Pesticides, Bees And Wax

An unhealthy, untidy mix

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This past Spring our lab, along with Clemson University, received a critical issues grant from the USDA to study the sub-lethal effects of miticides on honey bee colony health and performance, (bee population, brood production, honey production, and colony foraging rates), brood survivorship and adult longevity, and finally worker learning and responsiveness to queen pheromone. It is a two year study with our first season's data collection almost completed.

The study consists of six treatments with eight colonies per treatment for a total of 48 colonies. The treatments are Apistan™, CheckMite+™, Mavrik®, Taktic®, copper napthenate and a control (no chemicals). Treatments were inserted in the spring and fall. The chemicals used for the miticides are as follows: fluvalinate (Apistan™ and Mavrik®), coumaphos (CheckMite+™), and amitraz (Taktic®). All three chemicals control mites; however Mavrik® and Taktic® are not labeled for use in honey bee colonies but are used by beekeepers.

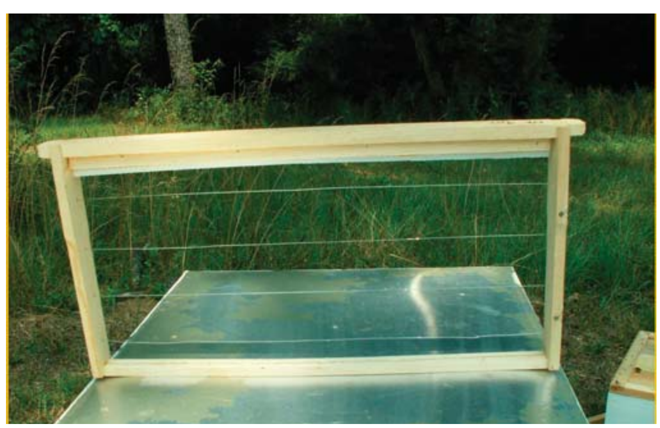
There were two main issues we had to address before the study could begin. First, we needed a source of "clean" (miticide free) wax foundation. We are examining the sub-lethal effects of different chemicals; therefore we needed to start with a clean slate, everything equal. If there are differing concentrations of chemicals unaccounted for, then what are we really measuring?

The first step was to analyze commercial foundation in order to find a source free of miticides. I quickly discovered this task had already been completed and the results were not good. Commercial foundation from the top five bee supply companies in the U.S. had been analyzed and residues of coumaphos, fluvalinate and the metabolites of amitraz were detected.

The next step in the venture was to ask several "chemical free" beekeeper friends for wax. Both were and had always been chemical free (including their wooden ware). Their samples were analyzed and again the news wasn't good. Both samples came back with detectable levels of coumaphos (512 & 870 PPB), a breakdown compound of coumaphos, coumaphos oxon (32 & 31 PPB) and fluvalinate (1820 & 2500 PPB). These compounds were detected at levels measured in parts per billion, which are miniscule amounts, but unfortunately still present. So, where did the chemicals come from? Here are a few ideas. Maybe bees from nearby apiaries, which have been treated with miticides, deposit chemicals onto the flowers they visit. When the "chemical" free bee visits these flowers she comes into contact with the chemical(s), bringing it back to the colony. Another idea, the miticides came in with the foundation that was purchased and placed into the hives. Both beekeepers used commercially bought foundation.

We kept searching and finally headed south, all the way to Brazil. Beekeepers in Brazil don't treat with miticides because of the Africanized bee population. They emerge in 19 days which is too early for the foundress mite's progeny to complete development before the adult bee emerges. Anyway, wax was collected and sent to our lab. It was analyzed and, unfortunately, there were so many other chemicals detected it wasn't suitable for our study.

Finally we gave up trying to find a source of untainted wax and settled on using a ½ inch strip of un-waxed, plastic foundation. Four wires were added to each frame to increase the strength and durability of the wax comb. The bees did a great job building the wax combs, however it was during a nectar flow. The only problem; if the colony was slightly tilted from left to right they would



Frame with strip of unwaxed plastic foundation and four wires.

A comb drawn out from the plastic strip.

build from say, the top bar of frame three to the bottom bar of frame four. Then it was quite difficult to remove and examine frames.

The second issue that needed to be addressed was *Varroa* mites. Miticides were being applied to four out of the six treatment groups so we assumed the mite population levels would be contained. But what about the control or the copper napthenate colonies in which no miticides were to be applied? Varroa could definitely take their toll on these colonies and affect the results. We needed a non-chemical treatment, so we turned to powder sugar.

Each time we applied powder sugar we inserted sticky screens to measure mite populations. The colonies which received miticides were given one treatment in the Spring and one in the Fall according to the label instructions. By November the coumaphos, fluvalinate and copper napthenate colonies had an overwhelming number of mites, well beyond the economic threshold level determined for the southeast. Interesting?

Let me give a quick background for each chemical we choose to examine. Fluvalinate, a synthetic pyrethroid, is the effective ingredient used in Apistan™ strips. It targets the axons or nerve fibers used for the transmission of nerve impulses. At one time it was the only chemical registered in the US for the control of *Varroa* in honey bee colonies. Since its introduction, the formulation has changed. The original or "racemic" form of fluvalinate has now been changed to tau-fluvalinate. The difference: it went from having multiple forms (racemic) to a single form (tau). By doing so, the toxicity levels have increased two-fold. The original median lethal dose (LD_{50} - the lethal dose it takes to kill 50% of a population) was 65.86 µg/bee but with the new formulation the LD_{50} is now 8.78 µg/bee. This new level is considered to be moderately toxic to honey bees. But the EPA reported back in the mid 1990s that the LD_{50} for fluvalinate is now 0.2µg/bee which makes it highly toxic to honey bees. Most of this information was reported by Maryann Frazier in the 2008 June issue of *American Bee Journal*.

Mavrik®, also a tau-fluvalinate product, is a broad spectrum insecticide/miticide used to control a whole array of insects including mosquitoes, ants, spiders, mites, ticks, springtails, cockroaches, fire ants, and aphids to name a few. It is used widely in residential and commercial settings plus nurseries and greenhouses. Since the active ingredient is fluvalinate, same as Apistan™, beekeepers use this product primarily because it is cheaper.

Coumaphos, an organic phosphate, is an insecticide used for the control of a wide variety of insects found on livestock. It is a cholinesterase inhibitor, which attacks the nervous system. It is used against insects that live outside the host animals, (ectoparasites) such as ticks, and mites. It was registered in this country for use in honey bee colonies under a Section 18 or emergency use registration because of the mounting resistance to fluvalinate being reported by beekeepers back in the 1990s.

Amitraz, a formamide acaracide-insecticide, is used to control red spider mites (deciduous fruit crops, citrus, cotton and certain other crops), and leaf miners, scale insects, whiteflies, and aphids in other agricultural settings. On cotton it is used to control bollworms, white fly, and leaf worms. On cattle, sheep, goats and pigs it is used as a topical spray or dip to control ticks, mites, lice and keds (wingless fly). Since it is an acaracide (pesticide that targets mites) and again cheaper, some beekeepers chose to use it to for controlling *Varroa* in their colonies. However, it is not registered for use in honey bee colonies and is therefore illegal.

Fluvalinate, coumaphos and amitraz are all contact poisons. It is transferred throughout the colony by bee to bee contact. The mite either comes into contact directly or from the bee. They are also lipophilic molecules which are more likely to be absorbed and detected in wax than in honey. Amitraz degrades rapidly because of exposure to sunlight (UV), low pH, metabolism by bacteria and solution properties. Degradation usually occurs within two to three weeks, and is not very stable in honey, which is good news. The bad news is the break down products or metabolites which form are 2, 4-dimethylaniline (2, 4-DMA) and 2, 4- dimethyl phenyl formamide (2, 4-DMPF). These products are apparently more environmentally stable, plus the 2, 4-DMA has mutagenic (causes changes to DNA), oncogenic (malignant transformation – tumors) and genotoxic properties (genetic mutations) (Osano *et al.*, 2002). Of course this is dependent on the levels present.

Because of mounting complaints from beekeepers about problems with queens (increasing supersedure rates, and colonies unable to re-queen themselves) researchers began investigating the sub-lethal effects of coumaphos and fluvalinate on queens and drones.

In 1999, Rinderer's group investigated the effect of Apistan™ on drones. Their findings showed a 9.4% reduction of drone survival in colonies treated with Apistan™. Other negative effects were observed as well: lower weights, mucus gland and seminal vesicle weights and the number of spermatozoa (Rinderer *et al.* 1999).

In 2002 a group of researches from across the U.S. examined the effects of queens reared in wax exposed to varying concentrations of fluvalinate and coumaphos. Queens weighed significantly less when exposed to high doses of fluvalinate than those reared in lower concentrations or controls. Even though these concentrations were higher than doses beekeepers would apply, the misuse or accumulation of fluvalinate in wax could lead to these higher concentrations within colonies. They also examined other effects of coumaphos and found that during queen development, body and ovary weight were both lower. Also, when one coumaphos strip was placed into colonies with developing queens, they suffered high mortality along with physical abnormalities and atypical behavior. Both of these findings conclude that when fluvalinate or coumaphos are applied during queen development there is a significant negative impact on the queen's health (Haarmann *et al.* 2002).

Two years later the effects of coumaphos on queen rearing was again examined. Known concentrations of coumaphos were applied to queen cups in which queen larvae were being reared. Queens exposed to 100 mg/kg of coumaphos (which, by the way, is the U.S. tolerance level allowed in beeswax) were rejected by colonies 50% of the time. If that exposure was increased 10 fold to 1000 mg/kg there was complete rejection (Pettis *et al.* 2004). There are two trains of thought here as to how the coumaphos may affect the queens. One the miticides are being passed around the colony from bee to bee and from bee to the nurse bees which are attending the developing larvae. The toxin is making direct contact with the developing queen. The bees detect this and therefore reject the cell or emerging virgin. The second thought is that coumaphos is being directly incorporated into the wax as the queen cell is being constructed, which the bees detect and reject (Haarmann *et al.* 2002).

Dr. Collins took the above study one step further. Beeswax cups in which queens were to be reared were exposed to known concentrations of coumaphos (0 to 1000 mg/kg). Young bee larvae were then transferred into those cups and allowed to mature. The cells were placed into mating nucs for 21 days and then into production colonies for six months, or they were dissected to determine mating success. Queens reared in coumaphos laden wax weighed less. All but one of the queens failed to develop after being exposed to 1000mg/kg. Greater than 50% of the queen cells were rejected in the group exposed to 100mg/kg. The number of queens still functioning in colonies after six months was reduced by 75% if they were reared in cells with the presence of coumaphos (Collins *et al.* 2004).

Queens aren't the only ones affected, drones are as well. A student from Virginia Tech recently investigated sperm viability of drones when exposed to miticides. Drones exposed to coumaphos (recommended dose on the label) during development and sexual maturation had significantly reduced sperm viability which continued to decrease over a six week sampling period (Burley *et al.* 2008).

At a point in our beekeeping history, fluvalinate and coumaphos may have served a purpose. You may remember the initial years of *Varroa* and how our colonies would not have survived without the use of these chemicals. However, over time, researchers, beekeepers and the bees themselves have found methods to reduce mite populations without the use of these harsh chemicals. Now with mounting evidence showing the negative impact these miticides are having on our bees, what more do we need to convince us? Sick, little, skinny queens mating with inept drones, which will soon be superseded by bees born in unhealthy, chemically laced wax; not something I want in my colonies.

We'll have more from this study when it has been completed and the results analyzed. Stay tuned. BC

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