This Week in Microbiology

With Vincent Racaniello, Elio Schaechter, Michael Schmidt, and Michele Swanson

Episode 187: Rounding up the bees

Aired October 19, 2018

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(music)

Vincent: This is TWIM, This Week in Microbiology, episode 187, recorded on October 11, 2018. I'm Vincent Racaniello and you are listening to the podcast that explores unseen life on Earth. Joining me today from Small Things Considered, Elio Schaecter.

Elio: Hello there, how are you?

Vincent: I'm well, how are you?

Elio: Okay.

Vincent: San Diego.

Elio: San Diego. What a place.

Vincent: Also joining us from Ann Arbor, Michigan, Michele Swanson.

Michele: Hello! Where it is fall, the leaves are starting to change color.

Vincent: And you are playing football.

Michele: Yes, we are. I'm not personally, but.

Vincent: Also joining us from Charleston, South Carolina, Michael Schmidt.

Michael: Hello, everyone!

Vincent: And Michael, is it raining out there?

Michael: No, the sun has come out, and hopefully we are gonna have fall like weather after Hurricane Michael blows its way through. So we had Hurricane Michael last evening and early this morning. It woke me up with the house shaking and it was blowing and by that point in time it was only tropical force winds, so I feel very blessed here in Charleston that we dodged two major bullets this year in hurricanes. The poor souls in the Florida Panhandle of course have gotten clobbered with Michael, that was a category 4 storm, and it came in like a juggernaut and it went as far as Atlanta where it had significant effects. So Mother Nature, never mess with her.

Vincent: Well, I hope that it works out for you. So you had winds but no rain, right?

Michael: We just had a little rain. The majority of the rain was in the middle of our state.

Vincent: You have a good connection today, maybe the hurricane does that, you know.

Michael: You never know.

Vincent: Alright, before we do some science we have, well, it's science but it is in the form of two follow up emails to our last episode. Or maybe two episodes ago. What was the one we did the alcohol?

Michael: That was the last episode that I think got published.

Michele: 185. Yeah.

Vincent: Alex writes:

Dear TwiM People!

I finished episode 185 "There's no moa Moa" during my weekend and needed to comment on your argument about the relevance of acquired resistance of bacteria against alcohol disinfection.

As I see it, Elio had a point when he said we do not have a problem regarding isopropanol resistant bacteria. At this point of time, isopropanol concentrations are sufficiently high to handle even most resilient strains. But, there are caveats. The authors of the paper in question were able to show a trend I consider similar to those we all wish we had seen before the rise of MRSA and others. It may be quite possible that, in future, we might indeed get those hard-to-disinfect superbugs Michele mentioned.

Some factors involved are for example:

– Inappropriate application of disinfectants, like using low dosages or shortening exposure times leads to decreased effective concentration or incomplete killing, respectively.

– Wrong handling and storage might lead to evaporation of alcohols and other effects which decrease the efficacy of disinfectants.

– These factors occur even more often because of the inflationary usage of disinfectants in households by people not familiar with their appropriate application and storage.

Especially the last point should be all too familiar from problems encountered in antibiotic medication. How often do we tell friends and family to just take them as the doctor ordered instead of quitting as soon as they feel better?

Last but not least I do want to point out that there is scientific data which suggests a connection between resilience against disinfectants and resistance against antibiotics. By acquiring the former, bugs could indeed passively acquire the latter as a side effect.

I do not fancy giving information the TWiM gods were not already aware of. But it's worth to try a lucky shot. Should some of this actually be helpful: you're welcome!

Continue the great work!

Best wishes, Alex.

Michele: Thank you Alex, that's great!

Vincent: They're good points, for sure.
Michael: They are, indeed.

Vincent: And, I mean, everyone is aware of this now, I think, or many people are, so we'll keep our eye on it. Elio, did you hear all that?

Elio: Yeah. I think she's got a point, it's true. On the other hand, I think mitigating the fact that one could misuse the alcohol is the fact that the dispensers are really sort of mindless. You can only get it from a dispenser one way, the same shot and it's gonna be pretty good against evaporation in pattern. So I'm not sure but it's certainly a big problem.

Vincent: Yeah, I also think that they really go through those bottles or dispensers pretty rapidly, so they're refilled on a regular basis, and so probably evaporation is not an issue.

Michele: What about people that are using them in their homes or carry them in their bags?

Vincent: Could be. Could be.

Michele: So that's a big burden.

Vincent: I went to a meeting the other day and they handed out to all of us on the committee little bottles of hand sanitizer, right, and it's sitting on my shelf. It's gonna sit there for ten years probably.

Michael: And then you're gonna use it and it won't have any alcohol in it. It'll just have hand cream.

Vincent: I mean, it's a little thing that you're supposed to carry with you, right, so… that's good points. And we have another email in the same vein.

Ayush writes:

Dear Vincent et al.,

I truly enjoyed your discussion on 'Increasing tolerance of Enterococcus to handwash alcohols'. Such lively discussions make doing science even more fun. This work also highlights how scientific findings get lost in translation by popular media. Thus we need more and more platforms like yours where scientists explain such findings to the public. One thought from this paper, do we know how fast the alcohol evaporates from hand sanitizer bottle? I looked at the hand sanitizer bottle in my office while listening to your podcast and turns out it is way past the indicated expiration date (which may or may not mean anything) which may mean that the actual alcohol concentration in this bottle may be less than the indicated 62% (which is what we seem to be using in Canada). Based on this study, the take home message for me was perhaps to make sure that the hand sanitizer that I use in my office is not too old.

Michael: Was he at the same meeting as you?

Vincent: (laughs)

Michael: Ten years ago?

Vincent: He's in Canada, my meeting was an institutional biosafety committee meeting.

Michael: Oh, that was nice of them.

Vincent: Where they said hey, we're gonna give everyone hand sanitizer and then they also gave us flu shots at the same time.

Michele: Wow, and by the way, put on this coat and glasses.

Michael: And you already had your flu shot!

(laughter)

Vincent: Sorry, Elio, what were you saying?

Elio: It seems to me that the take home point is in 25 years sanitizer out of the little bottle you carry with you, be careful how you use it. Use it according to instructions.

Vincent: Don't keep it too long, yeah. And Ayush says:

As always great job! Btw, everyone in my lab is big fan of your podcast. Our graduate seminar this week was inspired by the TWiM 170 (Ectoparasites and Plague).

Best, Ayush.

Ayush is an associate professor in the department of microbiology at University of Manitoba, which is in Winnipeg, which is in Canada, in case you didn't know that.

Michael: That's right above North Dakota.

Vincent: Right above it.

Michael: In the middle of the country, in the middle of our continent, I should say.

Vincent: Alright.

Elio: We love the message. Thank you very much.

Vincent: Yes, we appreciate it. Alright, on to some science, I have a snippet for you. This was published in Cell recently. It's called "Bacteriophage cooperation suppresses CRISPR-CAS3 and CAS9 immunity". It's by Borges, Zhang, Rollins, Osuna, Wiedenheft, and Bondy-Denomy, from the University of California San Francisco and Montana State University, which is in Bozeman. And so this is all about CRISPR-CAS immunity in bacteria, and if you need a little primer on that go to TWIM 184, where I spoke with Sam Sternberg all about CRISPR-CAS immunity and immune systems. Sam was a PhD student with Jennifer Doudna and he's really good at explaining things. So if you haven't listened to that episode you should check it out, it was really good. So just to recap, CRISPR-CAS immune systems in bacteria, the way they work is when the bacteria are infected or when DNA comes in, they make fragments of these incoming nucleic acids, they are stored in the genome in arrays, and then they are transcribed into small RNAs, CRISPR RNAs which guide destruction of incoming DNA by nucleases.

So these CRISPR-CAS immune systems can prevent phage infection, they can prevent lytic or integrative events in phage infection, they can prevent plasmids or other DNAs from coming into cells. Now, as we know, phage genes have arisen that antagonize CRISPR-CAS immunity. They're called anti-CRISPRs, ACRs, and these proteins either inhibit the CRISPR RNA from binding the DNA target on the virus or they inhibit the nuclease activity, and that is what this paper is all about, looking at how these anti CRISPRs work. And up until now what people have done is to put the coding region for these anti CRISPRs on high copy number plasmids, they are produced in the bacterium, and then they infect. The authors say this is not really reflective of what happens in nature, in nature phages infect the cell where you are making a lot of CRISPR RNAs already. In fact, Sam said they are made continuously. And so the phage gets in and you don't have the ability to make high amounts of anti CRISPR proteins right away. In fact, the author said that usually, when a phage infects an immune bacterium, the phage genome is degraded within two minutes. So that's probably faster than you can make the anti CRISPR protein.

Michele: Wow.

Michael: That's true.

Vincent: So what's going on, since they can work in certain scenarios. So that's what they did. They did a number of really interesting experiments. They have a series of phages containing ACR genes and the host they use is Pseudomonas aeruginosa which has a CRISPR-CAS system that targets these phages. They use an array of phages with different ACRs of different efficiency. They start by doing very straightforward plaque assays and showing that even with ACR in a phage it is not able to replicate very well, it does not plaque very efficiently on these strains that have CRISPR-CAS systems.

So they say ACR is imperfect. These phages are still sensitive to CAS immunity even though they have these anti CRISPR proteins in them.

Michael: Are they giving them cloudy plaques?

Vincent: They do an efficiency of plating with and without the ACR.

Michael: They're not getting a cloudy plaque where there's some sort of breakthrough.

Vincent: No, they actually said there is no breakthrough, there is no mutation, you just don't get plaques. You get few plaques made. So what's going on? They said well, what if we increase the multiplicity of infection? So they look at a system where they just get lytic phage infection, no lysogenic where there is integration, and they infected different multiplicities. And they find that as you increase the multiplicity, that is the number of phages you add per cell, you can then overcome the CRISPR immunity and the weaker the ACR in the phage, the higher MOI you need. And so what that means is you have multiple phages infecting a single cell. So under those conditions you make more ACR protein and that can overcome the CRISPR immunity. So that is very cool. And they can even do the following: they can infect with an infectious bacteriophage and then they can coinfect with a noninfectious phage, which they have been able to engineer. We don't have to say how. And the non replicating phage which they call a donor can supply enough ACR to let the replicating phage grow in the immune host.

Michele: So trans complementation.

Vincent: Exactly, exactly.

Elio: Why something not relevant, but historically it sounds like it, in the old days people started something called multiplicity of reactivation.

Vincent: Right.

Elio: You relight the phage that you relight, it will not grow unless there is a lot of… so vaguely, it's not connected to this but it sounds like it.

Vincent: Right, multiplicity dependent. The more phages you get in a cell the more likely you're going to fix whatever defect is in one phage, right. They also say, what about lysogeny? Does this hold for lysogeny as well? So they measure, they have a cute way, they mark phages with gentamicin resistance and they can measure integrated DNA in conferring that. And again they see that lysogeny is very inefficient even with phages that have these ACR proteins, and only when you increase the multiplicity or add a non replicating phage can you get overcoming of the CRISPR-CAS immunity. So both lytic and lysogenic events can be, you can overcome CRISPR-CAS immunity as long as you have enough phage which produce enough ACR protein.

And they also repeat all of this for a different CRISPR-CAS system. So far everything has been done with CRISPR-CAS3, so CAS3 is the nuclease that cleaves the target DNA, and they do CAS9 as well, which is from S. pyogenes. And they get the same effects that it is hard for phages even with ACRs to

replicate in CRISPR containing bacteria, but if you put enough of them in, it works. So they think this is a widespread principle that they have discovered that you need a higher multiplicity. So that is really the experimental data and I just want to spend a few minutes being philosophical, because I think this is really interesting. Here is another way to look at it. In this series of experiments, they have shown that if you put enough phage within a single cell you can overcome host immunity because you are making enough of the ACR protein. But if you just put one phage in or a few it is not enough to overcome it. So making ACR immunosuppresses the cell. One way to look at it is that in nature if a phage infects a cell, even if it fails it may make enough ACR to immunnosuppress the host so that the next phage that comes in will succeed.

Michele: Yeah. The first one takes one for the team.

Vincent: Exactly!

Michele: Makes it easier for the next. And they showed it beautifully with computational modeling.

Vincent: Yes. So they say even if phages don't replicate, they can remodel the host cell.

Michael: And this paper is open access, as well, which is unusual for Cell.

Vincent: So they called these ACR proteins "public goods", which is something we have talked about before. Remember the arbitrium system? There was a system where infected bacteria seem to communicate and guide the decision between lysis and lysogeny, we talked about that a number of TWIMs ago. And this is similar except, they say, it is completely altruistic. So as Michele said, many infections must fail before a few can succeed.

Michele: And the biochemical basis they point out there is a slow dissociation between the ACR protein and the CAS protein, so the first one goes in, the phage may not make it but its protein will lock on and associate, remain associated for some period, creating a window of opportunity for the next one to come along and infect.

Vincent: There's a window, for sure.

Michael: So it's the K-on and K-off, and as that protein or enzyme evolves with time it may change its specificity and you could see how you could get modulation of the effect in a population.

Vincent: So they say this is the first documented example of true viral altruism. Okay.

Michele: It's convincing.

Michael: First we debate whether viruses are alive, now we are debating whether they are altruistic.

Vincent: That's right, that's right. So let me read the last two sentences because I think this is really interesting. And they say: the sacrificial population level aspect of CRISPR inhibition, which is what we have just talked about, is reminiscent of manifestations of CRISPR adaptation in populations of bacteria. The majority of infected naive hosts cells die before a clone with a new spacer emerges. So in

other words, I asked Sam about this, too. When a phage infects a bacterium and there is no spacer to match it, why doesn't the bacterium just die? In fact, many do die, until one pops up that is resistant and then that grows out and it becomes a resistant population. So it is kind of the same thing, right? A lot of bacteria die to provide a resistant one that goes on, it's the same thing with this anti CRISPR many phages don't replicate in order to make one that eventually will spread throughout the community. So to paraphrase Star Wars, many phages died to bring us this information. (laughter)

Michael: Oh no, many droids.

Vincent: Yes!

Michael: Many droids! And you know, some of the droids do look like phages in Star Wars.

Vincent: They do, they do. In one of the movies someone says many something died to bring this.

Michael: Many droids!

Vincent: To bring us this information. I thought that was cool and I think they are right. This is real altruism which, you know, people wonder is there altruism in nature? How can you predict what's gonna happen? But in this sort of situation we know population has to evolve resistance to something, there can be altruism.

Michael: There has to be otherwise it would drive towards sameness.

Vincent: Yeah.

Michael: If anything, evolution has taught us it is driven towards diversity.

Vincent: Well I love it because it is full of plaque assays.

Michael: Right, and that's effectively your secret handshake to get into the Virology Society, right? You ask everyone have they done a plaque assay.

Vincent: Yeah, we said on TWIV once, you can't be a virologist unless you have done a plaque assay. Someone actually wrote in, they said I've done a plaque assay, can I be a virologist? Sure. (laughter)

Michele: I'm proud to say the President of University of Michigan has done plaque assays.

Vincent: Mark Schlissel.

Michele: Yes.

Vincent: Is that right? I think he said that at that ceremony, right?

Michele: Right, you were in the audience.

Vincent: Yeah, that was funny. Elio, what do you think of that paper? You like that?

Elio: Love it.

Vincent: Love it?

Elio: It does, it transcends the usual sort of one on one thoughts it comes up with between host and parasite. Here it is a population phenomenon that requires numbers and I like that a lot. It is a novel way of thinking of it. I am wondering if somebody is more clever than I that can put this together with multiplicity of reactivation. It's a phenomenon, recombination, frequency of recombination being higher when the numbers are higher. But on one hand this does come in in some fashion, as well.

Vincent: It might, sure.

Michael: We'll wait for our listeners to comment.

Vincent: There is a similar paper in Cell, it is August 9th, 2018, and the paper we just discussed is, when was that published...August 9th. Same issue of Cell. And it is titled "Anti CRISPR phages cooperate to overcome CRISPR-CAS immunity." And the authors are Landsberger, Gandon, Meaden, Rollie, Chevallereau, Chabas, Buckling, Westra, and van Houte. They are from University of Exeter, University of Montpellier. Both papers come to similar conclusions. Bacteria with CRISPR immunity are partially resistant to phage and sequential infections help overcome resistance.

Elio: What phages are in their paper? I haven't read it yet.

Vincent: The other phages are, let's see.

Michele: They're both Pseudomonas phages.

Elio: Okay.

Vincent: Both Pseudomonas, yeah. Okay. Alright, next, for a paper on honeybees, Michael.

Michael: Alright. So this next paper comes to us from one of our faithful listeners, from New Zealand, Dave Havel, who was alerted to the paper that we are going to discuss by one of his colleagues, Raj Palisame. So this goes to show you that you can send papers to your friends who can then send them to us and they can come to TWIM and both individuals are in New Zealand. So they are both our faithful listeners and they are part of a group that has a common listserv called Weeds. And so the paper today is "Glyphosate perturbs the gut microbiota of honeybees." It is from Motta, Raymann, and Moran, all at the Department of Integrative Biology at the University of Texas at Austin. And this is a very significant paper for the consequences it will have not only for honeybees but it may have other consequences, I'll speculate at the end.

Our listeners have likely heard of colony collapse disorder which is a phenomenon that occurs when the majority of worker bees and a colony disappear and leave behind the queen plenty of food and a few nurse bees to care for the remaining immature bees and the queen. This phenomenon has been

recognized for approximately greater than 10 years, when during the winter of 2006-2007 some beekeepers began reporting an unusual high loss, between 30 and 90% of their hives, and as many as 50% of all the affected colonies demonstrated symptoms inconsistent with any known causes of honeybee death. So this paper is looking at a mechanistic, a potential mechanistic cause, and it has a microbial physiology via chemical twist. Now many of us have heard of the herbicide glyphosate under its trade name which is Roundup. And Roundup or this herbicide is thought to be innocuous in animals including bees because it only targets an enzyme found in plants and you are likely gonna guess microbes as well, specifically the active ingredient in roundup is glyphosate and it targets the 5-enolpyruvylshikimate-3-phosphate synthase that they abbreviate EPS-PS.

This is an enzyme that is specific for the production of aromatic amino acids, phenylalanine, tryosine, and tryptophan. Now there are some bacteria that need this pathway in order to make their amino acids and others do not. And the EPS-PS enzyme transforms phospholinopyruvate plus 3-phospho shikimate into phosphate and the 5-enolpyruvylshikimate-3-phosphate, and the herbicide serves as a competitive inhibitor and inhibits this process and stops it cold. Now why is this herbicide bad for bees? Well, the workers rely on a specialized microbial community in their hindgut that benefits their growth and provides defense against pathogens. So the story is exposing bees to this herbicide alters the microbial community and thereby increases the animal susceptibility to infection by opportunistic pathogens. So it is a simple story. The bees are out there foraging. They pick up pollen. The plant has been exposed to the herbicide and the herbicide comes along with the pollen and it goes back to the hive, which then in turn affects the bees microbiota that then makes them more susceptible to an opportunistic pathogenic attack.

Michele: That's the hypothesis they are testing here.

Michael: That is, it sounds simple, and they go through a very sophisticated and very elegant way of demonstrating or testing their hypothesis. So the first question they ask is can this herbicide, glyphosate, perturb the honeybee bacterial community? So what they did is they actually, and this is part of the elegance of their system, they took hundreds of bees from a single hive. They separated them into three cohorts. The first cohort they fed sugar water plus five milligrams per liter of the herbicide. The second cohort they doubled the dose and gave them ten milligrams of the herbicide. The third dose is effectively the sterile control that they just fed sugar water to. They then painted the bees so that they could tell them apart, the three cohorts, so they fed them for five days and then they returned them to the original hive.

And then they harvested the bees, separated them into the respective cohorts, and day zero and three days, and they asked the question: what was going on? And they present this beautiful figure that shows you and they examine each bee so you can literally see the changes for each bee and then they give you the absolute abundance. So on day zero this is after they had fed the bees the herbicide for five days and then they are comparing to the control. At day zero there was a little effect on the bees, bees gut microbiome size, there were still the same numbers, so the herbicide is not effectively acting as an antimicrobial, but the absolute and relative abundance of one of the core species of the honeybees hindgut, Snodograssella alvi, was significantly lower and the cohort that was exposed to the highest concentration of the pesticide, or the G-10 group, which was fed 10 milligrams of the herbicide.

Now to give you an idea, they are using concentrations of the herbicide that are routinely associated with plants in nature, and plants, the pollen would typically pick up between 1.4 and 7.6 milligrams per liter of this glyphosate and so this is a typical concentration. So they are working with a, for a lack of a better expression, physiological concentrations.

Elio: Looking at the controls, I was a little bit surprised by the variety of numbers, although the proportional difference seems to be roughly the same in difference than the case ones. Some bees have lost more bugs than others.

Vincent: Yeah.

Michael: This then comes further on because these bees are going back into a hive. They are not contained in a lab. These bees are allowed to go out and forage and then come back to the hive. So this is really out there in the wild so to speak that the bees are flying around collecting pollen after they have been treated with the herbicide.

Vincent: Yeah. So you are saying Michael that that is the normal variety among these foraging honeybees to have different microbiomes, right?

Michael: Yes. I think that was the intent of showing us this, because you have to ask yourself, why did they display the 15 bees from each group?

Michele: They also point out, they are very transparent in their presentation, they point out that fewer than 20% of the bees actually come back to the hive and they can't rule out that there hasn't been some enrichment for particular fitness advantage that is not being tracked here.

Michael: And they go on and some of their subsequent analysis talking about one of the side effects of the herbicide could be that it alters the bee's behavior because that has been previously reported. So keep that fact in the back of your mind as we progress through this molecular pathogenesis story. So as time progresses, the effects of this herbicide become more apparent. When they appreciate that at day three the total number of bacteria decrease for both treatment groups, the five and the ten, but was only significant for bees exposed to the lower concentration of the herbicide. And when you look at the figure, you can see substatial compositional shifts in the types of bacteria present with the absolute abundances of the four dominant gut bacteria, the Snodograssella, the Bufidobacterium, and then two flavors of Lactobacilli, Lactobacillus FUR4 and FUR5.

One, however, one group increased in abundance and in the G5 group, or the low concentration of the herbicide, and this was Gilliamella apicola, and this microbe is in the order that I had never heard of before, Herbalus, which is in the proteobacter, and this, the taxonomists created to accommodate the novel bacterial species people were recovering from the guts of honeybees and bumblebees. So I thought that was an interesting group of trivia.

So we don't know too much about the microbes that are inhabiting probably one of the most important insect species for our planet. Now the weird thing was that in the guts of the bees exposed to the higher concentration of the herbicide, they only saw an absolute decrease in the Lactobacillus in the group from 5, and they repeated this using bees from different hives and different seasons, and

observe some more trends. So they were very careful in making their generalizations to make certain that it wasn't the time of year or the type of food that the bee was eating based on the pollination schedule. Now the authors did not offer an explanation for the relative lack of effects of the G10 treatment on the microbiota composition after their three day treatment other than the herbicide may be doing something else. And Michele also brought to our attention that only 20% of the bees come back.

Michele: I wonder if the more heavily dosed bees never left.

Michael: Mhm. They could have died.

Michele: Yeah. Or they were lethargic and so they stuck around.

Vincent: Sure, could be.

Michele: It's a challenging experimental system, no question.

Michael: It's effectively what is actually happening. So they are really doing a service to the honeybee community in that they are really trying to understand what the application of this herbicide and this herbicide, Roundup, is used everywhere. I mean, I'm sure everyone who has a yard with weeds has some in their garage today. The next aspect they investigated was that since this herbicide arrests bacterial growth without directly killing the cell, simply by preventing the production of amino acids, so then they hypothesized that it would have a greater effect on actively dividing cells present during early gut colonization. Now here is where they change gears a little bit. And now they are gonna introduce us to what they refer to as news, which are the newly emerged workers. And newly emerged worker bees are effectively coming out of their pupa state nearly free of gut bacteria and they acquire their normal microbial immunity orally through social interactions with other workers during their first few days after emergence.

Elio: Just like we do.

Michael: Just like we do, that's absolutely correct! Yes. So this type of bee was simultaneously exposed to an inoculum consisting of their normal microbial community and then the herbicide. They point out that this experiment is modeling what likely happens to the bees in the wild as the herbicide has been detected in hives and honey samples. So then I am sitting here reading this, leading me to wonder even if I buy a certified organic pesticide free herbicide free honey, how can I be certain that the bees have not been harvesting pollen that has been exposed to Roundup from the neighbor's farm or neighbor's yard for all of the pollen looks tasty to a bee, and there is evidence now that foraging bees can return and the Roundup comes with them coming back to the hive. And the scary thing is that this glyphosate is a stable, water soluble chemical that can persist in the environment for long periods of time. So that's out there as well.

Vincent: As you know, there was recently a big verdict in the Roundup case, right? 289 million dollar award for someone who claims that they were damaged by Roundup. So you don't probably want to eat honey containing Roundup.

Michael: Yes. And again, they assess the gut microbiomes and identified all 8 core gut taxa in this new, or these newly emerged workers, and both control and treatment groups, showing that the herbicide does not eliminate or prevent worker colonization by any of the core members in this newly emerged worker. They remarked, though, that the dominant species is this Alvi who is the most strongly affected member of the gut microbiota and it decreased in both absolute and relative abundance while the lactobacilli principally firm four increased in relative abundance. And they then do some very nice principle coordinate analysis showing us what is actually going on in this population of newly emerged workers, leading them to offer us a conclusion that this herbicide exposure during early development of the gut community can interfere with normal colonization by altering the abundance of these beneficial bacterial species.

Another interesting fact as I was reading this that then comes into play when you begin to dissect how they did their work is I learned that captive honeybees do not defecate, leading to the accumulation of dead bacterial cells and accumulated DNA in the gut of the bee. So now, considering that you are harvesting material out of these dissected bees and if you just isolate the DNA, you don't know if it is dead DNA or if it was from live DNA but that is why they are very careful in that they extract both DNA and RNA from the guts of treatment and control bees, and recall that they painted the bees to tell them apart. So again, as Michele pointed out, this is very very careful work that these workers have done for us. They also--

Michele: And just to be clear about that, the DNA is very stable even in a dead bug, but RNA turns over more rapidly so if you see RNA, you can deduce that it is a live bacterial cell.

Michael: Yes.

Elio: Even in constipated bees.

Michael: Even in constipated bees. I mean, this goes back to an earlier TWIM where you were looking at fossilized fecal material, from the last TWIM I think that we did.

Michele: No wonder bees seem so angry, buzzing around. (laughter)

Vincent: Yep, they're constipated all the time. Poor guys and gals.

Michael: Well, the other thing that they did in their careful analysis, they added another control group if you will where they used an antibiotic tylosin, which is heavily used in agriculture. This is a bacteriostatic macrolide antibiotic that has broad spectrum activity against Gram positive organisms and only a limited range against Gram negatives. And it is used as a feed additive. And use of this antibiotic in beekeeping was anticipated to perturb the microbiota of bees, but the decrease that they observed was only significant for RNA samples. Again, showing the whole constipation and which Michele just pointed out that RNA is likely responsible from live bees. So you have a bacteriostatic antibiotic that is gonna stop further growth of bacteria so you can actually detect what is actually going on. So then that experiment considered in total suggests that the DNA data are partially obscured from DNA from the dead bacterial cells although the effect did not entirely mask the shift in bacterial abundance, suggesting that it is really a pretty big shift that they are able to see even in constipated bees, so to speak.

So up to now we have a story that the herbicide can alter the microbiota of honeybees but then the question is does this matter? So they next determined whether or not exposure to this herbicide that then induced perturbation of the microbiota affected the host health, and the host health is the bee. They measured the bees susceptibility to an opportunistic bacterial pathogen, Serratia marcescens, and the experiment was set up by exposing these newly emerging workers to the herbicide at the time they would be acquiring their microbiota. After five days of exposure to the herbicide, the bees were challenged with a pathogenic strain of Serratia, which is not normally detected or if it is detected, it is detected at very low frequencies in the bee gut.

Michele: It's a gastrointestinal, so it is gonna be down there in the microbiota that has been perturbed by the herbicide.

Michael: Correct. So when challenged by Serratia, the bees lacking a gut microbiota, so they have bees lacking gut microbiota had an increased mortality relative to that observed for bees with conventional gut microbiota regardless of exposure to the herbicide. For bees with a conventional gut microbiota, the herbicide treatment resulted in increased mortality after the Serratia challenge. To determine then whether or not this increased mortality was due to the effects of the herbicide and the gut microbiota or to direct effects of the herbicide on the bees, they included control groups not challenged with Serratia.

And this is all very well depicted in one of their sub panels of their figures and it is a classic Kaplan-Meier death plot. And you can just look at it and you can see on the figure that there is three clusters, there's the cluster, the first cluster in which you effectively are having very little effect on that host health, and they are measuring death over eight days, and so you in fact have the bees that have a proper gut that they expose to the herbicide and then you have bees that have no microbiome and then bees without a microbiome plus the herbicide, and the death rate essentially follows along and it is not statistically significant. When you challenge newly emerged worker bees with Serratia you can ssee it is significantly different from that cluster, but when you have the herbicide added along with the Serratia, you see a remarkable collapse in the viability of the host in a very short period of time. It is quite remarkable.

Michele: It is a beautiful dataset, really impressive.

Michael: It shows you how important the microbiota is and it shows you how important exposure to the pathogen is as well as the herbicide. And it is really quite remarkable.

Vincent: You know though, Michael, technically you would want to add back and see that adding back the microbiome makes them resistant.

Michael: You wanna rescue these.

Vincent: You want to rescue it because you could argue that maybe the glyphosate is doing something else, right?

Michael: Yes.

Vincent: It's wiping out the microbiome or changing it, but how do we know that directly impacts Serratia susceptibility?

Michele: They do another set of experiments where they monocolonize. It's really a beautiful piece of work.

Michael: They're taking it to the wall, Vincent.

Vincent: Tell us about that one.

Michael: Let's see…

Vincent: If it's not next, that's okay. We'll get to it, I guess.

Michael: Yes.

Michele: Let's do it next.

Vincent: You're gonna mess up Michael, he doesn't like to go out of sequence.

Michael: Why don't you tell 'em, Michele, and I'll see if I can find where I…

Michele: No, no, no, you're more prepared than I am.

Michael: Alright, so what the authors are trying to do is they are trying to understand the role of S. alvi, and the herbicide reduces the effect, the protective effect of the gut microbiota against this opportunistic pathogen. And we know that alvi is the bacterial species most negatively effected by exposure to the herbicide. So they present an experiment in the supplements that I thought should have been in the main body, but you can take a look at the experiment because it, I believe this is again another open access paper, that showed that S. alvi provided some protection against pathogen challenge but not as well as a complete gut. Now they postulate a mechanism that some strains of alvi have a type 6 secretion system and they suggest that such a trait in a strain would contribute to colonization resistance through contact dependent inhibition of Serratia and/or host expression so the bees expression of antimicrobial peptides because that is upregulated after S. alvi colonization, so you get the idea that alvi may be essential in enabling the full microbiota to assemble properly, thus enabling greater protection. And again, that telling figure is 2G with the Kaplan-Meier death chart. So now they are going to move into this mechanistic basis of how the herbicide is potentially harming the bee by harming the microbes.

Vincent: Michele, is that the experiment you were referring to where they put back S. alvi?

Michele: No, they even take it another step and start to look at these two different isoforms of the enzyme.

Michael: And that is where we are going next. So this is a long and complicated paper. So the next experiment is they are trying to build this mechanistic basis of how this herbicide is working. So they show that the bee gut contains bacterial species with both sensitive and insensitive types of the enzyme. So this is again the EPS-PS enzyme that is competitively inhibited by the herbicide. Class 1 microbes, their enzyme is naturally sensitive to the herbicide, whereas Class 2 are not. And so these EPS sequences that they in silico analyze from alvi, this other Apicola, a whole list, clustered with those from other organisms containing the Class 1 EPS-PS enzyme system and thus the bacteria were predicted to be sensitive to the herbicide glyphosate.

Thus it is not surprising that alvi is going down simply because it has got a Class 1. But they next show that the bee gut bacteria vary in sensitivity to the herbicide at both the species and strain levels, and they did this by isolating and culturing the strains in vitro in the presence or absence of this high dose of the herbicide. And so most alvi and Apicola strains tested contain a Class 1 EPS-PS enzyme system. They either do not grow or they have a delay in growth when cultured in the presence of the herbicide, but no such effect was ever observed for strains containing the Class 2 EPS-PS enzyme system, and those are lactobacillus form four and the B. apis particular strains, and they go through that and their third figure in a series of figures in the appendices.

But there is always a twist in biology. Now, this S. alvi system, and there were two particular strains, WKB2 and 298, and despite the fact that these are Class 1 enzymes, so they should be sensitive to the herbicide, they grow well in the presence of the herbicide as well as they do in its absence with no initial delay in growth. And they reported that a previous study of the genes required S. alvi to be grown in the hindgut of the bee required the aromatic biosynthetic pathway and so this reminds me of what we see in our guts where butyric acid is very important and it is feeding other bacteria in our gut making certain that our gut GI microbiome is properly balanced.

So the aromatic amino acids that are produced by S. alvi may be very important in balancing the gut of these microbes. And the fact that S. alvi strains can become, if you will, resistant to or not sensitive, I think is the proper word, to the herbicide is I think interesting. And this resistance was independent of bee gut strains. So like all good microbial pathogenic studies, they tried to tease apart the mechanism behind how some of these bee gut strains are prevented from growing in the presence of the herbicide, and they did what any self-respecting geneticist would do. They complemented with the genes from E. coli, and they cloned them from the bee gut bacterial strains, I think this is the experiment that you wanted, Michele, right? Where they are doing it in trans?

Elio: At this point I think I need a take home lesson.

Michael: The take home lesson is S. alvi is very important to the development of the new worker bees guts and absent a properly functioning hindgut of the bee, the exposure to the glyphosate makes them susceptible to opportunistic pathogens like Serratia marcescens.

Vincent: These two S. alvi strains, right, with the sensitive and a more or less sensitive enzyme, they fed bees these two strains, right?

Michael: Right.

Vincent: But the results are not black and white.

Michael: No.

Vincent: Then when they gave these bees glyphosate it has a negative effect on both, and one was more affected than the others, right?

Michael: That is correct, and that is Figure 4.

Elio: From this, can you learn of a way of providing the bees with the right bacterium to be able to withstand the glyphosate?

Michael: I don't think we know enough yet.

Vincent: Yeah, I don't think so.

Michael: It's suggested that it is very important to understand this role of this keystone species, if you will. I think what we know is alvi is a keystone species in this ecological mix. I think they have to do some modeling in order to address this system. But when I was talking with--

Elio: Since glyphosate is not going away, we have to protect the bees.

Michael: Yes.

Elio: Would we find the right bacteria, we should allow one to grow in the presence of glyphosate.

Michele: I'd say it is hopeful that they found among the S. alvi strains two with very different, well, the different sensitivities to the chemical, and when they monocolonized these with those they saw a different sensitivity. So perhaps we'd better understand the normal microbiota in our honeybees that pollinate our foods. We could, I don't know, seed our flowers with bacterial species that are resistant to the chemical, maybe?

Vincent: So you don't think we're gonna stop using Roundup, right? It's never gonna happen?

Michael: Well, it's a very, very useful chemical for the controlling of weed species that impacts on the yield of our food crops. And with companies out there making Roundup ready seeds, and what the agriculture companies have done is they have engineered the plants and they have changed the EPS-PS of the plant from a Class 1 to a Class 2.

Vincent: Make it resistant, right.

Michael: To make it resistant.

Elio: It happened with honeybees.

Michael: When I was sharing this with one of my colleagues who works on coral, one of the significant pathogens that is affecting coral is again Serratia. And as she pointed out to her husband, we use Roundup by the tankful in the Caribbean to effectively make sure our golf courses aren't invaded by the jungles. And remember, those Caribbean islands are very, very porous. It's water soluble herbicide that literally will go through the lawn out into the ocean, and the question is, is the same herbicide affecting the coral microbiome in a similar sense?

Elio: It's an awful lot about impossible...I mean, glyphosate, it's really a very hard issue.

Michele: Yeah.

Elio: It implies that Nancy Moran has gotten into this business, she is known for her amazingly wonderful work about endosymbionts. This is not endosymbionts, so it's a new thing for her and she did it with aplomb.

Michael: With gusto!

Michele: And Eric Motta, the PhD student in her group was the first author, and he has been driving this project for his thesis. Eric is from Brazil, he earned a bachelor's in pharmaceutical science and then a master's in science, working with plants, specifically bioactive metabolites including some that have antimicrobial activity. So when he decided to go after graduate training, he was looking for labs that study host-microbe interactions, either plant-microbe, animal-microbe, and he was fortunate to be recruited by Nancy Moran who had been working with bees, aphids, and their symbionts. So as we saw in this paper, Eric focused on understanding how different environmental stresses, mainly pesticides, perturb the gut microbiota and of course, to do this work they had to work with bees. Hundreds of bees, which he said was an interesting challenge.

So first, before they could paint them, they had to immobilize the bees, so they chilled them out with cool temperatures. Once they were immobile and couldn't sting, then they would spend the rest of the day painting the thorax of the bees. So he said doing this by himself, experiment after experiment, would have been really tedious, so he was really grateful for the help he got from Casey Raymond, a former postdoc in the lab who is now a professor at University of North Carolina at Greensburg, who not only painted but also gave him constructive criticism in all steps of the project. They also were assisted by Zack Schaffer, a former undergrad research assistant, and he said it was a lot of fun and funny. Marking bees took a lot of time but Casey and Zack made the process go smoothly. They always were listening to music while they painted bees, and after a while the three of them decided they were bee artists. (laughter)

He was also grateful for help from Dr. Erik Quandt, especially cloning experiments he did to kind of link the phenotype to this particular enzyme. So now Eric is doing some followup experiments for this project before winter comes because of course they can't work with the hives. They have reduced brood production in the cold weather, and that's okay because he is homesick. After his productive year in the lab, he is going to have the chance to visit family and friends in Brazil this December. They'll celebrate Christmas together. He says they'll have a lot to talk about, including bees. So finally, Eric says he is passionate about the research he does and he believes that to succeed in academia, this is the first step: enjoy what you do.

Michael: And how.

Vincent: I am still puzzled about what to do with this, because clearly this glyphosate affects the microbiome. There is an effect on Serratia susceptibility, but how broad is that? The mildly resistant S. alvi is…

Michael: Are we gonna select out a community of bees that is resistant?

Vincent: What would you do? Would that be bad? I just don't know.

Michele: Or would that be good?

Vincent: Maybe that's one experiment to do, to select a microbiome in these bees that is resistant and then see how they fare, right?

Michele: Their fitness advantage, yeah. I mean, we have to remember we are not just caring about these bees, we rely on bees to pollinate our foods.

Vincent: It's wild bees as well as these hive ones.

Michele: So this is kind of the one health philosophy. We gotta bear it in mind.

Vincent: Boy, we mess things up, don't we?

Michael: When they did the test, they exposed lots of animals to the herbicide, and it didn't have any bad effects.

Vincent: Did they expose bees?

Michael: I don't know. It's been around for a long time. It's been around for a long time.

Michele: We've also been having bee colony problems for a long time.

Michael: Yeah. So the question is as this herbicide has become more ubiquitous in its use, is this responsible for the collapse that we are seeing?

Michele: And again, the authors are very careful and conservative in their language. They are not saying this is the problem but they are admitting that colony collapse syndrome is complex, lots of different stresses can contribute, but they are beginning to investigate a molecular mechanism for one, which hopefully we can then use to mitigate the problem.

Vincent: Well, I guess also, as we said, make some resistant flora and put them in bees and see how they do. That will be interesting to do. Alright. Very interesting, really cool, thank you Michael, thank you Michele. Let's just read three short emails, here, so we can get through some of them because we have a lot. The first is from Richard:

Dear TWIM team,

Thanks for all the great science. Thanks especially for the eureka moment when you recently described how a diminished and fragmentary intracellular bacterial parasite is distinguished from a giant virus. It would be delightful if an exception were found.

Richard

Richard is in Illinois. Michael, can you read the next one?

Michael: Sure. This is from Sonia, and Sonia writes:

Hello TWiM team!

I am so grateful to have found these amazing series!

Please enter my name into the book give away contest!

I have been working in a pharmacy as a pharmacy technician for the past 14 years and was always interested in researching bacteria, viruses, and parasites. I enrolled in university (York University in Toronto, Ontario, Canada) at age of 29 and am currently in my 2nd year of my biology undergrad. I am wanting to pursue a masters and maybe PhD in microbiology. This book would be of great interest to me! I wanted to thank the TWiM team for taking the time to make this series and others available for the general public! It's sad that microbiology is not in our high school curriculum I truly think the unseen world would spark interest in many young minds.

Thank you so much for all informative talks!

That's a lovely sentiment, and ASM through its education offerings on its website has exercises for high school that if you are a high school teacher out there you can download and incorporate into your lesson plans.

Vincent: Michele, can you take the next one?

Michele: Yes, it's from Mike.

Hello,

Thank you for always putting great effort and entertainment into the podcast. The Idexx VBNC question perked my ears up as I monitor wastewater and drinking water laboratories using the method. In my experience the Idexx methods have always seemed sufficiently sensitive (probably more so than membrane filtration alternatives), besides Enterolert. Hopefully I can win the book and learn some more about antibiotics here in sunny Hamilton, NJ where it is 29 degrees Celsius, and most importantly not humid.

Mike

(laughter)

Vincent: Well, today it is very humid here. In fact, I'm not too far from Hamilton, New Jersey, and all week it has been extremely humid. I don't know why, but for mid-October it is pretty weird. Alright. We have another email, we will save that for next time. That will do it for TWIM 187. You can find it wherever fine podcasts are distributed. If you listen on your phone or your tablet, you use an application, please subscribe to our podcast so you get every episode, and it helps us to have your numbers, to have subscribers, so we really appreciate if you can do that. If you use the podcast player Overcast there is a way to favorite episodes. We would appreciate if you could do that, as well. You just tap the star on every episode. And if you love what we do, consider supporting us financially. We would be grateful for your support. You can go to microbe.tv/contribute, we have a number of ways that you can do that. As always send your questions and comments to TWIM@microbe.tv.

Michele Swanson is at the University of Michigan. Thank you, Michele.

Michele: Thank you, all.

Vincent: Elio Schaechter is at Small Things Considered. Thanks, Elio.

Elio: My pleasure, thank you.

Vincent: We'll get you a new mic so you sound better next time. Michael Schmidt is at the Medical Univerity of South Carolina. Thank you, Michael.

Michael: Thanks, everyone.

Vincent: I'm Vincent Racaniello, you can find me at virology.ws. Thanks to ASM for their support and Ray Ortega for his technical help. I'd also like to thank Ronald Jenkees for the music you hear on TWIM. Thanks for listening everyone, we will see you next time on This Week in Microbiology.

(music)

Transcribed by Sarah Morgan