COOPERATIVE EXTENSION UNIVERSITY OF CALIFORNIA

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Honey Bee Brood and Pesticides

For decades many of us have been reminding regulatory staff at the California Department of Pesticide Regulation and the United States Environmental Protection Agency that determining the safety of pesticides to honey bees involves a lot more than simply making topical or oral applications of pesticides to adult honey bees and following their mortality over the next 48 hours. Chronic exposure to sub-lethal doses and effects on brood just were not on their radar screens. When pressed, they would respond that they were not familiar with published papers on those subjects. They said they rely very heavily on published data to support their decisions.

I provided them the reference to the 1986 published paper by Atkins and Kellum ("Comparative morphogenic and toxicity studies on the effect of pesticides on honeybee brood," J. Apicul. Res. 25(4):242-255). I was informed that those chemicals were mostly no longer in use. I sent them a copy of my paper published in 2004 ("Effects of selected fungicides on growth and development of larval honey bees, *Apis mellifera* L. (Hymenoptera: Apidae)," Environ. Entomol. 33(5): 1151-1154). No response was forthcoming.

For quite a few years, beekeepers have been noticing dead and dying brood following applications of certain fungicides on almonds, and in some cases on other crops. While Rovral® and Pristine® are most frequently mentioned, others also seem problematic. I did find Rovral to cause problems with larval development in my study, but I did not test Pristine. I have reviewed a well-done study of exposure to a 2X field dose of Pristine to nucs confined in large flight tunnels planted solid with *Phacelia*. No detrimental effects to bees or brood were seen for many weeks.

Then, why do we see problems in some commercial crops? Probably because our hives contain so many types of residues (121 found, so far) from exposure to agricultural crop production chemicals. Apparently, one or more of the residues is synergized by the fungicide, causing the impaired brood development.

Often labels on certain agricultural chemicals will list compatible products with which they can be tank-mixed. This information usually is based on phytotoxicity – some mixes injure, rather than protect the crop plants. Couldn't the same type of information be developed for honey bee brood toxicity? Probably not. The phytotoxicity tests involve just a very limited number of chemicals. At least 121 chemicals might be involved in a beehive.

The most recent publication in this area is an entry by Ales Gregorc and James Ellis titled: "Cell death localization *in situ* in laboratory reared honey bee (*Apis mellifera* L.) larvae treated with pesticides." The citation is: Pesticide Biochemistry and Physiology 99 (2011): 200-207. In that paper, the researchers relate their findings that ALL the pesticides they tested caused cells in developing larval tissues to commit suicide by programmed death, which is called apoptosis (the second "p" is silent).

They listed the percentage of cells being lost in the midgut, by apoptosis, for the following chemicals, at the concentrations they used:

1. untreated larvae – approx. 10 percent
2. simazine – approx. 77 percent
3. myclobutanil – approx. 69 percent
4. glyphosate – approx. 69 percent
5. chlorpyrifos – approx. 61 percent
6. imidacloprid – approx. 61 percent
7. fluvalinate – approx. 30 percent

Similar damage was observed in the salivary gland tissue and in the nurse cells of ovaries. In a queen honey bee's ovaries, two cells are involved with each egg: the eventual egg cell and a nurse cell that moves along with it providing nourishment for the developing egg.

Is it any wonder that queens are not performing as they should? Are poor queens simply a consequence of chemical exposure to larval queens during development? Or, can similar damage continue in the body of a mature queen exposed to the chemicals while she is laying? I think it is the latter, since beekeepers complaining about poor queen performance and early queen losses can contact other customers, who obtained queens from the same batch from the same breeder, and find that things are fine elsewhere. I believe that hive pollution is a highly significant part of our queen failure problem.

What can be done? I've mentioned this before, and it bears repeating. It is time to remove the brood combs with twenty years' (hard to believe that *Varroa* has been here that long!) worth of mite control product residues in them. It can't be done all at once, unless there are many drawn

honey combs looking for a new role. It is true, that when clean combs are inter-sorted with contaminated combs, contamination is tracked onto the new combs. But that secondary contamination is nowhere near as concentrated as is the primary level.

We have learned enough about chemical synergisms between certain, studied residues to know that even our two earliest registered varroacides, fluvalinate and coumaphos, synergize each other. We are finding synergisms between fungicides and other comb residues. It is time to provide our bees with a cleaner home.

Grooming for Varroa Mites

Various studies have suggested there are three major ways that honey bees might thwart varroa mite reproduction. One is an intrinsic biochemistry that prevents mite reproduction. Preliminary studies suggest that such a mechanism may exist, but trying to tease it out and into a breeding program has been truly frustrating.

The second way that bees can reduce mite populations is by sniffing out the mites while they are trying to reproduce on the pupae. Bees showing "hygienic behavior" remove the infested brood and the mites do not increase in numbers. We currently have a number of lines of hygienic bees in the United States.

The third way that bees can reduce mite populations involves grooming behavior. *Apis ceranae* is particularly good at removing mites from co-inhabitants in the hive, whether other *A. cerana* individuals or *A. mellifera* co-inhabitants in cross-fostered colonies.

Researchers Shahera Zaitoun and Abd Al-Majeed Al-Ghzawi, from Universities in Jordan, followed the seasonal variation in the number of damaged (presumably bitten) varroa mites in hives of *A. m. ligustica* (Italian bees), *A. m. carnica* (Carniolan bees) and *A. m. syriaca* (Syrian bees).

Damaged mites were found throughout the year, but all three subspecies peaked in damaging mites in June, when the bee populations were peaking. Dr. Marla Spivak also says that her hygienic bees root out *Varroa* best during honey flows in mid-season.

At the peak grooming season, the Syrian bees groomed off about 38 percent of the mites. The Italians and Carniolans tied for second at a distant 25 percent. However, by September the mite populations went into geometric expansion and the Italian bees had the highest level of infestation at 33 percent. Carniolan bees were a bit better, at 31 percent, and the Syrian bees were best, but still were infested at a 27 percent level.

The authors failed to mention whether they thought the grooming behavior actually was of value to the bees. If you wish to review this article, the title is: "Monthly changes in the natural grooming response in workers of three honey bee subspecies against the bee parasitic mite *Varroa destructor*." Jordon J. Ag. Sci. 5(2): 207-216 (2009).

Register for Referendum Vote

In July, the California Department of Food and Agriculture (CDFA) will be conducting a mailed referendum (vote) on whether affected beekeepers wish to pay an assessment to fund the potential California Apiary Research Commission. The assessment, not to exceed $1.00 per colony (rate set by commission directors), would be

assessed on **ALL BEEKEEPERS** operating 51 colonies or more in California "with intent to earn income."

Unlike the previous California-only beekeeper assessment, from which research received only about 20 percent of the fees, the Commission will have direct access to practically all of the funds, minus the required, minimal, agency administrative fees.

In order to be eligible to receive a ballot, a beekeeper must return a voter eligibility form **BY MAY 30, 2011**. That happens to be Memorial Day, so I would suggest getting it there at least a few days earlier. I have placed the two letters of CDFA information and the Beekeeper Registration Form on my Entomology Department website, in pdf, so that you can print out the form and mail it in. I anticipate that all the beekeeper names and addresses that CDFA can find will be used to try to mail a form to you. I know that CDFA will not be able to find records for a lot of you who probably really would like to vote on this matter.

To keep this assessment in perspective, by law the seven-member commission, including one non-CA beekeeper, will decide where to set the assessment. If it were set at 50 cents per colony, that equals 0.03 percent (three-hundredths of one per-cent) of a $150 almond rental fee. That rate could raise over $500,000 to be used for research, anywhere around the globe, and distribution of research findings to the beekeepers. To find this information on the Internet, go to: www.cdfa.ca.gov/go/bees.

Venerable Fumagillin

Do you have an old bottle of Fumidil-B stuffed in the back corner somewhere? Or some aged Nosem-X or Fumagillin B?

Apparently, Dr. Robert (Rob) Cramer, in the Department of Veterinary Molecular Biology at Montana State University, Bozeman, wishes to see what effects the older formulations have on *Nosema cerana*. Dr. Cramer works with fungal infections of domestic mammals, and he was the first to question our use of fumagillin to treat *Nosema* infections.

When we started using the antibiotic, we did not know that *Nosema* was a fungus. We knew that fumagillin was being tested as an anticancer chemical. But, we didn't hear that fumagillin wipes out mammalian immune systems.

Dr. Cramer wishes to determine how pure the fumagillin was. The original manufacturer, Pfizer, was having difficulty with patent infringements. They made a deal to get out of the fumagillin business and sell the patent, if the competitor promised to maintained rigid quality control over the product. Apparently, there is some question about this.

If you have some "old" fumagillin sitting around, please send a good-sized sample to Rob. Be sure to send either the original bottle--photocopy the label if you wish to keep the bottle--or write down as much information as you can from the label. If you wish to speak to Rob, his office phone number is (406) 994-7467 and his lab number is (406) 994-7468.

The mailing address is:

Dr. Robert Cramer
Dept. Vet. Molec. Biol.
Montana State University
P.O. Box 173610
Bozeman, MT 59717.

UN Report on Honey Bees

Periodically the United Nations Environment Programme (UNEP) publishes reports on emerging issues. For 2010, the report was on honey bees and how they are faring around the world. The report is on-line and titled: "Global honey bee colony disorders and other threats to insect pollinators." The color photo on the cover shows an adult worker honey bee standing on a comb with a varroa mite on the dorsum of her thorax.

The world view puts the value of animal pollination into perspective with charts and graphs about global food production and the contribution of native bees and honey bees to the food supply. While not down-playing the value of bees in food production, one graph shows insect pollination responsible for less than an eighth of vegetable production, about one-sixth of edible oil crops production, less than a fourth of fruit production, about 40 percent of "stimulant crops" production, and about a tenth of spice production. They totaled it up to about 150 billion Euros ($210 billion, U.S.), globally, or "about 9.5 percent of the total value of human food production worldwide."

The report includes a nice seasonal colony population graph for European honey bees located in temperate climates. Of interest to California almond growers, the data taken from the French Agency for Environmental and Occupational Health and Safety report, titled: "Weakening, collapse and mortality of bee colonies," suggests that the normal, expected size of a healthy over-wintering colony is **five frames of bees**.

There is another graph that shows the number of honey-producing colonies in the U.S. since 1945. The graph looks a bit like a roller coaster at a theme park. There is a quick climb from 1940 to 1950 (4.3 to 5.6 million colonies). Then things go rapidly over the top and down the slope, dropping to 4.1 million in 1978. In 1982, things seemed to be headed back up again with an increase of about 200,000 colonies.

The next data point is 1987. Tracheal mites were pretty well spread across the country and varroa mites were just being found. Colony numbers dropped by nearly 1 million colonies. A slower, continuous decline in numbers followed for about ten years. Numbers still had been decreasing, through the end of their data set, to around 2.8 million colonies in 2007.

Subsequently, I believe that colony numbers across the country have leveled off at about 2.25 million. Keep in mind that these numbers reflect only the number of honey-producing colonies for which beekeepers have voluntarily submitted data to USDA National Agricultural Statistics Service (NASS). There are large numbers of colonies in northern California that are never involved in honey production and are not included in the NASS data base.

Data for Europe is not extensive in the UN report, but overall decline in colony numbers is the rule. Here is the generalized statement on colony conditions: "Since 1998, individual beekeepers have been reporting unusual weakening and mortality in colonies, particularly in France, Belgium, Switzerland, Germany, the United Kingdom, the Netherlands, Italy and Spain. Mortality has been extremely high when activity is resumed at the end of winter and the beginning of spring."

In Asia the generality is: "In recent years, Chinese beekeepers have faced several inexplicable and complex symptoms of colony losses in both *Apis* species (*A. cerana* and *A. mellifera*). Certain losses are

known to be caused by varroa mites on *A. mellifera*, sacbrood viruses on *A. cerana*, and *Tropilaelaps* mites on both species. However, other factors and mechanisms are being investigated, although no data has been published to date."

Most African beekeepers have not encountered extra beekeeping problems. However, "Egyptian beekeepers based along the Nile River have reported symptoms of CCD. One scientific experiment involved moving certain affected colonies to another habitat. The results have shown that a clean environment with diverse vegetation, compared to the original location, has an important role in defeating the symptoms of CCD. Until now, there are no other confirmed reports of honey bee losses from Africa."

The report continues with systematic descriptions of the various stresses on honey bees and other pollinators, with which we are all too familiar. Varroa mites are singled out as the worst problem. They conclude with a strong recommendation, for crop producers and anyone else with an opportunity: **plant more forage for pollinators**.

Industry Supporting Academia

Joe Traynor from Scientific Ag Company in Bakersfield, California, sent me a link to an interesting article concerning the poultry industry. Apparently, the UC Davis School of Veterinary Medicine did not have anyone on the faculty working with poultry.

Two industry organizations, the California Poultry Industry Federation and the Pacific Egg and Poultry Association, pledged their support to the Vet School to fund half the expenses of employing a faculty member dedicated to poultry health. The new faculty member will teach vet students specifically about poultry health and well being, methods of prevention and control of diseases with particular emphasis on viral and other infectious diseases. The poultry veterinarian will work with comercial meat and egg producers, smaller producers, and backyard poultry owners. The poultry veterinarian will see patients at the Vet Med Teaching Hospital on campus.

They did not say how much it would cost, annually, to meet their obligations, but the poultry industry generated about $1.2 billion in 2009 in chicken, eggs, turkeys and other products.

That got me thinking. How much gross income does our beekeeping industry generate in California? We provide around 500,000 colonies for almond pollination each year. At $150 per colony, that is $75 million. An additional $90 million is generated from pollinating the rest of California's 90 bee-dependent crops. A few years ago we were producing about $14 million in the bee breeding segment of the industry and about $44 million in honey. That totals somewhere around $223 million in gross income (a fifth of the poultry income). That sounds like a lot of money, BUT – how much does it cost to keep those 500,000 colonies alive and populous, so that they can do all that work?

Many beekeepers now invest significantly more than $200 a colony just to keep it at a size that meets the desires of almond growers each February. There goes over $110 million. Of the remaining income, how much is directed toward academia?

The California State Beekeepers' Association annually makes available for research projects around $50-75,000. Many of our beekeepers devote a lot of "in-kind" time (which equates to real monetary expenses) to helping USDA and university researchers conduct experiments on their

colonies. The beekeepers usually hope I won't ask them to help me – I have a track record of proving how to lose colonies.

I have an aversion to fundraising. If someone asks me to help with that, I open my wallet but I do not offer my assistance. Recent reductions in the University budget have cut severely into the support funds that the University now provides for my extension work. I am attempting to operate on half of the support that I had when I arrived in 1976. Had it not been for an extremely generous donation from the Gimbal's Fine Candies of San Francisco and many contributions that I have received from California beekeeping clubs and individuals, I would have run out of operational funds two years ago.

So where is this headed? I have my hat in my hand and I would appreciate it if any individual, small organization, large organization, or corporation would make a donation in support of my extension program. If the decision is made to do so, please make the check payable to the **UC Regents.** Enclose the check in a letter to the chair of the UC Davis Department of Entomology, Dr. Michael Parrella (same mailing address as mine: Entomology, University of California, Davis, CA 95616), explaining that you wish to donate funds in support of my extension program.

Yes, the Department skims off a very small amount for handling the funds, but I receive nearly all of it. There are no restrictions concerning the expenditure of the funds, as long as they are used for appropriate University business, which, of course, they are. Recently, nearly all those funds have been spent on travel and meeting expenses. Thank you for considering my plea.

Honey Antibiotics

We have become familiar with most of the basic components that tend to confer antibiotic activity to honeys:

1. high osmotic pressure – sucks the water out of the pathogens
2. hydrogen peroxide – formed in nectar and diluted honey by an enzyme added to nectar by the bee
3. acidity of about pH 3.2-4.5 – inhibits growth of many bacteria
4. methylglyoxal – a chemical found in biochemical pathways of bacteria
5. defensin-1 – called "the most important antibiotic component in honey" [or, Editor's note – perhaps a synergist that modulates the potency of *Manuka* honey?]

A couple papers published recently pertain to this topic. In a 2011 paper by Katrina Brudzynski and Linda Kim from Brock University in St. Catharines, Ontario, Canada, researchers examined the intensity of antibiotic activity in various local honeys and the longevity of antibiotic properties in stored honey.

They found buckwheat honey to be most potent, followed by sweet clover that was only half as potent as buckwheat. Blueberry was about one-third as potent as buckwheat, and wildflower was just a bit behind. Sunflower and blackberry honeys had only about one-fifth the potency of buckwheat, while cranberry, pumpkin, alfalfa, linden and loosestrife were quite limited in antibiotic effects.

Antibiotic activity was attributed to phytochemicals. After three months in storage, all but buckwheat honey became darker in color, the concentration of UV-absorbing compounds changed, melanoidins appeared, and the honeys lost half of their antibiotic activity. Buckwheat honey

maintained its full activity for three months, but it was down to half in six months. The activity remains constant after six months. The authors assume that the beneficial phytochemicals became sequestered in melanoidin aggregates. The citation is: "Storage-induced chemical changes in active components of honey deregulate its antibacterial activity," Food Chemistry 126: 1155-1163, 2011.

The second paper, by Paulus Kwakman and four other authors, explains what they found when they inactivated the enzymes, the hydrogen peroxide and the methylglyoxal in honey. It still possessed significant antibiotic properties, so they conducted further analyses. Here is a quote from the media: "Ultimately, researchers isolated the defensin-1 protein, which is part of the honey bee immune system and is added by bees to honey. After analysis, the scientists concluded that the vast majority of honey's antibacterial properties come from that protein." To review this March 4, 2011, document, look up "Two major medicinal honeys have different mechanisms of bactericidal activity," PLoS ONE 6(3): e17709. doi:10.1371/journal.pone.0017709.

Sincerely,

Eric Mussen
Entomology Extension
University of California
Davis, CA 95616
Phone: (530) 752-0472
FAX: (530) 752-1537
E-mail: ecmussen@ucdavis.edu
URL: **entomology.ucdavis.edu/faculty/mussen.cfm**

Eric Mussen
Department of Entomology
University of California, Davis
One Shields Ave.
Davis, CA 95616