What the Bees Brought Home

A cooperative effort between beekeepers in Marin County California and Penn State University has resulted in the first suburban county map in the U.S. showing pesticides found in bee bread

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The beekeepers are into food, from creation to cuisine. That night they'd laid out a feast, pollinated by their tiny livestock, on the long barn table. But the mood of the gathering was not festive. So many of their colonies were dying, and they didn't understand why. Their dinner guest, entomologist Maryann Frazier, was on a Penn State University team that discovered significant chemical residues in US bee hives. Could that be their problem?

Marin County, like the rest of the country, has seen a surge of interest in beekeeping – bees being the new chickens. From some 15 beekeepers meeting monthly, membership in the Marin Beekeepers has grown to over 300; most came out of curiosity and stayed, fascinated by the insects that pollinate a third of our food.

Bonnie Morse, a member who keeps bees around the county with her husband Gary, had been gathering statistics on Marin losses yearly since 2009. At 39 to 53%, they are even higher than the news-making national averages of 30% that are widely agreed to be unsustainable. It's not a pretty sight: desiccated bees strewn across a hive bottom, abandoned brood decaying in their cells, or just a haunting vacancy.

It is quickly apparent to anyone keeping bees that "hobby" is not a term that applies to the level of husbandry required to keep a hive. And what might have begun as a pastime has connected most beekeepers in surprising ways to an interacting web – the biology and behavior of the creatures, the botany of forage, shifting weather patterns, our food and how we grow it.

Their bees, they have learned, are not individual insects buzzing about; a colony is a complete being, a superorganism, with each member functioning in concert with the whole. And that colony interacts intimately with nearly 28,000 acres of surrounding environment. So, when a beekeeper says "I miss my bees", he is grieving for more than the $150 or so that it costs to re-stock a colony; it is the loss of a taste of place, the breaking of a living link to surroundings, and a first-hand jolt of environmental disaster.

Frazier has worked with honey bees at Penn State for 25 years. When the first mysterious massive bee losses, called CCD, were reported to her in 2006, she wanted to know what the chemical contribution could be. She knew that finding out would take unique expertise and a collaborative effort, so she teamed up with toxicologist Chris Mullin; her husband, insect physiologist Jim Frazier; and technician Sara Ashcraft.

The reasoning was simple: pesticides kill insects; bees are insects. Most pesticides are lipophilic -- that is, they are attracted to lipids, which include fats and waxes. Pollen and beeswax contain lipids, making them chemoattractants. So, she reasoned, find out what is in the hives.

But it was not remotely simple to isolate pesticides – diverse chemicals at very low levels -- from wax, pollen and bees. To do it would require sophisticated equipment, little of which was available to the researchers: mass spectrometry to identify compounds and various types of chromatography to quantify them to parts per billion. What they set out to do was unprecedented, and not a few onlookers were convinced they were on a snipe hunt.

The flummoxed researchers were mentioned in testimony before Congress on CCD by Penn State entomologist Diana Cox-Foster. By chance, the story got to Roger Simonds, who runs a lab associated with the pesticide database program for the USDA in Gastonia North Carolina, equipped to monitor pesticides in agricultural commodities. He stepped forward. "Talk about our stars aligning," said Frazier, "He's a beekeeper."

Together, Simonds and Mullen identified a means of extracting pesticides from difficult matrices – wax and pollen. The team analyzed wax, pollen, bees and some flowers and royal jelly from samples sent by migratory to small scale beekeepers. Of the 171 pesticides and metabolites they were able to screen for, they came up with evidence of 121 of them. From over 1000 samples, just 1% had no detectable residues. The Penn State Center for Pollination Research (CPR) website publishes information on the projects (http://ento.psu.edu/pollinators).

The Marin beekeepers' dinner took a turn in tone with eager questions: Could such a study be done for Marin? Yes, said Frazier, but it would be very expensive – both in time and money. Morse began calculating on a napkin.

Marin would be the first urban area in the nation to be surveyed this way. Would testing prove its pride in environmental awareness? Organic farming in Marin is extensive, over 33,000 acres – mostly pasture for livestock and dairy operations in the west, and nearly all the row crops.

To get an accurate pesticide map of the County, Frazier calculated, it would require about 25 beekeepers taking pollen samples over each of eight months from hives distributed across the area. Penn State could help subsidize the cost. Even so, the lab work would cost the group $12,000. Morse stepped forward to coordinate the sampling. Jerry Draper began fundraising with the beekeepers through the non-profit SuperOrganism.

Quickly people stepped forward to support the project: Edible Marin and Napa Magazine, the San Geronimo Planning Group, Marin Organic pledged to sponsor hives. Celebration of the Bees, a fundraiser set in a pollinator garden with food, music and bee-learning stations, was underwritten by a grant. Proceeds from members' classes and honey sales were donated. Individuals supported hives. Funding was ready for sampling to begin in spring, 2012.

The Study Design

Morse enlisted 28 beekeepers from across Marin. These represented a total of 27 sites that were grouped by six geographic locations (see Table 1). Samples were to be collected monthly over an eight-month period from March through October. The 28th site was a control outside Marin County, established in an agricultural area in Brentwood with an experienced beekeeper.

When it came time for her to muster the army of volunteers for monthly sample collection, the concept proved easier than the execution. Beekeepers have a reputation for being independent, and they were required to conform to a specific protocol demonstrated in a film Frazier made for the project with honors student Brian Baits. Morse had to keep track of exacting scientific data conducted over eight months by amateurs whose lives had numerous other priorities. She'd had some experience to draw on: In Tanzania she gathered data for a baboon study by tracking individual troop members. So she tracked beekeepers until, at last, she packed 200 meticulously labeled vials of pollen on ice and shipped them to Penn State. Frazier then grouped them by date and site, creating a total of 42 samples submitted to the USDA for analysis.

Simonds' lab can detect 171 pesticides residues at the ppb (part per billion) level ranging from 1 to 500 ppb. Frazier likens this amount to a square of toilet paper in a roll that reaches from New York to London, a favorite analogy of her husband's. The lab was less persnickety when it came to scheduling, however; the analysis was on hold in favor of government work for months.

What Was Found in Marin

When the lab report for Marin finally arrived, Frazier summed it up: "We have a canvas of the landscape over time. There are definitely pesticides in the environment and some that are toxic to bees -- not nearly the number or levels found in agricultural environments, but nevertheless they are present."

What was found in the pollen? In all, 13 different pesticides were discovered in a total of 42 pooled samples taken from March through October, 2012, across the County and an outside agriculture site (see Table 2). Excluding nine samples from the agriculture site, the 33 Marin samples had 18 (54%) with no detectable pesticides and the average number of residues per sample was > 1. However, of these, six are considered toxic to honey bees (bifenthrin, cyhalothrin, cypermethrin, esfenvalerate, imidacloprid and methomyl). The other chemicals include fungicides (boscalid, captan, hydroxychlorothalonil and myclobutanil) and another insecticide (etoxazole) as well as residues from in-hive mite treatments (fluvalinate and thymol).

Of greatest concern is bifenthrin, a more potent and persistent pyretheroid which is highly toxic to bees, found in Marin across seasons and locations. The degree of toxicity is referred to as the LD50, this is the level that kills half of a test population; for bifenthrin it is 150 ppb. One of the Marin locations was at 140 ppb in June, which would be expected to have severe impacts if that level were found in a single colony. However, since the sample was pooled from nine sites, it is impossible to know the distribution. Still, that dangerous amount, measured by Frazier's paper rolled from New York to London, is so small it would hardly make it out the front door to begin the journey.

Bifenthrin is classified as restricted to use by professionals, but over 600 products with lower concentrations are available over the counter – Scotts LawnPro, Ortho Bug-B-Gone, Brigade, Capture, Torrant. They work by disrupting the insect nervous system, causing paralysis. Bifenthrin has the longest known residual time in soil of pyrethroids on the market -- up to eight months, and most native bees are ground nesting. Although the use of pyrethroids in California has reduced dramatically, applications of bifenthrin, the most potent and persistent of them, has decreased less – not enough to solve the problems it creates, which are not limited to bees (Quarles 2012).

Also at the bees' poison picnic was imidicloprid, a systemic insecticide absorbed into plants, often through a soil soak. It acts by binding to receptors in an insect's nervous system, leading to disorientation, paralysis and death. Because it binds much more strongly to insect neuron receptors than to those of mammals, and is considered safe for mammals (including humans) it has become the most widely used insecticide in the world. Although it was found in just one location at a low amount, 5.1 ppb, its presence cautions beekeepers. Just 3.7 billionths of a gram can kill a bee (oral LD50 = 3.7 to 81ng/bee or 280 ppb).

Other chemicals were brought home by Marin bees in low amounts – such as two additional highly toxic pyrethroids: cyhalothrin and esfenvalerate. Frazier's question is: "How much is too much?" A little bit might matter

Traditionally, chemical toxicity has been determined by lethal dose. The LD-50 is measured by topical or oral application on foragers, not chronic sublethal doses in pollen fed to larvae, particularly with newer systemic pesticides (Di Prisco 2013). Over the last 15 years, studies have revealed new insights into the effects of low levels on learning performance, behavior, and neurophysiology. "We are becoming increasingly concerned that pesticides may affect bees at sublethal levels, not killing them outright, but rather impairing their behaviors like communication and foraging or physiology; altering their ability to fight off infections, shortening their life span or impairing memory and learning" said Frazier.

Marin beekeepers, like those nationwide, experience most of their losses over winter. Winter bees, unlike their short-lived summer sisters, are biologically prepared to live for months. Thus they ingest more pollen; also, they may be more susceptible because they have greater fat deposits, allowing the lipophilic pesticides to accumulate in their bodies. In addition, cold temperatures make pesticides more toxic to bees (Decourtye 2003).

The EPA requires toxic analysis to be made over a 48 hour period, which is typically done by chemical companies. Frazier's Penn State team began experiments with two-day tests and then compared results when they were extended to four days, finding that observable effects could shift dramatically from no response to significant mortality during the additional two days. Other research found that the decline and death of a colony can occur months after exposure; for example, when summer bees were fed low levels of imidicloprid, the winter bees died (Lu 2012).

Because the average number of detections in Marin hives was >1, Frazier points out that synergetic interactions between them may not be a primary concern there -- although in Southern Marin in June, five pesticide residues were found in the sample, but again, pooled from eight sites. How these chemicals interact in the hive is significantly unknown. Adjuvants labeled "inert" are not required to tested for environmental impacts by the EPA, but, she notes, some have been found to be toxic to bees. "We have to ferret out what they are because they are proprietary information." Adjuvants work in the body of the bee by either interfering with metabolic pathways that break them down or enhancing penetration to increase the amount that reaches the target site.

The fungicides found in Marin hives are a concern as well, as their potential toxic interactions with pesticides are just beginning to be understood. In lab studies, certain classes of fungicides in combination with insecticides can heighten toxicity due to interactive effects of chemical components. For example, Johnson (2012) found that the SBI (sterol biosynthesis inhibitors) fungicide prochloraz produced an almost 2000-fold increase in the toxicity of the miticide tau-fluvalinate.

Raw pollen is not digestible by bees. It is converted into bee bread by fermentation through microbial action while it is stored in cells. It is further acted upon by the microbial community in the bee gut, and the diversity of these microbes may relate to the food value.

If there is good news for Marin, it is that the number and levels of pesticides bees are being exposed to is relatively low compared to those found in US hives in general and commercial hives used for pollination specifically. In the agricultural control site outside the County, more chemicals were found than in Marin (5 pesticides were detected, with 3>100ppb in a single site), more typical of such an environment. Unfortunately, samples could not be collected from a second agricultural site in Tracy; excessive spraying caused the beekeeper to pull the hives from that location.

In addition, the project showed that many Marin beekeepers are refraining from putting pesticides into their hives. Miticides, widely used by beekeepers over the last 20 years to control mites, were found to be the chemicals found most often in Mullin and Frazier's earlier studies. Brood nest wax and pollen were found to have very high frequencies and often high levels of fluvalinate and coumaphos. In Marin, although fluvalinate was the most frequently detected pesticides (12 detects) the amounts ranged only from 2.8 - 40.1 ppb and coumaphos was not found. What was surprising was that they were found at all in hives of beekeepers known to have quit using them many years ago or never used them at all. Frazier speculated that because the chemicals are so easily absorbed, it is entirely possible that they can be coming in from commercial wax on foundation or from purchased nucs. "Also worth noting is that these materials hang around for a long time", she said. "The half life for coumaphous is five years." And that is in sunlight; in a dark hive no telling how much longer it lingers (Stralka 2014).

A newer, "natural" mite treatment extracted from thyme, thymol, was found in the pollen. Beekeepers have been led to believe that this substance would not build up as much as other treatments, but "this data seems to suggest that it accumulates in the wax just the same", said Frazier.

Residue from a moth treatment, a dichlorobenzene metabolite, was found in one sample from a beekeeper who had thought that it would evaporate.

No study would be complete without the caveats that make good science so painstaking and inconclusive. Frazier pointed out that some of the Marin samples were 1-2 grams instead of the optimal 3 grams. Also, "because the samples are pooled, the levels in general are low." That is to say, the study was designed with multiple sites in each geographical area, all of which were tested together. Testing each hive would have been prohibitively expensive, but the results tend to average out, potentially diluting results for an individual colony that might have had more toxic exposure.

What We Get

Frazier cites this project as "a great example of a cooperative effort between a university research/extension specialist and beekeepers. It was the coordination of Bonnie Morse, the volunteer Marin beekeepers, and the community of Marin County, California "who really made this project happen".

So what do we all get for twelve grand and nine months of volunteer work? We get a local window into one of the interacting causes of the decline of pollinators worldwide. Marin deserves some self-congratulation, as it is not nearly as contaminated as agricultural areas. Less, yes, but it is not yet time to celebrate: even these bees do not live in a clean house. "In general, it appears these [Marin] bees are exposed to a small number and relatively low amounts of pesticides," said Frazier. "While the number and levels of pesticide residues are small, the number of pesticides toxic to honey bees is troubling. However, knowing what pesticides are involved, where and when they are being applied allows beekeepers to work within their communities to potentially change this scenario."

So what does it mean in the larger picture? "We have answered the first question," said Frazier, "Are pesticides potentially a problem for bees? Yes, we feel quite confident that they are. The next question is: how is it impacting them?" That is a complex inquiry. The Penn State group is working with Diana Cox-Foster who found immunosuppressive effects: Bees exposed to pesticides and subsequently challenged with disease had higher virus titers. With some chemicals it was found that genes that turn on detoxifying enzymes were inhibited.

Pesticides, it is widely acknowledged, interact with other factors influencing the precipitous decline of honey bees in the US and worldwide: they include Varroa mites, viruses, poor nutrition and decline of habitat (Bes 2006).

What We Can Do

"Clearly the phenomenon of pollinator decline is not limited to honey bees or to the US," concluded Frazier, "And the consequences are far reaching for agriculture and our food supply."

Many Marin beekeepers eschew using chemicals in their hives or do not breed from treated hives. Most report that they are mindful of reducing pathogen buildup by culling old comb; some are experimenting with guiding bees to create their own comb as well as using management techniques to control mites. A shared goal is to develop resistant traits in bees that can replace chemical treatment for mites and diseases. The Morses work as Bonnie Bee & Company to select regional survivor stock. Cynthia Perry helps beekeepers split and share strong hives, and members share progeny of survivor queens.

But the beekeepers cannot control where their bees forage. It turns out that the ideal of pest-free surroundings, often achieved with prophylactic perimeter sprays, is achieved at a significant environmental cost (Quarles 2009). A pesticide such as bifenthren is advertised as "pet friendly" but there are few pesticides that do not harm bees. In one Marin pollinator garden, sixty species of native bees have been identified, most of which nest in the ground: Newer pesticides can reach all the bees by their presence in the soil as well as in nectar in pollen.

What the beekeepers hope is that those who care for the landscape of homes and parks will take heed. Alternatives to harmful pesticides are available to combat pests (www.birc.org), and beekeepers hope they will be adapted – if not for the love of their corps of fuzzy humming vegans then for the fruits and vegetables they pollinate.

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