

# APIS



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## Varroa Resistance to Fluvalinate: Evaluating Current Status

AT THE MOST RECENT Honey Bee Technical Council meeting in Gainesville, Fla., there was much discussion about the probability that Varroa had become resistant to fluvalinate in Florida. Fluvalinate is the active material in the one registered product for the mite, Apistan®, which is formulated as a plastic strip. Sometimes fluvalinate and Apistan® are used interchangeably. This discussion was precipitated by Dr. Frank Eischen's article in the February 1998 *American Bee Journal* (Vol. 138, No. 2, pp. 107-108), "Varroa Control Problems: Some Answers." Dr. Eischen and James Baxter, who first noticed that mites were not responding to treatment, did several tests showing:

1. Colonies had plenty of mites after being treated with Apistan® strips and/or "home-made cardboard strips soaked in Mavrik® (active ingredient here is also fluvalinate). This was shown because many more mites were killed in other colonies in the same condition (controls) treated with chemicals known to work elsewhere in the world, but not labeled in the United States.

2. These same Apistan® strips were then taken to Texas. They killed as many mites as controls (the other chemicals) in two different outfits, one in south Texas and the other in east Texas.

THESE RESULTS, according to Dr. Eischen, left him feeling more comfortable using the term "fluvalinate-resistant" to describe Varroa in the Florida colonies he'd worked on. Although this might be the case, Dr. Eischen saw nothing "to get excited about just yet." This is because the resistance appears to be localized. There is little evidence that it is widespread. This mirrors other parts of the world where resistance has been found. In France, for example, Apistan® still functions in the majority of that country, even though resistance has been detected for over a year, particularly in the South<sup>1</sup>.

Development of resistance to fluvalinate by Varroa in some Florida honey bee populations is no surprise. I reported on an Italian study ("Valutazione dell'efficacia dell'Apistan®," *L'apicoltore Moderno*, Vol. 83, June, 1992, pp. 95-8) in the October 1992 *APIS* that concluded that *V. jacobsoni* easily develops resistance to chemicals, and that beekeepers should be careful not to lose the war while winning a battle when treating mites<sup>2</sup>. The investigators recommended using fluvalinate legally, scrupulously adhering to recommendations on the label, treating all colonies at the same time and same season (autumn), and employing other kinds of control measures in conjunction with the one material available that is effective and legal.

*Continued next page*

<sup>1</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis97/apmar97.htm#3>

Two other issues brought up at the Technical Council deserve attention. One was concern that Apistan® is somehow defective. The product is suspect to some because it has been perceived to have altered over the years. These changes are reflected in statements on the label and/or revealed in physical differences in the strips, packaging, labeling or all three. Mr. Doug VanGundy, technical product support manager at Zoecon, the manufacturer, stated at the meeting that there had been no change in production to his knowledge since it became registered for general use<sup>3</sup>.

**A**T THE American Beekeeping Federation convention in Colorado Springs, Dr. Bill Wilson discussed experiments with older Apistan® strips, manufactured earlier under a Section 18 label and formulated on a different plastic. The results, he said, were an enigma. They showed a better level of control for the older than the newer strips. The explanation for this could be that fluvalinate is released faster from the earlier plastic, putting more material in the hive to kill mites. However, this inserts an element of uncertainty into the resistance situation.

Section 3 strips have been on the market for a long time and appeared to provide excellent results in every region of the United States until very recently. In addition, there continue to be reports from most of the rest of the country that there are no control problems using new strips, the reason for Dr. Eischen's assertion that there is little reason to get excited. Finally, product failure is an explanation that has often been turned to when control measures that have worked in the past begin to fail.

In the November 1993 *APIS*, I quoted from an article by Drs. John Capinera, chair, and Majorie Hoy, eminent scholar, Entomology and Nematology Department, University of Florida, published in *Florida Grower and Rancher* about pesticide resistance found elsewhere<sup>4</sup>. The scenario these authors describe is eerily reminiscent

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Misuse of fluvalinate has probably occurred, as it has elsewhere in the world.

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of the current Varroa control situation in Florida:

“A grower observes that a treatment which formerly was effective for pest control no longer works quite as well. Blaming it on the weather, the applicator, or the product is the natural response. This is followed by increased frequency with higher rates of application, which prove temporary relief. But soon this also fails to provide satisfactory pest control. Eventually the problem is diagnosed as pesticide resistance. The grower scrambles to find another pesticide which controls the pests but in doing so experiences crop losses, higher pesticide costs and — increasingly — lack of alternative pesticides.”

Examples of this are legion. They include leafminer on celery, diamondback moth on cabbage, sweetpotato whitefly on tomato, green peach aphid on potato, broad mite on peppers and two-spotted spider mite on strawberries. The seriousness of the problem is indicated by the fact that as early as 1984, some 39 percent of 171 medical and 61 percent of 164 agricultural insects and mites showed resistance to pesticides. For more history, see the remarks by Dr. Robert Metcalf, considered the dean of pest control among entomologists,<sup>5</sup> and more recent information on a parallel situation, appearance of resistance to antibiotics by bacteria<sup>6</sup>.

In that article I concluded, “Whether alternative chemicals would become available for Varroa control is problematic. The authors of the article in *Florida Grower and Rancher* say that pesticides are increasingly concentrated in the hands of

only a few manufacturers that choose to market only to producers of large crops like corn and cotton<sup>7</sup>. Fewer, in some cases, no, options exist for developing chemicals for many minor uses. Thus, the risk of development of resistance to pesticides must be minimized in these minor crops.” For beekeepers, I said, this meant that “Apistan® should be treated like the rare commodity it really is.”

Another issue is that resistance is the result of “misuse.” This message is being widely broadcast as a major reason for the current situation. It appears to be why resistance in Italy developed as early as 1990<sup>8</sup>. However, it is not the only factor, as stated by Dr. Wilson in Colorado at the 1998 American Beekeeping Federation meeting. Resistance, he said, is not necessarily the result of unregistered formulations of fluvalinate or inappropriate use of Apistan®. It occurs in any situation where the same material (registered or not registered) is used year after year and there is no alternative. This is not the time, Dr. Wilson said, for beekeepers to blame chemical manufacturers or vice versa for appearance of this phenomenon. With reference to this, it is important to keep in mind that Apistan® is not necessarily a dead product when resistance appears. It could possibly be used again after use is discontinued for some period<sup>9</sup>.

There is, however, an element of truth in both scenarios discussed above. Beekeepers have been warned repeatedly to use fluvalinate according to the label to conserve Apistan®'s effectiveness, and most have. However, realistically it must be concluded that misuse of fluvalinate has probably occurred, as it has elsewhere in the world. Either way, as I quoted a German researcher in the February 1995 *APIS*: “It may not be a question of ‘if’ but only ‘when and where’ the first super-Varroa mites will show up in North America. This should serve a warning to all beekeepers to use control methods only as directed on their labels.”<sup>10</sup> ■

<sup>2</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis92/apoct92.htm#5>

<sup>3</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis90/apnov90.htm#1>

<sup>4</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis93/apnov93.htm#3>

<sup>5</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis88/apaug88.htm#3>

<sup>6</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis94/apnov94.htm#2>

<sup>7</sup> <http://www.css.orst.edu/herbgnl/tree.html>

<sup>8</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis90/apnov90.htm#1>

<sup>9</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis97/apmar97.htm#3>

<sup>10</sup> <http://www.ifas.ufl.edu/~mts/apishtm/apis95/apfeb95.htm#FL>

# Detecting Resistance

THE REAL QUESTION to be asking now is how bad is the situation for each beekeeper. Fortunately, Drs. J.S. Pettis, H. Shimanuki, and M.F. Feldlaufer have developed a user-friendly screening tool that can be used to detect miticide resistance in a Varroa population. Thanks to the authors and the *American Bee Journal* for providing permission to use this material in *APIS* prior to formal publication.

The assay is designed to screen Varroa for resistance to fluvalinate (Apistan®). It relies upon a comparison in mite fall between 2.5 percent and 10 percent strips using paired samples of live bees from the same colony. Two samples from each of six to 12 colonies should be collected in an apiary, and bees held in jars for 24 hours at 75 to 95 F. After 24 hours the mite fall is determined and a comparison is made between the number of mites that fall using the two dosages. If mite fall alone does not produce clear results then the number of mites remaining on the adult bees in each jar can be determined, which gives additional clarity and precision to the assay.

The authors define fluvalinate-resistant Varroa as a population that continues to increase in spite of the presence of Apistan® strips applied according to current label recommendations of one strip per five frames of bees. The assay was tested on four geographically separate Varroa populations, three of which were experiencing mite control problems. It can be modified to use a single sample per colony and can be read in as little as six hours. Apiary inspectors may find the use of a single dose advantageous, as it reduces the number of jars and allows more colonies to be sampled.

**Disclaimer:** This assay has been developed as a decision-making and management tool for beekeepers and apiary inspectors. The data collected to date are limited, but results have been consistent enough to provide guidelines for determining if resistance is present. We will con-

tinue to test the assay under various climatic and environmental conditions and against other Varroa populations. The decision scale may shift with additional data. The assay provides a screening tool but is not intended to provide precise information about the level of fluvalinate resistance. Mention of a proprietary product does not imply endorsement of that product by USDA-ARS.

## Materials Needed to Assay 12 Colonies:

1. 2.5 percent and 10 percent Apistan® strips, cut into 3/8 x 1 inch pieces (2.5 percent is sold as package bee strips, 10 percent Apistan® is the standard for colony strips)
2. Rubber gloves
3. 24 mason jars (wide-mouth pint jars are preferred)
4. Metal hardware cloth cut to fit jar lids, #8 mesh size (8 squares to the inch)
5. 24 sugar cubes, one per jar
6. 24 3 x 5 inch paper index cards
7. Cardboard box to shake bees into (copier paper box is ideal, 10 x 11 x 17 inches)
8. One-quarter cup measuring scoop for live bees (125 to 150 live bees) (40 to 50 ml beaker or 2 ounce scoop will also work to give 125 to 150 live bees per scoop)

## Assembly Instructions:

1. Cut circles of hardware cloth (#8 mesh) and press them into the ring portion of the lid; the screen provides ventilation and allows for mite removal after 24 hours. Window screening (#12 mesh) **is too small** to allow mites to easily pass through.
2. Cut the 2.5 percent and 10 percent Apistan® strips at 3/8 inch intervals along the length of each strip. This will give you 3/8 x 1 inch pieces, as the width of the strips is approximately 1 inch. **Use rubber gloves when handling strips.**

3. Staple a single piece of strip into the center of each 3 x 5 inch index card to yield 12 cards of the 2.5 percent and 12 cards of the 10 percent Apistan®, write 2.5 percent or 10 percent on each card. You need paired samples, so number each of the two-card sets 1 through 12
4. Place cards inside the jars with the Apistan® facing inward. The card should fit snugly along the side of the jar with the 5-inch length wrapping around the sides of the jar.
5. Place a sugar cube in each jar and cover with the screen lids.

## Apiary Instructions:

1. Select 12 colonies with Varroa mites (select colonies with five or more mites detected with ether roll). It may be necessary to test several colonies with the ether roll method to assure adequate Varroa levels for a good resistance assay.
2. The jars are numbered in pairs, so place two paired jars next to each colony to be tested. Avoid direct sunlight on the jars if possible.
3. Open the first colony, select a frame covered with bees from the brood nest, check for the queen and remove her if necessary. Shake the bees into a cardboard box. Quickly bump the box to one side and use a quarter-cup container (2 ounces) to scoop up bees, placing one scoop of bees in each of the two jars. Close the lid and place jars upright in the mason jar box with cardboard dividers. **Do not scoop bees directly from the frames**, as you can get different numbers of bees in each jar and increase bee mortality in the jars.

## Assessment of Mite Fall:

1. Once bees have been collected in the apiary keep them out of direct sunlight and find a place **to hold them for 24 hours between 75 and 95 F.**
2. Holding temperature must **not be too cold** — below 75 F — **or too hot** — above 95 F.
3. After 24 hours take each jar, invert it over a white piece of paper and hit the jar three times with the palm of your hand. The force of the blow will cause mites to fall through the screen onto the paper. Count all the mites (both live and dead) on the paper.
4. Compare the total number of mites that fell in the 2.5 percent jars with total mite fall from the 10 percent jars (totals from columns A). If mite drop is nearly equal

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between the two dosages, then mites can still be controlled with Apistan®. If the mite fall in the 2.5 percent jars is less than half the mite fall in the 10 percent jars, then mites will not be adequately controlled with Apistan®.

5. You may stop the assay at this point if results seem clear, but it is recommended to continue the assay by determining the number of mites that remain on adult bees within each jar and calculating a percent mortality (see assay modification and mite recovery techniques listed below).

#### Assay Modifications:

The assay as described above does not require the recovery of Varroa that remain on bees after 24 hours. However, the assay becomes more sensitive and additional information is gained if, after 24 hours, the mites that remain on the bees are also recovered and counted.

#### Mite Recovery Techniques:

**Heat Method:** To remove Varroa using heat, simply take the jars after counting the 24-hour mite drop and invert the jars, screen lids down, over sticky paper placed in the bottom of the cardboard box. Place box with live bees in an incubator or oven at 125 F. The Varroa will drop off the bees and through the screen before the bees die. When using heat, periodically check the jars to see if the bees have died. Once bees have died (in one to four hours), jars can be removed and mites counted on the sticky paper (adhesive shelf paper or butcher paper coated with PAM® or petroleum jelly will work). Greater than 98 percent of the Varroa mites can be recovered by using heat, but the bees will die in the process.

**Wash Method:** Before washing the index card should be removed by placing the jars in the freezer to chill the bees. The Varroa remaining on bees can be removed by adding water with either alcohol or soap (a few drops of liquid dish detergent per jar) through the screen, covering and shaking the jars to dislodge mites. The solution can then be poured through the screen and Varroa collected on cheesecloth or coffee filters. Collect the washing solution and rewash each set of bees within the jars to collect and determine the number of Varroa that remained on bees after 24 hours.

#### Percent Mortality:

To calculate percent mortality, determine the number of mites remaining on bees, then add it to the number that fell after 24 hours. Now divide the latter number (those that fell after 24 hours) by this total and multiply by 100.

#### Storage and Reuse:

The mason jar box with dividers can be used to transport and store jars. Jars containing cards with Apistan® strips should be transported and stored out of direct sunlight. Jars should be washed between uses and rinsed with hot water. No data has been collected on the reuse of strips, and this may decrease reliability of the assay. Thus, it is currently recommended that new cards and strips be made for each test.

#### Interpreting the Results:

If only the mite drop after 24 hours is considered and mite fall between the two doses are nearly equal, this indicates that mites are susceptible to fluvalinate. However, if the number of mites that fall in the 2.5 percent jars is less than half the num-

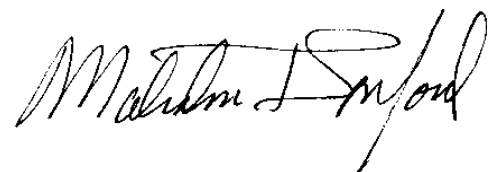
ber that fall in the 10 percent dose, then mites are resistant and alternative control measures should be considered when treating bee colonies against Varroa.

If percent mortality of mites is calculated, it gives you an accurate measure to compare with other locations and from year to year within your own operation. Don't base any decisions on the results from a single colony; consider the results from the apiary as a whole. When percent mortality is greater than 85 percent for either the 2.5 percent or 10 percent jars, then mites can still be controlled with Apistan®. If the percent mortality in the 2.5 percent is less than 30 percent, or in the 10 percent jars is below 50 percent, then you likely have fluvalinate-resistant Varroa. Remember, low temperatures during the 24-hour holding period can yield inconsistent results.

#### Remember These Four Precautions:

- 1) Don't base your decision on a single colony; consider the apiary as a whole.
- 2) Don't rely on small numbers. Five or fewer mites per jar from the assay can be misleading.
- 3) Use six or more colonies per apiary, and assay only colonies that you pre-screen with an ether roll test that have five or more mites present per sample.
- 4) Keep jars **warm (75 to 95 F)** to allow bees to move about and contact the plastic strip during the assay period. ■

Sincerely,



*APIS*, a monthly newsletter, is celebrating its 16th year of service to beekeepers. For subscription or other information, please write, phone, fax or e-mail.

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