**PROJECT TITLE**

**Title:** Honey Bee Health: Management of Varroa mites and Viruses

**ADF File Number:** 20130111

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**ABSTRACT**

Over the past decade, global honey bee populations have been under increasing stress from a variety of pests and pathogens resulting in economic losses for producers. Chief among these is Varroa destructor, a parasitic mite which weakens colonies and transmits a number of economically significant viruses. Nosema spp, species of microsporidian gut parasite of honey bees also has a negative impact on colony build up and wintering success.

Examining these factors and their interactions, study colonies were infected with Varroa mites and studied for two seasons. The presence and concentrations of viruses and Nosema spp. as well as colony performance were tracked. Four treatment groups were chosen based on treatment timing: spring, fall, spring and fall, and an untreated control. Nucleus colonies were also established at different times (early/late) and starting sizes (3 frames/5 frames) to determine effective methods for replacing dead colonies in a Canadian prairie environment. Colonies were assigned Varroa treatment groups, and were assessed for colony performance and Varroa infestation over two seasons.

This study clearly showed that Deformed Wing Virus and Varroa mites working alone or in combination are primary factors associated with colony mortality. Other viruses and Nosema (particulary *Nosema ceranae*) can influence colony health and are correlated with reductions or variability in colony size in colonies that survive winter. Timing and starting size both proved to be significant factors in successful nucleus colony establishment, with earlier made nucs with more frames of bees having the largest populations over time. The successful application of best management practices for the control of Varroa, viruses, and Nosema will contribute towards a sustainable and healthy honey bee stock. Since viruses cannot be controlled, they must be managed by monitoring and managing Varroa levels in colonies. The improved understanding of how interactions among parasites and pathogens affect colony health should result in more reliable production and maintenance of honey bee colonies and nucleus colonies.

**INTRODUCTION**

**Bee Health**

Over the past several years, honey bee populations in North America and across the globe have been under increasing stress from a variety of parasites and diseases resulting in devastating economic losses for producers (Neumann and Carrick 2010; Rosenkranz et al. 2010). Colony winter losses in Canada have averaged 30 to 40% in recent years (Currie et al. 2010) which is similar to the losses experienced with “colony collapse disorder” (CCD) in the United States (US) (vanEngelsdorp et al. 2009; Ellis et al. 2010) but far greater than our normal long term winter-loss average of 5 to 15%. Despite this similarity, the CCD found in the U.S. has not been identified as an important factor in relation to colony losses within Canada. Our losses have been attributed to a number of interacting stress factors linked in part to a particular parasite, the Varroa mite (*Varroa destructor*)(Currie et al. 2010; Guzman et al. 2010, Nasr et al. 2010). In spite of identifying possible causes of reported high bee colony kill in Canada, research initiatives have not succeeded in providing effective tools to stop bee health decline and colony kill. Moreover, researchers have not been able to fully understand the interrelationship between varroa, viruses, wintering conditions and bee mortality in Canada.

Substantial annual losses of honey bee colonies have resulted in a decline of number of bee colonies in many countries around the world including many regions across Canada. In order to replace killed bee colonies, beekeepers need access to healthy bee stocks and develop management practices to locally produce nuclei (3-5 frames of bees) with a queen (for stock replacement and increasing their number of colonies). These practices for production of nuclei need to be further researched and developed to sustain the health of the industry. Production of nucleus colonies would also require healthy disease free colonies from which to establish their populations further highlighting our need to understand pathogen parasite interactions and their effects on bee management.

**Viruses, Varroa and Bee Health.**

Of the secondary pests and diseases that are associated/interact with varroa to affect colonies, the ones that are the least understood are the viruses. This association between varroa and viruses varies under different management contexts and environmental conditions.

There are seven economically important viruses: deformed wing virus (DWV), black queen cell virus (BQCV), Israeli acute paralysis virus (IAPV), sac brood virus (SBV), chronic bee paralysis virus (CBPV), acute bee paralysis virus (ABPV) and Kashmir bee virus (KBV).These viruses are broadly distributed throughout regions of Canada where managed honey bees are kept and varroa is known to occur (Desai and Currie 2015, manuscript in prep). DWV, BQCV and IAPV are all very common, occurring in 40 to 100% of colonies with KBV, SBV, ABPC and CBPV being more sporadic in distribution. Prior to this study, the virus profiles and virulence level of the viruses in colonies in Saskatchewan was not well studied and this is of particular interest since many areas of Saskatchewan did not have a history of exposure to mites when this project was conceived.

Viruses linked to colony winter population and colony loss in Canada include DWV, IAPV, BQCV and SBV (Desai and Currie 2016, manuscript in prep). These are linked to some extent with Varroa but their impact appears to differ under different wintering management methods. Varroa is an effective vector and activator of many viruses and three viruses in particular (DWV, IAPV and ABPV) have been linked with large-scale winter losses in other regions at various times (Berthoud et al. 2010; Cox-Foster et al. 2007; Dainat et al. 2012a, Dainat et al. 2012b; Highfield et al 2009). Of these viruses DWV, is emerging as one virus that has a high impact on colony health under a broad range of environments (Chen et al. 2004b; Cornman et al 2012; Dainat et al. 2012a, 2012b; Francis et al. 2013; Highfield et al. 2009; Martin et al. 2012). Other viruses such as BQCV may interact with other viruses or pathogens such as Nosema spp. to impact colony health (Bailey et al. 1983a, 1983b; Cornman et al. 2012) although the role of these interactions remain uncertain (Dainat et al. 2012). In order to understand how to manage these viruses, the interactions between Varroa mites and viruses in different environmental and bee management scenarios need to be elucidated. This will allow for the development of effective management recommendations to manage varroa that should ultimately prevent high levels of viruses from damaging colony health, and enhance overwintering success under Canadian conditions.

Overall, the objective of this project was to understand and improve varroa controls and reduce the impact of associated viruses by studying the interrelationship between varroa, viruses, level of virulence of viruses, treatment time, and their impacts on bee health and colony survivorship under Saskatchewan conditions. This project, demonstrates effective sustainable methods for varroa and virus control so beekeepers can address their daily challenges with bee health and thereby mitigate some of the economic chalenges faced in the beekeeping industry.

**Varroa Management**

The primary challenge facing the beekeeping industry currently is the availability of effective, sustainable treatment measures against Varroa mites and viruses, as well as the availability of healthy bee stocks. In the recent past, Varroa have developed resistance to two widely used miticides (Currie et al 2010). Resistance develops quickly (Nasr et al. 2008)because of the beekeeper’s reliance on prophylactic and repeated use of a single effective registered miticide for mite treatment (there is presently a lack of approved alternative miticides for rotation). This factor is especially significant due to the short lifecycle of the varroa mite and allows biological resistance to outpace treatment. Currently only a single miticide, Apivar®, is effective (Canadian Association of Professional Apiculturists (CAPA annual report pub., 2011). However, it is expected varroa will shortly develop resistance to Apivar® and beekeepers will continue to suffer from high losses of bee colonies.

The presence of *Nosema* spp, vitellogenin and total protein of honey bees are important factors affecting wintering honey bees (Amdam and Omholt 2002; Botias et al. 2013). Nosema is a harmful gut microsporidian of honey bees and it can negatively affect wintering colonies as well as slow their spring build up (Botias et al. 2013). Analysis of vitellogenin and total protein in worker bees are excellent proxy measures for the wintering readiness of honey bees. Knowledge of these two factors, in addition to the Varroa and virus data already collected, work to provide a nearly complete picture of the condition of the bees as they prepare for winter. By having all these critical pieces of information together, a greater understanding of the wintering dynamics of honey bees can be obtained.

This project was designed to develop an integrated pest management program for varroa. It was to provide options for beekeepers for sustainable Varroa control, tools for managing resistance, ways to reduce the secondary negative impacts of viruses on wintering bees and improve bee health. Included in these goals was the development of best management practices for making healthy nuclei to replace dead colonies. This will increase beekeepers’ ability to successfully maintain colony health, significantly reduce winterkill, and reduce the cost of production. Varroa and virus research on honey bees by Currie in Manitoba and acaricide impact and Varroa control research in Saskatchewan by Parsons and the Technology Adaptation Team have shed light on the varroa and virus interrelationship complex and the efficacy of current treatments. This project looks deeper into how Varroa populations and treatment times affect viruses, colony health and survivorship of a hive.

**Objectives & Ministry-approved revisions**

1. Evaluation of virus presence and concentration in bee colonies throughout the year to establish their relation to varroa populations, treatment time impacts on bee population growth and colony mortality

This objective has been negatively affected by the failure of a collaborator to provide proof of use of funds. As a result this replicate has been removed from the project with Ministry approval as of July 4, 2016.

1. Assess and determine the level of virulence of different strains of viruses found in bee colonies that are varroa infested, mite free, and winterkilled within the Province of Saskatchewan

This component of the project has been previously removed with a revision to the project plan accepted by the ministry.

This project objective was removed in 2014 due to reports of incursion of Varroa mites to the previously identified mite-free region. The Provincial Apiarist, Geoff Wilson met with beekeepers on May 16th 2014 at which time they reported Varroa mites in the Meadow Lake area. He subsequently verified this report with a visit to the area in June. As a result there was not sufficient Varroa-free bee source for the project’s needs.

1. Evaluation of virus presence and concentration in bee colonies throughout the year to establish their relation to varroa populations, treatment time impacts on bee population growth and colony mortality
2. Developing best management practices for production of healthy nucleus bee colony (3-10 frames of bees) with acaricides’ rotation strategies to replace dead colonies

**METHODOLOGY:**

1. **Evaluation of virus presence and concentration in bee colonies throughout the year to establish their relation to varroa populations, treatment time impacts on bee population growth and colony mortality**

**Testing hypothesis:**

Varroa control application time does not affect viral levels and subsequently colony health and survivorship.

**Methodology:**

During the months of May and June, 2014 donor colonies for the project were collected from beekeepers throughout Saskatchewan. The collected colonies were placed in one of five bee yard locations in and around the Prince Albert/MacDowall area of Saskatchewan. During the spring and summer of 2014, these colonies were evaluated for strength and thoroughly assessed for disease. Numerous inspections were performed for the brood disease American foul brood (*Paenibacillus larvae larvae*) as such an infection would have severely hampered the project.

At the end of July, 2014 once the colonies were confirmed to be without any symptoms of American foulbrood, 50 were chosen for inclusion in the first year of the experiment. Fifty double brood chamber Langstroth colonies were split with queen excluders after having the bees driven out of the top box with Bee Repel®. Twenty four hours later, after workers had returned to the top box, it was removed to the location of the experiment. This left a queen right donor colony that was not used for the experiment and a queenless, brood and bee filled top box. The 50 top boxes were shook free of bees into two large screened bulk boxes. While the brood chambers were nearly free of bees the frames were sorted between colonies to roughly equalize the brood area of the boxes. Next the two large screened bulk boxes of known weight were then reweighed to determine the amount of bees they contained. Then each of the two bulk boxes was sampled in triplicate for the percent Varroa infection using an alcohol wash. The two bulk boxes were found to have a different % Varroa infection, so one scoop from each bulk box was placed in each of the experimental colonies. Each scoop of bees was weighed with a kitchen scale to 450 grams for a total of 900 grams of bees per brood chamber. The resulting colonies were then screened in for 24 hours. After 24 hours they received a 10 day old queen cell and were unscreened. The colonies were then left for three weeks until August 22nd to give queens time to hatch, mate and begin laying in the colonies.

One to two weeks after preparations, colonies were examined to determine colony strength and Varroa mite levels (for an initial base line reading). The bee colonies were then assigned in a randomized block design (into 4 treatment groups). These blocks were then considered based on Varroa infestation levels and colony strength (frames of bees). Treatment groups consisted of 10 untreated control colonies infested with Varroa, 10 colonies receiving spring treatment for Varroa, 10 receiving fall treatment, and 10 colonies that will receive both a spring and fall treatment. The treatment group without Varroa had to be dropped due to the lack of bees for this group (See objective 2 for details). In order to establish the timing of Varroa control, Apivar® strips (active ingredient amitraz) were used, as it is currently the only product with both good efficacy as well as only recently documented resistance concerns in Canada and could reliably create the desired mite populations in tested treatments at different times in the season. Treatments were applied according to the label. Standard beekeeping management practices were applied to all experimental colonies throughout the experimental period.

On September 9th and 10th, 2014 the colonies were first assessed and treated. Assessment included measurement of brood area, counting of bee spaces filled by the bee cluster, collecting 300 bees in alcohol for Varroa sampling, and collection of 30 bees into test tubes for later virus sampling by the University of Manitoba. Treatment groups that required treating (Fall treatment and spring and fall treatment groups) were treated to label directions with Apivar® (amitraz). Sticky board traps were placed under the colonies continuously from September 6th to October 20th, 2014 and changed every 7 to 10 days. For the 2014 season, colonies were assessed again on September 24th, October 6th, and October 20 and 21st. Colonies were weighed on October 23rd and scored for adult population by counting filled bee spaces top and bottom on October 24th.

**Colony sampling.** For the remainder of the study period, colonies were sampled to determine Varroa mite prevalence twice a month when possible during the spring and fall (approximately March, April, May and August, September, and October respectively) once monthly during the summer months (June and July)

**Evaluation of colony performance.** Colony performance was evaluated throughout the project using two parameters: bee population and brood area. These variables were measured at the same frequency as Varroa and virus sampling. Population was determined by measuring cluster size; the number of bee spaces covered while the colony is clustered was counted and assigned a score. Brood area in inches squared was determined by using a frame with a one-inch grid overlain and counting the area of capped brood in each colony. Winterkill of bee colonies was determined each spring.

**Addition of Nosema spp sampling.** With the loss of the expected Varroa-free colonies in 2014 (details in Objective 2), additional sampling to determine levels of the microsporidian parasite *Nosema spp* was added to the project. The alternate sampling included collection and processing of bees from colonies currently in Objective 3. These samples were collected and processed for the presence and quantity of *Nosema apis* and *Nosema ceranae* as well as vitellogenin and total protein, and were collected for the remainder of the first replicate of Objective 3 started in 2014 and the second replicate started in 2015. Sampling coincided with the twice monthly assessments already conducted during the fall and spring. Samples of 30 bees from the outer edges of the colony cluster were hand collected into test tubes, flash frozen with liquid nitrogen and shipped on dry ice to the labs of the University of Manitoba. These samples then had their Nosema species and quantity determined with rtPCR. Vitellogenin and total protein were quantified by collaborators at the U of Manitoba. Pathogen levels (virus and Nosema) were also correlated with colony health and economic impact where possible, as well as with other pathogens and parasites. Developing a method for quantification of protein levels that could work in conjunction with viral analysis studies was also attempted, and this objective was partially met. Although not planned as a component for the University of Manitoba, the relative prevalence of two forms of DWV, (DWV-A and DWV-B) were quantified that are thought to vary in virulence.

The experiment was replicated twice with each replicate running for approximately two years. The first replicate of the experiment began in June 2014 and continued with sampling and evaluation until April 2016. The second replicate of the experiment began in April 2015 and continued until November 2016; both replicates followed the sampling and assessment schedule outlined above. This design allowed for greater confidence in the data and provided more opportunity to capture the variability that can exist from year to year due to weather and environmental conditions.

Colonies were moved into indoor wintering facilities in late October each year of the experiment when weather dictated. There, they were maintained at approximately 5°C and 60% relative humidity throughout the season. During the experiment all colonies were managed as commercial colonies would be. This included placing and removing honey supers as needed as well as feeding with sugar syrup in preparation for the winter.

**University of Manitoba Sample Processing**

Honey bee samples were collected from colonies in Objective 3 by Graham Parsons, Hannah Neil and other members of the Saskatchewan Technology Adaptation Team, from 43 colonies in each experiment and shipped to Manitoba on dry ice. Samples of 30 bees per colony were stored in -80 °C for further analysis. For experiment one, colonies were followed and assessed through two winters. Samples were collected in the fall of 2014, spring, summer and fall of 2015 and in May of 2016. As described above, for experiment 2, colonies were only followed and assessed through one winter thus samples were processed only from the fall of 2015 and spring, summer of 2016.

Samples were analyzed following a new protocol that was developed by Currie at the University of Manitoba to allow direct quantification of bee viruses (deformed wing virus (DWV), black queen cell virus (BQCV), sac brood virus (SBV), Israeli acute paralysis virus (IAPV), Kashmir bee virus (KBV), acute bee paralysis virus (ABPV), and chronic bee paralysis virus (CBPV)) concentrations. The protocol utilized a bead crushing method that provided efficient extraction of RNA for quantification of the viruses in bee samples. As viral quantification was standardized using a standard curve with virus standards made from g-blocks it allowed for cross comparison of viral loads between years and seasons and should allow results to be translated to equivalent measures in other labs. To quantify virus load, the frozen bee samples were taken from the -80 crushed in bead extractor in cold buffer according to our standard lab protocol. The total RNA was extracted from 30 mg of ground honey bee material using an RNeasy Mini Kit (Qiagen, Valencia, CA, USA) following the manufacturer’s instructions. RNA samples were dissolved in molecular grade water in the presence of ribonuclease inhibitor and stored at -80 °C for further analysis. The RNA quantity and purity was determined by a nanodrop spectrophotometer by measuring the absorbance at 260 nm and 280 nm. An average of 2 μg of total RNA was reverse transcribed to produce cDNA using iScript™ Reverse Transcription Supermix for RT-qPCR (Bio-rad) according to the supplier’s recommendations. Real Time PCR reactions were performed on triplicate samples for each virus and compared to a standard curve to quantify the number of virus particles per microgram of RNA. The efficiency of each primer set and gBlocks was validated prior to analysis. The virus quantification by real time PCR was carried out in triplicate on a CFX96 Touch™ Real-Time PCR Detection System (Bio-rad) using SsoFast™ EvaGreen® Supermix kit (Bio-rad). Viral gene copies will were calculated using gBlocks as the standard curve in Bio-rad CFX manager software.

A protocol for sampling levels in bees homogenized for virus was also developed (Objective 2). It was designed to solubilize proteins taken from homogenate that resulted during crushing of bees for viral analysis. The protocol developed involved the following steps:

* Step 1: Add 500 ul trizol reagent to crushed bee stored at -80C
* Step 2: Vortex sample thoroughly to ensure it is mixed
* Step 3: Incubate on ice for 10 min.
* Step 4: Centrifuge @ 13,000 rpm for 15 min and separate the protein containing layers
* Step 5: Store supernatant @ -20C.
* Step 6: Dilute 10ul of supernatant in 190ul of PBS buffer (1:20) in a 1.5ml Eppendorf tube.
* Step 7: Set up three strips of 0.5ml tubes with 10ul of commassie reagent for every 8 samples being measured. (ie. run in triplicate)
* Step 8: Add 10ul of diluted sample to each appropriately labelled tube.
* Step 9: Vortex tubes gently to ensure they are mixed.
* Step 10: Tap out bubbles and briefly spin down with centrifuge.
* Step 11: Using 8-loader pipette measure samples using a Nanodrop 8000.

The protocol was successful in quantifying protein but there appears to be some interference between the binding dye, Commassie and the Triazol detergent used to soluabilize membranes. This interference caused some variation in ability to replicate samples and generate a standard curve. In order to improve the assay we intend to replace the Triazol with Triton. This should resolve the interference issue and allow more accurate and reliable determination of protein levels in these samples crushed for viral analysis. This will provide a comparatively cheap potential way of assessing the relative health of winter bees.

**Statistical analyses.**

Data analysis was carried out using proc mixed in SAS 9.3 statistical analysis software to run repeated measures ANOVA (except where stated otherwise). For Objective 3, repeated measures designed to determine if the timing of the application of acaricide impacts virus levels and colony performance was used.

1. **Developing best management practices for production of healthy nucleus bee colony (3-10 frames of bees) with acaricides’ rotation strategies to replace dead colonies**

**Testing hypothesis.**

Nucleus colonies made of 3 and 5 frames of bees can form a healthy unit to replace killed bee colonies.

**Methodology.**

This experiment was conducted in Saskatchewan; split plot design was used for this objective of the project. Factor 1 was determined as nucleus colonies being started with 3 frames of bees vs 5 frames of bees. Factor 2 was evaluated as treatment of varroa (7 treatment groups) and number of replicates (6 nuclei replicates) per treatment.

During the months of May and June, 2014 donor colonies for the project were collected from beekeepers throughout Saskatchewan. The collected colonies were placed in one of five bee yard locations in and around the Prince Albert/MacDowall area of Saskatchewan. During the spring and summer these colonies were evaluated for strength and thoroughly assessed for disease. Numerous inspections were performed for the brood disease American foul brood (*Paenibacillus larvae larvae*) as such an infection would have severely hampered the project.

On July 24th and 25th, 2014 the nucleus colonies (nucs) were created from the donor colonies. For the 3 frame nucs, 1 frame of bees with brood, 1 frame of bees with honey and one empty brood frame were combined in a 3 frame nuc box. In the 5 frame nucs, 2 frames of bees with brood, one frame of bees and honey were combined with one honey filled dark comb frame and one empty drawn dark brood frame. Frames and bees were screened into the colonies for 24 hours and had 10 day old queen cells added on July 27th, 2014.

On August 14th and 15th, 2014 the nucs were assessed for their queen-right status. The cell was first checked for hatch success, then the colony was checked for all brood stages including eggs, larva and capped brood. All queens, if successfully raised, were found in the colony and marked with green paint on the abdomen for easy identification.

On September 10th the successfully raised nucs were evaluated for brood area, queen-right status and had 300 bees collected into alcohol for Varroa sampling. On September 11th the remaining nucs were randomly assigned to one of four treatment groups: non treatment control, Apivar treated, formic acid treated and oxalic acid treated colonies. All treatments were applied to label direction or to current best management practice. Adult population was assessed by counting the number of bee spaces filled. Colonies were sampled again for Varroa on October 10th, and weighted on October 24th before being placed indoors for winter. Throughout the experiment the colonies were managed as commercially operated nucs would be. This included moving the nucs to 5 or 10 frame boxes as they expanded, and feeding them sugar syrup in preparation for winter.

Bee population and brood area were determined once monthly from the beginning of the beekeeping season until October, with Varroa prevalence being determined at the same time as the determining the bee population. The nuclei were treated in the fall and were first established according to their respective treatment groups (see above). Relative acaricide treatment effectiveness was monitored with standard alcohol wash methods. All study nuclei were wintered at indoor facilities in Melfort, SK for all winters throughout the course of the project. Survivorship and colony strength were determined as they were brought out of the indoor facilities each spring; Colony development was followed through July.

Two more rounds of nucs were started in 2015. “Early” made nucs were started on June 9th and 10th. As in the first year, three and five frame nucs were made up from donor colonies. The three frame nucs were established with one frame of bees and brood, one frame of bees and honey and one frame half honey, half empty. The five frame nucs were started with 2 frames of bees and brood, one frame of bees and honey, one frame of honey, and one empty frame. The nucs were given a queen cell 24 hrs later. “Late” made nucs were started, with the same three and five frame configuration, on July 13th. These too were given a queen cell 24 hrs later and left to establish. Three weeks following making the nucs their first assessment was performed to determine their queenright status and the health of the colony. The Early made nucs were given honey supers if required, the Late made nucs did not require honey supers during their first summer.

The first experiment was started in June 2014 and continued until October 2015, while the second experiment began in June of 2015 and continued until August of 2016.

**Statistical analyses.**

Data analysis was carried out using proc mixed in SAS 9.3 statistical analysis software to run repeated measures ANOVA (except where stated otherwise).

For Objective 4, brood area, Varroa percent infestation, honey yield, and cluster size were measured at varying intervals throughout the study period, and analysed to determine effects from treatment group, nucleus colony start size (3 or 5 frames), and interactions between the two

**DISCUSSION**

1. **Evaluation of virus presence and concentration in bee colonies throughout the year to establish their relation to varroa populations, treatment time impacts on bee population growth and colony mortality**

*\*For Objective 3, ‘Experiment 1’ shall refer to study colonies started in 2014, and ‘Experiment 2’ shall refer to study colonies started in 2015. Both ‘Experiments’ were run for two seasons.*

**Experiment 1**

**Varroa, Experiment 1:**

In experiment 1, the initial starting infestation of Varroa mites when colonies were first sampled in fall, ranged from 6.2 + 2.3 to 11.1 + 2.3 mites per 100 bees (Fig. 1). For Varroa there were significant overall treatment (P <0.0001) and date effects (P <0.0001) and the relative mite levels in each treatment varied with date (P <0.0001). Acaricide treatment was very effective in supressing Varroa on each of the treatment dates in spring and in fall. The only date on which mite levels were similar after the application of acaricides was on July 21, 2015. The different treatment regimens resulted in differences in the level of Varroa in the different treatments across season resulting in combinations of relatively high and low mite infestations at different times in the beekeeping season (Fig. 1).

**Viruses, Experiment 1:**

Only three viruses were affected by the acaricide treatments, DWV, BQCV and ABPV. DWV was found in all samples that were tested (100% prevalence) in experiment 1. DWV levels at the start of the experiment in September 2014 where high mite levels were present ranged from 1.2E8+2.08E8 to 3.7E8+2.08E8 virus copies per µg of RNA. For DWV there was a significant effect of acaricide treatment schedule on virus concentration that varied with season (Fig. 2). DWV levels varied with treatment only on May 15, 2015 and in the period from mid-August (Aug 10, 2015) to late fall (22 October, 2015) (P <0.004). Significant seasonal fluctuations in DWV level were observed in each of the three acaricide treatment scenarios (P <0.04) but not in the untreated control group where virus levels remained high throughout the experiment (p >0.09). DWV levels peaked in mid fall of 2014 and decreased in October in both treated and untreated colonies. However, virus levels remained at comparatively high concentration in May of 2015 relative to early fall 2014 (6.76971E7+ 2.0805E8 to 5.1748E8+2.1931E8 virus copies per µg of RNA) even in colonies that had mite levels reduced by acaricide treatment.

BQCV was also common and found in 100% of samples tested. For BQCV there was also a significant overall treatment effect (P < 0.0305) that varied with season (P < 0.0305 but this virus did not show any interactions between treatment and season (P < 0.4515) (Fig. 3). Concentrations of BQCV were higher in colonies treated in spring or spring and fall than in untreated controls (P < 0.05) but concentrations in fall-treated colonies were between the controls and other treated colonies and did not differ from either of these ( P > 0.05).

ABPV was relatively rare with a prevalence of 2.4%. However, ABPV also showed an overall treatment effect (P < 0.0239) (Fig. 4). Although the overall influence of date was not significant (P > 0.1452) the relative influence of treatment did vary with date (P < 0.0441). Only the spring and fall treated colonies varied significantly over time (P <.0001) and there were no differences in virus concentration between untreated colonies and those treated in spring and fall.

*Fig.1* Effect of acaricide treatment and season on VARROA level, *Experiment 1*

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Fig.2 Effect of acaricide treatment and season on DWV level, Experiment 1



Fig.3 Effect of acaricide treatment (left panel) and season (right panel) on BQCV level, Experiment 1



Fig. 4 Effect of acaricide treatment and season on ABPV level, Experiment 1



Three viruses, IAPV (P >0.919), SBV (p > 0.7988) and CBPV (p > 0.6999), were not affected by acaricide treatment but did show significant seasonal variation. IAPV had a prevalence of 37.8% of bees infected with detectable levels, but showed no interaction between treatments and season (P >0.7485) (Fig. 5). Seasonal fluctuations in virus were apparent with a large peak in concentration occurring in 2015 (P <0.0001).

SBV was also common and found in 55.8% of samples. SBV concentration was not affected by acaricide treatment schedule and there was no interaction between treatments and season (P > 0.9812) (Fig.5). Seasonal fluctuations were significant but different from those seen in IAPV. For SBV, there were apparent with “peaks” in concentration appearing in fall of 2014 and 2015 with another prominent peak in May, 2015 (P < 0.0001).

CBPV was relatively common, occurring in 33.6% of samples. Its concentration was not affected by acaricide treatment schedule and there was no interaction between treatments and season (P > 0.5004) but seasonal fluctuations different from those seen in IAPV (Fig. 6). For CBPV, there was a small peak in the fall of 2014 and very high levels in the first sample after bees were moved from winter quarters in 2016 (P < 0.0023).

KBV was rarely found in detectable levels and only detected in 2 samples (prevalence 0.59%) in experiment 1. Its concentration did not vary with treatment (P > 0.4388) or date (P > 0.2976) and there was no interaction between treatment and date (P > 0.1334) (Fig. 6).

**Nosema, Experiment 1:**

Nosema spore level was quantified on only three sampling dates in fall of 2014 and one on May 16, 2016. Sixty nine percent of colonies had detectable levels of spores in 2014 but only 28% of colonies were infected on May 16, 2016. Mean Nosema spore counts per bee averaged over samples of 30 bees showed Nosema spore levels were not significantly influenced by acaricide treatments ( P < 0.07) and there were no significant interactions over time. Nosema level did vary with date (P < 0.0001) showing an increase in spore level from early to late fall (Fig. 7) in 2014 and were at much higher levels following the second winter. In the fall of 2014, Nosema spp. spore samples which were not analyzed for species composition showed a significant correlations with BQCV in the same fall (R2 0.52 P= 0.003). October 2014 samples were also correlated with KBV and IAPV the following spring (R2 0.55 P= 0.0005 and R2 0.45 P= 0.007 respectively) In the single sample taken in spring of 2016, sores of *Nosema spp*. and gene copies of *N. apis* and *N. ceranae* were not correlated with each other or any other pathogens measured on that date, or colony size.

Fig. 5 Seasonal change in SBV (left panel) and IAPV (right panel) concentrations, Experiment 1.



Fig. 6 Seasonal changes in CBPV (left panel) and KBV (right panel) concentrations, Experiment 1.



Fig. 7 Seasonal changes in Nosema spore levels per bee (left panel) and species composition (right panel), Experiment 1 (no samples were estimated from 15 Nov, 2014 to 15 May 2016).

**Colony Performance, Experiment 1:**

The different acaricide treatments created a range of parasite and pathogen conditions that were assessed for their impact on colony performance and so that potential relationships between viruses and colony loss could be examined under different levels of parasite pressure. Brood production varied seasonally throughout the fall of 2014 and summer of 2015 (P < 0.001) but was not affected by treatment in either period (P>0.918) and there was no interaction between treatment and time (P>0.938) (Fig. 8). Winter weight loss, which is indicative of the number of bees in the colony throughout winter (larger colonies consume more food) also did not vary in the first winter indicating good survival through the winter months (Fig. 9). Colony cluster size was assessed three times in the fall of 2014 and twice in the spring of 2015(Fig. 9 & 10). Cluster size did not vary with treatment in either fall of 2014 or spring of 2015 (P>0.0826). Cluster size did vary by season (P<0.001) but there was no interaction between season and date (P >0.05).

Honey production was measured only in the summer of 2015 and was not significantly affected by acaricide treatment schedule whether colonies that died were counted as zero for production (p>0.566) or excluded from analysis (P>0.882) (Fig. 11).

Fig. 8: Seasonal Changes in Brood Area in fall 2014 and summer 2015, Experiment 1.



Fig. 9: Fall cluster size in 2014(left panel) and colony winter weight Loss 2014/15 (right), Experiment 1.

Fig. 10: Spring cluster size in April (left panel) & May (right) following 1st winter 2014/15), Experiment 1.



Fig. 11: Honey production per colony in summer of 2015, Experiment 1.



**Worker population following 2nd winter, Experiment 1:**

Worker population in May of 2016 varied with acaricide treatment schedule (P < 0.02 ) and was higher in colonies treated in spring and fall than in untreated colonies (Fig.12). Colonies treated in fall or in spring had population levels that were intermediate between the other two treatment groups and did not differ from them or each other. There was also significant variation among treatments and colonies treated in fall only showed significantly more variability in worker population levels than in colonies treated in spring and fall where populations were consistently large.

Fig. 12: Effect of acaricide treatment schedule on mean worker population size following winter of 2015/2016, Experiment 1. Means followed by the same letter are not different. Horizontal bar with “8” indicates difference in variance among treatments.



**SASK YEAR 2**

**Varroa, Experiment 2:**

In experiment 2 the starting infestation rate of Varroa was higher than in experiment one and ranged from 11.4 + 3.1to 16.1+ 3.1 mites per 100 bees on the first sample collected on 9 September. Acaricide treatment schedule still had a significant overall impact on Varroa mite infestation level (P < 0.0025) which varied throughout the season (P < 0.0025) (Fig. 13). Mite levels also varied showing a typical pattern of increase in the proportion of bees infested as brood rearing ceased in the fall (P < .0006). However, under high mite loads, the spring treatment was not as effective in reducing Varroa and the lowest infestation date in spring to fall still had 8.6 +5.3 mites per 100 bees. In contrast, colonies that had been treated the previous fall still had only 0.98 + 3.7 mites per 100 bees. With that exception the infestation curves showed a similar trend to that found in experiment 1.

**Viruses, Experiment 2:**

In experiment 2, there was only one virus that showed significant effects associated with acaricide treatment or seasonal interactions and that was CBPV that had shown no interactions with treatment in experiment 1. For DWV, trends in seasonal virus levels in different treatment groups showed trends similar to that in experiment 1 but were not statistically significant. This is likely due in part, to the lower sample size resulting from high winter mortality in the 1st winter of experiment 2. Since virus levels were significantly correlated to production data these values are plotted by treatment and date for DWV below.

**DWV, Experiment 2:**

Deformed wing virus was found at detectable levels in all but two samples and had a prevalence of 99.1%. In experiment 2, deformed wing virus concentration was not significantly affected by acaricide treatment (P < 0.77) and treatment showed no significant interaction with date (P < 0.28) (Fig. 14). DWV showed seasonal variation (P< 0.0001) with concentration increasing in the fall, declining over winter and remaining stable throughout the summer or increasing slightly towards fall. In experiment 2, initial DWV levels were higher in spring than in experiment 1 ranging from 5.7E8 + 3.0E9 to 9.0E8 + 3.0E9. As was the case in experiment 1virus levels rose in the fall however, in experiment 2 the level did not decline prior to winter as we found in experiment 1. There was however, a reduction in DWV level over the winter that mirrored that found in experiment 1. Despite this, virus levels were still very high in spring with 100% of samples having detectable virus and levels that ranged from 6.6E8 + 3.7E9 to 2.3E9 + 5.7E9. On June 2 virus levels appeared to have dropped in all treatment group and were 3.6E8 + 4.0E9 particles in untreated controls and 8.3E8 + 5.7E9 in the fall-treated colonies, however, these samples thawed in shipping and may underrepresent true virus levels. Virus levels subsequently “rose“ in untreated colonies in late August at the time of the last virus sampling period but remained relatively low in the other treatments. In late, August of 2016 the DWV concentration in surviving colonies averaged 3,482,427,200 virus particles per ug RNA in all colonies.

**CBPV, Experiment 2:**

CBPV

Chronic bee paralysis virus was found at detectable levels in 30% of samples tested. Although no overall treatment effects were found with chronic bee paralysis virus, there were significant interactions between treatment schedule and date (P < 0.0024) (Fig. 15). CBPV did not vary with season in spring-treated bees (P > 0.2535) but did in all other treatment groups (P < 0.002). Significant treatment differences were observed on 19 May and 2 August and had the opposite trend to that shown in DWV. When DWV was high, CBPV was low and vice versa.

Fig. 13: Effect of acaricide treatment and season on VARROA level, *Experiment 2*



Fig. 14: Effect of acaricide treatment and season on DWV level, Experiment 2.



Fig. 15: Effect of acaricide treatment and season on CBPV level, Experiment 2.



Four of the remaining viruses (BQCV, IAPV, SBV, and ABPV) did not show any treatment-related effects but did show variation in the seasonal phenology.

**BQCV, Experiment 2:**

Black queen cell virus was very common and detected in 98.7% of samples. Unlike experiment 1, BQCV was not affected by acaricide treatment schedule (P > 0.8029) and there were no interactions with treatment (P > 0.9860) (Fig. 16). The virus concentration changed over the time (P <.0001) and showed a pattern similar to that of CBPV.

**IAPV, Experiment 2:**

Israeli acute bee paralysis virus was found in 23.6% of samples. Acaricide treatment schedule did not affect IAPV concentration (P > 0.7269) nor was there a treatment\*date interaction (P > 0.4954). IAPV concentration (P < 0.0001) varied with date but was lower than in experiment 1 and showed a different seasonal phenology in experiment 2 (Fig. 16). In experiment 2 it was at its highest levels in the fall of the first fall after mites were introduced whereas in experiment 1 it peaked in the late summer of the following season.

**SBV, Experiment 2:**

Sacbrood virus had prevalence similar to that of IAPV and was found in 21 % of samples. SBV did not vary with treatment (P > 0.1432) and there was no Treatment\*date interaction (P > 0.7146) (Fig. 17). There were seasonal changes in concentration (P <.0001) with levels being highest in early fall of the first year and remaining low and stable throughout the remainder of the season.

**ABPV, Experiment 2:**

Acute bee paralysis virus was more common in experiment 2 than in experiment 1, and was found in15.5% of samples compared to a prevalence of only 2.4% in experiment 1 (Fig. 17). There were no significant treatment effects (P > 0.896) or interactions between treatment and date (P > 0.992) but there was significant seasonal variation with the highest levels being found in the fall preceding the1st winter (P < 0.049).

**KBV, Experiment 2:**

KBV was also rarely detected in experiment 2 and had a prevalence of 0.9%. There were no significant treatment effects on virus concentration (P > 0.7807) nor were there any interactions between treatment and date (P >0.928) (Fig. 18). Concentrations were very low and did not vary significantly with season (P>0.7674).

Fig. 16 Seasonal changes in BQCV (left panel) AND IAPV (right panel) concentrations, Experiment 2.



Fig. 17 Seasonal changes in SBV (left panel) AND ABPV (right panel) concentrations, Experiment 2.



Fig.18. Seasonal changes in KBV concentrations, Experiment 2.



**Nosema**

*Nosema spp.* spore load (P > 0.73), gene copies of *N. apis* (P > 0.47) and gene copies of *N. ceranae* (P > 0.84) were not affected by the acaricide treatment schedule targeted at Varroa mites. The spore load measured by traditional techniques indicated that Nosema spore load differed among dates throughout the beekeeping season declined slightly in June and was highest in mid-May and early August (Fig.19). The two species that make up the composite *Nosema* spore load as well as *Nosema* in body tissues differed in their seasonal patterns (Fig. 20). *Nosema apis* showed a significant reduction from May to August and was at very low levels by the time of the last sample. In contrast, *N. ceranae* did not vary significantly among dates throughout the season. *N. ceranae* as well as *Nosema* spore levels (which include both *N. apis* and *N. ceranae*) had high positive correlations with all other pathogens (SBV, BQCV, DWV, ABPV, CBPV and KBV) except IAPV. *N. apis* which showed reduced concentrations throughout the summer season, showed similar positive correlations with BQCV, DWV ABPV and CBPV, but not DWV, SBV and IAPV (Fig. 20, Table 1). Correlations with Varroa, which was manipulated through acaricide use, were inconsistent and sometimes positive and sometimes negative.

Table1. Simple correlations between parasites, pathogens, brood production and measures of worker bee population in the fall of 2015 (October cluster score), spring of 2016 (April worker population), and fall of 2016 (September worker population).

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Nosema spp*. Spores | R2 | P= |  | *N. ceranae* gene copies | R2 | P= |  | *N. apis* gene copies | R2 | P= |
| **May-16** |  |  |  | **May-01** |  |  |  | **Jun-06** |  |  |
| SBVjun2116 | 0.88 | 0.0004 |  | KBVmay1616 | 0.61 | 0.0069 |  | CBPVjun0216 | 0.87 | <.0001 |
| BQCVmay1616 | 0.84 | 0.0021 |  | CBPVoct615 | 0.51 | 0.0186 |  | CBPVaug3016 | 0.65 | 0.0034 |
| **Jun-06** |  |  |  | **Jun-06** |  |  |  | VARsep9 | -0.62 | 0.0027 |
| DWVjun0216 | 0.72 | 0.0011 |  | BQCVmay1616 | 0.54 | 0.0205 |  | VARaug16 | -0.51 | 0.0497 |
| BQCVoct615 | 0.71 | 0.0101 |  | VARaug16 | -0.53 | 0.0418 |  | BQCVaug0216 | 0.51 | 0.0454 |
| DWVaug3016 | 0.65 | 0.0045 |  | KBVsep815 | 0.50 | 0.0234 |  | **Aug-02** |  |  |
| DWVaug0216 | 0.61 | 0.0094 |  | **Jun-21** |  |  |  | VARsep22 | 0.95 | <.0001 |
| BQCVjun0216 | 0.57 | 0.0269 |  | KBVmay1616 | 0.94 | <.0001 |  | KBVaug3016 | 0.95 | <.0001 |
| VARjun2116 | 0.49 | 0.0454 |  | CBPVoct615 | 0.93 | <.0001 |  | ABPVaug3016 | 0.95 | <.0001 |
| **Jun-21** |  |  |  | CBPVjun2116 | 0.68 | 0.0029 |  | CBPVaug3016 | 0.82 | <.0001 |
| DWVjun0216 | 0.82 | 0.0001 |  | KBVaug0216 | 0.61 | 0.0127 |  | CBPVjun0216 | 0.73 | 0.0033 |
| DWVaug3016 | 0.75 | 0.0006 |  | BQCVaug3016 | 0.53 | 0.0245 |  |  |  |  |
| DWVaug0216 | 0.63 | 0.0067 |  | **Aug-02** |  |  |  |  |  |  |
| VARjun2116 | 0.54 | 0.0241 |  | KBVaug3016 | 0.98 | <.0001 |  |  |  |  |
| **Aug-02** |  |  |  | ABPVaug3016 | 0.98 | <.0001 |  |  |  |  |
| DWVaug3016 | 0.87 | <.0001 |  | VARsep22 | 0.97 | <.0001 |  |  |  |  |
| DWVjun0216 | 0.83 | <.0001 |  | CBPVaug3016 | 0.78 | 0.0001 |  |  |  |  |
| **Aug-30** |  |  |  |  |  |  |  |  |  |  |
| ABPVaug3016 | 0.94 | <.0001 |  |  |  |  |  |  |  |  |
| KBVaug3016 | 0.94 | <.0001 |  |  |  |  |  |  |  |  |
| VARsep22 | 0.94 | <.0001 |  |  |  |  |  |  |  |  |
| CBPVaug3016 | 0.81 | <.0001 |  |  |  |  |  |  |  |  |

Fig.19. Seasonal changes in *Nosema spp.* spore loads, Experiment 2.



Fig. 20. Seasonal changes in *Nosema apis* concentration in homogenized bee tissue (left panel) and *Nosema ceranae* (right panel), Experiment 2.

**Brood Production**

There was an effect of treatment on the overall level of brood production (P < 0.045) with untreated control colonies having lower overall brood production than spring treated colonies (Fig. 21). The other acaricide treatment groups had brood areas that were similar to the spring treated group but they also did not vary significantly from the untreated control (P > 0.05). Brood production also varied seasonally (P < 0.00010) showing typical patterns, declining in late fall and remaining at a consistently higher level throughout the spring and summer seasons. Fall brood production was not negatively correlated with pathogens in the first fall. Summer brood production from May to August was negatively correlated with Varroa and DWV (Table 2). Unexpectedly, brood production was positively correlated with CBPV the previous fall (possibly because stronger colonies were better able to resist effects of this virus).

Table 2: Simple correlations between parasites and pathogens and summer brood production.

|  |  |  |
| --- | --- | --- |
| Summer Brood Production/16 | R2 | P= |
| Varroa, Oct. 9/15 | -0.67 | 0.002 |
| DWV, Aug. 02/16 | -0.66 | 0.004 |
| *N.* Spores, July 4 | -0.499 | 0.049 |
| *N. ceranae*, July 4 | -0.5193 | 0.0393 |
| Varroa, Aug. 16/16 | -0.51 | 0.046 |
| CBPV, Sept. 8/15 | 0.81 | 0.002 |

Fig. 21 Effect of acaricide treatment schedule on overall brood production (left panel) and seasonal pattern of brood production in fall of 2015 and spring and summer of 2016 (right panel), Experiment 2.



**Population Assessment:**

As was the case in experiment 1, fall cluster size in Experiment 2 of the first winter was not affected by treatment (P >.81) (Fig. 21). The effect of acaricide treatment schedules on worker population size in 2016 varied with date (P < 0.0389). In April 2016, there were no differences in worker populations among surviving colonies (excluding dead colonies) in the four acaricide treatment groups (Fig. 22). However, in September, surviving colonies that had been alive in spring had significant differences in their cluster scores. Untreated colonies had lower worker populations in September of 2016 than either the fall-treated, spring-treated or spring +fall-treated colonies (P <0.05). Treated colonies did not differ with respect to worker populations in the different treatment groups (P>0.05). When the colonies that died over winter were included in the analysis colonies treated in spring and fall had more worker bees than untreated control colonies or colonies treated only in spring (Fig. 23). Fall treated colonies had populations that were intermediate in size and did not differ from the untreated or spring and fall treated colonies (Fig. 23). Correlations among viruses, Nosema and colony size related metrics in different seasons are shown in Table 3.

Table 3. Simple correlations between parasites, pathogens, and measures of worker bee population in the fall of 2015 (October cluster score), spring of 2016 (April worker population), and fall of 2016 (September worker population).

|  |  |  |
| --- | --- | --- |
| Oct. Cluster Score | R2 | P= |
| BQCV, Aug. 02/16 | -0.45 | 0.045 |
| Varroa, Sept. 9 /15 | -0.52 | 0.003 |
| Varroa, Oct.9/15 | -0.43 | 0.004 |
| April Work Pop | R2 | P= |
| Varroa, Sept. 9 /15 | -0.36 | 0.044 |
| Brood, Oct. 9/15 | -0.48 | 0.016 |
| Brood, May 19 | 0.6 | 0.006 |
| Varroa, Oct.9/16 | -0.55 | 0 |
| Sept. Worker Pop | R2 | P= |
| Brood, Aug. 2/16 | 0.52 | 0.026 |
| Brood, Aug. 17/16 | 0.61 | 0.007 |
| Total brood/16 | 0.69 | 0.001 |
| DWV, Aug. 02/16 | -0.59 | 0.008 |
| DWV, Aug. 30/16 | -0.59 | 0.007 |
| N.Apis, July 4/16 | -0.57 | 0.014 |
| N. ceranae, July 4/16 | -0.58 | 0.012 |
| N. spores 5 Aug/16 | -0.57 | 0.013 |
| N. ceranae,5 Aug/16 | -0.67 | 0.003 |
| Varroa, Oct.9/15 | -0.52 | 0.008 |
| CBPV, Aug. 30/16 | -0.57 | 0.009 |
| KBV, Aug. 30/16 | -0.54 | 0.015 |

Fig. 22 Effect of acaricide treatment schedule on worker bee cluster size in October, 2015 (left panel), and worker bee population of surviving colonies on April 2016 and September 2016 (right panel), Experiment 2.



Fig. 23 Effect of acaricide treatment schedule on spring 2015 worker bee populations in all colonies that were alive the previous fall October, 2015, (Dead colonies were recorded as zero bees) Experiment 2.

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**Honey Production**

Total honey production in surviving colonies was affected by acaricide treatment schedule in 2016 (P < 0.0038) (Fig. 24). The colonies with the highest honey production on a per colony basis had been treated with acaricide the previous fall and in spring and produced more honey than colonies treated only in fall (P <0.05) but not those treated only in spring (P >0.09). As expected honey production was positively correlated with brood production and negatively correlated with Varroa infestation level (Table 4). The honey production was positively correlated with KBV level but since the prevalence of this virus was so low (only two positive colonies were found), this is likely not a causal relationship. KBV was also correlated with other pathogens. Although no pathogens were directly negatively correlated with honey production through simple correlations, several were negatively correlated with brood production which in turn, is correlated to honey production.

Table 4: Simple correlations between brood production, parasites and pathogens on honey production.

|  |  |  |
| --- | --- | --- |
| Total Honey | R2 | P= |
| Brood, May 19/16 | 0.60 | 0.008 |
| Brood, Aug. 2/16 | 0.65 | 0.005 |
| Brood, Aug. 17/16 | 0.62 | 0.008 |
| Total brood/16 | 0.63 | 0.005 |
| KBV, June 02/16 | 0.61 | 0.034 |
| Varroa, July 16/16 | -0.62 | 0.014 |

Fig. 24 Effect of acaricide treatment schedule on honey production in summer of 2016, Experiment 2.

**Multivariate Analysis: Effect of pathogen combinations on Winter Mortality/Survival:**

Multivariate statistics were used to look at major predictors of colony death (logistic regression) and effects of combinations of parasites and pathogens on colony population size in spring. For predicting colony death, DWV and Varroa were identified as the best predictors of colony mortality when colonies were exposed to high mite loads, varying mite loads over extended periods. When sampling colonies for Varroa and virus in early September, DWV alone was sufficient to predict colony death over winter. The maximum level of virus that would provide less than 15% winter loss varied with the level of Varroa mite control. Colonies with consistently low Varroa or those treated in fall could tolerate higher DWV concentrations without experiencing loss than treatments with consistently high mite levels (controls) or fluctuating mite levels (spring treated colonies) (Figure 25). Levels of DWV that would have low and high probabilities of causing colony death are shown in Fig. 25.

For colonies sampled in mid to late September that had previously been exposed to high or fluctuating mite levels Varroa mite infestation level (mites per 100 bees) was the best predictor of colony mortality (Figure 26). When colonies had been previously exposed to high levels of mites, it predicts that the number of mites in mid to late September must be very low if colony mortality below 15 to 20% is desired (Fig.26).

For colonies sampled in mid-October, a combination of Varroa mites and deformed wing virus provided the best predictions of colony mortality (Figure 27). The figure shows that in order to have high colony survival through winter bees in mid-October would have to have very low levels of Varroa with moderate DWV levels, very low levels of DWV with moderate Varroa levels or low levels of both the pathogen and parasite.

When considering the maximum concentration of any of the seven viruses, DWV was also the best predictor of colony death over winter (Figure 28) when colonies had been exposed to high or fluctuating mite levels over a long period of time.

Figure 25: Logistic regression to predict major factor(s) resulting in colony death over winter based upon colony sampling in early September for Varroa, DWV, IAPV, BQCV, SBV, CBPV, ABPV, and KBV under four Varroa mite treatment schedules. Controls were untreated, Fall was treated only in fall, Spring was treated only in spring and Spring and Fall were treated in both spring and fall. Data include Year1 experiment, 1st fall, Year1 experiment 2nd fall and year2 experiment 1st fall. Red lines indicate virus level where probability of survival would be equivalent to 85% which is equivalent to what is generally considered an acceptable winter loss of 15%. Control and spring treatment did not differ from each other but had lower survival probability expectations than fall-treated or spring and fall treated.

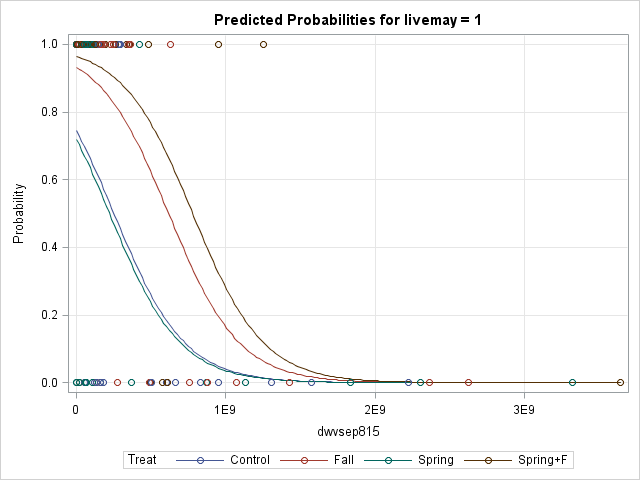


Figure 26: Logistic regression to predict major factor(s) resulting in colony death over winter based upon colony sampling in mid-September for Varroa, DWV, IAPV, BQCV, SBV, CBPV, ABPV, and KBV under four Varroa mite treatment schedules. Treatments were not significant predictors of colony death in the model. Controls were untreated, Fall was treated only in fall, spring was treated only in spring and spring and fall were treated in both spring and fall. Data include Year1 experiment, 1st fall, Year1 experiment 2nd fall and year2 experiment 1st fall. Red lines indicate virus level where probability of survival **(with a combination of viruses + Varroa)** would be equivalent to 85% which is equivalent to what is generally considered an acceptable winter loss of 15%.

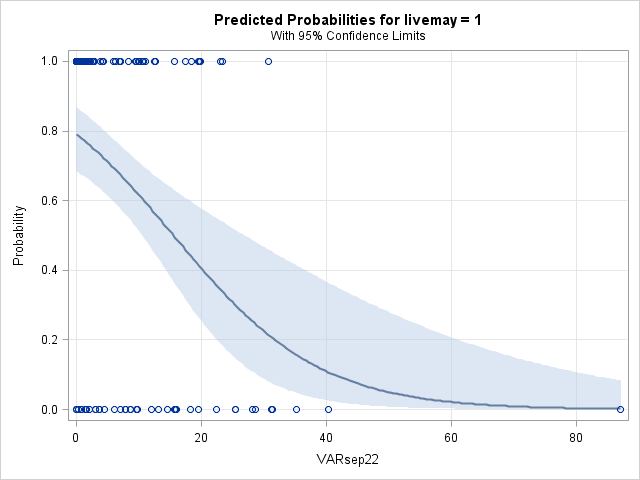


Figure 27: Logistic regression to predict major factor(s) resulting in colony death over winter based upon colony sampling in early October for Varroa, DWV, IAPV, BQCV, SBV, CBPV, ABPV, and KBV under four Varroa mite treatment schedules. Combinations of both Varroa and DWV were significant predictors in the model. Treatments were not significant predictors of colony death in the model. Controls were untreated, Fall was treated only in fall, Spring was treated only in spring, and Spring and Fall were treated in both spring and fall. Data include Year1 experiment, 1st fall, Year1 experiment 2nd fall and year2 experiment 1st fall. Red lines indicate virus level where probability of survival **(with a combination of viruses + Varroa)** would be equivalent to 85% which is equivalent to what is generally considered an acceptable winter loss of 15%. Bottom Axis indicates percentage of Varroa on adult bees (mites per 100 bees) vertical axis indicates DWV virus level in virus particles per ug RNA. Lines and colours on interior of graph indicate probability of colony death over winter (red is higher survival, blue is lower survival). Lines at 45 degree angle indicate 80%l, 60%, 40% and 20% survival.

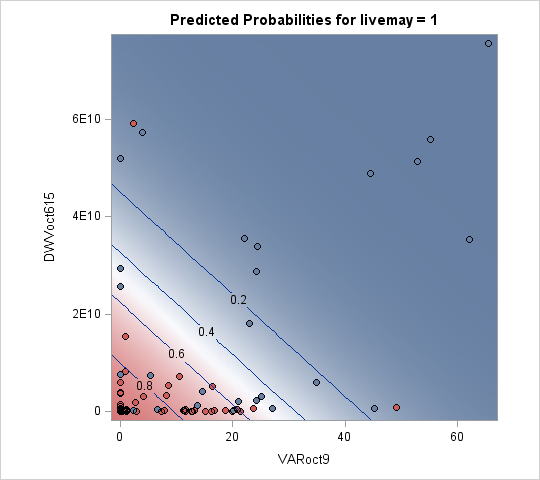
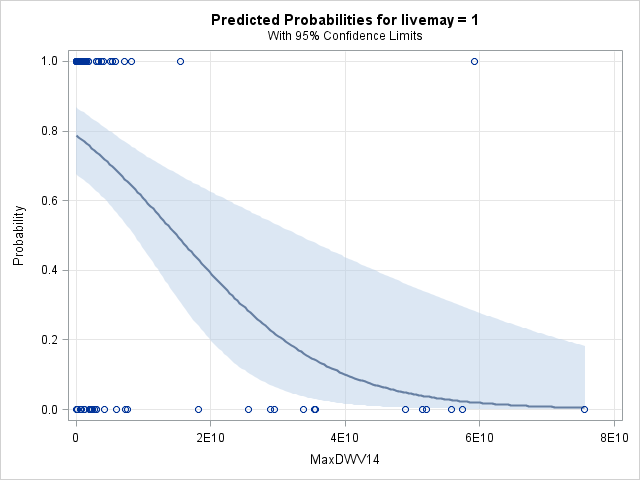


Figure 28: Logistic regression to predict major factor(s) resulting in colony death over winter based upon colony sampling over all dates in fall where colonies had been previously exposed to high and variable Varroa mite levels and examining the **Maximum level of DWV, IAPV, BQCV, SBV, CBPV, ABPV, and KBV** in any treatment period **and late October Varroa** under four Varroa mite treatment schedules. DWV was the only significant predictor in the model. Treatments were not significant predictors of colony death in the model. Graph indicates probability of survival based upon DWV concentration in virus particles per ug RNA. Red lines indicate virus level where probability of survival **(with a combination of viruses + Varroa)** would be equivalent to 85% which is equivalent to what is generally considered an acceptable winter loss of 15%.



**Multivariate Analysis: Effect on Spring Population Size of *Surviving* Colonies:**

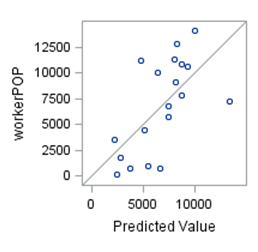
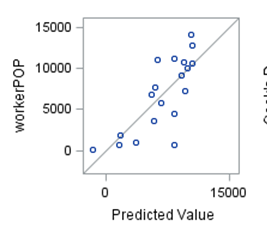
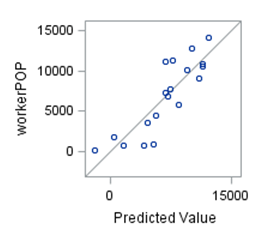
Spring population size varied considerably in the three years when it was measured across the two experiments. In year 1 experiment, spring 1, colony survival was very good and colony sizes did not vary significantly in the year 1 experiment, spring 2, there were significant differences in both survival of colonies, the size of surviving colonies and the variation in colony size within the different combinations of Varroa and virus created by the treatments. Finally, in experiment 1, spring 1 there were differences in colony mortality from the high mite levels the year before but variation in the size of spring populations that survived was not as high at the time it was quantified. Therefore separate multivariate regression analysis were carried out on each of the three fall pathogen and spring population size combinations to quantify the factors most highly correlated with variation in population size. Stepwise regression was used to reduce the number of parameters selected in each model.

Beekeepers wanting to estimate the impact of Varroa and pathogens on their colonies would most likely sample either in early fall or late fall to estimate what the potential impact of pathogens is on winter survival. Therefore, we compared prediction models for predicting spring population of surviving colonies on: (1) samples taken only in early fall for Varroa and the seven economically important viruses; (2) the minimum level of Varroa found throughout the fall sampling period with the maximum level of virus detected in any of the three fall sampling periods and (3) the maximum level of Varroa found throughout the fall sampling period with the maximum level of virus detected in any of the three fall sampling periods.

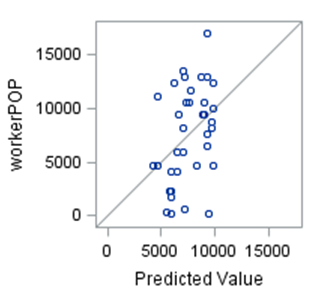
The relationships and most important parameters selected for each case are shown below. Making predictions based upon sampling in **early September for Varroa** and all other virusesgave a correlation R2 for the model of 0.4 with a significance level of P= 0.017, where the two most important parameters were Varroa and deformed wing virus with partial correlation coefficients (controlling for all other factors) of 0.16 and 0.19 respectively (Fig.29). Using the **minimum Varroa** level and maximum virus concentrations throughout fall the overall model had an R2 of 0.58 P= 0.0009 and the two most important parameters were DWV and BQCV with partial correlation coefficients (controlling for all other factors) of 0.48 and 0.17 respectively. Finally, using the **maximum Varroa** level and maximum virus concentrations throughout fall the overall model had a R2 of 0.74 P= 0.0009 and the three most important parameters were Varroa, partial correlation coefficient R2 =0.38, DWV partial correlation coefficient R2 = 0.52, and BQCV partial correlation coefficient R2 = 0.18.

For experiment 1, fall 1 and experiment 2 fall 2, surviving colonies showed no significant differences in colony size so it is not unexpected that multiple correlations among the suite of pathogens and spring population size were not significant (even though dead colonies were associated with DWV see above). The lack of correlation in experiment 2 might also result from the small sample size of colonies left alive in spring. Therefore, we pooled the results from experiment 1 and experiment 2 to see if there were any subtle effects on population size of survivors. The overall model had a R2 of 0.23 P= 0.007 and the three most important parameters were SBV, partial correlation coefficient R2 = 0.17, KBV partial correlation coefficient R2 = 0. 0.11, ABPV partial correlation coefficient R2 = 0.06, and DWV partial correlation coefficient R2 = 0.05.

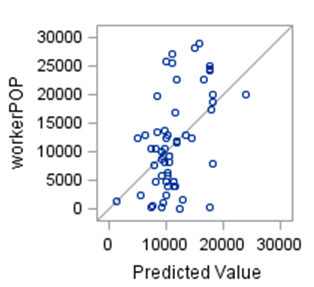
**Fig. 29. Fall of 2nd season of exposure to mites and varying acaricide treatments. Left panel = Estimated relationship based upon mite and pathogen Samples all collected on 9 sept.; Middle panel = Estimated relationship based upon minimum mite level found throughout the fall period and maximum virus concentration of all seven viruses at any time throughout the fall; Left panel = Estimated relationship based upon maximum mite level found throughout the fall period and maximum virus concentration of all seven viruses at any time throughout the fall;**

**Fig. 29. Fall of 1st season of exposure to mites and varying acaricide treatments experiment one. In addition to Varroa and the seven viruses, Nosema spore counts were included in the multivariate analysis. Colony survival in this season was very good, and there was little variation in colony size of surviving colonies. In examining the partial correlations and multivariate models there were no significant correlates with the spring colony size. There were also no significant relationships between pathogens and the size of surviving colonies for Experiment 2. Shown are the correlations with samples from the maximum mite infestation, virus infestation and Nosema spore level samples.**



**Fig. 30. Fall of 1st season of exposure to mites and varying acaricide treatments pooled for experiment 1 and experiment 2. Varroa and the six of the seven viruses were included. Nosema spore counts were not included in the multivariate analysis because they were not represented in both experiments and could not be modeled; BQCV was not included because it was highly correlated with DWV in experiment 2. Surviving colonies had some variation in colony size; the best predictors in this case were SBV followed by KBV, ABPV and DWV.**



**DWV Virus strains in this Study**

Virus strain is thought to affect the relative impact on colony health. Little is known about the effects of viral strain on bees in Canada. Twenty four samples of bees from experiment 1 taken on 15 and 25 September were analysed for the type of DWV strain present. All samples contained the DWV A strain. One sample contained both the DWV A and DWV B strain and within that sample DWV A was at numerically higher concentrations than DWV B.

1. **Developing best management practices for production of healthy nucleus bee colony (3-10 frames of bees) with acaricides’ rotation strategies to replace dead colonies**

**Brood Area:** In the first assessment of brood area after establishment, which occurred in September and October of the year they were made, colonies showed significant differences. For late made nucs, those made with 5 frames showed more brood in September, 2014 (Pr>F=<0.0001), October, 2014 (Pr>F=0.01), and again in September, 2015 for the second replicate (Pr>F=0.018). Early made nucs in 2015 followed this pattern, with 5 frame nucs producing significantly more brood than 3 frame nucs (Pr>F=0.031). Colonies would have only had a few months to build up after establishment, and 5 frame nucs had more space and resources to expand.

Across all replicates, brood area showed the most pronounced differences between starting sizes in late spring and early summer; this is around the peak of brood production in the season. Colonies followed a similar pattern of growth for the year after establishment until the following spring, regardless of timing.  In June and July, nuc start size was found to be statistically significant, or nearly so, in all three replicates of the experiment. In July 2015, late-made colonies started with 5 frames from 2014 produced significantly more brood (Pr>F=0.0002) than 3 frame colonies (Figure 31). By September, the difference becomes less pronounced and colonies went into autumn with similar levels of capped brood regardless of starting size. In 2016, 5-frame nucs started early in 2015 followed this pattern, producing significantly more brood than 3-frame nucs (Pr>F=0.017) (Figure 32). Finally, late made nucs with 3 frames were found to produce more brood in their second June, but the effect was not quite significant (Pr>F=0.077) (Figure 33). In June of 2016, 5 frame late made nucs from 2015 experienced a noticeable decrease in brood production (Figure 33); this replicate was the only one where 3 frame nucs surpassed 5 frame nucs in brood area during their second summer. A further look into the field notes from that assessment date showed that there was a large amount of swarming during that spring for this replicate. Six out of the seven colonies that experienced confirmed swarming (demonstrated by hatched swarm cells, virgin queens, and/or decreased populations) were 5 frame nucs, resulting in a dip in average brood production during that time.

Throughout the course of the project, Varroa treatment type did not have as great an impact on brood area as starting size. However treatment did show significant interactions with starting size in July, 2015 (Pr>F=0.0097) for late made nucs from 2014. Three frame nucs treated with formic acid and Apivar had late season peaks in brood production in August and September. This caused the significant gap in brood area between these and the oxalic acid-treated and control colonies in July, 2015. Treatment also showed significant interactions with starting size in September, 2015 for early made (Pr>F=0.019) and late made (Pr>F=0.01) nucs from 2015. Significant treatment effects were also shown in September, 2015 for early made nucs from 2015 (Pr>F=0.0082) and late made nucs from 2014 (Pr>F=0.01). At this point in the season, Varroa levels start to rise sharply and this highlighted differences between the treatments. However these effects occurred before first treatments were applied between September 18 and October 1, 2015, so the impacts and interactions of acaricide treatment groups are null.

Fig.31: Brood area for 2014 late made nucs by starting size

Fig. 32: Brood area for 2015 early made nucs by starting size

Fig. 33**:** Brood area for 2015 late made nucs by starting size

**Varroa:** Percent infestation of Varroa mites was analyzed for treatment and starting size effects across the length of the experiment. No major patterns were repeated across replicates; however a few nearly significant effects were noted. Treatment type did not prove to be an important factor in Varroa infestation for this experiment, with the only nearly significant treatment effect occurring in October, 2015 for late made nucs established earlier that year (Pr>F=0.088). Control colonies had high mite loads going into their first winter, with colonies treated with Apivar, formic acid, or oxalic acid showing lower levels. This effect was shown in both 3 frame (Figure 34) and 5 frame nucs (Figure 35). Starting size for nucs showed an effect on Varroa infestation for late made 2014 nucs in July, 2015 (Pr>F=0.053), but had no other significant effects throughout the project.

Fig. 34**:** Varroa percent infestation by treatment type, 3 frame late made nucs.

Fig 35**:** Varroa percent infestation by treatment type, 5 frame late made nucs.

Repeated measures analysis showed no significant difference between treatments; however at the final assessment date in August 2016, large biological differences appeared. Too much variability was present in the data to generate significant difference, with Varroa % infestation ranging from 1.45%-44.22% for late made nucs from 2014 (Figure 36), 11.41%-41.39% for early made nucs from 2015 (Figure 37), and from 32.26%-60.67% in late made nucs from 2015 (Figure 38). A larger sample size in future studies could help to reduce this variance and help illuminate differences between acaricide treatments. The average final % Varroa infestation values for treatment groups are found in Table 5, showing the Apivar treatment for late made nucs from 2014 having the lowest ending value at 1.45%. All study colonies displayed mite levels above the treatment threshold of 1% infestation, so all colonies would have required treatment at the end of the experiment.

Fig. 36: Average Varroa % infestation by treatment group for late made nucs from 2014

Fig. 37: Average Varroa % infestation by treatment group for early made nucs from 2015

Fig. 38: Average Varroa % infestation by treatment group for late made nucs from 2015

Table 5: Final average Varroa % Infestations by treatment across all years.

|  |  |  |  |
| --- | --- | --- | --- |
|  | **2014 Late Made** | **2015 Early Made** | **2015 Late Made** |
| Apivar | 1.454545 | 11.41349 | 35.62687 |
| Control | 44.21976 | 41.39247 | 43.98116 |
| Formic | 8.262724 | 31.44325 | 32.25681 |
| Oxalic | 23.17895 | 30.31522 | 60.67181 |

**Honey:** A Proc GLM was used to analyse the honey yield data. Neither Size nor timing of nuc establishment proved to be a significant factor for honey yield in this project. Yield was measured in the first and second years after establishment for early made nucs, and in the second year for late made nucs. A significant interaction was found for treatment and size at establishment (Pr>F=0.038) in the 2015 season for nucs established earlier that season (Figure 39). This is likely due to the small proportion of colonies that produced honey in their first season, which was only 32.26% of the early made nucs. Treatment effects should be null as honey yield was measured before treatments had been assigned and applied in 2015.

Fig. 39**:** Honey yield for 2015 early made nucs by start size

**Cluster Size:** Late made nucs from 2014 were assessed for cluster size across autumn and spring, and early and late made nucs from 2015 were assessed only on one date in autumn. The late made 2014 nucs showed significant effects from starting size across all dates, September, 2014 (Pr>F=<0.0001), October, 2014 (Pr>F=<0.0001), and May, 2015 (Pr>F=0.0005). Nucs started with 5 frames had significantly larger clusters of bees than 3 frame nucs (Figure 40).No observable trends regarding cluster size were demonstrated in the other replicates of the experiment.

Fig. 40: Cluster size for 2014 early made nucs by treatment and start size

**Winter Survival:** Colonies in Objective 4 were followed through two winters for nucs made in 2014, and one winter for those made in 2015. Winter survival was calculated as the percentage of colonies put into wintering facilities that were alive for the first check the following April. Due to study design, it was not possible to conduct statistical analysis of winter survival data between experiments in this Objective. However, it was noted that early made nucs showed the greatest percentage of surviving colonies overall (88.89% in 2016) (Table 6). Among the colonies in this replicate, nucs started with 5 frames had higher survival rates (90% in 2016) than those started with 3 frames (88.88% in 2016). Late made nucs from 2014 consistently had the lowest winter survival success for both years they were in the experiment (64.10% in 2015 and 20.00% in 2016) (Table 6). The “very late” timing of establishment for these nucs (made on July 25, 2014) could be a factor in their wintering success. Earlier “late made” nucs from 2015 (established July 13, 2015) had much better survival rates (86.36% in 2016), suggesting that the two week difference in “late” establishment timing had a noticeable effect on winter survival.

Table 6: Percentage of surviving colonies after winter for different nuc starting times

|  |  |  |
| --- | --- | --- |
| Nucs | 2015 | 2016 |
| 2014 Late made | 64.10% | 20.00% |
| 2015 Late Made | n/a | 86.36% |
| 2015 Early Made | n/a | 88.89% |

**CONCLUSIONS AND RECOMMENDATIONS**

This project has demonstrated that the successful application of best management practices for the control of the Varroa mite and viruses will contribute towards a sustainable and healthy honey bee stock. Of the seven viruses tested, DWV, BQCV, ABPV and CBPV showed reductions in virus levels and/or altered seasonal patterns in response to Varroa control. The other four viruses typically showed changes in seasonal phenology but were not directly influenced by mite treatments. Levels of some viruses such as DWV were found to remain at very high levels long after Varroa levels were reduced and can remain at high levels during critical periods in the fall when colonies are physiologically shifting and preparing for the production of the wintering population of bees. Several viruses as well as Nosema showed negative correlations with colony health and production parameters.

This study clearly showed that Deformed Wing Virus and Varroa mites working alone or in combination are primary factors associated with colony mortality. Other viruses and Nosema (particulary *Nosema ceranae*) can influence colony health and are correlated with reductions or variability in colony size in colonies that survive winter. DWV and black queen cell virus (BQCV) appeared to have the largest influence in the second winter as determined by multiple regression analysis. Other viruses SBV, KBV, ABPV appeared to have contributed to lower spring populations of bees but in the case of these pathogens, although negative partial correlations determined through multiple regression analysis were significant, they were comparatively weak relationships relative to those found for DWV.

DWV was shown to persist for long periods in colonies following colony exposure to high mite levels. This has major implications for management as the virus levels remain near that which are economically damaging so that even fairly low mite levels the following winter can result in higher probability of colony death over winter. Levels of this virus that have the potential to cause economic damage were identified and could be used as a tool to guide producers when making management decisions to mitigate colony loss.

This study highlights the need to keep parasites and pathogens under control and to ensure that Varroa, in particular, does not exceed economic thresholds. In order to keep bees in a sustainable fashion beekeepers will have to consider the impact of not only Varroa mites at the time of sampling but the history of past exposure to Varroa. Judgments made on thresholds for Varroa alone, without consideration of the virus level in the hives may lead to higher probabilities of winter loss. Regular management of Varroa to ensure it does not reach high levels should be a priority, but if for example, mite control failures occur or infestations are acquired from neighboring operations, this may be difficult to prevent. In these instances, particular care should be taken to have very good mite control in the following fall as this study shows viruses can still be very near the level where they can cause economic damage, and furthermore, the combination of moderate infestations of Varroa with these levels of virus could increase the probability of winter loss. Since controls are not currently available for viruses beekeepers need to rely on good integrated mite control practices and consider other cultural control options like the use of resistant stock, high genetic diversity, better nutrition and possibly comb management to help bees deal with the combinations of Varroa and viruses that may affect their hives.

This project has also demonstrated that the development of nuclei of 3-5 frames of healthy bees to replace dead colonies will help in developing a sustainable industry and alleviate concerns of the public regarding bee kill and colony collapse disorder. If losses to honey bee colonies are reduced to the long term historic level of 15% loss over winter, the Saskatchewan beekeeping industry could increase its economic stability, having favourable outcomes for beekeepers. This includes what is saved by not needing to pay to replace dead colonies and the loss of potential honey production due to high bee hive mortality in Saskatchewan. The improved understanding of how interactions among parasites and pathogens affect colony health should result in more reliable production and maintenance of honey bee colonies and nucleus colonies (Objective 4). Making nucs earlier in the season with more frames of bees create hardy, productive colonies in our prairie climate that can be used to replace winterkilled or dead colonies. These factors combined should alleviate pressure to maintain their stock for beekeeping operations and allow them to expand and/or provide excess bees for sale or to fulfill expanding needs for colonies used in crop pollination.

The knowledge gained through this research has been broadly distributed to beekeepers directly through talks at their industry meetings (eg. Saskatchewan, Manitoba, Ontario). Provincial Apiculture specialists and other technology transfer teams were also informed of the results so they can better inform their clients and stakeholders.

**SUCCESS STORIES/PRACTICAL IMPLICATIONS**

Producers interested in testing colonies for virus levels should consider the implications of indoor vs. outdoor wintering **(see “follow up research”)** when judging the potential impact of viruses on their colonies. If high virus levels are detected in their colonies in fall they might also consider wintering indoors as a potential management technique to help mitigate their potential losses.

It is important to note that the late started nucs in 2014 and 2015 were established almost two weeks apart; 2014 late made nucs were started on July 25, 2014 but in the next replicate were started earlier on July 13, 2015. This difference in date seemed to have an impact on the success of the colonies; only 5 out of the originally established 39 nucs from 2014 survived into the spring of 2016. Starting size also showed effects on cluster size into the second season, showing that 5 frame nucs have greater populations than 3 frame nucs when starting them after July 1st. The increased wintering success of the “earlier” late made nucs from 2015 (having much higher winter survival than the “later” late made nucs from 2014) add to this picture. Based on these observations, July 25 is likely too late in the season to build nucs that will last multiple seasons in a northern Saskatchewan climate. With the significant success that the 5 frame nucs had over 3 frame nucs in this replicate (brood area, cluster size, and first season winter survival), it is important to make your nucs as strong as possible (5 frames over 3) if you are making them later in the season.

**PATENTS/IP GENERATED/COMMERCIALIZED PRODUCTS**

N/A

**TECHNOLOGY TRANSFER ACTIVITIES**

Currie Rob, et al. 2017. Costs and Benefits of Using Marker Assisted Selection for Breeding Resistance Stock and the Impact of the Suite of Pathogens Bees Face. Manitoba Beekeepers Association. Winnipeg Manitoba. Airport Hilton Hotel, February, 17, 2018.

Currie, Rob 2017. Varroa and viruses.Joint meeting of the Ontario and New York Beekeepers association in Niagara Falls on Nov 16 and 17. Invited symposium speaker.

Currie, Rob 2017 Current status and trends in honeybees in Canada March 21 Kelowna BC science and stewardship of pollinators. UBC Okanogan, Kelowna BC

Currie, Rob 2017. Implications of long term effects of viruses on the management of bees. The annual general meeting of the Manitoba Beekeepers Association, Winnipeg, Manitoba, 24 February, 2017.

*Rob Currie,Graham Parsons, and Zoe Rempel.(2017).* Manipulating varroa mite and virus levels on a colony scale to quantify their impact on honey bee colony winter Survival. American Bee Research Conference, Galveston, Texas. 13 January, 2017

Currie, R.W. 2016. The impact and control of *Varroa* and virus on honey bee colonies and their survival in winter. Saskatchewan Beekeepers Association annual meeting. Saskatoon, Sk. 1 December.

# Currie, R.W., Parsons, G and Z. Rempel. The impact of different combinations of *Varroa destructor* (Anderson and Trueman) and viruses on honey bee, *Apis mellifera* (L.), colony survival in winter. International Congress of Entomology, Orlando, Florida. September, 2016

# Currie, Rob. 2016. ***Going, Going, Gone? Can the World Survive the Death of the Honey Bee?*** Seniors' Alumni Learning for Life Program - Spring 2016 Sessions

Currie, R.W., G. Parsons and Z. Remple, 2016. Effects of viruses on bees. Manitoba Beekeepers’ Association, Winnipeg, Manitoba. Feb 27

Currie, Rob. 2015. Managing Colony Stressors Using Thresholds Joint Annual Meeting of the Saskatchewan Beekeepers and Canadian Honey Council, Saskatoon, SK, Dec 3, 2015

Neil, Hannah 2016. Technology Adaptation Team Update. Saskatchewan Beekeepers rk Beekeepers Association Convention. Radisson Hotel Saskatoon, November 30, 2016.

Parsons, Graham 2016“Spring 2016 SBA TAT Update and Information”, Saskatchewan  Beekeepers’ Association Spring 2016  Newsletter

Parsons, Graham “TAT Update and Preliminary Findings”, Saskatchewan Beekeepers’ Association Fall 2015 Newsletter

Parsons, Graham “Honey Bee Health: Management of Varroa Mites and Viruses”, presentation at the Saskatchewan Beekeepers’ Association Annual convention, November 19-21, 2014

Parsons, Graham “Exploring the Relationship Between Varroa Mites, Viruses and Treatment Timing”, presented at the Eastern Apiculture Society, Guelph Ontario, August 14th, 2015

Parsons, Graham “TAT Future” presented at the Saskatchewan Beekeepers’ Association Annual General Meeting, March 2016

**INDUSTRY SUPPORT**

The investigators and collaborators of this project wish to acknowledge and sincerely thank the groups and individuals that contributed to this project’s success. The Saskatchewan Beekeepers Development Commission provided logistical and financial support to this project at $20 000/year over three years, and in the form of donated honey bee colonies for study and project administration. The Alberta Beekeepers Commission also donated $15 000.00/year over three years toward the completion of the project. Finally, the individual beekeepers that donated colonies and equipment to the project include Murray Hannigan (Hannigan Honey), Neil Specht (Sweetheart Pollinators), Megan Specht (Sweetheart Honey), Lalonde Honey Farms, Ron Glendenning, Dani Glennie (Glory Bee Honey), Calvin Parsons, Chris Warriner (West Cowan Apiaries), Jake Berg, Stacey Zosel (SJBeez), John Sochaski, and Shawn Meckelborg.

**FOLLOW UP RESEARCH**

This study also provides good information on the levels of DWV virus that are damaging to bees. However, it should be noted that although the primers used would pick up both strains of DWV the tests for strain differences indicate that the ones found in this study consisted primarily of DWV-A. DWV-B is more common in other parts of Canada and its effects on wintering under Saskatchewan conditions or Canadian conditions in general have not been studied. Shifts in the distribution of viruses could alter the economic injury level based upon virus concentration.

Several of the viruses analyzed as well as Nosema showed negative correlations with colony health and production parameters in this study. A better understanding of the impact of these combinations of parasite and pathogen interactions will inform the development of management practices to best mitigate their effects and help identify pathogens that have the greatest impact on colony health so that sampling efforts and resources can be focused on the most serious factors affecting colony health.

It should also be noted that indoor wintering was used in this study. Desai and Currie (2016) and Bahreni and Currie (2015) have shown that the negative impacts of Varroa and viruses may be more pronounced in colonies wintered outdoors. Thus, levels of virus that were found to not be damaging to indoor wintered colonies in this study could still be damaging to outdoor wintered colonies. Establishment of economic injury levels for viruses on outdoor wintered colonies requires further research.

Further studies into specific late and early nuc starting times in a Saskatchewan climate would yield further results to determine optimal timing in this area. The differences between the two late-started replicates occurred in less than a two week time frame, proving that colonies are sensitive during this time of growth and small differences in starting time can have an impact on success.

**ACKNOWLEDGEMENTS**

The Ministry of Agriculture of the Government of Saskatchewan as well as Growing Forward 2 were acknowledged as the primary funding source for this project in all written reports and presentations given by Hannah Neil, Graham Parsons, the Technology Adaptation Team, and Dr. Rob Currie. We would like to further acknowledge and thank these groups for their support in the completion of this project in this report.

Past members of the Technology Adaptation Team, Jess Morris and Nadine Hines, should also be acknowledged for their hard work in the field and the lab during the course of this project. Graham Parsons, former TAT lead researcher, conceptualized this project as well as completing field and lab work for the 2014 and 2015 seasons; since his transition to a position with the Ministry of Agriculture as Apiculture Intern, he has continued to provide support and advice to the project. Zoe Rempel, Suresh Desai, and Cole Robson-Hyskai were also important members of Rob Currie’s team in the lab at the University of Manitoba doing sample analysis. We thank them all for their work.

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