COOPERATIVE EXTENSION UNIVERSITY OF CALIFORNIA

from the U. C. APIARIES University of California

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Newsletter Emailed to You

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Antibiotic Resistance

I reported earlier on a quick study that Dr. Brian Johnson and I conducted on some newly established packages that we started in new equipment on commercial foundation. We fed 20 of the 80 packages sucrose syrup, *ad libitum*, throughout the first two months after installation. We fed 60 other packages a cup of sucrose syrup at installation that contained 200 mg of each of the following: oxytetracycline hydrochloride, tylosin, and fumagillin. Those doses were the amount normally fed to full-sized colonies for disease prevention or treatment. Following that treatment, we fed the 60 colonies a 50:50 blend of sucrose and high fructose corn syrup, *ad libitum*, for the two months.

In case we eliminated some important microbes from the honey bee intestines, we fed a dog-designed probiotic containing 18 supposedly helpful microbes, at varying periodicities, including daily, to the 60 colonies.

Over the two-month period, we observed that the colonies fed the sucrose blend out-performed the sucrose-fed colonies. I questioned whether the intestinal microbes were really important to honey bee food digestion. This was not a new idea to me, since it seems that honey bees plow

right through freshly collected almond pollen daily and don't wait for microbial action in storage to assist digestion. Perhaps those intestinal organisms were not so critical, either.

However, I had forgotten to put two and two together. Had not *Paenibacillus larvae larvae* been treated with oxytetracycline hydrochloride (oxytet) for decades which eventually resulted in selecting for a strain of bacterium resistant to the antibiotic? That is why we had to start using tylosin and lincomycin. Thus, it might be possible that the intestinal organisms, required to assist in obtaining nutrients from pollens in the honey bee intestinal tracts, also are resistant to the medications.

A recent publication by Baoyu Tian, *et al*., working in conjunction with the Nancy Moran lab, currently at the University of Texas, Austin, reported on studies to determine the mechanisms of resistance in intestinal microbes of current U.S. honey bee colonies. In comparison, they examined colonies of honey bees from countries, in which antibiotic use is prohibited, and some bumble bees for resistant genes in their intestinal organisms.

The laboratory findings were very interesting. Not surprisingly, genes for oxytetracycline "efflux" were found in the intestinal organisms. Efflux is the name for a type of cellular pump mechanism that pumps antibiotics out of the bacterial cells quickly enough so that they are not toxic to the microbes. A number of these pumps can confer multi-drug resistance be-cause they are not too specific about what they move through the cell wall: metabolic inhibitors, organic solvents, and molecules involved in bacterial cell to cell communications, among others.

The researchers in this study found that after years of being subjected to relatively frequent exposure to oxytetracycline, there are at least eight different loci that have genes for resistance, including efflux genes *tetB*, *tetC*, *tetD*, *tetH*, *tetL* and *tetY*, as well as ribosome protection genes *tetM* and *tetW*. I included the list not to expect you to analyze your bees' microbes for resistance, but to show how many different genes have been selected for, apparently at different times and places. But, now, they are occurring together.

In the honey bee colonies that had not purposefully been exposed to antibiotics, and the bumble bee samples, there were resistant genes. They were very few in number and not replicated in the genome as extensively as are the U.S. resistance genes. The authors speculated that the few resistance genes they found in untreated honey bees and bumble bees are likely due to natural mutations or to chronic exposure to sublethal levels of antibiotics in the environment.

Thus, it might very well be that the reason our heavy dose of antibiotics did not seem to faze our commercial package bees was because the intestinal microbes can handle the antibiotics due to previous exposure to oxytetracycline, sulfathiazole, and fumagillin. Multiple drug resistance could also mitigate the effects of tylosin to these microbes. One way or another, it did not appear that feeding that cocktail of drugs negatively affected our colonies. But, did they really change anything in the bees?

The research article reviewed is: Tian, B., *et al*. 2012. Long-term exposure to antibiotics has caused accumulation of resistance determinants in the gut microbiota of honeybees. Doi:10.1128/mBio.00377-12.

ELAP in CA – Wazzup?

Beekeepers from various locations in the state have been contacting me asking what is going on with the USDA FSA ELAP Program. In a few words, it has been modified at the state level. That was done right here in Davis, so I didn't have to look very far to find the answer.

The document that includes the alterations to the program is "Livestock Disaster Assistance Program 1-LDAP (Rev.1), CA Amendment 1."

"Paragraph 816 – Eligibility Criteria
L. Eligible Adverse Weather of Loss Conditions for Honeybee Colony and Hive Losses
1) Colony Collapse Disorder (CCD), as an eligible cause of loss shall be limited to 35% of actual claimed losses.
2) COC shall determine all colony losses, which are claimed due to CCD, in excess of 35% as ineligible losses."

[How did they decide that? If you look at the only extensive set of data, to which only a few commercial beekeepers ever submit data, you will note that non-commercial beekeepers, describing their opinions of what killed their colonies, reported CCD in only about 7 percent of their responses. So CA FSA feels it is being generous with their 35 percent. Go to the website "beeinformed.org" and look at the colony loss data, etc.]

"Paragraph 845 – F Report of Colonies
Honeybee producers **must** also provide the following information on the printout of the automated FSA-578 within 30 days of occurrence:
Date and method of establishment of new colonies throughout the year.
Methods of establishment
a. Splitting existing colonies using
- Cell queens
- Mated queens
b. Purchase of bulk bees (any amount) with queens
C. Purchase of "nucs"
d. Purchase of completely mature and fully established colonies

Note: Producer shall sign and date after each entry."

[Why those questions? The FSA staff asked around to determine the expected success of various types of starting colonies or results of requeening. They are comparing their "expected" losses with the ones that you report.]

"G Adjustments to Honeybee Inventory
Adjustments to inventory numbers for honeybee producers must be made when less than completely mature and established colonies are purchased. Adjustments should be explained to the producer and amounts entered into items 12A, 12B, and 12C. If revised figures are not entered into item 12A-C prior to completion of application, COC shall enter adjustments in items 12H through 12J and appeals rights shall be provided according to CA Exhibit 1.
Adjustments may not be delegated.
Adjustments will be made based on the method of colony establishment and the length of time each method requires before reaching full maturity.
The following figures **shall** be used in making adjustments:
Method of colony success rates:
Splitting with mated queens – 90% success
Bulk bees (packages) – 90%
Nucs – 95%
Length of time to develop into commercially valuable colonies based on method of establishment (minimum length of time for colonies to count for any purpose)
Splits with mated queens – six weeks
(splits with cells not recognized)
Bulk bees plus mated queens
2#/4# packages – eight weeks
Nucs – four weeks."

[The intent – to give credit for all colonies that had the chance to be productive, but not for lost sub-income-producing (too young) colonies.]

"Application:
First, determine if the colonies are eligible based on the length of time that is customary for development based on the guidelines above. If the colonies have not existed for the mini-

mum duration as outlined above, zero credit for those colonies shall be given.

For those colonies, that have existed the minimum length of time, factor the quantity by the success rate based on the particular method of establishment as outlined in this paragraph."

[So, do what you can to keep those new colonies alive, because they are not likely to be covered unless you started them pretty early in the season.]

"D Loss Adjustments for Honeybee Colony Loss to CCD
CCD as an eligible cause of loss shall be limited to 35 percent of actual claimed loss
All additional lost colonies in excess of 35 percent of total lost colonies shall be determined by COC as ineligible honeybee colony losses during the program year and are not eligible for payment unless ALL the following apply:

An additional cause of loss occurred AND
The additional cause of loss truly caused the losses AND
The cause was timely filed on CCC-934."

[This may be the place to plea for some assistance for losses due to continued severe drought. It is obvious that your colonies would be a lot better off if they had been placed on some "natural" pastures occasionally during the year. Honey bee colonies do not persist well living on human-concocted pollen substitutes and supplements alone. See Randy Oliver's series of articles in the four latest issues of the American Bee Journal.]

BMPs from the Almond Board

The Almond Board of California responded strongly to the damage to, and loss of, honey bee colonies over the last few years attributed to being present in almond orchards. But, the suggestions relate to honey bees and use of pesticides in ALL crops. Their effort is quite extensive (10 pages) and too much to include in a newsletter issue. I will outline what is included but you will have to go to the following web address to find the set of documents: www.Almonds.com/BeeBMPs. The site will be up and functioning on Oct 17 if all goes smoothly. I will move them into my "Bee Briefs" when the photos are incorporated into final product: http://entomology.ucdavis.edu/Faculty/Eric_C_Mussen/Bee_Briefs/.

1. Why should growers and others involved in almond pollination care? First, honey bees and almond pollination have become dependent upon each other for a very large proportion of commercial beekeepers across the country. Second, those bees go off to pollinate more than 90 other crops in orchards and fields across the country. The bees have to remain healthy all year to provide those services.

2. The communication chain. It seems that beekeepers who have developed good relationships with their growers and regulatory personnel seem to have better "luck" when it comes to satisfaction and reduction of colony damage than those who just show up for a check. The details of this topic are written as they pertain to almond pollination, but are equally applicable to other crops.

3. Honey bees and insecticides. This portion of the materials speaks to insecticides, fungicides, and other chemicals likely to be used when the bees are foraging in the vicinity. There is a good deal of emphasis on an IPM approach to problem control: monitor field conditions to determine when problems first begin to develop; watch the problems develop and determine if interventions are required; If something must be done, try to determine what secondary non-targets are apt to become involved; try to determine the likely consequences following the application of various treatments – to beneficial insects, predaceous mites etc., and, finally, when to remove the colonies from the orchards.

Hive Products and Humans

We are well aware that various honey bee-related "products of the hive" have been used, probably since bees and humans met each other, for "folk remedies" for all sorts of problems. Earlier, hive products and herbs were about the only things available, so they likely were tried on a lot of ailments. Some appeared to have pretty good results, so they became part of "folk medicine." They still

are being used in many parts of the world, but we for the most part have a truly skeptical group of medical professionals. The Hippocratic Oath contains a "do no harm" element that prevents willy-nilly experimenting on patients, but has also slowed down obtaining some potentially useful information from hive products studies. Here are a couple examples:

Dr. Seung Min Lee and his team of associates in the Departments of Apiculture and Moxibustion and Korean Medicine, College of Korean Medicine, Kyung Hee University, Seoul, Republic of Korea, used bee venom as a last effort to try to find an analgesic (pain reliever) and anti-inflammatory for a patient with postherpetic neuralgia. Postherpetic neuralgia is intense or stabbing pain along the course of a nerve, which persists after a bout with shingles.

The 72-year-old patient had chronic severe pain and hypersensitivity where a herpes rash had developed two years earlier. Treatments with anti-virals, painkillers, steroids, and analgesic patches did not help. The patient decided to try the East-West Pain Clinic to receive collaborative treatment. After determining that he was not sensitive to bee stings, subcutaneous injections of diluted bee venom (1:30,000) were administered along the margins of the rash, once a week for four weeks.

Pain levels were evaluated on each visit. The patient chose "8" of 10 as the beginning intensity. In the fifth week, he reported the pain had decreased to a level "2." The patient encountered no negative side effects. Periodic phone calls from the clinic determined that the positive results persisted up to a year, when this report was published. With an "n" of one, this is hardly a definetive study, but it should encourage a well-controlled, double-blind experiment to determine the effect of bee venom on this type of pain.

This study was supported by grants from the Korean National Foundation and the Undergraduate Research Program of the College of Medicine at Kyung Hee University. But who will fund the large study? It is unlikely that big pharmaceuticals will be interested.

The reference is: "Bee venom treatment for refractory postherpetic neuralgia: A case report." Lee, S.M., *et al*. 2014. Journal of Alternative and Complementary Medicine, March: 212-214.

A second study on honey bee venom by Mohamed A. Amin and Ihab T. Abdel-Raheem, of the Department of Pharmaceutics, Pharmacy, Al-Azhar University, Assiur, Egypt and the Department of Pharmacology & Toxicol-ogy, Damanhouyr University, Danmanhour Egypt, repsectively, was designed to determine if the addition of honey bee venom to a specific wound-healing preparations would enhance its curative and physical handling properties.

The motivating problem for this study is the impaired healing of flesh wounds of diabetic patients. In diabetics one of the normal contributers to the problem is hypoxia (lack of enough oxygen) that prevents tissues from healing prpoerly. Not enough oxygen is being provided by the patient's tissues and the topical medications tend to block access to atmospheric oxygen. The lack of oxygen causes further inflamation. Other disturbances trap microbes in the wounds. The question was: could the addition of honey to one of the better medicants inprove its effectiveness?

The experiments were conducted on diabetic rats. The basic wound dressing film was formulated by freezing a polyvinyl alcohol (PVA) and chitosan (Chit) hydrogel matrix. The hydrogel holds a lot of moisture and mimics intact skin in many ways. The base components were increased and decreased with various levels of honey added. Honey was included due to its reputation as having anti-inflamatory and antibiotic properties, missing in the current hydrogels.

The reseaerchers determined that adding up to four percent honey to 10 percent PVA and 0.6 percent Chit hydrogels had some positive results. The gels became more elastic and could be used in thinner layers, but they had to be frozen and thawed three times to get adequate mixing. The honey gels were less inflamatory at the wound sites. Although thinner, the gels still prevented bacterial penetration. The pH of the gels were increased with the addition of venom. There was an "accelerator" effect on wound healing with more collagen production. Finally, their hope was that patients would be happier to use the dressing since it should hurt less when applied and was very easy to peel

off, suggesting less patient discomfort. Human studies remain to be attempted.

This study is: "Accelerated wound healing and anti-inflammatory effects of physically cross linked polyvinyl alcohol-chitosan hydrogel containing honey bee venom in diabetic rats." The Pharmaceutical Society of Korea, 2013. Doi: 10.1007/s12272-013-0308-y.

And finally, a finding that is a bit negative, from our point of view. Spanish doctor Leticia Vila and her associates became associated with an 11-year-old girl who was admitted to the hospital with an inability to talk, aand experiencing coughing, wheezing and eyelid swelling. She seemed to be having an anaphylactic response and her symptoms resolved with adminis-tration of the active ingredient in Benadryl® and steroids.

Two hours before the reaction, the girl had ingested a beverage containing crude royal jelly and fructose, and about 30 minutes previous to that, she had taken a dose of ibuprofin to treat an upper respiratory tract infection. She had taken ibuprofin previously with no problems. It was the first time she had ingested royal jelly. However, she had been on medications (antihistaamines and intranasal sterols) to reduce allergic rhinitis (hay fever ?) symptoms. To her knowledge, she had never been stung by a honey bee.

The doctors conducted skin prick tests to determine the extent of her allergies. She was checked against house dust mites, storage mites, latex, cat and dog dander, two fungi and pollen from timothy grass, English plantain, *Parietaria judaica* (sticky weed), and dilutions of the royal jelly. A positive reaction (wheal) was defined as 3 mm or greater in diameter. She tested "positive" to dust mites, timothy, and royal jelly. Her royal jelly reaction was 6 mm.

The doctors waited a week and gave the patient an "open challenge" (OC) with ibuprofen. No effects were noted. A week later, the patient was openly challenged with 2 ml of the royal jelly liquid beverage. She experienced itching in her mouth and swelling in her eyelids. OC was terminated and she was treated with oral antihistamines. As a follow-up in the lab, they drew blood and found that incubating her blood with house dust antigen eliminated a binding interaction between royal jelly extract and IgE. Immunoglobulin E (IgE) is considered a prime instigator in anaphylaxis reactions.

The final conclusions were that people with allergic responses to other allergens are more likely than others to react to royal jelly. In this case, there appears to be a cross-reaction between house dust mite allergens and royal jelly components. Since the mite allergy probably developed first, that would explain how anaphylaxis could have occurred with the patient's first exposure to royal jelly.

The article is: "Cross-reactivity between royal jelly and *Dermatophag-oides pteronyssinus*." Vila, L., *et al*. 2013. J Alllergy Clin Immunol: In Practice 2013; 200-1. http://dx.doi.org/10.1016/j.jaip.2013.01.003.

Annual Colony Losses – 2013-14

The summary data from this spring's survey on winter colony loss is available for review on "beeinformed.org," the public's entry to information from the Bee Informed Parnership (BIP). Since it is called winter loss, it does not necessarily record the total losses in many operations because colonies are lost over the entire year, picking up considerably in fall and winter. Until recently the summer losses, often replaced using colony splits, were unreported.

The good news is that the national average loss declined to 20.7 percent, the best in about a decade. Not many beekeepers blamed CCD (no logical explanation) for their losses, but mites and starvation were leading explanations.

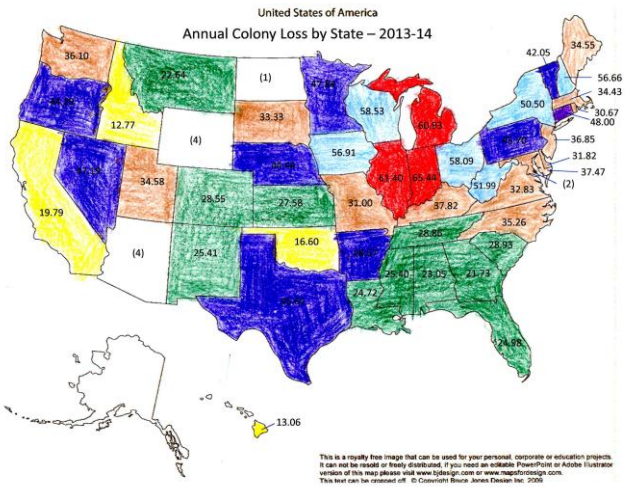
Since the data was listed by state averages, I wondered if that data were placed on a map of the U.S. could we see some sort of regional patterns. Using colored pencils and scribbling like a kindergartner (or at least like I did in kindergarten and still do), I did not see much of a pattern that stuck out. The states with the highest average losses (over 60 percent) did form a cluster (Illinois, Indiana and Michigan). The states with losses in the 50 percent range were all east of the Mississippi River: Iowa, Wisconsin, Ohio, West Virginia, New York and New Hampshire. States with losses in the 40 percent range were spread equally all over the country: Oregon, Arizona, Nebraska, Texas,

Minnesota, Arkansas, Pennsylvania, Vermont and Connecticut. States with losses in the 30 percent range filled in a swath of states just south of the 50 and 60 percent losses, as well as Washington, Utah, South Dakota, Massachusetts, Rhode Island, and Maine. States with losees in the 20 percent range included seven of our southeastern states: Montana, Colorado, New Mexico, and Kansas. California, Idaho, Oklahoma and Hawaii showed state average losses below 20 percent.

There is a whole lot more that can be compared in the data, such as average loss by size of operation. It appears that small-scale beekeepers may have greater percent losses than commercial beekeepers. But that may be easily explained simply by numbers. A small-scale beekeeper with three colonies loses one and has 33.3 percent loss. A commercial beekeeper might lose 10 colonies and still have only a one percent loss.

The organizers of the Bee Informed information have combined data by regions of the U.S. and by man-agement decisions. But, the data remains quite heavily biased toward non-commercial sized operations (average colny numbers for many responses were twenty-some colonies). With the 2013-14 data, the organizers hoped to have enough responses from commercial beekeepers to analyze their data separately. We will see how that turns out.

Graphic of 2012-13 U.S. Colony Winter Loss



Honey Bees and Pesticides, Again

Twenty-six years ago (1988) Dr. Kenneth F. Haynes wrote a comprehensive article for the Ann. Rev. Entomol. 33:149-68, titled "Sublethal Effects of Neurotoxins on Insect Behavior." Dr. Hayes devoted a good portion of the article to pesticides and their effects on nerves, but the behavioral changes that are most intriguing. Remember, this was long before most of our current products were on the market.

Under the heading Reproductive Behavior, one of his generalities was that exposure to every class of pesticides, up to then, decreased the production of offspring (19 references). While some of the effects might not be too important to honey bees, spermato-genesis and sperm mobility were mentioned, and we appear to be having problems with sperm viability today. Another observation was that insects exposed to low levels of pesticides had difficulties following pheromone plumes and finding their mates. At least with permethrin, this was a temporary change lasting about 48 hours. Permethrin also reduced the ability of male moths to maintain flight. Carbaryl disrupted the normal zigzag flight upwing to mates, and chlordimeform disrupted male flight, and the male's courtship display. In an interesting contrast, chlordimeform made one species of male moth 1,000 times more sensitive to its mating pheromone.

Switching to food finding and feeding behavior, he gave many examples and then paid special attention to honey bees. The first observation was: just because you do not see dead bees does not mean that the colony is healthy. Parathion at sub-lethal doses disrupted the ability of waggle dancing bees to send proper directions. Their compas directions were off from 7.5 to 29 degrees. Within 24 hours the dances were back to normal. When placed on a horizontal dance surface, the dance compas directions were accurate. The affected bees could not properly translate photomentotaxis (directed movement at an angle to a light source) to geomenotaxis (directed movement at an angle relative to gravity). However, the distance information was off. Recruited foragers and the dancers themselves stopped short of the goal they were seeking. Those effects lasted only 5.5 hours. Also, the bees lost their ability to seek their food at the time of day it usually was present in the field. That problem lasted at least 24 hours.

Thus, recovery times vary depending upon the chemical, the dose, and the behavior selected for study.

Continuing in this learning vein, sublethal doses of pyrethroids slowed learning rates and memory ability for proboscis-extension learning way down. Remember that minuscule doses of imidacloprid sped up learning. As doses of imidacloprid increased, learning returned to normal, then dropped off as with pyrethroids. Other examples included permethrin-treated bees that spent more time cleaning themselves and doing tremble dances and less time moving around, foraging, and sharing food than untreated controls. Both parathion and methyl parathion reduced brood production and Smirle *et al.*, 1984, developed a bioassay for sublethal effects of pesticides on honey bees.

In the final conclusion, Dr. Haynes suggests that observing honey bee communication changes should be considered one of the better indicators of sublethal effects of exposure to insecticides for honey bee colonies. That is quite a step from our acute toxicity to adult worker honey bee – and sometimes brood – approach. It would be more time-consuming and certainly more subtle in data interpretation.

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