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SPEAKERS

Jamie, Guest 2, Guest, Serra Sowers, Amy, Stump The Chump

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. Hello, everyone, and welcome to another segment of Two Bees in a Podcast. We've got a really great topic to discuss today, it's how honey bee queen health is impacted, or potentially impacted, by contact exposure to pesticides commonly found in beeswax. This is actually a new paper that came out. We'll make sure and link the paper in our show notes so that you can find it. We're joined by two of the authors on this manuscript, the first of those being Dr. Allison McAfee, who's a postdoctoral research scholar who has a dual appointment both in the Department of Applied Ecology at NC State University, as well as the Department of Biochemistry and Molecular Biology from the University of British Columbia. We're also joined by David Tarpy, who's a Professor of Applied Ecology at the NC State Apiculture Program, also in the Department of Applied Ecology at NC State University in North Carolina. Allison, David, thank you so much for joining us on this episode of Two Bees in a Podcast.

Guest 01:46

Thanks for having us!

Jamie 01:48

Yeah, so you guys co-published this manuscript. It's going to be very interesting to the beekeepers who are listening to this podcast here around the world. I want to ask you guys if you could tell us a little bit about the background of this project. How did you come up with the idea, what led to the research, and a little bit about the setting? And what got you to publishing this manuscript in the first place?

Guest 2 02:09

Yeah, so I think that I think I'll take this question, because it really was a follow-up study to a bigger project that was funded by project Apis M. And the goal of that project was to come up with a way of developing a molecular test to diagnose different forms of queen stress. And the ones we were looking at were cold exposure, heat exposure, and pesticide exposure. And in this previous research we did, we exposed queens to these different stressors. And then what I did was look at the proteins that changed in abundance in their spermathecae. What I was looking for was sort of like a molecular fingerprint that was specific to each, where the response was specific to each of the stressors. So we found a couple of proteins that were unique to each form of stress. And then the next question was, can we find some kind of marker or indicator that we can measure in, say, the queen's progeny, so without destroying her but sampling some of her eggs, or maybe even adult workers, that can also indicate queen stress. So that's why we're doing this field trial was to, okay, now we've done these sort of shorter term experiments where we stress the queens in the lab and then look at proteins that change in her tissues and found some things that changed. But now what happens when you stress the queen, and actually put her back in the colony? Also, that yields the opportunity to measure any changes in her performance. So like her egg-laying pattern, for example, or maybe her weight would change over time if the workers treat her differently, or something like that. So it was like doing a deeper dive into the pesticide exposure tests that we had done previously in the lab. And also, in that sense, doing a deeper dive into the components of that pesticide exposure itself because we were mainly using this cocktail of different pesticides that was a mixture of quite a few different things that were found that are commonly found in wax. And so some of the work in this paper was actually like dividing up that cocktail into its components and looking for short-term effects of each one of those components to see if there was one that was causing, say, the majority of the response. We ended up not finding a response so that's the answer to that question. But that was the motivation going into it.

Amy 05:18

So that was actually my next question for you was about the methods. So let's dive a little bit more into the methods used for your study. And I know that in the paper you were talking about acute exposure. And so just to let our listeners know, can you explain what that actually means within your methods?

Guest 2 05:36

Yeah, so acute exposure is really just a short-term exposure. You usually hear it contrasted with chronic exposure, which is a long-term exposure. So let's try to think of a common example. I guess, yeah, maybe I don't have one on the top of my head. So maybe we'll come back to the example. But acute exposure is short-term, and chronic is long-term. So something that happens quick, like dispensing a pesticide onto a queen's body would be acute, and then chronic would be like if that exposure was actually occurring over the timeframe of, say, days or weeks.

Amy 06:22

So let's say like on a farm, if there's someone applying a pesticide, they're applying it maybe once versus once every other day, or once every week. So would that be kind of an example of acute versus chronic exposure? Or did I totally get that wrong?

Guest 2 06:41

It could be, but maybe a different way of thinking of it is like how long something hangs around is also part of it. So if, say, a farmer was to apply a pesticide, and then that pesticide can persist in the environment for a long time, then that could still be a chronic exposure. So there's also that to think about is not only the application but then also how quickly does that pesticide disappear or break down after it's out there?

Jamie 07:19

Well, let's zero in specifically to the method that you used in your study. You wanted to expose queens to pesticides, and then follow various parameters. So how did you expose the queens to pesticides in your research study?

Guest 2 07:31

Yeah, so this exposure method was a topical exposure, and that just means that we are literally dispensing a very small drop of this solution containing the pesticides onto the queen's body, and we chose to dispense it onto her thorax, just to kind of match up with a lot of other toxicology research that's done out there. For insects, as far as I understand, that exposure to the thorax is kind of like the standard method for topical exposures.

Jamie 08:09

I'm curious, though, as well, with regard to not only the exposure scenario, but how did you choose which concentrations to use or which doses, I guess, in this particular case?

Guest 2 08:19

Yeah. So it's always hard to choose a dose when you're doing pesticide research because you want to try to make things as realistic as possible. But in a lot of cases, like this one, knowing exactly what the doses that the queens are exposed to is very difficult to gauge, because, well, I'll just run you through our line of thinking here. So we know from Kirsten Traynor's really awesome paper, documenting the levels of pesticide residues in different hive components, there's a pretty wide range of what is out there in commercial bee colonies. And what we did was look at that data and take the median amounts that were found in wax. The reason for that is because wax is probably the hive component containing pesticides that queens are most in contact with. It obviously is not the only thing in the hive that has pesticides in it, but if you're thinking about contact exposure, that's, I think, really going to be what would yield that. So we took the median, actually, hazard quotient, which is now a whole new can of worms, and maybe we can come back to what that is and how it's calculated later. But we took the median hazard quotient of, actually, the wax from colonies where there was known to have been a queen event. And this is all data from the Traynor paper I was talking about. So that seems to be a good estimate of the level of exposure that might be sufficient to cause problems with queens. And

then, there are all kinds of other questions you can have from that because this is the median concentrations found in wax of colonies that had queen events, but then how much of those compounds is actually being, say, absorbed by the queen as she's going about her business laying eggs and walking on that wax and whatnot? So that is a big open question. That level of exposure does not directly translate to what we did, which was apply a two-microliter dose of the solution containing pesticides at that same concentration directly to her body. There are so many unknowns. Firstly, you don't know what the queen's actually absorbing from the wax as she's exposed to it day-to-day. And then you also don't know exactly how much of the pesticide is absorbed from that drop that we put on her. So it's really, there's a lot of guesswork in it. And it's definitely not perfect, but we just kind of tried to use the data, use the information that we have as best we could to try to create a realistic dose.

Guest 11:47

Yeah, and if I can jump in here real quick, Alli, you had said a couple of things about why the pesticides in wax being tested on queens. The other co-first author of this paper is Joe Milone, who was a former PhD student in our program, and you guys have done a lot of very similar work. And so this is a great example of where both of your paradigms kind of came together and made a great collaborative effort. So he was funded through a grant through the Foundation for Food and Agriculture Research. And he was looking at following up on that Traynor et al. paper that you just referred to, looking at the exposed zone that queens are exposed to inside the colony. And he was looking at the two different routes, the wax, as well as the food. They're always fed royal jelly by the nurse bees. And what his other papers had shown, not really alluded to so much in this particular paper, but the exposure, the potential exposure route through food is very minimal because the worker bees, the nurse bees that are feeding the queen royal jelly, are kind of filtering out the vast majority of any pesticides that might be coming in from the pollen, which by the way, is a completely different kind of cocktail than the pesticides that tend to be found in wax, according to that Traynor paper. And so looking at just the pesticides in the wax made a lot more sense because it doesn't seem that queens are actually exposed to pesticides in food as much as we might assume. So that gives another kind of context of why this particular set of pesticides were explored in this paper because there isn't much of a direct exposure to queens through the food route.

Jamie 13:58

I will say too, just listening to you guys talk about this, the wax matrix is very interesting because a lot of pesticide work, they're looking at pollen as an exposure route and nectar as an exposure route. And I've struggled with wax. We've got an assay that we'll be submitting soon for testing chronic exposure and acute exposure of pesticides to adult worker bees through wax. But this wax thing is fascinating to me, specifically because if a compound is found in wax at high levels, it's lipophilic. So, for the listeners out there, lipo is wax, philic is loving, so wax-loving. So I wonder, for the biochemists out there, is there a reason to believe a lipophilic compound would leave wax to move to something that is not wax and so, to me, a lot of people will point at wax residues and say, "Well look how bad the residues potentially are in colonies," but it may be, in fact, the other way around, the fact that it's trapped in wax and not accessible to the bees in the hive. So there's a lot of biochemical work that will have to definitely be done in the future to look at the pesticide movement into wax, and certainly, maybe even more

importantly, out of wax to see what levels we could expect to move to bees in the first place if it's trapped in wax. So it's very interesting to see that you guys are trying to tackle this head-on, given that not much work is done from the wax perspective. People look at residues in wax but don't often treat bees with residues found in wax.

Guest 2 15:30

Yeah, that's a really good point, Jamie. And, yeah, there's really not very much known about how these compounds might exit wax, and then kind of make their way into the bee. One kind of interesting thing to think about, though, is that honey bees do have these cuticular hydrocarbons that are also kind of like fatty molecules that are coating their body. So that coating the outside of their body, so those are involved in things like nestmate recognition because the bees can smell these different compounds on each other. I don't know if there are enough of them to kind of promote that transfer from wax to the bee. But that's maybe one potential mechanism.

Jamie 16:21

Yeah, it's interesting you mentioned that, I thought about that as well. And I was thinking bees are kind of covered in these cuticular hydrocarbons, this like, thin wax layer, are they providing a route of exposure or accumulation of pesticides for lipophilic pesticides? It's just hard to know. And I'll tell you, I can't wait to see folks tackle those questions. And these are great questions. And wax exposure is so interesting because wax is just there. The food cycle's in and out of the hive, but the wax is there. And so it's really neat to see you guys take advantage of that.

Amy 16:54

Yeah, I think it took me a couple of years after I became a beekeeper to even know that there are pesticides in wax. I think a lot of, especially the beginner beekeepers that are listening into the podcast, there are lots of places that hold pesticides. And so it's always interesting because when people feel like they have a pesticide kill, they'll immediately collect bees. But now, there are so many other ways to look at pesticides and the different components of the colony. So I think that's super interesting.

Guest 2 17:26

Back to the original question, too, of what concentrations did we test, I was speaking about how we chose the treatment for the field trial. But there was also the other part of this paper, which was the exposures that were done in the lab. And in those experiments, a whole range of different doses were tested all the way from the, well, from no dose, which was the control, of course, all the way up to 32 times the median concentration found in wax. And this was for, again, the short-term laboratory exposures where this was actually mainly Brad and Joe in David's group did this work over in North Carolina. And then after two days, measured the sperm viability and a whole suite of other standard queen quality metrics after exposure to that range of doses, for, I think it was, I don't remember actually how many of the compounds they tested, but for all the major components of this pesticide cocktail. Well, I think it's important maybe to say that 48 hours was the time that we chose because in my previous research, I found that after 48 hours there was this elevation in certain subsets of proteins. So that implies that it was long enough for the queens to have some kind of molecular response to the

stress. And also from the research on temperature stress we've done, we know that two days or 48 hours post-stress is long enough to see that reduction in sperm viability. It could be that it was too short for a pesticide exposure but those were kind of the reasons why we chose that timeframe.

Amy 19:32

Yeah, so something that Jamie did not mention when he was introducing the title was your title actually says, "Honey Bee Queen Health is Unaffected by Contact Exposure", and so you've already mentioned this earlier on that there really wasn't anything found as far as any effects on the queen. And so can you just kind of go through a little bit more on what you were examining during your methods and and because your results say it's unaffected, what were you looking at?

Guest 20:03

Yeah, thank you for bringing that up because there's always the case where you can measure a bunch of things and find no effect among the things that you measured. But there are always limits to what you can measure. So then you start wondering if there were things you missed that might have been important. So I'm happy to clarify what exactly it was we were measuring. So in the field trial, the field part of the study, we applied this pesticide cocktail to the queen, like we were talking about before, and then put her back into her colony and we measured her laying pattern. So her egg-laying pattern, we measured her mass, and at the end of the experiment, we measured the proteins within her spermatheca because that was the tissue that I had used in my previous research to identify these markers of queen stress. And so I was looking for the same markers that I saw before. And we also measured the mass of the emerging workers and measured the proteins that were present in the eggs that the queen laid. So that was the idea behind that was to try to look for transgenerational effects of the queen's stress having some kind of impact on the proteins in her eggs because it would be really convenient if there was some kind of marker or indicator we could measure in the eggs without killing the queen. And I should say also, before applying these stress treatments to the queen, before applying the pesticides to the queen, we measured her laying rate before and after and her mass before and after. And obviously, you can't measure proteins or spermatheca before or after because that will kill her. But the idea there was to try to get like the queen's baseline performance rate, and then measure a change in that because, as probably everybody who's worked with bees knows, there's a lot of variation from queen to queen and hive to hive.

Jamie 22:31

Like Amy said, in the title of your manuscript it says queen health is unaffected. So I would ask, there were no impacts of exposure on any of these parameters that you measured?

Guest 22:42

Yeah, we didn't find any significant impact. And I actually missed one parameter that that in that list of ones that we measured. So we also measured sperm viability, and that was part of the field trial, as well as the lab work. So at the end of the lab experiment, where queens were exposed to a range of different doses of each of the components of the cocktail, as well as the cocktail together, there was no impact on sperm viability after a two-day recovery period. And there was no impact on the queen's

mass in that experiment, either. Of course, there are more things that we could measure, and you can tweak things, try different concentrations, try a longer exposure duration, or, let's say, I had something on the tip of my tongue a different scenario, oh, yeah, giving them a longer time to respond to that pesticide treatment, for example. But since everything we had measured so far really didn't yield any significant changes, we kind of chose to stop pursuing that aspect of it, which is also relevant, this work that Dave was talking about earlier about the exposures that Joe Milone was doing. It was pretty clear from that work that actually chronic exposure via feeding colonies pollen was actually having some pretty major impacts on queen health there. I think that the message from our work is not that pesticides don't matter for queens or queen quality. That's definitely not what I want to portray. It's that this particular exposure route under these conditions that we tested really didn't have an impact but there are other exposure routes that definitely do. That's a critical point, Alli, because I think a lot of people, and Jamie was alluding to this, a lot of people, they say, "Oh, we find these pesticides in the wax. We also noticed that a lot of our queens are becoming drone layers or becoming sterile, and their sperm viability goes way down. And so the queens don't live as long, as we're having all these problems." And they equate the two. They're saying, "Well, exposure to pesticides in the wax is sterilizing our queens. And that's the reason." And so the fact that we treated the snot out of these queens with huge levels of these pesticides that are in the wax, but it's not affecting the sperm viability, at least in the near term, suggests that the queens are quite resilient to these stressors. It might have effects on them in other ways. But it's probably not the simple connecting of the dots of pesticides in the wax sterilizing our queens. There's something else going on that's more complex here. So that's exactly right, Alli, we shouldn't have the takeaway that pesticides are benign to queens. That's certainly not it. But this immediate or direct effect of the pesticides, at least in the wax, may not have such profound and obvious effects on the queen's reproductive capacity.

Jamie 26:27

So I think this is really interesting. So my team and I published pesticide research before, and Allison, just like what you said, I mean, you sounded like a scientist when you said it, all we found here is consistent with what we did under the experimental paradigm that we used, that the doses we used, etc. But every time I talk to beekeepers about pesticides, they often will say, "Okay, that's what you found. But what about all these other exposure scenarios?" For example, you guys focused very closely on acute impacts. Queens get exposed to an acute dose, boom, it hits them, they're given just a recovery period, and they're put straight back into hives. And a lot of what you measure, for example, sperm viability was over a very short period of time. So anytime, I would see a title like yours, "Queen Health is Unaffected by Contact Exposure," on and on and on, it's all the what ifs. "Well, what if they're living every day in contact with this pesticide? What if it affects their this gland or that gland or this organ, or that organ, and maybe the acute exposure that we're used to with pesticides isn't simply giving the pesticide enough time to do its thing? Really, queens are getting exposed to these chronic levels over time, and it's these things that worry us as beekeepers. Downstream, maybe they don't burn out today, but maybe six months from now they burn out." And so you both have really touched on this quite a bit, this idea that you're looking at acute exposure, and what you found is what you found for what you did, and it doesn't necessarily give pesticides a free pass from the chronic perspective. So I'm just curious, maybe I'm jumping ahead here, but what are some projects that you have planned for

chronic, or do you have planned any projects from a chronic perspective? Maybe what you found with acute is enough based on, Allison, just what you said a moment ago. So what is your future in that regard?

Guest 2 28:28

Well, since my motivation for doing all this was to try to come up with a way to diagnose queen stress, and then, I found from this work that those markers that I had identified before as being related to pesticide stress, in the first experiments I did, they were upregulated in queens after they'd been exposed after a short-term exposure. And in this experiment, which gave, in the field, the queens a longer time to recover from the treatment, I found that those same proteins were no longer upregulated in the exposed queens. So for me, since my kind of guiding motivation was to come up with this protein fingerprint or diagnostic test I could do on queens, then that's kind of a dead end for me because I find that under a more realistic timeframe, and under field conditions, that these protein markers are not effective. So, I actually don't currently have plans to pursue some different experiments with chronic exposure or different timeframes or that kind of thing. But I think that the exposure routes to chase probably are going to be the pollen exposures because, like what Joe found in his experiments, it really had some serious impacts on the colonies' ability to rear queens. So they found that when colonies were exposed to pesticides via contaminated pollen patties over a long period of time, their ability to rear new queens, like the viability of those queens, this is viability of the queens, not of their sperm, was lower compared to the control colonies. And he also found that the workers, they're the ones eating this pollen and producing the royal jelly that's fed to developing queens, he also found that the composition of the royal jelly was different when the colonies were fed these pesticide pollen patties. So I think to me, like looking at the big picture of all of this data together, sure, maybe there are longer term effects of chronic exposure to like, chronic contact exposure to these pesticides and wax, but it's pretty clear from the work that's already done that this pollen exposure is having some serious effects. So, to me, I would think that would be maybe the better scenario or better exposure route to focus on.

Guest 31:07

And again, to clarify, there were those effects on royal jelly, but the pesticides were not in the royal jelly, right? So that's why I think it's really important to not assume that they're the direct effects, that there can be indirect effects. So pesticides can be coming in on pollen, the nurse bees are consuming that, as they're synthesizing the royal jelly, they're kind of filtering out a lot of those pesticides as they're feeding the queens. But because they themselves are affected by the pesticides, the quantity and the quality of that royal jelly is not as good, which does have an impact on the queen, right? So queens are being impacted, but it's not from the direct exposure or secondary exposure of the pesticides themselves. It's because it's affecting the entire system of the colony. And the queens are really the recipient of it, rather than the main target or the main individual that's being influenced. So we've got to look at it from the entire system and all of the different pathways, not just the obvious direct one because that's not always the case.

Amy 32:58

I have so many thoughts and I have so many questions. As you're speaking I'm seeing an infographic in my mind of the exposure routes of where that could go throughout the colony, I think that would be really something fun to work on. But it's just amazing to me that really the workers act as like the liver. They're like the filter to make sure that the queen is as healthy and as great as she can be, although they're having their own impacts. I mean, I'll just leave it at that. But really, it's just how fun is honey bee research? There are just so many factors that go into it and just so many things to take into consideration, so I'll just stop there. But back to what Jamie was asking, we do have a lot of different seminars that we do with beekeepers. I'm sure you all have your extension talks with beekeepers too. Beekeepers are always wondering, okay, so what? So what does this mean for me? And so the last question I have for you all, is what are some take-home messages you want to share with beekeepers?

Guest 34:03

Well, I can jump in, if you wish. One is kind of what we were talking about before. Just because we're looking at direct acute exposure to individual queens and not finding profound, immediate effects doesn't mean that pesticides are benign to queens, right? So we need to keep that in mind that they're like, as Jamie was saying, doing some more chronic exposure, looking at these things over a longer period of time is really what needs to be done. As a result, it's still probably a good idea for you to cycle out your combs more than you do. It's almost like you can't do it enough. But as beekeepers, we tend to be pretty bad about cycling out our combs, not just for pesticides, but for pathogens and other things that can be harbored and sequestered in the combs. So doing that fairly frequently is always good advice. The other is to not just jump to conclusions. Just by finding a given compound in a given hive matrix doesn't necessarily always mean that's the cause of any problem that you can ascribe. This is a very complex system. And so we need to not have that knee-jerk reaction of finding something and immediately blaming everything that's gone wrong on that one thing. I think we need to take this holistic approach of looking at not just one pesticide at a time or kind of one pathogen at a time, but everything in the aggregate to try to understand what's going on with our colonies, especially when it comes to the queens. It's a focus of what we do because a lot of people are studying the effects on colonies and therefore, workers, but the queen and especially the drones tend to be largely ignored when it comes to looking at bee health. And so trying to understand this very complex system as it applies to the reproductives can really help our overall understanding of the different stressors that affect colonies so that we might be able to mitigate them.

Guest 2 36:25

Very well said, David. Thank you for jumping in. I think the point about not jumping to conclusions is a really good one. The only thing, maybe, that I'll add is this work is really just a small piece of a much bigger project that is aimed at really trying to figure out what can cause queen quality to decline the way that a lot of people are seeing. Just talking to beekeepers, so many of them say that queens aren't as good as they used to be, or they don't last as long as they used to. This is a really, really complicated problem. And there are endless things you can think of that could be contributing to it. So what we try to do in our work is really pick those apart, like pick one issue that we think queens could be having, and really dive into it, like this work on topical exposure to pesticides. Because, surprisingly, that was actually a gap that needed to be filled. There's not very much work on topical exposure to queens. The

things that we measured and the data that we gathered show that for these methods there was no effect. But there are all kinds of other things that queens are dealing with, like these extreme temperatures, or like viral infections. We're sort of looking at this in the bigger picture of these different forms of stress that queens could be having. So there are lots and lots of other things yet to be investigated among those as well.

Jamie 38:18

Allison, David, thank you so much. I really appreciate you joining us and discussing the results from your research.

Guest 38:24

Thanks for having us, guys.

Guest 2 38:26

Yeah, thank you.

Jamie 38:27

Everyone, that was Dr. Allison McAfee, who's a postdoctoral research scholar in the Department of Applied Ecology at NC State University with a second appointment in the Department of Biochemistry and Molecular Biology at the University of British Columbia, and Dr. David Tarpy. He's a Professor of Applied Ecology at NC State University where he manages the NC State Apiculture program in the Department of Applied Ecology.

Amy 39:12

Well, that was really cool. I always really like talking to Dr. Tarpy and Dr. McAfee. I feel like they're so knowledgeable in honey bee queens in general. And they just have so much knowledge about queens. So I'm happy they were both together on the podcast today.

Jamie 39:28

Yeah, David has built a big program at NC State. And Allison was able to be a postdoc there and also has that dual affiliation. And of course, David, one of the things he's known for is just queen research. We had him on before. Talking about his queen diagnostic clinic that he's got there at NC State. And that's also really neat. So it's really cool that he's working on behalf of the beekeepers, especially the queen producers and Allison was able to make the contributions that she's made to that research as well.

Amy 39:40

Yeah, I know. Right. Yeah. So I mean, there are a couple of things that are just in my mind about that episode. They were talking about the exposure to queens. And I totally understand the oral exposure from feeding through pollen. I think I'm a little bit more confused about the beeswax. I mean, so a queen is like stepping over beeswax and let's say that the wax has pesticides in it. I don't understand

how the exposure could have come in. And so do you know what they were kind of talking about with that?

Jamie 40:23

Yeah, Amy. So that's like the million-dollar question. I alluded during the interview to the fact that Dr. Jack and I had thought years and years ago, this idea that yeah, bees will eat pesticide-laden pollen or pesticide-laden honey but they're also living in and on a matrix that itself has really some of the highest residues recorded. Many of the compounds that are out there are lipophilic, as I said, wax loving, which means you get really-

Amy 40:49

They attach on to it, right?

Jamie 40:50

Yeah, they just get right into it. So this idea is that yeah, they're consuming pollen and nectar or honey, but they're touching wax. So just think about it from the queen. If she's living, let's just arbitrarily say two years, and she's touching it every day. And every day, she sticks her abdomen into a cylindrical wax cell and her abdomen is touching it. So they were essentially asking the question, if there was a residue level that's high enough in wax to produce an acute exposure, Amy, that gets back to that acute versus chronic. Acute exposure is a high dose that gives a pretty immediate effect, and by immediate, usually within 48 hours. If you look in the books of the old days about bees dying from pesticides, it was always acute exposure. Bees go out in the field, they get a super high dose, they come back, everybody eats from that pollen or nectar, whatever, and just all the bees die.

Amy 41:49

Yeah.

Jamie 41:49

So they were asking, is there a possibility of queens being exposed to pesticides in wax acutely that would produce any of the number of parameters that they were investigating?

Amy 42:05

Yeah, so it's like, with humans, we always kind of try to think like, okay, like, how would I make sense of this? So let's say like lead in paints, would that be kind of an example of something similar, I guess, to just the exposure?

Jamie 42:19

Yeah. So the way that I would explain it is, let's say that while you were painting with lead-based paint, the whole bucket just falls on you.

Amy 42:27

Right.

Jamie 42:28

That's an acute exposure. Now, let's just say you're living in a house that was painted with lead-based paint, that's a chronic exposure, a small amount, over an extended period of time that you might see an effect, whereas the bucket falling on you and fully covering your body is an acute exposure.

Amy 42:46

Right.

Jamie 42:47

-to a lot of it at one time. And so they were looking at it more from the bucket falls on your whole body perspective, rather than the, "I'm living in the house that's been painted with lead paint," what might have happened to me six months from now, eight months from now, a year, two years from now, which is why I think, when Allison was talking about this idea of, yeah, we were wanting potentially to consider chronic impacts but other folks who were looking at pesticide exposures with queens, they kept citing this other paper, they were seeing more of an impact through pollen exposure. So her argument is, if we're not seeing it, necessarily, through an acute exposure that would mimic what you see in wax, we might default back to pollen exposure since that may be a more relevant exposure scenario for queens than, let's say, a topical exposure through wax.

Amy 43:35

Yeah, for sure. The other thing that I really enjoyed about it is I feel like is their research projects are very applicable to beekeepers. Something that Alli had mentioned was that beekeepers say that their queens just haven't been as good as they used to be. Quite honestly, I hear that pretty often. I mean, people say that all the time. And I'm hearing that more and more these days, or that they're having either queen issues or queen quality problems. Have you heard that? What are your thoughts on whether queens are worse or better or the same as they used to be, quote, unquote, back in the day?

Jamie 44:13

it's definitely something I hear beekeepers talk about all the time. And even if you look at the Bee Informed Partnership surveys from beekeepers asking beekeepers what's the principal stressor on their honey bee colonies, every year queen quality is in the top five, right? So beekeepers recognize it. They talk about it. You and I've had these conversations before, off the air, about my beliefs about this whole, "as good as it used to be" or "as bad as it used to be." What I would argue is that we don't really know what queen quality used to be.

Amy 44:45

Sure.

Jamie 44:45

People didn't measure queen longevity as much as it's being measured today or people didn't look at queen quality as much as it's being looked at today. So it's really hard to compare today to what was

yesterday, and 30 years from now, people are going to be able to make those comparisons because people are collecting a lot of data now to which 30 years from now can be compared. So maybe queen quality is worse than it's ever been. Maybe it's exactly what it's always been. But there are still things that we need to address. And that may be the perspective that Allison and David were taking, "Hey, even if it's the same or worse, they're still clearly things that impact queen quality. And when queen quality is impacted, we know that could have downstream effects on the entire colony." I think there's a lot of value in that type of research. And it's neat that they were doing it the way that they were doing it.

Stump The Chump 45:37

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

Amy 45:48

So in this question and answer time, actually in the last question and answer segment that we had were three questions about swarms and queens. And so the next three questions that we have for today, Jamie, are also regarding queens and requeening and splits. So this is basically just a continuation of what we did last week.

Jamie 46:10

Yep, I love these topics. They're really management centric, and I look forward to discussing them.

Amy 46:14

Alright, so for the first question that we have, this person is wondering, so they have a split, they're wanting to requeen a split from queen cells from another hive. So I guess what I'm imagining is there are two different hives, two different colonies, and so they're wanting to introduce queen cells. Is this something that people do?

Jamie 46:37

Absolutely. So I think in this question, this individual has a strong colony, they made a split, but that strong colony itself didn't have queen cells. And so with that split, let's just say they split it into a nuc for the purposes of our discussion, can they re-queen that nuc, that split using queen cells from another hive? You absolutely can. In fact, that's one of the first things I learned as a beekeeper years and years ago when I first started keeping bees. I'll even double, in fact that. In fact, on top of that, I do it all the time. And this is kind of my strategy: Imagine having 10 colonies in your apiary, and let's say that you've got a strong production colony and you make a split. And let's just call it colony number one for the sake of argument. Let's say that colony number seven is your best colony in the apiary, the bees are nice, they're productive, they're strong, they're pest-resistant, etc. So you wanted to make a split from colony one, maybe it was strong so you had enough bees to do it. But you don't necessarily want to produce a new queen from that queen's offspring. Well, you can move over queen cells from colony number seven into that split and allow the bees in that split to make queens from those queen cells rather than from ones that it would have produced from its own brood cells. You don't even have to move over queen cells, for that matter, you can just move over frames of eggs or incredibly young larvae from one colony into your split so that they can begin making queen cells off of that frame as

well. But I think in this particular case, the beekeeper is wanting to be able to move in established queen cells to take a week or two off the timeline. And you absolutely can do that. It usually works best if you don't move over the bees with them. So for example, if you're going into colony B to remove a frame of queen cells and put it in colony A, you'll want to remove the bees from that frame. You don't want to shake that frame too hard because that can damage the contents of the queen cells. You might just slightly brush the bees off, but then you can move that frame over with cells and all into your split into a queenless colony. Really however you want to deal with it.

Amy 48:45

Isn't that a common practice anyway? I mean, you can purchase mated queens, you can purchase virgin queens, but you can also purchase capped queen cells, right?

Jamie 48:53

Absolutely. It's super duper common. In fact, in a lot of management in the backyard, folks do this, and I did it all the time. Basically, what I was saying is, "Look, you're strong enough for me to make a split from but I don't necessarily want to use your queen to make my next queen. Instead, I want to go to this colony because it's such a great colony." So you're basically being a breeder in that case. You're selecting. You like colony X and you're using its queen cells to requeen splits from colonies Y and Z. And that's perfectly normal. And just like you said, Amy, you can even purchase queen cells rather than mated queens, often at a cheaper price. Think about it too, the bees do this themselves, right? They make new queens this way. I think the real catch is, are you comfortable, are there any anticipated problems moving queen cells from one hive into another hive? It's usually not a problem at all and it's a very common way of dealing with requeening.

Amy 49:49

Okay, so for the second question, this person has this queenless hive. So they purchased a queen from somebody else and so they're wondering, in that queenless hive, there are queen cells in there, and so is introducing a mated queen enough for the workers to take care of the queen cells that are already there? Or does a beekeeper need to go in and remove these queen cells either before or after introducing their mated queen?

Jamie 50:17

Easy answer to this question. Always remove the queen cells first. If you listen to the way the questioner asked this question, if I put a mated queen into a colony that already contains queen cells, will that cause the bees, upon acceptance of that new queen, to just go in and get rid of the queen cells? And that certainly can happen. But you actually run a potentially greater risk the other way around, where the presence of queen cells in that colony makes it less likely that they'll accept that queen. So inevitably, what I tell folks, anytime you want to introduce a mated queen, you want to make sure there are no other queen cells in that colony because that might cause them to carry through the production of their own queen, and kill the one that you've purchased. So absolutely, always remove all the queen cells when you are introducing a mated queen, and don't even let it come to the opportunity for that new queen to emerge, and the worker bees going, "Oh, we got a queen, we can go take out

these other cells," because it almost always works the other way around. So I would prefer you take them out. And I will say too, you might need to hear that when a queen is released, sometimes in the process between you removing the cells, putting the new queen in, and then releasing her, that could take two or three days, at which point they may make new queen cells. And so sometimes after her release, I might go through and just double-check that they haven't made new queen cells. Less of a problem for them to accept her because they released her, they've accepted her, and she's in there, but more of a problem if you're in peak production season, they can be tempted to swarm, maybe, with that new queen that just emerged from the cage because there are some cells leftover. So I remove cells beforehand, before introducing, and then even once I've discovered that she's been accepted, I remove queen cells.

Amy 52:13

Cool. All right. So for the third question. So in our last Q&A segment, we talked a little bit about secondary and tertiary swarms. And so the questioner is wondering, how long does it take workers to start laying after they swarm if they're unsuccessful with requeening? So, I guess there are lots of different ways to see that. But I'm thinking about maybe a secondary swarm. And let's say that their queen goes out on a mating flight and doesn't come back. Workers, what do they do?

Jamie 52:43

Yeah, so if you're listening to this podcast, then you know that honey bees are the most amazing organisms not named humans on planet Earth. I mean, think about it this way. The worker bees are really quickly able to know that they are queenless because the queen produces pheromones, and the workers can cue into those pheromones, "Hey, we have a queen, life is good." The moment she's gone, within 12 to 24 hours, they're already in replace queen mode because they no longer smell her. Her scent is not there. They know that there's a problem and they start producing queen cells. But most workers have a biological clock, this kind of cushion that says, "We'll give this process two to three weeks. And if we don't smell new queen pheromone in two to three weeks, some of us are going to have our ovaries develop and we're going to become laying workers." And that pretty remarkable if you think about it, because in theory, just 48 hours later, there's very little indication that there was ever a queen in there in the first place. They can't smell her, she's not there, etc. So why don't workers' ovaries start developing that quickly? But it doesn't. I mean, they don't typically develop that quickly. Typically, with laying workers, it's usually, you can safely requeen colonies at two weeks, sometimes up to three weeks. But that kind of two-and-a-half to five-week window is when colonies become hopelessly queenless and some workers in that hive will become laying workers and at that point, it's difficult to address. Difficult, not impossible, but difficult to address. So it can just take somewhere in the neighborhood of two-and-a-half to five weeks. Usually, if a colony is queenless after two weeks, then I know that it's going to be a little bit more of a struggle for me to try to get a queen in there before the laying workers start.

Amy 54:27

Alright, well, there we have it. Those are our questions and answers for the day. Keep the questions coming. We're excited to have them in our email, on social media. If you give us a phone call, I cannot



promise you that Jamie and I will pick up our phones, but we do check our voicemails. You can try that way.

Serra Sowers 54:49

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. If you have questions you want answered on air, email them to us at 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.