

# Episode 107\_mixdown PROOFED

Thu, Aug 25, 2022 10:07AM • 50:41

## SUMMARY KEYWORDS

bees, beekeeping, queen, beekeepers, colony, hormone, larval, jh, research, bee, insects, pesticides, development, honey bees, pollen patties, insect, pollen, rearing, juvenile, workers

## SPEAKERS

Serra Sowers, Guest, Stump The Chump, Jamie, Amy

### Jamie 00:10

Welcome to Two Bees in a Podcast brought to you by the Honey Bee Research Extension Laboratory at the University of Florida's Institute of Food and Agricultural Sciences. It is our goal to advance the understanding of honey bees and beekeeping, grow the beekeeping community and improve the health of honey bees everywhere. In this podcast, you'll hear research updates, beekeeping management practices discussed and advice on beekeeping from our resident experts, beekeepers, scientists and other program guests. Join us for today's program. And thank you for listening to Two Bees in a Podcast.

### Amy 00:51

Hi, everyone. Welcome to today's episode of Two Bees in a Podcast. Today, we have Dr. Julia Fine, a research entomologist with the Invasive Species and Pollinator Health Research Unit with the USDA ARS in Davis, California. She will be discussing a paper that just came out called 'The Behavioral Toxicity of Insect Growth Disruptors on Apis mellifera queen care. Thank you so much, Dr. Fine, for joining us today.

### Guest 01:19

Yes, thank you for having me, Amy.

### Amy 01:21

All right. So the first thing we do with our speakers is always ask them how they got into honey bee research in the first place. So tell us just a little bit about yourself.

### Guest 01:31

Yeah, sure. I think when I first started getting into honey bees, I was still going to school doing my undergrad at the University of Akron. And at the same time, I was working at a chemical company that was just outside of Cleveland. So I was doing a lot of analytical testing of polymers, actually. But I ended up taking my first entomology class at the University of Akron with Professor Randy Mitchell.

And I can remember when we started learning about insect behavior, and we learned about fixed action patterns, and Hymenopteran behaviors specifically. There was this solitary wasp that he was teaching us about that parasitizes larva and buries it. And if it gets interrupted during this process, it just starts the whole thing again. My thoughts were that this is such an elegant and intriguing process. And from there, he started teaching us about social insects, and their behaviors. And again, I thought, this is so elegant and so complex and straight out of science fiction. So I decided that insects were a lot more interesting than polymers and I was going to go to grad school. So I ended up meeting Professor Chris Mullin, who was a toxicologist at Penn State. He's retired now. But toxicology turned out to be a really good field for me. And I ended up pursuing my PhD at Penn State with Chris Mullin in toxicology, but by pursuing toxicology, I was able to meld two of my interests, that would be honey bees and also, I got to use some of the skills that I picked up in analytical chemistry along the way.

**Amy 03:31**

I always think it's so fun when you have a professor in undergrad that really influences you that much. I mean, you really are his success story. It's pretty neat to hear.

**Guest 03:42**

Yeah, he was great. He really took his time and taught all of his students and was able to pass along some great knowledge to us, and also his passion for insects and for nature.

**Jamie 03:57**

Julie, I really liked that story about how you got where you are. I mean, that's really amazing. Amy, I second what you say, that idea that this individual, this professor who was teaching at the undergraduate level really lit a fire in you and now here you are doing this great work. And that's what we want to talk about in this podcast episode today. So you're joining two of your interests, social insects, specifically honey bees, with toxicology. And you and your colleagues, specifically, were looking at how workers, when exposed to certain compounds during larval development, will be affected downstream, their developmental time, their weight, their longevity, their queen pheromone responsiveness as adult bees. Now, you were looking specifically at a group of compounds referred broadly to as insect growth disruptors. Before we talk about your specific research, can you give us a brief overview of what an insect growth disruptor is and how they are used?

**Guest 04:55**

Yeah, sure. So insect growth disruptors or IGDs as I might refer to them are pesticides that are used to disrupt the growth and development of pests. So, the two most common categories of IGDs are chitin synthesis inhibitors, which disrupt the formation of insect cuticle, and insect hormone mimics that are going to simulate hormones that are involved in insect growth and development. Insect hormone mimics can disrupt the normal growth processes of insects, often resulting in delayed, impaired, or premature molting.

**Amy 05:36**

So can you tell us a little bit about your project specifically, and the insect growth disruptors? How they were tied together? So what were you looking at in your study?

**Guest 05:48**

Yeah. So first of all, when I was doing this work, when I started this work, I was working with a technician who is now my student, Eliza Litsey, who was the first author on this paper. And I have to, first of all, give her a shoutout because she and I designed the experiment together, and she took it and ran with it. She did the bulk of the day-to-day work with this experiment, organized it, and wrote up the results. And this was her first author paper, and I think she did a tremendous job. But when we started thinking about IGD and honey bees, we realized that these hormones, specifically that these insect hormone-mimicking IGDs often have multiple functions during honey bee development and in honey bee adults too. So for example, queens during development have higher juvenile hormone titers than workers do. And if you introduce exogenous juvenile hormone, you can cause worker larvae to develop more queen-like characteristics. So, if some of the hormones that insect growth disruptors mimic can have these effects at small doses, Eliza and I wanted to know what effects these IGDs themselves could have on certain behaviors that could differ amongst castes. So we chose to look at queen care behavior, or queen pheromone attraction by workers because it's one of the most important behaviors for supporting colony-level reproduction, but also because queen pheromone responsiveness is something that can be manipulated by parameters like reproductive potential, which we know can be hormonally manipulated during development.

**Jamie 07:50**

So, Julia, we're going to ask you specifically about your research more in a second, but I want to kind of double back to these insect growth disruptors. So we have these compounds, but how are they used? I mean, I'm assuming they're applied to crops out there in an attempt to cause problems for pest insects.

**Guest 08:11**

Yes, so they would target immature insects, primarily. So the IGDs that we looked at in this study were diflubenzuron, which is a chitin synthesis inhibitor; pyriproxyfen, which is a juvenile hormone mimic; and methoxyfenozide, which is an ecdysteroid agonist. They are going to be used a lot in almonds. And of course, almonds are a huge crop for beekeepers in the United States. It's the largest pollination event in North America. So it's likely to anything that bees encounter in almonds is likely to affect the bee population in the United States.

**Jamie 08:59**

Yeah, so that sounds like a really good reason to study that right? If 60% of the nation's bees are moving to almonds every year to provide pollination services, then a lot of bees might be exposed to these three compounds that you were talking about. So knowing that those are the ones that made your study design, could you tell the beekeepers who are listening to us around the world what study design did you use? How did you conduct this research? How did the experiment look?

**Guest 09:27**

Yeah, so we used some standard methods to rear bees in a laboratory setting. Actually, we used an in vitro larval rearing method that, Jamie, you published with Dan Schmehl, I think back in 2016. Yeah. So that allows us to really precisely expose bees to different pesticides and control the conditions that

they're exposed to during development. So, so that way was sort of an off-the-shelf method, and we introduced pesticides at sublethal doses into their diet. Now, when they eclosed as adult bees, that's when we had to start getting a little bit creative just because bees that eclosed from this system tend to be a little bit delicate. So some things that I've noticed over the years working with in vitro reared bees is that sometimes they can still be tallow upon emergence, which means that their cuticle isn't quite hardened. So they can dehydrate really quickly and they'll be very weak. So in a hive, they might hang out a little longer in their cells so that they can sclerotize, and then they'll come out later. So what we ended up doing is, for a little bit after they emerged, we gave them a plastic comb. And what you'll see, it looks like they're sick, but actually, they're just crawling back into their cells to finish the sclerotization process. And then they come out when they're done, and they're great. They're ready to go. So we kept them in those kinds of cages for a little bit. And then, during that time, we also exposed them to their gut microbiome. So this is another important piece that we realized while we were thinking about in vitro reared bees: they don't have a gut microbiome like they normally would in a colony because they don't have any contact with any hive matrices or with any nurse bees, which is where they acquire those bacterial colonies. And without it, we see that they tend to have a really reduced lifespan, and they're just not normal bees. So again, I have to say, I have to give credit to Eliza for taking a lot of initiative with this insight. She knew that this was going to be a really important piece for this study because she'd worked on a similar project or a project looking at the microbiomes of native bees. So she was the one who said, "Nope, we need to do this and give them something." So we ended up grinding up nurse bee guts and feeding it to them while they were in this plastic comb sclerotizing. So early on, they could acquire their microbiome like they might in a colony. So this is how we made them as normal as possible while they were reared almost entirely in a laboratory as opposed to a colony. So from there, we took these bees, and we placed them in petri dishes for the behavioral assay. And this approach was inspired a lot by Hagai Shpigler's nursing assay. So the bees were in these petri dishes. And then we would introduce a queen pheromone lure or just a plastic lure that had been impregnated with this synthetic queen pheromone. And we recorded the number of times that they would contact it, so it was simulating a queen. And we were trying to see how they might respond, how attracted they were to a pheromone that queens produce.

**Jamie 12:45**

That's right.

**Amy 13:21**

I have so many questions for you. I feel like you just shared so much information especially with in vitro. I mean, part of my job is to go out and teach the public about what we're doing here at the lab, what researchers are doing with honey bees, and we do have a student that works with in vitro rearing here, and people are always like, "Well, what's the point of in vitro rearing? Like, why would you want to do that?" And a lot of times, we say that it's a lot of pesticide work. The fact that you were looking at the pesticides and also the microbiome of the bees, I mean, that plus the bees, how do they know? I just have so many questions like, how do they know that it's time that they need to go harden their cuticles a little bit longer? Right? I mean, do they just know that naturally? Or is it something that you kind of forced them to do? I have so many questions on what you just said.

**Guest 14:15**

Right. Well, I think with respect to your question about the sclerotization process, normally, I think the bees will hang out, they want to close right after their final molt, they'll just hang out in their cell a little bit longer before they chew through the wax capping. But in an in vitro setting, we don't always have the option to let them hang out because you might be ready to check on them, like you check on them twice a day or something, and if you leave them in too long, they'll starve.

**Amy 14:54**

Right.

**Guest 14:55**

So you might have to take them out a little bit longer, but even in a colony, after they do eclose and chew through their wax capping, you see them go back into cells. People think it's cell cleaning. And I think there's some recent research, I can't remember off the top of my head who published it. But there is recent research showing that actually, they might not be just cleaning cells, they might be trying to complete their sclerotization process.

**Amy 15:29**

That is so cool. That's really neat. All right. So I went a little off track. But I'll get back to my next question. So you and your team, you were looking at the insect growth disrupters and the concentrations on the worker larva and so can you tell us how you determine that concentration that you're testing in your study?

**Guest 15:51**

Right, so the question about the doses that we use is super important, and I think it deserves a nuanced response. But the shortest response is that we were looking for sublethal doses. So for methoxyfenozide, we used 16.1 micrograms per mil, which is about 16 parts per million. And this is 10-fold higher than what's been recently reported to be found in honey bee colonies. So, from methoxyfenozide, we can say that this is not really a field-realistic exposure. For pyriproxyfen, we used 164 parts per billion. Diflubenzeron around was 167 parts per billion. And so these concentrations are going to be on the high end of what's been found in pollen taken from bee colonies in recent surveys. And larval bees only eat a very small amount of pollen during development. So it's probably not common for larva to be exposed residues this high through diet in the field. But that being said, wax tends to sequester pesticide residues. And we're not sure if this can result in larval exposure, either through contact with wax, or through pesticide residues leaching from wax into the diet. I think, either way, our engineered exposure scenario, where we just put pesticides into the diet directly, isn't very realistic. And we'd need to do more testing to determine what levels larvae are actually exposed to. I love the in vitro larval rearing assay. But this is, I think, a common criticism that just putting pesticides directly into larval diets might not be the most realistic way to expose them. So, I would hypothesize that the true levels that they'd be exposed to would be lower than what we tested here. But again, the reason that we chose these doses was not necessarily to test a field-realistic dose, we wanted to test a sublethal dose. So our intent was to push ourselves as scientists and as toxicologists to go beyond this idea of an LD50, and to demonstrate that just because we don't see a pesticide knocking down half of

the bees in a test population doesn't mean that it's not necessarily having a really detrimental effect. So for honey bees, more than solitary insects, we really need to go beyond this sort of thinking because bees are social, and they have this colony structure that depends on coordinated behaviors of thousands of bees. So if you have a brood cycle that emerges, that seems healthy, but, for instance, they won't cooperate with each other, this could be really deadly, maybe more deadly than losing maybe 10% of the colony to pesticide exposure.

**Jamie 19:08**

So, Julia, this is a very elegant design. You guys put a lot of stuff together into this research project. Can you tell us a little bit about some of the key findings from your study?

**Guest 19:18**

Yeah, so the most noticeable results that we got were that when you expose larva during development to pyriproxyfen, which is a juvenile hormone analog, and to methoxyfenozide, which is an ecdysone agonist, you see reduced responsiveness to queen pheromone in adult workers. And what this means is that in a colony, these bees might be less inclined to care for and provision a queen. I thought it was notable, too, that we didn't really see any effects on the treated larva up to and including adult eclosion. So we didn't really see effects on larval survival or adult weight and morphology. So if we had just stopped the assay there, we wouldn't have seen anything.

**Jamie 20:11**

So, Julia, that's really neat. And you've got these three different IGDs, I've got to remember it because I'm so used to calling things IGRs, but these IGDs, these insect growth disruptors. You have one that's a juvenile hormone analog, and I'm pretty familiar with juvenile hormone. I was actually doing research on JH when I was a high school student. So it's kind of near and dear to me. And then you had one that's an ecdysone agonist. And so ecdysone, among other things, is responsible for molting in insects. So you've got these two things that, in my mind, can picture how they might affect developing bees. But I'm curious what you guys speculated, how they might impact the behavior of the emerged adult. So, you made the point to say you didn't actually see any impacts on larvae up to eclosion. In other words, you didn't see any impacts on the development process. But instead, you saw these impacts own their responsiveness to queen pheromone when they were adults. So how might a JH analog and an ecdysone agonist do that?

**Guest 21:17**

Yes, and actually, I have to correct myself just briefly, because I did say that we didn't see any effects during larval development, but we did see one effect. It didn't appear to be detrimental. We saw that the juvenile hormone analog, pyriproxyfen, significantly reduced the length of the pupation period for the exposed larva. And overall, we hypothesized that for this juvenile hormone analog, pyriproxyfen, this reduced response to queen pheromone that we saw might have been because exposure to the juvenile hormone mimic during larval development could have pushed them towards a more queen-like state, meaning that they would have higher reproductive potential. And this is supported by this shorter development time that we saw for pyriproxyfen-exposed bees. So compared to workers, queens have a shorter pupation period. Now, for methoxyfenozide, which is an ecdysone agonist, we're not really sure



what's going on. We know less, frankly, about what ecdysteroids are doing during development besides affecting molting. So we need to do some follow up, I'd say, for both of these treatments to investigate further what's actually happening with these bees.

**Amy 22:53**

Yeah, so that was actually my next question. What is next, from a research perspective?

**Guest 22:58**

Well, immediately, I'd say that we need to follow up on this work and investigate the physiology of these bees that we reared. And we need to look at the expression of various genes related to caste differentiation to get a handle on why we observed what we observed to see if our hypotheses are correct, or if it's something else that's going on. So I'm happy to say that this is work that's in progress right now. And we're getting some very interesting results to be published soon, I hope. But in a broader sense, we really need to do more to investigate some of the more subtle effects that agrichemicals can have on bees in field settings so that we can better understand how and if, in a field setting, they might be disrupting behavior and affecting colony survival.

**Jamie 23:54**

So, Julia, all this is great. It's near and dear to me. I really like in vitro rearing bees. It's always fun to be able to see what we can do in this process. It was neat that you were able to look at these and this particular class of compounds and how that affects the behavior of the resulting adult bee, rather than just the developmental parameters of the developing bee. So I'm curious in the grand scheme of things, as you think about what this research says for bee colonies and bee health, what are some take-home messages that you think you could share with beekeepers based on your research?

**Guest 24:25**

I would say just to keep in mind the cascade effects that certain stressors can have and to really consider that just because you're not seeing an immediate response or decline in colony populations doesn't mean that an introduced stressor is not going to, down the road, have an effect on colony longevity. Because honey bee colony dynamics can be kind of a delicate system and if what we saw in this study and in other lab studies can be reflected in the field, we might find that certain stressors that appear to be harmless in the short-term can have some, potentially, long-term consequences.

**Amy 25:21**

So, Julia, this is all really, really, really great information. And we're super excited that you were able to join us today.

**Guest 25:28**

Yep, thank you so much for having me. It's been a pleasure.

**Amy 25:31**

All right, everybody. That was Dr. Julia Fine, a research entomologist with the Invasive Species and Pollinator Health Research Unit with the USDA ARS out of Davis, California. Thank you so much for

listening to today's episode of Two Bees in a Podcast. Alright, Jamie, I must admit, pesticide research is not my specialty. So I just wanted to follow up because I'm like, okay, there are some things that I know that people in the pesticide world understand, but I just didn't understand some of what was going on. So I thought that maybe we could talk just a little bit about the hormones of the different types of compounds that are being used and why they're being used. Dr. Julia Fine, it sounds like she did really great work and it sounds like their methods were really great. And their findings were great. I don't know, should we start from the beginning?

**Jamie 26:48**

Yeah, let's do that. So she was specifically talking about a group of pesticides or in this case, insecticides, known as insect growth disruptors. And it really kind of helps out that because what they do is in their name, insect growth disruptors. I'm very familiar with juvenile hormone because, showing my nerdiness here, my nerdi-ocity, when I was in high school, I did a lot of science fair projects using juvenile hormone mimic with honey bees. So I learned a lot about JH at the time. So let me just tell you briefly about juvenile hormone. As the name implies, it's a hormone that's found in the developing bees. And for that matter, it's actually found in adult honey bees as well. And juvenile hormone is partially responsible for their progression through the developmental stages. And in honey bee workers, there's some evidence that suggests that it's also responsible for their progression through their behavioral stages. So as they age, the JH levels go up or down, and that changes what task they'll be moving into. There's actually a paired set of glands in the head of the bee called the corpora allata and these glands produce JH. And I've known people who are good enough to remove those glands from adult honey bees surgically, and the adult bees remain alive. And then they can cause bees to jump or regress in behaviors based on placing juvenile hormone on the worker bees' backs. So we know that JH is partly responsible for the development of the bee, but also the development of the cascade of the behaviors that they have as adult workers, at least there's strong evidence for it. JH is also partly responsible for differences between workers and queens, based on how much JH is produced while those larvae are developing. Again, all of that being influenced by what they're feeding on, worker jelly versus royal jelly. So JH is very important. So a juvenile hormone analog would be a compound that is very similar in structure to juvenile hormone, therefore, it produces similar results in juvenile hormone. So if you're trying to control an insect with a juvenile hormone analog, what you're doing is you're hoping to throw this stuff out in the environment and cause disrupted development. A lot of insects that get exposed to high levels of juvenile hormone analogs, for example, methoprene or the one that she was studying, they never pupate. They never make it through to adulthood. Now, there are two others, and I'm really bad with pesticide names, so she had three different pesticides. I'll butcher the names, but I'm really good with what they do. And so one was the JH analog. Another was a chitin synthesis inhibitor. So chitin is an important material that goes into their exoskeleton.

**Amy 29:45**

Okay.

**Jamie 29:46**

If it's a chitin synthesis inhibitor, it is something that inhibits the synthesis of chitin. So, therefore, they never harden, it again disrupts their development. And then the final one was an ecdysone agonist. So



an agonist is anything that works against something. Ecdysone is a very important hormone while insects in general are developing. And as I shared during the interview, it's partially responsible for molting. We know that all insects molt, as they age, as they grow, and that's simply the shedding of their exoskeleton. So ecdysone is partly responsible for triggering these molts. So if you've got an agonist, something that's working against, or inhibiting, for example, ecdysone, then it's got a problem molting. So the idea is that you can put these insect growth disruptors out there in the environment and target the specific things that you're looking at, trying to control, she said, on almonds or whatever. So that's why it's a little surprising to me that she didn't really find a lot of effects of these things on the development of the bees, the physical development, whether or not they pupated successfully, that it was more on downstream behaviors. They were exposed to this as immatures but we saw this behavior as an adult. And that was particularly intriguing to me.

**Amy 31:17**

Yeah, and I know something that she had highlighted over and over was the student that she actually was working with who was the lead author on this paper. So Eliza Litsey, and so she's just started her master's degree, but it seemed like she was really just a go-getter to run this project and get it to the finish line for this research. That was pretty cool to hear, as well.

**Jamie 31:38**

Amy, you're spot on. If you're a professor or a scientist at the USDA, or somewhere around planet Earth, we are rarely the ones who are able to get to the bench or get into the field anymore to do this great work that we end up talking about at conferences or to beekeeping clubs or even in podcasts. It's often graduate students, undergraduate students, postdocs, technicians, who are the ones that are really creative, coming up with amazing ideas, doing elegant studies. And I just love the fact that Dr. Fine was emphasizing Eliza's contribution all the way through. We all have our own Elizas as I know for sure. So it's really neat that she was doing this work and making such great progress with it. I was also excited, too, that she was using the in vitro rearing method that Dan Schmehl and others and I were able to do. Just briefly about that for you listeners out there, the in vitro rearing method is simply taking incredibly young worker larvae somewhere in the neighborhood of 12 to 36 hours old, we graph them and put them in these plastic tissue culture plates, these plates that we take into incubators, and every day of the larval bee's life we feed them, then we can carry them through pupation to adulthood. And this little assay that we were able to codevelop or at least make better is something that's now used around the world to do exactly these types of tests that Dr. Fine was talking about. So it's something we're proud of here at the University of Florida Honey Bee Research and Extension Lab, because prior to us tinkering with that assay, it just wasn't very repeatable. Lots of folks around the world had their own tweaks, but it just wasn't repeatable. And now that we've got this assay out, a lot of labs all around the world are using it to look at the impacts of pesticides on honey bees, as well as look at the impacts of a lot of things on the development of honey bees and the future behavior of the adult bees that come out. So we're really excited to have that published and out there and are happy that folks around the world are using it to make bees healthier and beekeeping better.

**Amy 33:36**

Absolutely. Well, I'm excited to see what Dr. Fine and her group continue to publish and it'll just be exciting. I love hearing what people are doing just around the world, around the nation, with the USDA labs. It's always fun to hear what's going on.

**Stump The Chump 33:57**

It's everybody's favorite game show, Stump the Chump.

**Amy 34:08**

Okay, we are at the question and answer time. And Jamie, the first question that I have for you is by a listener who reads publications and journal articles, which is really cool. But this person was reading an article about how queen cup sizes and queen caging influence the size of queens that are produced. So let's talk about this, because I think I've heard this too. And I think maybe at one of our graduate lunches we were talking about queen cup sizes and whether that actually influences or impacts the queen quality that we have. So what are your thoughts on the article?

**Jamie 34:43**

Yeah, Amy, so there are some neat projects coming out these days about queens and queen rearing that, at least, I have never really thought much about, factors that might influence the quality of queens. Normally when our queen producers produce queens, they will go and graft the youngest available female larvae into these queen cups, either plastic cups or wax cups, they put those cups into a cell builder colony that will go in and develop out those queen cells, feed those young larvae. Sometimes they'll move them from there into these finisher colonies where the bees will cap over those cells. And then, of course, once the cells are capped over, the breeder will take those cells and put them into mating nucs and allow the new queens to emerge and go off and mate, blah blah blah, ultimately cage them and sell them to you. Well, this questioner found a research article where some scientists were looking at the size of the queen cup into which they were grafting young larvae, how those sizes influenced the size and quality of the queen that emerged, and they were also looking at something that I've never thought about doing before at all with rearing queens. What they did is the mother queen, whose offspring they were going to graft, they caged them, zero days, two days, or four days to release them from those cages at those time points, and allowed the queens to lay eggs for six hours. And then they measured the size of those eggs, as well as the size of the queens that were produced from those eggs through the grafting process. This second part, caging the queen is not something that we do as part of queen breeding. But the first part grafting in different size cell cups is something that we do so I'm going to talk about the results from both half of those. Yeah, so in the first half, the long story short is all the things that they measured -- the weight of the queen, the overall size of the queen, various aspects of her physiology -- all of those improved when the larvae, when she was grafted as a larvae, was grafted into a larger queen cell cup. So basically, what they found is when you graft young female larvae into different size queen cell cups, you get different size queens, which is kind of, I suppose, intuitive. If they're being grafted into a larger cell cup, they have more room to grow, so workers might take care of them better. But the implication behind it could be, in fact, this might be a follow up study that they're wanting to do someday, the implication could be that if you graft into larger cups, you'll get larger and potentially better queens. So my guess is that they're probably working on this behind the scenes, and we'll know about that soon.

**Amy 37:45**

Well, then the other thing too, I mean, there must be a size that's too big, right? I mean, you can't just have a huge cell, right? Well, I'm excited for that. So actually, you mentioned us putting it on the show notes. And I had a couple of emails, people asking where the show notes are. And if you go to our website, Ufhoneybee.com, you will be able to access the show notes directly onto our website. And we also have recorded episodes on there as well. So there you have it.

**Jamie 37:53**

Absolutely. And I was thinking the same thing. But it looks like what they did is 9.4, 9.6, 9.8, and 10-millimeter cell cups. And I'm guessing that they had not hit the threshold yet at 10 millimeters. So it's possible that there is a size where it's just too big, and you shouldn't do it, just like what you mentioned. But it doesn't appear that they had hit that limit. So the other thing that they did, again, going back to caging the mother queen zero, two, and four days, then releasing those queens, and taking the eggs the first six hours they were released from those cells, they found that the egg sizes were bigger if the queens were caged longer prior to laying those eggs. So if you don't cage at all and you instantly look at her eggs, they're smaller than if you cage her for two days, and then four days making the biggest eggs. And not only that, but they found that the queens resulting from those eggs from queens caged for four days were bigger queens, almost as if they had been reared in these larger queen cell cups. So both of these mechanisms, rearing the queens in larger cell cups, as well as caging the mother queen for a longer period prior to grafting her offspring, both of these lead to bigger queens. So we will make sure and link this paper in the show notes so that you guys out there listening can go in and have a closer look at it. But my guess is, and this is totally just a guess because I don't know these scientists personally, but my guess is that this is all part of an effort to improve the quality of queens and they're just looking at what factors are possible. And it's one of those things that you just never think of, "Hey, maybe just grafting into bigger cell cups makes better queens. Maybe caging the queens a little bit beforehand, forcing them to lay, quote, larger eggs, however that happens because they're caged longer." All of these things could possibly lead to bigger queens and it's things like this that sometimes make a big difference in the bee world, things that we don't think about. Just boom, come back, and improve things. So we'll see where this goes. That's a really good point because the show notes aren't on any of the other podcast platforms, right? You have to go to the podcast on our website to be able to see that. So you click on an episode, it opens up, and it shows you who we're interviewing, what questions will be asked, links to all these things that we're discussing. Good point, Amy.

**Amy 40:39**

All right. So the second question that we have today. So bees go out, and they forage and they bring in pollen, and they bring in nectar. Sometimes we don't have pollen and nectar and they basically go through a dearth. And so beekeepers will supplement that by using either sugar water and/or pollen patties. And so the questioner is asking, are these colonies more susceptible to diseases if they're being fed sugar water and pollen patties, instead of bringing in some of those resources that they have out in nature?

**Jamie 41:12**

Well, it's easy to talk about the first half of this. If bees do not have available resources, stored honey or incoming nectar or stored pollen or incoming pollen, then they are absolutely more susceptible to diseases and pests. So then the second question is, let's say that none of that's happening. None of it's coming in, no nectar, or no pollen. So then, as beekeepers, we're having to supplement the nectar and pollen through sugar water or pollen patties. Does that compensate, from the disease and pest perspective, for the lack of incoming pollen and nectar? And I would say, collectively, the research suggests yes, but only to a degree. I have never seen researchers compare, for example, colonies that are fed sugar water and pollen patties to those in the same apiary that are allowed to forage for naturally occurring pollen or nectar. But I do know that a lot of commercial beekeepers and beekeepers, for that matter, in general, when they are experiencing nectar or pollen deficits will feed colonies to avoid some of the disease and pest problems that occur. Now, I will also say that feeding certain things can actually promote certain things. For example, pollen patties, if you live in an area where small hive beetles are present, pollen patties can cause beetle populations to explode. If the pollen patties are positioned in the colony in some way that beetles can get to it but bees can't keep those beetles cleaned out from those patties. There's some conflicting research that pollen patties may increase virus loads or Nosema loads or decrease virus loads or Nosema loads. Ultimately, this question is one for which there needs to be more research. But I would say in the absence of incoming nectar and pollen, and if your colony otherwise has a storage deficiency of those, then feeding to overcome those deficiencies better positions the colony to handle diseases and pests.

**Amy 42:58**

I think that's fair. All right. So the last question. So, Jamie, I started my beekeeping journey about eight years ago, and you started yours, what, like 70 years ago or something like that, right?

**Jamie 43:26**

Yeah, 812 years ago. It started when I was 12. At the moment I'm speaking I'm 44. So what? 32 years ago? 32 happy years.

**Amy 43:35**

32 years? Yeah, I was close. Okay. Anyway, when I go and speak to beekeepers associations, I always tell them, the longer I've been a beekeeper, the less I know. And I think that's really true. I mean, after a couple of years, you think you're like kind of an expert when you've had bees for two or three years. And then all of a sudden your mind starts opening up to all these other possibilities and bees do crazy things sometimes. And so this questioner is asking, they don't know what they don't know. And so they're wondering where can they go that offers different quizzes, maybe a quiz or a test that can help them find the holes in their knowledge? And I really do appreciate and like this question, because again, the longer we've been beekeepers, the less we know about bees, I think.

**Jamie 44:28**

So, Amy, I love this question too. And I really have a strong opinion about it. So I'm going to say that beekeeping is one of those things that you can know the basics and go out and manage bees and for the most part, do okay, but there are a lot of beekeepers, myself included, who are just hungry for more knowledge about bees and need to make themselves aware of things that are happening now. New

discoveries, new treatments, new feed supplements, whatever, and there are really only a few ways you can do that. And I love the way this question was posed. If you don't know what you don't know, how can you find out what you don't know?

**Amy 45:11**

Exactly.

**Jamie 45:11**

So there are a couple things that are really important to me in this regard. Number one, I think every beekeeper should be a member of a beekeeping club. For example, I think you should be a member of a local club. Now, local clubs come with the benefit that you're around other beekeepers who know a lot, but you might hit a critical mass of knowledge, even hanging around your local club. But local clubs can do things like invite out-of-town speakers, other beekeepers, and scientists from the state or region, wherever you are. So they have access to some resources that maybe you can't get on your own. But similarly, moving up the chain, state or provincial clubs or regional clubs, and then national clubs, and there are even international clubs. Amy, one of the ways that I get the newest information about bees and beekeeping most is by attending international meetings on bees. And that includes things like Apimondia or Eurbee or the American Association of Professional Apiculturists meeting, things like that. Going to meetings where internationally or nationally noticed speakers are is a really great way to expand your bee horizon. That's honestly the way that I learned most is when I go see other speakers speak. Number two, you must subscribe to your nations' beekeeping periodicals. Here in the US, that's the American Bee Journal, Bee Culture, ones like that, because there are authors in those magazines who are writing about the current things related to bees, beekeeping, and bee science. So that is a great way to stay on top of that. Number three, I will say and I believe this wholeheartedly, one of the things that has happened over the last couple of years is a huge migration to online education. We say we're seminared out, right? But I'm telling you through Zoom and other platforms like that we now have access to bee speakers who we never could access before. I know that there are lots of online seminars. For example, here in the southeastern US, Auburn University runs one stay-at-home beekeeping series and they have a different bee scientist speaking from all over the southeastern United States once a month so people can sign up. Hundreds of people go and watch that. It's absolutely free. I know a lot of other clubs around the world do something similar when they're bringing in speakers online. Over the last two years, I've been speaking online to clubs as small as 30 people, but as many as 700 people will attend. So finding these online real-time classes is a great way to expand your horizons. Now, the last thing that I'll mention, taking advantage of things like online learning or more broader learning, technological learning, things like listening to podcasts like ours. Obviously, I'm biased. I love our podcast. But it's a great way to do things. But I know this individual was talking about quizzes and tests and things like that. It's hard for me not to talk about the one we do. We have an online master beekeeping program. Maybe I'm biased, but I think it's probably the most thorough program in the world. We have over 200 lectures on bees and beekeeping in our program. And it's a structured four-tier system. There are tests, examinations, there are requirements, and expectations as you move through the program. And it is an incredibly rigorous program that teaches you a lot about bees and beekeeping and help fills in a lot of gaps. But there are a lot of similar programs offered by other institutions that can help you know more, but there's so much to take

advantage of today. Doing things like podcasts, and online learning all this stuff really puts the world of bee scientists and beekeepers at your fingertips. And all of this helps you discover what you don't know.

**Amy 48:59**

You know what? There's one thing that you missed that I was thinking about as you were responding to this, and that was teaching kids. Sometimes kids, Jamie, sometimes kids will ask questions, and I'm like, I didn't even think of that question. I don't even know why, like someone asked me, "What is a honey bee? And why are honey bees black and yellow? Where's the king bee?" I mean, there are so many questions that kids have asked me that I'm like, "That's a great question. I need to go figure out what the answer is to that."

**Jamie 49:29**

Amy, brilliant comment. I mean, today's podcast episode's a very good example of that. Two questions ago, you were asking me about the effects of queen cell size and caging because someone asked that. I had no clue. So I had to go read this manuscript myself to be able to answer it. So getting asked questions, I answer questions on this podcast, I answer questions for the American Bee Journal, I answer questions everywhere I go. And that has made me more knowledgeable because it's forced me to try to find answers to questions I didn't know. So that's a really good point. Good point.

**Serra Sowers 50:04**

Thank you for listening to Two Bees in a Podcast. For more information and resources on today's episode, check out the Honey Bee Research Lab website at [UFhoneybee.com](http://UFhoneybee.com). If you have questions you want answered on air, email them to us at [honeybee@ifas.ufl.edu](mailto:honeybee@ifas.ufl.edu) or message us on social media at UF honey bee lab on Instagram, Facebook and Twitter. This episode was hosted by Jamie Ellis and Amy Vu. This podcast is produced and edited by Amy Vu and Serra Sowers. Thanks for listening and see you next week.