Red River Apiarists' Association

The Bee Cause

Volume 13, Issue 5 May 2016

Next general meeting is 7:30 Tuesday, 10 May 2016 at the **The Elmwood Legion 920 Nairn avenue , Winnipeg.**

Speaker: Club bee yards Spring and summer bee-keeping strategies

Inside this issue:

POLYADENYLATION OF RIBOSOMAL RNA IN RESPONSE TO PICORNAVIRUS INFECTION IN HONEY BEES (APIS MELLIFERA) by JOHNNY YU THESIS Submitted in partial fulfillment of the requirements for the degree of Master of Science in Entomology in the Graduate College of the University of Illinois at Urbana-Champaign, Urbana, Illinois. 2012

Abstract

At least eighteen different picornaviruses can infect honey bees (Apis mellifera). The combination of the virulence of these pathogens as well as their transmissibility through Varroa destructor, an ectoparasitic mite, can cause severe declines in honey bee populations. Polyadenylated (polyA) rRNA has been associated with honey bees infected with viruses in past studies. In bacteria, yeast, and some mammals, polyA rRNA serves as a molecular marker for the degradation of rRNA. Honey bees may be utilizing polyA rRNA in the same fashion to degrade cells that are infected with picornaviruses. In this thesis, I attempted to correlate viral infection and polyA rRNA using qRT-PCR and RNA-seq. Five polyA regions were identified with RNA-seq. One region (28S 2070-2130) was weakly associated with viral infection. I used quantitative RT-PCR to more accurately measure the 28S 2070-2130 region and again found a weak association between viral infection and polyA rRNA. Also, significantly more polyA rRNA was found in the abdomen than in the head. The high expression levels of polyA rRNA in the abdomen may be a consequence of picornaviruses that infect mostly the abdomen.

A. mellifera is challenged by a variety of pathogens and pests, including bacteria, fungi, and other invertebrates. Invasive species that originally parasitized the Asian honey bee, A. ceranae Fabr., have become even more destructive after they shifted hosts to A. mellifera colonies. One such invasive pest, Varroa destructor Anderson & Trueman, an ectoparasitic mite, has been suspected to be a contributing factor of CCD (Highfield et al., 2009; Rosenkranz et al. 2010). Mites infest a colony and feed on hemolymph from honey bee pupae and adults. This parasitic interaction not only drains the honey bee of bodily fluids, but also transmits pathogens (Chen et al. 2006). During the feeding process, the mite vectors many honey bee viruses belonging to a group of single stranded RNA (ssRNA) viruses called picornavirus. At least 2 of the eighteen viruses in this family have been found to cause sickness in the honey bee, the most ubiquitous being Deformed Wing Virus (DWV) (Bowen-Walker et al. 1999). DWV has had a sublethal impact on honey bees in the past, but now in conjunction with V. destructor, DWV and other picornaviruses have even more of a deleterious impact on honey bee health and together serve as an indicator for CCD (Chen et al. 2005; Dainat et al. 2012). V. destructor and DWV appeared in the western hemisphere years before the first occurrence of CCD (Nordström, 2003), but only recently have been associated with CCD. The presence of the mite and virus before and after CCD insinuates that they cannot be the only factors in CCD. Nonetheless they have a significant negative impact on honey bee health. The Varroa mite also suppresses the honey bee immune system, which leads to increased viral replication (Amdam et al., 2004; Gregory et al., 2005; Yang & Cox-Foster, 2005). Insect cellular and humoral immune responses, including antimicrobial peptides and enzymes involved in degrading foreign substances, are down-regulated in honey bees infested with the Varroa mite. The down-regulation of these invertebrate immune responses facilitates infection by picornaviruses. The reduction of general cellular and humoral responses has been linked to viral amplification via

(Continued on page 5)

Presidents Comments for May, 2016

Seeing that no report was provided I have inserted mite information from an article of Randy Oliver's (2006-2009). I expect we are into the state of mite infestation they were and with the increase of new beekeepers the need to be informed and have action plans. I am sure all readers will have read this on Randy's sight, however, it's worth the re-read.

IPM I Fighting Varroa I: The Silver Bullet, or Brass Knuckles? Randy Oliver 2006, 2009

In preparing for our brass-knuckled assault on our enemy, we must know its weak spots (I've put them in **boldface**). Unfortunately, during the period that mite populations are building, about two thirds of the mites at any time are safely hidden in sealed brood, protected by the silken bee pupal cocoon. Only a few volatile miticides can penetrate the cocoon—notably formic acid and thymol. In the process, however, these two treatments also kill a percentage of the bee brood.

So, other than using a cocoon-penetrating fumigant, we must hit the mite when it is most **vulnerable—in the "phoretic" (hitchhiking) stage.** There are two times to do that: **when the colony is broodless** (during winter, or made broodless by manipulation), or when the mite is feeding on adult bees prior to entering a cell.

Here's the biology: a female "foundress" mite enters a cell when (or just before) the bee larva is in the propupal stage, but before it spins its cocoon. This occurs about day 8-10 after the bee egg is laid. The foundress mite and any mature daughters emerge with the adult bee on day 21 (if the bee survives until then). Therefore, the mite is hidden for only about 10-12 days. The adult female must then spend from 4 to 15 days sucking the blood of adult bees (**usually on nurse bees** in the brood nest area) before she is ready to enter a cell and start egg laying. A female mite can live for 3-4 breeding cycles. Reproductive success averages roughly 1-2 viable offspring in worker cells, and **2-3 in drone cells.** Because of this low rate, in order for the mite population to increase, female **mites must invade a cell and reproduce more than once** during their lifetimes. Most all methods of beating up the mite focus on its vulnerability during the phoretic stage.

The varroa life cycle takes about 11-12 days in the cell, then several days in the phoretic stage on adult bees.

Update May 2012: USDA ARS scientist Jeff Harris narrates close-up video of bee and varroa development at http://video.google.com/videoplay?docid=-7304562435786960616

One other aspect of mite biology is of note: the foundress mite is more **attracted to, and successfully rears more offspring in, drone brood** than in worker brood (on its natural host, Apis cerana, the mite reproduces only on drone pupae), due to the drone's larger size and longer developmental period. We can use this fact against our enemy.

Another biological aspect of varroa worth noting is its amazing ability to quickly develop genetic resistance to chemical miticides (at least the synthetic ones that have only one mode of action). Once in a cell, the foundress mite lays a male egg first, then female eggs thereafter. The male mite mates with his sisters, and dies when the cell is opened. This inbreeding locks in successful (cnt'd pg 4)

Red River Apiarist's Association
Minutes of the Regular Meeting
APRIL 12, 2016

Chairman: Waldemar Damert
Recording Secretary: Art Quanbury

Approval of the Minutes of the previous General Meeting
Motion: That the minutes of the March 2016 meeting be accepted
Moved: Armand St. Hilaire
Seconded: Ken Feher
Carried

Treasurer's Report
Chequing account $1311.00, Savings account $4221.00
Most membership renewals are in.

Bee Yards Update
The discussions for a yard at the zoo are ongoing. A meeting with the director is being arranged. Space has been identified and photos of it are being sent to RRAA.
The Hay Road yard is definitely a go and can accommodate a lot of colonies.

Bee Day
June 4 has been chosen as the day for the Celebration of the Honey Bee at the Forks. The vendor will be there. Volunteers will be needed and a sign up sheet will be circulated at the next meeting.

Coop Meeting
The atmosphere is not good for commercial beekeepers. Honey prices are low because of imported diluted honey. There is a 290 M lb surplus on the market now compared to the usual 90 M lb. Bulk price is $1.40/lb. Bee Maid is sold out of packages of bees. These are not as good as local nucs because the bees in the packages are often too old and the queen has been held for too long. Packages should be given a frame of brood. NZ has lost many native pollinators as well as many species of birds. It has been admitted that neonics are the problem. France is the first country that will go neonic free.

Late Spring Management
Main issues now are feeding and nosema control. Initial feeding should have been 3 weeks ago however feeding should not be done until bees have had a cleansing flight as it will encourage nosema. It is important to feed enough but not too much because space needs to be left for egg laying. Can't always go by weight of brood chamber. Wait until weather gets warmer and then look inside. (temperature at least + 12 C.). 1:1 concentration of sugar syrup is OK and there should be 3 or 4 frames of food. The rest should be brood (4 to 5 frames). Pollen is also an issue. There needs to be enough but worker bees can raise one generation of bees from their own body resources. Start feeding in the fall when there is still some foraging going on. Bees will pack in pollen.

Nosema is highest in the spring. Boxes can be washed with a power washer and left in the sun to dry. The uv light will kill the bacteria. It is important to eliminate other diseases as well. Viruses are attached to mites so getting rid of mites is important. Bacteria can be killed by high temperatures. It is important to evaluate every hive every year. Check all symptoms and write them down then find out why the bees have died. An early start to the season means a long season for viruses and diseases. White crystals in the hive are defecation of mites meaning that the treatment was too late. Mites can be treated with a flash treatment of formic acid before honey flow. You can also do a flash treatment in the summer because no residue is left in the honey. Temperature is critical in flash treatment. It is possible to kill the queen. Put formic acid pad on top of hive if temperature is less than 25 degrees and on the bottom if it is above 25 degrees. The amount can be decreased if it is very hot. Top and bottom entrances must be fully open. Can put acid on an open pad for faster action or on thick cardboard (like shoebox material for slower action. Slower action is better. Use 65% acid here (85% in Europe). Put cardboard in a ziplock bag with an X cut on it. Treatment can be effective at 5 to 7 degrees but top entrance must be closed. At 25 degrees leave for 24 hours. It is estimated that 90% of losses are due to diseases and 10% due to starvation. Bees take a break in breeding cycle during the winter but food consumption increases with breeding.

Check bottom board for mites or use the icing sugar technique. If mites are found treat with formic acid on top of frames. To and bottom entrances should be open 3.5 inches. Oxalic acid is good treatment in fall if there is no brood. Can treat at end of November after there has been 2 to 3 weeks of cold weather.

American foul brood can be detected by smell. Use the toothpick test to see if you have it. (Stick a toothpick into dead brood cell. Stir it around and then withdraw the toothpick. Brood killed by AFB will rope out about ¼ inch and then snap back like a rubber band.
Check for queen. If no queen you can buy now from Bee Maid. If your equipment is old you should replace it with new. Shake the bees out of old equipment and let them walk into new equipment.

Looney Draw winners
The following were winners of the loonie draw. Keith Bamford (2), Alex Remkes, Ron Rudiak, Dave Weselak, Barry Briscoe, Monica Wiebe, Jim Uttley, Hans Borst, Tim Kennedy, Christos Argiriou, Joelle Boucher.

Adjournment
The meeting adjourned at 9:45 pm

The next meeting will be held on May 14 at the Elmwood Legion on Nairn Avenue at 7:30 pm.

Next Meeting
The next meeting will be on September 13, 2016.

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MBA Report April 2016
Margaret Smith, RRAA MBA Representative
No report

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(From pg 2) genetic mutations that might have allowed a particular foundress mite to survive a chemical treatment.

You may have noted that I have not discussed our allies in this battle–the bees (who have a vested interest in surviving). I will cover their contributions further along.

Our Contribution to the Problem

You're all familiar with selective breeding for better bees. Be aware that we have also been breeding for "better" mites. That is, mites that can survive our repeated Silver Bullet attacks. Specifically, we have been applying selective pressures to favour mites that:

1. Evolve resistance to miticides

2. Rebuild populations quickly in mite-depopulated colonies (mites with low reproductive success cannot recover from regular treatments)

3. Kill colonies, rather than coexist with them, since colonies that collapse best disperse mites.

In other words, we've been inadvertently selecting for the most virulent, rather than the most benign mites. One could argue that it would be better to change our strategies to reverse the above selective pressures, and promote mites that are less virulent, and that can coexist at a low level in our colonies.

Our Fighting Strategy: Integrated Pest Management

I already mentioned that the Brass Knuckles approach means that we're going to accept the mite as a permanent resident in our hives, but we're sure going to make its life miserable. If it starts to build up to a level that hurts our bees, we're going hit it from several directions, so that it doesn't have a chance to build a defense (resistance) against any single tactic or weapon. The strategy we're going to use is called Integrated Pest Management (IPM). A search of Google definitions for IPM gives us:

"The use of different techniques in combination to control pests, with an emphasis on methods that are least injurious to the environment [read that, the honey bee colony, the wax combs, and our honey]."

"A combination of biological, cultural, and genetic pest control methods with use of pesticides as the last resort. IPM considers a targeted species' life cycle and intervenes in reproduction, growth, or development to reduce the population."

"An approach to pest control that includes biological, mechanical and chemical means."

"Maintaining pest populations below a level at which economic damage results by using the least toxic methods."

An Overview of IPM for Varroa

O.K., so what does IPM mean for the beekeeper? In a nutshell, learn about varroa's strengths and vulnerabilities. Then develop a strategy to thwart its strong points (especially its ability to evolve resistance to miticides), and exploit its weak points, attacking it from several different directions. Allow me to give you an overview of the rest of this series:

First, you don't have to fight the mite single-handedly. There are bees out there with the genetics to fight the mites themselves—they'll just need your help sometimes. I'll talk about these fightin' bees in **Choosing your Troops.**

VSH (Varroa Sensitive Hygiene) breeder queen. This line was developed by the USDA Agricultural Research Service to fight varroa. Breeder queens are available from Glenn Apiaries. Photo Glenn Apiaries.

Second, stop freaking out if you see a mite! Understand your enemy, so you're not irrational with fear. The bees can handle a certain level of mites fairly well. Find out for your area, just what load of mites your colony can safely carry at any time of the year. The level that starts to hurt the colony is called the Economic Injury Level. So monitor your mites to make sure they stay below that level. If the mites are at a lower level, relax–you can sleep at night. However, if they are starting to approach injury level, start hitting them "softly." Only if they are over that level would you consider hitting them hard with some sort of strong chemical (you want to save your strong chemicals like a pistol in your back pocket, just in case things start to get out of hand). I'll cover monitoring under **Reconnaissance.**

A sticky board monitors mite levels; other monitoring methods are more accurate in the short term, a typical sticky board after a day or two. The mites and hive trash fall in lines between the frames, and mites are often mostly in one area. The 1/8" screen keeps the bees from removing the mites.

Third, let's make it generally miserable for the mite to survive, reproduce and disperse; and do all we can to help our bees fight the mite on their own. These tactics fall under **Biotechnical Methods.**

One biotechnical method is trapping mites in sacrificial drone brood. I'm inserting a drone trap frame in February in an almond orchard.

(this is 2.5 pgs of 8 more in September-IPM task chart on pg 10)

the fact that the honey bee becomes so immunocompromised due to unchecked invasive bacteria and toxins that viruses have the opportunity to rapidly multiply (Shen et al. 2005).

The spread of V. destructor through migratory beekeeping has increased the incidence and virulence of deleterious honey bee viruses (Sumpter & Martin, 2004). Viral pathogens have been less of a concern to beekeepers relative to the honey bee threats mentioned above, but recently, viruses in combination with the Varroa mite have been so deadly that they even have been implicated as a cause for CCD (Bromenshenk et al., 2010; Cox-Foster et al., 2007). Honey bee viral infections typically are latent as well as unapparent, meaning that bees are symptom less even though they possess the virus, and only become acute and lethal if the bee becomes immunocompromised (Ribière et al., 2008). At least eighteen different viruses infect the honey bee, the majority of which are picornaviruses; positive strand single stranded RNA viruses. The most prevalent of these are Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Sacbrood Virus (SBV), Kashmir Bee Virus (KBV), Acute bee paralysis virus (ABPV), and Chronic Bee Paralysis Virus (CBPV) (Chen & Siede, 2007; vanEngelsdorp et al., 2009). They can infect the larval, pupal and adult stages of the honey bee (Aubert, 2008). Multiple viral infections can be present in a single bee (Chen et al., 2004). BQCV and DWV are the most prevalent because they can be vertically transmitted through an infected queen that is responsible for the reproduction of an entire colony (Chen et al., 2006). BQCV gives rise to dead queen larvae in black cells (Bailey & Woods, 1977). Many nurse bees carry a low level of BQCV. Several nurses that together feed a queen larva transmit a high enough quantity of BQCV that the queen becomes heavily infected. Queens either die from infection at this stage or mature into an adult queen. The queen can live on, not showing any sign of infection, until the bees she mothers eventually nurse a future queen (Nordström et al., 1999). A mature infected adult queen carries the virus in her gut as well as her ovaries, meaning that she can vertically transmit the virus to her offspring (Chen et al., 2006). Like the other honey bee viruses, BQCV can be transmitted fecally, orally or through the vector V. destructor (Chantawannakul et al., 2006). The most common and virulent of all the picornaviruses that infect honey bees is DWV (Miranda & Genersch, 2010). DWV is most apparent in adult honey bees, but can infect all life stages of the honey bee. The symptoms are most noticeable in adult bees that have "shrunken, crumpled wings, decreased body size, and discoloration" and a decreased lifespan (Chen & Siede, 2007). DWV was once considered to be a virus of low pathogenicity because of its persistence in colonies at high titers, but in combination with parasitization by V. destructor, DWV has become more virulent (Bowen-Walker et al. 1999; Gisder et al., 2009; Shen et al., 2005).

All viruses use the host ribosome to synthesize their own proteins, but picornaviruses can synthesize the necessary viral proteins with fewer steps than some other viruses, as they consist of a single-stranded RNA which is sense (+) strand and thus immediately capable of translation once within the cell. In picornavirus genomic RNA, the 5' UTR contains the Internal Ribosomal Entry Site (IRES) that allows the virus to enter the ribosome more quickly than the host mRNA and synthesize its proteins. Usually ribosomes recognize the 5' methylated cap of mRNA transcripts and subsequently use eukaryotic initiation factors (EIFs) to carry out translation (Martínez-Salas et al., 2001). Picornaviruses, on the other hand, use the IRES to gain entry into the ribosome, need fewer EIFs to start translation, and even cleave EIFs needed for host mRNA translation (Pestova & Hellen, 2006). To this date, no conserved IRES sequence has been established, but the IRES sequence is assumed to undergo high mutation rates as with the rest of the viral genome (Fernández-Miragall & Martínez-Salas, 2007). Part of their virulence may be attributed to the RNA virus's high mutation rate (Drake et al., 1998). Picornaviruses use an RNA-dependent RNA polymerase to replicate their genome. RNA-dependent RNA polymerases are more error-prone than DNA-dependent DNA polymerases, which most organisms use to replicate their genome. The high mutation rate due to the lack of proofreading activity by RNA polymerase lends RNA viruses the ability to adapt to host responses.

Insects lack the adaptive immune system seen in vertebrates. After the invasion of a foreign microbe or virus, insects respond with innate immune responses, not an adaptive 12 response that uses the memory of a past attack. The insect innate immune system has two responses: the cellular response and the humoral response. The cellular response consists of phagocytes that circulate in the hemolymph and engulf invading bacteria and fungi (Strand, 2008). The humoral response consists of the Toll and immune deficiency (Imd) pathways, which activate antimicrobial peptides (AMPs) that also attack bacteria and fungi (De Gregorio et al., 2002). To this date, most immune responses studied in A. mellifera have been in relation to bacteria and fungi. The immune response to viruses remains an active field of study. Research in the field of insect virology has been limited by the lack of understanding mechanisms behind the defenses observed in insects.

Molecular techniques for pathogen analysis Molecular techniques exist to identify the RNA in present in a honey bee sample. Serological and histological techniques can detect viruses, but molecular techniques are more quantitative, sensitive, accurate, and cheaper to perform (Miranda, 2008). RNA extractions from a honey bee can give information about the bee itself and also the pathogens infecting the bee. To identify and measure the types of RNA present in a honey bee, the RNA must first be reverse-transcribed in to cDNA, a more stable molecule that can be analyzed in downstream processes such as PCR, microarrays, and DNA sequencing, namely high-throughput sequencing (HTS). In quantitative reverse transcription PCR (qRT-PCR), cDNA is used to measure specific genes that can be targeted with primers. A fluorescent dye binds to the double-stranded DNA 14 that is detected by

Red River Apiarists' Association

Editor's Note & musings by Ken Rowes

Bees have been working well but the declines have increased. 2015 has been a high winter loss for many. And bees are in short supply.

Sorry for the high tech articles in the newsletter. Just not enough time to summarize and condense. But the substance of the articles are focused on the viral infections and once you see the mites its almost too late so you need to control the infestation. Interestingly even import bees can carry the viruses.

It is great weather now for outdoor work, especially fixing and painting equipment, and of course oil changes and gardening. More importantly managing bees. Pollen, water and propolis even some nectar is coming in.

My newsletter time is short and time-lined so have tried to inform for the spring and summer bee season. Queen rearing is just weeks away.

If you have concerns come to the meeting May 10 it will be your last chance. Beekeeping tasks will increase and many will not have time for you.

Bee Well

Your Beefriend Ken RRAA editor

PS.

If you were wanting or selling please consider the RRAA Classifieds

The Bee Cause is the official publication of the Red River Apiarists' Association for distribution to its members and their colleagues in the beekeeping industry. It is published eight times a year on a monthly basis except December and the summer months of June, July, and August when membership meetings do not occur.

Articles can be best submitted in word documents as email attachments. Though they may be edited for spelling and basic grammar, no changes will be made to their contents, message and opinions. They are those of their originator and not of the Red River Apiarist Association.

Deadline for any submission to this newsletter is the second Saturday preceding the membership meeting to allow for publishing and mailing delays. Regular membership meetings are normally scheduled 7:30 PM on the second Tuesday of every month at the **Elmwood Legion 920 Nairn Avenue** in Winnipeg except the months as noted above.

The Red River Apiarists' Association, formed in 1963, represents the beekeepers of the Red River Valley and environs in southern Manitoba. The association provides a forum for the promotion of sound beekeeping practices through education, networking opportunities, meetings, field days, workshops, presentations by local apicultural experts, as well as the dissemination of this monthly newsletter.

We are on the web!
www.beekeepingmanitoba.com

CLASSIFIEDS

1 For Sale: Plastic queen excluders $3.50 each. **Contact, Lance W. Phone # 712-6783, Email; lancewld@gmail.com**

2 For Sale: Nucs with 4 frames full of bees. Lots of brood on 2 of them. All nucs have 2015 raised queens from winter hardy, mite tolerant, own local stock. No foul brood in my apiary. Price TBD. 2) New inner covers 7/8" x 7/8", pine rimmed with 3/8" solid plywood. $10.75 each Contact Ted Scheuneman: 204-338-6066

3. For Sale: Insulated hive boxes with Metal Lid and Bottom Treys $20; Honey-Frame Display Case $20,; Cobana boxes for comb honey $20; Nuc Boxes $10; Super shells with damaged frames $15; Boxes with wired frames but no foundation $10; Supers and Brood Boxes -$ 20 - $40; Honey pails- various sizes, Hive stands $5; Lids with metal or wood top $10; Bottom boards $5 / Screened Bottom Boards $8; Bee blowers $75-$150; Skunk prevention plates $1; Screened Plastic bottom boards $15; Inner covers $1; Frame building jig and Wiring jig and pre-cut wood pieces for building boxes and frames; Pure beeswax foundation $120 ; Boardman feeder trays, jars and lids $4; Beekeeping suits, gloves, veils, tools -all in excellent condition. Smokers $20-$25; Fencers for bear protection $75 -$200; Metal Fence Posts, Fencing Wire; Bee Cozies Winter Wrap [new] $15; Mann Lake 3" pro feeders [new] / $25 case of 5.; Misc. Charles Polcyn at 204 284-7064 or at vernapolcyn@yahoo.ca **Contact Charles_polcyn@ymail.com or Charles 204-284-7064 Wpg. Or farm 204-348-2506.**

4. Wanted: Honey **contact: John at**

204-943-0166 Email:honeyb@mymts.net

5. For Sale: Three frame nucs for sale with new Carniolan queen ; can deliver to Winnipeg. Price is $180, deposit required ; **paul@interlakeforageseeds.com, Interlake Honey Producers Ltd. 204- 372-6920**

6. For Sale: Four frame nucs after mid May; contact **Chris Argiriou at 204-296-4848 or e-mail christos-a@shaw.ca**

7. For Sale: Four frame nucs with young local queens. Queens available in July contact**: Waldemar Ph 204-755-2340 or Email: wdamert@yahoo.ca**

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the instrument. The amount of fluorescence (cont'd on pg7) **(From pg 5)** in a sample reflects how much PCR product, or gene of interest, is present in the sample. This method allows for accurate quantification of honey bee genes as well as viruses present in a sample (Miranda, 2008).

Conclusion This study gives strong evidence for the presence of polyA rRNA fragments in virus infected bees, as first suggested by Johnson et al. (2009). While the association between viral load and polyA rRNA expression level was not firmly established, the use of polyadenylation as a signal for the cell to degrade the ribosome may play a role in insect immunity. Cellular defenses to RNA virus infection by inhibition of translation have been observed in the past in HeLa cells (Bonderoff et al., 2008). Viral IRES elements undergo strong selection to have mutations that change its secondary structure, which could imply that the viruses are adapting to ribosomes (Martínez-Salas et al. 2001; Fernández-Miragall & Martínez-Salas 2007). Polyadenylation of rRNA could add to the suite of cellular defenses that inhibit viral replication in the cell. Nonetheless, no strong association was found between virus load and polyA rRNA in this study. These rRNA markers were first found in association with CCD, but they can be associated with a number of debilitating conditions that other pathogenic and environmental stresses (Johnson et al., 2009). In future research, honey bees with other pathogens associated with CCD, such as IAPV and N. ceranae, can be tested for up regulation of the observed polyA rRNA fragments. As noted earlier, these markers arise from degradation intermediates from intracellular processes that stress initiates. Honey bees can endure stress from any number of factors, including parasites, insecticides and poor cultural practices (Oldroyd, 2007). PolyA rRNA 34 fragments were initially suggested as a marker for CCD, but they could also be general stress markers. In either case, the rRNA markers can be used to measure stress, CCD or otherwise, in which case they can be used when beekeepers are concerned about the health status of their bees. To minimize the effect of other pathogens and environmental conditions, the RNA-seq analysis should be performed again with honey bees from Varroa-free and Varroa-infested islands of Hawaii. In the RNA-seq dataset used to compare polyA rRNA between virus-low and virus-high honey bees, half of the bees were inoculated with N. ceranae, which could have affected the outcome of the attempted association between polyA rRNA and virus load. DWV abundance is negatively associated with N. ceranae spore loads (Costa et al., 2011). To this date, BQCV abundance has a positive association with N. apis infection, but not N. ceranae infection (Bailey et al., 1983). Whether or not honey bee virus and N. ceranae quantities have a positive association, N. ceranae still presents a confounding factor in this experiment. Bees infected with N. ceranae have an altered immune response, so the polyA response expected in this study may have been suppressed (Antúnez et al. 2009; Chaimanee, et al. 2012). Future experiments testing for polyA rRNA should ideally use honey bees that are infected only with picornaviruses and no other malady in order to best control for immunosuppression. Honey bee cell lines may be a better test of the significance polyA rRNA in disease progression. Cell culture offers a more controlled environment where viral quantity and cell quantity can be better measured. Protocols for creating and maintaining honey bee cell lines exist. Isolated viruses, on the other hand, do not exist and are required for proper rigorous testing of viral infection according to Koch's postulates (Hunter, 2010). 35 Genomic studies can help to identify genes for selective breeding that will confer a better immune response to viral infection. Gene expression studies reveal which genes play a role in pathogen defenses, such as the hypothesized polyA rRNA response. After the genes that confer immune responses are identified, beekeepers can focus their breeding efforts on bees that react to viruses. Currently beekeepers use acaricides to control Varroa and other chemicals to control for other threats to honey bee health. In the long term, selecting for bees with stronger immune defenses will ensure the future of honey bee health (Moritz & Evans, 2008).

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Argentine Ants Carry Virus Deadly to Honeybees
By SINDYA N. BHANOO New York Times SEPT. 11, 2015

The Argentine ant, already known as one of the world's most widespread and damaging pests, may be infecting honeybees with a deadly virus, a new study finds.

Alexandra Sebastien, a biologist at Victoria University of Wellington in New Zealand, analyzed Argentine ant populations in New Zealand, Australia and Argentina as part of her doctoral research.

She and her colleagues found that ants from all three locations can carry the deformed wing virus, a pathogen linked to colony collapse in honeybees. The new study appears in the current issue of the journal Biology Letters.

Argentine ants have flexible diets that allow them to thrive in many climates and on multiple continents. They eat other ants and insects, but also prey on larger animals like skinks and geckos.

"These ants, when they establish in an area, they become very widespread and they forage in the same places as bees," said Phil Lester, a biologist at Victoria University of Wellington and a co-author of the new study.

The researchers also discovered that some Argentine ants carry a second virus that could be useful in controlling the ants. "It could save us from utilizing large amounts of pesticides," Dr. Lester said. —//\\—

Viruses, Mites and Black Bees, Oh My!

Dr. Humberto Boncristiani is a post-doc in the vanEngelsdorp bee lab at the University of Maryland. He is currently studying a variety of honey bee viruses, with a particular focus on picorna-like viruses. His interest in honey bees came from his father, who keeps bees in Brazil, and his virology background comes from his work on human viruses (**Cont'd pg 8**)

at the University of Sao Paulo. The inspiration for his transition from humans to honey bees came in 2007 when Diana Cox-Foster published a paper documenting an association between Israeli acute paralysis virus (IAPV) and colony collapse disorder (CCD). IAPV just so happened to belong to the same group of viruses that Humberto was studying in humans. This prompted him to make the jump to studying honey bee viruses.

Picornaviruses have a wide range of hosts, from honey bees to humans. They are of particular interest because of the manner in which they replicate. Picornaviruses have an Internal Ribosome Entry Site (IRES) analogous to that of their host's mRNA. The IRES is a complex part of the viral RNA genome used to confuse the cellular machinery and inciting the ribosome to start translation of the virus proteins. In order to prevent the cell from producing its own proteins, the picornavirus cleaves an important protein from the cellular mRNA making it unrecognizable to the ribosome (Boncristiani et al., 2009). Finally, the picornavirus uses the cell's ribosomes to replicate itself by the thousands. The virus transcriptase lacks the ability to proofread itself, leading to higher mutational rates in the copies it makes. This results in a cloud of genetically diverse viruses, most of which can cause physical symptoms and infect other cells. The incredible genetic diversity in these viruses makes them dangerous pathogens that hosts have a difficult time evolving resistance to, which is why they have such a profound effect on bee colonies.

Some of Humberto's current work is based on a phenomenon that has come to be known as "The Black Muscle Bees." These bees were discovered after processing a set of USDA-APHIS National Honey Bee Survey samples of adult honey bees. The samples of bees were crushed in water to prepare a solution that could be analyzed for *Nosema* sp., a fungal pathogen that is commonly detected in honey bee colonies across the country. The solution turned a dark shade of black, as opposed to the normal brown. This unusual observation warranted further investigation, so more bees were collected from multiple colonies in the same yard of the original "Black Bees." Upon dissecting these bees it was discovered that the tissue inside of symptomatic bees was entirely black when compared to the standard pink tissue of healthy bees. It is thought that this darker colour is caused by an increase in pigmentation or melanin formation. The melanin formation process is induced by a serine protease cascade involving the enzyme prophenyloxidase (PPO). The symptomatic "Black Bees" have a high level of PPO compared to asymptomatic bees from the same apiary. These bees were screened for other viruses and it was discovered that Deformed Wing Virus (DWV), a picornavirus, was more prevalent in the colonies with symptomatic bees when compared to healthy bees from the same yard. Additionally, the genetic diversity of these DWV strains was much higher in the colonies containing "Black Bees". DWV is a virus transmitted by *Varroa destructor*, a parasitic mite that was introduced to the United States in the 1980s. *Varroa* has since been documented as a vector of a variety of honey bee viruses. It transmits viruses directly into the haemolymph, the insect equivalent of blood, of the bee as the mite feeds. This mode of inoculation bypasses the typical GI detoxification pathway of the honey bee. *Varroa* mites also provide these viruses with an additional place to replicate and diversify.

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Blog post written by:

Olivia Bernauer is a first year Master's student in Dennis van Engelsdorp bee lab working with wild, native bees. Olivia is currently working with volunteers to monitor the floral preference of Maryland's native pollinators.

Andrew Garavito is a Master's student in Dennis van Engelsdorp's Lab. He is studying honey bees, with a focus on the diversity of pollen types brought in by foragers, and the effects of different pollen diets on bee health.

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Viruses in Manitoba and the Latest Research on Bee Health David Ostermann, MAFRI

Date of publication I believe 2012. I haven't read updates lately, however, these facts show the critical state of Manitoba bees under the mite impacts which implies mite checks and control is essential in Manitoba Apiculture.

The latest research - There have been some important and interesting findings in honey bee health this year. For example, studying colonies with CCD recently, researchers in the U.S. found large amounts of unusual ribosomal RNA (rRNA) fragments in the guts of honey bees. Compared to non-CCD bees, these abnormal fragments were "conspicuously more abundant in the guts of CCD bees" and "may be a possible consequence of picorna-like viral infection" (Johnson et al. 2009).

Ribosomal fragments - The fragments are unusual and concerning since "if the bees' ribosomes are compromised, then they can't overcome exposure to pesticides, fungal infections or bacteria or inadequate nutrition because the ribosome is central to the survival of any organism" (Kaplan 2009). So the consequences seem quite severe yet varied. (Cont'd pg 9)

Picorna-like viruses - While the fragments of ribosomal RNA in honey bees have been confirmed, there could be a number of causes for this, and the relationship between these picorna-like viruses and ribosomes is not clear. Yet it's suspected there may be a link given these findings and since "picornavirus infection in mammals both reduces protein production and causes strings of translating polyribosomes to break down, and these idle ribosomal subunits may be more susceptible to degradation" (Johnson et al. 2009).

The name "picornavirus" is derived from pico meaning small, and RNA referring to the ribonucleic acid genome, so it literally means *small RNA virus*. Picorna-like viruses include deformed wing virus (DWV), Israeli acute paralysis virus (IAPV) (Kaplan 2009), Kashmir bee virus (KBV), and sacbrood virus (SBV) (Shen et al. 2005).

Viruses in Manitoba - Seven viruses have been found in Manitoba to date, including picorna-like viruses DWV, IAPV, KBV, and SBV (Desai et al. 2009). In U of M research involving 22 colonies, DWV was found in 17 of the colonies (77%), IAPV was found in 12 (54%), KBV was found in 2 (9%), and SBV was found in 2 (9%). Interestingly, DWV was often found in combination with other viruses. The U of M continues to work very hard on the identification and prevalence of viruses in Manitoba.

Viruses and varroa - The link between varroa and viruses is well established and we know that varroa transmits picorna-like viruses (Shen et al. 2005). Speaking generally about the relationship between varroa and viruses, Todd et al. (2007) writes that "studies have shown that adult female mites acquire viruses from infected bees and transmit them to healthy adult bees or pupae on which the mites subsequently feed. Infection with virus during the bees' larval or pupal stages can result in death of pupae or reduced longevity or deformation of newly emerged adults, depending on the type of virus transmitted and the amount of virus replication."

In Manitoba the past 2 years we've seen a significant increase in the proportion of samples analysed at the Apicultural Diagnostics Lab with more than 1% varroa mite. This has been written about extensively in the Manitoba newsletters the past couple years.

Importance of nutrition – These recent findings and what we already know about honey bees and stress suggest a greater focus on nutrition these days, including checking on pollen stores and providing supplement or substitute as needed, may be warranted. I'm sure we'll be learning more about these relationships in the coming months and years. For more information, see the references below or contact David at 945-3861 (Winnipeg).

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Humans Are Spreading Deadly Bee Virus, Study Says
By SINDYA N. BHANOO New York Times FEB. 8, 2016

The deformed wing virus is decimating bee populations worldwide, and it is spreading because of human trade and the transport of bees, a new study reports." It's largely a man-made problem," said Lena Wilfert, an evolutionary geneticist at the University of Exeter and an author of the report.

Dr. Wilfert and her colleagues analyzed genetic data from honeybees and Varroa mites, which infect the bees with the virus and feed on their larvae. After gathering samples from 32 locations and 17 countries, they traced the major routes by which the virus spread.

The researchers found that the virus, which originated in Asian bee populations, first spread to Eastern and Western Europe, and then moved to North America, Australia and New Zealand.

"It was driven by the trade and movement of honeybee colonies," Dr. Wilfert said. According to her study, which appears in the journal Science, the virus is spreading largely because of the transport of European honeybees.

On its own, deformed wing virus does not seem to be a major threat to hives. The infection typically results in deformed wings and other developmental abnormalities in infected bees. When a hive also has Varroa mites, however, the combination is deadly: The mites eat larvae and infect high numbers of bees with the virus. "We rely on pollinators both for crops and for biodiversity," Dr. Wilfert said. "We need to consider our impact not on just honeybees but so many other pollinators that have no one to take care of them." —//\\—

Here is a timeline chart Randy Oliver uses in his Integrated Pest Management especially for mites.

A Typical IPM Plan—Northern California Foothills

Month [1]	Mite Threshold [2]	Monitor [3]	Drone Removal [4]	Treatment [5]	Management	Actions	Seasonal Strategy
March	2		✂	####		Inspect broken drone brood for first mites	Springtime: Use biotechnical or "soft" treatments to retard early mite population growth. Use grease patties for tracheal mite control if indicated. Don't allow robbing of weak colonies or deadouts! Minimize swarming to avoid stocking the feral colony mite reservoir.
April	5	🖋	✂	Soft	RQ	Requeen with mite-resistant stock	
May	7		✂	Soft	RQ	Honeyflow on—remove supers if necessary to treat. Make late splits.	
June	10	🖋	✂	Soft	Split		Good time to make mite-free splits or nucs. ***Watch for curlywing virus*** [6]. You can relax a bit until the drone brood starts to disappear.
July	16		✂	Soft	Split		
August 15th	30	🖋		Soft [7] Strong		Multiple soft, or strong treatments, if needed	***Aug 15 is the critical date in most areas get mite levels down below threshold so colonies can raise healthy "winter bees." No excuses!*** [8]
September	20	🖋		Strong	Feed	Strengthen colonies by feeding pollen substitute	Recheck mite drop after brood emerges. Keep brood rearing going to raise fat "winter bees."
October	10	🖋		Soft	Feed	Feed for buildup. Treat for Nosema?	Watch for reinfestation from robbing. Remove collapsing colonies from yards. Must monitor!
November/ December	0.5	🖋		OA		Once colony is broodless, use oxalic to eliminate mites. Give optimal wintering care.	This is your chance to get a clean start, by eliminating mites when they are most vulnerable. Don't allow robbing of weak colonies or deadouts

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