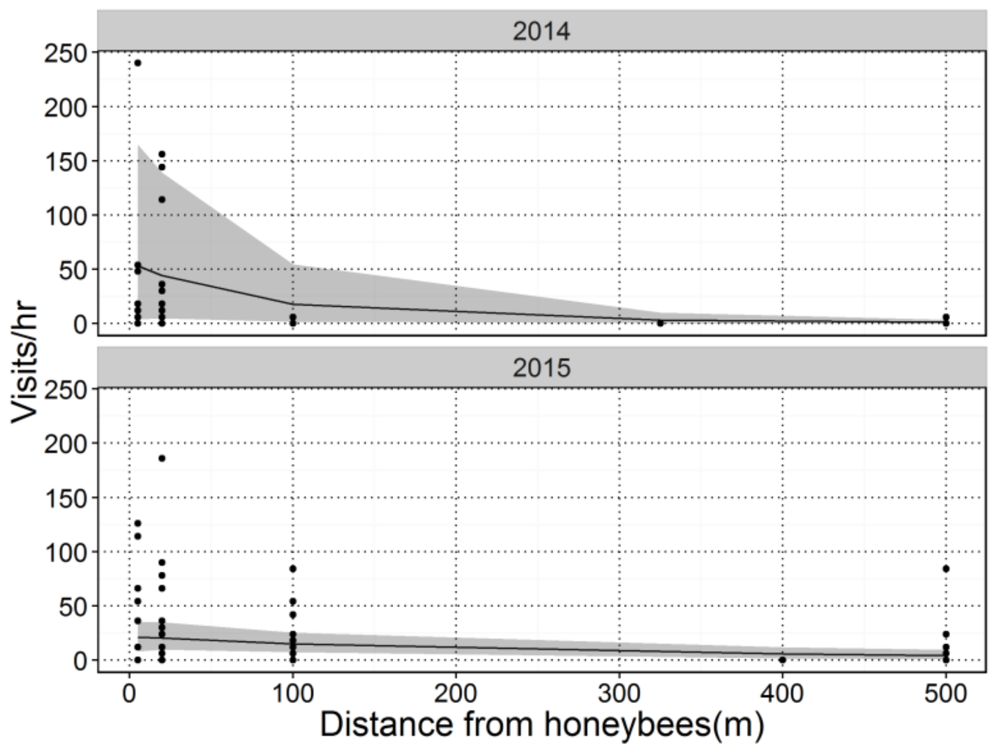

Figure 3. Honey bee visits to experimental flower plots at different distances into commodity canola fields. Bee abundance declined rapidly with distance.

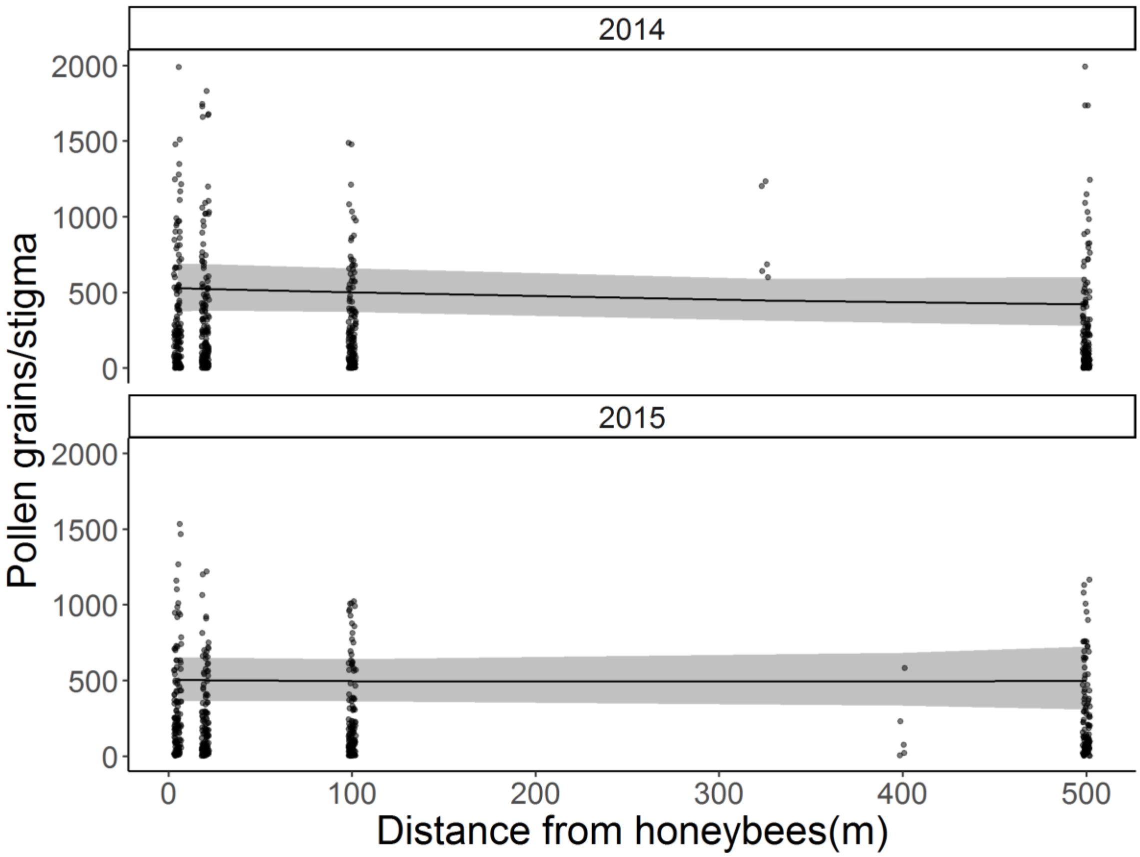

Figure 4. Pollen deposition on stigmas with increasing distance into the field in 2014 (530 near the field edge vs 420 grains at 500 m into the field), and away from honey bee hives. While bee abundance declined with distance into the fields, pollen deposition did not. In 2015, there was no decrease in pollen deposition with distance into the field (505 vs 499 grains).

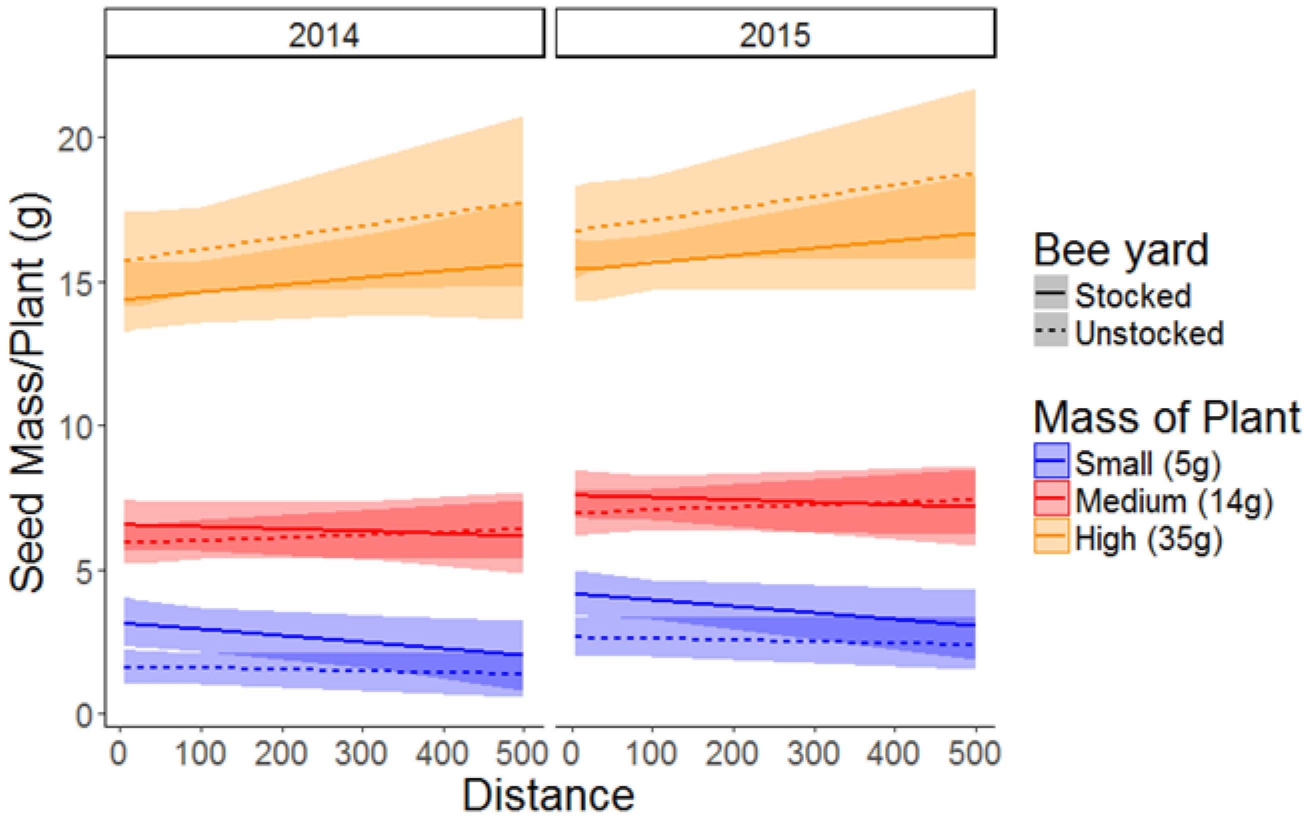

Figure 5. Seed yield per plant with increasing distance into the field, for a variety of plant sizes (5-35g), under stocked (honeybee hives present) and unstocked conditions, for both 2014 and 2015. Plants in 2015 produced significantly more seed, and there was a decline in seed yield with distance from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

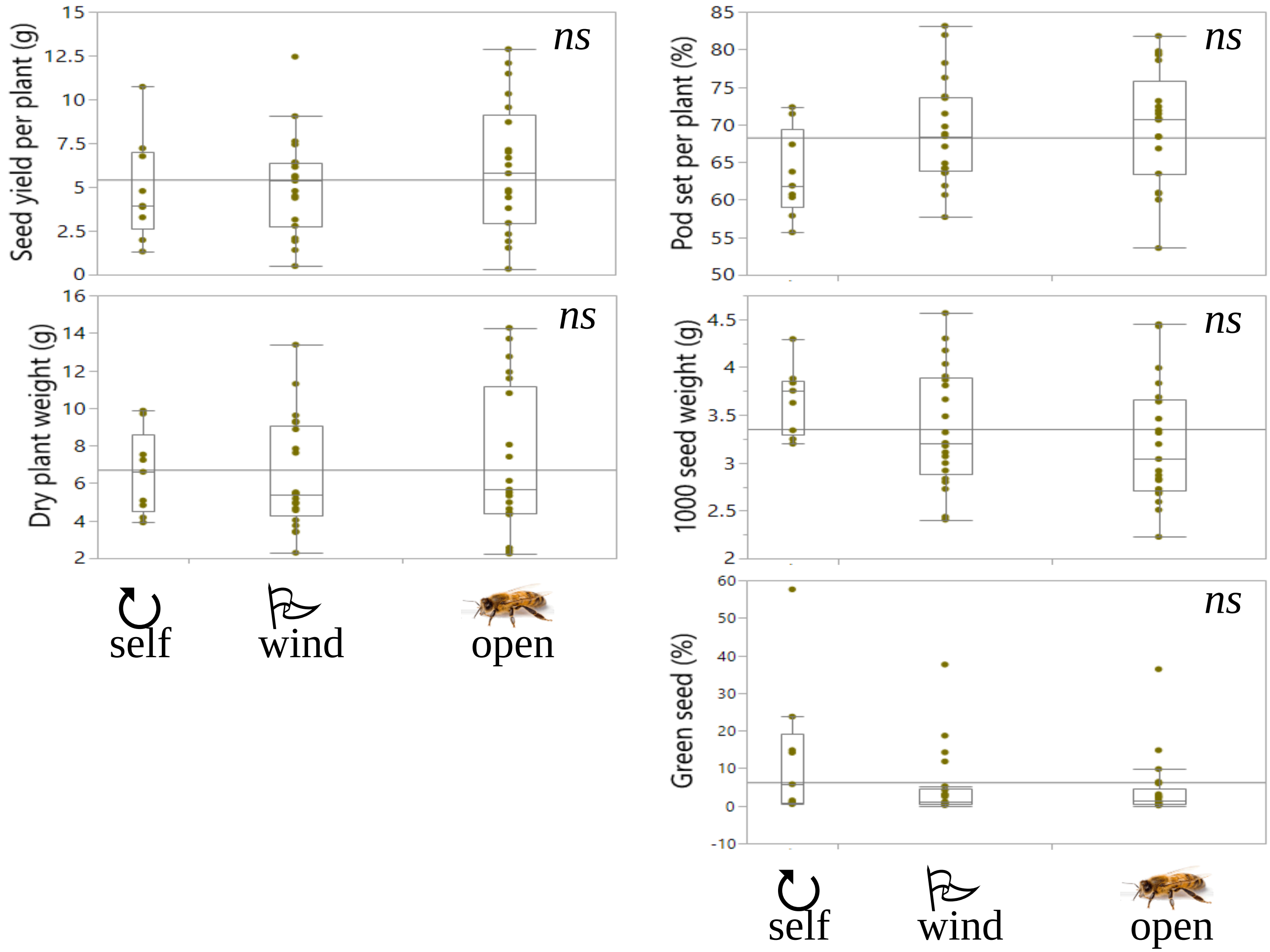

Figure 6. Box and whisker plots of seed yield per plant, dry plant weight, pod set per plant, 1000 seed weight and green seed incidence from canola plants located in one of three treated plots in 2016: 1) self-pollination (fine mesh cage to exclude both pollinators and the wind) (n=9 fields), 2) wind-pollination (plants placed in a coarse mesh cage during bloom to exclude pollinators but not wind) (n=21 fields) and 3) open-pollination (uncaged plot) (n=21 fields). For all measures there were no

significant treatment differences (ANOVA, P>0.05). The grey horizontal line in each graph indicates the global mean for all three treatments.

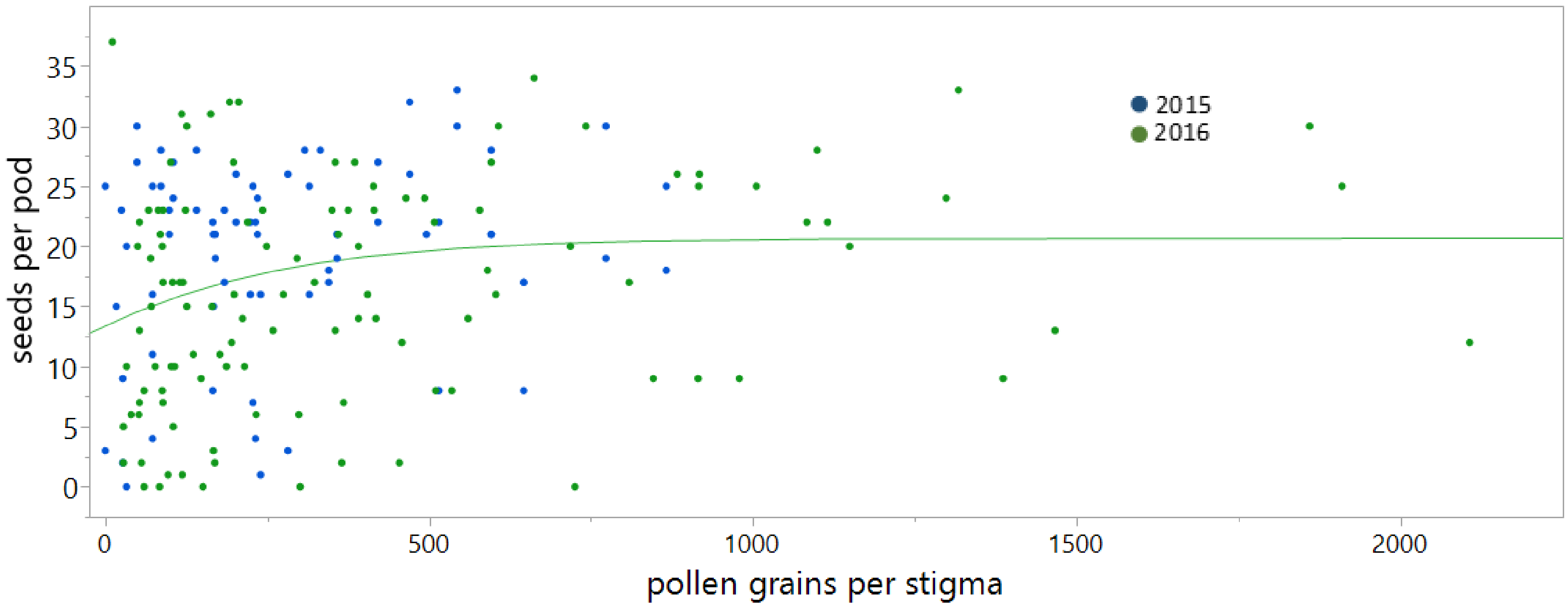

Figure 7. Nonlinear sigmodial curve fit of the number of pollen grains per stigma versus the subsequent number of seeds produced per pod using the Gomperz 3P Model in the statistical program JMP. Stigmas were harvested from tagged flowers immediately following petal drop and after ovule fertilization and mounted on stained gelatin slides for microscope counting. Prior to harvest, the seeds in these same pods were counted. Data from 2015 and 2016 were combined for the analysis. The model had a R^2 fit of 0.06 and reached an asymptote after 20.7 ± 1.8 seeds were set. The model predicts that approximately 16 seeds will be set after 122± 47 pollen grains are delivered to the stigma and 18 seeds after 265± 89 pollen grains are delivered.

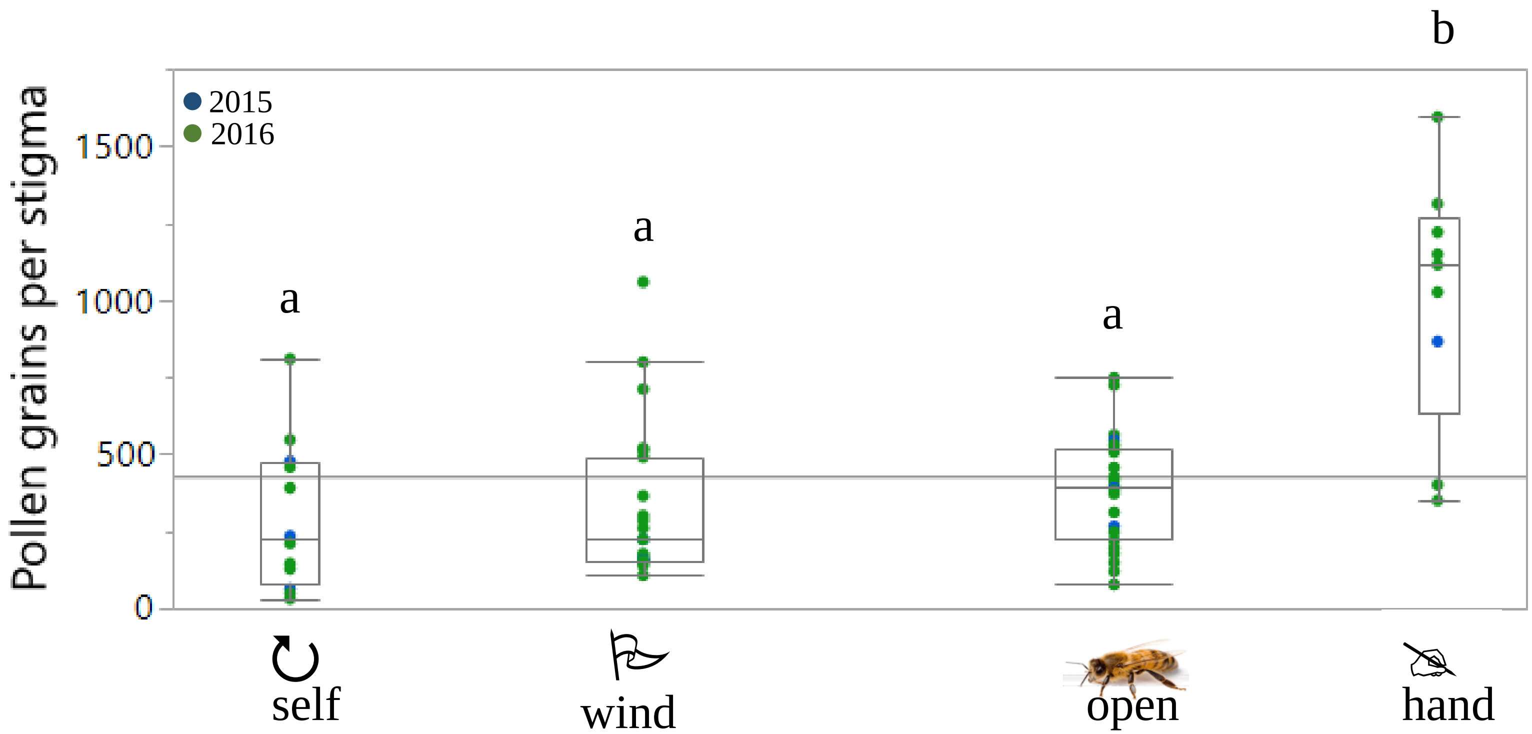

Figure 8. **Box and whisker plots of the pollen deposition on the stigmas from canola plants located in one of three treated plots in 2015 and 2016: 1) self-pollination (fine mesh cage to exclude both pollinators and the wind) (n=12 fields), 2) wind-pollination (plants placed in a coarse mesh cage during bloom to exclude pollinators but not wind) (n=24 fields) and 3) open-pollination (uncaged plot) (n=24 fields). Moreover, a selection of flowers in each plot was saturated with pollen by applying pollen from adjacent plants to directly to the stigma (hand, n=8 fields). Means followed by the same lower-cased letter indicate no significant differences (Tukey-Kramer HSD, α=0.05) and the grey horizontal line in indicates the global mean for all four treatments.**

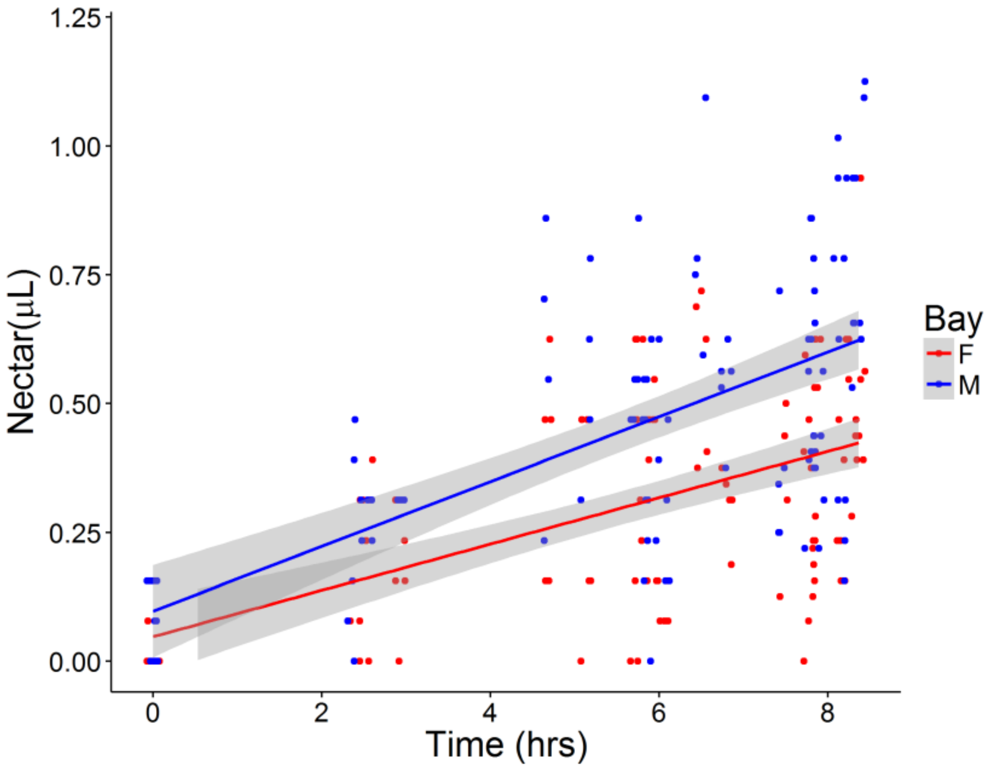

Figure 9. Nectar production over time in netted flowers inaccessible to bees. 'Male' plants produced more nectar than 'female' plants per hour.

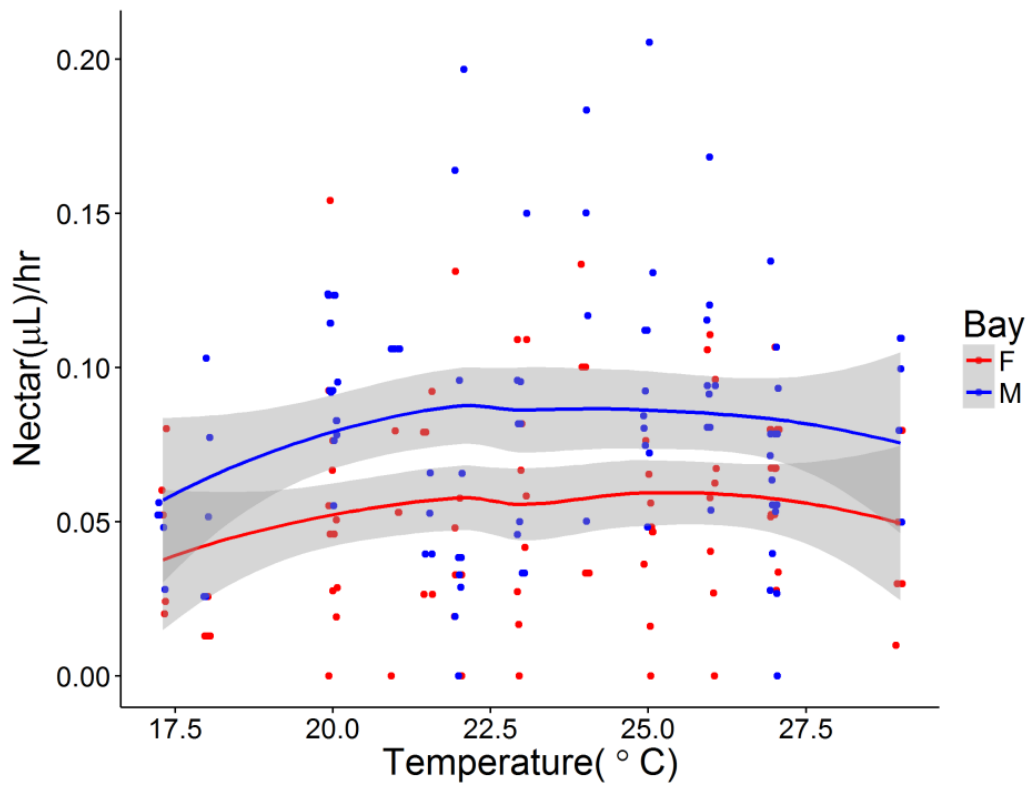

Figure 10. Nectar production per hour in male (blue) and female (red) plants, at different temperatures.

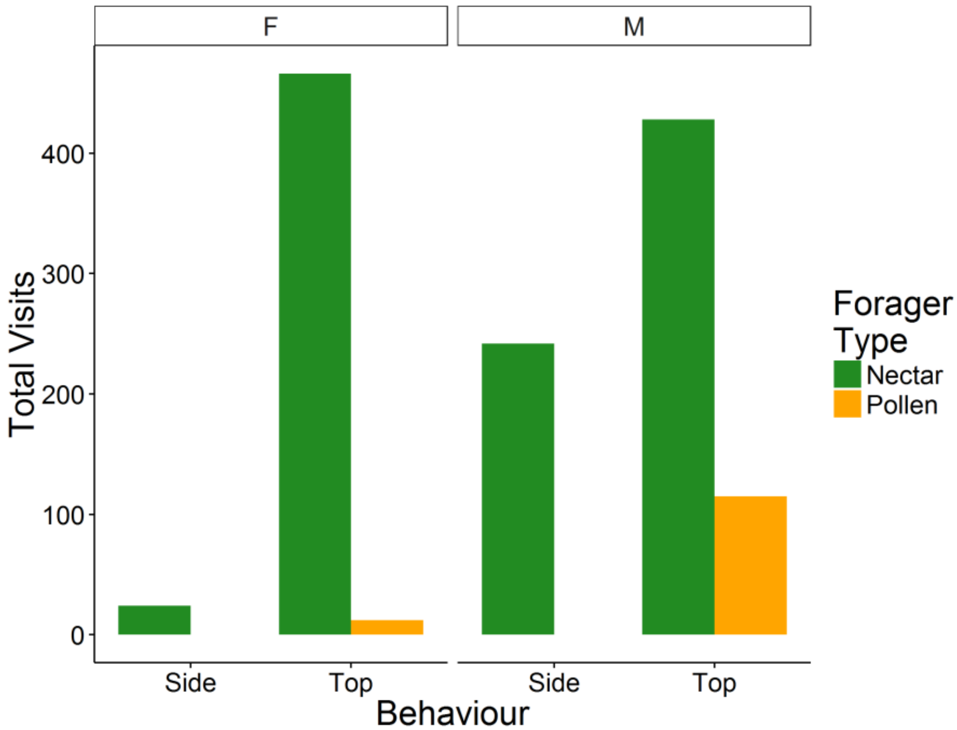

Figure 11. Total visits of honey bees per plot in seed canola fields for both pollen (gold) and nectar (green) foragers in both male (M) and female (F) bays; foragers either accessed the flower from the top (thus coming in contact with pollen) or side-worked them (nectar robbing).

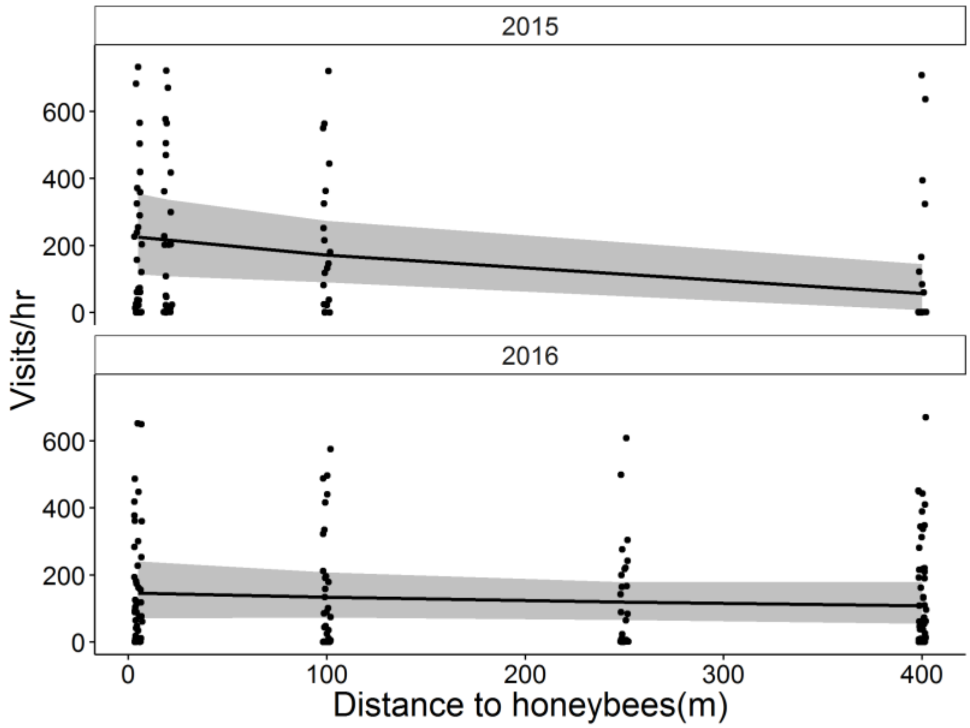

Figure 12. Honey bee visits per plot with increasing distance into the field (and away from hives).

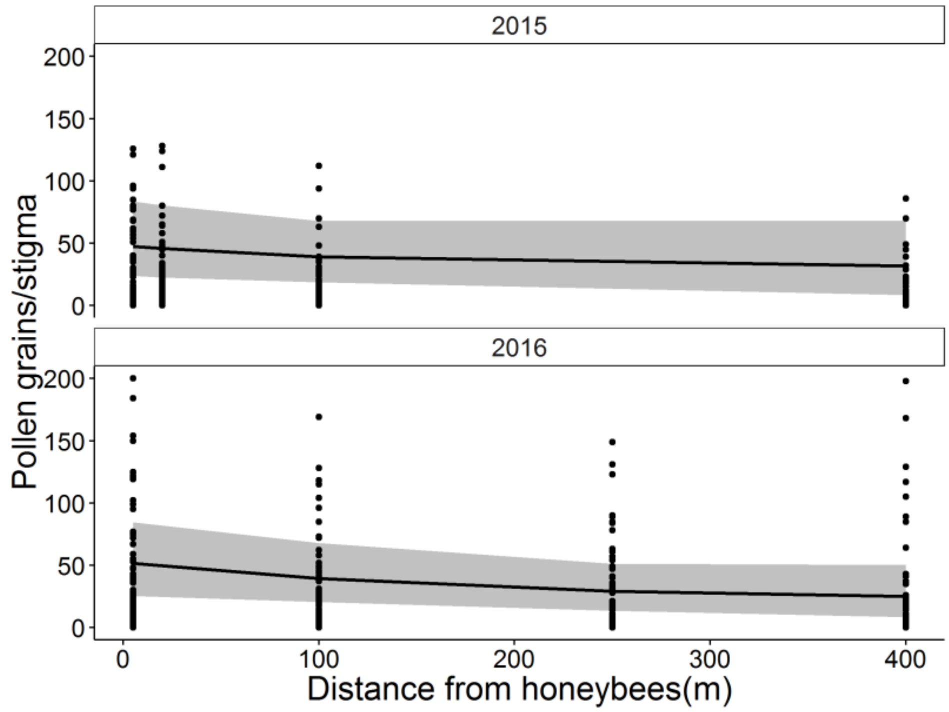

Figure 13. Pollen deposition on stigmas with increasing distance into seed fields / away from hives.

Figure 14. Seed yield at increasing distances from honey bee hives in seed production fields in 2015.

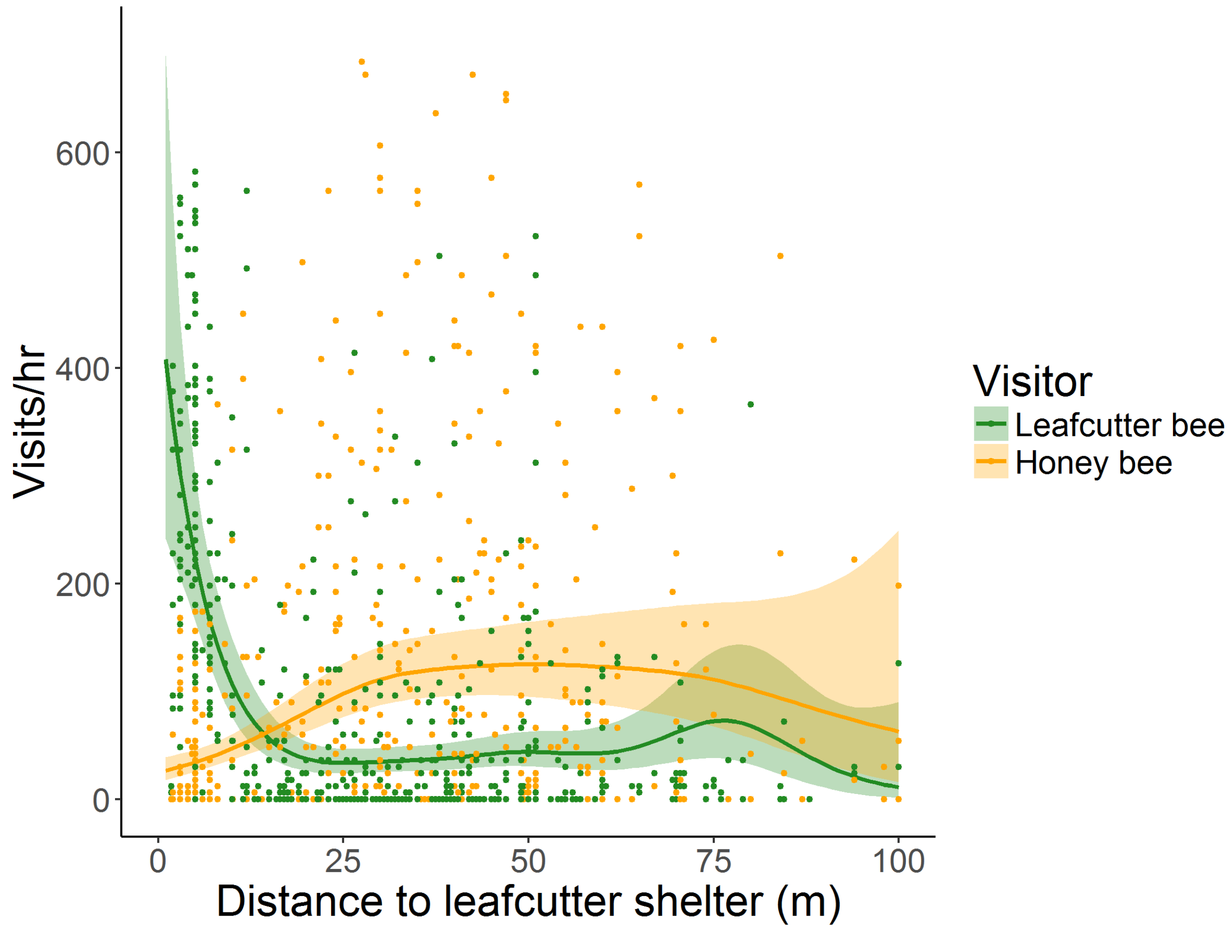

Figure 15. Leafcutter and honey bee abundance with distance to nearest leafcutter bee shelter. Leafcutter visitation drops very quickly away from the shelter, plateauing at about 20m from the shelter. Honey bee visitation increases away from the shelters, but the plateau starts at about 30m, meaning that honey bees appear to feel the effects of leafcutter shelters at further distances. (Adjusted R^2 = 0.21), but both the honeybee (χ^2=47.33, p<0.0001) and the leafcutter splines (χ=145.78, p<0.0001) were highly significant.

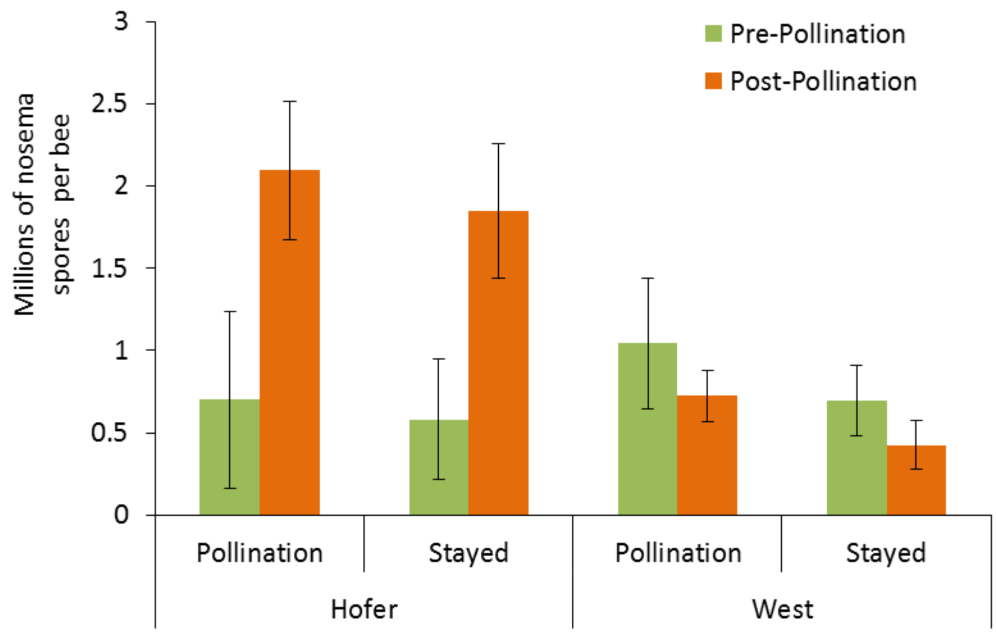

Figure 16. Nosema infection (an indicator of colony health, mean spore count ± SE) as determined pre-pollination (green) and post-pollination (orange) compared between the colonies that went to pollination, and those that stayed, for each of the yards Hofer and West.

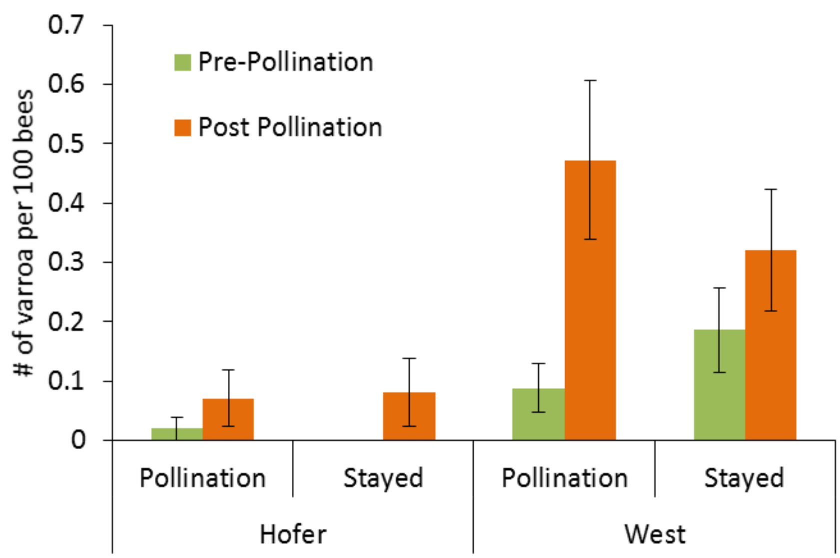

Figure 17. *Varroa* infection (an indicator of colony health, *Varroa* / 100 bees ± SE) as determined pre-pollination (green) and post-pollination (orange) compared between the colonies that went to pollination, and those that stayed, for each of the yards Hofer and West.

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 apiaries ('Hofer' and 'West').

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

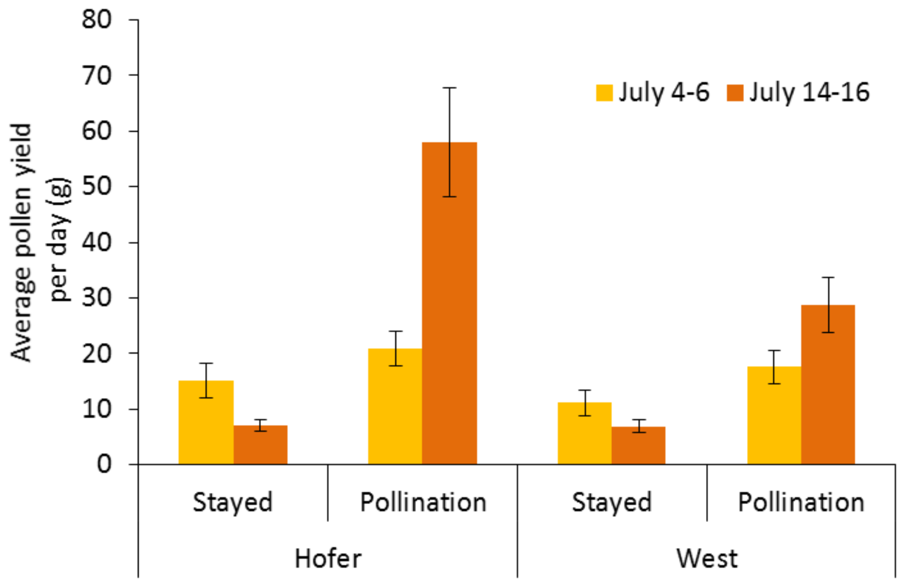

Figure 18. Average ± SE pollen yield per day (an indicator of colony productivity) as determined July 4-6 2015 (yellow) and July 14-16 2015 (orange) compared between the colonies that went to pollination, and those that stayed, for each of the yards Hofer and West.

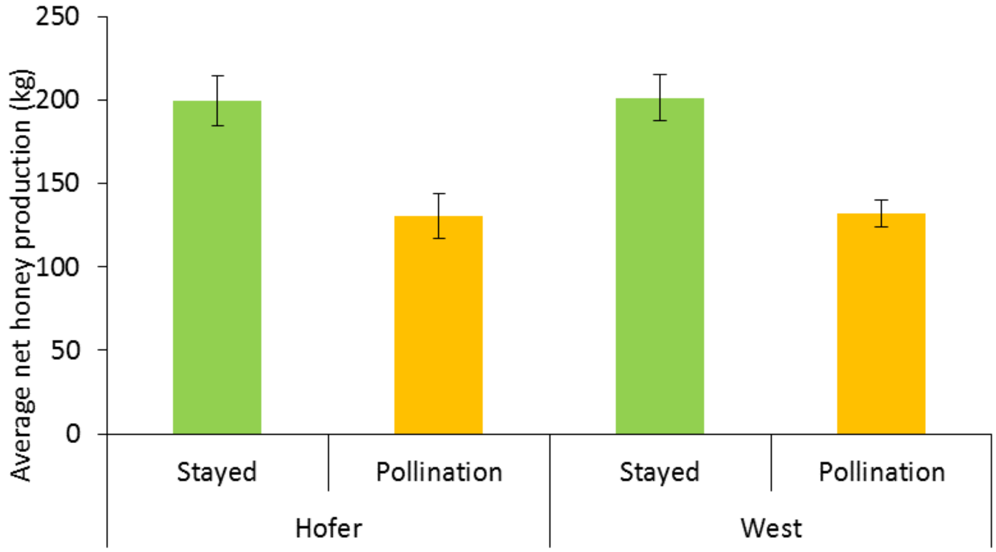

Figure 19. Average ± SE net honey production (an indicator of colony productivity) as compared between the colonies that went to pollination (yellow), and those that stayed (green), for each of the yards Hofer and West.

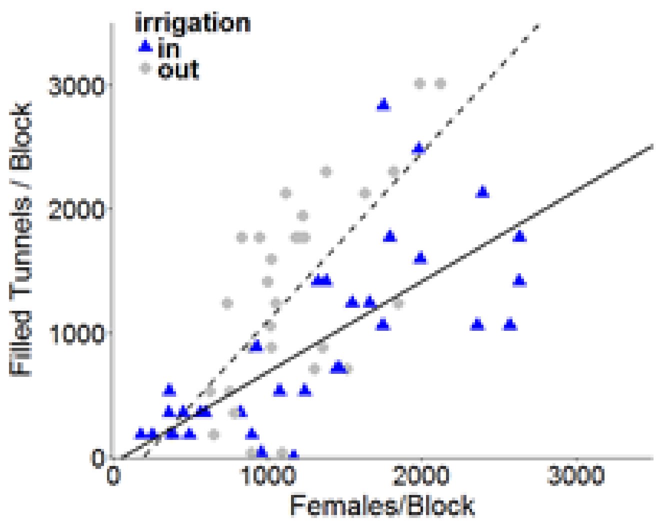

Figure 20. Leafcutter reproductive success (measured in terms of the number of tunnels filled with cocoons) among shelters located outside the irrigation drip-line in canola fields compared to outside the drip-line.

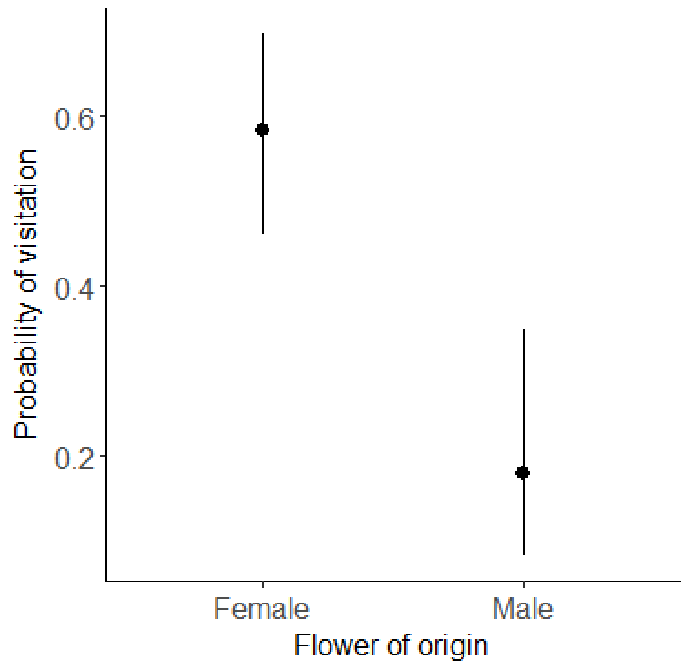

Figure 21. The effect of the type of flower foragers were originally on ('flower of origin', male or female) on the probability that leafcutter bees or honey bees would visit a female flower (n=294) (β = -2.02 ± 0.35; χ^2 = 38.93, *DF* = 1, *P* < 0.001). Points represent means and lines represent the 95% CI. Leafcutter bees also 'accepted' female inflorescences more than honey bees (β = 0.74 ± 0.34; χ^2 = 5.00, *DF* = 1, *P* = 0.03).

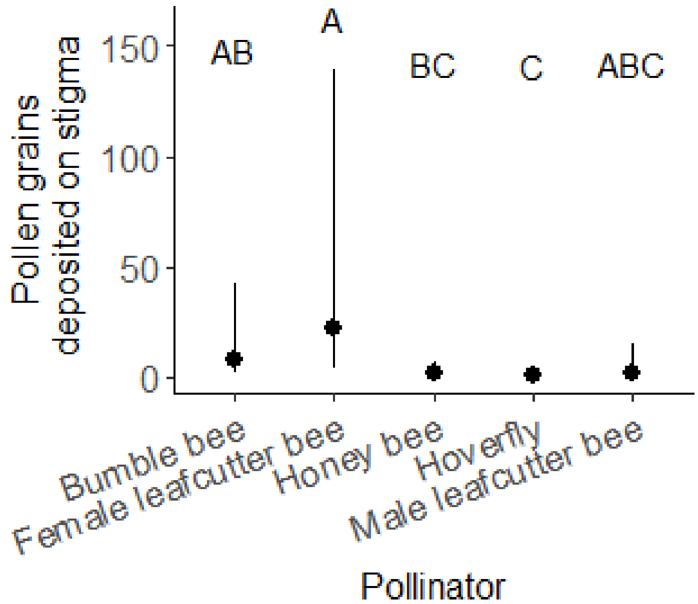

Figure 22. The type of pollinator (χ^2=27.30, df=4, p<0.001), the amount of pollen a pollinator had on its body (χ^2=4.17, df=1, p=0.04), the flower it was originally foraging on (χ^2=33.38, df=1, p<0.001), and the time it spent on a flower (χ^2=12.79, df=1, p<0.001) all significantly affected the amount of pollen deposited on a stigma. A post hoc Tukey test showed that female alfalfa leafcutter bees deposited significantly more pollen on stigmas than honey bees (p<0.001) and syrphid flies (p<0.001), but not more than male leafcutter bees (p=0.11). 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). 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.

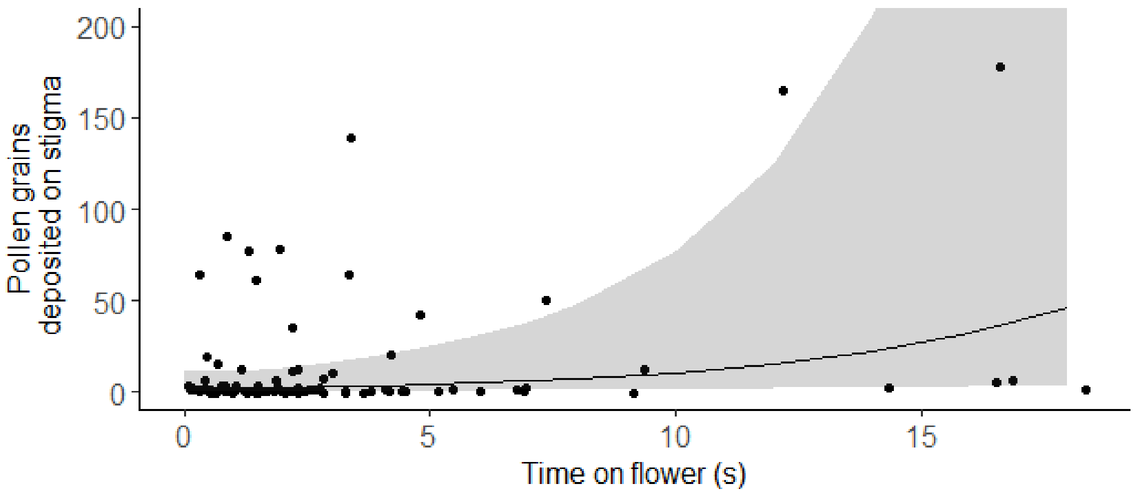

Figure 23. Relationship between time on flower and pollen grains deposited, with predicted trend line plotted against observed (non-adjusted) points. For trend line, variables pollinator type (5 levels), flower of origin (2 levels), and pollen on body were held constant. Shaded lines represent the 95% CI.

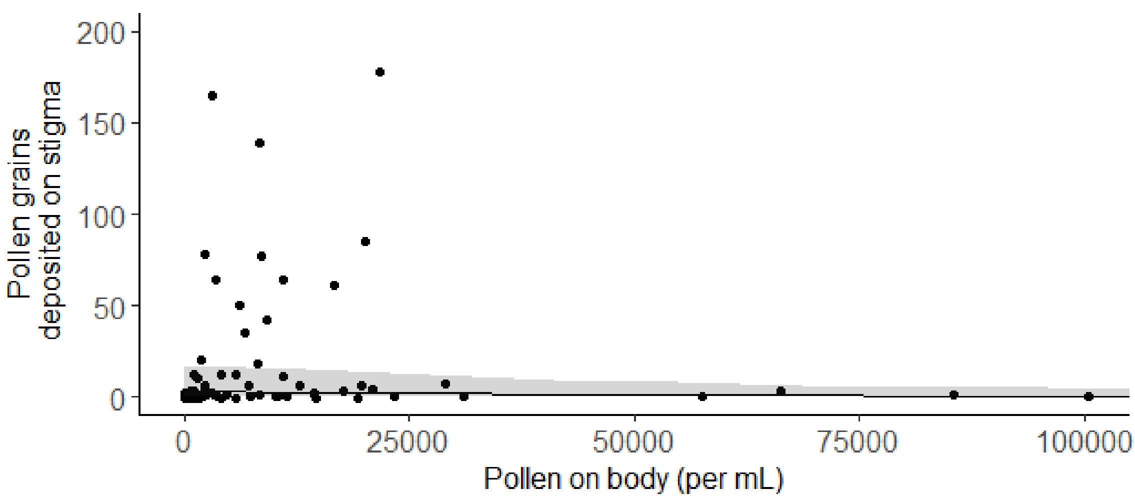

Figure 24. Relationship between average pollen grains on body (per mL) and pollen grains deposited on stigma, with predicted trend line plotted against observed (non-adjusted) points. For trend line, variables pollinator type (5 levels), flower of origin (2 levels), and time spent on flower were held constant. Shaded lines represent the 95% CI.

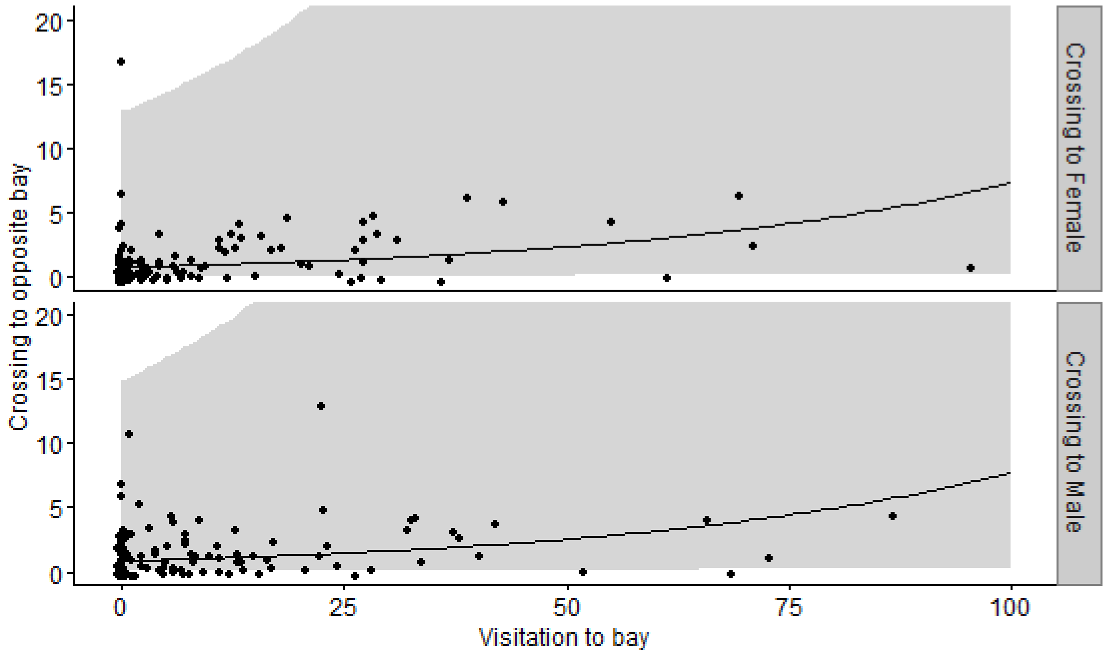

Figure 25. Relationship between honey bee visitation to the male or female bay (X) and crossing to the female (top) or male (bottom) bay (Y), with predicted trend line plotted against observed (non-adjusted) points (n=121). An increase of honey bee visitation to the male bay prompted crossing to the female bay (df=6, ΔAIC=1.49). Δ profit, temperature, and Julian day were held constant for trend line. Shaded area represents the 95% CI.

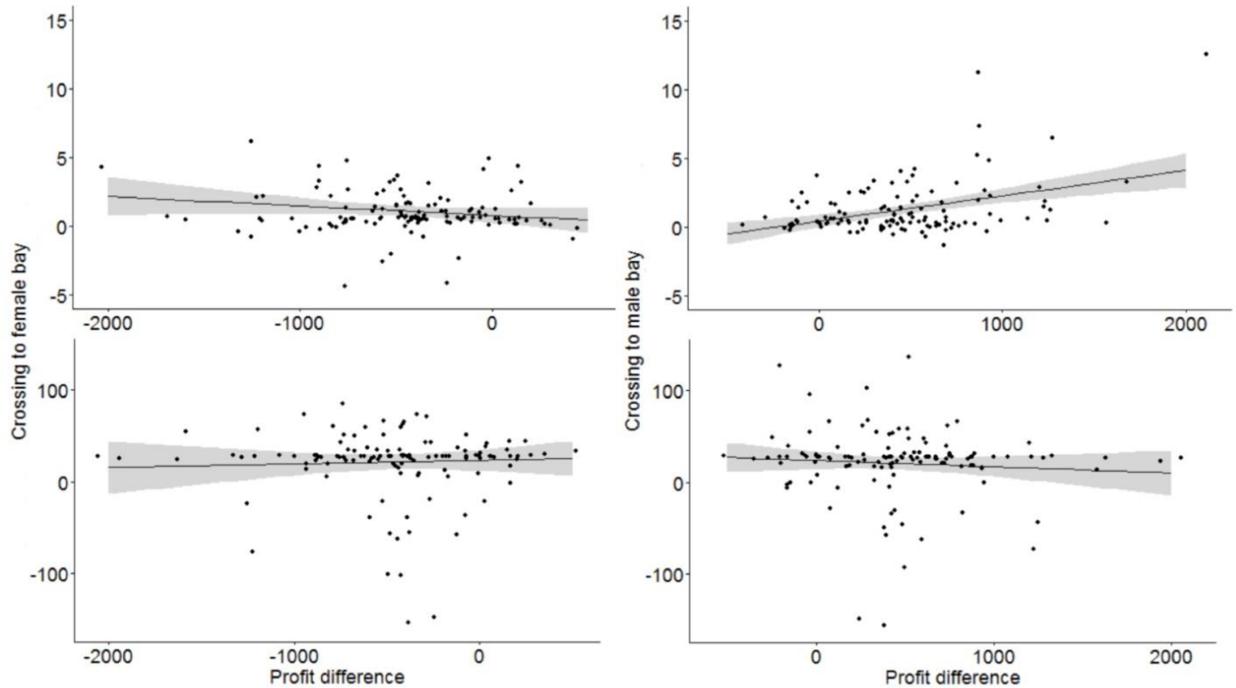

Figure 26. Partial regression plots of honey bees (top; n=121) and leafcutter bees (bottom; n=122) crossing between bays as influenced by Δ profit between the bays, adjusting for the effects of visitation, temperature, and Julian day. The figures on the left represent female profit minus male profit, while the figures on the right represent male profit minus female profit. Shaded area shows the 95% CI.

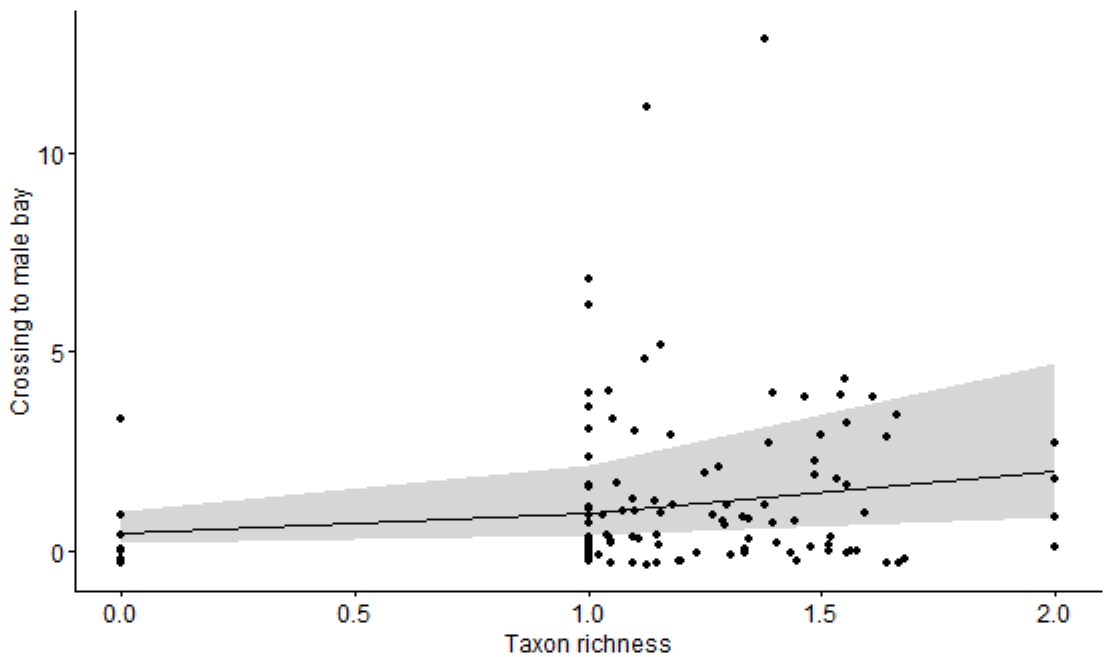

Figure 27. Frequency of honey bees crossing to the male bay as influenced by rarefied taxon richness (n=121), with predicted trend line plotted against observed (non-adjusted) points. For trend line, variables Δ profit, visitation, temperature, and Julian day were held constant. Shaded area represents the 95% CI.

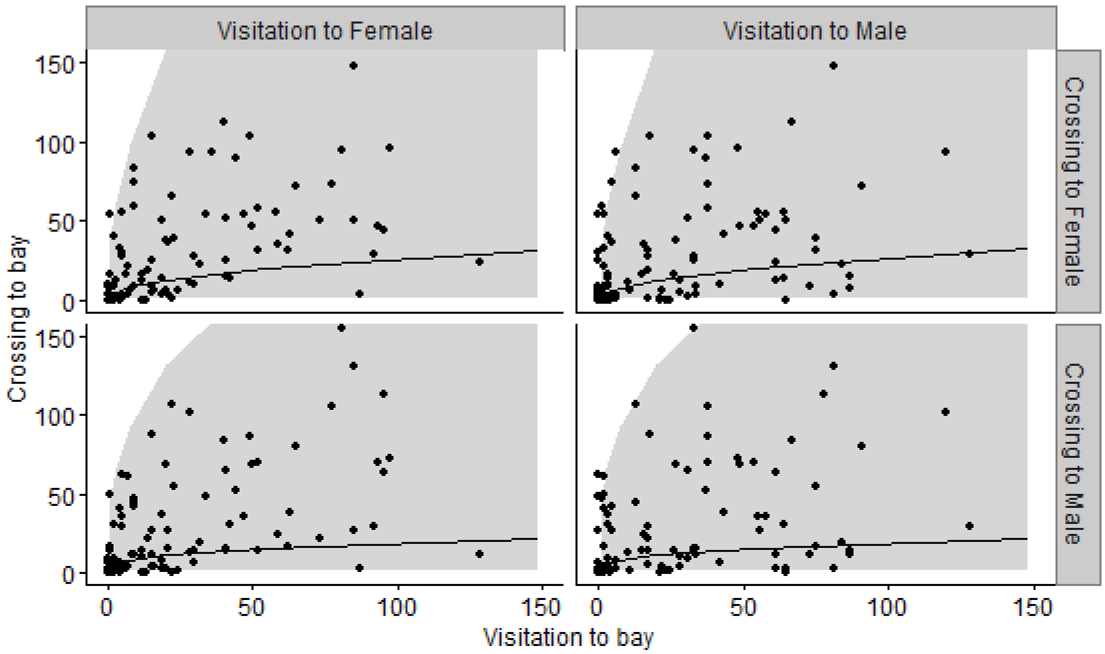

Figure 28. A partial residual plot of alfalfa leafcutter bees crossing to the female (top) and male (bottom) bays, as influenced by conspecific visitation to the female (left) and male (right) bay, with all other variables held constant. Shaded area represents the 95% CI.

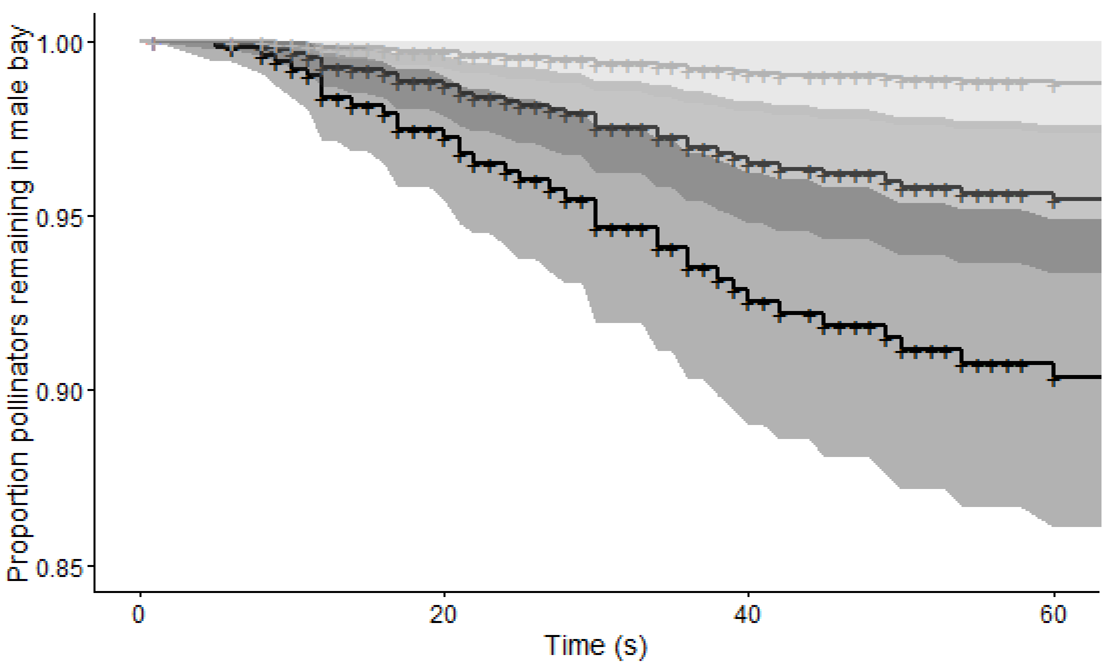

Figure 29. Propensity of leafcutter bees (black line), honey bee nectar foragers (grey line), and honey bee nectar foragers (light grey line) to remain in the male bay for 60 s (n=728). Slopes are Cox model coefficients (see Table 3.4). Shading around lines represents the 95% CI.

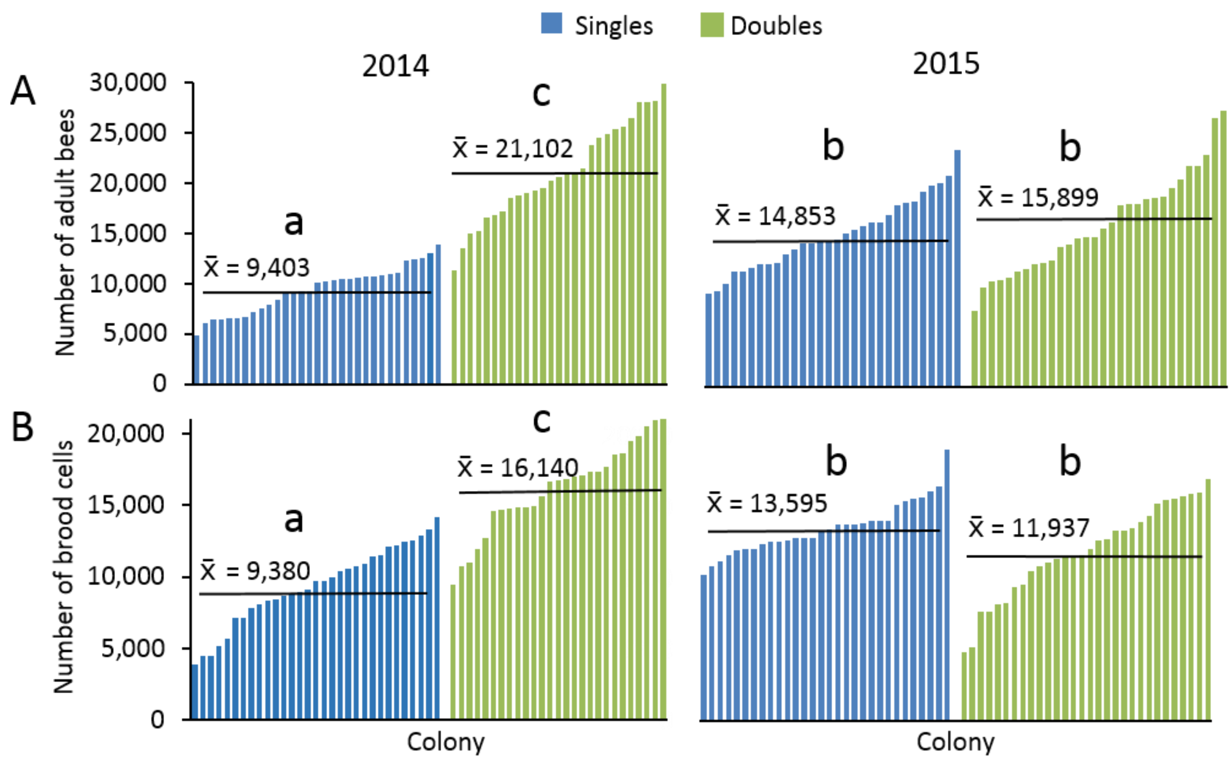

Figure 30. Number of adult bees (A) and number of brood cells (B) in the single brood-chamber (blue) and double brood-chamber (green) colonies in 2014 and 2015. Each bar represents an individual colony (sorted from smallest to largest), and the lines represent the population mean (?) of each grouping. Different letters above the groupings indicate significant differences (within and between years) according to Factorial ANOVAs with Tukey LS Means separation (*P* < 0.005).

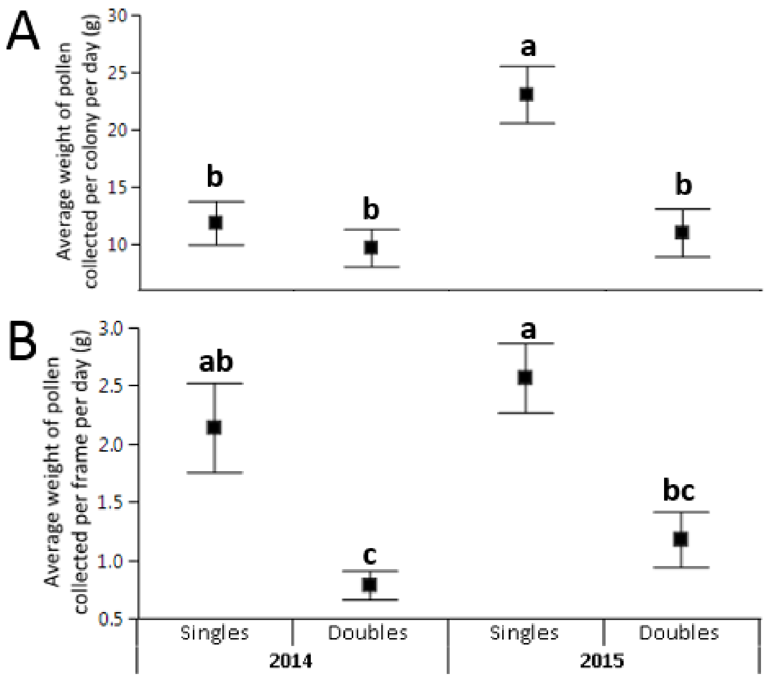

Figure 31. Average weight of pollen collected per day ± SE at the colony level (A) and at the frame level (B) in the singles and doubles in 2014 and 2015. Different letters above the groupings indicate significant differences (within and between years) according to Factorial ANOVAs with Tukey LS Means separation (*P* < 0.005).

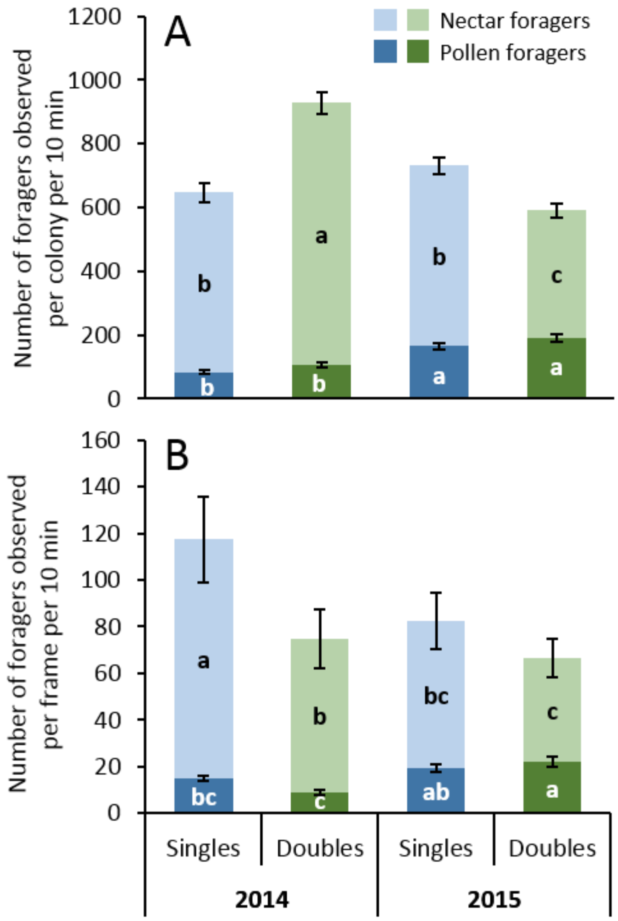

Figure 32. Mean ± SE number of nectar and pollen foragers observed in 10 min at the colony level (A) and the frame level (B) in the singles (blue) and doubles (green) in 2014 and 2015. Different letters above the groupings indicate significant differences (within and between years) according to factorial ANOVAs with Tukey LS Means separation (*P* < 0.005).

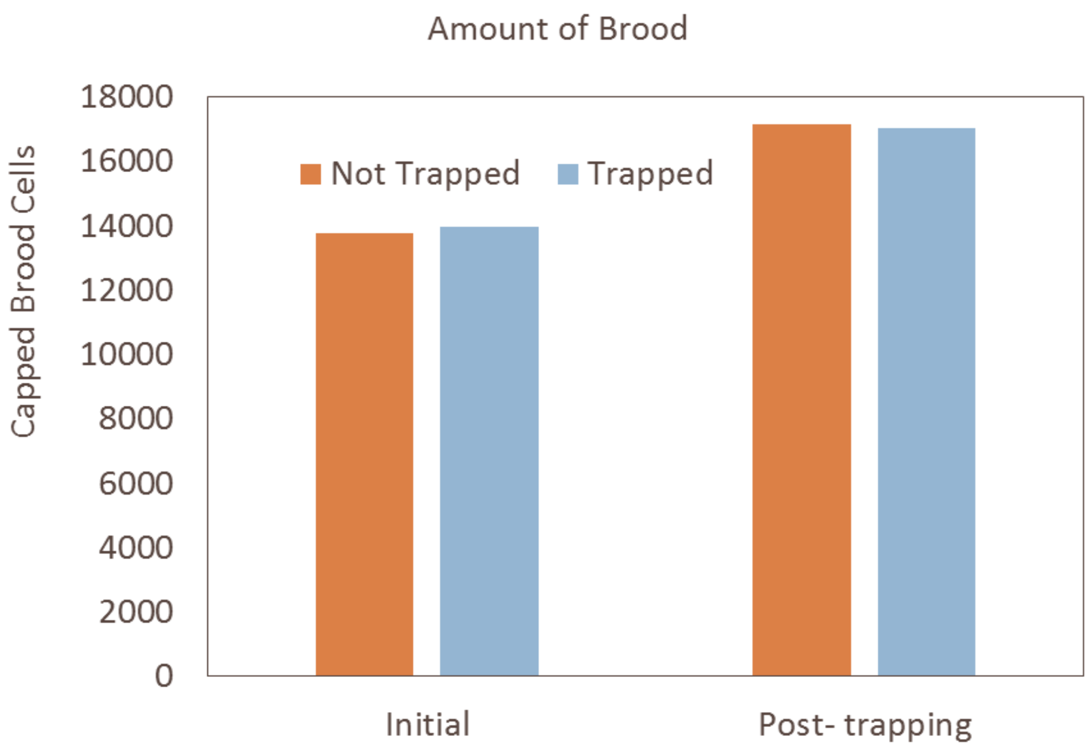

Figure 33. Amount of brood in colonies with (blue bard) and without (orange bars) pollen traps. There was no effect of collecting pollen on brood production ($T_{1,114}$=10.17, P= 0.87).

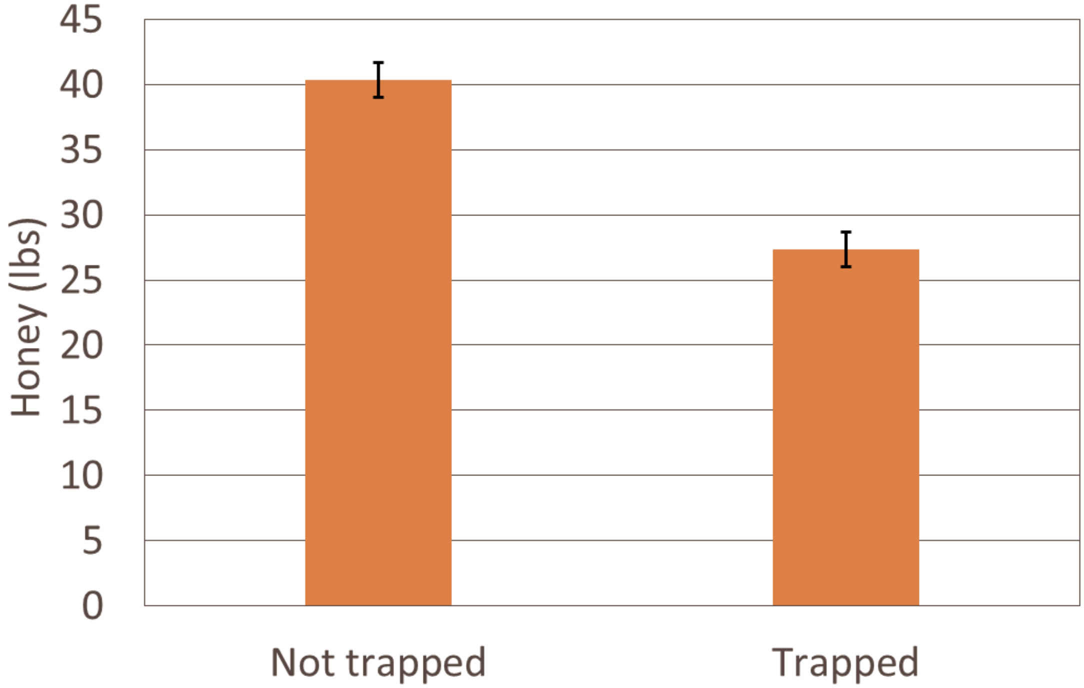

Figure 34. Honey production (mean per colony ± SE) in trapped (right) and untrapped (left) colonies. Trapped colonies had a pollen trap on their entrance while in canola pollination. Pollen traps decreased honey production ($T_{1,113}$=3.11, P= 0.002)

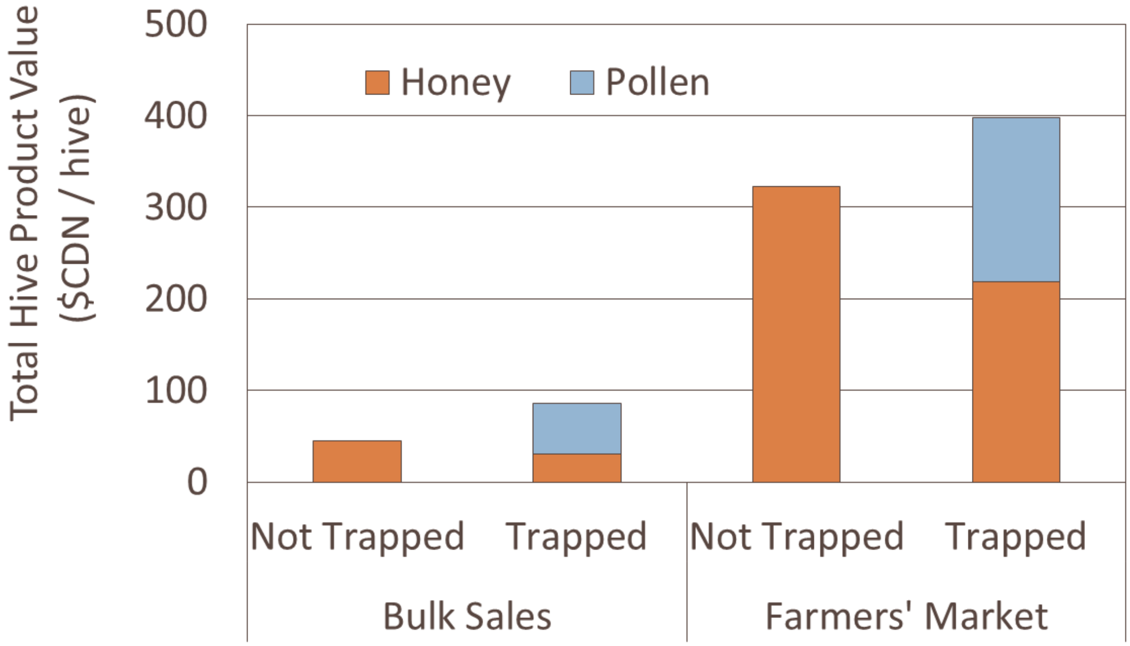

Figure 35. The total value of hive products was greater for colonies that had pollen traps (pollen + honey) compared to colonies with no trap (honey production only), regardless of whether the products were sold at current bulk or farmers' market prices.

Appendix 2 Photographs

Photo 1. Typical hybrid canola seed production field set-up with honey bee hives placed in one or two corners of the field, and leaf cutter bee shelters placed throughout the field. Bays of male-fertile ('male'_ and male-sterile ('female') plants are visible in the crop.

Photo 2. Dr. Melathopoulos hand pollinating flowers in a coarse-mesh tent (used to create the ‘wind only’ pollination treatment that excluded insect visitors but allowed air movement through the tent material.

Photo 3. Using a microcapillary tube to collect nectar from a canola flower.

Photo 4. Using forceps to collect a stigma from a canola flower to mount on a slide for subsequent pollen load analysis.

Photo 5. Taking photographs of each side of each frame in a honey bee hive to determine colony population.

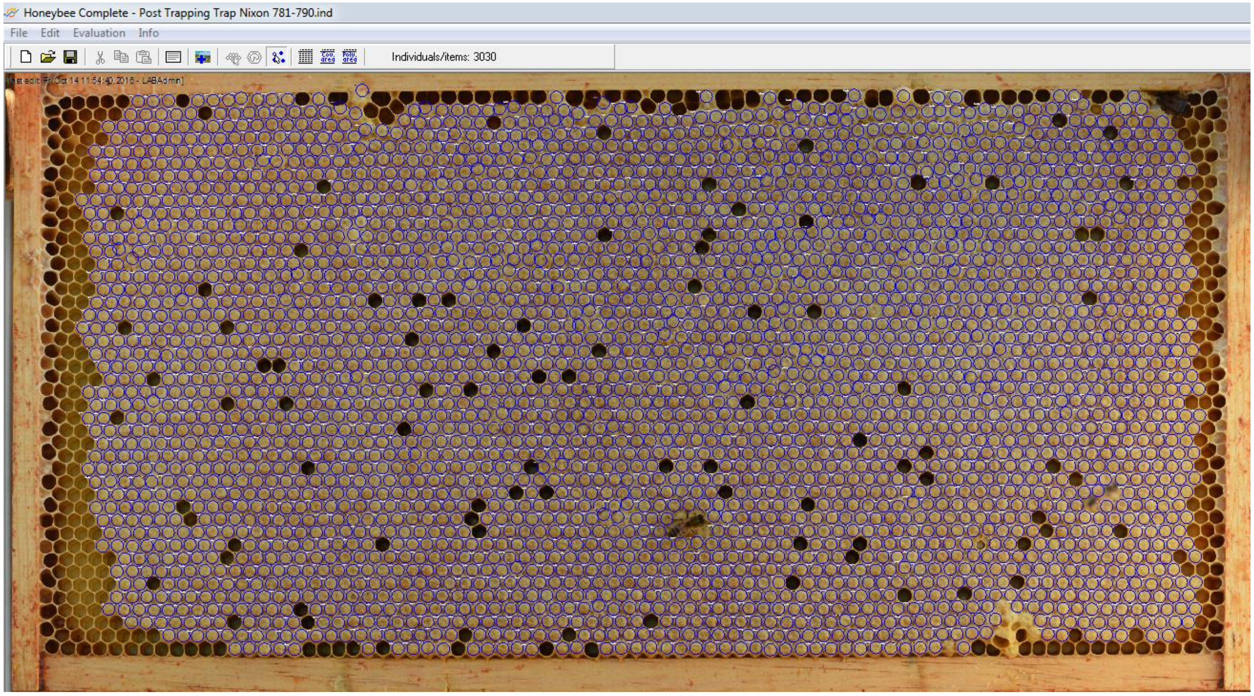

Photo 6. Honey bee brood comb analysed by HoneyBee Complete ™ software to count each brood cell. This method was used to determine total brood per colony before and after pollen trapping during pollination in canola seed production fields.

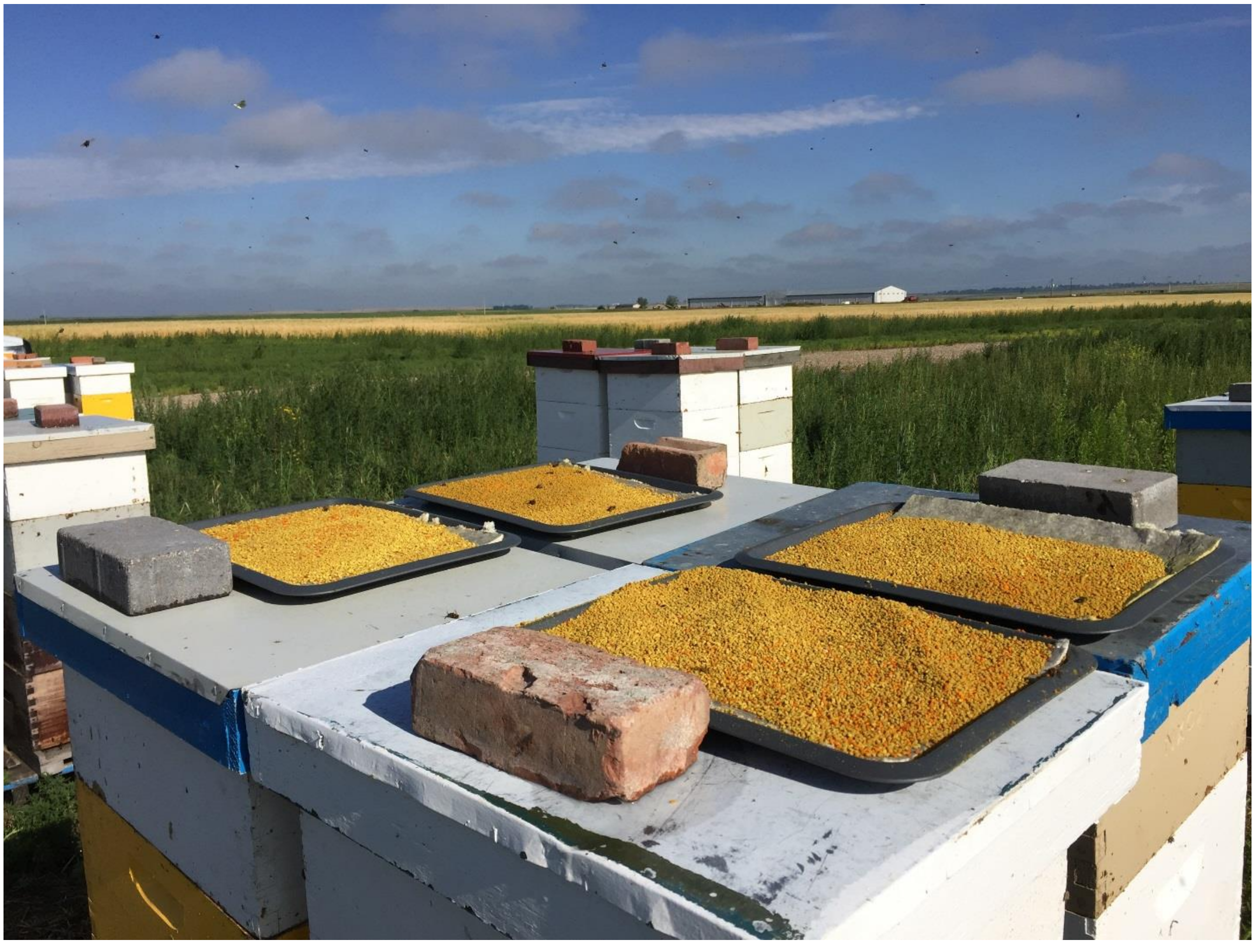

Photo 7. Pollen collected from four hives during canola pollination on one collection date.

Supplementary Video 1: Bee responses to interview bouquet

Response.mp4