Carl Bradley

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SPEAKERS

Brandon Kleinke, Ed Zaworski

B Brandon Kleinke 00:01

I see that plants podcast shares the stories of people in plants, pests and pathogens and the conflicts among them. Join us as we speak to the folks who are helping the rest of us live healthier, more productive lives through pest management research. We strive to make science accessible. I see dead plants created by the Crop Protection Network and hosted by Ed Zaworski. The Crop Protection Network is a product of land grant universities.

E Ed Zaworski 00:25

Welcome back to the ICD plants podcast. As always, I'm your host, Ed Zaworski. And today, I'm joined by Carl, Dr. Carl Bradley. And just to give you a little bit of background on Carl, before we get into the subject matter at everything, Carl got his bachelor's in Plant and Soil Sciences at SIU in Carbondale, got his master's at the University of Illinois in Urbana Champaign, as well as his PhD in plant pathology. So how're you doing today, Carl?

00:58

I'm doing good. How are you? I am doing pretty good. Can't complain. Heat subsided a little bit. But talking to you earlier, it sounded like it might be pretty hot down there in Kentucky. So it's extremely hot. We really need some rain. And we really need this, this temperature to kind of just drop about 10 degrees, that would be great. How hot is it? Um, well, right now it's about 85 degrees. But it'll get up. It'll climb up close to 100 Today, I think. And so it's, it's not great. Yeah, it's a good day to try to work on some inside stuff. It's a great day to record a podcast. Absolutely, yes. So today, we're going to we are we're going to get into something a little bit different. We're

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not going to talk about a particular publication, but rather, we're going to tell the story of

fungicide resistance. And we're going to use a disease called frogeye leaf spot as kind of our our case study to talk about it. But before we jump in to that, I do want to ask Carl, like, you know, we talked about your background a little bit from from Illinois. How did you get where you are today? How did you get into plant

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pathology? Yeah, absolutely. So So I grew up in a little town called Ridgeway, Illinois, that's in the southeastern part of the state. The county, I grew up in borders, both Indiana and Kentucky. So that gives you an idea where it is way down there. I grew up on a on a grain farm, we grew corn, soybean and winter wheat. So I got accustomed to agriculture, through our our family operation really didn't know anything about diseases until this disease called Sudden Death Syndrome showed up in some of our soybean fields, back in the 1980s. And at that time, we were one of the fortunate or unfortunate, I guess, to be one of the first fields in Illinois to have sudden death syndrome. So and that, at that time, nobody really knew exactly what was causing it. So it really kind of bothered me that we had some really nice looking soybeans, and then, you know, they got to a certain stage and they just started dying. And, and that side, that was sort of what made me realize that there were diseases that could affect plants and crops. So you know, I went on, in, like you said, plant soil sciences at Southern Illinois University and worked for soybean breeding program. While I was there, I had some internships with a couple of different companies and, and decided that plant pathology was was what I wanted to go into. So I went to University of Illinois, and, and that's what I worked on. And so I've been working on diseases of crops for number of years now. Excellent. I don't know that I've had like, a real life a call to action story like that yet, or somebody, you know, you literally grew up, you know, facing this, this plant disease. And that's kind of what spurred you into. Yeah, I mean, I don't I, you know, I, it was, like I saw, it's like, hey, I want to be a plant pathologist. But that that is sort of what at least kind of triggered my mind and like, wow, you know, this is, this isn't great. And there's nothing we can do about it. You know, that was, that was sort of a shocking thing. And I remember walking down into the soybean field, where we had this big patch of soybeans that were that were dying, basically. And it just really bothered me that there was nothing we could do about this, you know, this is just going to happen. We were going to have to suffer those losses. And so I think that Did you know, as I look back, I think that did probably move me into this direction a little bit. Yeah. Excellent. Also, as we were talking about earlier, You know, Southern Illinois a lovely place, folks, if you ever get a chance to visit it, it's a very different part of Illinois than the Chicagoland area in Chicago suburbs, which I think, you know, most people think of maybe when they think of Illinois, so ever get a chance to go down there. It's a wonderful natural area. Yeah, Shawnee National Forest is in southern Illinois, there's lots of hiking, lots of fishing, lots of outdoor type things to do. It's a it's an I'm not too far away from there. Now actually, as, as I work for the University of Kentucky over in the western part of the state, I can flip over to Illinois to some of my routes pretty easily. Now. Was that was that by design? Did you want to stay close to home when you found your job at Kentucky or? Well, it was certainly a part of that decision making process. Yeah. Good deal. Good deal. Well,

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thanks for telling us a little bit about your your story and how you got into plant pathology. Let's talk frogeye I guess. Can you give us an overview of frogeye leaf spot as a disease? What

06:03

causes it? What does it look like? You know, all that good stuff? Yeah, absolutely. I can tell you maybe first I'll just tell you how I got interested in working on frogeye leaf spot. So I was I was a plant pathologist and a faculty member at the University of Illinois and prior to that, I was at North Dakota State University. And when I was at NDSU, up in Fargo, I worked on some fungicide resistance projects asked to kind of bite of chickpea, actually. And that was some of my first experiences with working on fungicide resistance. So when I moved to the University of Illinois back in 2007, I think I wanted to work on fungicide resistance. And that was at a time right when foliar fungicides were kind of starting to be marketed on soybean. And that was mostly due to the appearance of soybean rust. In the first the first appearance of soybean rests in the continental US which I think was 2004. And then it showed up in Illinois actually, in gosh, I think it was right before I came so I think it must have been 2006 showed up pretty late. So there was a lot of worrying about soybean rust. And then at that point in time, the only way to control it was was through fungicides as as, as we know, soybean rust did not end up being a problem for much of the US, it's still a problem in the southern US and parts, but it never really made it up to you know, some of the Midwestern states that grow a lot of soybean at least early enough to cause Machiel loss. So anyway, so I was interested in in fungicide resistance and fungicides were starting to be a thing on soybean. So I looked at frogeye leaf spot. So I knew that was a disease that would occur down in Southern Illinois some. And so we started gathering isolates and things like that and working on fungicide resistance. And then when I moved to the University of Kentucky in 2015, you know, it's a disease as you go further south, it's a disease that's that has been historically more important. And so I've continued to work on that particular disease in my current role as well. So yeah, frogeye leaf spot. It was first found in the US back in the 1920s. So it's been around for in the US for about 100 years now. But it took a while for really to be a problem in much of the soybean production in the US. Historically, it's been an issue down in the southern US. I remember going to a lot of meetings were Dr. Melvin Newman, who who passed away unfortunately last year. But he was a plant pathologist at the University of Tennessee and he was he was sort of the frogeye leaf spot guy he would he would talk about frogeye leaf spot a lot. Another plant pathologist, Dan Phillips, who is retired from the University of Georgia was another one that had done a lot of work. So that kind of gives you some idea of where you know where this disease had been important for a number of years. It's been moving northward, though, and in causing more problems in states like Illinois, it's ITT Tech. We've we found it all the way up in North Dakota with Dr. Sam Markel, who's the plant pathologist there had sent us some some samples a couple of years ago, and it's all the way in North Dakota now. So it's got a lot larger geographic footprint than it used to. Right. A lot of things I suppose at this point, right? Yeah, yeah, I think that's probably right. So it causes spots on leaves. I mean, it's called frogeye leaf spot. I think it's, you know, the ideas that those thoughts look like a frog sigh. I think that's a little bit debatable. I'm not exactly sure that that's that's correct. I was gonna ask you if you knew that because I never knew and I, yeah, yeah what separates a frog die from a different right. But it is a unique name. So that is you know, that is something that's that's kind of cool with it. It's caused by a fungus. It's called Sir prosperous. So Gina are so Dinah counts either way. And it affects soybean, it doesn't have a real wide host range, but it can affect some other species of plants as well. In fact, we've done a little bit of work on that that we still haven't published, it's one of those things that when I get a chance, we'll get around to publishing that looked at some different hosts that this can affect a little bit little bit more than what we thought actually, there's a few more hosts than than what we thought but soybean is the most important economically, same same symptoms on other hosts. I know you haven't published it yet, but I'm just curious. Yeah, it can be on some hosts that there's also some hosts where we can we well, some of the work that one of my former postdocs had done when I was at University of Illinois, she had found that we

could take it could infect, usually using some molecular techniques, she could find that that fungus could could actually infect some other plants that may not see symptoms. So there, there's also some asymptomatic posts as well, apparently. But yeah, on the on the hosts that it does cause symptoms, the symptoms are pretty similar to what you see on soybeans. And which those those symptoms look like kind of a tan, or light gray colored lesion or spot. And then that spot is surrounded by kind of a dark border, usually kind of purplish colored border around that spot. And it's there normally, you know, they can be kind of circular, but lots of times they meet may be sort of irregular, ly shaped. And so those can occur on the leaves on the pods. And on the stems, they look a little bit different when they're on the pods and stems, but the leaves are where you typically will see those symptoms. And soybeans can actually withstand a pretty good level of of leaf damage, you know, a lot of a lot of entomologist sort of work this out as to how much leaf damage, how many, how much area can be gone, due to insect eating or whatever. And, and still have a decent yield. And so it actually takes a bit of lesions to kind of cause yield loss, but it can certainly happen. So, you know, for some of my work, and you know, this probably isn't anything official, I guess. But looking at some of our fungicide data, it looks like anytime you get, you know, over 10 to 15% of that leaf area, in the upper canopy being affected, that's when you're probably going to have some level of loss that might be noticeable when you're out there harvesting stuff. And we've had disease severity in some of our research trials up to about 50% of the area affected in the upper upper third leaf. So it can be pretty severe on susceptible varieties. So there are different levels of resistance in soybean varieties. And, and we still have some susceptible ones out there. So it can be a pretty important disease. As I said, it's been historically more of a southern disease. And that's mostly because I think of the weather and the humidity. So really, you know, high humidity, warmer temperatures, anything that's going to keep dew on those leaves, those leaves wet for an extended period of time, it's really going to help that fungus in fact, and for that disease spread and be a problem. So as you go up and go up a little further north, where you may not quite have the humidity, or the warmer temperatures, this disease does not tend to be a problem. But as I mentioned, we are seeing it in areas that we haven't seen it before. And I think that probably has to do with at least you know most likely weather is is changing. I think we can all agree on that and things are getting warmer in places and that's part of maybe why we're seeing this. But this fungus now also has fungicide resistance to specific chemical and that may give it a little bit of advantage as well on being able to compete with some other microbes that that may still be sensitive to this particular fungicide. So So yeah, that's a little rundown on frogeye leaf spot. That's a that's a great segue Carl. I was just my The next thing I was going to ask you about is could you kind of lay out for our listeners. What is fungicide resistance in general, what is it? How does it happen? And what what are obviously issues with having it? Yeah, so I think, you know, fungicide resistance could be defined as, as when a fungicide is doesn't work as well as it used to. So that may mean that it just does not work at all, like, you know, this, this fungicide use to control this disease caused by a fungus. And now, it does not work at all. Or maybe it might also mean that it, it still works. But it doesn't work as well as it used to. So there's a couple of things there that have that, you know, it goes along with that, how it happens, it's, it's sort of basic biology, you have to have some genetic diversity in the fungal population. So let's, we've been talking about frog every spot, right, so let's just kind of continue down that line. That fungus is called Sir Cosper. So Gina, we know that there's a lot of genetic diversity in that, in that pathogen populations. So that means that

E **Ed Zaworski** 16:06

you know, if I were to, to take a leaf in from one field, and Kentucky and isolate that fungus from that leaf, and then maybe go to a different perhaps even in the same field, get a different

leaf and make an isolation there. If we looked at those two isolates, genetically using some molecular techniques, we would, we would find that, that they're probably not going to be the same, they're not a clone of each other, that they're, they are genetically different in some ways. So when you have a pathogen population that has a lot of that genetic diversity, that's, that's part of the, of the of the, of the thing of fungicide resistance. The other part is, has to do with the fungicide. When we apply a fungicide, we apply a pressure onto that pathogen population. And, and we're select we're, you know, we're controlling a big portion of those, but we're also selecting out some of those that are just a little genetically different, that may be less sensitive or resistant to that fungicide, that's called selection pressure. And so a lot of that has to do with the the type of fungicide that we're applying, we do have some fungicides that are what we call multi site, fungicides. So they act on the fungus and a lot of different ways. Okay, in sometimes we don't even have all those weights figured out. We don't have very many of those types of fungicides anymore, but that there are a few that we still have. And you know, when you have a multi site fungicide, it's less likely to select out individuals in that pathogen population that are resistant. That's because this fungicides working on that, that fungus a lot of different ways, right, so maybe it can overcome a little bit of one way, but it's got these other ways it's attacking the fungus. A lot of our fungicides now are sort of single mode of action. So we know how they're they affect the fungi. And lots of times it's just in one particular spot. And in one particular way, right? Right. So when you have that situation, and you have a really genetically diverse fungal population, then that's sort of the worst case scenario for fungicide resistance, that's where it's going to be highly likely. And that's kind of where we are with a lot of our fungicides like our strobe alert and fungicides, for instance, we also call those the QI are quite known outside inhibitor fungicides. And when I was talking about fungicide resistance in the frogeye, leaf spot pathogen, that's the one I'm really talking about. That's, that's the one that we've identified and have found in a number of areas down. Could you could you talk about that a little bit like, what is a QI or show boiler and what does it do to the to the fungus? Yeah, so basically, like I said, it's a, we call it by a couple of different names. Strobel urn is sort of the the name that we use when I talk to farmers. And, you know, when chemical companies are sort of marketing their products, you know, at least in the corn and soybean and wheat world, that's what we kind of have referred to referred to them. They're also called Qi or fly known outside inhibitor fungicides. It's the same group. And just to throw in one other term here, there's a group called the fungicide resistance Action Committee, and that abbreviated that's FRAC fr AC and the threat has developed some codes to identify the different chemistry classes that we have in fungicides. And that particular code for for struggler ions is fret group 11. So that's another way to classify these. These work on the fungus by inhibiting respiration. So fungal respiration So that's that's kind of how they worked on the fungus. So, yeah, and you know, and the problem is, it's just one little single mutation that has to happen one little nucleotide shift, which ends up meaning that you have a different amino acid in a certain certain place. And that one little shift means that you now have resistance to the strobe learning fungicides. And as I mentioned, there's so much diversity and a pathogen population likes to prosper, So Gina, that those shifts happen naturally. So it's not like the fungicide causes that mutation, right? That mutation just happens naturally, I mean, that mutations happen in fungi, about, you know, one in 1 million. Okay, that seems like a lot. But when you think about like a soybean field, let's say you have like the 40 acre field or so right? You know, you've got, you know, these these lesions, these spots on soybeans, and within those lesions, spores are created. And so you start doing the math and like, 1 million, you know, one in 1 million is nothing, you know, that's that's easily can happen. And so you're gonna have these natural mutations that are occurring out there. And some of these natural mutations will be this, this amino acid shift this nucleotide shift that turns into a different amino acid. And so when you apply that fungicide, then you're selecting out those individuals and over time, you know, at first you don't notice it, and it's, you know, it's out there. This struggler and fungicide is still

working great. Like wow, this, this has been, you know, this is the best thing I've used to control frogeye leaf spot ever. But you keep doing that year after year, and it may be year two, it may be your three, it may be your six, it's you know, we don't always know. But eventually, you have selected out enough of that resistant population where the majority is now resistant. And when you spray that fungicide, it is not working, maybe at all or not as well as it used to. So that's kind of how this happens over time. For for those of you who are visual learners, there, Carl turned me on earlier to some great resources. So there's like a, what was it the FAQ the fungicide resistance FAQ, and Crop Protection Network and out anything we talked about, I'll obviously post links in our additional resources section. But there's a good visual there that kind of illustrates this. I think that you know, you start off with a diverse population. And then as you apply these selective pressures, applying the fungicide like Carl says, you end up with a completely different population, one that's not being affected as much or not being affected at all by these fungicides.

B Brandon Kleinke 22:52

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23:05

So that's I think that's a good overview of resistance. Can I flip you back to Frog guy pretty quick? And can you give us like the, you know,

E Ed Zaworski 23:13

we talked about frog eyes disease. Now we've talked about resistance. But when did we start? What's the history of resistance in frogeye? Like, when did we start seeing I know you mentioned kind of a first appearance of it, maybe. But well, or were you saying no, no,

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you're talking about where frogeye first popped up. But where did resistance first happen? And kind of how has it progressed over time? And where are we now? Yeah, yeah, great question. So I'll go back to my story. I guess when I clicked to the university or started working as a faculty member at the University of Illinois and working on fungicide resistance. I think 2008 We started a project that was at that time funded by the Illinois Soybean Association. And what we did is we developed a baseline sensitivity level of the struggler in fungicides. Our other costs were So Gina to the struggler in fungicides, so we had to take isolates that had never been exposed to struggler and fungicides. And so, I had mentioned Dr. Dan Phillips from University of Georgia earlier, he had a collection of circuits for so Cina that had started back in the I think maybe the 1970s or 80s, which was prior to struggles or in fungicides ever existing. So we were able to get that set of isolates and and then look in the lab at the sensitivity of that what we call a baseline population to the struggler and fungicides and so we were able to see you know, that this baseline population was sensitive at I don't remember what part per million

but anyway, we had a part per million of like azoxystrobin, which is a struggler and fungicide of that baseline population. So what we started to do after we developed that baseline was to go out into the field Old and collect isolates from collect samples and then get isolates of the fungus from across from Illinois. But then we also asked our colleagues all over to send us samples and in 2010, so we did that a couple of years and then 2010 weeks that we got our first samples where we had isolated isolates of the fungus that were resistant to struggler and fungicides from some of our petri dish assays, so we would basically have different concentrations of the fungus, part sorry, of the fungicide in, in kind of mixed in the in the media in the agar, of the of the petri dishes. And then we would then take these spores of these isolates we collected and spread it out, we'd look at at spore germination. And I like to say we, because it was really like my, my graduate student that was doing this, but But anyway, so pretty tedious work. But we found that in some cases, some of these spores just germinated and acted like that fungicide wasn't even there. And then we did some things to confirm that, you know, this, these were truly resistant. So these came from Tennessee, West western Tennessee was some of the first isolates that we found. There are a couple of counties in Tennessee where we found it in 2010. And then actually on my home farm, where my dad and my brother were farming at that time. And in southeastern Illinois. There was a there was an instance of we were found isolates that were resistant in that farm. And then my dad, right, yeah. The interesting thing is my dad had not really been spraying fungicide. So you know, that just goes to show you that they were there naturally. But you know, there were farmers in the area that were spraying fungicide, so there was some selection pressure that was happening there. And then my predecessor, Dr. Don Hirshman, who's, who was at the University of Kentucky, I had also sent me samples from from Western Kentucky. So those were the three areas that we found it in 2010. And so at that point, time, this turned into the United Soybean Board project that was USP was funding it because it became bigger than Illinois, it was it was the kind of multi state and this was something that was becoming more important. And so we continue to look and, and then other labs were doing the work as well, Dr. Tom Allen Downs, Mississippi State University, and then Heather Kelly at the University of Tennessee where a couple of others and and now there's others that are doing it as well. So that if you kind of add it all up, where I think we're over, we're over 20 states, it might be 22. States, I'm not sure if I have the number correct, right off, but it's over 20 states now that we found a strobe learn resistant isolates for customers. So Gina, so, you know, this is the started back in 2010. So we're about 12 years into it. And, you know, over 20 states, so it's kind of crazy. I'm assuming that it's mostly in the southern US, but how far north and west has it moved? I guess. Yeah, it's so it's pretty much where soybeans are grown now. We're refining it. So yeah, you know, up in North Dakota, Nebraska. Canada, and Canada. Yeah. Alberta nuda, who's a plant pathologist up there in Canada? Don't don't think they've published it yet. But they were kind of scooping. Oh, well, that's okay. Albert, I think as we get getting the message out. But yeah, we we've provided some some advice on how to do some things. And it looks like they've they've been able to identify it up in Ontario as well. And then and then we haven't published this yet. It's it's in the works. But Dr. Gary Bergstrom from Cornell University had sent us some things last year. And it looks like we've got resistance in New York as well. So basically, as far east as New York is part West is I guess, I'm not looking at a map we're asking Nebraska, North Dakota. So yeah. And then, you know, again, I'm not looking at a map. So I think yeah, New York might be further east, the North Carolina and some of those other coastal states, right. Anyway, it's it's, it's, it's spread across the soybean growing areas, right? Pretty much and what we find now at least around me in Kentucky and you know, states around me is that it's really easy to find the resistant strains. So if you have frogeye leaf spot, it's likely that some of those the isolates that are causing that disease are resistant to scribbler and fungicide. So

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very widespread at this point time. So, I guess from there, why are these fungi and I'm assuming frog a Probably not the only one with resistance to Qi at this time, right?

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Yes, that's right. So yeah. Why are these fungi becoming resistant to puis? Is there? You know, was it that this chemical was like

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early developed? Or was it the use the most to use more frequently? Why? Why would you say? Well, so, you know, soybeans were not the first crop that struggler and fungicides were used on and, and actually, it didn't take very long when those struggles are in fungicides were first registered on some other crops where fungicide resistance started to occur. And so a lot of this has, like, you know, its fungicide resistance is made up of two components, things that happen in the pathogen population, and then characteristics of that particular fungicide. And so in cases where there's, you know, a lot of genetic diversity in that pathogen population, that's important part of this. And then the other part is the fungicide. It says it's a single mode of action, it's very targeted. And so it's, it's makes it relatively easy for that fungus kind of overcome it genetically. So that's, that's sort of what's happening here. Dan, to go along with that. Edie, though, that's a great question you asked because, you know, some of the people are listening in might might remember some of the first fungicides that were being marketed on soybean, and those were all struggling fungicides. So, when we, you know, we actually didn't have we had very few fungicides registered on soybean in the early 2000s. And at some point time in the early 2000s, we had headline and Cuadras were registered and both of those are struggler in fungicides. We had some section 18 Emergency exemptions for some of the triazole fungicides because of soybean Ross and a lot of those then kind of rolled over into section three regular registered registrations. And so but but there was struggler in was was either you know, there was there's a lot of people that were just spraying the street struggler in product, or they may have been mixed with the triazole at one point time. Now we have some more mixes out there. But that's also the other thing is, is you know, if somebody was going to use a fungicide, it was probably going to have a struggler and fungicide in it. And you kind of start doing the math over how many acres of soybean we have. And, and even though not every acre is sprayed with a with a fungicide, there's still a pretty good portion that are and, you know, that's that's a lot of potential exposures happening and a lot of selection pressure and a lot of selecting for these resistant isolates. Gotcha. Yeah, that's kinda, I hadn't thought about that. Yeah, I talked to Ed Secura. A while back about the sweeping rust history. And yeah, he mentioned that there weren't Yeah, what you just said there weren't a lot of fungicides labeled for soybeans at the time. And so yeah, that's that's just kind of interesting. Because you said what 19? You said 1920 was the first time we saw frogeye? Yeah, like 1924, I think was the first time it was reported in in the US. And I think that was in the South. I can't remember which state it might have been South Carolina, but I don't remember now. But yeah, it's it's sort of that disease has been around in the US for 100 years. Yeah. Yeah.

E Ed Zaworski 33:31

It's that, I guess. Yeah. I hadn't realized in my, my relatively short career in plant pathology. Yeah, it's the disease has been around a long time and soybeans have been around, but fungicide applications weren't, I guess, as popular until relatively recently, in what history of us growing soybeans, right. So yeah, really,

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in the last, you know, 1015 years as that become more mainstream, makes it even a little crazier than that it all happened so fast. And now, you know,

E Ed Zaworski 34:03

I suppose, you know, I don't know, apples or an apple production very well, but I would imagine that, you know, something like that, it would be even crazier. For folks that don't know, like apples and apple production. There's a lot of a lot of spraying of fungicides, you know, many, many, many times in one season, so it kind of even ramps up a situation like this with resistance even faster. But anyway, I don't want to get too far down to a sidetrack there, but um, so, yeah, that kind of got into I think, the next question I would have asked you was, why are there so many instances of fungicide resistance happening in recent years, but I think, you know, that kind of a kind of gets into it. So you he talked a little bit about how you determined if a if a what, let me get the terminology here. The Not the race, but the the isolate, sorry, the Isolate frog I was resistant to Q allies. Yeah. You mentioned that you use like, Would it be the poison plate assay? Is that the, the determining the concentrations? Or is that something else? Or?

35:17

Yeah, no, that's one of the wait. Yeah, so we've got a little more efficient in our methodology now, but when we were first doing this, it was the poison plate assay. And, and what we would have is, we would, for one, one isolate, we would have, I think, six different concentrations, that we would test that isolate again. And when we do that, that lets us calculate what we call an EC 50, which stands for the effective concentration, in which you get a 50% inhibition of the fungus relative to a, a non treated control. So if so that was a lot of work, that was not very efficient to do that. But that was sort of necessary at the beginning. And then we developed something that we that we use now a lot more often is just the discriminatory dose. So what we found, by doing some of that work across all these different concentrations, we could kind of figure out, okay, so we'll use this dose of this particular fungicide that sort of characterizes anything that still grows, is going to be resistant, anything that doesn't grow at that concentration is going to be sensitive. So that's a lot quicker way. Now we can just use basically one plate or a couple, we also use a control so, so two plates, to kind of figure that out, rather than, you know, half a dozen plates. So there's also some molecular methods that we can use, one of my postdocs had developed a specific primer. So you can use PCR and use a specific primer to determine if this particular mutation is present. We call this the mutation that we find in insert customers. So Gina that causes this resistance is called the G 143. A mutation. And that's actually when fungi are resistant to struggler and fungicides, that is the most commonly observed mutation that's out there, there are two others that can that can that can be there as well, once the F 129 l. That's found sometimes in some fungi. And there's another one called

the G 137. r, which I think so far is just sort of been found in the lab. But the G 143 A is the most most commonly observed. That's what we've got in this particular pathogen. And that unfortunately, that mutation also means that it's highly resistant. So you could, you could use like 100 times the regular dose of a struggler and fungicide and it still wouldn't really work. So, so so we can use molecular methods. And then we also just use sequencing to so so we know what part it's all these mutations occur in what we call the site of the cytochrome B gene of the fungus. Okay, and so we now have some primers that let us kind of cut out, you know, a particular part of that cytochrome B gene. And then we can sequence it. And then we can do some alignments, and look for, we know where those mutations occur. So we can look for all three of them now. So there's a little bit more streamlined ways that we can look for this, these particular types of resistance now, that's really interesting. That you are, you're asking all my questions for me, I'm just sitting here and listening. No, I

E Ed Zaworski 38:43

was gonna ask you, you know, is the g 143. A, is that the only mutation and but the fact that it's the most prevalent is really interesting, right? Because it kind of, I mean, tell me if I'm wrong, but to me, it kind of suggests that what that there was that this pad, this resistant population of the pathogen has kind of moved around quite a bit, right? Like, if, if we have the one mutation, do you think I know I'm just spitballing? And I'm asking, I'm gonna ask you a wild question. Now.

39:15

Do you think that it happened? And it's kind of centrally located somewhere, you know, a back in 2010 and then kind of radiated outward? With that same generic question? Yeah, we had those same questions. And, and we weren't the only ones. I think Dr. Heather Kelly, Tennessee has kind of done some this work too. So we have some work that we had done while I was at the University of Illinois kind of asked him that question because we wonder too like, hey, maybe this happened. Maybe this happened in that field in Tennessee and you know, and then like, he's like sluts kind of went out. They got up you know, they, you know, this is not a disease that can move like a like a soybean rush or southern rust of corn, right, but it can move a little bit you know, it these spores can can move over. little bit. And so you kind of over time, you kind of look at that. And so maybe this happened. And then somehow this fungus these particular isolates just kind of spread out. So what we found, though, is, you know, we took some isolates from some different areas that were had the G 143, a mutation and, and then kind of looked at the genetic diversity using some different molecular tools to do that. And what we found is, even though they all had the same mutation, or fungicide resistance, is that in other ways, they were genetically diverse. And it was, it seemed unlikely that it originated from one place that these were probably separate selections that occurred elsewhere. And remember, you know, this is a highly diverse fungus. And these mutations happen naturally. So these were probably just happening out there. And it really took the fungicide to select those individuals to become more prominent, and that these selections were probably happening independent from each other, and occurred in different places. So we don't think that it was a case where this happened one place, and then this thing kind of got moved around different ways. We think that these collections were taking place independently, and happened in different places, if that makes sense. Yeah, it's that's really interesting. You know, like, yeah, just spatially, they're kind of having a similar while they're having the same mutation, right, but

for the similar outcome. Yeah, cuz yeah, exactly. Well, I can't remember off top, my head is sick. Aspera. So Gina, Can it move via seed, as well? So it's possible. Yeah, it can be seaborne, we have done some work. Looking at how, how well it transmits from seed to seedling so that for instance, if you planted a seed that was infected and and planted it out in the field, or in our case, the greenhouse, how often would that turn into a plant that ended up having a leaf lesion? Right. And so we found that that was an extremely low percentage. And although you know, when you start doing the math of you know, how many soybean plants are in an acre soybean, yeah, okay, maybe maybe to do that. But I, we don't think that that really probably played much of a role in how it's spread. You also nowadays, it's hard to find soybeans that you're planting out in the field that aren't pink, or green or purple. Right, that have these different fungicide seed treatments on them. And in lots of times, there's multiple fungicides that are that are in those seed treatment mixes and some of those would have activity against sir Cosper. So Gina, so we don't think that that's a way that's really contributing much to its spread. Gotcha. Well, thanks. Thanks for addressing my random curiosities. And thanks, folks, for bearing with me and listen. Okay, well, can you Carl, could you describe some of the research that you're that you've been working on that you are working on that you plan to work on with regards to resistance and frogeye? Yeah, I mean, we kind of touched on it a little bit. So, you know, we've my lab has sort of continued to be a place where you when states have not yet observed resistance to strike struggler and fungicide resistance. And yet, in the frog, at least, but pathogens. We are, have kind of served as a hub. So we're kind of helping do that. Also teaching other labs how they can do it on their own to that, you know, strobe learns aren't the only fungicides that we're using now. Right. So we're also using a lot of triazoles. Those are also known as the methylation inhibitors or demise. Frag group three. We're also now starting to use them SDH eyes are fractured seven, and there's still some benzimidazoles out there, that's prac group one, actually, it's the only we only have one of those it's diabetic methyl and it's the active ingredient that's in Thompson and T methyl and others a lot of other products that that that use, if any methyl so we have these three other chemistry classes that that are now being used and you start thinking about okay, so I'm a I'm a farmer, I've got brought I'm worried about frogeye leaf spot size, spray a fungicide, and let's say I spray a fungicide that is a struggler in triazole mixture, right and I'm still getting good control, but we know that that struggler is not working right. It's really literally doing nothing to control frogeye leaf spot out there. So the triazole components was doing all the heavy lifting. So now they're A lot of selection pressure with the triazoles. Right? So we're worried about how long they're going to stick around. So that's where some of my work has shifted to is looking at sensitivity to triazoles. We're also looking at the H eyes. And like I said, the benzimidazoles. So we're our work is shifting a little bit in that direction, we've we've done some of that baseline sensitivity work I talked about earlier. And I guess the good news so far is that we haven't seen any major shifts and sensitivity. One thing about resistance to triazole, you know, so when you think about the struggle around, it's like a light switch, you know, it can go from this is working great, great. And to like this no longer works. It's like a light switch, you know, it's just that one single little one step mutation that has to happen with the, for resistance to trials, that's actually more of a multi step process. And that happens over years. And so it's sort of like a decline in efficacy over time. Okay, and it's like a slider instead of a light switch. Yeah, that's a slider. That's a good way to go. Yeah, I'm gonna use that. That's great. Yeah, that's great. Yeah. So. So we've been looking at that over time, because you have really have to take over time to look at that, right. And we have not really seen much decrease in sensitivity. But I do have a graduate student who's taken a bunch of multistate fungicide data, and looked at the efficacy over time. And if you graph that out, it does show that with the trials that they don't seem to be working quite as well as they used to over time. So we're kind of in that area. Now. We're, even though the trials are still working. You know, we think that there may be some, some sliding happening there on that light switch, that maybe maybe, you know, in another 510 years, we're going to

see that they may not work as well as they used to. So, you know, all this really means like, what I tried to stress to when I'm out talking to farmers and others, in ag industry is that, hey, let's not forget about all these other tools that we have in our toolbox. In managing diseases fungicides is not, that's not the only tool we've got, it's one of a few tools. I mentioned earlier on that there are resistant soybean varieties. So we have varieties that have a pretty high level of resistance to frogeye leaf spot. And if you're in an area, where you, you know, you've noticed that you get probably at least about that, that's one of your major diseases, you really should be selecting varieties that have a high level of resistance, okay? Crop rotation is very important, this pathogen overwinters and soybean debris. So if you're rotating away from from soybean for a year or two, that's going to help that that soybean debris break down and along with that, that fungus will break break down with it. And so that's going to help. So that, you know, those are some other ways to manage planting date can have an effect, sometimes we find that later planning swiping fields might have a little bit more disease. But that's, you know, that's something that we don't always control. I know farmers try to plan as soon as they can. But Mother Nature kind of prevents us from doing that same year. So but yeah, and then and then using fungicides only when we need to. Right. You know, there's there's a lot of talk about fungicides just giving a yield bump, a yield response, right. And not always kind of putting that disease component in there. And there are, you know, some of these fungicides can have some physiological effects on plants. There's no doubt about that, right? That's scientifically shown. And sometimes those physiological effects can have an effect on yield, take away the disease situation, sometimes we can see a yield response to a fungicide.

48:57

What we find in, in our research, when you take it to the field, and I have so many data points now from Illinois and Kentucky, for over a dozen years. And what we find is when we get those big yield responses, it's usually almost 95% of the time it's due to having a disease problem, right? Right. And there's a little blip occasionally, where we get a yield response that would be profitable enough to pay for that fungicide, where there was very low disease pressure. So the odds are with you on getting that profitable fungicide application is when you're really thinking about disease. So if we're only applying when we really need to, and, and not applying this all the time, that's going to help keep these fungicide products around longer. So we're not going to get as much exposure on that pathogen population when we're only using these when we really need to. So that's, that's something that I preach as well and that's going to help us keep these fungicides around for a longer period of time. How would a girl or know if they if they needed to play devil's advocate on you? But yeah, what is it? Is it simply get out there and scout? Or what would you say are Scouting is a big part of it. You know, something, Scouting is not the only thing you have to think about risk as well, okay? I think sometimes I feel like the entomologists have an advantage, they can go out with sweep nets, and they can say, Oh, I've got you know, this many insects, right. So we can't really we can do that with spores, but you'd have to, you really have to have some specialized equipment to trap scores and be able to identify and count them. And so we're not able to really do those kinds of things. But you can see that the level of disease that's, that's on a plant and, and I think it's important to scale it, you know, once you kind of go switch over into those are stages, those reproductive reproductive stages to r1 would be flowering, that's a good time to really start scouting, we have found that if the most profitable fungicide applications are usually right around our three, that early pod development, so that gives you a little time to make that decision, if you are seeing some disease out there. That can give you an idea, if you're just seeing nothing at r1, it's very unlikely you're suddenly going to have an epidemic of frogeye leaf spot, this is going to be a huge problem that you need to spray. Right. Right. The other thing is thinking about risks,

though. So some of those things I mentioned, well, you know, the disease triangle, so we can't have a plant pathologist without talking about disease trying right. Disease trying disease, strangle, you got the susceptible host, which is soybean, in this case, right, you have the pathogens present. So, you know, we know that soybean fields, it's probably going to be there. The biggest part, though, is whether like we have no control over that. But in this case, you know, very humid, warm weather on a rainfall, you know, anything that's keeping those, those leaves wet, moist for extended period of time would be important. So thinking about risk, there's actually a, what we call a score sheet that's available on this website called I will take action.com. And I think you're going to have that listed in those resources. But you can go to that score sheet. And you can you know, it's got questions like, you know, is this field rotated? Is this a susceptible variety? You know, kind of all these things, and you can kind of assign these points based on your answer. And if it gets to the certain threshold level, then then then the risk is greater for disease, and you might need to spray. So there's some things like that, that you can do. But I think for me, most important is the variety is in a susceptible variety, or is it a resistant variety, and then the weather? Probably two of the most important things and then followed by continuous soybean versus rotated soybean. Gotcha. Those are things to consider along with scouting. So, I got another question for you if, you know,

E Ed Zaworski 52:58

there's any growers listening, and I hope they are, and they wanted to see if they you know, if they have frogeye consistently and they wanted to see if they had a resistant population. Are there any resources for them available to

53:12

check that have it tested that kind of thing? So right now, there's, I don't think there's any commercial labs that are that are doing these testing. So most of it are individuals research projects, right. So this is where it would be important to get to know your, your extension personnel. So I know not every state is the same here in Kentucky, we we still have county agents when I worked in Illinois, that was a little bit different situation and more of a regional thing, but you can you can talk to your local extension and they can they can they can get you to your your soybean disease specialist in the state. And and that would kind of be the route to take. I will tell you though, that it's if you've got frogeye leaf spot in your field, no matter where you are in the US, it's highly likely that some of those isolates are resistant to strugglers. Okay. So, and really, as far as fungicide resistance management, we really need to think about this before we even have the resistance out there. So the idea is to slow down that resistance developing. You can't really keep it from happening because it's mother nature, but we can slow it down. So you know, doing things like only spraying a fungicide when you really, really have to managing diseases using some of those other practices that I mentioned. And then you know, when you if you decide to spray a fungicide, be sure that it's a product that contains different modes of action, different chemistry classes, or you can take mix you can also take mix here and there. You can get different products that are maybe single active ingredient and take mix those together. Another really good resource Ed is the fungicide efficacy guide on the Crop Protection Network. so that that can not only show you what products work against certain diseases, but there's also some color coding that's on there and some different things on there that shows you what chemistry classes or fungicide groups each of these belong to. So you can kind of say, Oh, well, here's a product that works here. And you can look over and see,

oh, well, gosh, that contains three different active ingredients from three different chemistry classes. That would be a great one to use, because I'm going to get really good efficacy, but it's also going to help with some of that fungicide resistance management as well. Yeah,

E Ed Zaworski 55:34

if you guys haven't listened to the podcast that I did with Dr. Kiersten wise, a few episodes back at this point, go check that out. We talked about that fungicide efficacy guide. And, of course, we'll have it in the link here as well, it'd be a great tool for helping you with this.

B Brandon Kleinke 55:54

Are you tired of seeing dead plants everywhere? Wish you could help but don't know, how do you sometimes wonder what life would have been like if you had gone to school to be a plant doctor or an insect expert? It's not too late, make up the last time but he's hitting the Crop Protection Network for your pest management information. Remember, every plant dies, but not every plant truly lives.

56:15

Carl, are there any other? What are some of the other diseases, I guess that are being affected by resistance that you know of? I mean, we've talked a lot about frog guy, but I'm just curious. I mean, I'm not. We don't need a comprehensive list. But just for our listeners if they might be curious frog eyes, not the only thing Correct? Yeah. So sleeping. There are some other pathogens that we now know there are resistant to struggling and fungicides and I will give you a comprehensive list. Okay. Didn't want one. I'll take one if I can get it. I just don't want to put you on the spot. No, no, no, I think I have them all. So the pathogen that causes for cost per leaf whites, that's a difference for cost per then the one that causes frogeye leaf spot. There's been resistance to to the Scribbler, and fungicides that have been reported in Louisiana. We actually in Kentucky, we actually sent some samples to some LSU researchers last year, and they did some testing and now we know that some of our isolates are resistant to scribbler and fungicides in Kentucky as well. The septoria brown spot pathogen pathogen septoria glycines is the same. Not too long ago, we published a paper where where we were able to identify resistance in Kentucky and Illinois. And then Dr. Heather Kelly from Tennessee and sinister my slits, and they were some of those were resistant. And Dr. Darren Mueller at Iowa State had sent us some isolettes as well. And we found those resistance so so there's that the target spot pathogen poor and desperate, because sicula that's one where it was resistance to struggle, earnings was first reported in Alabama. And now it's been recently reported and a few other southern states we can throw Kentucky in that list as well. So there's about a half a dozen states where that's been reported. And then the the I think the last one or I don't think I'm forgetting one, there's there's a disease called rhizoctonia aerial blight. And there's been resistance to the struggler and fungicides in Louisiana. to that to that pathogen as well, I should also mention that for prosper leaf blight pathogen. In Louisiana, they also did find some resistance to frack group one the, the benzimidazoles, as well. So so there's some different things happening out there, I think, probably at least what's probably getting the most press because it's been, you know, over 20 states and, but there's, there's these other pathogens that are that are being resistant and in the background as well. So we're finding that the

struggle learn fungicides over time are not working as well as they used to just because of resistance building up not only in the frog, at least pathogen but in some of these other bullier pathogens as well.

E Ed Zaworski 59:07

Yeah, it's it. It's a good cautionary tale the story of frogeye leaf spot on it's something it's real folks. fungicide resistance is real. It's becoming more prevalent, like Charles mentioned, you know,

59:19

it's on more diseases than just one on many actually. So be aware and in, like Carl talked about take those steps to help mitigate the development of resistance, Kenny. I'm gonna brainwash all my listeners at some point IPM IPM IPM, integrated pest management. Silver Bullets are oftentimes they might be good in the short term, but in the long term, use it an integrated approach of all those different management practices is the way to go based in my opinion, I

59:51

think CRO would agree 100% Yep. Well, with that, I think we've we've covered Resistance pretty good. We've covered frogeye. And I guess I'll ask you my closer question, the tough one. So

1:00:10

what is this? What does fungicide resistance and your research and what's happening with frogeye? on soybeans? What does it mean, to the general public? Why should they care? What can I do about it? You know, that kind of thing? Yeah, well, you know, I think soybeans are extremely important crop to us, right? It's economically important. There's, there's, I think, last year, the US produced over 4 billion bushels of, of soybean, that's, that's about a third, a little over a third of, of the, the soybeans produced in the world. Okay, so economically, soybean is extremely important to the US, and, and you may not be a farmer, right? But you think about farmers spending money, right, they have to make money to spend money. So all of this contributes to our local economies, and then the bigger picture, right? Food supply, soybeans are a very critical part of our our food supply. Now we're, you know, a lot of us may not be eating straight up soybeans, but there's a lot of products that we use that have soybean in it, soybean oil, you know, so many others, that that we're using products, soybean is also used as far as food for livestock. And if we can, if we're eating pork, or or beef, you know that this is all that's important for all of that as well. So you start thinking about food prices, how does this affect what you're paying for, for food at the grocery store. All of this is related, believe it or not, the fungicide resistance and how good of a job we're doing to manage diseases, how much money farmers have to spend on managing diseases. So if we have products that are no longer working, that means that we have to go to other management practices, we may have to spend more money on certain ways to manage this disease. So all of it eventually could could end up being a problem in people's pocketbooks. Just your local consumer that's buying food at the

grocery store, right. So I think we can make a lot of connections that way and the work that we do, but soybeans being such an important economic crop to us that it's extremely important in how we how we produce that and, and how we're able to sustainably produce. So if we were losing a bunch of products, things are no longer becoming, you know, no longer working. That kind of reduces our sustainability as well. There you have it, folks. Let's try to be a little bit more sustainable. With that, Carl, it's been wonderful having you on, I'm very grateful that you were willing to do this. At this point.

E Ed Zaworski 1:03:04

You have any acknowledgments. People you want to thank organizations you want to mention. Resources, you want to mention any of that stuff, you have the floor, go for it. Oh, great.

1:03:15

Yeah. Thank you so much. Well, obviously, I'm one person and I, there's I talked about a whole lot of work that was being done and, and so I probably cannot, I'm running, but I attempt to acknowledge everybody, because I'll leave people off. But certainly there's been graduate students and postdocs in my lab, both at Illinois and now at University of Kentucky, who's who's contributed to a lot of the work that was talking about, but also my colleagues. So you know, I have had so many colleagues that have sent me samples that has shared information with me that's that's doing a lot of this research is published it very important research. So, you know, we couldn't do this on my own. So I'm very grateful for all my colleagues from across a lot of different states that have worked with me in this work and doing their own work. That's also really important here. Funding wise. I mentioned the Illinois Soybean Association, kind of got this project started when I was at the University of Illinois, United Soybean Board is still funding this work. And it's been very important to for that funding to to come in and of course keep this work going. The Kentucky Soybean Board has funded some my more local work here in Kentucky and I again all my colleagues that are across the least different states they are a lot of them are getting funding from some of their local Soybean Board. So the soybean checkoff has been extremely important in in funding this research and, you know, I've mentioned some of the resources that crop prediction Crop Protection Network. I know you're going to have that website up and everything. But that's that's been a great place for me as an extension person to work with a lot of other extension people from across different states to put up really Good information. There's one that's getting a little age on it, we probably need to, to kind of redo it here in the next year or two. But it's still really good if there's a publication on frogeye leaf spot that's on the Crop Protection Network. You mentioned the fungicide resistance FAQ that's on their fungicide efficacy guide. And so there's lots of good resources there on the on the Crop Protection Network. And the other one I'll mention is they I will take action, I will take action.com that's another place where you can go and there's some good information about fungicide resistance, there's a that score sheet I talked about. And then there's a poster on there as well, that you can print off it has some of the different products and shows what chemistry groups they belong to. So for the fungicide so anyway, yeah, that's, I think that covers it, hopefully. But like I said, there's there's been a lot of other organizations and people that have that have really helped with a lot of this work. Yeah, absolutely.

E Ed Zaworski 1:06:03

I mean, the breadth of this, this research and this Yeah, it'd be really hard to mention everything, but um, but they knew who they are. Right? Right. Awesome. Well, with that. Remember, folks, if you want to help us out with icy dead plants, remember to subscribe to the podcast. Obviously, if you haven't already, rate, leave some comments, you can always email us at crop protection network dot our crop protection network@gmail.com with any questions, emails, if you want to hear about something if you want to have Carl back on here to talk about something else, let us know. And with that, thanks again, Carl. Hopefully,

1:06:46

it doesn't get too hot today down in Kentucky. And with that, we'll see you next time folks.

B Brandon Kleinke 1:06:52

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