1. Project name and contact information
Testing two selection assays efficacy for *Varroa*-mite-tolerant Queen Bee Production

Project Number: FNE08-631

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2. Goals
This project will illustrate and quantify the results of two selection practices small-scale queen breeders may potentially utilize to select breeder queens whose workers exhibit VSH and Hygienic behavior. Both assays exist in the literature today, but have never been applied together as a selection practice. By comparing a *Varroa* mite tolerant queen line with a normal line, utilizing this selection practice under controlled experimental conditions, we plan to determine if breeding for mite tolerance may be facilitated if breeders were to adopt these two assays, as a selection practice. We will make the experimental data and results available to all beekeepers and follow up with a step-by-step description and summary of the assays.

3. Farm Profile
Our enterprise is small-scale. We have approximately 125 colonies that we use to produce queens and extracted honey as a part-time, sideline endeavor with Adam and Kelly each working part-time. We market our honey locally and are developing more distant markets; we label our honey as being from bees managed without chemicals. Our queen sales have grown well as our reputation for producing productive, mite tolerant queens becomes known throughout the beekeeping community. Beekeepers are very fickle about purchasing queen bees. In this limited market, a good reputation is extremely important. We've experienced healthy growth and have limited our laying queen production this year to ensure our quality remains consistent. We're working on expanding our inseminated breeder queen market. Despite two poor honey seasons, our enterprise has been successful.

4. Participants
Our technical advisor, Dr. Dewey Caron, helped with outreach strategies and experimental design. Adam Finkelstein (owner) and Kelly Rausch (owner) performed the research and made outreach presentations. A part-time laborer performed equipment building.

5. Project Activities
Initially we proposed to test two selection assays in a scientifically controlled experiment

comparing the results between two different types of production honey bees. The results were to be analyzed to determine if there were any significant differences between the two lines and to discuss the selection assays.

After a poorly performing establishment period and generally poor beekeeping year (2008), almost all of one of our test lines had either swarmed or superseded and the resulting colonies were unfit to use in the original experiment. The following year (2009) we decided to concentrate on one assay, and test known and trial colonies with it, thus utilizing the assay for selection. We chose to use the alcohol wash assay (AWA) because it is simple and inexpensive to conduct. Samples may be taken and analyzed later at a more convenient time. The freeze killed brood assay (FKBA) requires procurement of liquid nitrogen, arrangement to obtain or rent (we were going to rent) a storage dewar, and two trips to each colony tested to obtain results. Our poor season last year coupled with the time constraints that queen production put on our schedule, influenced us to eliminate the FKBA from the project. That it was more difficult to set-up and run, is data to consider when planning out selection assays in one's breeding program.

We conducted three reps of the AWA, testing twenty colonies each time. We performed the tests in two different locations.

6. Results

A brief review of the Alcohol Wash Mite Assay (AWA) used:

Collection:

Live bees are collected from brood combs by sliding the entrance of collection vessel across the comb in a fashion to force bees into ~ 75ml or ¾ cup Isopropyl Alcohol (70% worked well. Ethanol (70%) may be used although it is more expensive). Samples may be collected and held until convenient to test.

Below is the step by step procedure:

Starting with the bottom of the frame, draw the open jar lid across the comb to collect the sample bees, ending at the top of the frame (see Pictures 1-5). Select two or more frames with mixed aged bees (open and sealed brood) as needed to collect a total of 100-200 bees per jar, slightly less than will fill the level of alcohol in the jar as seen in Picture 6.

Picture 1

Picture 2

Picture 3

Picture 4

Picture 5

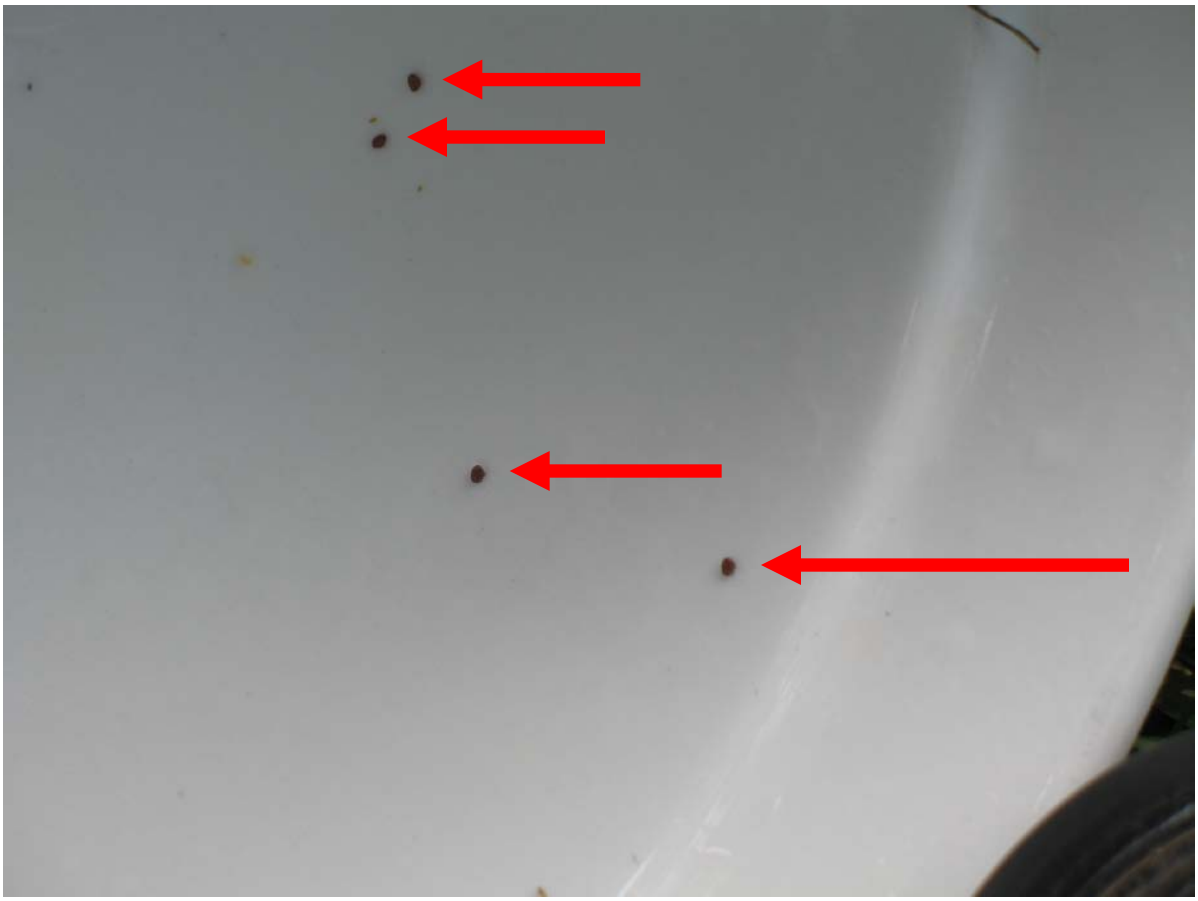
Picture 6

Testing:

The sample of dead bees and the alcohol in the jar are poured carefully through a coarse strainer into a light colored bowl (Picture 7).

Picture 7

Once poured, the sample container (jar) is checked for any adhering *Varroa* mites—if any are found, this number is recorded. Mites seen in the bowl with the alcohol are then counted and recorded (mites are identified in Picture 8 with red arrows).



Picture 8

The alcohol in the bowl can now either be discarded, or, the mites can be removed from it and it can be returned to the jar for reuse at another sampling time. Note that ALL mites must be removed from the alcohol if it is to be reused.

The bowl is then quickly rinsed with water to ensure no mites remain in the bowl. The sample of bees in the strainer is then sprayed with a moderately forceful water stream to "wash" the sample of bees and the resulting "wash" is collected in the light-colored bowl (Picture 9). This is done for ~ 5 -10 seconds.

Picture 9

The wash is then observed and the mites are counted and recorded. The bowl is rinsed clean with water**. The wash step is repeated twice more for a total of 3 washes**, counting and recording any mites washed into the bowl each time.

The total number of mites per sample is counted; the total number of bees in the sample are then counted.

The calculation for determining % of mites is:

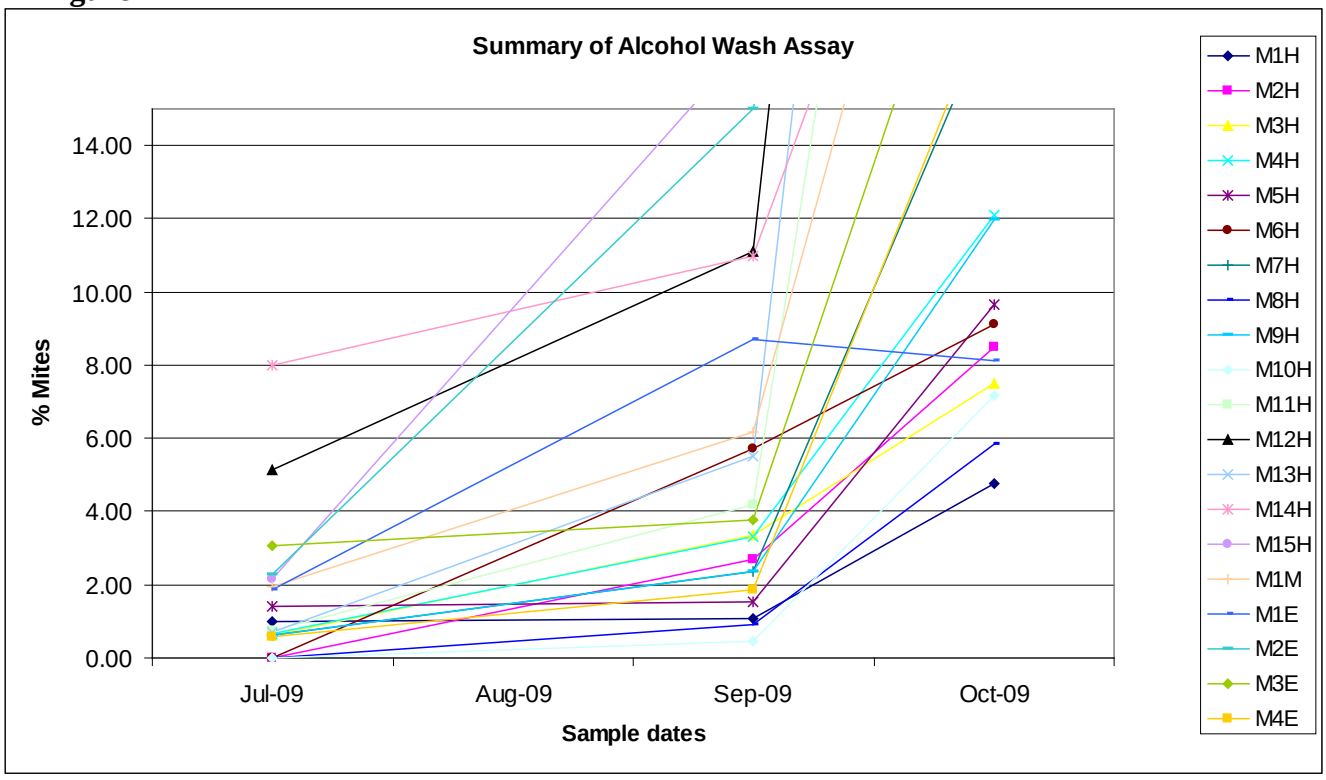
Total mites per sample/Total bees in sample X 100 = % Mites or mites/100 bees

We found that the most mites are observed after the first wash. Shorter washes are better than longer ones. Be careful when counting: stingers and pieces of pollen/hind-gut contents can look like mites. Look for immature mites. If there were no mites in a sampling, we did not count number of bees in the sample. We did not distinguish between drones and workers, but if the sampling is done on correct combs (active brood combs with mixed bees and brood) very few drones will be collected.

Twenty colonies were sampled three times over the season. We sampled when we felt we'd see the most accurate representation of the *Varroa* mite population, which occurs during time when the colonies have an active brood cycle. In our area, this usually corresponds to dates between May and October. This will differ depending on the local environmental conditions where the hives are, and the season (some seasons begin or end earlier due to weather conditions). We felt that an assessment taken 3 times during the period of the active brood cycle was sufficient. One can choose to sample more frequently than this, as long as the sampling points are spread through the time frame when the brood nest is active in the colonies.

All hives sampled had increasing numbers of mites over the season (see Figure 1). Many hives had a higher then optimum index of mites/100 bees or % mites. However, on some test samples not included in our dataset taken in the late Fall, mite populations fell by 50%, most likely as a function of broken brood cycles (see Figure 1). This verified that sampling in times of the season when there is not an active brood nest, or when the colonies are shutting down their brood nests in preparation for overwintering, was not an accurate timeframe for sampling.

Figure 1



Discussion:

We have set a threshold mite percentage for selection in our breeding program to be 5-7% (~ 5-7 mites/100 bees on average over the season). We report much higher mite levels with this data and found that the 2009 season had an extremely high mite population: we observed high mite loads in our production colonies. Do we then eliminate all the colonies tested this year since they do not fit into our selection criteria? This point is important (see Figure 1). Test colonies M1H, M8H, M10H, and M3H were all at or under 7 mites per hundred bees at their highest infestation level. Test colonies M1E, M2H, M6H, and M5H were slightly higher than 7 mites per hundred bees at their highest infestation level. For a breeder to maximize the value of the Alcohol Wash Assay, he/she would use these results in conjunction with other selection metrics (gentleness, honey production, comb building, i.e. any criteria you prefer to select for), as a screening mechanism. For example, from the first set of colonies at or below 7 mites/100 bees in an extremely heavy mite infestation year, colony M1H ranked the highest in our overall selection criteria. Thus, factoring in a low mite infestation percentage with an already outstanding candidate, ensures accuracy when selecting. We plan to use queen M1H this year as a breeder for test crosses. The AWA when combined with other rigorous selection metrics will facilitate accurate selections in a mite tolerance breeding program.

7. Conditions

2009 was another in a series of poor weather years for optimum nectar flow for honey bees. We also noticed an unusually high number of *Varroa* mites present in our colonies, as much as 75% more than has been the seasonal norm over the last few years. This high population has certainly affected our results in that we collected many more mites, but it also corroborates the validity of the results. 20% of the colonies tested showed 7% mite load at the heaviest infestation. That met our selection criteria for mite tolerance.

8. Economics

By choosing to eliminate the Freeze Killed Brood Assay from our research, we were able to spend significantly less capital and still select for desirable stock. Conducting Freeze Killed Brood Assays takes time (two trips to each colony tested, two or more times per season), and requires liquid nitrogen and liquid nitrogen storage equipment, which is costly.

The AWA, however, is a cost-effective method for use by anyone from a hobbyist to apiculturists with larger scale operations. A breakdown of the feasibility costs is as follows—this assessment is based on an *established* apiary, eliminating any start-up costs:

For each colony you choose to test, determine how many samples you will collect and have these spread throughout the season during times when there is an active brood cycle in your locale. We recommend 3 or more samples per season.

Time spent for each colony (sample) is estimated below. This will, of course, depend on the number of colonies you will sample, and the travel time to each apiary/colony.

- Time to prepare for each sampling period (date): 15 minutes, to include filling mason jars with alcohol, labeling them to match the colonies you have chosen to test, and

preparing a notebook to record the colonies you've sampled and that date the samples were taken.

- Time to open colony and find frames with active brood for sampling: <10 minutes
- Time to collect samples of bees: <5 minutes
- Time to test each sample in the AWA: <15 minutes to include testing, counting and calculating results
- Time for cleanup: <5 minutes

This is a total of <35 minutes per sample, and most likely less than this.

Therefore, if sampling on 3 dates through the season, it will take approximately 1.75 hours per colony.

Costs associated with the performing the AWA are minimal. An estimate of expenses is below.

Mason jars and lids (pint size):	~ $ 1.33 per jar w/lid
Isopropyl alcohol (70%):	~ $ 0.20 per sample
Strainer:	~ $ 15.00
Light colored bowl:	~ $ 5.00
Baking sheet:	~ $ 5.00
Garden hose with spray nozzle:	~ $ 20.00

Many of these components are usually already available in a household, *and are all reusable*, including the alcohol for up to 3 samples, so this is a *very* cost effective and time effective test for the results it can produce, combined with other selection data.

Using the AWA in our breeding program to aid in accurate selection will possibly increase our chances of developing good breeding stock more quickly, but we will not know this until the next season. However, selecting two queens from a group of twenty, who show desirable production traits *and* who have an overall seasonal high mite load of 7% is certainly going to enhance our breeding stock for this season, and make the queens we produce desirable to beekeepers.

9. Assessment

Utilizing any selection tool that facilitates the propagation of desirable traits, is a boon to breeders. Selection tools for breeding hardy, mite-tolerant bees are welcomed by any honey bee breeder if they provide accurate measures of mite tolerance within a population. The AWA is a very simple means to compare members in populations within a given time-frame to facilitate screening and culling. As an added benefit, if performed over the season several times, a breeder will gain insight into the mite population growth in his/her area leading to more informed IPM decisions.

Several research papers are forthcoming that could provide more information on how to craft new VSH assays that could be used in conjunction with the AWA. Breeders will hopefully use the AWA in their breeding programs now, adding the new assays to their

existing selection techniques, as they become available.

10. Adoption

The results we obtained from this research are clear: using the Alcohol Wash Assay in a mite-tolerant bee breeding program is a simple and effective method to help in selection for ideal breeding stock. Testing stock three or more times throughout the season is recommended. Layering the results on a conventional performance evaluation dataset will make breeding candidate selection for the subsequent breeding program very straightforward. We will use the AWA every year in our breeding program.

If we had had a better season the first year, and our test population had fared better, testing the Freeze Killed Brood assay in conjunction with the AWA hopefully would have led us to a recommendation for adopting both assays in a selection practice for mite tolerance. However, even though we did not use the Freeze Killed Brood assay, we can state that it is more expensive and time consuming to perform then the AWA. If one had the resources, we'd recommend using both assays, however.

11. Outreach

We are performing our outreach program in two ways. We've given four presentations to regional beekeeping associations and one presentation to a National Beekeeping association (Eastern Apiculture Society annual meeting, August, 2009). We're arranging to present again at one of the National Summer meetings this year, either EAS again or perhaps another one. Our Technical advisor will assist us with the necessary arrangements.

Our website has a section devoted to this research: www.vpqueenbees.com/awa and also we discuss this research on the VSH bee breeding site: www.vshbreeders.org we created for VSH breeders and other bee breeding topics.

12. Report Summary

Testing two assays for their efficacy in a mite-tolerant breeding program was the goal for this project. We concentrated on the alcohol wash assay to measure a colony's phoretic mite level in the brood nest three times over the season. Colonies that had a low percentage of phoretic mites and that had other desirable economic traits were found and will be used for breeding. Bees were sampled from the active broodnest in alcohol. When convenient, these bees were put into a strainer and the alcohol drained into a container where the mites were counted—then the bees were washed three times with a water spray through the strainer and mites counted again to determine total mite count per sample. Mite counts were converted to a percentage. This assay is simple and compliments a performance evaluation selection program. The low measurement of phoretic mites in a colony throughout the year is a good indicator that the colony shows the VSH traits. Colonies that have low mite percentages and that do well will become excellent candidates for breeding stock. We recommend this assay to anyone working on a mite tolerant bee breeding program.

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