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MANAGEMENT OF VARROA POPULATIONS WITH SASKATRAZ BREEDING STOCK AND SELECTIVE TREATMENT STRATEGIES

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INTRODUCTION

The aim of the Saskatraz project is to breed gentle productive honey bees with resistance to mites, viruses, microsporidia and brood diseases. We have made progress in selecting families with good productivity, wintering ability, resistance to tracheal mites and chalk brood, but only varying degrees of tolerance to varroa mites. In the last few years we have looked at the variability in susceptibility to viruses (Deformed Wing Virus (DWV), Kashmir Bee Virus (KBV), and Israeli Acute Paralytic Virus (IAPV)). In general, colonies which better suppress varroa population growth are less susceptible to virus infection. In 2010 we began screening our Saskatraz breeding lines for resistance and/or susceptibility to nosema infection.

In the last three years we have initiated progeny analyses of traits, such as grooming behaviour, thought to be associated with varroa tolerance. Considerable variability in the expression of grooming behaviour has been observed between daughters of breeder queens selected for varroa tolerance. To further enrich for mite tolerance we are reselecting the most tolerant daughters from each family for propagation. Some examples of this re-current selection process will be described in this article. In 2010, Meadow Ridge volunteered to test 16 breeding lines from an Australian queen breeder for tolerance to varroa. Australia does not yet have varroa, so it was of interest to compare these breeding lines with Saskatraz stock.

Experiments with selected stock and treatment strategies with organic acids will be described. Another observation affecting honey bee tolerance to varroa has been noted. Colonies subjected to synthetic miticide treatments (Apivar) may be losing some degree of the defence mechanisms needed to resist the mite. This has led us to investigate the efficacy of using organic acids (formic and oxalic) in combination with Saskatraz breeding lines, selected for varroa tolerance. Some preliminary experiments with oxalic acid liquid in combination with Apistan, and fall and mid–winter treatment with oxalic sublimation will be described. The effectiveness of miticide treatments is often dependent on weather conditions, particularly temperature. Outdoor tests were performed on three synthetic miticides and a formic acid flash treatment in the fall of 2010. Some studies on fall outdoor and indoor oxalic treatments were initiated.

RESULTS AND DISCUSSION

1. COLONY GROOMING ASSAYS

In late October of 2008, 24 daughters from six Saskatraz families were selected for analyses of grooming behaviour during indoor wintering (2008-2009). Colonies were evaluated for uniformity (population, brood, stores, etc.) and normalized for mite populations by Apistan treatment in September 2008. Colonies having high varroa infestations (90 to 100%) from our varroa nursery were used to infect each of the test colonies on November 4, 2008 .The colonies were infected with 300 adult, phoretic, varroa mites. Varroa drop was monitored on sticky boards every 24 hours on the 24 test colonies for 21 days. Out of the six (SAT-23, 34, 65, 84, 86, 88) Saskatraz families tested, SAT 88, 34 and 84 showed the highest cumulative varroa drop between November 5 and 26, 2008 (Figure 1).

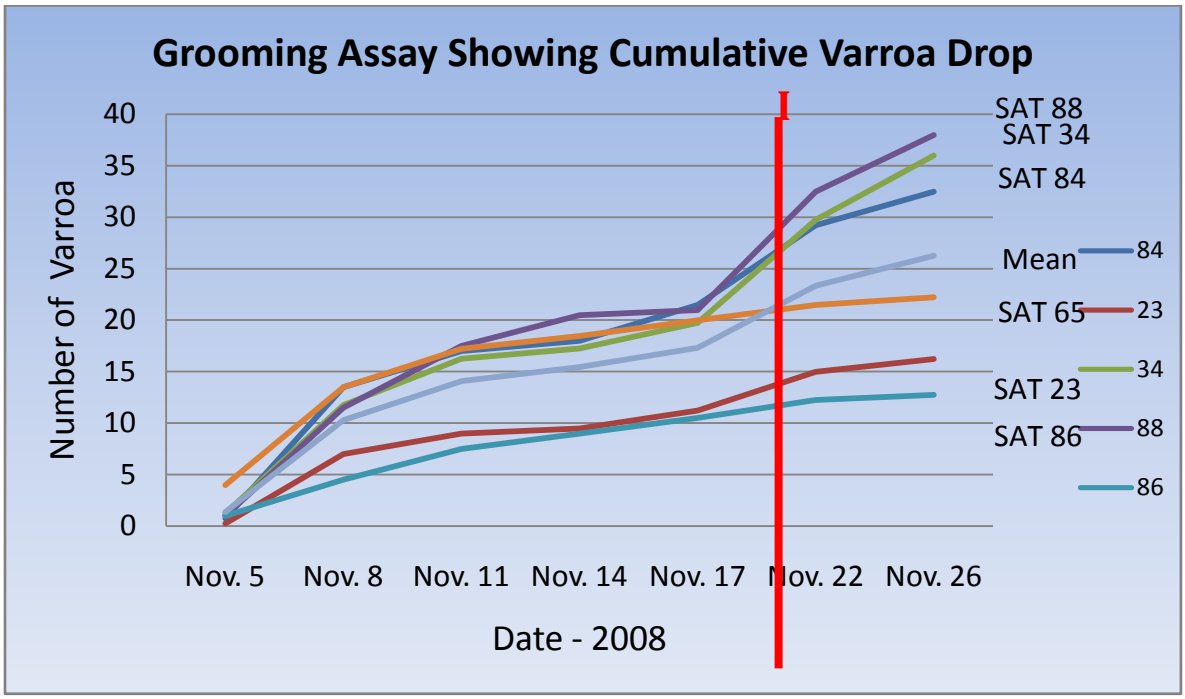


Figure 1. Cumulative varroa drop in six Saskatraz families, moved indoors (I) November 20, 2008.

SAT-65 had cumulative varroa drop similar to the mean value for all 6 families. However, there is considerable variability within families (ie. SAT-88),as shown in Figure 2.

This type of assay helps us identify and re-select daughters expressing the best grooming behavior. In this example 88d would be selected. We have been performing whole colony grooming assays for three years now with eight daughters of eight families being assayed in 2010-11. In addition to varroa drop we assay the adult varroa infestations, virus and nosema status of each colony. This allows us to evaluate the susceptibility or resistance of each colony to pathogens associated with varroa infestation.

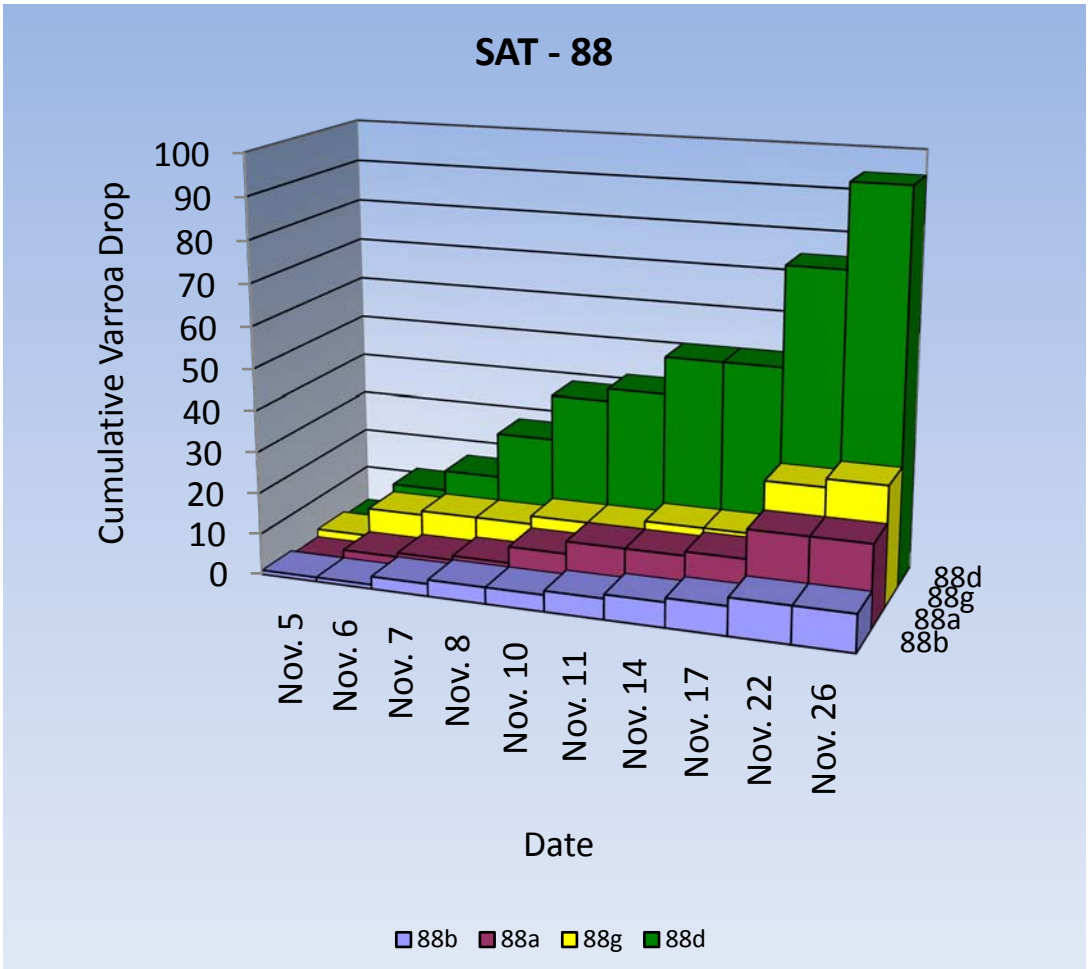


Figure 2. Cumulative varroa drop in SAT-88 daughters.

2. EFFECTS OF VARROA TREATMENT STRATEGIES ON SASKATRAZ COLONIES

(a) Formic acid treatments

After seven years of using natural selection to select colonies for resistance to varroa, we have found colonies showing varying degrees of tolerance, but none which we would define with heritable varroa resistance. SAT 88 is the longest surviving colony (42 months, Sept. 2010) without synthetic miticide treatment. Daughters from this colony show considerable variability in grooming behavior, varroa sensitive hygiene, varroa tolerance and honey production. We are using recurrent selection techniques to try and improve the heritability of these traits, in this family, as well as several others. In addition, we have studied the effects of using organic acids (formic and oxalic) on a Saskatraz apiary with colonies showing high, intermediate and low varroa infestations. In the fall of 2008 we treated Saskatraz-Q (Figure 3) with formic acid (mite wipes, 60% formic) on September 30th and October 17th. The varroa drop rate showed a decline, until the second treatment on October 17th, where an increased drop rate was observed. The colonies were winterized in insulated wraps at the same time as the second formic treatment. On October 25th , the colonies were treated with oxalic vapour. The outside temperature was

4°C. A massive increase in varroa drop rate occurred over the next few days falling back to pretreatment levels within 14 days. In the spring of 2009, only three colonies out of the 12 survived. The three surviving colonies had mite infestation levels below the apiary mean % adult bee infestation of 20%, but three colonies with the lowest mite levels (SAT-96,7.5 %; SAT 93, 4.5%; SAT-84, 5.3% also died). All colonies with mite levels above the apiary mean, 25% died. Scanning electron microscopy of dead bees from colonies treated with the formic-oxalic treatments showed reduced hair coats when compared to untreated bees (data not shown). Oxalic treated bees showed oxalic crystals on their hair coat. Oxalic fall treatment at 4°C outdoor temperatures was very effective at killing varroa on adult bees. However, the combined formic-oxalic treatment may have caused excessive stress to the colonies, adding to colony mortality.

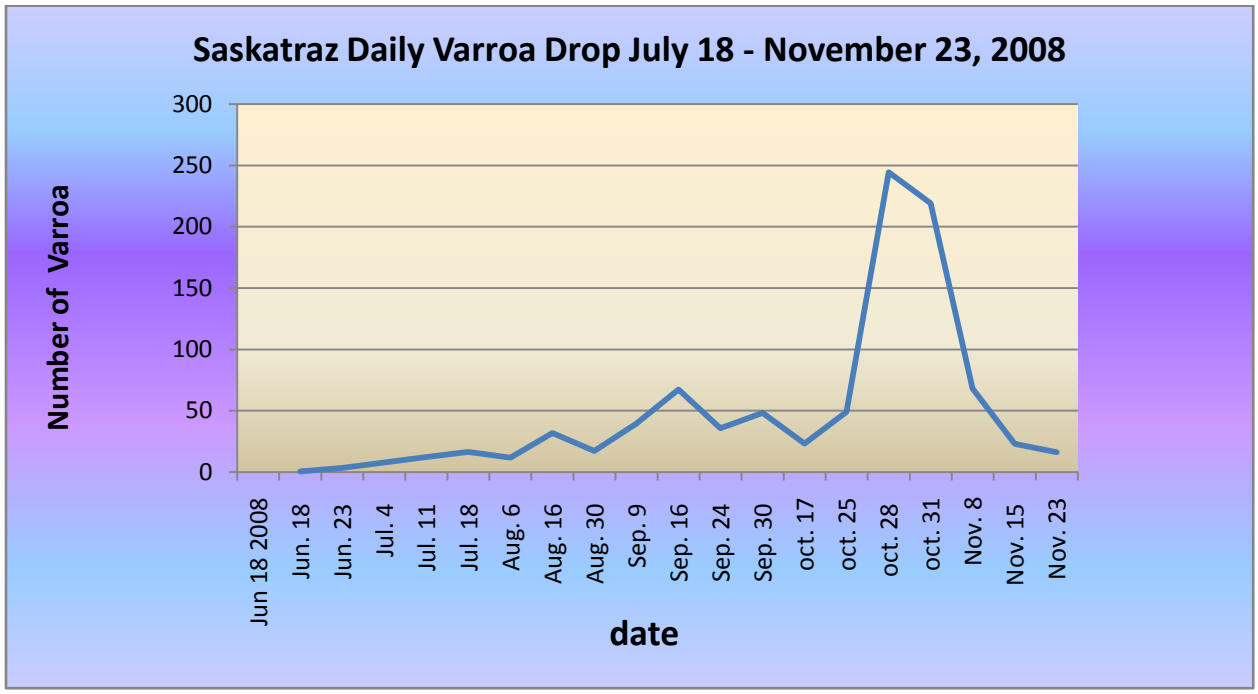
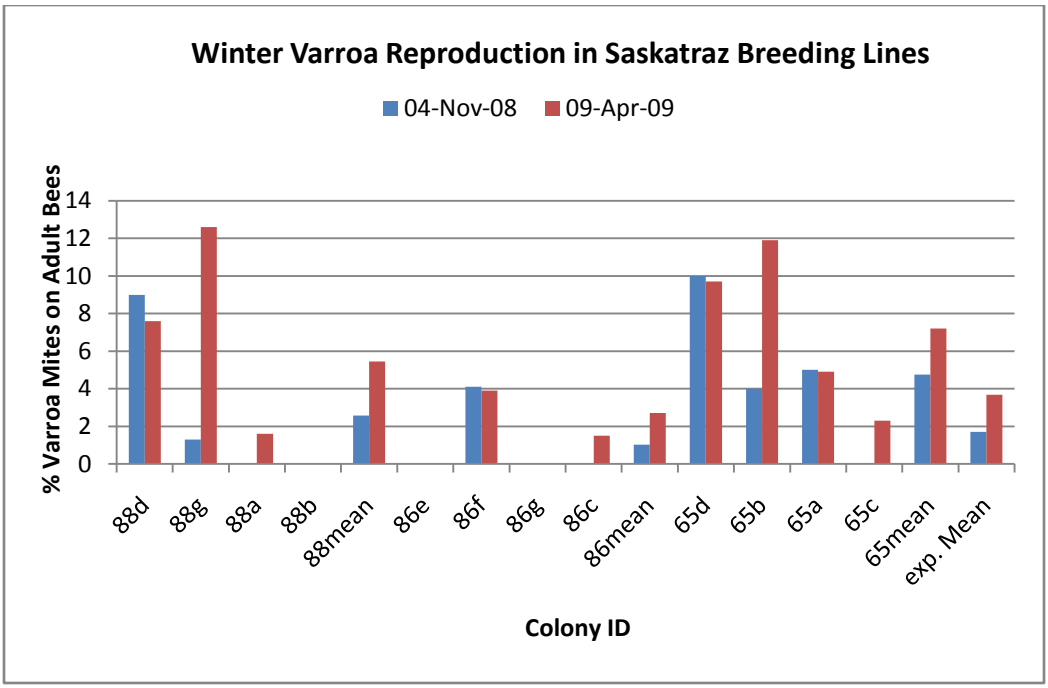


Figure 3. Mean varroa drop per day from June 18 to November 23, 2008 at Saskatraz-Q. Formic acid treatments (mite wipe pads, 60% formic) were made on September 30th and October 17th. Oxalic vapour treatment was performed on October 25.

(b) Apistan followed by oxalic liquid drench

In April 2009, after completion of indoor grooming assays on four daughters of the six selected Saskatraz breeding lines described in section 1, we decided to try a combined treatment approach with the synthetic miticide Apistan, followed by an Oxalic liquid drench .The percent varroa infestations on adult bees was determined on April 10, 2009, Figure 4(a) and (b) (red bars) after adding 300 varroa mites on November 4, 2008.



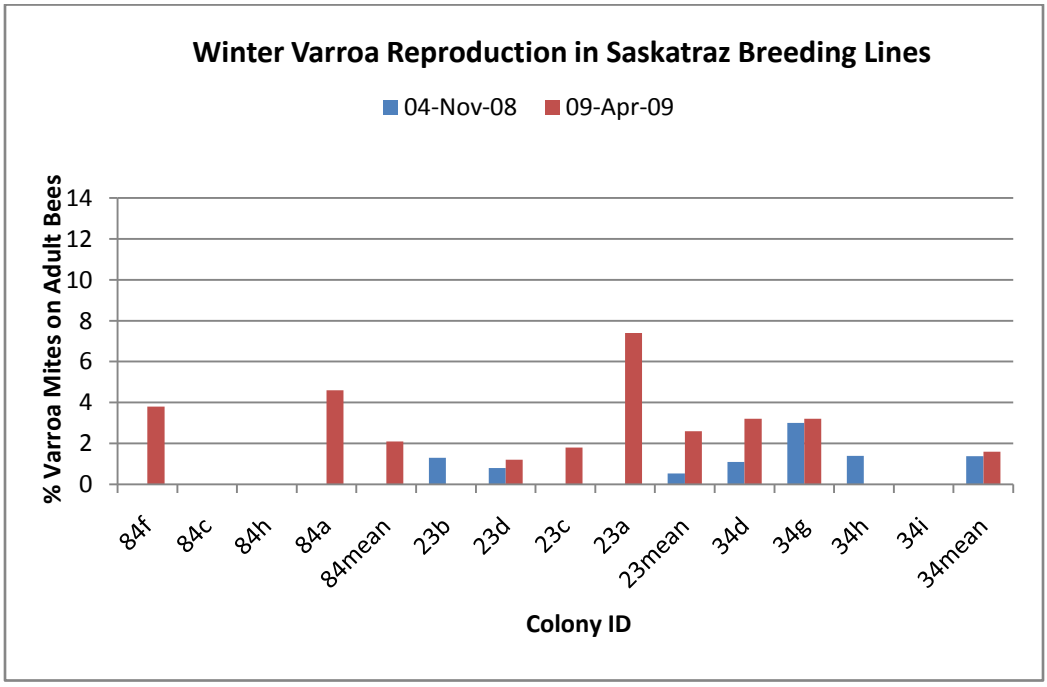


Figure 4. Comparison of fall (November 4, 2008) and spring (April 9, 2009) varroa mite levels on adult bees in six Saskatraz families. Colonies were treated with Apistan in September to normalize mite populations, and 300 varroa mites were added to all colonies on November 4, 2008.

These colonies were all treated with 2 strips of Apistan for 42 days. After 36 days of Apistan treatment they were treated with 50 mls of a 3.5% w/v oxalic solution, dissolved in 50% sucrose. The solution was dribbled between the frames with a 50 ml syringe .On August 9, 2009 all colonies tested negative for both tracheal and varroa mites.

Figure 5 shows the percent varroa infestation on adult bees at Saskatraz PW 17 months after treatment. After mite treatment these colonies were moved to an isolated apiary and monitored for varroa population growth as well as honey production. Almost all of the families except SAT 23, maintained the varroa mite population well below 10%. One SAT 23 daughter SAT 23C showed a 40% infestation after 17 months without treatment. The SAT 84 family showed the lowest mean infestation at 3% with some colonies(SAT 86C, 65C, and 88 G) showing no detectable mites (less than 1%). Some of these observations are consistent with varroa population growth during the 2008-2009 winter experiment (Figure 4(a) and (b)), and others are not. For example after innoculation with varroa in November 2008, SAT 86C and 65C maintained low winter levels, where as 88G showed higher levels (12%) in the spring. We have observed that a individual colony phenotype can go from tolerant to varroa population growth to sensitive within one season. We think this could be due, at least in part, to supercedure or different subfamily worker populations (patrilines). In the spring of 2011 all of the colonies in the apiary will be assessed for varroa population growth. These observations are encouraging, and suggest that using varroa tolerant breeding lines and treatment strategies that reduce varroa levels to less than one percent plus moving apiaries to areas with some isolation can significantly reduce miticide treatment frequencies. Recent observations suggest this should be possible by using tolerant breeding lines and oxalic sublimation treatments.

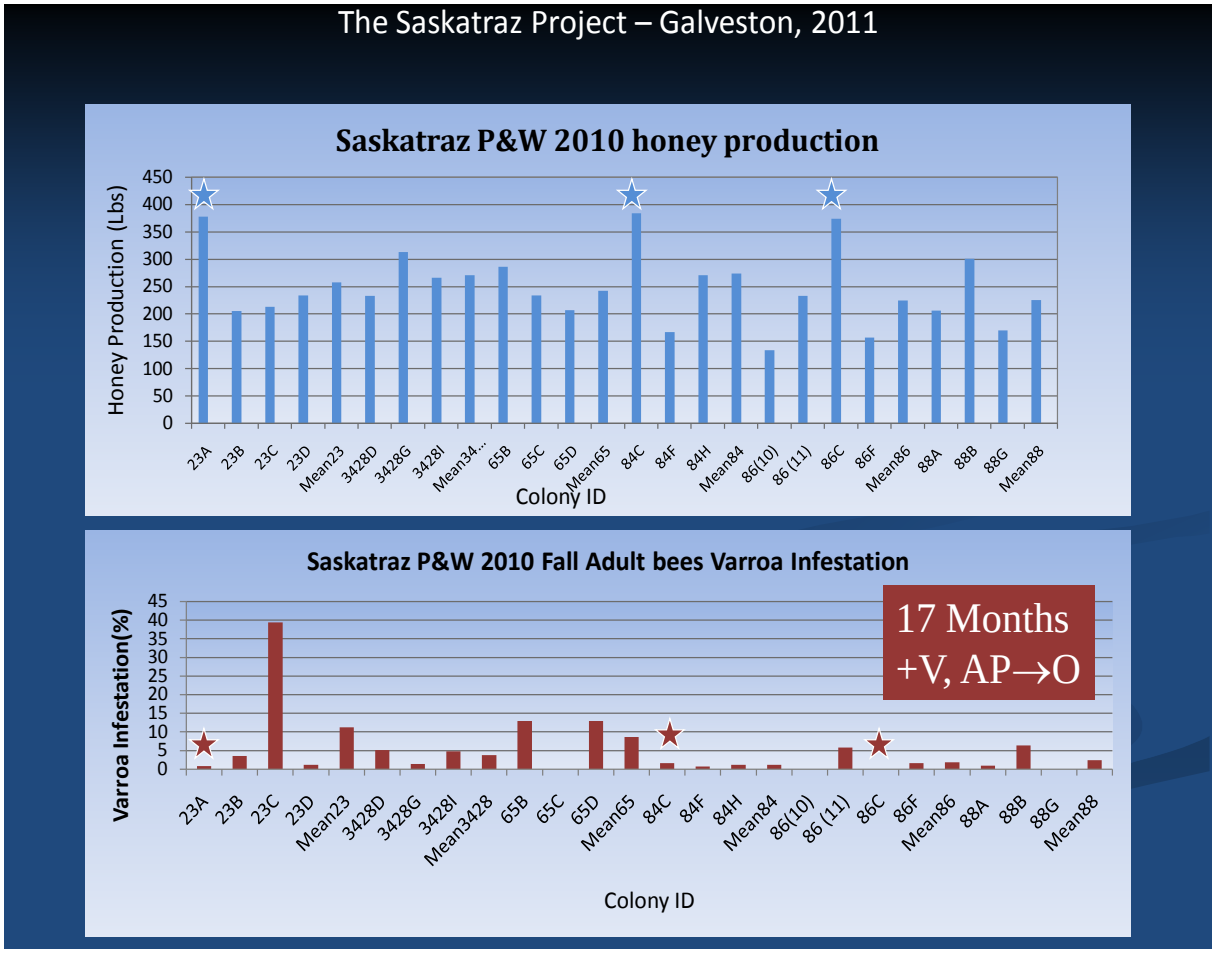


Figure 5. Percent varroa infestation on October 14 ,2010 at Saskatraz-PW. 17 months after treatment for varroa with Apistan –Oxalic liquid.

3. FALL VARROA TREATMENT TRIALS

(a) Oxalic sublimation

In September to December 2010 experiments on the efficacy of oxalic acid, formic flash and synthetic miticides (Apistan, Apivar and Checkmite) were initiated. Figure 3 shows an experiment with oxalic vapour (fan driven, 2 to 3 grams of oxalic crystals) applied to winterized colonies in late October,with an outside temperature of 4°C. In this study (Figure 6) we look at the effect of Oxalic sublimation using an apparatus purchased from Heilyser Technologies. Two grams of oxalic crystals was added to the heating pan and heated for 1 minute using a 12 volt battery. The hives were sealed with foam strips after inserting a heating pan into the bottom center of the hive entrance. After 10 minutes the foam and apparatus were removed. Figure 6 summarizes the results of oxalic sublimation on 16 test colonies on December 7, 2010. The oxalic treatment data for one (1ox) and two(2ox) consecutive treatments, three weeks apart is shown.

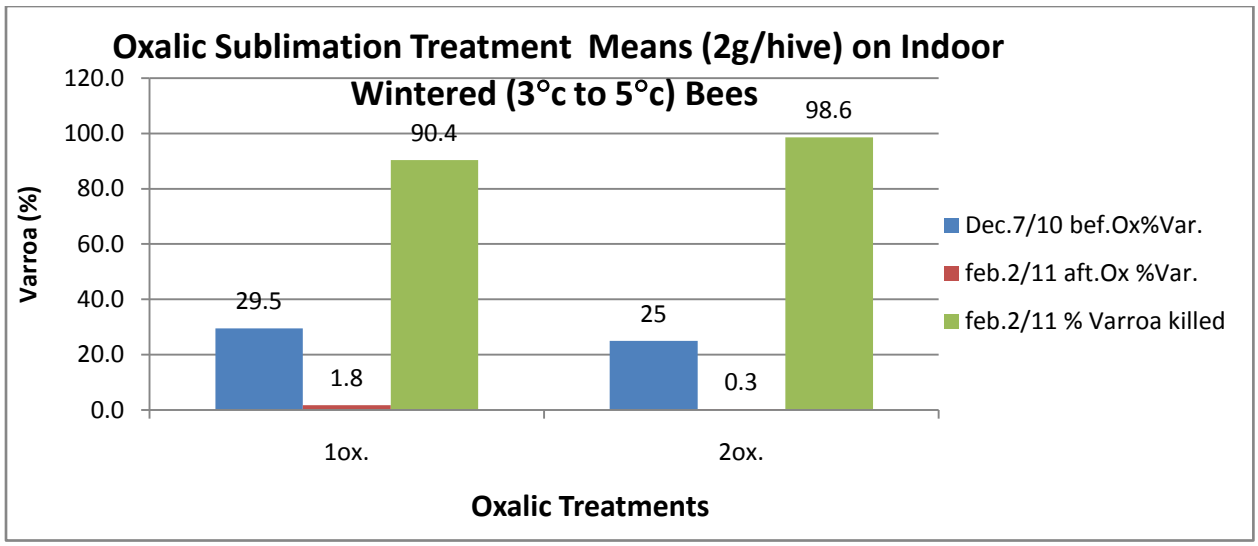


Figure 6. Effects of one and two consecutive oxalic treatments 3 weeks apart.

The blue bars give percent varroa infestations on adult bees prior to treatment, red bars 54 days after first treatment. The green bars show that oxalic sublimation is very effective at removing varroa mites from adult bees, with two applications removing over ninety percent of the phoretic varroa from the adult bee population. Analyses of varroa drop on sticky boards indicated the varroa were all dead. It has been suggested that oxalic crystals condense from the hive vapors on to varroa mites and honey bees. We have observed the crystals on bee hairs by scanning electron microscopy after fan driven oxalic sublimation and have experiments in progress looking at the effect of sublimation on the honey bees external surface. Oxalic acid is thought to kill varroa mites because of the dehydration effects of the attached crystals. In addition, depending on sublimation conditions and temperature of the heating pan some formic acid may be released.

Figure 7 shows the varroa drop over time after oxalic sublimation experiments. The blue line shows varroa drop in untreated colonies, red in oxalic treated. The highest kill rate occurs in the first 6 or 7 days, decreasing between 7 and 14 days and leveling off after three weeks. Our second oxalic treatment

was based on these observations. Preliminary investigations into the effects of oxalic sublimation on varroa mites in sealed brood indicated some varroa kill on pre-emergent adults. The efficacy of these treatments will not be known until spring.

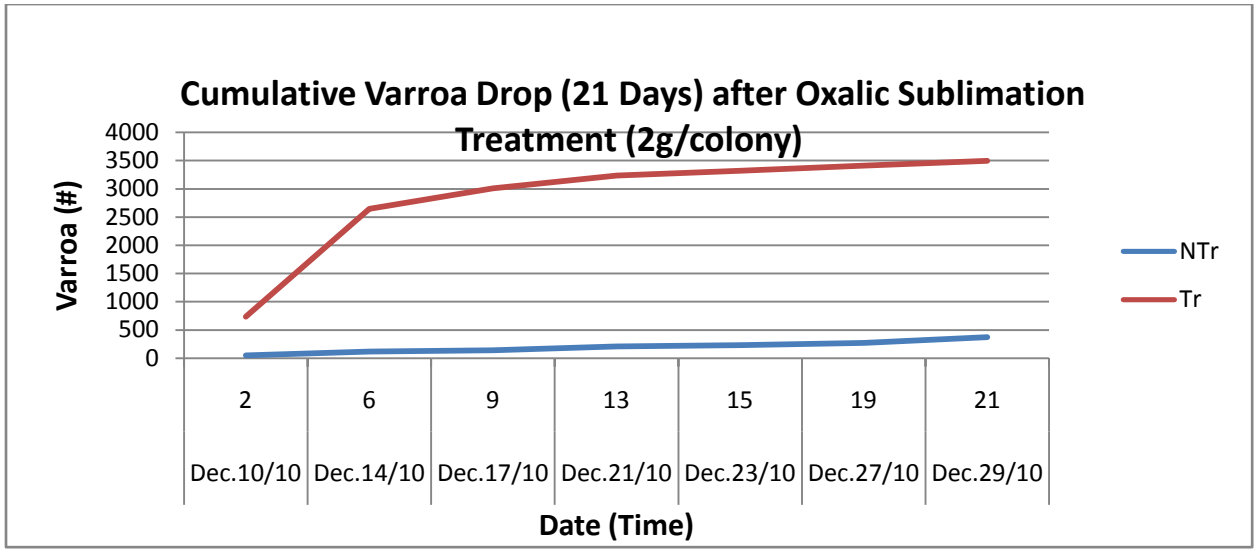


Figure 7. Varroa drop over time after oxalic sublimation (2 grams/colony). Treated (red); untreated (blue).

(b) Fall Apistan and Apivar miticide treatments

In September 2010 we found some unexpectedly high varroa levels in our commercial apiaries .These apiaries were treated in the spring (May) of 2010 with 2 strips of Apivar, for 42 days . Follow up testing indicated the treatments had been initially effective (Figure 11). This was the first exposure to Apivar for these colonies. We were surprised with the rapid increase in varroa levels in the fall of 2010,which could have been the result of a number of factors ,including fall brood rearing ,honey flow ,environmental conditions ,or possibly a post treatment effect of Apivar.

These observations prompted us to investigate the effectiveness of both Apistan and Apivar as a late fall treatment, for varroa mites (figure 8). Five randomly selected colonies from the same apiary were used per treatment. Plotted values are mean plus or minus SE. In the control group (NT) there was an 86 % increase in varroa infestation between September 29 and November 1. Apistan reduced the adult bee varroa infestation by 79 % and Apivar by 77%, indicating both miticides were about equally effective .In the Apivar treatment group there was a small increase in adult bee varroa infestation between Oct. 11 and Nov.1, 2011.Two to five percent varroa infestations after these treatments is not acceptable, and it indicates fall treatments may be less effective because of environmental effects (temperature, clustering etc.).

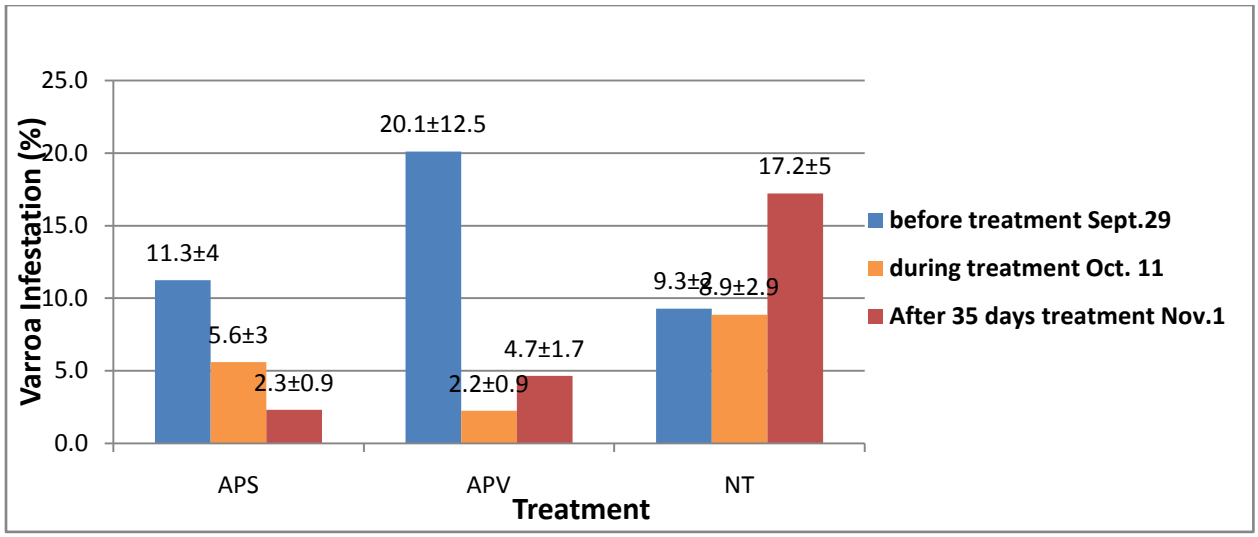


Figure 8. Effects of Apistan and Apivar miticide treatments for 35 days (September 29 to November 1, 2010) on the percent varroa infestation on adult bees (alcohol wash).

(c) Comparison of fall Apistan, Apivar, Checkmite and formic flash miticide treatments.

In this experiment (Figure 9) 5 colonies per treatment were selected, all with low varroa infestation levels (2.3 to 4.2 %). Mean values plus or minus SD are plotted. This treatment trial was initiated 10 days later than that described in Figure 8. There was a 10 % increase in the control group with no treatment. The most effective treatments under these conditions were Check mite and Formic Flash, which maintained mite infestations at starting levels. The temperature at the time of formic flash treatment was in the high teens, and showed an effective mite kill from analyses of sticky boards. Formic flash treatments on some of our varroa nursery colonies with 40% infestations, showed a 73% varroa mite kill. The data presented in Figures 8 and 9 indicates that later fall synthetic miticide treatments are inefficient, and leave significant varroa mite populations present on adult bees going into winter. These types of treatments may also contribute to the development of resistance mechanisms. More studies are required in this area, especially the effect of temperature on the effectiveness of synthetic miticides.

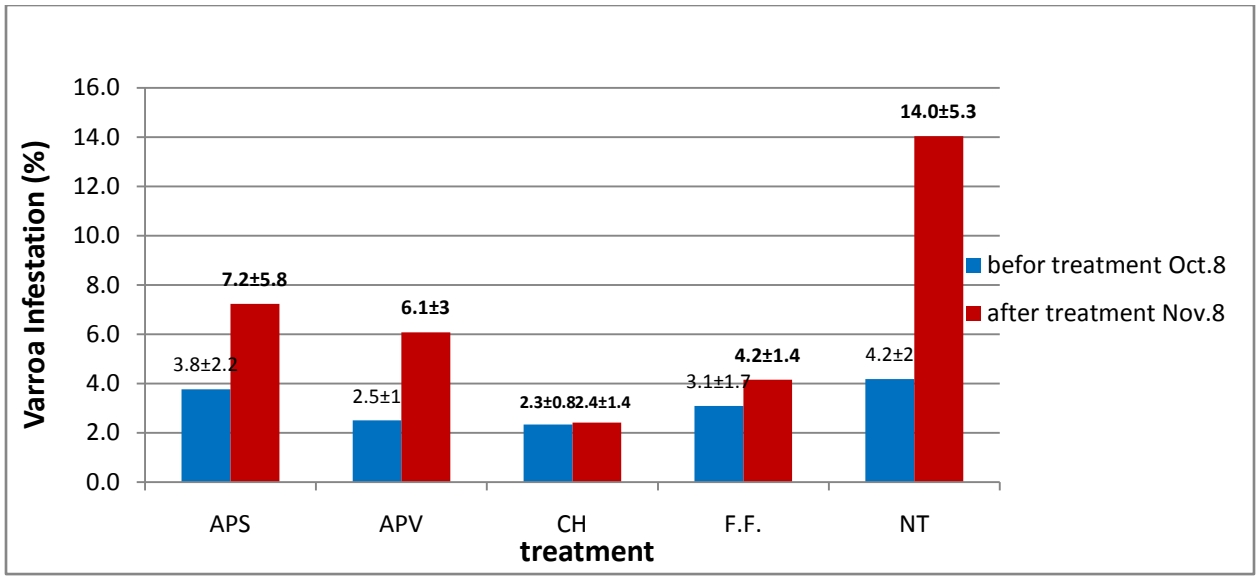


Figure 9. Effects of three synthetic chemical miticides, Apistan (APS), Apivar (APV), Checkmite (CH) and Formic Flash (FF) treatments (October 8 to November 10, 2010) compared to the control (NT-no treatment) on the percent adult bee varroa infestation.

4. COMPARISON OF VARROA TOLERANCE IN SASKATRAZ AND UNSELECTED STOCK

In the spring of 2010 Meadow Ridge volunteered to test 16 queen breeding lines and 20 commercial Australian queens for tolerance to varroa, honey production and brood diseases in Saskatchewan. Since Australia does not have varroa the bees have not been exposed to any selection pressure. The Australians expect it is only a matter of time before they become infected by varroa and would like to get an idea of what tolerance or susceptibility exists in their breeding stock. This analyses also gives us a control to measure our Saskatraz stock against. Saskatraz stock has been subjected to several natural selection cycles under varroa and tracheal mite pressure. Australian stock, has not been exposed to varroa.

Figure 10 shows considerable variability between colonies in honey production and varroa infestation levels. Each queen was introduced into a 5 frame nuc in the middle of May. The bees and brood used to make the nucs were previously treated with Apivar to normalize varroa levels to less than 1%. We tested all the attendant workers sent with the breeder queens for viruses and Nosema. We did not find any viruses consistent with no varroa pressure. We did find a trace of Nosema apis. You will note that after 5 months all colonies are showing significant varroa infestations; however, there is considerable variation between lines. We selected JH-2-10 for honey production, JH-12-10 for honey production and varroa suppression, and JH-10-10 for best varroa tolerance of the breeding lines tested. On August 24, 2010 tracheal mite analyses showed low level infestations (2 to 8 %) in JH1 to 8, and JH 15. No tracheal mites were detected in JH 9 to 14 and 16. It may be of interest to know that JH 4 to 9 are derived from Hastings Caucasian queen lines imported from Canada between 1975 and 1980.

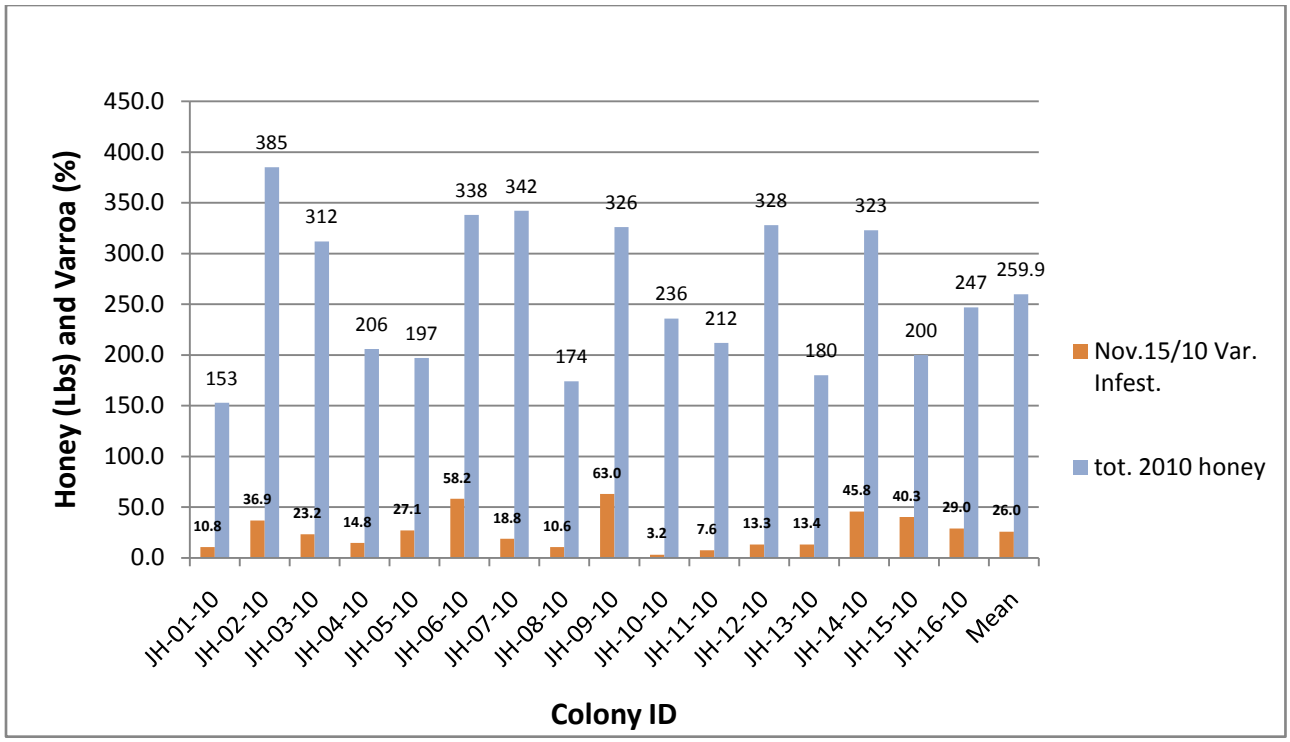


Figure 10. Honey production and varroa infestation in 16 Australian breeding lines.

Figure 11 compares the mean percent varroa infestation on adult bees from a number of different apiaries. Red bars describe colonies not treated with miticides for 16 to 42 months. Blue bars are apiaries that were treated with Apivar (2 strips/colony) starting in early May for 42 days. The brown bars indicate percent varroa infestations on adult bees determined by alcohol washes, made in early May, yellow bars varroa infestations determined in mid to late June, after treatment. Final varroa infestations were determined in late October 2010. The Saskatraz apiaries were not treated with any synthetic miticides for the months indicated (red bars). Saskatraz –D colonies (progeny analyses) were established from nucs in May of 2009 using bees from colonies previously treated with Apistan and formic acid. They were inoculated with 200 varroa mites on October 25, 2009 and showed mean apiary varroa infestation levels of 0.9% in early May, 1.2%, in June and 18 % by late October 2010, 19 months after the last varroa treatments. Saskatraz –PW colonies were inoculated with 300 varroa mites on November 4, 2008 and treated with Apistan and oxalic drench in April 2009. Figure 12 shows that in May and June mite levels were not detectable (below 1%) and showed only a 3.3% varroa infestation in late October, after 17 months with no treatment. SAT-88 is the longest surviving Saskatraz colony, without synthetic miticide treatment for 42 months, but formic and oxalic in October 2008. SAT-88 showed a 1.8 % varroa infestation in May, which increased to 4.2% in June and dropped to 3.1% in October. The original Saskatraz apiary, Saskatraz-Q was re-stocked (20 new colonies) in the spring of 2009, and mite levels were normalized amongst all new colonies by Apistan treatment, before initiating another round of natural selection. These colonies were from diverse sources, some of which had been subjected to re-current selection, others were new selections from new sources. SAT 85, 88 and 96 remained as survivors from 2007, and were not treated. In the spring of 2010 ten of the best colonies were dead. Analyses of the dead hives did not identify any cause, except possible queen loss in the fall of 2009. One hive was found with an injured queen that turned into a drone layer. The mean colony varroa infestation

level was 10.9% in May, 8.3% in June and 11.4% in October 2010, 16 months since the last treatment. The VSS (Varroa Sensitive Selections) are colonies we select for sensitivity to varroa in the spring of the year. These colonies were last treated in May 2009, showing mean infestation levels of 5% in May, 8.2% in June and 41% in October 2010 (16 months from last treatment). These selections make up our experimental varroa nursery, which we use as a source of varroa for grooming assays and molecular work. Most of these colonies are now dead. The trucker apiary (16 Australian breeding lines, figure 10) was established in May from 4 frame nucs. The bees used to make the nucs were previously treated with Apivar, to reduce and normalize varroa mite levels, but the Australian queens were not exposed to Apivar. No varroa were detected on adult bees in May, but 0.8% was detected in June and a mean apiary varroa infection of 20.7% was detected in late October, 4.5 months after the last treatment(figure11). Comparing this apiary to Saskatraz apiaries clearly shows the increased tolerance to varroa gained by natural selection cycles and re-current selection. The greatest difference was between SAT-88, which showed a 3.1% infestation after 42 months and Saskatraz PW showing a 3.3% infestation after 17 months, compared to a 21.7% infestation after 4.5 months in the varroa sensitive Australian bees. The original Saskatraz –Q apiary showed a mean varroa infestation of 11.4% after 16 months, without varroa treatment. This apiary included a number of new selections not previously selected for varroa tolerance, as well as some colonies that had been selected in the past and re-selected for another natural selection cycle at Saskatraz-Q. Saskatraz-D showed an 18.9% infestation 19 months after treatment, and 12 months after having 200 varroa added in October 2009.

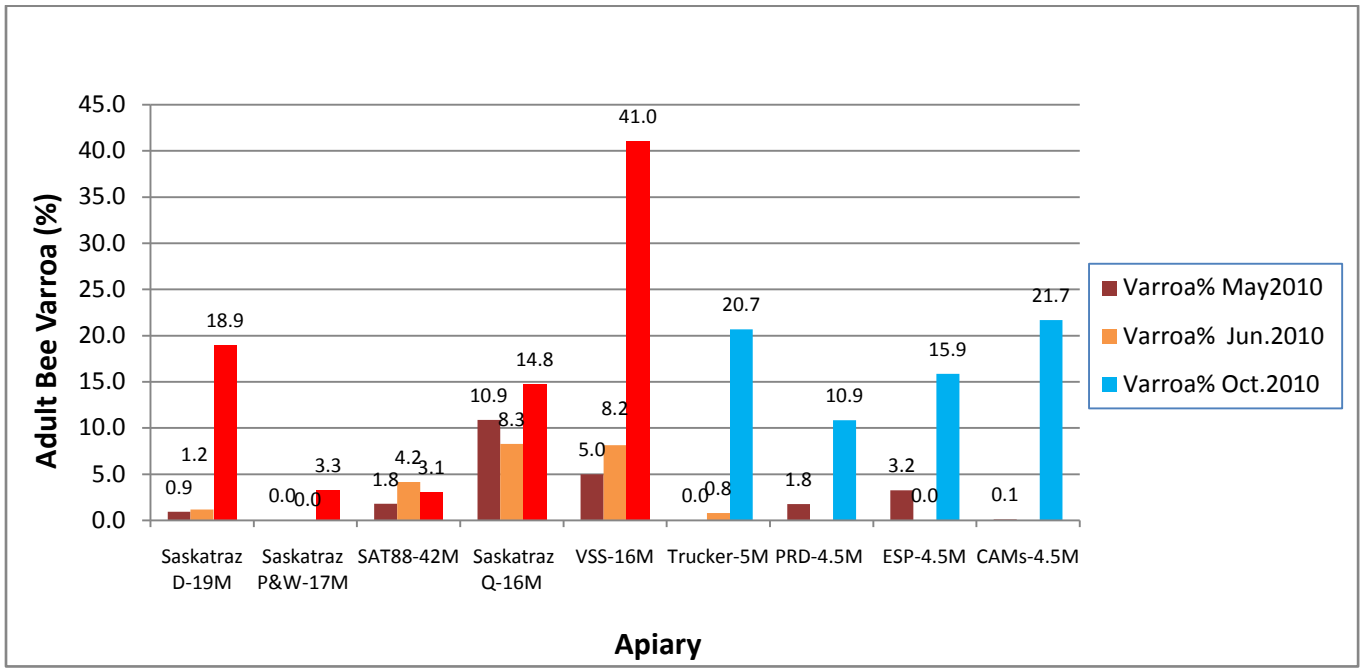


Figure 11. Mean percent varroa infestation on adult bees at 8 different apiaries and SAT-88 (2010). Blue bars designate colonies treated with Apivar for 42days in the spring (May-June), 2010.

PRD and ESP are apiaries established over the last three years for close population mating purposes. These apiaries are made up of out crossed Saskatraz family members re-selected for honey production

and low varroa population growth, under commercial beekeeping procedures. All colonies are reselected each spring for wintering, brood diseases, etc. Sub standard colonies are removed. Colonies with varroa levels over 2% are also removed and some are replaced by reselected outcrosses. This helps maintain the quality and diversity of Saskatraz breeding stock and serves for close population mating procedures, as well as breeder queen construction. Both of these apiaries were treated with Apivar in May, showing 1.8 and 3.2 percent varroa infestations, respectively, prior to treatment and removal of colonies with over 2% varroa. After treatment (42 days) with Apivar, no varroa mites were detected; indicating levels were below 0.1% showing the treatment was very effective at killing varroa mites. It was surprising to see a rapid increase in mean apiary varroa infestation (10.9 to15.9 %) 4.5 months after Apivar treatment, where as Saskatraz colonies, such as Saskatraz –PW, not treated for 17 months, showed mean varroa levels of 3.3%.CAMs apiary, a commercial apiary with mixed stock, showed low levels after spring treatment with Apivar, but an average infestation of 21.7 percent varroa on October 25th, 2010. The percent varroa infestation in this apiary was determined by sampling bees from all colonies for alcohol wash instead of individual colonies.

The rapid increase in varroa populations after Apivar treatment of Saskatraz colonies, compared to those Saskatraz apiaries not treated for varroa (Figure 11) is of concern. This suggests that some synthetic miticides may be making our bees more sensitive to varroa in both selected and non-selected populations. It is also possible that removing the stimulus (varroa mites) may reduce expression of tolerance mechanisms. We are planning more experiments to further test these hypotheses in 2011.

5. DIAGNOSTIC SERVICES AND UPDATE ON EXPORT OF CANADIAN BREEDING STOCK

In 2007 we began developing tests for pathogens in honey bee colonies, to help understand colony losses associated with varroa infestations. This was made possible by funding from ACAAF between 2007 and March 31, 2011, and our collaboration with the Saskatchewan Research Council and the Veterinary Infectious Disease Organization, at the University of Saskatchewan .We have successfully developed RT-PCR tests for viruses (Deformed Wing Virus [DWV]; Kashmir Bee Virus, [KBV]; Israeli Acute Paralytic Virus [IAPV]; Sac Brood Virus, [SBV]; Black Queen Cell Virus, [BQCV]) and PCR for Nosema apis and ceranae. More than 20 case history studies have been performed for commercial beekeepers with high colony losses. We have developed tests (figure 12) for adult bees, pupae, bee feces, varroa, varroa feces, hive products (bee bread and honey) and commercial pollen. These tests can be performed post-mortem, and can help explain colony losses and determine future action .We have found that the presence of 2 or more viruses (DWV + IAPV or KBV) and microsporidia (either and/or both species) causes high colony losses. High virus levels were always correlated with high varroa infestations. Case studies of bees having high Nosema spore counts of both apis and ceranae resulted in high colony mortality and continued colony dwindling in the spring. Follow up testing showed that Fumidil B was an effective treatment with ceranae infection being cured before apis. We also found that hive products (bee bread and pollen) can be infected with viruses and microsporidia in colonies showing high levels of infection. Although the infectivity of these pathogens in brood comb is not known, there are some treatments available, such as radiation and acetic acid application. Acetic acid treatment is effective for the control of nosema and methods for treatment and more information can be found in Pernal et al. 2011. Integrated

Management of Nosema and detection of Antibiotic residues, HiveLights, in press, and at the following website prepared by Medhat Nasr
http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex11780.

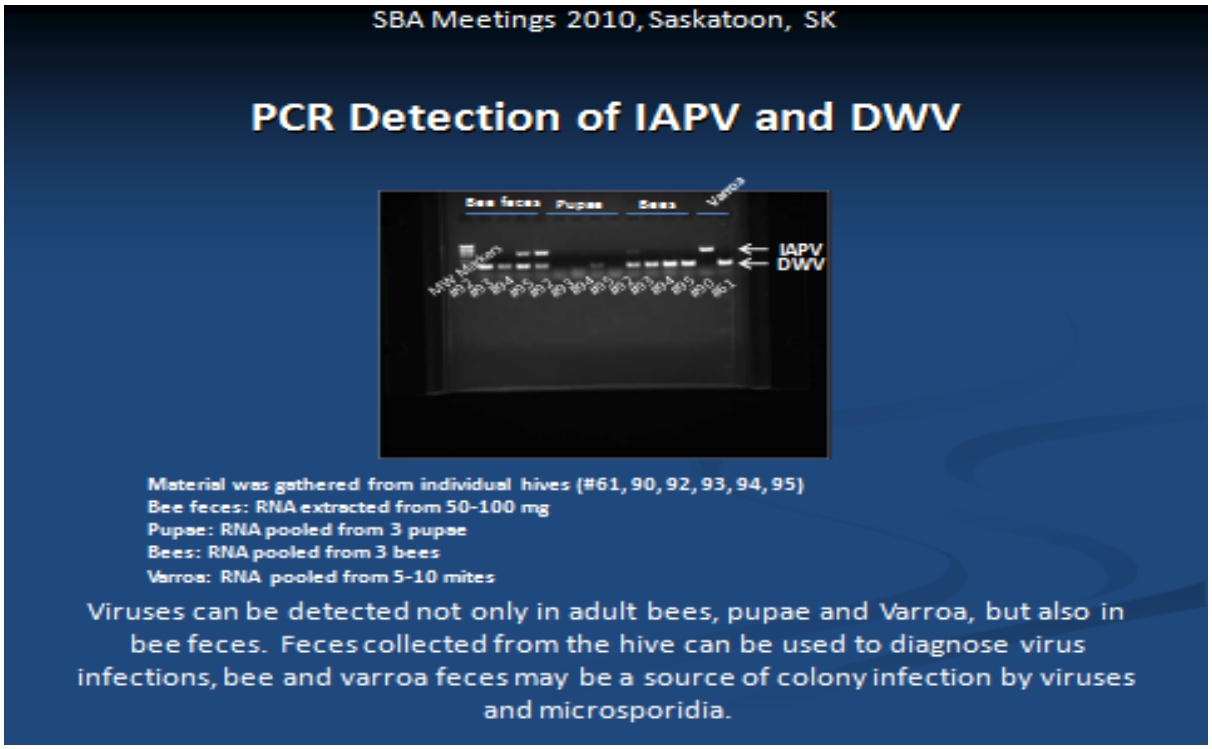


Figure 12. Shows the results of an RT-PCR screen of bee feces, pupae, bees and varroa.

We will continue to provide testing services for bee keepers this spring. However, our funding ends with ACAAF, as of March 31, 2011. A meeting was held with Prairie Diagnostic Services (PDS), at VIDO, in November, 2010 and transfer of the diagnostic methods developed over the last 4 years to PDS was discussed. PDS is a non-profit organization which provides testing services at cost. They are jointly operated by Saskatchewan Agriculture and the University of Saskatchewan, and housed at the Veterinary College. Last November Drs Pierre Lafortune and Albert Robertson met to draft an export document for export of queens to Chile. The possibility of PDS being accredited or approved by CFIA, for official testing of honey bee pathogens was discussed. This service would be of use by anyone looking at exporting honey bee breeding stock to other countries. Whether or not PDS becomes involved in testing for honey bee pathogens will depend on demand, as well as what molecular testing might be needed by countries importing Canadian bee breeding stock.

The final export certificate drafted for export of Canadian breeding stock to Chile, in November, 2010 was reviewed by the Hive Health Committee, Canadian Honey Council and CAPA, the Canadian Association of Professional Apiculturists and an official document was sent to CFIA counterparts, SAG, in Chile in late January, 2011. CFIA is waiting for comment.

6. BIOMARKER ANALYSES

Microarray analyses for identification of genes involved in the expression of varroa tolerance and honey production.

This project was initiated last fall with the arrival of a graduate student, Sanjie Jiang to work on this project as part of his master's thesis. The following information was provided by Sanjie jiang as a progress report on the microarray project.

Investigation of possible molecular mechanisms involved in conferring tolerance to varroa mites in domestic honey bees is being approached by comparing gene expression profiles (microarrays) of sensitive and tolerant Saskatraz breeding lines. Honey bee pupae at different developmental stages were collected, as well as from adult worker bees in the fall of 2010. All bees were collected from Meadow Ridge apiaries by Sanjie Jiang with the assistance of the Saskatraz field research team. Brood frames were removed from sensitive and resistant colonies, and brought to the field laboratory for harvesting pupae. They were stored at 32 C, 80% humidity until collection was complete. Collection involved carefully opening capped brood cells, and removing pupae at the described stages under a 10× stereo microscope. Pupae with and without varroa were collected from both sensitive and tolerant colonies. Adult honeybees (approximately 200 per sample) were collected from sensitive and tolerant colonies by hand catching live worker bees with and without varroa infestations. Rubber surgical gloves were worn to prevent contamination with nucleases and keratin, etc. Samples were frozen in liquid nitrogen before being stored at −80°C.

Total RNA was extracted from the heads of two bees (dark-eye pupae) using RNeasy kits (Qiagen, Valencia, California) as described by manufacturer and treated with DNase (Rnase free DnaseI, also Qiagen). RNA purity and integrity were checked by electrophoresis (1% agarose gels) as shown in Figure 13.

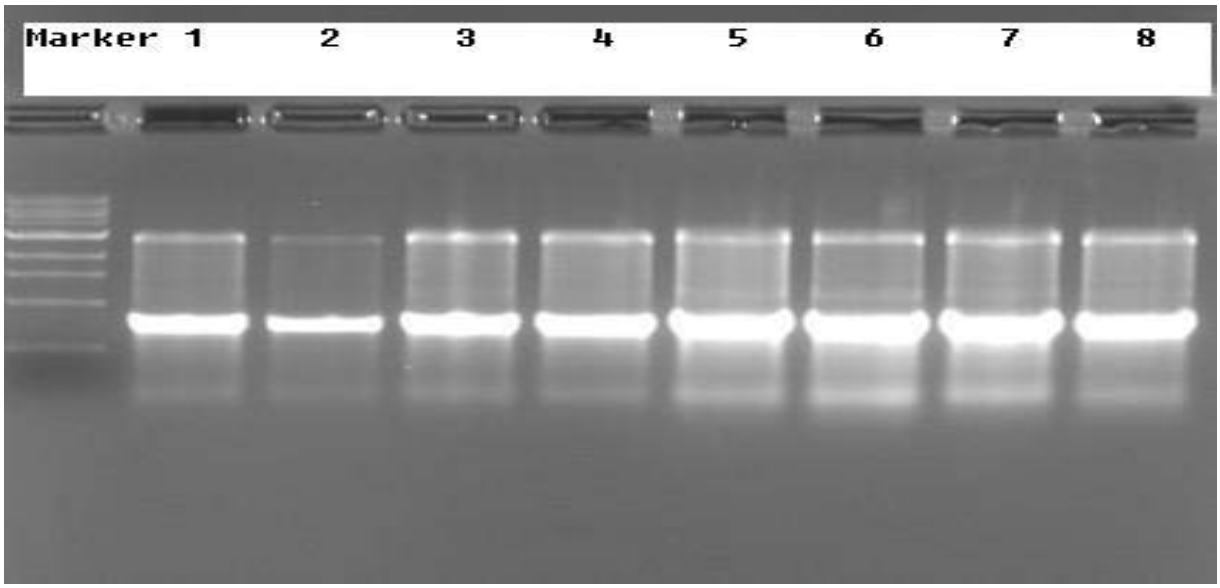


Figure 13. The RNA extracted from the bees heads shows excellent quality, and is being used for microarray analyses.

7. CERTIFIED SASKATRAZ STOCK AVAILABILITY IN 2011

Saskatraz stock will be available to any beekeepers or queen breeders interested in 2011. Collaborating queen breeders with certified breeders or queen cells purchased from the breeding program will be encouraged to out cross, re-select and return selections back to the program (initiated in 2006). New selections for testing in the Saskatraz breeding program are also welcomed. New selections or stock returned for evaluation will be purchased or traded with new Saskatraz selections, and evaluation results will be shared with contributors. This process was started in 2004 with Saskatchewan and Manitoba queen breeders to initiate the Saskatraz project.

Prices for breeding stock in 2011 will be as follows:

1. Out crossed Saskatraz breeder queens $80, sold with four frame nuc $250.

2. Closed population mated Saskatraz breeder queens $300.

3. Queen cells from extensively tested Saskatraz breeding lines $20. These breeder queens are never sold, just queen cells derived from them. Many of these families are described in the Saskatraz Review published in 2009 and available at saskatraz.com.

We began re-current selection procedures in 2007 to help maintain selected Saskatraz families. The re-selected colonies were set up for closed population breeding. We have established over the last three years apiaries with re-selected Saskatraz colonies for closed population mating procedures. We consider these valuable gene pools and will provide custom breeding services to interested queen breeders. We will close population mate virgin queens to the drones from these apiaries. The queen breeder could supply either queen cells or virgin queens less than six days old.

We suggest anyone interested in adding Saskatraz breeding stock to their operation purchase at least 10 queen cells from several families of interest. Out crossing this stock to your drone population and evaluating the daughters will give you an idea of the cross ability and what families work best with your bee population. Some queen breeders re-select some of the best daughters, as breeders for their own operation. Others have re-constructed their own Saskatraz apiaries for multiplying queens by closed population mating procedures. Please contact us for advice on re-constructing Saskatraz breeding families. Make sure the Saskatraz stock you purchase is certified .If it is not certified the origin is uncertain .We will be looking for qualified queen breeders in the future, who we will assist in distributing certified stock. Recent microarray results demonstrate that we will soon be able to verify certain traits in breeder queens.

In 2010 we tested 16 breeding lines from an Australian breeder for honey production and varroa tolerance, and re-selected 3 of the best performing lines for re-evaluation in Canada this year. We will not know about wintering ability until late April. Some commercial production queens are being offered in May from these re-selected Australian breeding lines, at competitive prices.

Proceeds from all stock sales are used to support the Saskatraz breeding program.

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The authors do not recommend any of the chemical treatment procedures used in this article, there usage here was for research purposes only. This article is dedicated to the memory of Randy Lawrence, a long term, dedicated employee of Meadow Ridge Enterprises Ltd. who passed on April 15, 2011.

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