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Bee-Kill Survey on Acute Bee Kills

Limited response to their formal bee kill report solicitation prompted the US EPA Pesticide Program Dialog Committee (PPDC) to enlist the services of the Pesticide Research Institute (PRI) to tease out some information dealing with the topic. The environmental consulting firm tried to contact as many beekeeping organizations and beekeepers as possible to convince them to contribute information.

PRI recently released the results of the first survey for beekeepers to peruse. The report was written in five sections: (1) Survey Results (2) Crops and Pesticiderelated Bee Kills (3) Economic Impacts and Bee-Kill Investigations (4) Additional Comments and (5) Descriptions of Methods Used. I will mention only some of the findings from the summary pages and specific crops pages. To read the entire report, use this link:

http://pesticideresearch.com/site/?page_id=2_4.

The authors carefully explained that these reported losses were from high impact, immediate deaths caused by acute exposure, not losses that might have resulted from long-term exposure to sublethal doses of pesticides. PPDC assembled a PPDC Pollinator Workgroup that designed the survey and suggested how to best contact the beekeepers. Members of the committee

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included commercial beekeepers Darren Cox, Jeff Anderson, Rick Smith, Bret Adee, and Steven Coy. The beekeepers reviewed the data, as did external reviewer Erik Johansen, from the Washington State Department of Agriculture, and Iain Kelly of Bayer Crop Science.

A total of 365 non-commercial beekeepers submitted data covering 2,597 colonies. Only 62 commercial operators (50 or more colonies) responded, but they reported on 244,171 colonies. Commercial beekeepers reported bee kills more often than non-commercial beekeepers, as would be expected. Commercial beekeepers also reported longer term, abnormal bee mortality that was not clearly related to an acute bee kill. Sixty-eight percent of the noncommercial beekeepers reported no notable bee kills, while only 12 percent of the commercial beekeepers reported no problems. Commercial beekeepers felt that 76 percent of their bee kills were due to pesticide applications to crops that their bees were not pollinating. Seventy-four percent of the losses were attributed to corn or other crops that did not require bee pollination. Noncommercial beekeeper felt that their losses originated mostly on golf courses, landscape areas and roadside weeds, but 24 percent cited pesticide use on crops not dependent on bee pollination.

The authors of the report split out the data in three different ways, but I am going to provide details only on the commercial crops that were most dangerous to pollinate. The commercial beekeepers listed the following visited plant sources in descending order of what the bees visited: nonag weeds (about 76 percent); ag weeds (about 73 percent); corn (about 65 percent); alfalfa hay (about 63 percent); almonds (about 55 percent); apples and pears, urban plants, soybeans, cherries, sunflowers,

melons, blueberries, summer squash, peaches, winter squash, caneberries, citrus, cotton, specialty crops, cucumbers, alfalfa seed, canola, cranberries, walnuts, and other nuts. Asked in a different way, "What percentage of the total number of colonies ever foraged on the following crops?" almonds bested ag weeds (46 to 45 percent) with the rest staying in just about the same ranking as the previous data.

When asked what crops were likely to result in bee kills "occasionally" or "frequently," cotton led the pack with 70 percent likelihood of problems. Corn followed, at about 54 percent. Next down the list were melons at 37 percent, which was quite similar to citrus, almonds, alfalfa seed, and soybeans. Slightly better (about 35 percent problems) were alfalfa hay, ag weeds, cucumbers and sunflowers. The list trickled down from there to practically no total exposures on nuts and cranberries.

Asked, again, in a different way, cranberries suddenly became the worst of the bunch. Those who reported going to cranberries related that around 92 percent of the time, their bees were badly damaged or killed. Alfalfa hay was next, with about 87 percent. Soybeans and other nuts were next at about 75 percent. Half of the remainder of the list tallied above 50 percent likelihood of bee kills. Only peaches, apples and pears, cherries and caneberries were "safer" at 5 to 15 percent.

Another way the authors analyzed the data was by bee kills per acre of crop pollinated. Cranberries still led the pack with about 24 colonies killed for every acre pollinated. Second was winter squash, but quite a bit better (13 colonies lost per acre). Melons lost about seven hives per acre, cucumbers and alfalfa seed about five colonies per acre; summer squash, other

nuts, and caneberries about four per acre; berries and walnuts at three colonies per acre; and with peaches, cherries, almonds, sunflowers, citrus, canola and apples and pears practically no colonies were lost per acre.

Next the data switched over to commercial beekeepers' noticeable bee problems in the field. Researchers ranked the signs of intoxication as "Frequently," "Occasionally," "Rarely" and "Never." "Dead and Dying Bees in Front of Hive" totaled 64 percent occasional or frequently, with 32 percent stating rarely or never. Four percent did not answer. "Rapid Substantial Drops in Hive Population, Including Loss of Entire Hive" totaled 52 percent occasionally or frequently, and 48 percent reporting rarely or never. Finally, Dead Bees with Extended Proboscis (tongue) totaled 46 percent occasionally or frequently and 47 percent rarely or never. Seven percent did not respond.

Only 39 percent of non-commercial beekeepers noted dead and dying bees around their hives occasionally or frequently. Even fewer (32 percent) noted rapid drops in hive populations. And, dead bees with extended probosci were seen only 11 percent of the time.

When asked to describe the "contributing factors" to their acute kills, some of the previously mentioned factors were reiterated, but some new ones were given, also. Pesticides on blooming crops not being pollinated 75 percent commercial and 22 percent non-commercial. Pesticide use on non-pollination-dependent crops 74 percent commercial and 24 percent non-commercial. Pesticides used on comercially pollinated crops 52 percent commercial and seven percent non-commercial. Exposure to seed coating dusts 37 percent

commercial and 20 percent non-commercial. Exposure to contaminated water 32 percent commercial and 15 percent non-commercial. Exposure to mosquito control products and "other" pesticides were problematic to just under 30 percent of the commercial operators, but scored about 15 percent for the non-commercial beekeepers. In only one category did the non-commercial folks report exposure levels more frequently than the commercial beekeepers, non-ag use on landscaped areas, golf courses, roadsides, etc. 29 percent to 21 percent. Both groups reported about seven percent of their exposures were in forests. Only seven beekeepers thought that their bees were acutely affected by neonicotinoid chemicals and all were non-commercial.

Although the report meandered from the acute poisoning theme, a couple of survey questions dealt with abnormal bee mortality not thought to be due to immediate toxic effects. Beekeepers who thought that they saw such effects less than five percent of the time totaled 20 percent of the commercial operators and 46 percent of the non-commercial folks. About 22 percent of the commercial and 18 percent of the noncommercial operators observed abnormal colony mortality in five to 20 percent of their hives. Twenty-eight percent of the commercial operators and 15 percent of the non-commercial folks felt that 20-40 percent of their colonies died for abnormal reasons. Equal percentages of large-scale and smallscale beekeepers (about 12 percent) attributed 40-60 percent of their colony losses to abnormal causes. And, for those losing more than 60 percent of their colonies to unknown causes, 18 percent of commercial operators and 10 percent of non-commercial operators reported those substantial losses. Twice as many (16 versus 8) non-commercial beekeepers reported queen problems as did commercial operators (but that is 16 out

of 365 ((4.4 percent)) versus 8 out of 62 ((12.9 percent)) by the commercial operators). Failure to build up rapidly was reported by 11 (3 percent) of the small-scale operators, while 6 (10 percent) of the commercial operators filed that complaint. The commercial beekeepers seemed to have worse control over "hive pests:" 13 (3.5 percent) small scale operators reported problems, while four (six percent) of the commercial operators had difficulties.

I believe that the take-home message is that honey bees can become exposed to lethal doses of pesticides despite where their apiaries are located. However, such exposures are more likely to occur in agricultural environments. It also appears that very few growers who rent bees for crop pollination poison their bees on-site. But when the bees are not on-site, honey bee-toxic products are frequently used in commercial agriculture. It is difficult for bees, in those settings, to avoid lethal exposures from time to time.

<u>APHIS Disease/Pest Survey – 2011-2012</u>

I mentioned a few times that a number of surveys were being conducted on beekeeping across the country and that you should participate. It all seemed a little nebulous at the time, but now the data is being reported.

Through the combined efforts of the University of Maryland, USDA APHIS, USDA ARS, and California Cooperative Extension, a report was released: "2011-2012 National Honey Bee Pests and Diseases Survey Report." This is important because the national surveys are what will be used to allow or disallow imports of live honey bees from other countries into the U.S. Additionally, the data gives us a much better picture of what our disease and mite

populations are like. You may examine this complete report at the following web site: http://www.aphis.usda.gov/plant_health/plant_pest_info/honey_bees/downloads/2010-2011-Limited_Survey_Report.pdf.

The report begins with an executive summary, which assures us that we have not found any Tropilaelaps mites, Apis cerana, or Slow Bee Paralysis Virus (SBPV) in our sampled bees. Tropilaelaps is an Asian mite that is very small and looks like a quickmoving, whitish dot on the combs. When you put them into a vial of clean alcohol, they are a very dark gray. Apis cerana is a native Asian honey bee that currently seems to have become well entrenched in Australia. Slow Bee Paralysis Virus has been found to have at least two different strains and has been isolated from samples of bees from England and Switzerland. There also are documents suggesting that SBPV has been identified in Fiji, Western Samoa, and Australia. The Australians firmly believe that the virus is not in their country. Not surprisingly, SBPV has been documented to be transmitted by Varroa destructor.

These results come from an everincreasing data base that reflects findings from bees collected in 34 U.S. states: AL, AR, CA, CO, DE, FL, GA, HI, ID, IL, IN, IA, LA, MD, MI, MN, MT, NE, NY, NM, NH, NJ, NC, ND, OH, PA, SC, SD, TN, TX, UT, VA, WV and WI. Some samples still are being collected and more are still being analyzed. When completed, the analysis will cover 875 representative apiaries containing 7,000 colonies. As the data accumulates, it is being posted, with timely updates, on the web site:

www.beinformed.org, operated by the Bee Informed Partnership.

"Survey Kits" were distributed to the Departments of Agriculture in each cooper-

ating state in June of 2011. The 2012 kits were sent in April and May. The details of how the samples were collected and processed can be found in various protocols linked internally in the report.

Not all known honey bee viruses were targets of the "new, high-performance chemistry" used this year to identify them. Acute Bee Paralysis Virus (ABPV), Deformed Wing Virus (DWV), Israeli Acute Paralysis Virus (IAPV), Chronic Bee Paralysis Virus (CBPV), Black Queen Cell Virus (BQCV), Slow Bee Paralysis virus (SBPV), Nosema ceranae and Nosema apis were targets. Subsamples were sent to other labs for Nosema spore counts, Varroa mite load determinations, and possible Apis cerana. A few pollen samples were collected for pesticide residue analysis at the USDA AMS food lab in Gastonia, NC.

For me, it is easiest to look at the graphs to see the major results. Nosema (not by species) was fairly prevalent during the first three years of the survey (2009-2011). The respective average U.S. infection levels were about 86 percent in 2009, 50 percent, and 58 percent the next two years. Interestingly, spore loads averaged 800,000; 430,000; and 580,000 respectively. When that data is broken out in more detail, the monthly prevalence still shows the trend toward higher counts early in the season, backing off in the summer and picking up again in the winter. A similar monthly breakdown showed that spore loads very infrequently were above one million spores per bee, the level at which we (Furgala and Mussen) suggested that Nosema apis be treated with fumagillin. There also is a very interesting figure (#16) in the report dealing with Nosema spore counts and positive Nosema counts based on molecular methods. There are publications explaining how much more sensitive the molecular methods are

compared to microscopy. But, the figure shows that microscopic examination determined spores of undetermined species in 0.57 percent of the samples, while polymerase chain reaction (PCR) detected only 0.01 percent of *Nosema apis* and 0.08 percent of *Nosema ceranae* in the same samples. (Editor's Note: perhaps they intended to have the decimal point two digits to the right.)

The Varroa mite data is very disconcerting. The percentage of samples of adult honey bees in which mites were found totaled 88, 92, and 91, respectively, from 2009, 2010, and 2011. Worse yet, the average mite load per 100 adult bees has increased over those three years from 2.5, to 4.2, to 5.3. Most of the sampled colonies should have had at least 20,000 bees in them. So, the mite loads must have been between 500 and 1,600 mites per colony. The more we learn about the relationships between Varroa mites and virus diseases. the more we should try to find ways in which to keep the mite loads as low as possible. The current monthly Varroa prevalence data shows only a bit of a depression in mite numbers in November through January. Otherwise, practically all the sample bees came with mites. As we have seen in many previous studies, the average monthly mite load per 100 bees starts lowest in January and increases slowly through August, then increases quickly going into winter. That timing is coincidental with many of the late season colony losses that have become so vexing.

What about the viruses that *Varroa* vectors? In the three survey years, Deformed Wing Virus was by far the most common, found in 78 to 90 percent of the bees sampled. Since not much Chronic Bee Paralysis Virus was found during the first two years, the target was switched to Black Queen Cell Virus. In the 2011 samples, BQCV

occurred in about 67 percent of the bees sampled. The somewhat feared, quickkilling viruses Israeli Acute Paralysis Virus, Acute Bee Paralysis Virus, and Kashmir Bee Virus were not that prevalent, remaining mostly below 20 percent on average. By month, depending upon the year, DWV seems to build from January to June, and then sometimes becomes less prevalent the next half of the season. ABPV is consistently most prevalent (50 percent) in December. In 2011 it remains quite prevalent from December through April. But, in 2010 and 2012 it was considerably subdued from January through November. The two years analyzed so far for BQCV demonstrates that it remained prevalent (50 percent of the bees or more) all year and was most prevalent in April and May (reaching 100 percent) and at or above 60 percent in every month other than July. If high virus prevalence is related to unexplained colony losses, then DWV and BQCV would be the two leading candidates as potential problem causers.

The pollen pesticide residue samples were rather limited in number, and the results do not closely reflect the larger data set that was published a while back. The fungicides, herbicides, and insecticides are grouped, then presented in alphabetical order of their chemical names. The only fungicide to be found in ppm (7.060) residues was tetrahydrophthalimide, a breakdown product of captan. The next largest fungicide residue was fenbuconazole (335 ppb) found in Indar[®] and carbendazim (233 ppb) which used to come from Benomyl[®].

The herbicides were relatively low in residues. The two insecticides that stuck out like a sore thumb were coumaphos, one sample of which was 1.110 ppm in pollen, and thymol, which was 39.700 ppm in one pollen sample. Imidacloprid was found in 9 of 99 samples at levels between 3.5 and 216

ppb. Since the oral LD_{50} for adult honey bees is around 192 ppb, some of the pollen could have been directly toxic to the bees consuming it in the hive.

I would strongly suggest that you take the time to peruse this document. The more you know, the more persuasive your arguments can be.

Nosema Counts and Controls from Canada

If you have wondered about where, and how many bees, to sample for *Nosema* spore counts, researchers in Canada tried to resolve those issues. They took samples of 30, 60, and 90 bees from the brood nest, the outer honey frames, and the bottom of the inner cover of naturally infected colonies.

In April, the brood nest and inner cover sample counts were similar and less than those from the outer honey frame samples. As the season progressed (May), the brood nest bees' spore counts remained low. The inner cover bees began to have higher counts, but the outer honey frame bees had the highest counts. When they completed the analyses, the researchers determined that the brood nest counts were always the lowest, the inner cover counts seemed to represent the mathematical average of the brood nest and outer honey frame bees, which always had the highest counts. For those of you who pay attention to these things, the error bars for the means of the counts on the graphs were quite small for brood nest bees, a bit larger for inner cover bees, but were really lengthy with honey comb bees. This suggests that although the oldest bees were most apt to contain spores, their variation in countable spore numbers was quite considerable. The researchers decided that the inner cover samples best represented the colony average and will use

them in further studies, similar to the sampling method used by Furgala and Mussen in the 1970s.

In a separate study, 60 New Zealand packages were installed in equipment containing 192 full-depth Langstroth drawn combs that had been previously sprayed with *Nosema ceranae* spores at the rate of 451 million spores per hive body. Eleven sets of contaminated combs were treated as follows: (1) acetic acid fumigation (2) heat treatment (3) irradiation and (4) contaminated, only. A twelfth set of combs (5) were neither contaminated nor treated (controls).

Molecular tests determined that the bees had only *Nosema apis* in them in May, even the colonies that had been inoculated previously with *N. ceranae* spore suspensions. In June some residual *N. apis* remained in the fumigated, irradiated and inoculated colonies, but nearly all the colonies demonstrated mixed infections favoring *N. ceranae*. In October, infection levels were low, but *N. apis* became predominant, again. In August, most of the treatments still had fairly low levels of *Nosema* spore counts, except the irradiated combs, where levels of infection with *N. apis* were more than 90 percent.

Related to mortality, after 16 months of no further treatments, 17 percent of the non-inoculated control colonies perished. Forty-two percent of the colonies that were simply inoculated, or when the combs were heat-treated, had died. Fumigation was a bit better, with about 33 percent mortality. But, none of the colonies in the irradiated treatment died. It appears that the irradiation treatment may have compromised some other microbes that were harming the bees.

The lab researchers were very busy and ran some studies on the mode of action

of fumagillin, some analogs of fumagillin, and carbendazim (major breakdown product of Benomyl®) on Nosema control with caged bees. Briefly, caged bees were fed solutions containing ten million spores per ml of syrup for 48 hours. Then they were fed sugar solutions containing 0, 0.04. 0.4, or 4 mmol concentrations of fumagillin (only 0.04 mmol, since it works at that level), carbendazim and three fumagillin analogs. The carbendazim treatments did little to reduce spore counts, so it was discounted. (I wonder, however. This is the same lab that decades ago told Dr. Furgala and me that we missed the boat with our Benomyl treatments. We saw spores and gave up on Benomyl. They told us that Benomyl-treated bees produced only nonviable spores. It is surprising that nothing is said about that in this study.) The fumagillin analog, that has an aspirin attached to it, proved quite effective in reducing spore counts and will be studied further.

The last question studied was: "Which is better, spring or fall treatment of Nosema with fumagillin?" Thirty-six naturally infected colonies were divided into three groups: (1) 97.5 mg fumagillin in 250 ml drench (2) 97.5 mg fumagillin in 2 liters of feeder syrup or (3) 2 liters of nonmedicated feeder syrup. They were fed twice, a week apart, at those doses. A graph shows the spore counts for bees in the various treatment groups. Things looked pretty similar at the beginning. The spore counts went down a bit, then rose a bit, similar to the findings of Dr. Zachary Huang and his lab at Michigan State University. But, the unmedicated bees had spore counts that rose to five million spores per bee in October and 20 million spores per bee in December and February, after a bit of a tapering off in January (10 million). The drench treatment resulted in moderate control, with levels around seven million

spores per bee in February. The stored syrup treatment showed the lowest levels of spores per bee, about four million in February. The spring feeding data is not presented. The researchers stated that the results were pretty similar: highest counts in untreated bees, lower counts in drenched bees, and best results with stored syrup feeding treatments.

These research results were not extracted from research publications, but from the August, 2012 (Vol. 25 #3) edition of "Hive Lights," the periodical published by the Canadian Honey Council. The organization has given me permission to republish their information with appropriate references. In this case, the title of the research project is: "Integrated Management of Nosema and Detection of Antibiotic

Residues." This progress report was submitted by Abdullah Ibrahim, Andony P. Melathopoulos and Stephen F. Pernal. They work for Agriculture and Agri-Food Canada, Box 29, Beaverlodge, Alberta, Canada TOH 0C0.

Sincerely,

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