July/August 2007

Newsletter E-mailed to You

If you wish to have this newsletter sent directly to your e-mail address, when it is published, please follow the instructions below.

Send an e-mail addressed to **listproc@ucdavis.edu**. Leave the Subject line empty. In the body of your message put in the following: sub ucdavisbeenews <your first name (without these “brackets” around it)> <your last name>. On the next line, insert two hyphens, not underscores (underlines). If I were subscribing, it would be:
sub ucdavisbeenews Eric Mussen
--

The hyphens are there to tell the subscription software on the server not to be confused by any following information that occurs, such as a “signature frame” (or signature block, as I call it).

If you wish to be removed from the list, then you do the same thing, but instead of **sub**, you use **unsub** or **signoff**, then the name of the list and your first and last names followed on the next line by hyphens.

Viruses and Bees

Although trying to cover everything about honey bee viruses would require writing a book, the publications are out there, already, and worth reading if you wish to be more knowledgeable about bee viruses and honey bee diseases. However, some new things have developed, recently, in this area of study that are worth considering.

RNA viruses are quite small viruses with a small amount of genetic material (ribonucleic acid) wrapped up in a protein coat, when they are “virions” (virus particles). I had an opportunity to study one of those viruses for my PhD dissertation – sacbrood virus.

In order to get a “pure” (which I am sure that it wasn’t) batch of virus with which to work, I accompanied the local bee inspector when he checked apiaries for colonies with AFB. If we noticed any sacbrood disease, I took those larvae back to the lab and processed them. Eventually, there was enough virus to inoculate more larvae and adult bees in the lab and to layer on a cesium chloride density gradient and centrifuge with an ultracentrifuge. That virus prep was inoculated into rabbits and they produced anti-serum (antibodies) so that I could track the virus.

I processed the larvae and adult bees and looked for crystalline arrays of virus that had replicated in various tissues. It worked well enough to earn my PhD. The virus showed up in just about every tissue that I examined. My major purpose was to demonstrate that sacbrood virus, which doesn't stay infective very long once it is out of its host, existed in adult bees over the winter as inapparent infections. Remember, I was looking for virions.

About a decade earlier, Dr. Leslie Bailey in England started a multi-decade study of virus diseases in honey bees. His contention, right from the beginning, was that viruses could lay in wait in honey bees as "latent" infections and be stimulated to become "active" and replicate in the bees if they were properly stimulated (injections of hemolymph from other bees, or even milk). However, the proof of this theory was always circumstantial. Dr. Bailey refereed my sacbrood publication. He let it go to press, but stated that he thought that I had activated acute bee paralysis virus when I injected the worker bees with sacbrood virus. All those RNA viruses are about the size and shape of ribosomes, so it would be very difficult to prove his contention, one way or the other. The only evidence I had on my side was the positive sacbrood antibody reaction to the virus taken from tissues of worker bees. The antibody was to the virus that I had taken from sacbrood larvae.

Now, decades later, the world of virus studies has changed dramatically. In most cases, samples of bees are collected and processed so that the nucleic acids can be extracted and analyzed. You don't need to dissect the bee, prepare its tissues for electron microscopy, and check for the presence of virions with serological tests. All you have to demonstrate is that the diagnostic RNA strands are there. What you don't know with these new techniques is whether the RNA came from a latent or active infection.

One complication is trying to make sure that the strand selected for diagnosis can be found only in the virus of interest, and not in others. I'll let the experts determine how that is done. This is such an important line of study, today, that the US Army is trying to determine, not just for viruses but for all pathogens, what the proper diagnostic genetic code is for every single one. There is an interesting tidbit in the September issue of Popular Mechanics (page 16) that describes a 50-pound Integrated Virus Detection System (IVDS) that can be used to separate even the extremely similar RNA viruses by THEIR SIZES! The instrument was developed at the Army's Aberdeen Proving Grounds in Maryland under the direction of Dr. Charles Wick. Yes, it is being used in CCD studies.

Many virus researchers around the country and the world have been contributing information about adult honey bee RNA viruses, since one of them, deformed wing virus, causes signs (bees with stumpy wings) that we can see. It now seems that Dr. Bailey was very prescient in the 1960's. Lots of honey bees have latent viruses in them. If they don't replicate, the viruses don't appear to cause any physical damage to the bee. But, they are showing up in honey bee eggs, larvae, pupae and adults. They are in the reproductive organs and products of queens and drones. They are in the stores of honey and pollens in the hive. We seem not to be able to get away from them.

Years ago, virologists used to talk about infectivity and receptors on potential host cells. In some of our first molecular manipulations, researchers would try to add to a potential set of instructions to the host genome so that the host cells would produce a product that would bind with the receptors and prevent the virus from attacking.

In a recent experiment by Ilan Sela it was reported that an RNA virus in honey bees in Israel, named Israeli acute paralysis virus, included four major sets of instructions in its genome – three for making parts of the protein coat and one for reproducing the RNA strand of instructions. He further found that if one or more of the coding segments for the protein coat became integrated into the honey bee genome (naturally), the bee cells were resistant to infection. That could be very useful in bee breeding programs. By testing and selecting for bees with that partial RNA code, you could breed bees resistant to the disease.

But, the research uncovered one more interesting fact. The WHOLE GENOME of the virus could become incorporated into the bee's genome. Then, under the right circumstances, the instructions for virus replication could be activated right from within the bee cell, itself. Is this the way that honey bees maintained "latent" viruses in Dr. Bailey's context? If so, any tests upon which to base stock selections would have to search for bees with only partial codes for protein coat, but making sure that the whole code didn't come along with a portion of its parts. This information is on the Web at: "BARD US-3205-01R Final Scientific Report."

It appears as though we are likely to hear much more about Israeli acute paralysis virus. It seems to have accompanied the Australian packages into the U.S.

Disease Resistance in Honey Bees

Hosts and their parasites wage a continuous tug-of-war in their inter-relationships. The parasite has to be able to overcome the defenses of the host enough to keep reproducing and spreading to new hosts in order to avoid extinction. Meanwhile, the parasite has to be gentle enough not to wipe out all its hosts. That would lead to extinction as well. On the host side, there have to be mechanisms to prevent them from becoming infected, and for dealing with the parasite if it manages to get past the defensive barriers. Honey bees like all other animals live in a sea of microbes. Most are considered benign, but we do know that some microbes are pretty hard on the bees, like American and European foulbrood, chalkbrood, sacbrood, black queen cell virus and the two *Nosema* species.

As in football, the old adage "the best offense is a good defense" works quite well for honey bees. The exoskeleton and its cuticular surface are a pretty formidable barrier against microbes. The outer layer of the exoskeleton is covered with a waxy layer that includes unsaturated fatty acids. Besides preventing loss of water (prevents dehydration) and penetration of water (prevents tissues from becoming "water logged"), the waxy layer makes it difficult for microbes to become attached to the surface. It also resists enzymes (chitinases) secreted by microbes that might other-wise be able to eat their way into the insect. While we think of the exoskeleton as protecting the outside of the bee, it extends well into the tracheal system and lines the fore gut and hind gut of the intestinal tract. It has to be sloughed each time the bee molts. The mid gut does not have cuticle protecting it, but just behind the proventricular valve there are cells that secrete the peritrophic membrane that surrounds food masses as they move through the digestive tract. The epithelium and muscle tissues of the intestine also might slow the movement of fungal mycelium into the body cavity and hemolymph (bee blood).

Despite that protection, microbes do make their way into honey bees. In the case of fungi, a number have been listed as being pathogenic. Some are regarded as named "bee diseases" like *Ascosphaera apis* (chalkbrood); *Aspergillus flavus, A. fumigatus*, and other *Aspergillus* species (stonebrood); and *Nosema apis* and *N. ceranae* (nosema disease). Other fungi have been recorded as agents of "opportunistic" infections. They include *Aureobasidium pullulans, Trichoderma lignorum, Mucor hiemalis, Rhizopus* species (black bread mold), and various species of *Candida*, a yeast.

Honey bees are forced to rely on some rather simple mechanisms to deal with fungi that start growing in their bodies. They have not been found to produce antifungal immune peptides similar to drosomycin in *Drosophila* or thanatin in a plant bug. Their first internal defense is phagocytosis. Invading fungi are detected, physiologically, and a number of hemocytes (blood cells) are attracted to the area. The cells will engulf and digest the fungus. Besides the effects of lysozyme (N-acetyl-muramylhydrolase), which acts as an opsonin and reduces negative charges on microbes, phagosomes (more valuable in fighting bacterial infections) and melanins appear to be involved in the process. When melanins accumulate, black spots develop in the tissues.

Larger amounts of fungus (diameter larger than 10 microns) can be too much to consume and digest. Then the hemocytes accumulate in larger numbers and "encapsulate" the fungus by completely surrounding it. A special type of hemocyte, called granulocytes, releases an attractant that signals other cells to join in the task. Often melanins are involved in this process and black spots develop in the tissues.

Honey bees are a bit better at dealing with bacterial infections. All life stages of honey bees have detectable levels of lysozyme, an enzyme that dissolves bacteria. There are other immunity-related enzymes: phenol oxidase, glucose dehydrogenase, and glucose oxidase (makes hydrogen peroxide). The level of naturally occurring ("constitutive") lysozyme is especially high in larvae. But, if the lysozyme is not getting the job done, then special cells in the immune system of the bee respond by releasing "inducible antimicrobial peptides." Peptides are short chains of amino acids that, if accumulated in larger amounts and bonded together, would form proteins.

The best known and most active peptides in honey bees belong to the apidaecin-family. This is a large group of small (about 2-4 kiloDalton), proline-rich compounds. Abaecin (4 kiloDalton) is another, similar peptide. Hymenoptaecin, a glycine-rich polypeptide (10 kiloDalton), has activity against most bacteria, also. One other peptide, a defensin, is cysteine-rich and has some activity against fungi as well as being bactericidal.

Now that you have learned all these names of chemicals, how can you use that knowledge? The ability of honey bees to respond to infections is likely to determine whether or not they can survive the rigors of living through a year in the 20th century. By being able to read their genetic code, and determine which bees have the appropriate genes, bee breeders will be able to graft queens and provide drones that, when crossed, will give the most disease resistant bees that we can produce.

Feeding Winter Bees

It seems like many California beekeepers, and others who bring colonies to this state, tend to feed their colonies right through the winter. However, in nature, temperate climate honey bees tend to take at least a portion of the winter off, or at least reduce brood rearing to a minimum.

I have explained, before, what winter bees are and how they are expected to live from October to about March. Normally, in cooler climates, they would suspend brood rearing completely until the end of December, when the days start getting longer. I don't have any proof, but I sense that once the winter bees really become involved in brood rearing, they start a clock that leads to death well before they reach six months.

That doesn't really matter under natural conditions, because only a few bees are needed to feed patches of early brood. They won't make a big dent in the population when they leave. As more winter bees become involved in late winter / early spring brood rearing, they are hastening their demise. However, the summer bees that the winter bees are rearing will replace them and they won't be missed. Eventually, over a relatively short period of time, the winter bees fly off to die and the summer bees take over.

Beekeepers wishing to "beef up" their colony populations over the winter are going to be trying to entice winter bees out of their rest period into a brood rearing period. Stimulative feeds of pollens, pollen substitutes and supplements, and various sugar syrups can sometimes prompt them to rear brood. It seems to me that this practice begins the aging of winter bees too early. Unless brood rearing goes really well, the number of bees leaving is going to be a lot greater than the number of newly emerging bees. The colonies will dwindle instead of building up.

Colonies kept in locations commonly referred to as The Coast, and in way southern California, usually have winter weeds and winter flowering trees (especially *Eucalyptus* species) available to them when it doesn't rain (a time period that seems to be expanding to the detriment of nectar and pollen supplies). The weather is warmer and flight conditions are better than in the Central Valley. With luck, somewhat similar conditions can be encountered in the upper foothills on the western slopes of the Sierra Mountains. Natural food encourages the bees to brood up early, and the colonies can become quite strong before almonds.

Valley Bees are another story. The weather is much cooler, and usually it is very damp (foggy). Flight intervals are severely limited. Winter weeds and trees probably will bloom, but the bees often are not able to utilize them. Or, the weather turns unexpectedly warm and bright. The bees fly like crazy, but there is little or no food to collect. So, the bees just blast through their winter stores. Maybe, cold and damp isn't so bad!

Early loss of winter bees leads to colony shrinkage. Shrinkage can lead to many trips to the bee yards to "combine" colonies. They can be combined over and over, again, and still be too small to use for almonds or even dwindle right down to nothing.

The time to feed the bees was last month and this month (September). The bees are doing their best to rear a special group of long-lived bees as you read this. Did you luck out on a late honey crop and let the bees fill the brood nest with honey? Honey bound brood nests will not hold enough brood to get you through the winter with a population size that you wish to have next "spring" (almond time). By October, your feeding should be completed and the bees should be able to kick back and rest until the end of December.

New Bee Diets

It is too soon to have comparison data, but new diets for honey bees have come onto the market. One product, FeedBee, doesn't sound new. It has been available for about two years. However, like most products, this year's formulation is "New and Improved" – sort of FeedBee III. You can read much more about this product at www.feedbee.com.

The long-awaited launch of MegaBee took place in August of this year. Begun as an attempt by Dr. Gordon Wardell, who is affiliated with the Tucson bee lab, to formulate a "liquid protein diet," the ingredients have been refined many times. The formulation has been modified to contain only ingredients labeled GRAS (Generally Regarded As Safe). Apparently, because it is milled so fine, the product - formulated as a powder - can be fed dry, as a patty, or suspended in syrup. I still suggest strongly that this type of feeding be done in patties. Try to use an "inverted" type of syrup to help the patties maintain moisture. Is high fructose corn syrup OK? Yes, as long as it hasn't been heated too hot and has not been stored very long. Left over HFCS that has been sitting around for a while could be toxic to your bees.

Seedless Citrus

It has been a rather tortuous road leading to a possible "compromise" between beekeepers and growers of seedless citrus in the San Joaquin Valley. Beginning as threats of lawsuits against beekeepers, Citrus Mutual introduced AB 771. The bill has been amended often from a "spot bill" to its current language that mandates that the Secretary of Agriculture "promulgate regulations" to protect the citrus, if the beekeepers and growers can not come to a mutually acceptable solution.

In order to better explain the complexities of how citrus integrates into CA beekeeping, the editors of a University of California Cooperative Extension publication, Topics in Subtropics Newsletter, asked me to write an article about the matter, explaining the beekeepers' point of view. I wrote the article, and it was first published in the Jan-Mar 2007 issue. You can find that issue on the Web by putting the name of the newsletter into a browser. The next issue, Apr-Jun 2007, has been published, but isn't up on the Web, yet.

I was chastised for my inaccurate calculations of how much land would become unavailable to our beekeepers if there were a two mile restriction implement-ted on placement of beehives around every planting of six acres or more of potentially seedless citrus. A physicist and citrus grower in southern California graciously recalculated my statistics and made the information available to the newsletter editors so that an erratum could be printed. Instead, the editors decided to publish the full article a second time, with the accurate data in bold print. In my previous attempt and in the new article, the conclusion was still the same. If all six acre plantings of seedless citrus were surrounded with a two-mile bee-free zone, honey bees could be excluded from all the citrus producing acres in Tulare, Fresno, and Kern Counties.

Since Citrus Mutual is pretty well connected with top agricultural administrators, the California State Beekeepers Association's Board of Directors felt compelled to enlist the services of a professional lobbying agency to be certain that its point of view was being disseminated accurately and adequately around Sacramento. As I stated in a former newsletter, the idea of being forced to move from every location that was unacceptable to someone, for some reason, would soon lead to very few places in the state (or country) were colonies could be located.

Brazilian Propolis

Dr. Marla Spivak at the University of Minnesota, St. Paul, told us, a while ago, that she was winding down her hygienic bee breeding program and redirecting her focus to the value of propolis to a honey bee colony in respect to reducing infectious diseases and parasites. She is particularly interested in "green propolis" obtained from *Baccharis dracunculifolia* that grows in Brazil.

Besides its possible therapeutic effects in beehives, the March 2007 issue of the Journal of the American Apitherapy Society contained three summaries of recently published reports of the beneficial values of green propolis (already used extensively in Brazilian medicine) in dental care and as an adjuvant in immunization.

Periodontal disease can occur in a number of ways – tartar, gingivitis, bleeding, fluid accumulation, receding gums, loose teeth, pus formation, and bone loss. Green propolis was dehydrated under vacuum, ground to a fine powder, then used directly or as a tincture of 8% by weight in 80% ethanol. Propolis was added to tooth-paste for brushing, then the tincture was used as a mouthwash. In more severe cases, propolis was introduced into periodontal pockets once a week for five weeks. Nothing is perfect, but good results were seen in 95% of the patients. The authors of the paper suggested that a 10% tincture might be better. In any case, the propolis is free for collecting, so the patients should have ready access to a very effective medicine.

Denture wearers sometimes have problems with oral infections with the fungus *Candida albicans*. Eighteen patients with that problem were treated with either propolis or an expensive fungistat, Nystatin®. The twelve patients treated with propolis had their oral mucosal regions dried and swabbed four times a day with the same tincture used in the previously reported study. All 18 patients were well healed by both treatments in less than three weeks. Eleven patients were better in seven days and six were better in 15 days.

Finally, immunologists have known for decades that vaccines tend to work better when the allergen is mixed with an "adjuvant." In this case, Suid herpes virus type 1 (SuHIV-1), which is a disease agent in swine, was combined with green propolis and aluminum hydroxide, then injected into mice. The mixture worked well as long as the antigen was absorbed in a particulate adjuvant. So, green propolis may become an ingredient in many vaccines.

All the above-mentioned studies can be found on the Web. The first is “Periodontal treatment with Brazilian green propolis gel.” Cairo de Maral *et al*., *Pharmacologyonline* 3 (2006), pp. 336-341. The next two are at www.interscience.wiley.com. They are: “Oral candidiasis treatment with Brazilian ethanol propolis extract,” V.R. Santos *et al*., Phytotherapy Research 19 (2005), pp 652-654; and “Immunodomodulation produced by a green propolis extract on humoral and cellular responses of mice immunized with SuHV-1,” Geferson Fischer et al., *Vaccine* 25 (2007), pp. 1250-1256.

Sincerely,

Eric Mussen
Entomology Extension
University of California
Davis, CA 95616
Phone: (530) 752-0472
FAX: (530) 754-7757
Email: ecmussen@ucdavis.edu
URL:entomology.ucdavis.edu/faculty/mussen.cfm

Eric Mussen
Entomology
University of California
Davis, CA 95616