ORGANIC FARMING RESEARCH FOUNDATION

Project report submitted to the Organic Farming Research Foundation:

Project Title:

Natural Products for Control of Parasitic Honey Bee Mites

FINAL PROJECT REPORT

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Summary:

We began by examining a number of management techniques. Our work with formic acid has contributed to the registration of Mite-Away II, a formic acid product very similar to the ones we worked on. Our data were provided to the manufacturer to assist in the formulation of their product.

Our examination of mite resistant stocks has led to the establishment of a breeding population being selected for mite resistance, hygienic behavior, honey production and wintering ability. Due to the two-year cycle required for evaluation, selection and mating, this is a long-term project that is not expected to yield results for 6 years.

We completed an evaluation of two non-chemical methods for managing *V. destructor*:

1. Drone comb traps: the objective of this experiment was to determine whether the application of the drone trap method would maintain mite levels at acceptable levels throughout the summer until the end of the late summer nectar flow (goldenrod). The method is based on an understanding of the biology of the mite. Mites are found most often on drone brood where they produce about twice as many offspring as on worker brood. Therefore, by removing capped drone brood from an infected colony, one removes a disproportionately large number of mites without affecting the worker population, and one removes those mites with the highest fecundity. We found drone comb traps to be highly effective in suppressing the growth of mite populations in honey bee colonies.

2. Screen bottom boards: the objective of this experiment was to determine whether the application of the drone trap method would maintain mite levels at acceptable levels throughout the summer until the end of the late summer nectar flow (goldenrod). Many studies have shown that mites fall off of bees at relatively high rates, even when no chemical treatment is present. Many of these mites are still alive and manage to reacquire a host. It is commonly believed that mite populations can be suppressed if these fallen mites could be removed from the colony before they reacquire a host. The screen bottom board allows mites that fall from bees to fall out of the hive. Since they cannot re-enter the hive, they cannot reacquire a host and they cannot contribute to the growth of the mite population. Screen bottom boards are being heavily promoted to beekeepers by suppliers of beekeeping equipment and by various extension apiculturists. However, there is little data available upon which to base recommending or not recommending the use of these devices. We found no evidence that screen bottom boards would be helpful in suppressing mite population in honey bee colonies.

Previously, we had identified a number of essential oils that killed mites in laboratory bioassay, but we have not been able to develop these compounds into functioning treatments. Our efforts at incorporating essential oils into smoker fuel did not yield positive results.

Introduction to Topic:

The western honey bee, *Apis mellifera*, was introduced to the US from Europe in the 1600's and is the only species of honey bee present in the US. The honey bee provides the majority of pollination for over

90 commercial crops grown throughout the US. The value of honey bee pollination to US agricultural production is estimated at $14.6 billion annually. In addition, US beekeepers produce between 170 and 220 million pounds of honey each year, more than 50% of total US consumption. Hence, a sustainable supply of healthy and affordable honey bee colonies is a critical factor affecting farm productivity and the stability of farm incomes and food prices.

The parasitic honey bee mite, *V. destructor* (Fig. 1), is considered to be the most serious global threat to beekeeping and to the sustainable production of crops that rely on *A. mellifera* for pollination. *V. destructor*, which kills honey bee colonies of European descent within 1-2 years, was detected in the US in 1987. Since then, it has killed millions of managed colonies and has greatly reduced the number of wild colonies. The synthetic pesticides Apistan® and CheckMite+® have provided some relief, but control has always been unpredictable due to the fact that mite populations often rise rapidly during the honey producing season when treatment is proscribed by label restrictions. Consequently, colonies often suffer serious damage while the beekeeper waits for a legal treatment window to open. The threat from *V. destructor* has become a matter of increasing concern as mite populations in the US have developed resistance to both Apistan® and CheckMite+®.

To continue as a viable enterprise, beekeepers require sustainable management practices that keep mite populations below the economic injury level. The best approach to achieving this goal is the development of management programs that rely on multiple tactics, rather than solely on chemicals. One such approach is referred to as Integrated Pest Management or IPM. The IPM approach incorporates chemical and non-chemical tactics; however, for several reasons, IPM seeks to minimize the use of chemicals whenever possible. First, chemicals add a recurring cost to a beekeeper's management program. Second, chemicals inevitably show up as residues in hive products; and that jeopardizes their reputation as pure and natural products. Third, chemicals can be injurious to the applicator and may pose a risk to the consumer. This also raises the issue of liability, especially for beekeepers with employees. Fourth, the less a pest population is exposed to a pesticide, the more slowly it develops resistance to that pesticide. So, by minimizing the use of a pesticide, its useful lifetime is extended.

Rational for IPM Program:

In order for a colony to survive the winter in good condition, it must have a strong population of healthy worker bees in the fall (Fig. 2). A colony exhibiting early stages of parasitic mite syndrome (the end stage of mite infection; Fig 3 and Fig. 4) in mid-summer can usually be saved by the application of an effective miticide because it still has time to produce two or more generations of healthy workers in a low-mite environment. However, in the northeastern US, these symptoms usually occur during or just prior to the fall nectar flow when chemical treatments are proscribed by label restrictions. By the time the flow is over, mite populations have increased dramatically and colonies have suffered severe damage. The result is a loss of colonies during the fall flow or shortly thereafter. This phenomenon is known as 'fall collapse', although it may occur in late summer or winter. The lesson is simple. One cannot assume that colonies will survive if treatments are delayed until the end of the fall flow to apply a pesticide. Mite levels must be kept at low levels during the summer in order that colonies can rear healthy workers during late summer and early fall. Two methods that offer promise for achieving this goal are the drone trap method and the screen bottom board.

Objective Statement:

My objectives were:

1. to provide beekeepers with safe, effective, reliable and affordable alternatives to Apistan and Coumaphos for control of parasitic mites. Currently, these chemicals must be applied twice each year to ensure colony survival, and that is often insufficient. I will investigate alternative strategies that either reduce the use of synthetic pesticides by ½ or that eliminate them all together.
2. to develop a means of keeping mite levels at relatively low levels throughout the summer, in order that colonies are healthy when it is time to apply the fall treatment.

Materials and Methods:

1. **Drone comb traps:** Experimental colonies were treated with CheckMite+ in the fall. The following spring, quantities of bees and brood were equalized, but colonies were not retreated. The brood nest of each colony consisted of 18 full-depth worker combs and 2 full-depth drone combs (Fig. 5) housed in two, 10-frame hive bodies. Each worker comb had < 12.9 cm^2 of drone cells (Fig. 6 and Fig. 7). Drone combs were kept in the 2^{nd} and 9^{th} positions of the upper brood chamber. Standard management practices were used throughout the season, including the addition of honey supers above a queen excluder. Colonies were randomly assigned to one of two groups. In the control group, drone combs remained in place throughout the season. In the treatment group, drone combs were removed on 16 June, 16 July, 16 August and 16 September and replaced with empty drone combs (16 June) or with drone combs removed on the previous replacement date.

   Mite-to-bee ratios. The ratio of the number of adult mites per adult bee in each colony was estimated from samples of worker bees collected from brood combs in October according to the method of Calderone and Turcotte (1998).

   Fall worker population. The number of combs of adult bees at an ambient temperature of -2 ^oC to 0 ^oC, was estimated in the fall using the method of Nasr et al. (1990).

   Weight gain. Colony weight gain, primarily a measure of honey production (McLellan 1977), was determined by weighing colonies on 11 June, 18 August and 25 September and calculating weight gains/losses after adjusting for the weights of supers added and removed.

   Analysis. Data were analyzed with PROC MIXED (SAS 1996) using a fixed-effects, factorial design with treatment and apiary modeled as main effects. Significant interactions between main effects were analyzed with the Tukey-Kramer test (SAS 1988).

   Number of cells of capped drone brood removed from treatment colonies.
   Drone combs were photographed after being removed from treatment colonies on each replacement date, and the number of cells of capped drone brood removed from each colony was determined by counting. Data were collected for informational purposes.

2. **Screen Bottom Boards:** The study extended over three years. In the first year, 64 colonies were randomly assigned to one of two groups: a treatment group in which colonies received screen bottom boards and a control group in which colonies received regular, solid bottom boards. Equal numbers of colonies from both groups were randomly

assigned to four apiary sites for evaluation. In both the second and third year, 32 colonies were randomly assigned to one of the two treatment groups, but colonies were kept in a single apiary each year. The same three types of data collected for the drone comb traps were collected for the screen bottom boards.

Project Results:

1. **Drone Comb Traps:**

   Mite-to-bee ratios. Mite-to-bee ratios in the two groups at the end of the fall nectar flow (October) were significantly different. Colonies in the control group had an average ratio of 0.109 ± 0.017, compared to a ratio of 0.025 ± 0.016 in the treatment group ($P \leq 0.0001$). The lowest and highest ratios were 0.012 and 0.441, respectively, in the control group, and 0.000 and 0.070, respectively, in the treatment group. The interaction between treatment and apiary was significant ($P \leq 0.0121$). Tukey-Kramer tests revealed significant treatment effects in the Cole Grove and Durfee Hill apiaries. In the third yard, mite levels remained low in both the treatment and control group.

   Fall worker population. The average worker populations in October in the two groups were not significantly different. The average number of combs of bees in the control group was 6.24 ± 0.68, compared to 6.71 ± 0.63 combs of bees in the treatment group ($P \leq 0.6204$). The interaction between treatment and apiary was not significant ($P \leq 0.4009$).

   Weight gain in period 1. Average weight gains in the control and treatment groups were significantly different. The average gain in the control group was 20.87 ± 3.12 kg, compared to 30.76 ± 2.89 kg in the treatment group ($P \leq 0.0188$). The interaction between treatment and apiary was not significant ($P \leq 0.440$).

   Weight gain in period 2. Average weight gains in the control and treatment groups were not significantly different. The average gain in the control group was 36.02 ± 2.72 kg, compared to 38.09 ± 2.52 kg in the treatment group ($P \leq 0.5129$). The interaction between treatment and apiary was not significant ($P \leq 0.9543$).

   Seasonal weight gain. The average seasonal gain in the control group was 56.89 ± 4.83 kg, compared to 68.84 ± 4.47 kg in the treatment group ($P \leq 0.0709$). The interaction between treatment and apiary was not significant ($P \leq 0.8421$).

   Number of capped cells of drone brood removed. The average number of capped cells of drone brood removed from treatment colonies in each apiary on each replacement date are given in Table 1.

2. **Screen Bottom Boards:** Mite-to-bee ratios were estimated in the early fall each year. The average mite-to-bee ratio in the treatment group was not significantly different than the corresponding ratio in the control group in any year. Screen bottom boards did not adversely affect colony health as measured by the size of the worker population or by honey production. Fall worker populations were similar in the two groups. Similarly, seasonal honey production was similar in the two groups. These data demonstrate that screen bottom boards do not provide any benefit as a mite control tactic during the honey producing season.

Table 1. Number of cells of capped drone brood (mean ± SE) removed from colonies in the treatment group in each apiary on each replacement date.

Date	Cole Grove	Durfee Hill	Nelson
16 June	2,860.56 ± 192.74	1,711.75 ± 344.75	1,587.50 ± 563.39
16 July	1,599.50 ± 270.87	2,247.29 ± 193.70	1,497.83 ± 261.11
16 August	1,863.00 ± 503.34	1,965.14 ± 442.21	2,678.17 ± 270.70
16 September	1,272.89 ± 250.22	1,501.14 ± 398.34	1,350.50 ± 276.99
Total[1]	7,354.50 ± 1,014.03	7,506.14 ± 762.40	7,114.00 ± 961.89

n = 9 for Cole Grove, n = 7 for Durfee Hill, n = 6 for Nelson

[1] The average number of cells of capped drone brood removed from each colony during the entire experimental period

Conclusions and Discussion:

Drone brood removal holds significant promise as a major component in an IPM program for managing *V. destructor* in honey bee colonies. Drone brood removal suppressed mite levels throughout the summer and early fall. Mite-to-bee ratios in colonies in which drone combs were removed four times during the spring and summer were relatively low in October (average ratio < 0.03 in each apiary) compared to colonies in which drone combs were not exchanged (average ratio of 0.10 for the three apiaries). The lowest and highest ratios were 0.012 and 0.441, respectively, in the control group, and 0.000 and 0.070, respectively, in the treatment group. The average mite-to-bee ratios in the treatment colonies in the three apiaries remained below 0.03, regardless of the mite levels in the corresponding control colonies. This suggests that the amount of drone brood removed was more than sufficient to trap the available mites. The maintenance of low mite levels during the summer and early fall will greatly reduce the incidence of late summer and fall collapse, which is typical in mite-infested colonies. This will ensure that colonies going into the winter have healthy workers.

The drone brood removal method has implications for the frequency of miticide application. Colonies in this experiment were not treated in the spring, the last miticide application being made the previous fall. The low mite-to-bee ratio in the drone comb treatment colonies after am entire year without pesticide treatment suggests that drone brood removal may eliminate the need for a spring treatment. In addition, colonies with mite levels below the economic threshold in the fall may skip an additional pesticide treatment. The limits and long-term value of this method still need to be investigated.

Drone brood removal did not adversely affect colony health as measured by the size of worker populations or by honey production. Fall worker populations were similar in the two groups. Average honey production was significantly greater in the treatment group than in the control group during period 1, similar to gains in the control group during period 2 and, perhaps, greater overall when measured over the season.

The screen bottom boards did not provide any control of *V. destructor*. The reason for this is unknown, but it may be because the fallen mites are sick or old and no longer able to reproduce.

While disappointing, this finding is important because of the widespread belief among beekeepers that this method is effective. Based on three years of data, there is no reason to recommend the use of these devices for control of mites.

Outreach:

Reviewed publications:

Calderone, N. W. (in press) Evaluation of Drone Brood Removal for the Management of *Varroa destructor* (Acari: Varroidae) in Colonies of the Honey Bee *Apis mellifera* L. (Hymenoptera: Apidae) in the Northeastern USA. J. Econom. Entomol.

Calderone, N. W. (in prep) Evaluation of Screen Bottom Boards for the Management of *Varroa destructor* (Acari: Varroidae) in Colonies of the Honey Bee *Apis mellifera* L. (Hymenoptera: Apidae) in the Northeastern USA. J. Econom. Entomol.

Calderone, N. W. (in review) Integrated Pest Management for *Varroa destructor* in Colonies of the Honey Bee *Apis mellifera* in the Northeastern USA. *SARE Agricultural Innovations* (This is a new publication series for USDA Sustainable Agriculture, Research and Education (SARE) Program who also provided funds for this project)

Extension publications:

Calderone, N. W. (in prep) IPM Methods for *Varroa destructor* in Honey Bee Colonies. Bee Culture. (This is one of the two major trade industry trade journals) (scheduled for May 2005 issue).

Calderone, N. W. (in prep) Using Drone comb traps for managing *Varroa destructor*. The Bee-Files (electronic fact sheets available from masterbeekeeper.org) (to be available this year).

Calderone, N. W. (in prep) Using Drone comb traps for managing *Varroa destructor*. Printed Fact Sheet for distribution to beekeepers by state apiary inspectors in the northeast) (to be available this year).

Calderone, N. W. (in prep) IPM for *Varroa destructor*. The Bee-Files (electronic fact sheets available from masterbeekeeper.org) (to be available this year).

Calderone, N. W. (in prep) IPM for *Varroa destructor*. Printed Fact Sheet for distribution to beekeepers by state apiary inspectors in the northeast) (to be available this year).

Other venues:

The information developed from this project is being used as the basis for talks with beekeeping groups and is being incorporated into the Cornell University Master Beekeeper Program Workshops. It will also be used in an upcoming presentation to the Eastern Apiculture Society in August at their Annual Meeting to be held in Kent, Ohio (600+ participants anticipated).

Sample talks:

Integrated Pest Management for *Varroa destructor* in Honey Bee Colonies

Empire State Honey Producers Annual Meeting, Owego, NY, November 13, 2004 (69 participants)
Washington State Beekeepers Association Annual Meeting. October 16, 2004 (63 participants)

Addenda:

Figure 1. *Varroa destructor* on adult worker honey bees.

Figure 2. Strong colony of bees in the fall.

Figure 3. Worker with deformed wing virus, a symptom of parasitic mite syndrome.

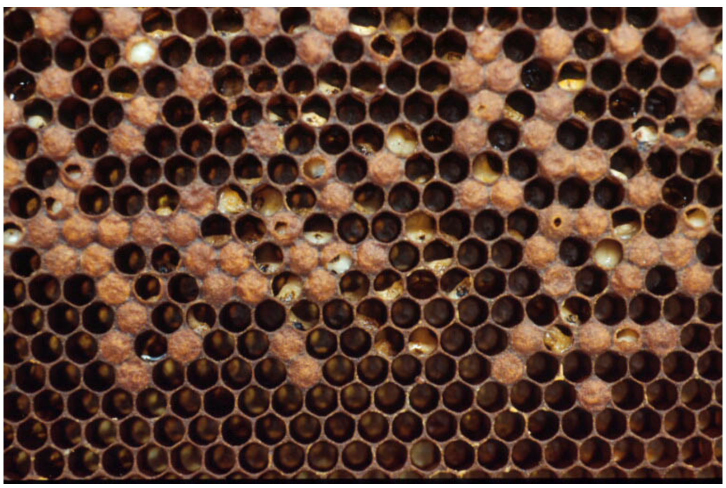

Figure 4. Deteriorating honey bee brood, a symptom of advanced parasitic mite syndrome.

Figure 5. Drone comb with capped drone brood used to trap mites.

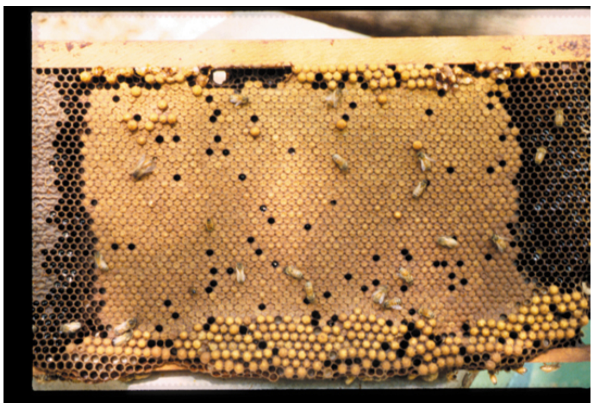

Figure 6. Worker comb with too many drone cells (the large cells) for the drone comb exchange.

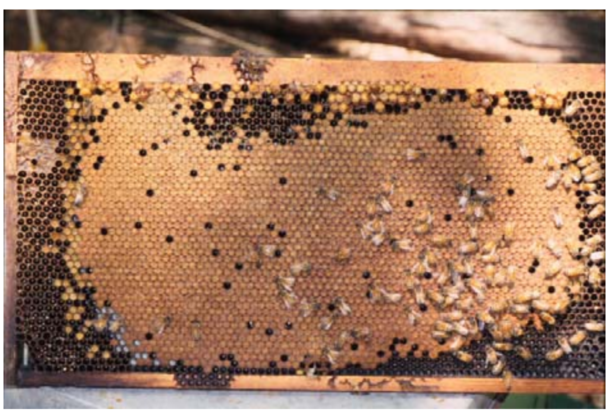

Figure 7. Ideal worker comb (few or no drone cells) for the drone comb exchange method.

References:

Amdam, G. V., K. Hartfelder, K. Norberg, A. Hagen, and S. W. Omholt. 2004. Altered physiology in worker honey bees (Hymenoptera: Apidae) infested with the mite *Varroa destructor* (Acari: Varroidae): A factor in colony loss during overwintering? J. Econ. Entom. 97: 741-747.

Baxter J., Eischen F., Pettis J., Wilson W.T., Shimanuki H. 1998. Detection of fluvalinate-resistant varroa mites in US honey bees. Am. Bee J. 138, 291.

Calderone N. W., and R. Turcotte. 1998. Development of sampling methods for estimating infestation levels of *Varroa jacobsoni* (Acari: Varroidae) in colonies of the honey bee, *Apis mellifera* (Hymenoptera: Apidae) J. Econ. Entomol. 91: 851-863.

Calderone N. W., and L.P.S. Kuenen. 2001. Effect of Western Honey Bee, *Apis mellifera* L. (Hymenoptera: Apidae), Colony, Cell Type and Larval Sex on Host Acquisition by Female *Varroa destructor* (Acari: Varroidae). J. Econ. Entomol. 94: 1022-1030.

Calis J. N. M., W. J. Boot, J. Beetsma, J. H. P. M van den Eijende, A. de Ruijter, and J.J.M van der Steen. 1999. Effective biotechnical control of varroa: applying knowledge of brood cell invasion to trap honey bee parasites in drone brood. J. Apic. Res. 38: 49-61.

Charriere, J. D., A. Imdorf, B. Bachofen, and A. Tschan. 2003. The removal of capped drone brood: an effective means of reducing the infestation of varroa in honey bee colonies. Bee Wld. 84: 117-124.

DeJong, D. 1990. Mites: *Varroa* and other parasites of brood. *In* R. A. Morse and R. Nowogrodzki (eds.) Honey bee pests, predators, and diseases, 2nd edition, Cornell University Press, Ithaca, NY. pp. 474.

Elzen, P. J., F. A. Eischen, J. R. Baxter, G. W. Elzen, and W. T. Wilson. 1999. Detection of resistance in US *Varroa jacobsoni* Oud. (Mesostigmata: Varroidae) to the acaricide fluvalinate. Apidologie 30: 13-17.

Elzen P. J., F. A., Eischen, J. B., Baxter, J., Pettis, G. W. Elzen, and W. T. Wilson. 1998. Fluvalinate resistance in *Varroa jacobsoni* from several geographic locations, Am. Bee J. 138: 674-676.

Elzen P.J., Westervelt D. 2002. Detection of coumaphos resistance in *Varroa destructor* in Florida, Am. Bee J. 142: 291-292.

Fries, I., and H. Hansen. 1993. Biotechnical control of varroa mites in cold climates. Am. Bee J. 133: 435-438.

Fuchs, S. 1990. Preference for drone brood cells by *Varroa jacobsoni* Oud. in colonies of *Apis mellifera carnica*. Apidologie 21: 193-196.

Harbo, J. R., and J. W. Harris. 1999. Selecting honey bees for resistance to *Varroa jacobsoni*. Apidologie. 30: 183-196

Harbo, J. R., and J. W. Harris. 2001 Resistance to *Varroa destructor* (Mesostigmata: Varroidae) when mite-resistant queen honey bees (Hymenoptera: Apidae) were free-mated with unselected drones. J. Econ. Entomol. 94: 1319-1323

Harbo, J. R., and J. W. Harris. 2003. An evaluation of commercially produced queens that have the SMR trait. Am. Bee J. 143: 213-216.

Harris, J. W., J. R. Harbo, J. D. Villa, and R. G. Danka. 2003. Variable population growth of *Varroa destructor* (Mesostigmata: Varroidae) in colonies of honey bees (Hymenoptera: Apidae) during a 10-year period. Environ. Entomol. 32: 1305-1312.

McLellan, A. R. 1977. Honeybee colony weight as an index of honey production and nectar flow: a critical evaluation. J. Appl. Ecol. 14: 401-408.

Nasr, M. E., R. W. Thorp, T. L. Tyler, and D. L. Briggs. 1990. Estimating honey bee (Hymenoptera: Apidae) colony strength by a simple method: measuring cluster size. J. Econ. Entomol. 83: 748-754.

Rosenkranz, P., and W. Engels, 1985. Consistent drone brood removal, an effective biotechnical measure for reducing the damage caused by varroa disease in honeybee colonies. Allgemeine Deutsche Imkerzeitung 19: 265-271.

Santas, L.A., and D. M. Lazarakis, 1984. Using drone brood in the control of the varroa disease of bees in Greece. Entomologia Hellenica 2: 63-68.

SAS Institute. 1988. SAS/STAT user's guide, release 6. 03 edition. SAS Institute. Cary, NC.

SAS Institute. 1996. SAS/STAT software. Changes and enhancements. SAS Institute. Cary, NC.

Shimanuki H., N. W. Calderone, and D. Knox. 1994. Parasitic mite syndrome - I. The symptoms. Am. Bee J. 134: 827-828.

Strange, J., and W. S. Sheppard. 2001. Optimum Timing of Miticide Applications for Control of *Varroa destructor* (Acari: Varroidae) in *Apis mellifera* (Hymenoptera: Apidae) in Washington State, USA. J. Econ. Entomol. 94:1324-1331.