Honey Bee Stock Performance Trial

Final Report to Alberta Beekeepers Commission

Applicant: Dr. Shelley Hoover

Co-applicants: Drs. Marta Guarna and Steve Pernal, AAFC; Patricia Wolf-Veiga, GPRC-NBDC

1. Key Objectives

- To assess the performance of commercially available honey bee stocks in Alberta, using both domestic and imported stocks.
- To correlate queen traits with colony-level performance.

2. Deliverables

(A) Summary of the relative performance of commercial honey bee stocks at two locations in Alberta: Beaverlodge and Lethbridge, for numerous colony-level performance measures including: disease loads, honey production, population build-up, and survival.

(B) Summary of quality parameters of commercially available queens (a) at shipment and (b) after one year in production; including: disease loads, sperm count, and viability.

(C) Correlation of queen quality parameters and colony-level performance measures.

3. Introduction

The apiculture industry in Alberta continues to grow alongside Canadian and global demand for hive products and pollination services. Alberta currently has over 315,000 honey bee colonies, representing over 40% of the honey bee colonies in Canada. Alberta honey bees and produced 40.5 million lbs of honey valued at $71 million in 2017 (Emunu 2019). In addition to honey production, many of these Alberta-based colonies provide pollination services to hybrid canola seed production fields in Southern Alberta, or to berry and fruit tree pollination in British Columbia.

Each honey bee colony is headed by a single female queen bee. Early in life, each new queen takes several mating flights over a few days, then stores and uses the spermatozoa (sperm) acquired during those mating flights for the remainder of her life. A queen is the mother of all the workers in the colony, therefore the quality of the queen and her mating plays a pivotal role in determining the success and productivity of the colony. Canadian beekeepers, including those in Alberta, frequently cite “poor queen quality” as a top-four contributing factor to colony loss (CAPA 2018); poor queen quality can also contribute to sub-lethal losses in colony productivity (Amiri et al. 2017). Beekeepers replace queens either proactively, introducing a new young queen to a colony with an older queen, or as required to replace queens that they determine are ‘failing’ according to criteria that they determine.

Beekeepers have two options for obtaining new queens: they can either rear their own queens, or purchase queens from commercial queen breeders. Canadian beekeepers import over 250,000 queens annually worth $7.7 million CDN (AAFC 2016). Domestic queen production exists locally throughout Canada but is limited as queen rearing and mating requires reliable summer conditions, and queens are generally replaced in the months of April through June, with a limited number replaced later in the season, as needed. Therefore, most queens imported to Alberta are bred in warm parts of the USA (Hawaii, California) or in contra-season countries in the southern hemisphere (New Zealand, Australia, Chile), and are installed in hives in Canada in spring. As a result, these queens were selectively bred in a

very different environment than Alberta, where their colonies will be subjected to harsh winter conditions.

While beekeepers appreciate the contribution of having quality queens to colony health, they currently have insufficient information to support their management decisions. For example, there is little empirical information available to Alberta beekeepers on the performance of different queen stocks in Alberta or Canada, particularly in comparison to commercially available domestic stocks which are available later in the spring/early summer. In addition, there are no standard criteria against which beekeepers can evaluate their queens (either at shipment or queen performance in colonies).

The goal of this project was to examine the variability that exists among imported and domestic stocks in initial queen quality, relate these measurements to colony productivity as measured in the field, and provide data to support management decisions as they pertain to queen quality. Queens were evaluated by measuring morphological characteristics, sperm counts, and sperm viability of new queens. The quality of imported and domestic stocks under Alberta conditions was evaluated by measuring colony level parameters including brood solidness, hygienic and defensive behaviour, disease tolerance, honey production, adult bee and capped brood population, and cluster size and colony weight before and after winter. These analyses provide valuable data which enable beekeepers to communicate with queen breeders regarding the performance of their stocks under Alberta conditions. Additionally, this project provides data on the productivity of mid-season splits over their first and second summer, and field data to determine the importance of measurable queen quality parameters in predicting colony-level performance.

4. Methods

4.1 Colony Management

Stock assessment apiaries were established in May of 2017 at three sites: one in Beaverlodge managed by the AAFC bee team, and two near Lethbridge managed by the AF bee team (Lethbridge AF), and by a participating beekeeper (Lethbridge BK). At each site, 32 (Lethbridge BK), 37 (Lethbridge AF), or 60 (Beaverlodge) newly-established small queenless splits were prepared, each with similar comb composition and bee populations. Colonies were maintained as single broodnest colonies and managed according to normal beekeeping practices for the region. Supplemental feeding was provided with pollen patties and sucrose syrup at establishment and in the autumn. The Lethbridge colonies managed by a participating beekeeper were moved within 50 km of Lethbridge as necessary to pollinate hybrid seed canola. In fall 2017, Lethbridge AF and Beaverlodge colonies were treated for *Nosema* with Fumagilin-B. Colonies at Lethbridge AF and Beaverlodge were also treated for Varroa with Apivar® following label instructions in fall 2017. Colonies at Lethbridge BK were not treated for *Nosema* in fall 2017 and were treated for Varroa with oxalic acid sublimation as they were managed by a participating beekeeper. All colonies were overwintered indoors.

Measurements evaluating the performance of the stocks commenced once there was sealed brood from the new queen in the case of brood solidness measurements (approximately 19 (Beaverlodge) or 20 (Lethbridge) days after queen release), and after the population of bees had turned over from the original splits in the case of all other measurements (greater than 45 days). Colonies were removed from the experiment of they died (no adults bees or queen), or if the original queen was lost or replaced.

4.2 Queen Shipments

Queens were shipped from British Columbia (BC), Hawaii (HI), and New Zealand (NZ) from 16 May to June 2, 2017. Each shipment from NZ and HI to Beaverlodge and Lethbridge was accompanied by a temperature data logger inside the shipping box to record the temperatures experienced by the queens during transport. Queens were marked a unique color for each stock and were kept in a queen bank until they could be introduced to colonies (up to 17 days). 15 queens of each stock were used for laboratory testing at the National Bee Diagnostics Centre (NBDC) in Beaverlodge upon arrival (or the subsequent morning if the shipment arrived too late in the day to permit evaluations that day). Queens were introduced to splits at all sites on June 1-2, 2017. During the following week the queens were released, the splits checked for eggs, and adult populations were equalized to counteract drift.

4.3 Queen Measurements

Morphological measurements

Each queen to be analyzed by the NBDC was temporarily immobilized by placing it in a freezer and then its fresh weight was recorded to the nearest 0.1mg on an analytical balance (CPA 224S, Sartorius, Goettingen, Germany). The thorax length, thorax width and head width were measured to within 0.1mm using digital calipers, and then each queen was sacrificed by decapitation. The queen's abdomen was dissected and spermatheca removed. The diameter of the spermatheca, without tracheal net, was measured using a calibrated ocular micrometer in a dissecting microscope and its spherical volume calculated. To analyze ovaries, the abdomen of each queen was dissected while being viewed through a stereomicroscope (Model SMZ1000, Nikon). Right ovaries (defined as the ovary on the right side of the queen pinned ventral side down) were carefully removed with forceps and transferred immediately to a slide. Ovary wet weight was measured to the nearest 0.1mg. The number of ovarioles per ovary was immediately determined by visual counting under stereo microscopy using a fine needle to isolate individual ovarioles.

Sperm counts and viability

Immediately after dissection, each spermatheca was ruptured in 0.5 ml of Buffer D (Collins and Donoghue 1999) in a glass vial. The vials had 15ul of SYBR 14 and 8ul of propidium iodide added and were incubated at room temperature for 15 minutes (adapted from LIVE/DEAD Sperm Viability Kit, Molecular Probes, Eugene, OR, USA). The stained sperm was immediately loaded onto a Thoma counting chamber. A fluorescence microscope (Fluoview FV10C-W3, Olympus, Tokyo, Japan) was used to visualize both live and dead sperm. Live sperm fluoresce at green wavelengths (EM 520-570 nm) while dead sperm fluoresce at red wavelengths (EM 650 nm). Live and dead sperm were counted and the percent of viable sperm present from each spermatheca was calculated and the average sperm count was corrected for dilution to calculate number of stored sperm.

4.4 *Varroa* and *Nosema* Sampling

To monitor disease pressure and determine any differences in disease tolerance amongst stocks, colonies were repeatedly sampled for *Nosema* spp. (approximately 100 bees collected from honey frames into a plastic centrifuge tube which was subsequently placed on dry ice) and *Varroa* (approximately 300 bees collected from brood nest into a 120 mL sample jar filled with ethanol). In 2017, *Nosema* and *Varroa* samples were taken in mid-June to establish the disease load of the colony prior to the population turning over, and then in mid-August, and mid-October prior to wintering. In 2018, *Nosema* and *Varroa* samples were taken April 24 after wintering, and mid-July prior to the end of the trial.

Other Sampling

In mid-July of 2017, approximately 100 bees were sampled into ethanol for paternity testing. In Late-July of 2018, 25-30 adult drones were sampled from each of the remaining colonies and placed into a plastic centrifuge tube on dry ice to facilitate identifying queen alleles in paternity analyses by the NBDC. Ten purple-eyed drone pupae were also sampled into plastic centrifuge tubes and placed on dry ice for genotyping by the Zayed lab, Department of Biology, York University.

Upon completion of the trial in August 2018, 13 queens from the Beaverlodge site were located, positively identified, and placed onto ice to be analyzed to determine the amount and viability of sperm remaining in the queens' spermathecae. High mortality is often observed in shipments of older queens (unpublished data), therefore to preserve the queens from the Lethbridge sites, and their spermathecal contents, all queens from Lethbridge were frozen immediately after collection, and shipped on dry ice to the NBDC. While this method preserved the spermathecae for accurate sperm counts, it precluded sperm viability analyses. At the Lethbridge sites, 20 queens were located and positively identified, and shipped to the NBDC for sperm count analyses.

Due to the recent construction at the NBDC, paternity testing of the queen progeny is partially completed, and should be finalised in 2019.

4.5 Brood Pattern and Brood Diseases

To assess the performance of the queens, the quality of a queen's brood pattern was measured by evaluating the solidness of the sealed brood in each colony. A rhomboid pattern (Figure 1), cut to outline 100 cells (10x10), was overlaid on a patch of sealed brood and the number of cells not containing sealed brood ("misses") was counted. Four patches were evaluated per colony, on the areas of greatest sealed brood continuity, on at least two separate frames. The solidness of the brood pattern was expressed the number of misses subtracted from 100. The brood solidness measurements began once capped brood was present after queen introductions, and were repeated approximately every two weeks for a total of 3-6 measurements throughout June-August in both study years. The 3-6 individual measurements were averaged and expressed as overall brood pattern quality ("solidness") within each year. During each brood pattern measurement, the colony was also inspected visually for brood diseases (American foulbrood, European foulbrood, chalkbrood, or sacbrood) and the presence/absence of adult bees with deformed wings. The number of affected cells across all brood frames was recorded for each brood disease.

Figure 1. *Rhomboid shape that holds 100 cells overlaid on a patch of solid brood - four misses are counted here which translates to a brood solidness measurement of 96.*

4.6 Hygienic Behaviour and Defensive Behaviour Assays

To assess the hygienic behaviour of each stock, one frame containing a solid patch of capped brood with purple-eyed pupae per colony was selected. Two PVC tubes (2-inch diameter) were pressed into the brood and filled with approximately 300 mL of liquid nitrogen to freeze the brood. The tubes were removed once the brood was thawed and the number of empty cells ("misses") in each circle were recorded. The frame was then returned to the broodnest and checked 24 hours later (+/- 5 min), when the number of cells that remained capped or partially removed was recorded. The assay was repeated one week later. The hygienic behaviour score was then calculated as the percentage of dead brood completely removed by the colony within 24 hours, averaged across the two assays. Two hygienic behaviour assays were performed after the honey flow in Mid-August 2017, and an additional two in June of 2018 (Lethbridge sites: June 6-7, June 12-13; Beaverlodge: June 18-19, June 28-29).

The defensive behaviour assay was performed after the completion of the hygienic behaviour assay on the same dates as described above. A black suede leather patch (7.6 cm x 7.6 cm) suspended from a pole on a white string was gradually waved 5 cm above the brood chamber for 2 minutes, after which the number of stings on the patch was recorded. Colonies on the same pallet were tested simultaneously.

4.7 Colony Strength Measurements

Honey Production

The weight of honey produced by each colony was measured in 2017 and 2018. For Lethbridge AF and Beaverlodge, honey supers were weighed prior to being placed on the colony to establish a tare weight, which was subtracted from the full weight of each super. The Lethbridge BK colonies were managed by a participating beekeeper, and as such an average empty super weight of 8.9 kg was used instead of a tare weight. Honey supers were removed throughout the honey flow and their full weights recorded. The honey production for each colony was summed. As the project ended at the end of July, the 2018 honey production represents the honey production to the end of the experiment at each site: July 24 for Lethbridge AF, July 25 for Beaverlodge, and August 13 for Lethbridge BK as it was managed by a participating beekeeper.

Adult Bee and Sealed Brood Population

In early August of 2017, and July of 2018, all the frames containing bees in each colony were removed and photographed in the early morning, before bee flight. To determine the population of adult bees, the photo ladder method described by Ovinge and Hoover (2018) was used, where the adult bee photos were viewed on a computer, and compared to a wall of representative photos to estimate how many bees were in each photo. For the 2017 photos, a 10% subset of photos were manually counted to validate the estimation, which showed that the estimation was 97% correlated (R^2) with the actual count of bees and within 50 bees of the actual count 83% of the time. For the 2018 photos, a 7% subset of photos were manually counted, which showed that the estimation was 94% correlated (R^2) with the actual count of bees and within 50 bees of the actual count 82% of the time.

After the pictures of adult bees had been taken, all the frames containing sealed brood were photographed after the bees were carefully brushed off. The sealed brood photos were later analysed with the software HoneyBeeComplete 5.4 (WSC Scientific, Heidelberg, Germany) to determine the number of sealed brood cells per frame side. The total number of sealed brood cells for each side of each frame containing worker brood was summed to determine the number of capped brood cells per colony.

Cluster Size and Colony Weight

The size of the bee cluster and the weight of the colony were evaluated prior to winter in October 2017, and after winter in late April 2018. The size of the cluster was measured by viewing the top and bottom of the box and visually estimating the number of inter-frame spaces filled with bees (Figure 2). The total number of inter-frame spaces of the top and the bottom was averaged and expressed as the cluster score. The entire colony including lids, entrances, and bottom boards was placed on a scale and weighed. In April 2018, the Lethbridge BK colonies had been barrel-fed for one week before they could be weighed, so weights were not taken.

Figure 2. *View of the cluster from the top of the box. By counting the number of inter-frame spaces, this view would have a cluster score of 7.5 frames.*

4.8 Statistical Analyses

Separate factorial ANOVAs were used to determine the main effects of site and stock as well as their interaction on all colony level measurements with Tukey's HSD post hoc analyses (α=0.05) where appropriate. One way ANOVAs followed by Tukey's HSD post hoc analyses were used to compare queen morphological measurements among the three stocks. Correlations were performed using the pairwise method in the multivariate platform within JMP. All statistical analyses were performed with JMP Version 14 (SAS Institute, Cary NC). For all box plots, the center line represents the median, the box the 25/75th percentile, the bars the 5th/95th percentile, and the dots are outliers.

5. Results

5.1 Queen Shipments

Overall, there were no high temperature events that would be of concern during the queen shipments. The New Zealand queens shipped to Beaverlodge were kept at an average temperature of 18.5°C during transport (min 4.9°C, max 26.4°C), and the Lethbridge ones were similar, averaging 17.1°C (min 4.4°C, max 30.4°C). The minimum temperature was due to the queens being placed in a cooler at the airport to prepare them for fluctuations during takeoff; they were cooled from ~15^{o}C to 4-5^{o}C, where they remained for 8-10 hours, with the exception of a brief warm (15^{o}C) spike. After that point, both shipments were never below 8°C. The maximum temperatures show up at the end of the flight, and are likely due to fluctuations during landing and transport thereafter. The prolonged low temperatures experienced pre-flight may be of concern, as low temperatures (4^{o}C) can result in reductions in sperm viability after even relatively short exposures (1 hour) (Pettis et al. 2016). However, the actual temperatures experienced by the queens may be less extreme as the attendants would cluster and warm the queen, whereas they would not do so for a data logger in the box.

The Hawaiian queens shipped to Lethbridge were kept at an average temperature of 23.6°C during transport (min 16°C, max 29.5°C). After the queens were removed, the data logger was left in the truck for three days, where it recorded daily fluctuations of 10°C to 37°C in early June, demonstrating that queens should not be left in trucks due to fluctuations in temperatures that can occur. No temperature data were available for the British Columbia shipments, due to logistical difficulties.

5.2 Queen Analyses

Measures of queen variability are listed in Table 1. We detected differences in overall queen size and shape among stocks. NZ queens weighed significantly less than the BC and HI stocks, and the HI stock had significantly narrower heads than the NZ and BC stocks. The NZ stock had longer thoraces than did the HI queens, and the BC queens had intermediate thoracic lengths but the widest thoraces.

Ovarioles are the basic units of egg production of which insect ovaries are composed. Typically, female insects will have two ovaries, each composed of numerous constituent ovarioles. Each ovariole contains a germarium (mass of undifferentiated cells that give rise to egg and nurse cells) and set of follicles (containing individual egg cells). Ovariole count is therefore related to reproductive potential. We found no significant differences in ovariole count amongst the stocks we sampled, but New Zealand queens' ovaries weighed less than the other stocks when we received the shipments (Table 1). Queens are physiologically capable of partially resorbing their ovaries. For example, in the week prior to swarming, queens are fed less, and their abdomens "diminish" (Winston 1987 and references therein). New Zealand queens are often banked longer prior to shipping than BC or HI queens, and therefore their ovaries may have been smaller when we received the shipment for this reason.

The spermathecae, the semen storage organs of queen bees, also varied among the stocks with the HI queens having the smallest diameter and volume of these organs. Importantly however, this did not result in differences in sperm count (the amount of spermatozoa stored in the spermathecae) among the different stocks (Table 2). The maximum total sperm count observed was 10.4 million in a BC queen, whereas the minimum was observed in a NZ queen with only 3.1 million sperm. Overall, the new queens purchased for the experiment had an average of 5.9 million stored sperm per queen. This is similar to or greater than reported values of sperm count in production queens (e.g. $\bar{\chi} = 3.99$, Delaney et al 2011; $\bar{\chi} = 4.37$, Tarpy et al. 2012; $\bar{\chi} = 5.07 - 6.74$, Lee et al. 2019). It is important that note that the drone production in mating areas and the mating and shipping conditions queens experience change over time, and the values of sperm count and viability will not be constant for any stock, even within a single season.

There were, however, differences in the viability of the stored spermatozoa among stocks, which could be due to lower drone quality, or to a decrease of sperm viability in banking, queen transit, exposure to pesticides, or other factors. Despite the low temperatures experienced prior to shipping, the NZ stock had the highest sperm viability; the BC stock had intermediate viability, and the HI queens had stored sperm with significantly less viability than the NZ queens (Table 2). The NZ queens were mated in the southern hemisphere late in summer, or early autumn, whereas the BC queens were mated early in the northern spring, and it is possible the seasonal availability of mature drones affected sperm quantity or viability. Queen failure due to low sperm viability has been documented between the ranges of approximately 35-78%, and 80% viability has been proposed as an acceptable level (Pettis et al. 2016). The minimum sperm viability observed in this study was for a NZ queen with only 69% sperm viability, however the highest viability was also observed in a NZ queen at 98%. Seven percent of the NZ queens, 20% of the BC queens, and 27% of the HI queens in these shipments had less than 80% sperm viability. Importantly, however, there were also no differences among the stocks in the number of viable sperm the queens had stored ($F_{2,41}$=1.43, P=0.25).

We found no relationship between any measure of queen size (body weight, head width, spermatheca volume, thorax width, thorax length, Table 1) and number or viability of stored sperm (P > 0.05), however the number of viable stored sperm across all sampled queens in the initial shipment did increase with spermathecal diameter and volume (P = 0.046, r = 0.30).

Table 1. Mean (± SE) queen measurements for each stock and ANOVA results. 15 queens of each stock were destructively sampled upon arrival of the queen shipment. All measurements were performed on 15 queens with the exception of the two ovary measurements were performed on 10 queens. Within each row, means followed by different letters are significantly different at α = 0.05 (Tukey's HSD).

	BC	HI	NZ	ANOVA
weight (mg)	209.47 ± 3.68 **a**	203.4 ± 3.51 **a**	183.31 ± 3.51 **b**	$F_{2,42}$=14.70, P<0.0001
head width (mm)	3.8 ± 0.02 **a**	3.65 ± 0.03 **b**	3.79 ± 0.05 **a**	$F_{2,42}$=6.03, P=0.0050
thorax length (mm)	4.88 ± 0.05 **ab**	4.73 ± 0.04 **b**	4.96 ± 0.07 **a**	$F_{2,42}$=4.99, P=0.0113
thorax width (mm)	4.72 ± 0.02 **a**	4.47 ± 0.04 **b**	4.59 ± 0.04 **b**	$F_{2,42}$=12.28, P<0.0001
spermathecal diameter (mm)	1.14 ± 0.01 **a**	1.08 ± 0.01 **b**	1.17 ± 0.01 **a**	$F_{2,42}$=16.16, P<0.0001
spermathecal volume (mm^3)	0.79 ± 0.02 **a**	0.67 ± 0.02 **b**	0.84 ± 0.03 **a**	$F_{2,42}$=15.56, P<0.0001
ovariole count per ovary	157.2 ± 5.26 **a**	145.7 ± 5.83 **a**	148.2 ± 5.65 **a**	$F_{2,27}$=1.17, P=0.3247
ovary wet weight (mg)	21.78 ± 0.74 **a**	22.91 ± 0.97 **a**	16.24 ± 1.4 **b**	$F_{2,27}$=11.13, P=0.0003

Table 2. Mean (± SE) queen measurements for each stock. Fifteen queens of each stock were destructively sampled upon arrival of the queen shipment. Within each column, means are significantly different when followed by different letters. "Poorly mated" is defined as the percentage of queens with less than 3 million sperm (Lee et al. 2019) and low viability as the percentage of queens with less than 80% sperm viability (Pettis et al. 2016).

	BC	HI	NZ	ANOVA
Sperm Count (millions)	6.48 ± 0.44 **a**	5.93 ± 0.27 **a**	5.3 ± 0.37 **a**	$F_{2,41}$=2.6, P=0.085
% Poorly mated	0	0	0	
Sperm Viability (%)	84.54 ± 1.66 **ab**	83.3 ± 1.58 **b**	89.22 ± 1.8 **a**	$F_{2,41}$=3.49, P=0.040
% Low Viability	21%	27%	7%	

Two Beaverlodge queens were sampled in May 2018 as they were failing after winter 2017/18. The sperm counts were 6.6 and 8.2 million, with sperm viabilities of 89.3, and 90.2%, suggesting that the queens were "failing" for reasons other than sperm count or sperm viability. All other queens which could be found and verified as original were sampled at the end of the trial in July 2018 (Table 3). End-of-life queen sperm count data was available for a subset of the colonies in which we could locate and positively identify the original queens. Counts did not vary by stock ($F_{2,16}$=0.44, P=0.52), but did vary by site ($F_{2,16}$=11.9, P=0.0033) with the queens from Beaverlodge having lower sperm counts. It is possible that this is related to the higher populations of the Beaverlodge colonies, however these results should be considered preliminary due to the low number of queens available at the end of the experiment. It is interesting to note, however, that at Lethbridge, the mean sperm counts of the queens remained high after nearly two full production seasons.

Table 3. End-of-life sperm viability and sperm count for individual queens from each stock (BC=British Columbia, HI=Hawaii, NZ=New Zealand) sampled at the end of the trial in July 2018. Individual data is shown for queens to show variation, the mean for each stock is listed in bold at the bottom. The Lethbridge sites have been combined. No sperm viability was available for the Lethbridge queens due to the risks associated with shipping older queens.

Queen Source:	**BC**		**HI**		**NZ**	
	Viability (%)	sperm count (millions)	Viability (%)	sperm count (millions)	Viability (%)	sperm count (millions)
Beaverlodge	92.4	6.28	75.5	3.53	66.7	1.39
	98.2	4.56	78.4	5.56	48.0	1.14
			66.9	0.74	91.7	2.93
			76.2	0.66	91.9	3.17
			94.2	4.16	82.9	0.81
			88.5	2.40		
mean	**95.3**	**5.4**	**80.0**	**2.8**	**76.2**	**1.9**
Lethbridge		5.00		3.25		5.00
		4.50		4.25		4.50
		4.25		6.13		4.25
		6.88				6.88
		6.38				6.38
mean		**5.40**		**4.54**		**6.05**

The 2018 measures of brood solidness were negatively correlated with both the sperm count and sperm viability of the queens that were sampled at the end-of-life in summer in 2018, after two summers (pairwise comparisons, $P<0.05$, count $r= -0.56$, viability $r= -0.32$); end of life sperm count was positively correlated with sperm viability ($r = 0.59$).

5.3 Varroa and Nosema tolerance

The initial (June 2017) *Varroa* and *Nosema* disease load of the colonies did not vary significantly by stock, which was to be expected as the splits had been made from a mixed bee source, and the bee population had not yet turned over to include only offspring of the new queens. However, stock also had no effect on disease loads at any of the next four sampling dates: August and October 2017, and April and July 2018, indicating that the stocks were equally susceptible to *Nosema* and *Varroa.* The recommended treatment threshold in Alberta for *Varroa* is 3 mites per 100 bees in late summer, and *Nosema* is one million spores per bee (Nasr and Muirhead 2018). As Table 4 shows, there were significant differences in *Varroa* and *Nosema* levels among sites at some sample dates, but all sites were below the treatment thresholds when sampled with the exception of 3/5 *Nosema* sample dates at Beaverlodge. Had higher *Varroa* and *Nosema* disease loads been present at all sites, it is possible that significant differences between stocks may have been detectable in pathogen loads.

Table 4. Mean *Varroa* infestation and *Nosema* infection (± SE) at five sampling dates during the trial. No significant differences were found among stocks (factorial ANOVAs). Where significant differences exist among sites, they are indicated with an asterisk (*) or letters to distinguish multiple means (means vary significantly when followed by different letter).

Site	June 2017	August 2017	October 2017	April 2018	July 2018
			***Varroa* infestation (# of mites per 100 bees)**		
Beaverlodge	0.59 ± 0.13 **ab**	0.88 ± 0.17	0.14 ± 0.09	0.05 ± 0.03	0.13 ± 0.07
Lethbridge BK	0.09 ± 0.06 **b**	0.66 ± 0.17	1.86 ± 0.47*****	0 ± 0	0 ± 0
Lethbridge AF	0.85 ± 0.25 **a**	1.32 ± 0.29	0 ± 0	0.02 ± 0.02	0.06 ± 0.04
ANOVA	$F_{2,97}$=3.99, P=0.02	$F_{2,72}$=2.12, P=0.13	$F_{2,69}$=12.42, P<0.0001	$F_{2,53}$=1.21, P=0.31	$F_{2,37}$=1.39, P=0.24
			***Nosema* Infection (millions of spores per bee)**		
Beaverlodge	2.39 ± 0.26*****	0.13 ± 0.04	0.62 ± 0.16*****	3.6 ± 1.1*****	2.19 ± 0.59*****
Lethbridge BK	0.62 ± 0.17	0.1 ± 0.03	0.01 ± 0	0.09 ± 0.05	0.13 ± 0.04
Lethbridge AF	0.6 ± 0.15	0.03 ± 0.03	0 ± 0	0 ± 0	0.05 ± 0.03
ANOVA	$F_{2,97}$=17.4, P<0.0001	$F_{2,72}$=1.24, P=0.29	$F_{2,68}$=6.25, P=0.0032	$F_{2,53}$=2.26, P=0.0369	$F_{2,8}$=2.38, P=0.04

5.4 Brood Solidness and Brood Diseases

The solidness of the brood pattern is a metric commonly employed by beekeepers who are evaluating the quality of their queens as it is readily apparent, and solid brood patterns are anecdotally associated with strong colonies. Poor brood patterns are associated with the removal of dead or diseased larvae, but can also be due to the removal of pesticide-killed larvae or diploid drones (vanEngelsdorp et al. 2013), back-filling brood cells with nectar or pollen, or even a failure to lay.
In both years, colonies of the NZ stock exhibited significantly less solid brood than the HI stock, and in 2018 the NZ stock also had significantly less solid brood than the BC stock (2017: $F_{2,78}$=4.86, P=0.01; 2018: $F_{2,47}$=20.8, P<0.0001). As seen in Figure 3, brood patterns among colonies of the NZ stock were also highly variable, especially in 2018, with individual colonies having less than 75% solid sealed brood patches. van Englesdorp et al (2013) found brood pattern to be associated with brood diseases (chalkbrood and sacbrood), and identified patterns less than 80% solid as “poor”. In 2017, according to that criteria, 5/96 colonies had “poor” patterns: 3 NZ (11%), and 2 BC (7%), and 0 HI (0%). In 2018, 11/56 had “poor” patterns: 3 BC (16%), 1 HI (5%), and 7 NZ (44%).

Colonies at the Beaverlodge site had the most solid brood patterns; in 2017 colonies at Lethbridge AF had significantly less solid brood patterns than did those at Beaverlodge (with Lethbridge BK intermediate between the other two sites ($F_{2,78}$=3.76, P=0.03), and in 2018 Beaverlodge colonies had the most solid patterns, followed by Lethbridge AF, with Lethbridge BK having the least solid brood patterns (2018: $F_{2,47}$=26.7, P<0.0001). There was no significant interaction between site and stock on brood pattern.

At the Lethbridge sites, across both years of the trial, 6 BC colonies had brood disease observations (13 separate instances) and the average highest chalkbrood count per colony was 18 chalkbrood mummies observed across all frames. Similarly, brood disease was noted in 7 HI colonies (13 instances) with an average of the highest chalkbrood count being 19 mummies. In sharp contrast were the 10 NZ colonies

(24 instances) reported as having brood diseases, where the average of the highest chalkbrood count observed for each colony was 138 chalkbrood mummies (maximum 466 mummies). Overall, across both years, the NZ-led colonies had greater incidences of brood diseases observed (Table 5).

Table 5. Percent of colonies with no signs of brood disease (sacbrood or chalkbrood) observed in 2017 and 2018. Lethbridge sites only

% of colonies (count) exhibiting no signs of brood disease			
	HI	BC	NZ
2017	82 (n=17)	64 (n=14)	50 (n=12)
2018	40 (n=10)	70 (n=10)	30 (n=10)

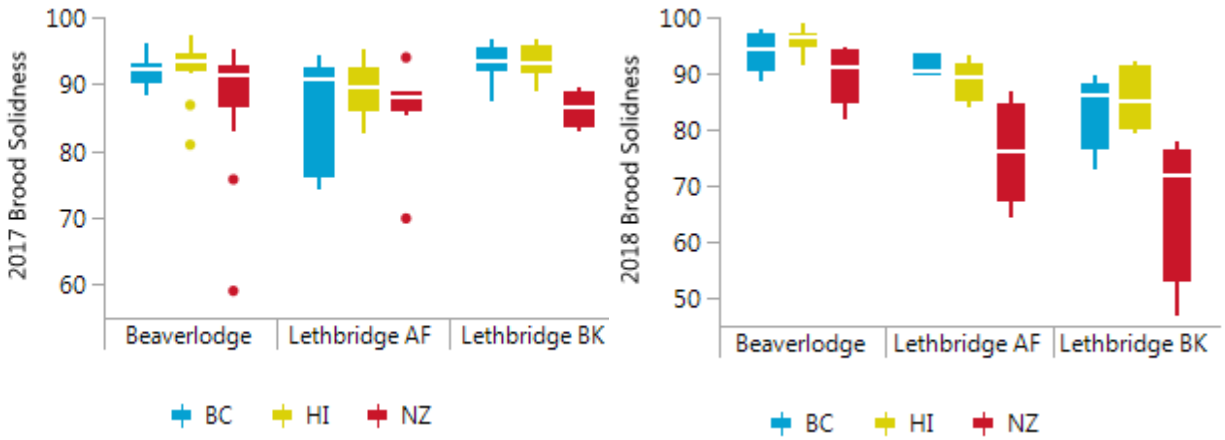


Figure 3. *Median overall brood solidness for each stock (BC: British Columbia, HI: Hawaii, NZ: New Zealand) at each site in 2017 and 2018 averaged across multiple readings. Lethbridge AF managed by Alberta Agriculture, Lethbridge BK managed by Beekeeper. The y-axis indicates the number of capped brood cells observed in a solid patch of 100 cells. n=4-16 (2017), n=3-10 (2018) for each stock within each site.*

5.5 Hygienic and Defensive Behaviour

In 2017, levels of hygienic behaviour did not vary significantly by site ($F_{2,72}$=2.11, P=0.13) or by stock ($F_{2,72}$=0.21, P=0.81), with an average (±SE) hygienic behaviour score of 67 ± 1.6% . In 2018, hygienic behaviour did not vary by stock ($F_{=2,47}$=0.17, P=0.85) but it did vary by site ($F_{2,47}$=171.0, P<0.0001), with Beaverlodge colonies exhibiting the highest levels of hygienic behaviour (80.3 ± 2.15%), then Lethbridge BK (65.8 ± 3.7%) and Lethbridge AF (53.6 ± 3.5%). It is likely that the difference observed among sites in 2018 is due to the assays being conducted later in June in Beaverlodge than Lethbridge. Colonies in Beaverlodge were experiencing nectar flow conditions, which can cause increased nest cleaning and therefore hygienic behaviour (Spivak and Downey 1998). There is a relatively strong genetic basis for hygienic behaviour (Lapidge et al. 2002), so it is interesting that no significant differences were found among these three queen stocks from widely different areas.

In 2017 the interaction of site and stock on defensive behaviour was significant ($F_{*stock4,72}$=2.96, P=0.03), suggesting that stocks behave differently in different environments ($F_{2,80}$=2.80, P=0.07; $F_{2,80}$=1.48, P=0.24). Weather conditions and nectar flows, and adult population sizes are likely to have contributed to this result. The defensive behaviour assay resulted in an average (± SE) sting rate of 1.6 ± 0.43 per colony with 50/81 total colonies not stinging the patch at all. This is consistent with anecdotal reports

from beekeepers mentioning that certain queen stocks are more likely to sting in some areas of Alberta than others, at some times than others, and reports that Africanized bees are less defensive in some regions than others.

In 2018, the colonies were far more likely to sting than in 2017. This defensive behaviour did not vary by stock ($F_{2,47}$=0.08, P=0.92) but it did vary by site ($F_{2,47}$=3.40, P=0.0418) with Beaverlodge having on average (± SE) significantly more stings per colony (15.9 ± 3.2) than Lethbridge AF (4.6 ± 2.7), while Lethbridge BK was intermediate (14.8 ± 3.4). 12/56 total colonies did not sting the patch at all in 2018. The highest number of stings observed on a single patch in either year was 81 stings.

5.6 Colony Productivity Measurements

The BC and HI colonies had consistently higher adult bee populations than NZ colonies in both years of the study, but the magnitude of this effect of stock varied by site (2017: $F_{2,75}$=8.41, P=0.0005, 2018: $F_{2,35}$=12.4, P<0.0001; Figure 4). As a result, there was a significant interaction between the effects of site and stock on adult bee populations (2017: $F_{*stock4,75}$=2.86, P=0.0291, 2018: $F_{*stock=4,35}$=3.28, P=0.02) demonstrating that stocks have different levels of productivity at different locations (Figure 4). The adult bee population also varied significantly by site overall, with Beaverlodge colonies significantly more populous than the Lethbridge ones in both years (2017: $F_{2,75}$=56.72, P<0.0001; 2018: $F_{2,35}$=35.2, P<0.0001). In 2017, Lethbridge BK colonies had significantly more adult bees than Lethbridge AF, but in 2018 the Lethbridge sites were statistically similar.

Given the lower populations in the NZ colonies, lower honey production would be predicted for this stock, and for each site. In 2017 there was a trend for NZ-headed colonies to produce less honey than the other stocks at both Lethbridge sites in 2018, however it was not observed in Beaverlodge. Nevertheless, honey production is highly variable among colonies, and it did not vary significantly by stock in either year of the trial (2017: $F_{2,73}$=0.32, P=0.73; 2018: $F_{2,35}$=0.58, P=0.56; Figure 5).

Honey production was significantly higher in Beaverlodge than at the Lethbridge sites both years (2017: $F_{2,73}$=132.02, P<0.0001; 2018: $F_{2,35}$=8.92, P=0.0007). In 2017 Lethbridge BK produced significantly more honey than Lethbridge AF, whereas 2018 they were statistically similar (Figure 5). The Peace Country generally produces more honey per colony than the Alberta average (Laate 2017) and as such, the difference in honey production is to be expected, and is consistent with previous research.

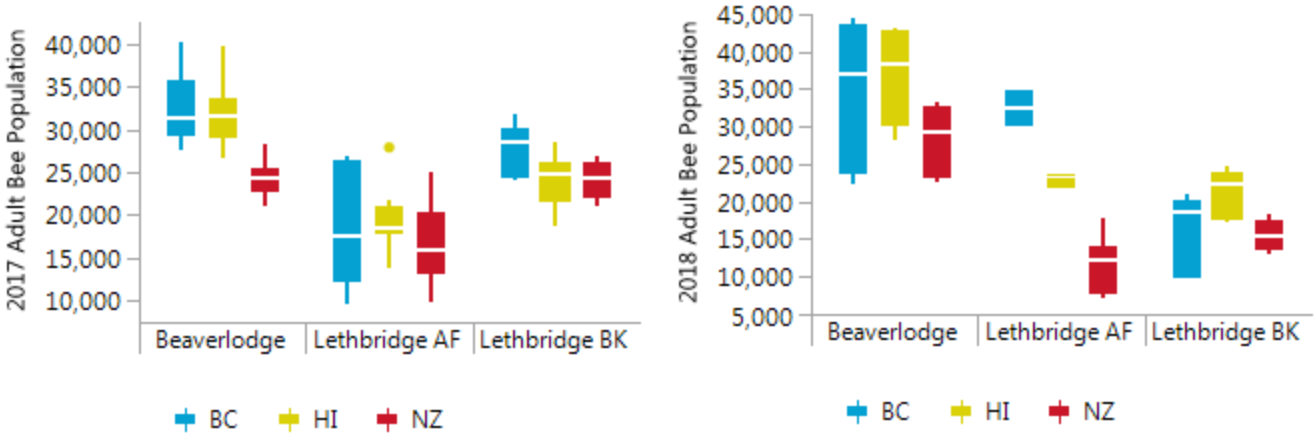


Figure 4. *Median colony adult bee population for each stock (BC: British Columbia, HI: Hawaii, NZ: New Zealand) at each site in August 2017 and July 2018 as determined by analysis of photos of all frames with the photo ladder method. Lethbridge AF managed by Alberta Agriculture, Lethbridge BK managed by Beekeeper (n=4-14 (2017) n=2-7 (2018) for each stock within each site).*

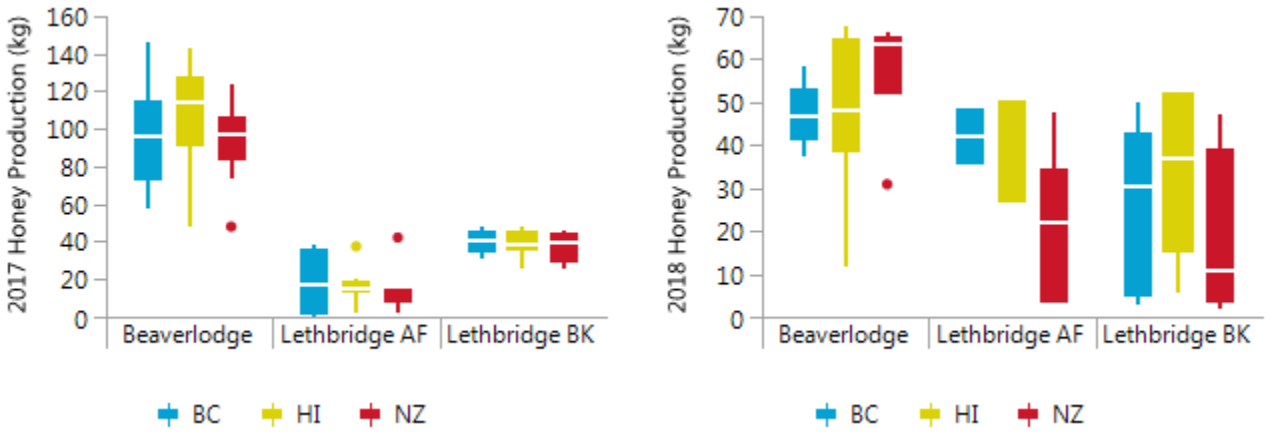


Figure 5. *Median honey production (kg) per colony for each stock (BC: British Columbia, HI: Hawaii, NZ: New Zealand) at each site in 2017 and 2018. Lethbridge AF managed by Alberta Agriculture, Lethbridge BK managed by Beekeeper (n=4-15 (2017), n=2-7 (2018) for each stock within each site). In 2017 the y-axis represents the total honey production, in 2018 it represents the honey production current to the point at which the trial was ended (details in methods section)*

BC and HI-led colonies had significantly more capped brood cells than did NZ-led colonies in both years (2017: $F_{2,76}$=10.82, p=<0.0001; 2018: $F_{2,35}$=9.59, P=0.0005), which is consistent with the aforementioned findings of adult bee population differences amongst stocks. The number of capped brood cells per colony did not vary by location in 2017 ($F_{2,76}$=0.27, P=0.76), but did vary significantly in 2018 ($F_{2,35}$=5.88, P=0.0063) with significantly more brood cells in Beaverlodge colonies than in Lethbridge AF, with Lethbridge BK intermediate (Figure 6), which is also consistent with the adult bee population results in 2018.

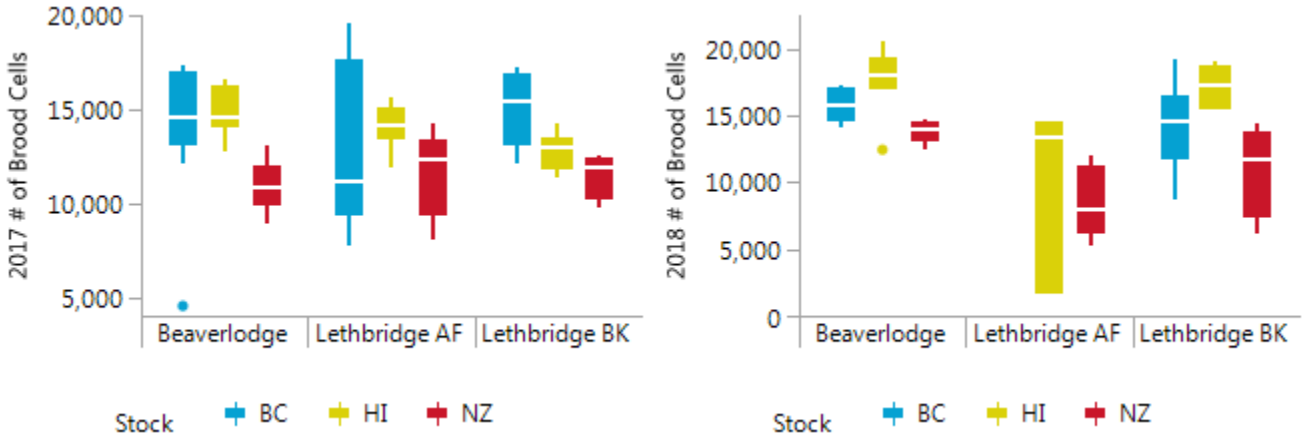


Figure 4. *Median number of sealed brood cells per colony for each stock (BC: British Columbia, HI: Hawaii Kona, NZ: New Zealand) at the three sites in August 2017 and July 2018 as determined by analysis of photos of all frames containing brood using the software HoneyBeeComplete 5.4. Lethbridge AF managed by Alberta Agriculture, Lethbridge BK managed by Beekeeper (n=4-14 (2017), n=2-7 (2018) for each stock at each site). The 2018 BC boxplot for Lethbridge AF is missing as there were only two colonies (16,854, and 15,492 brood cells).*

The interaction of site and stock on fall cluster score was also significant ($F_{*stock4,69}$=25.09, P=0.0073; $F_{2,69}$=84.6, P<0.0001; $F_{2,69}$=7.31, P=0.0013; Figure 7); similar to the effects seen for adult bee population, stocks exhibit variation in cluster scores differently by location. This makes intuitive sense, as cluster scores are inherently related to adult population size. After winter, in April 2018, there was significant variation in cluster score in the ANOVA model overall ($F_{8,54=2.30}$, P=0.03), but it was not significant for either site or stock ($F_{2,54}$=1.58, P=0.22, $F_{2,54}$=2.91, P=0.06).

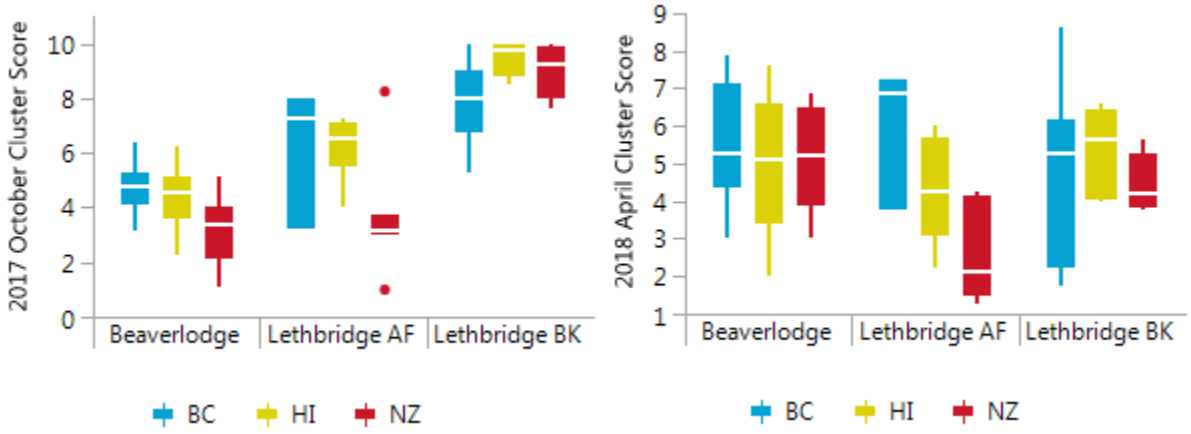


Figure 5. *Median cluster score for each stock (BC: British Columbia, HI: Hawaii, NZ: New Zealand) at each site prior to winter in October 2017 and after winter in April 2018. Lethbridge AF managed by Alberta Agriculture, Lethbridge BK managed by Beekeeper. The y-axis indicates the number of inter-frame spaces covered with bees averaged across the top and bottom of single brood chambered colonies. n=3-15 (2017), n=3-11 (2018) for each stock within each site.*

Colonies in Beaverlodge weighed more in October 2017 than did their southern counterparts ($F_{2,69}$=145.02, P<0.0001, Figure 8). The Lethbridge BK colonies also weighed significantly more than the Lethbridge AF colonies. Some variation in colony weight is to be expected between sites due to varying hive equipment but the 5-10 kg difference between Lethbridge and Beaverlodge is likely due to Beaverlodge colonies being fed far more fall sugar syrup in preparation for the longer winter they experience. Beaverlodge colonies were still heavier than Lethbridge AF the following spring (April 2018) ($F_{1,39=45.8}$, P<0.0001). Finally, the colony weight loss over winter also varied significantly by site ($F_{1,39}$=58.5, P<0.0001), with Beaverlodge colonies losing significantly more weight than the Lethbridge AF colonies. Given the harsher and longer winter conditions in Beaverlodge, it is expected that the colonies would consume more stored honey and therefore lose more colony weight.

Colonies headed by BC queens weighed significantly more than NZ or HI colonies in the fall of 2018 (Figure 8, $F_{2,69}$=5.40, P=0.0066), however by spring 2018 NZ *and* BC colonies were both heavier than HI colonies ($F_{1,39}$=8.64, P=0.0008). The weight lost by colonies over the winter also varied significantly by stock ($F_{2,39}$=13.4, P<0.0001), with the HI and BC colonies losing significantly more weight than the NZ colonies. As the NZ colonies tended to have smaller populations than the other stocks prior to and after winter (Figure 7), it follows that these smaller populations likely consumed less feed over winter, and therefore weighed more in spring and lost less weight than HI and BC.

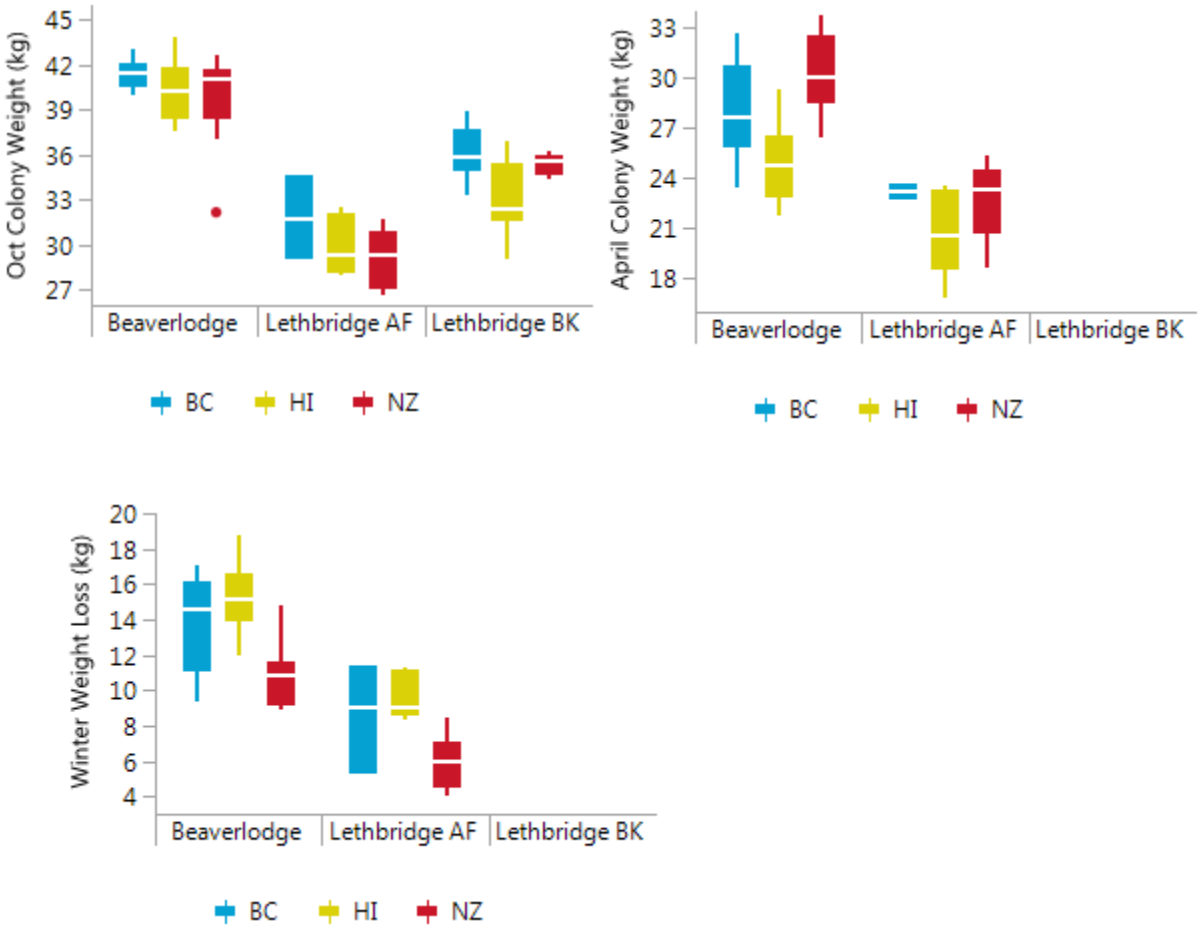
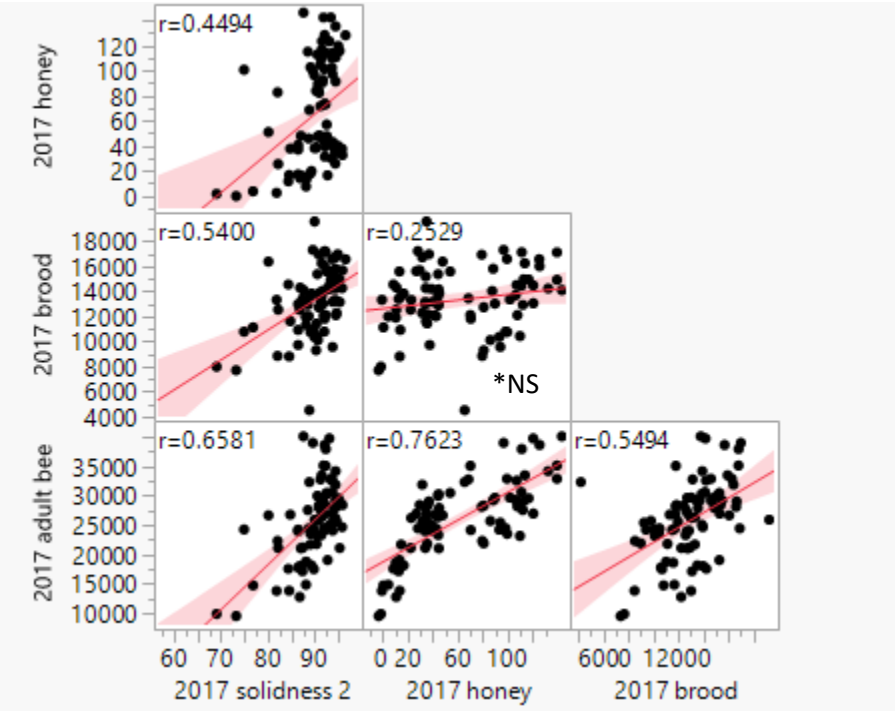


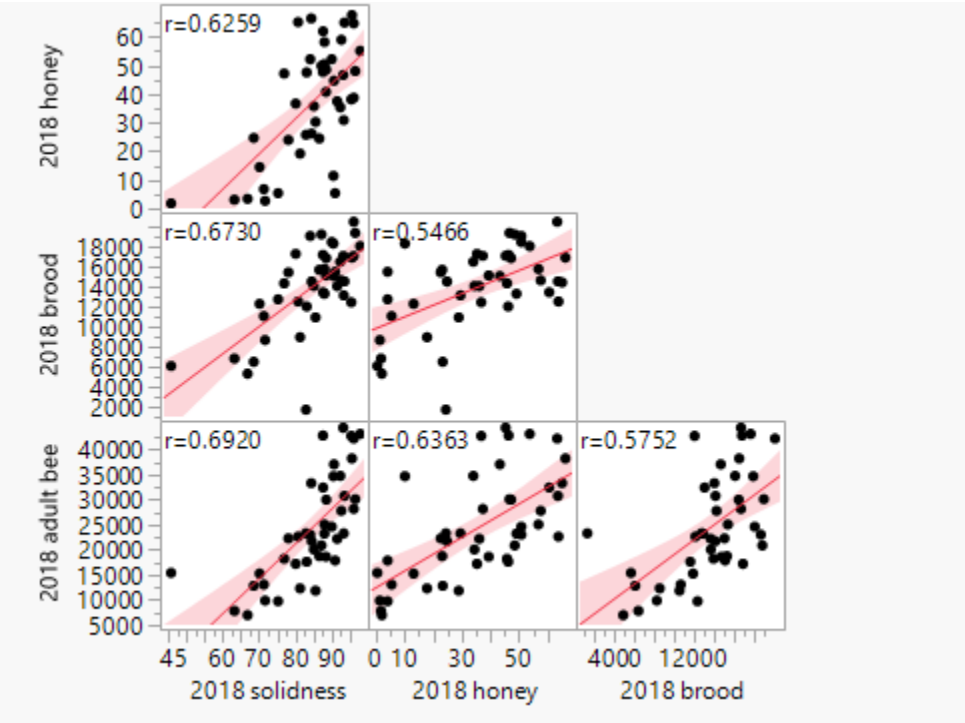
Figure 6. *Median colony weight (kg) for each stock (BC: British Columbia, HI: Hawaii, NZ: New Zealand) at each site prior to winter in October 2017, after winter in April 2018, and the median weight lost by colonies over winter. Lethbridge AF managed by Alberta Agriculture, Lethbridge BK managed by Beekeeper. n=3-15 (Oct 2017), n=3-11 (April 2018, Winter Weight Loss) for each stock within each site. Note that April data for Lethbridge BK was not taken due to the participating beekeeper barrel-feeding colonies prior to the time they could be weighed.*

5.7 Correlations among colony measurements

Across all stocks and sites, there were strong correlations in both 2017 and 2018 between pairs of a number of the colony level measures (Figure 9), including brood pattern solidness and adult bee population, brood population, and honey production, and adult bee population and honey production or brood population. In 2017 the correlation between brood population and honey production was not significant, whereas it was in 2018.



(a)



(b)

Figure 9. *Correlations between several 2017 (a) and 2018 (b) colony level measurements. The r represents the pairwise correlation coefficient between each pair of variables. 2017: all p<0.0003 except honey versus brood, which was not significant. 2018: all p<0.0001.*

5.8 Colony Loss

The number of days a colony was in the project (prior to removal due to colony death or the loss of the experimental queen) was not dependent on site ($F_{2,120}$=1.95, P=0.15) or queen stock ($F_{2,120}$=0.85, P=0.43). There was high colony attrition from the experiment due to queen loss, and it was difficult to keep large numbers of colonies headed by original queens intact through the second year of the trial (Figure 10). Eighteen percent of queens did not successfully introduce, and a further 18% did not survive their first summer. Winter loss was moderate with 14% of colonies overwintering dying. Thirty percent of the colonies surviving winter did not survive their second summer. It is possible that experimental assessments exacerbated queen loss, although beekeepers also anecdotally report high rates of queen loss and supersedure in non-experimental colonies.

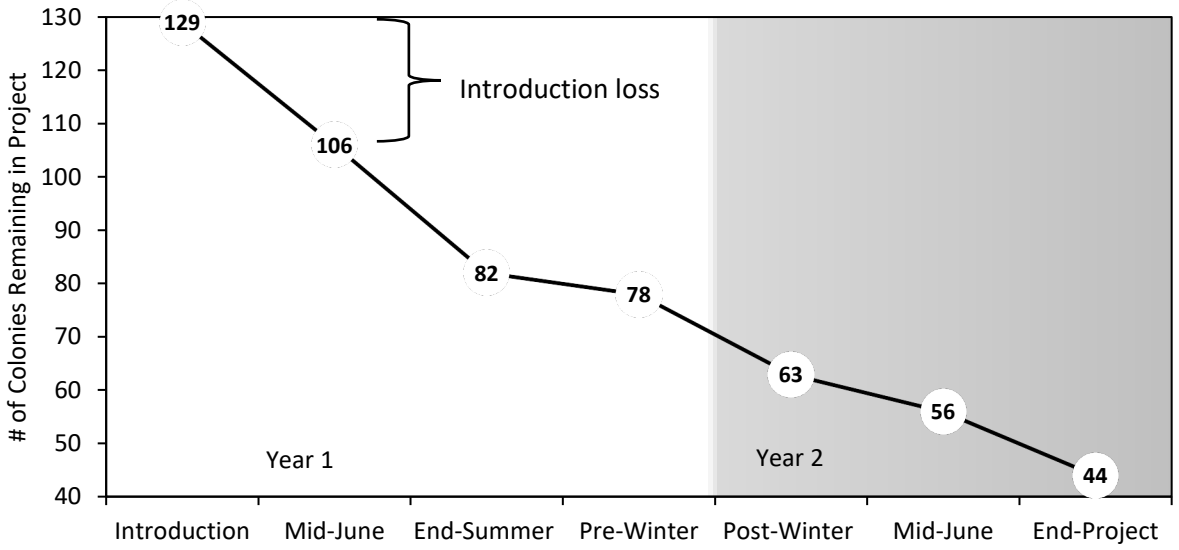


Figure 10. *Number of colonies remaining in project throughout the trial across all sites. Colonies were removed from the trial when queenless or when original queens had been superseded. The shaded section represents year 2 of the trial.*

6 Conclusions

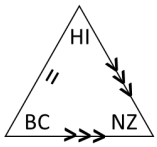
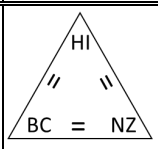
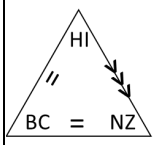
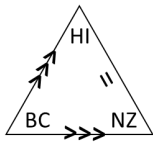
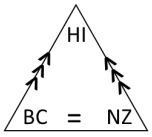
The shipments we received were of queens that were generally well-mated. There was variation in sperm count and viability among the subsamples of queens initially tested within each shipment, and higher sperm viability overall in the NZ stock compared with the HI queens, despite the low temperatures pre-flight in New Zealand. While queens from HI had smaller spermathecae than those from other stocks, they still stored substantive numbers of sperm. Across all sampled queens, there was significant correlation between spermathecal size and the number of stored, viable sperm. This indicates that, while in general queens with larger spermathecae are capable of storing more viable sperm, that other factors such as those governing mating success at the point of origin may have a greater influence on sperm number per queen.

The NZ stock repeatedly performed poorly compared with the other stocks across multiple productivity measures (summary Table 6). The NZ queen-led colonies had significantly less solid brood patterns, despite the queens having higher sperm viability and equivalent sperm count; this was likely associated with the higher incidence of brood disease (chalkbrood) observed in these colonies, especially in 2018.

These results agree with recent findings of Lee et al (2019), who found that poor brood patterns in commercial colonies were not related to sperm viability. Instead, they found an influence of the colony environment, including pesticide levels, on brood pattern. van Englesdorp et al. (2013) found poor patterns to be related to brood diseases rather than queen quality per se, and this was also evident in the high levels of chalkbrood observed in the colonies headed by NZ queens in this study. The susceptibility to chalkbrood had important colony-level impacts, as the NZ colonies had significantly fewer capped brood cells per colony, fewer adult bees, and smaller fall clusters than the BC or HI stocks.

Anecdotal reports from beekeepers have frequently suggest that stocks perform differently depending on the location of the apiary. Significant interactions between site and stock for adult bee population, chalkbrood, defensive behavior, and cluster score suggest that these stocks were performing differently at our different sites, and confirm the anecdotal reports by beekeepers. These results are not unexpected, as previous reports, including our own research, have typically indicated higher honey production, increased brood, and larger populations in the Peace region compared with southern Alberta.

Table 6. Significance triangles showing the relationship between the three stocks for each field measurement. (**>>>**) indicates that one stock is significantly greater than the other, (**=**) indicates that stocks are statistically similar.

HI and/or BC significantly greater than NZ	Stocks equivalent or BC / NZ greater than HI
 -2017/2018 adult bee population -2017/2018 # of capped brood cells -2018 brood solidness -Overwintering colony weight loss	 - 2017/2018 *Varroa* infestation - 2017/2018 *Nosema* infection - 2017/2018 hygienic behaviour - 2017/2018 defensive behaviour - 2017/2018 honey production
 - 2017 brood solidness - 2017 October cluster score  - 2017 October colony weight	 -2018 April Colony weight

Lee et al. (2019) found that after being introduced to new colonies, queens with poor brood patterns initially had improved patterns; the converse was also true, queens with good brood patterns initially had patterns that declined when they were introduced to colonies that had previously been rearing poor patterns of brood. They concluded that poor pattern alone is not a reliable indicator of queen quality and not necessarily (although it can be) an indicator of a failing queen. In some cases, queens with poor patterns have high sperm count and viability (such as the case of the two “failing queens” in 2018 at Beaverlodge). These symptoms can be attributed to many causes, and as such, finding the actual cause is important and necessary to remediate the problem. It is also critical that beekeepers carefully examine the brood frames and bottom board for signs of brood disease. In some cases, the cause of the poor brood pattern may be related to disease susceptibility. There may still be a benefit to replacing the queen in this case, if a genetic disease susceptibility is suspected, but there may be additional management measures that should be undertaken (such as comb replacement, for example).

Nevertheless, sperm viability is believed to decline as queens age (Lodesani et al. 2004, Tarpy et al. 2014), therefore knowing the age of a queen is important and can assist in determining the actual cause of the poor brood pattern. In addition, Pettis et al. (2016) found significantly lower sperm viability (<45%) in queens from colonies that beekeepers identified as "failing", whereas those that were identified as "healthy" has higher sperm viability (>70%). They also found that even relatively short exposure to extreme temperatures (4°C or 40°C) were detrimental to the viability of sperm stored in the queens' spermathecae, although this does not agree with our findings in the NZ shipments. Placement of temperature loggers in shipments is an important consideration to accurately gauge the temperatures that the queens themselves experience.

Our results suggest that (1) high sperm count and viability may be required for good brood patterns, however high sperm count or viability do not themselves guarantee good brood patterns or high colony productivity, (2) sperm count and viability may be more important indicators as queens age, if they begin to decline. While we found no initial relationship between sperm viability and productivity across stocks (with the NZ stock having the highest initial viability but least solid brood patterns), this may be due to the overall high quality of the queens we received.

We did find post-hoc relationships between sperm count, sperm viability, and brood solidness among individual queens at the end of the experiment. At the end of the experiment, queens with higher sperm counts also had sperm with higher viability. It is important to interpret the end-of-life sperm data carefully, partially because of the low sample sizes, but also because it is a non-random subset of the queens. Queens that survived to the end of the experiment were those that did not die or become superseded. For example, the two surviving BC queens that we were able to measure sperm viability for had a mean of 95% viability, whereas the mean of the initial subset of queens that were sampled was only 84% viability. The end-of-life NZ queens (n=5) at Lethbridge had an average sperm count of 6.05 million, whereas the mean initially was only 5.3 million. It appears likely that the surviving queens were well-mated and healthy, and less well-mated or healthy queens did not survive. Additional research is needed to examine these effects, which are difficult to untangle as they require destructively sampling of large numbers of queens.

It is also very important to consider that sampling a subset of queens in a queen shipment gives only a "snapshot" picture of the measures of those queens at that time. Earlier or later in the season the results may be different, as they would be for queens in different shipments or from different mating yards. Very large shipments will include queens from many mating yards or queen "pulls", and may be highly variable. Given that the testing is labour-intensive and time-constrained, it is important to consider how many queens would need to be sampled to give an accurate estimate of the shipment mean, and the value of the information gained.

Conclusions and Recommendations:

- **Measuring sperm viability and count of queens at shipment can be useful to identify problems, such as those caused by poor mating conditions or temperature stress during shipment, but high sperm count and viability are not of themselves reliable indicators that queens will perform well.**
- **Queen measurements are variable even within a single shipment, and good average measurements do not preclude poor-performing outliers.**
- **Poor brood pattern is not a reliable indicator of a lack of viable sperm. Disease, pesticide, or other issues can also result in poor patterns.**
- **Data loggers in queen shipments should be standard practice to identify events of extreme temperatures. Such events would be indicators that sperm viability may have been compromised in the queens in the shipment. Standards should be developed to indicate the temperatures and durations that are of concern.**
- **Quality of brood pattern (solidess) is a useful indicator for beekeepers to use to predict brood and adult populations and honey production, but not to gauge sperm count or viability.**
- **The majority of the queens we tested from commercial stocks did not survive through two production seasons, whether through lack of acceptance, supersedure or death. No differences were observed among stocks or testing sites.**

7 References

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8 Research Team Signatures and Employers' Approval

By signing as representatives of the research team leader's employing organisation, 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:** Apiculture Unit Head, Alberta Agriculture and Forestry
Signature: 	**Date:** February 25, 2019
Team Leader's Employer's Approval	
Name: Dr. David Feindel	**Title/Organisation:** Director Plant and Bee Health Surveillance Section, Alberta Agriculture and Forestry
Signature:	**Date:**

Team Members' Organisation

Team Member	
Name: Dr. Marta Guarna	**Title/Organisation:** Research Scientist, Agriculture and Agri-food Canada
Signature:	**Date:**
Team Member	
Name: Dr. Stephen Pernal	**Title/Organisation:** Research Scientist, Agriculture and Agri-food Canada
Signature:	**Date:**
Team Leader's Employer's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**
Team Member	

Name: Patricia Wolf-Viega	**Title/Organisation:** Acting Scientist Manager, National Bee Diagnostics Centre, Grande Prairie Regional College
Signature:	**Date:**
Team Leader's Employer's Approval	
Name:	**Title/Organisation:**
Signature:	**Date:**