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SPEAKERS

Guest, Stump The Chump, Amy, Jamie

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 episode of Two Bees in a Podcast. Today, we are joined by Dr. Frank Rinkevich who's a Research Entomologist at the USDA ARS Honey Bee Breeding Genetics and Physiology Laboratory that's located in Baton Rouge, Louisiana. Frank has been doing some really good work here in the US that's not only relevant to us beekeepers, but also beekeepers around the world. That work is focused on amitraz resistance in the US Varroa population. Frank, thank you so much for joining us on this episode of Two Bees in a Podcast

Guest 01:23

Jamie and Amy, thank you very much for the invitation. I'm really excited to tell everybody about the work we've been doing for the last five years now on amitraz resistance in Varroa.

Jamie 01:32

Yeah, Frank, Amy and I've had the ability to be able to contact you and hear some of your talks behind the scenes, so I know what you're going to be saying to us today is very, very important. But before we get into all of that work, our listeners just want to meet you. Could you tell us a little bit about yourself, how you ended up where you are, and how you ended up working with honey bees?

Guest 01:49

I wish I could say it was my childhood dream to work on honey bees, but I kind of just fell into it based on my research interests. So I did a master's and PhD at Cornell, and I studied insecticide resistance in house flies and fruit flies and all these other pest organisms, and I was trying to find out how better to kill them. So I used molecular biology and biochemistry techniques to find out how resistance develops in house flies, for example. And I did some population genetic studies and biochemistry. And then that led to my PhD where I did transcripts of nicotinic acetylcholine receptors and how that really affects toxicity to neonicotinoids in fruit flies. And then I did my first postdoc at Michigan State looking at neurophysiology of sodium channels. And it was really cool because we used frog eggs to express these sodium channels and we did some really interesting neurophysiology studies. And then, I finally landed a job at LSU as a postdoc to study the effects of mosquito adulticides on honey bees. So basically taking that same toolbox, but now applying them to honey bees for toxicity reasons. When I was at LSU, we did a lot of research on how products used for mosquito control may affect honey bees. And then all that work we were doing was in conjunction with the USDA lab in Baton Rouge, and when a position opened up at the lab in 2016, I interviewed and got hired as the resident toxicologist at the lab. But my research has taken a little bit more of a swing from looking at honey bee toxicology to looking at the same principles in Varroa because, as we all know, miticides are the most commonly used method for Varroa control. And so now, applying everything I know that I learned from house flies, fruit flies, sodium channels, and all these biochemistry, neurophysiology techniques and now applying them to probably one of the most important pests we have in domestic agriculture, the Varroa mite.

Amy 03:41
Yeah, absolutely Frank. It sounds like your background definitely set you up to be a great person to do work on resistance with honey bees, specifically. And as Jamie mentioned, as you mentioned, we are talking about amitraz resistance and Varroa. So this is something that beekeepers are quite familiar with. I think whenever we talk about resistance, when we talk about amitraz resistance specifically, I think a lot of the listeners are aware of amitraz resistance. We hear about it, we talk about it, but can you elaborate a little bit more on why it's important that beekeepers understand whether or not their colonies are resistant to amitraz?

Guest 04:17
it's really important because every beekeeper has Varroa mites, no matter what kind of bees you raise, or where you do beekeeping in, at least, the United States and, well, now, in the world, now that Australia has Varroa. Varroa is just a part of beekeeping and honey bees are livestock, so they're not very different from cattle or sheep or any other sort of livestock. They get pests and they get parasites and they require medical interventions, right? So if a cow or a dog gets fleas or ticks, you don't just let them figure it out and take care of themselves. No, you have to intervene with some medicines. And so that's exactly what happens here with Varroa mites and honey bees is that when infestations get high, people typically use miticides to reduce their populations. However, whether we're talking about Varroa mites, or two-spotted spider mites, or corneal worm, or cornwire worm, or any sort of agricultural pest, whenever there are these insecticides or miticides used to control them, under certain conditions where we typically see overexposure, so frequent applications and over-reliance on one material being the sole measure of control, in many cases, resistance will develop to it. And that's whether you're talking about pyrethroid insecticides, organophosphate insecticides, and even with transgenic crops that express the bacterial proteins, the resistance can develop. This is just a natural evolutionary outcome of an organism that has variation in their genetics that allows this sort of selection to find ones with the resistance mutations. So resistance is the defined ability of a group of organisms to withstand a dose of a poison, or protein in case of BT, that normally kills most of the susceptible population. And so

typically, that happens either through enhanced detoxification or target site resistance mutations. And so when we know that that's the background in which resistance operates, we can just apply those principles to Varroa. Varroa mites have a number of biological characteristics that make them highly susceptible to developing resistance, such as their breeding schemes, their genetic diversity, and so on and so forth. But also, too, so that's a little bit of the biological characteristics, but in the United States and a lot of places worldwide, there's a limited toolbox of miticides that beekeepers can use for effective Varroa control. So here in the United States, and I know this happens a lot of other places, amitraz is widely used for Varroa control. And when I say amitraz, I mean, that's the only thing most people do to keep mites under control. There's a lot of IPM measures that can be done, there are other miticides besides amitraz, but amitraz is the lion's share of miticide applications here in the United States and in some other places. With that being said, when we're only using amitraz, and using it excessively, taking your strip out, putting a strip in kind of treatment regime, when the wax accumulates amitraz, or its break down molecule DMPF -- I'm sure many people have heard of -- that kind of provides a constant selection environment, which requires the Varroa mites to have some sort of variation in their genes that allows them to survive. So what we've been seeing here over the last 20 years since resistance was first reported, we saw that amitraz was used pretty consistently, pretty widely across the United States. But about five years ago, you talk to a lot of beekeepers, and you find out what's happening in the real world. I think that's really important for bee scientists to talk to beekeepers because it's really important to find out what's happening in the real world. A lot of them kept on saying that, I use amitraz, and I treat 40 colonies in the yard, it works great on 35 of them, but these other five, the mites are still a problem, they're still really an issue. Or you hear that it doesn't work like it used to. And then, when I'm trying to find out why, I heard some interesting explanations about the bad batches, the product, or improper placement or some environmental issue. But the only thing I kept on coming back to based on my background with resistance was that these treatment failures are simply due to resistance. And so we're trying to find out how we can answer that question. So we did two different things, we tested the toxicity of just straight-up amitraz, just the technical material, as well as the ability of Apivar, the formulator registered product, to knock out mites in our field bioassay. So that way, we know, functionally, how this material might be leading to treatment failures in the field with, I call it the Apivar resistance test, and then coupling that with the amitraz resistance testing that I do in the lab with a technical grade amitraz. What we found was that whenever amitraz performed poorly in our bioassay in the field, it was always connected with a lower sensitivity to technical grade amitraz. So therefore, we know that a field bioassay with Apivar and the lab bioassays with technical grade amitraz were telling us the same information. So there was this consistent agreement. What we found in the first few years of our test, we found limited pockets of resistance, not very much, but over the past five years with help of beekeepers across the country, we're able to have a huge number of samples that we can start making some very definitive conclusions with. We've been finding that resistance potentially is increasing across the country. We're finding that the genetics of the resistance are quite well-defined and kind of simple, actually. This is all new data that just came out this year. And so what we have to do is try to find out how and why resistance evolved and manage it accordingly.

Jamie 10:13
So Frank, I think this is obviously a very important topic for beekeepers, not only here in the US, but around the world, at least beekeepers who rely on particular treatments, in our case amitraz. So I

listened to you talk about how resistance is developed in the Varroa population. You're telling us a bit about your research about how you're trying to map that resistance around the US. What are some of the long term impacts you think this will actually have for beekeepers, directly, but also on the health of honey bee colonies?

Guest 10:40
I think this has really important implications for the whole industry, whether you're a large commercial beekeeper with 10,000 colonies or someone who's just treating for colonies in your backyard. I think this is really important because the miticide market in the United States is extremely limited. We have relatively few compounds registered for use for controlling Varroa mites in honey bee colonies. Unfortunately, resistance has evolved to tau-fluvalinate, or Apistan, coumaphos, which is sold as Checkmite, and now amitraz, which is sold as Apivar, and those are the widely used synthetic miticides. But then we have formic acid, oxalic acid, thymol, and those make up a majority of what people are using. If we lose amitraz as a molecule to control Varroa, that's going to be really impactful because it really will reduce the amount of treatment options people can use. It will really limit the ability to control Varroa. We have a lot of research that shows that Varroa mites are one of the most important causes of colony losses. While many things are important, Varroa seems to be the most consistent and ubiquitous threat to colonies. So by removing amitraz from the toolbox, it really makes beekeepers scramble. I know that, especially the largest commercial beekeepers I typically work with, they can have some problems acquiring alternative miticides. I know that there were some other products that were in very limited supply the past few years because the efficacy of amitraz-based treatments was really complicating their ability to survive. I think that's been reflected in the high rates of colony losses we've been seeing. I think last year, we had phenomenally high colony losses, whether you're talking across the country. From Florida to California and every place in between, it was potentially an issue. And that's exactly what we're starting to see in the data is that whenever we find resistance in these colonies, we find that amitraz-based treatments do not control Varroa adequately. So we could tie the results of our bioassay, the resistance testing, to treatment failures at the colony level. That was some new data that we just got in this year that shows that if these mites are resistant in the test, amitraz-based treatments will not control them. So I think that could potentially be leading to higher colony losses, and also to really important implications for how beekeepers manage their colonies because a lot of it focuses around Varroa control. So I think that losing amitraz will have profound implications for long-term beekeeping miticide applications and beekeeping operations across the country.

Amy 13:19
Yeah, definitely Frank. So as you mentioned, there are not many products out there for beekeepers, and so many beekeepers do use amitraz-based products. And with everything that you've been saying, everything that you've studied, what recommendations do you have for beekeepers who use amitraz-based products? What are their options?

Guest 13:37
So what's interesting is that while we do have limited miticides, there are many other things that beekeepers can do to reduce Varroa populations. And it's something that I think we need to start thinking about earlier and earlier and earlier in the year and not just in October when Varroa populations explode. So using integrated pest management techniques, such as using brood breaks,

so that way the Varroa don't have a place to reproduce, you can take advantage of that in the springtime when making splits. Treating colonies that are broodless with miticides is typically more effective. So oxalic acid in the springtime when you're making the split and you don't have a lot of brood in the colony is a great time to knock Varroa levels low using resistance stocks of honey bees. So I know a lot of people have heard of the Pol-line honey bees that have Varroa resistant hygenic behavior that we have at the lab. We're going to work on getting that material distributed more widely over the next five years as part of our research plan. But there are also other people who are raising and doing their own selections for these Varroa resistant honey bees. Like I mentioned, I started at the lab in 2016. So I'm relatively new to the beekeeping world. Well, the whole time I've been in beekeeping, I typically use those Pol-line bees with Varroa-sensitive hygiene, and I don't treat for Varroa mites in those colonies, which is interesting because I tell people that and they look at me like I'm crazy. Like, what do you mean you don't treat Varroa? No, these Varroa resistant stocks, when they are maintained at a high level and you use rigorous selection and you have a closed mating population, they can provide very good performance in terms of keeping Varroa populations very low. So that's a little bit about the IPM measures that we could be using. So when we're using miticides, we could rotate miticides, so that way, if resistance develops amitraz, and you come in with oxalic acid, the probability of that mite being resistant to both amitraz and oxalic acid is practically zero, for all intents and purposes. We all have, whether here in the United States or even in Europe where they've used oxalic acid for a longer time, we don't see resistance developing with oxalic acid. So I think the future mite treatment regimes will need to incorporate some oxalic acid to rotate these modes of action, these different miticides, so that way, if resistance develops to one compound, we can use this effectively to others in order to manage them properly. Changing the labeled application intervals for these things are also gonna be really important. The oxalic acid label to be able to treat while honey supers are on is going to be another important option to keep Varroa populations low over the summertime because, pretty much, from when honey supers go on till they come off, none of the synthetic miticides are labeled for treatment. However, certain changes with oxalic acid could be providing some treatment control over those summer months during honey production. So I think we need to start thinking of Varroa management as less of a post-honey fall sort of issue. And I think we need to turn that clock back and start focusing on Varroa control in April when you're making your splits and introducing new queens. So throughout the whole beekeeping year, we need to focus on Varroa, as opposed to, oh, September, let's throw some strips in those colonies.

Jamie 16:47
So Frank, I want to think about some take-home messages for beekeepers who are worried about amitraz resistance in their colonies. Should they be testing for it? And if so, how and how often? What should they do if they find it?

Guest 17:01
So amitraz resistance detection is actually pretty simple. In fact, in 2020, we were shipping these kits across the country for people to do the testing on their own. So I would not be able to acquire the 1,565 samples that are in my dataset without beekeepers across the country doing these tests. If you are interested, I could send you a package that includes materials to test 10 colonies in your apiary. I send you the protocol, the materials, data sheets, but then, also, we have a video online showing the process. The first couple of samples might take a little bit of a learning curve. But once you do a few, it

gets really, really easy. There are ways that we could do the resistance testing in the field remotely by shipping kits. Well, now that we got this new grant, over the next three years, I'll be on the road a lot, typically in the Dakotas over the summer, and then wherever else we have beekeepers. Also, if you contact me, I can incorporate it into my trips. I could do it in-person out in the field. But I think we're going to start to see a big pivot towards genetic testing for resistance. As I mentioned before, we found that there were mutations in the receptor for amitraz that are associated with resistance. So this work started with Joel Gonzalez-Cabrera over at University of Valencia in Spain, and what they found was that there are these specific mutations in the receptor for amitraz. The octopomine receptor, specifically the beta2 version. And what we found is that we could really associate those with resistance United States. And what we hope to do going forward is to develop a pipeline where beekeepers can send in their Varroa, you go out anytime, you collect some Varroa with a sugar shake or your alcohol washes, however you collect your Varroa, ship them into the lab, we're going to start doing it here at the honey bee lab. And then we could tell you, based on our genetic testing, what proportion of those mites have resistant genes. What we really wanted to do, though, is turn this over to the National Ag genotyping service up in Fargo who does this as a fee-for-service so that you can guarantee a quicker turnaround time. So that way you ship them in, a couple of weeks later, you get your results, so that way we can kind of take pre-emptive measures to find out what are the levels of resistance genes in the population. So that way you could predict treatment success or potentially use something besides the amitraz if resistance levels can be high. We actually, ultimately, want to basically develop a strip testing kit where you grind up some Varroa in a little vial in the field, either stick a dipstick in or you apply it to a little test kit, kind of like a COVID test, and it'll tell you if you have resistance or not in the field. So we really want to make this accessible to beekeepers and not just something that is done in the lab for intellectual purposes. We want this to be used as a functional tool in the field by beekeepers in the real world, whether we're talking about bioassays or genotyping, there are a lot of ways that we can determine resistance levels.

Amy 20:10
Yeah, definitely. So Frank, we'll have to make sure to put your email and also your website on to our additional resources, which is located on our website. But is this service open and available to all beekeepers?

Guest 20:23
That's the thing is, like I said, now that we have Varroa mites in Australia, they're across the world. I think the better that we understand what this mite is made of, the better we could manage this pest across world, whether it's here in the United States, Canada, Europe, Australia, now New Zealand. I've been talking to people all across the world about this test. I think it's really important that we get as many samples as we possibly could. So this came out because we already have 500 individual genomes from the United States sequenced. So we said, well, if we get some Varroa mites from here, we can have a nice outgroup for comparison, or what if we got something here. The next thing you know, it's going to turn into, well, if we just did all these Varroa from across the world next year, we could probably get a very good contemporary picture of Varroa genetic diversity, potential resistance mutations, so on and so forth, from anywhere in the world. Because like I said, Varroa is a worldwide problem. So we started to talk with some people, and we got samples potentially coming in from New Zealand and Korea, in Europe, especially Western Europe. But the better we could sample across the

world, wherever we have Varroa, the better it'll provide a more complete picture on the status and trends of this pest genetically across the world. So yeah, it would be open to everybody worldwide. We could put my contact information, and we could hopefully get some samples from far and away and provide a very complete picture.

Amy 21:46
Yeah, absolutely. So what can beekeepers expect as far as a turnaround time of you getting back to them with any sort of results?

Guest 21:53
The first phase of the project where we're gonna do the worldwide genotyping, that's more for research purposes. So there's not gonna be a very quick turnaround time on that because we're gonna have to procure these mites, and shipping things across the world is a little tricky, especially when we have these pests. That's going to be more for the foundational studies about the diversity of Varroa mites. Whenever we have this fee-for-service arrangement with the National Ag genotyping lab, hopefully, we can get that funding and get that protocol worked out soon, you'll have to contact them about what they think about for turnaround time, but probably less than two weeks, I think, would be a reasonable expectation. I would love to do this from my lab. But there are so many other things we have going on that I think that it would really clog up our system and get in the way of our research. But I also want people to use this in a reasonable time, so that way, they can make real-time management decisions based on the fee-for-service, potentially, at the National Ag genotyping service. There's also another thing that we're working on too, where there are these, believe it or not, there are these kits that you could take out into the field and do some genotyping with, but they're a little expensive. And we really want to work on the protocols to get them under development. So that way, you can even do the genotyping within a few hours in the field if you have the means to purchase this equipment. Like I said, the other thing, too, is that we can also ship out these kits. So if you could spend half a day to do these bioassays in the field, it does take some time, but the results that you get are pretty, pretty valuable. So the interesting thing that we're finding with our data, though, is that amitraz resistance and Varroa tend to be similar across beekeeping operations, where Varroa mite management practices are similar. So let's say you have an operation with 10,000 colonies, right? And you're all treating Varroa mites kind of on a schedule and you know when, where, and how much they're getting treated. Resistance tends to be similar from apiary to apiary within a beekeeping operation. So the good news is you don't have to test a lot of Varroa from your operation to get a really good picture of what's going on across your operation, if Varroa are being managed the same way. Now I know that some beekeeping operations have different outcomes. So whether you're doing honey production, pollination, or making packages, those aspects of your operation may use miticides a little bit differently. In that situation where you might be making packages over here and sending bees for pollination services over there, the miticide application regimes really dictate resistance levels. If you can figure out how you're using this in your population, you might not even need to send hundreds of samples, it might just be only a few.

Jamie 22:10
Frank, given everything that you've said, what can we do to overcome this resistance issue that we are seeing?

Guest 24:47
With every resistance problem, whether we're talking about any agricultural pest, there are some really interesting resistance management programs that can minimize the impacts of resistance. So how to restore susceptibility in these systems. And that's really important because if you look at something like transgenic corn, there are some refuge strategies where they plant non-transgenic corn, so that way, they could foster a sort of refuge environment in order for susceptible alleles to survive and persist and then hopefully dilute out the resistant genes. A lot of times they have to be homozygous and they're recessive and all this kind of really interesting stuff. There are some very high level resistance management programs. With Varroa mites, this is something I'm developing and working on trying to integrate, biology, and the use of amitraz in order to overcome it. And I think the biggest thing that we can do is use rotations. So for example, early on when we were doing this research and we would identify amateurism populations, beekeepers would ask, "Well, what should I do? What miticide should I use?" And I didn't have any data on it. So I said, "I don't know. Do whatever you feel like. Probably don't use amitraz because they're probably resistant to it. And then let me know how it worked. And most importantly, document it, take notes, record the numbers." That's really important to knowing whether these treatments work. So the thing I always tell beekeepers, you want to start being a better beekeeper today? Take good notes. So when I worked with these beekeepers that had resistant populations, turns out they all use oxalic acid vapor. What was really interesting was that when we resampled those colonies the following year, in almost every situation, we found that susceptibility to amitraz was restored within one year. Now, that begs the question, well, how did susceptibility become restored after one year? I think it's a little bit about mite population dynamics. So usually in the fall, winter, late September, early October, mite populations tend to be their highest. I'm speaking very generally here. That's the time where mites become the largest. In that time, between the peak of the population in late September through the winter, mite populations crash dramatically, mostly because there's not a lot of brood. Varroa mites need brood to reproduce and maintain longevity. But when you don't have that over winter, the populations tend to decline. So hypothetically speaking, let's say you start September with 100 mites in your colony, and let's say there's some level of resistance. Just based on natural population dynamics alone due to brood patterns, let's say that population, on its own,crashes from 100, mites down to five, because it's all based on brood levels, the five hypothetical mites that might survive in this scenario, just by chance, might be amitraz susceptible. So then, that way, the following spring, when brood starts to become available in the colony, reproduction can resume, all the mites in that colony are descended from those few susceptible survivors. So I think it's population crashes, population bottlenecks in the wintertime, have this kind of founder effect the following spring, that just by chance, some of those colonies survive with susceptible mites. So using these different miticides can help foster that number of mites that decline in the winter, to get very, very, very low. So instead of five hypothetical mites arriving, maybe one or two mites might survive. So using rotations kind of assists in that founder effect, population bottleneck sort of dynamics of Varroa populations, that just by chance, the following year, they might be restored with susceptible mites. I don't think it's a genetic reversion. I don't think that the amitraz resistance mutation goes back on itself and reverts the susceptibility. While that can happen in other systems, as we found in things like the Colorado potato beetle, and even in house flies, where over time the resistance mutation reverts, we have genetic evidence to show that, but in Varroa mites in one year, that is definitely not enough time, enough generations in order for that resistance mutation to revert back to susceptibility. We don't have any evidence to support that yet. That's why I think it's mostly a population dynamics, population

bottleneck sort of dynamic that's really driving the whole system. So using miticides with different modes of action will be really important.

Amy 29:31
So Frank, you've been working on this, you mentioned for about five years now. I'm wondering what is next for you and the work that needs to be done?

Guest 29:38
Oh, there's so much more work that needs to be done. And I wish there were eight of me to get all the crazy ideas that I come up with for projects to get done. But as we know, there's only so much time and effort. But what we really want to do is to have a better understanding about the genetics of this pest. That's really important for a number of reasons. One is that if we can find out that mites across the country are all basically the same, which I think the data kind of supports that there's not a tremendous amount of diversity in mites, much like we would expect because of their sibling mating regimes, that can allow us to really have a nationwide generalized resistance management program. When you start to see these kinds of subpopulations develop, like we see with house flies, for example, where the flies in Florida are very genetically distinct from ones in, let's say, New York, for example, if we can show that Varroa in United States are all kind of the same, then that really allows those principles to apply to all populations. So that's what I think the future I'm going to be doing is in some of the genetic work in collaboration with Arian Avalos at my lab, whether we're talking about mites here in United States or across the world, that's going to be really important. The global Varroa genomics aspect that we're going to be working on is really important so that way we can track where Varroa may have been introduced from, so that way we could track potential routes of introduction, etc, and find out how this is going across the world. So what's driving the dispersal of Varroa? That's a big part of my future research. Other part of my future research is, I, along with, I think there's like 15 of us on this huge grant to study prevalence of resistance to not just amitraz but also other miticides such as coumaphos and fluvalinate to find out can we resurrect those materials for us. I think there might be an opportunity to use them in rotations for limited amounts of time. In fact, I've seen a lot of beekeepers applying strips of fluvalinate the colony this past year. We want to find out how widespread those mutations are and resistance levels. The fortunate thing with fluvalinate resistance is we have a genetic marker for pretty well-defined. So that really makes that resistance monitoring simple. But for coumaphos, we don't really have a very well-defined genetic marker for it. So that will be a challenge in itself. Another thing that we want to do with this grant is find out how we can apply oxalic acid in a more consistent manner across colonies across the entire United States because it appears that there are some regional variations in oxalic acid's efficacy. So understanding the environmental conditions that may be causing that variation is extremely important so we can provide consistent performance. Also, too, working to find new materials, because miticide agrochemical manufacturers are not developing new materials. There's just simply not enough miticides applied to make it beneficial for them to develop very new chemistry from the ground up. This is going to be a lot of looking at materials that are on the shelves that can potentially be used for Varroa mites. I know there are lots of people across the country looking at this, and there's some exciting new potential possibilities. However, that whole area of research, while it is exciting, and there's some really interesting work being done, registering miticides is expensive and it takes a long time. I think we need to start that research now and get those processes moved along in order to have more materials used. So that's kind of something that huge grant is looking at. So that's a

little bit about the miticide work we'll be doing in the future. But a large majority of my research going forward will be focused on developing and distributing stocks of honey bees with Varroa-sensitive hygenic behaviors, whether that's looking at the Pol-line bees that we have at the lab, or working with commercial queen producers to integrate that into their stock is gonna be really important. Like I said, this is all I know, are these stocks with Varroa-sensitive hygiene. For me, I always keep saying, "Why doesn't everybody use this?" And I understand that there are some reasons and hesitancy to incorporate this resistance stock. But like I said, I can tell you that we don't treat for Varroa mites at the lab with these hygenic stocks. I just went through some colonies a few weeks ago, I found zero mites on the adult bees, and zero mites in the brood. That's pretty impressive without any sort of chemical intervention. We really want to get that into beekeepers. We're going to take a top-down and a bottom-up approach by working with large-scale commercial queen producers in order to integrate those genetics into their existing stock. So that way, they could have resistance in the stock that they've developed and nurtured for many years that has the characteristics that they like. We could just introduce it to them. Then, also, working bottom up with small regional beekeeping groups. So that way, everybody in that region or area is using queens and producing drones with these genetics so it can perpetuate in the population so that way, if we start with the top-down approach and the bottom up, we can hopefully distribute those genetics in a much more widely available manner. That's part of it. But then, also, working on Varroa-sensitive hygenic genetics is going to be really important. And then, also, working with collaborations and people across the world. This research doesn't stop at our borders, it goes everywhere. Fortunately, there are a lot of people around the world working on it, and we've all been very collaborative in our research. I published a paper this year with Joel Gonzalez-Cabrera from Spain and now we're developing collaborations across the world. So that way we could work on this project collaboratively, whether it's miticide applications, resistance management techniques, or these Varroa resistant honey bee stocks, it's really going to focus a lot on not only what can we develop at the lab under scientific conditions, but how they apply to the real world so that way beekeepers could focus more on maintaining and being productive with their bees, as opposed to worrying incessantly about Varroa. I think this is a problem that we could really make a big dent on if we collaborate, share data, and speak openly about what we're finding works, what we're finding doesn't, and also producing data. As scientists, we all love data. So numbers talk. So write down what you see and document, measure as much as you can. If you're a beekeeper, whether you have two colonies in your backyard, or you're a migratory beekeeper with 10,000, better notes will make you a better beekeeper starting tomorrow. So that's a little bit about what we're going to be working on. But hey, the future changes. Who knows? If you asked me that question five years ago, I thought I'd be working on honey bee toxicology, but here we are doing Varroa. So it's a very dynamic world. And we'll just see where the beekeepers take us.

Jamie 36:16
Frank, I really want to thank you for joining us and giving us such an enlightening look at Varroa resistance to amitraz and all the exciting things that you'll be working on in the near future. This is definitely stuff that I think is going to help the beekeeping industry as well as improve the health of honey bee colonies not only here in the US, but elsewhere. So thank you so much for joining us on this episode.

Guest 36:32

Well, I really appreciate it. I look forward to talking with people about this in the future, whether it's at meetings, in the field, at your colonies. So yeah, very much interested in talking with everybody about this project going forward. And I'm always willing to listen to what's going on in the real world. You have my contact information so I look forward to chatting more.

Jamie 36:48
Thanks, Frank, and good luck with your work.

Guest 36:50
Alright, everybody, have a good day.

Amy 36:58
Jamie, it's always really interesting to speak with a specialist who's doing toxicology work, especially someone like Frank, who really is working a lot and knows a lot about resistance to chemicals. It's just really interesting, especially here in the United States, we have so few active ingredients that we can use that are labeled and legal for treating mites. I wanted to just kind of talk about that a little bit. What are your thoughts on where we are with miticides and resistance?

Jamie 37:24
So, Amy, we've talked about this before, the difficulty in controlling Varroa on honey bees. My grandfather was a dairy farmer and any particular pest that he had to deal with with his cows, there were multiple, really efficacious options for every one of those pests. And furthermore, they were trying to deal with an arthropod on a mammal, which gave them even more opportunity for new compounds that would hurt the arthropod or kill the arthropod but not kill the mammal in our situation. Everybody knows you're trying to control an arthropod on an arthropod with an arthropodicide and so it really limits the number of compounds that can be used. Frank said this a lot. Our industry is built on amitraz. Yes, I know there are other active ingredients formic acid, oxalic acid, thymol, fluvalinate, but a lot of these are so restricted in their use. Formic has a very tight temperature window that you've got to meet, but we don't meet that much of the year in Florida and that would be similar for other warm climates. And if you exceed that temperature window, then the formic can be damaging to colonies. Oxalic, as good as it is, you really need to be brutalist so that all the mites are adult bees and can get exposed to it. Fluvalinate, the active ingredient in Apistan, the reason we don't use it much is because of the same issue with amitraz. Varroa has developed a lot of resistance to it. So we're, at the moment, kind of stuck with with amitraz being the primary active ingredient. And it's just hard to find new compounds to add to that list. I know that there's labs around the US and around the world, for that matter, who are looking for that next compound, but they have that double whammy of needing to ensure that it's efficacious against Varroa at very low doses and innocuous to bees at those same doses. And that's the trick is finding something that works against Varroa but doesn't harm bees.

Amy 39:20
Yeah, I think what's also interesting is it is predictable, but kind of unpredictable about what the industry is going to do moving forward with the Varroa issue. I mean, Frank had mentioned integrated pest management, also just monitoring before and after treatment to see whether it's effective or not. He brought up just looking at the genetics and so that's another take on looking at Varroa resistance and

incorporating a Varroa plan into your beekeeping world. But you had mentioned something, and Frank had mentioned it earlier as well, you had discussed OA, and Frank also had mentioned OA, and how mites haven't really been showing resistance to that. In my mind, I'm just thinking, how long did it take for mites to show resistance to amitraz? And is that even a possibility with other active ingredients as well?

Jamie 40:08
It usually, in my experience in the honey bee world, takes you around a decade or so, or a little bit more, for Varroa to become resistant to the key active ingredient they're using. I saw in the case of fluvalinate, I don't have the hard numbers in front of me so this is kind of me just guessing out of the air, probably a couple of decades. And then similarly with amitraz, maybe 10 to 15 years and coumaphos was even worse. It was in between our use of fluvalinate and amitraz and Varroa seem to get resistant to it, I don't know, it seemed like it was quick, less than a decade. And so there's always these issues, potential issues with Varroa developing resistance to these compounds. I know with oxalic acid, I think we've had Dr. Cameron Jack on our podcast before talking about this, the likelihood or unlikelihood of developing resistance because it has a different or maybe slightly different mode of action. But in theory, Varroa can become resistant to any chemical, ultimately, that's used against them, especially if the chemical is the sole chemical being used and it's being used off label. You said something in your prelude to the question to me, this idea of of monitoring. We harp a lot on monitoring for Varroa populations to make a decision whether or not to treat, but what you said is key. You also need a sample after treating to make sure your treatment worked. Honestly to me, that's as important, maybe even more important than determining what population you have in order to treat is to sample afterwards to make sure it worked. Because in our own research here, when we look, a lot of the things that we think should be efficacious just aren't. We don't get the level of control, or within a month or two, Varroa populations have rebounded to pretreatment levels. And so you've got to monitor. You've got to monitor. You've got to monitor when making these treatment decisions.

Amy 42:09
Yeah, absolutely. So I would love to ask our listeners if they've listened to this podcast and now they are convinced that they need to monitor before and after treatment. I would love to hear your thoughts. I would love to hear whether this podcast has helped change that behavior or not. Let us know by sending us an email or messaging us on social media

Stump The Chump 42:38
it's everybody's favorite game show, Stump the Chump.

Amy 42:48
We are back at that question and answer time. Jamie, I feel like the podcast listeners might not know this, but it's been a while since you and I have actually done a Q&A segment together. So they won't know it but now they do. Alright, so for the first question that we have. This person emailed us and said that they have heard that beekeepers mentioned using nematodes on the soil around hives to try to target the pupation of small hive beetle larva and using it as a control method. Let's just talk generally about nematodes and the purpose of why a beekeeper would consider doing this.

Jamie 43:26
Yes, nematodes are really interesting critters. They are worm-like creatures that are really small and most of the species are microscopic. Unless you have a microscope you won't be able to see them. And nematodes, I am told by biologists who study them, are among the most abundant organisms on the planet. They're absolutely everywhere and a lot of gardeners know about nematodes because there are some nematode pests of vegetables, trees, things like that. So the good news about nematodes, because I always get this question next, well, gosh, nematodes are a problem in my garden. I don't want to put them out to control small hive beetle, they destroy my garden. But the key is that nematodes that eat plants eat plants; nematodes that eat insects, eat insects. They don't overlap. So there is a branch of research that deals with using nematodes to kill or control insects. It is called entomopathogenic nematodes, which basically means insect-eating nematodes. And the way that this works is that there are some nematode species that will go into the body of an insect, they will burrow into it, they will regurgitate a bacteria that helps digest the inside of the insect and then they will, in turn, feed on that digested part of the insect and you get nematodes going into the body, you get nematodes and their babies coming out of the body, and these nematodes can then go on to infest to other insects in the environment. This is already happening in the soil around your colonies for lots of other organisms. There are lots of species of nematodes, and they broadly fit into two branches. One of those, I kind of described as the heat-seeking missiles. So they will go through the soil looking for a host. And there are also nematode species that are good ambush predators. They sit in the soil, waiting for a host to crawl by them. When I was doing research years ago on nematodes and small hive beetle, we did screen a number of species of nematodes looking for their impact on small high beetles in the soil. And basically, my colleagues and I boiled it down to two different species, Steinernema riobrave and Heterorhabditis indica, and we found in very controlled research projects, that these two species of nematodes could almost completely control the pupating beetle. So we put these things in the soil, we would add beetle larvae to that soil, sometimes, it was just single applications of larvae. Sometimes, we put larvae on the soil every week, for I think, up to 15 or 17 weeks, we did this. We were still getting continued control. So this is not new for small hive beetle. There are nematode products out there available to control other beetles and other insects. But we found really good results. I will caution, however, and just say that all of that was in very hyper-controlled settings. There still need to be large-scale field trials to demonstrate efficacy of the beetle control. So we know that they're capable of controlling beetles. The question is, are they ready for primetime? We just don't know because no one's ever taken that next research step. We have someone here at UF who's going to look at nematodes and small hive beetles. And of course, beekeepers listening to me now might say, "Well, gosh, won't they impact bees?" But that's the key is to find something that's very effective against beetles or small hive beetles, specifically, and not effective at all or harmful at all to bees. We also are trying to find nematode species that are already native to the area so we're not introducing a different species of nematode that doesn't exist here. So long story short, in theory, they're very capable of controlling small hive beetles. We've got lots of data on that. It's just that no one's taken that final step and demonstrated at the full-scale field level yet. So it's one of those things that you can try. It's just hard to know, at this point, without lots of data backing those big field trials to know with certainty that they're going to be efficacious at kind of that economic level. So, nematodes are microscopic. I'm wondering Jamie, when you add nematodes to soil, what does that look like? Is that like a liquid, a powder? Like how do you apply nematodes to an area? That's a good question. The two ways that we did it in our research project is you can put them into water and water them into the soil. Or, in our controlled studies, our

colleague was rearing nematodes in mealworms, which are a larvae of another beetle species. We were burying the mealworm cadavers in the soil so that when the nematodes finished feeding in the mealworm, they would come out and be in the soil, so that when small hive beetle larvae came in. So from a practical standpoint, burying cadavers is probably not the way to go. More commonly when there are nematode products available on the market, they are usually sold to be diluted in water before it's ministered to the soil.

Amy 48:39
Okay, cool. All right. So for the second question that we have, so I usually make smoothies at home, I don't go out and I don't normally purchase a smoothie for like $10 because I think that's ridiculous, but sometimes, if you go into a smoothie place, they have bee pollen that they add on top of your smoothies. And what they say is that it's really good for your health. The questioner is asking if bee pollen products are good for you and if there are some health benefits.

Jamie 49:06
I do love this question. We get lots and lots of questions related to anything related to bees and health.

Amy 49:17
Human health.

Jamie 49:18
Yeah, human health. We have this general umbrella term that we use called apitherapy, and people take honey for allergies and pollen for this and bee stings for that. So, I do love and appreciate this and the other questions related to human health and bees. But I'm not going to answer the question. And the reason I'm not going to answer the question is that these are questions for PhDs or medical doctors who study human health-related issues. We don't engage in apitherapy here at the University of Florida Honey Bee Laboratory. I don't study that. It's not my field and we are constantly advised to be careful about making human health comments with regard to anything since it's a bit outside of our field. So what I would say in return is that I'm very aware of people eating bee-collected pollen for what they claim are a variety of health reasons. But I suspect their research is not strong in that field. I'm guessing there are some studies where people have looked at it, but my guess is like, which is the case with many apitherapy claims, that there's not a lot of rigorous research to support it. Now, I know if you're an apitherapy enthusiast out there, you're going to vehemently disagree with that statement, which is why we don't usually wade into these debates and discussions. But those are certainly great questions, but they're better suited for people who study human health and nutrition rather than bee health and nutrition. So it's a great question. Look it up on the internet, you'll see all the claims. But you might reach out to a human nutritionist, not a bee specialist. But great question. I certainly appreciate you sending it our way.

Amy 51:01
The last question that we have, this person is asking about drone comb. And as some of our listeners know, putting drone comb in for Varroa control is a best management practice and part of an integrated pest management. The questioner is asking if there's any research that's been done on pre-treating artificial drone comb to deter, weaken, or kill Varroa that are going to be entering there. The questioner

is asking just about any research done on treating drone comb, but then also, we do use drone comb to attract the Varroa. What are your thoughts on that?

Jamie 51:38
This is an interesting question. For those of you out there who are unfamiliar with the use of drone comb, you can buy sheets of foundation that are pre-sized for drone production. So the worker bees don't build worker-sized cells on these sheets of foundation, they build drone-sized cells on these foundation sheets and the queen, correspondingly, lays drone eggs or unfertilized eggs into the cells and you get a whole comb that's dedicated to the production of drones. And historically, this has been used to augment drone populations in colonies that you want to provide drones for selection purposes. So if you run a queen mating yard, you're selecting good mother queens from which to graft eggs, and good mother queens from which to produce drones, so that copious amounts of drones will be available to mate with queens in these mating systems. Well, these drone combs have a second use. A lot of folks, when Varroa were first discovered, noticed that Varroa preferentially go to drone brood. So someone said, "Well, gosh, why don't we just put drone comb in colonies, allow queens to lay eggs in the cells, and right before those cells are capped, the Varroa in those colonies will preferentially invade those cells on that one comb rather than the worker cells and the other combs." And so basically, we create a sink for drones, that's what it would be called kind of from a research perspective. It would be a sink, where all of the drones kind of go into it, then, once the cells are capped, you can remove that comb, freeze it, then thaw it, then put it back in the nest. The workers will remove all the dead bees and the dead drones and the Varroa queen will lay in it and start the cycle over again. So the idea is since you're constantly taxing the Varroa population through drone brood removal. So the question then is saying, well, gosh, maybe that's a lot of work. Is there a way that we can make an entire drone comb rather than give them a sheet of foundation on which bees have to construct cells, maybe we can pre-construct the cells for them? And, oh, by the way, if those cells can be impregnated with maybe a chemical or treatment in some way so that we don't have to freeze the comb, it just kills the Varroa that are in those drone cells developing. So I'm not aware of any research that's ever been done on that. I do know there have been similar thoughts for wax molds. There have been ideas about pre-treating combs with a wax moth product, which is basically a BT product, Bacillus thuringiensis, one of those bacterial derivatives that if the wax moth caterpillars eat the wax, they would die, if it's got this derivative on it, but the honey bees would be just fine. So the premise was the same there. You're protecting the combs from destruction by wax moths. In this case, you'd be putting something on the combs to kill Varroa. I think it's a good idea. I don't know that it's ever been tested. But the catch-22 is if it's permanently in the nest, and these residues are permanently in the wax cells, then the developing drones will be permanently exposed to it as will all bees walking around the net. So I do think it's a feasible idea. But I think it would take a lot of research to get it to the point where it would be very beneficial. So I liked the idea. I appreciate the question and suggestion. Maybe someone studied it. I'm just not aware of it. But I do think it would take some thought and some very close study to ensure that it's efficacious against mites, but essentially innocuous to the bees.

Amy 55:19
There's always a never-ending list of questions. Right, Jamie?

Jamie 55:23

Amy, let me let me expand on that. You know that I answered questions for the American Bee Journal, as well. Someone literally asked me within the last two weeks, "Jamie, I noticed that in your questions of the American Bee Journal, hardly an article goes by without you saying, we don't know this. This would be great to study in the future." And they asked me, they said, "Jamie, is there a national database into which questions like those go so that researchers know what to study?" I'm like, "Well, there's no national database. But the questions that I find very intriguing, I do write down on a sheet of paper on my desk with the intent of maybe studying it someday." So these are great questions. And it really illustrates the point that we just don't know everything and we've not studied everything, and there's a lot of room to grow in our industry.

Amy 56:07
I think someone should just go through all of our Q&As from all of our episodes, and every single time you say there needs to be more research on this, they can compile that database. Think it would be fun.

Jamie 56:18
I think it would be great. Now we just need to find the people to do the research.

Amy 56:22
Exactly. All right, everybody. Thank you so much for your questions. Hopefully, we've answered them, and if we can't answer them, we'll lead you in the right direction. But keep those questions coming. Be sure to send us an email or on one of our social media pages. Thanks for listening to today's episode. This episode was edited and produced by our podcast coordinator Mitra Hamzavi. Thanks, Mitra.

Jamie 56:53
Visit the UF/IFAS Honey Bee Research and Extension Laboratory's website, UFhoneybee.com, for additional information and resources for today's episode. Email any questions that you want answered on air to honeybee@ifas.ufl.edu. You can also submit questions to us on X, Instagram, or Facebook @UFhoneybeelab. Don't forget to follow us while you're visiting our social media sites. Thank you for listening to Two Bees in a Podcast.