

"Okay. Hi, everybody. Welcome to the second event of our Making Soils Data Actionable webinar series. I'm Dani Gelardi, senior soil scientist at the Washington State Department of Agriculture, and I am so excited to welcome you all here today to talk about soils data. If you have been working in this space, you've probably noticed that more soil tests and data are available to farmers than ever."

"And while this can be empowering, it can also be really confusing. New information on soil chemistry, biology and physics can sometimes be difficult to translate into action. And so the Washington soil Health Initiative has put together this series to showcase just how useful and necessary soils data can be to making in-field decisions. Every Wednesday at noon this month, we will be showcasing projects from across Washington that are using real data to make real world on farm decisions."

"Today, we have Lindsey Du Toit here to help us understand how to make soil biological indicators actionable. Lindsey is a professor and extension plant pathologist at Washington State University and is the current chair of the U.S. Department of Plant Pathology. Her vegetable seed pathology program focuses on the causes, epidemiology and management of diseases affecting spinach. The vegetable seed production in the Pacific Northwest, primarily in seed crops such as spinach, beet, chard, brassicas, carrot and onion."

"And so for everyone in the audience, there will be a Q and A at the end. But feel free to post your questions into the chat feature as we go. You can find the chat feature at the bottom of your screen in a little thought bubble icon. And with all of that, thanks so much for being here today, Lindsay."

Feel free to take it away.

Thank you very much. Let me right now get going on this. Thank you very much. Really appreciate the opportunity to share with you some of the perspective on biological data related to soil health that I'm hoping will guide you in your work. And I'm using a case study of a disease that we have worked on for many years in my program.

"This is Spinach Fusarium wilt. And you'll see as we go through this, why I think this is a very good case study for looking at biological data. All right. So see if I can hold my slide. All right. Just to give you a background on why we do our work with seed crops and why I was hired specifically to focus on diseases affecting vegetable seed production in Washington State."

"Washington State is a very important region climatically for production of vegetable crops of a number of species, and actually not just vegetables, but many species because of the very distinct climatic requirements to produce high quality seed. So for best production, the two the three areas that are called of right here are important for production of seed crops. The species such as the crops that Danny mentioned in the introduction."

"And so these are the two areas that I work primarily north in Washington and the Columbia Basin. To give you an idea of why these are so important, if you look at this map of the world, I've circled and read the regions where spinach seed crops are grown, and these are

the only areas of the world that have the right climatic conditions for spinach seed production, because in order for spinach to grow to flower and produce seed, you have to have long day length."

So it needs to be high latitude. You have to have mild summers because it's not a heat is a heat. Sensitive species is not tolerant of high summers. And you need dry summers because you don't want high disease pressure from fungal and bacterial pathogens infecting that seed. So these are the only regions of the world that have those three combinations that are necessary for production of spinach seed.

"And as a result, at Western Washington and western Oregon are the only region in the U.S. where we can grow high quality spinach production. So we produce about 20% of the world's supply of spinach seed in western Washington and western Oregon. So that seed is moving all over the world from the Pacific Northwest to us. And that's really important not to be carrying pathogens with that seed."

"This is the photo of a spinach crop. Spinach is delicious, meaning it naturally has separate male and female plants, which really facilitates hybrid seed production because you naturally have the separation of the genders. This photo shows the male rows, which are the lighter color, and they are. Let's see if I can put on my laser pointer point options."

"Thanks. There we go. So the lighter color, male rose versus the female rose. Females don't produce pollen, but there is a small percentage of the female rose that will produce pollen. And those lines have to be road crews work. Walk through and remove any female lines that stop producing pollen because otherwise you get inbred seed. So this is typical to wind pollinated species of these crops have to be separated from each other in order to prevent unwanted cross-pollination, which further limits the amount of land that you can use to grow up and see crop area."

"But we have the right climatic conditions for this. As I mentioned this, the crops have to be isolated because of wind and that creates further restriction on land options. And these crops all get pinned at the beginning of the season to determine where they're located and making sure that male lines that are close to you see crops that are close together have the same male line."

"Just so you're aware that other crops grown in this area that are really important, for example, cabbage seed crops where you can see the photos, I'm showing how the cycle through that 16 months process from producing the seedlings in the greenhouse, putting them out in the field in August, covering the winter, if it gets too cold, sneaking them in the spring when they open up and go to full flower, bringing in honeybees and then harvesting the seed."

"And that's a six month process and only can be done in Western Washington because these biennial brassica species don't tolerate the cold on the east side of the mountains. But the east of the mountains is really important for crops like carrot seed production. This is also a biennial. Many of the inbred lines are not cold tolerant, so they grow the roots down in the Imperial Valley of California, dig them up, fertilizing in cold storage, and then transplant them in the

Columbia Basin or central Oregon in the spring."

"And you'll crops which are also a very cold, tolerant biennial species. That seed is grown from in Columbia Basin, and these species I work with are mostly small seeded. But the Pacific Northwest is also really important for crops like sweet corn seed being seed and seed production. And a really important criteria for the seed industry is to produce seed that is true to type genetically."

"So making sure these crops are isolated, high quality seed germination and vigor, freedom from pathogens, freedom from noxious weed seeds and other debris and the ability to compete on the market with labor price can be pretty high, and the cost of producing these crops is very high. And when you look at the demand for seed, this is really interesting."

"The crop like spinach, there's been a just a tremendous increase in the demand for baby inexpensive because of the popularity of this and convenience and the health of crops like baby spinach and these baby leaf crops are seeded the populations of 7 to 9, 7 to 9 million seed per hectare at 3 to 4 million per acre. And they're harvested."

"And then 20 to 40 days after planting and the sequential plantings. So there's a very, very high demand for seed of spinach because of the up into the baby leaf industry. And you can see the intensity of this production means that a hectare of a hybrid spinach seed crop only produces enough seeds on ten hectares of baby leaf."

"So a 1 to 1 ratio, whether you're talking acres or hectares because of the huge amount of seed using these baby, these crops, which has really increased the demand for seed and put pressure on areas like the maritime region of western Oregon to western Washington to provide seed. So even though we have a really ideal climate, one of the big problems is that soils in this area naturally are acid and low in calcium."

"And these conditions are highly conducive to a sort of pathogen called seeds are amongst this from from a special spinach. So it's specific strains of our system that are pathogenic to spin to spinach. So this disease, fusarium wilt is very recalcitrant in our acid soils in Washington and Oregon. I took this photo in 2018 of a hybrid spinach crop in Sedgwick County, and you can see the female row is very, very susceptible to preserving."

"While she's dying, the male row has some resistance. This field had been out of spinach for 18 years and growers normally would feel like as they try to find fields that have not had spinach for a minimum ten, ideally 15 plus years. But even then, we have scenarios like this where despite 15, 18 years, we see significant wipeouts to preserve world."

And that's been a real challenge to see how how can we manage such a recalcitrant disease in this limited area that is suitable climatically for production of spinach seed. So this pathogen is widespread in the soil. It produces these dormant survival structures called criminal spores. The infection occurs through the find roots and then gets into the vascular tissue.

"You can see it blackens the vascular tissue. You can get well to the

seedling stage or at a mature stage, this lower right picture, the photo was taken of a seed crop that had been out of spinach for 50 years. And again, you can see that female is the female blind is really, really susceptible. And then when a spinach seed crop is harvested, it has to go through full senescence to get that seed to finish maturing and drying."

"And all those dead residues get colonized by fusarium. I'm not sure if you can see this salmon colored base to the stem, and that's just full of thousands, hundreds of thousands, millions of spores shown on this lower left picture forming on that senescent tissue because fusarium is a really good, what we call a neck. It likes to feed on dead tissue."

"So you get this massive increase in the amount of spore production when they see crops are drying down and all that gets incorporated back into the soil and as a future load of inoculum. So, you know, there's been a real question about for growers, how do we manage this? How is there a way to reduce that rotation interval even just by 50% to go to 8 to 10 years without significant increasing losses to preserve world?"

"And one of the questions I got from a grown up early on when I started this job, this grower had traveled internationally. He's also a field rep for companies, said, Why is it that in Denmark they can grow spinach the crops on a five year rotation and they don't see four zones? And that was a really good question."

"As I dug into aspects of soils in Denmark. So one of the things that really jumped out of soils in Denmark are alkaline and highly calcareous. And when I dug into all the literature, found some papers from the seventies on fusarium wilt up tomato and Florida and what can be done back then looking at agricultural limestone amendment to suppress tomato preserve."

"So we started a series of trials looking at agricultural limestone amendment in those fields in western Washington, fields that had been out of spinach for as little as 5 to 6 years, which normally would be suicide for most of these varieties that are grown to see production. And you can see this is a photo I took in 2006, the first year we did this."

"And you can see that highly susceptible female line on a five year rotation with no limestone is just decimated and behind it it has some different rates of Limestone Amendment versus a partially resistant female. But even then, that's very, very poor growth. And you can see the male line is there's just nothing left. It's so susceptible. So we've done some amazing work showing that agricultural limestone amendments can help suppress Fusarium, while it's a transitory suppression, not long term, but it makes a difference."

"So you can see in 2006 we saw this on a highly susceptible female line represented by the blue line over these different rates of limestone amendment and the marketable seed yield at the end of the season. And not just this very interesting phenomenon with this partially resistant female where we increased the seed by about 1000 tons per pounds per acre, sorry, not tons per acre."

"And then the seed plummeted dramatically. And as we were monitoring so nutrient status and plant nutrient status, about every every three weeks, we saw this tremendous decline in certain essential micronutrients, particularly zinc and manganese. And so we subsequently did trials looking at if you make a fully replicate version of a zinc and manganese either related or sulfate form at that present stage when the plants were starting to both transition to reproductive growth, you can eliminate the net negative impact of tying up those micronutrients as this piece was pushed off by the calcium carbonate."

"So you can see in 2008 how these high rates of limestone, we continue to see an improvement in yield because we were able to counter that, tying up with the micronutrients by putting all those high rates of limestone that was depriving the plant from what it needed, but also continuing to deprive the pathogen. And we had a Ph.D. student, Emily Gach, did a long term for you at trial."

"Well, not long term, but for your trial, looking at can you optimize the use of agricultural limestone to suppress the pathogen? You don't eradicate that threat. It's still there. You don't prevent it from growing, but you suppress it significantly. And so she did a trial where we grew spinach in 2009 in a growers field after five years out of spinach, and the grower came back the following year, planted potato and then went to wheat."

"And then we came back and part of the spinach trial 2012 and she put on limestone at the beginning of the 2009 trial and then again every year around the growers rotation and then again at the beginning of the 2012 spinach trial, which was only four years after the previous trial and the limestone was put on it, either one ton per acre or two tonnes per acre every year or not put on at all."

"And what you'll see is the results have saved yield, which reflects the amount of disease pressure with tens of thousands of hectares. This is equivalent to one or two tonnes per acre at the initial trial in 2009 for three different mines that were either highly susceptible, moderate or partially resistant. And again, to those same three female lines in 2012, the four years later where we had put on these applications and only one or two tonnes of limestone per acre, and look at that tremendous boost in yield by putting on agricultural limestone."

"So again, we've not elected to eradicate the pathogen, but we've created less conducive conditions that the inoculum in that soil is less effective. So it's really a good reflection of how inoculum amount of, and I can say is not always the most accurate predictor of disease risk. It's also the conditions around that inoculum like calcium and we can amend that with things like limestone."

"So this was a wonderful way to demonstrate we have the capacity to reduce the rotation interval to finish by optimizing limestone applications and basically doubling the carrying capacity for speed of sea production in the U.S. And one of the things Emily did was to document the degree to which this suppression is related to micronutrient availability. If she grew the spinach, preserved more

pathogen in these flies with different levels of these micronutrients, manganese and iron."

"So this is showing how much fungus grew at these concentrations of the micronutrients. And you can see zinc, for example, if you get deficient in zinc, this pathogen does not like to grow at all also with iron and manganese. And as the concentration increased, the growth rate of the fungus increased very significantly and the amount of spore production was also very dramatically impacted by the amount of micronutrients."

"So so what you're doing by adding agricultural limestone is you are tying up those micronutrients so they're not available to fungus. You also have to be careful that you don't provide the crop. What it needs by doing foliar feed. So the crop gets those micronutrients from above ground application without supplying it to the pathogen in the soil. And our really important question that a grower came up to me early on when I started this position, I remember it was around 2005, six growers said to me, I've had this wipe out in a field that had been out of spinach for 15 years."

"Why is this field not safe yet? How can we how can you help us know when a field is safe to go back to? I had two or three fields that could have chosen this field I thought was safe because being 15 years out, I could have chosen the field next door. And yet it wasn't safe enough for her to wipe out."

"And then a couple of years later, the president of the Seed Growers Association showed up at my office about five gallon bucket of soil he planted on my desk and they said, Lindsay put the soil and pots in the greenhouse and let's plant a highly susceptible spinach line to see what happens. So I want to know whether I can go back to the field."

"And that was the onset of this long series of trials we've been doing now for 15 years to develop a soil, I want to say, to help growers accurately quantify the risk of reserve world. And so we have been developing this bio. I say we started developing in 2010 as part of Emily Gaston's project. We published the first results of this, the first five years of this in a paper plant disease in 2015, and we've continued to offer this bio assay every year."

"And you can see them as a photo of some of these plants. Everything's dying and some of the things look really healthy. So what happens is the growers bring us soil in pots. We process that soil, we print it out and replicate 12 parts of the greenhouse. Four of them get planted with a highly susceptible female, four with a moderately resistant female."

"And so it is a partial resistant female. We randomize it in the greenhouse, so we're blind, we don't know what we're rating. And then we have an open house in January when we randomize everything. So the grower can come and take a look at the 12 parts of that particular field and that you can see early on we used to do five replications, now we've cut it down to four."

"But he has the pots that were planted with that partially resistant female, the moderate and the highly susceptible female. Everything died. This is a growers field that they were considering planting that

this this is the classic high risk scenario. We get some intermediate risk fields where like so I'll be with us if you have some good resistance in the male and female line, you could be okay."

"But if you've got high susceptibility like we saw on some of those photos that showed you you're going to have a wipe out. And that's one of the aspects of risk besides looking at the bio assay and what's in the soil and the conditions of that soil conducive to the disease, this is part of that risk equation is the susceptibility of the lines that growers get asked to grow."

"And most of the time these are proprietary inbred lines that are confidential. They don't know what they are, they don't know the genetics, they don't know the level of susceptibility and that's a huge part of the risk equation we've been trying to quantify for growers. And sometimes you'll see songs like the song See, which is perfectly safe and how susceptible a female was."

"Everything grew really well and this is what we've been trying to quantify for growers. And I'm showing you the results of the first two years of the bio asset that we did for the growers in the top figure from 2009 ten. So we start that assay in December, we finished it in January, February. The growers have results by March 1st, which is the date when they been on the map, one of these crops will be planted to avoid cross-contamination and you can see the physician index."

"One means everything died in the pot. The green bars represent the pots planted with the partially resistant spinach line, the orange, the moderate and the red the highly susceptible on. And you can see that first year large percentage of the fields we tested were highly susceptible, very high risk. If they were planted with the susceptible line in every one of these fields was being considered for planting that next year."

"And based on the success of the trial, you can see the second year we had a tremendous increase in the soil samples that we received. And you can see a lot of those fields are very safe. But you still have a significant number of fields that were very high risk. If either the male or female is susceptible, you might say, well, what if the male dies?"

"Is that not an issue? It is an issue because the male dies The female. When female plants are not pollinated, they will sex revert, so they'll start producing their own pollen. Now you have a bunch of inbred seed you can't sell it that doesn't have the correct genetics. The female dies, then clearly you're not going to have seed to harvest."

"So this is why both male and female lines need to be able to grow and to have a marketable spinach seed crop. And we did a lot of validation of the bio, I say, using other tests, going out to these fields, planting these same three female lines and seeing if the results in the field reflected what we saw in the biopsy."

"And we had very strong correlations between field results and the biopsy results for these different soils and again, just different data that's in this paper published in 2015, validating what we're seeing in the greenhouse to make sure what we're observing is

reflecting the actual physical world risk out in the field. One of the questions we came up with was, can we just test all kinds of properties of these cells?"

"We looked at peels, we looked at various micronutrients, macronutrients, we looked at organic matter, carbon exchange capacity. We looked at years out of spinach, we looked at texture of the soil, and we looked at quantifying just preserve access from not for Xerox's business because there is no way to test soils directly. And there wasn't at the time and also burn and die, which is another Fusarium fungal wilt pathogen that can infect spinach."

"So in all these cells that we test, we also run all these other tests and we ran a regression analysis for the first time in 21 fields from those first five years to see if we could get a strong prediction of the quantitative risk of zero and that's why which is your risk of zero world. And then these models, you can see the different variables that have the biggest influence on how much disease are things like number of years out of spinach soil."

"Sometimes it's texture depending on the parent line, sometimes it's clay, sometimes it's ammonium, which alkaline soils suppress and so on. But you can see the R-squared, which is a prediction of how strong you correlate these variables with the yield loss to preserve. As you can see, it was very poor, 0.33 maximums one. So you've got at most a 34% accounting for that risk of reserved world with all these variables that we try to plug into the model."

"So really if none of these variables along or even in combinations comes even close to quantifying that risk. So we still need to do this fairly tedious labor and time intensive biopsy to get an accurate prediction of risk and a really important feature I want to point out to many of you, even though you may never grow spinach in your life, is I see a lot of people using soil testing labs that offer the ability to test for zero desirable risk."

"And very often they are presenting just reserved to genus or sometimes two species for their access. And I think it's really important to recognize that there are many, many different for Zarina and amongst those in soil, and many of them are not pathogenic to the host plant that you're growing, many of them are even beneficial. So getting a solid test result that just tells you how many colonies of reserve you have per gram of soil or how many colonies of reserve mixes from is completely meaningless when it comes to knowing disease risk."

"You have to know in the case of zero, well, you have to know what the subspecies level is that's pathogenic to the plant that you're growing. So we looked at, for example, in 2018, here's all the songs we tested in 2018, from lowest risk to highest risk on that susceptible female. So again, that's the severity World index at the end of the season, the bio assay and I'll check lines of the India textiles at low, medium and high risk."

And this is the same soils with the amount of reserve system that we quantified by just plating that's going out on a argaman ham and petri plates that allows you to quantify preserve access from it doesn't



tell you if it's been easy. And you can see there's zero relationship between the amount of reserve system and the soil and the amount of fish there and what risk.

"And we have done this almost every actually not 12,014 out of the 15 years we've tested for the virus, we have shown there's no significant correlation between testing cells with zero of reserve access from and knowing the risk of spinach preserved world. And I see growers in Washington state too, especially in the Columbia Basin, do grid sampling of entire fields on two acre basis."

"So every two acre is represented by a small sample that gets tested for zero, say, for example, for onion growers. And they get a test back that says X amount of zero per gram for each to a grid. And they're making decisions around things like fumigation, which is not an inexpensive treatment. And yet those results are meaningless because they have so many fusarium."

So they're ubiquitous and there's so much diversity and even beneficial species. So this is a really important consideration to think about when you're making decisions around some of the biological soil data risk management I mentioned to that we also test these soils for and its forces for deciding worth of spinach. It's a very important pathogen because it can be born.

"And this is just one example of soils. We tested one year with the amount of sodium dye, which is a pathogen and protozoan tract corpus, which is not a pathogen and spinach, again, making sure that labs that offer these kinds of tests can differentiate the pathogenic species or subspecies from the non pathogenic. And you can see some very high levels."

"Obviously, we've even had fields up over 200, 200. And for a crop like potatoes, the threshold is is zero. They're about 30 or 40 C a few. So when you see some of the extremely high levels that are coming back here and you see some of those fields that do get planted to spinach, you can see what happens with the spinach crop in a field that at 120 colonies, obviously per gram of soil, this should be a bright, green, thriving crop and is showing this classic fertile soil."

"Well, sometimes it's not. Well, what totally kill your crop. You still get seed, but you get a significant reduction in yield. If you have over about 50 years of verisimilitude per gram of soil and you get infected seed the harvest will be infected. So this is another service we offer with this virus. So now I mentioned to you that part of your risk for seed grow is not just how much inoculum is there, the amount of disease pressure, but also how susceptible on the parent line to growing."

"So one of the things we now offer for growers and have for about eight years with the seed companies is to test the level of susceptibility of those parent lines that they're contracting to see growers to plant. And we now know and we didn't at the time we started this, but we now know from a Ph.D. student in my program that there are two races of the spinach because they're a real pathogen."

"So we actually now screen for responses to Rice one and Royce two. So

these are data from one of the years of testing all these spinach lines, this disease severity index against race one either in a high risk where we have a high level of inoculum in the soil represented by the white bars or a moderate level of inoculum in the soil represented by the black box or no inoculum represented by this tiny little gray bar."

"And you can see in for race one these fields on the left side, for example, in red, three or not fields the parent lines in red three is had really good resistance to just growing in the control with no inoculum with race one at medium and high rates of inoculum with race two at medium and high rates of inoculum."

"So you can see no by race one because no disease when in the presence of race one regardless of the rate of inoculation. But quite susceptible to race. Two if you look at inbred eight, it had some partial resistance in Brant eight to race one and maybe a little bit of resistance to race two. And you can see that's reflected in these photos."

I took it the medium and high rate of inoculation was race one in Howard's race to look at inbred 17 highly susceptible to both races. Everything died even at the moderate rate of inoculation. So this has been important information for companies to try and match up the more susceptible lines with the lowest risk fields that we detect in the bio testing.

"And so we offer this every winter. We now charge growers \$250 per field or companies to prepare it, and we give them the complete soil nutrient analysis. And in late January I have an open house where each grower can come and take a look at the plots for their field and without other growers around. And I have this incredible opportunity."

"Every year we offer this virus to have a 30 minute conversation with each of these growers about the risk with the representative of the biopsy, what might have contributed to that risk in terms of crop rotation and other practices and how they're making decisions around management based on the information we provide them. And that's been an amazing exchange of expertise for me to learn from growers, for growers to learn from me as a pathologist."

"And we want to be one of the most valuable aspects to me as a proper biologist with this biopsy is to have this direct dialog and exchange about risk area that we offer the service to growers. And as I said, we now do this as well with tailored line screening and we have tested over six 600, six, almost 650 fields over the last 15 years."

"And we've published another paper looking at the lessons we've learned from ten years of stakeholder adoption of this biopsy. So we now up to 15 years. But that paper was published a few years ago. We also had a question as we've been looking at these limestone Amendment trials and doing all this work with spinach preserved, well, it became apparent to me that some of the difficulty of spinach seed crops in combating fusarium in the soils is still structural and small to health and so on."

"Health soils in western Washington are heavier. They get worked pretty hard, particularly for some crops like fresh market potatoes,

where you want nice good soil structure around your potatoes to get my shrimp tubers for fresh market options. And that means these ground, the soil structure gets damaged every year from the degree of cultivation of these soils. And I've seen many spinach seed crops where the ground has a hard pan layer, not too deep down."

"And there's a lot of generating in some of these fields. So I wanted to look at the degree to which practices that might help improve soil structures on TILT could also provide a benefit to managing preserve well. So we've done a series of trials looking at compost amendment. We happen to use a biosolids compost because these are nonfood non feed crops, they're seed crops."

"So you can use the biosolids, compost the city of Thomas to provide a donated biosolids compost for us to do this work and you can see us putting the compost on that treated non treated plants prior to planting a spinach crop. And so if you take a look here, this is driveway of spinach per plant in the middle of season and seed yield at the end of the season and pounds per acre for those same three female lines except for moderate and partially resistant to frozen wilt the white bars on the absence of any treatment."

"The green bars that we were looking at a fungicide or proline to see wouldn't help. And the brown bars represent the parts of the treated with compost. So in the susceptible female, we saw a 20% increase in seed yield when we applied a single application of compost just before planting, the Margaret had a 48% increase in yield and the partial resistant had a 30% increase in yield compared to the non treated."

"So this is a really significant increase showing how in not only looking at Limestone amendment but also looking at quality of the soil and providing good soil structure. Nutrition can really help further reduce that impact of work on spinach production. Now, one of the key things that we also want to look at is why is it that certain strains are preserved like this from a pathogenic of spinach, these from a specialist see strains and others are not."

"What's the genetic basis behind this? So we have a Ph.D. student, Alex, working on this, and we work closely with folks at the University of Amsterdam that are specialists in fusarium pathogens, the genetic basis of zero isolates that are pathogenic on different hosts. And he was trying to figure out, don't have to worry about the details of these two questions, but basically what is the genetic basis behind spinach as a pathogen of spinach?"

"And so he does a lot of work to give you a snapshot of what he ended up showing. He took isolates of Xyrem that we knew were pathogenic on spinach based on running tests themselves, also isolates that came out spinach that were not pathogenic. Like just because you get preserved with a wilting plant doesn't mean that isolates cause the wilt."

"So it has these non pathogenic spinach isolates. And there's been spinach these since we call them for short. And he compared them the whole genomes of these isolates with isolates of other the said pathogenic on other hosts like radish peas, banana melons, tomato, cucumber, onion and so on that banana. And looking at this genetic relationship of these isolates, what became clear very quickly is we

have two very distinct groups, obstinacy."

"We now know after further testing, it turns out these are races, race one and two, and they differentiate, differentiate very clearly from these non pathogenic isolates that we can get off spinach seed of spinach plants of soil where spinach has been growing. And Alex was able to go in there. And this is really complicated, but this is a 120 different isolates of reserve accessed from Alex's spinach."

"The isolates shown in green and as non pathogenic spinach, rice and brown. And down all these rows that you cannot read is different. What we call putative effect is potentially genes that might be involved in that interaction between the host and the pathogen. And you can see basically blue means that gene is there, brown, gray is absence. And you have a very unique genetic fingerprint for this beneath the eyes from all these other isolates and from the non pathogenic isolates."

"And when you look up closely at some of these genetic factors that might be driving, the ability for Spinoza to infect spinach allows us to nicely isolate race two in race one and the non pathogenic isolates. And when Alex inoculated these isolates onto two different parent lines, this is now highly susceptible melanin that we've been using in all these monster trials and isolate so on."

"And this partially resistant line, what you see is with race one, this is several times really quickly, the pasta resistant line does develop symptoms compared to the control, but doesn't get completely killed. But he noticed that these these other isolates, which we now call race two that actually parent be this what we were calling passive resistance actually more susceptible to parent egg and this is what classifies what we call a differential host or differential varieties that can separate these races."

"This has been an important basis of identifying these two races of the spinach pathogen. So Alex then went ahead and tested 68 different commercial spinach varieties to see if we see some differences. So this lower figure here on the top, group one, this is race one, the 68 varieties ranging from the most resistant to the most susceptible and these in the same order."

"This is their response to the race. Two isolates and you can see there's no relationship correlation wise between the response of these 60 cultivars to race one versus race two. This has been really important information to provide to seed companies, to provide to finish breeders, to recognize when they're trying to screen for resistance to preserve, they need to be screening for these two races separately because cultivars can respond very differently to these races."

"And this is just an illustration. Have, for example, cultivar. If here is growing in the control and with race one and race two, everything's resistant. We've got something here. Cultivar e has good resistance to race one, but not race two and cultivar A has no resistance to race one that good resistance to race two. And you can see consistency across the paper."

And this was published in 2020 with one of the really interesting things that Alex noted as he was looking at this and repeating these

trials to look at can you get consistent results when repeats? So this is results preserved. What severity for some of these varieties in the fall of 2020 and also in the spring of 2020? He repeated the trial exactly as he had done with the first time.

"If everything was consistent, the response would be along the straight lines. So things like the baboon that are really susceptible to race one in this are very resistant to race one. And spring were also resistant ways to have a head kill or highly susceptible. Doesn't matter which time of year Alex run these experiments. So you're not just all these black cultivars with black circles are represented along lines, which means they persistently display the same reaction, whether susceptible or resistant to intermediate when the trial was repeated."

"But there was this really interesting phenomenon with some of these varieties when they're tested with race two, for example, have a head in the spring looked really, really resistant because it's down on the end of the of the x axis. But in the fall it would had a 75% severity. So it looked really susceptible. And at first we thought something went wrong to mix up ISOs to make some mistakes."

"What's going on? Why are these race to isolate the cultivars showing these change in response to race? Two isolates and not all of them. Some of them like this, they were consistently highly susceptible and this sugar beet line was consistently very resistant. What is going on that these lines are changing their action? When we repeated this experiment and one of the things Alex noted as he looked at all the things that might have influenced this was there were some very distinct differences in sunlight, intensity, daylight intensity when you ran these two experiments."

"So you ran the test to see if the amount of light influences the response of the spinach varieties to the two races. And sure enough, you saw a very, very big difference when he grew that thing. For example, baboon. And it was race one that shade or was shade very similar responses. Race two without shading and with shading."

"And you can see every time he shaded the varieties that were more susceptible, particularly to race two and again, this has been really useful information to say to growers and to seed companies the response of variety to one race in different depending on the amount of light intensity under which they varieties growing. So for breeding purposes, this has really practical application to trying to screen and identify good resistance because if you think something's resistant, then you plant in an area where there's a lot of cloud cover and a lot of rain, it may suddenly look highly susceptible."

"So another side benefit of this work that Alex has done is he's been able to identify some unique genetic areas of these race one or two isolates that separate them from the non pathogenic isolates and separated from each other. And he's been able to design a molecular DNA based assay that can identify the Spanish isolates because as I mentioned before, and not have the ability to tell which isolates was been taken out without actually growing in a petri dish, growing spinach plants, inoculating and seeing if they develop wilt."

"But we now have the ability to test infected plants, to test live cultures of the fungus to tell is that's been easy to tell which race it is and can detect it in the plants as early as seven days after planting in infected soil. So we now have a tool to be able to tell more efficiently than having to run the six week pathogen escape test."

"And just to wrap up some of the other work we've been doing, we had a post-doctorate, Sanjay Agrawal, who started screening the USDA spinach Germplasm collection, as well as wild spinach collections from the Dutch germplasm germplasm center to see if we can find sources of resistance frozen wells. And we have found some excellent sources of resistance. And this is just complicated genetic data, how you can ignore that."

"But we found a number of these what we call plant introduction lines that are in these germplasm collections that had very good resistance and most of them were Asian spinach types, not your typical baby leaf type of spinach. One of the very interesting things, as Alex Dreyer looked at the genetic diversity of these lines, the center of origin of spinach, this area here and Speck has done Tajikistan, Pakistan, Afghanistan area covers Kurdistan."

"And as we went back and looked at the G.P.S. coordinates of where these lines were collected by curators for these germplasm centers, you can see that the different colors here represent the genetic groups to which these lines belong, and you have this very distinct geographic separation of the the pieces that mostly were green versus mostly blue. These are split nicely Turkistan to wild ancestors to spinach."

"And this really reflects there's a big, big mountain range that runs up right down here through Kyrgyzstan, Tajikistan, Uzbekistan, that physically separates these lines to the north, on the plains, to the south. And over the thousands of years, we believe this has driven some of this genetic differentiation. The really good thing is that today I was able to find sources of resistance in both of these populations of spinach."

"The textiles that can now be introduced into the cultivate is finished and I shoot spinach all raised here. So we hope that that presents a lot of potential for getting better resistance into spinach cultivars. So to summarize some of what we've done in working on spinach, preserving what now for almost 20 years is we have demonstrated that there is the capacity to double the carrying capacity for spinach production in the U.S. through careful use of some of these management practices."

"We have shown that highly acidic fertilizers like ammonium, can really help, unfortunately make source more conducive because of putting so fine fertilizer right then for planting. Whereas nitrate, we can't use nitrates in high rainfall areas because they leach. So looking at alternatives such as you can be really useful for trying to minimize that disease pressure in my children because my business low on charge."

"Well, thank you. We've also looked at a number of fungicide

treatments to see if they can help, particularly seed treatments to reduce the risk of having preserved one seed that might contribute. But really the bio assay and the soil amendments like limestone on the mount most important management practices that we've been able to provide to growers and screening of parent lines to match that risk to reduce the likelihood of wipe out."

"I wanted to mention that a big part of what I do in my job is applied research and also extension, and I try to take that information that we and those practices we've learned through my job to a class I teach called Field Pathology to graduate course. And a big objective of this course is to get students out of the classroom and into the world of agriculture and production and to learn about managing diseases and detecting plant diseases and working with stakeholders in the real world environment."

"So we take them around the state for two weeks in the summer when we can June one week, July, in direct directory with people involved in production regulation, you know, chemical side, the organic side, the cemetery certification side from the State Department of Agriculture, the USDA, and just really getting the context of all that they've been learning in their courses and how it fits in the real world."

"And this just helps students really get passionate about the careers of top pathologists and other fields of agriculture as they see just how much science help drive improvements to agricultural production. So that I just want to acknowledge the various agencies that have funded the work I've been presenting also the steep growers and feed companies, the students, the technical staff in my program who are critical to being able to do this kind of work and make a difference."

"But I really believe the importance of interacting with growers in what we do to make our work more effective. So I don't know, Danny, if that's time for questions now."

"Yeah, Thanks so much. We have a few questions in the chat and some that folks have already submitted while registering. So let's start with the question from Chloe, who asked about those photos you showed with greenhouses full of of the soil samples and the bio assays. She asked why aren't they contaminating each other? And could a small farm replicate this work without the risk of contamination?"

"Yeah. So. So freedom is not a foliar pathogen. It's a full blown pathogen. When we handle this. Also, as I said, the grower brings us soils in a five gallon. Put a lid on, we keep the soil sealed. When we're processing the soil, we have a big concrete wash pad area. Every soil gets handled very, very carefully. So we use we dry the soil out too."

"If it's too wet when it comes in to get a little bit dry, to put it on tops that can be sterilized and washed between soils. Every soil gets passed through soil spreader to make sure we don't have big clods. THOMPSON Get it mixed really, really thoroughly. And then if the growers are planning on putting a limestone that spring, we will amend that soil with equivalent right to grow plans to use so that our risk reflects what risk they are likely to encounter if they go ahead and plant that field."

"So we do that in the cement mixer and we burn after every piece of every soil has gone through every piece of machinery, everything gets pressure, wash with hot water and everything gets sterilized with alcohol. And so we are extremely careful. I am very particular about who I allow to do this work because I do not want to be contaminating between soils, but the fact that the soil born and not airborne and we're very, very careful about how we handle this when we plant the pots, everyone's sterilizing hands are wearing gloves that they can't get so along with the nails."

"So we're not causing cross-contamination between fields. So we're very, very careful about this. We have control soils that we also randomize and replicate. So if we are causing cross-contamination, we will see it. But it's shocking when you can see, you know, you have one part with everything completely healthy right next to a pot, with everything dead and they're in that greenhouse for 40 days."

"We don't cause I'm very, very careful about watering. We don't let anyone water the trials. So we have really tight control. But to answer Chloe's question about could a small farm do this in a shed and that risk of spreading, I do believe a small farm could do this. It might not be quite as well controlled as we're doing because we're researchers and we're aware of the risks."

"But I think just the fact that the growers showed up at my office in front of five gallon bucket on my desk, I think you could do this, take fields. You're thinking about planting, put them in a pot pots or flats. So we'll never behaves well in pots. So you have to also be really careful about how you manage and work with soil in containers, making sure you have the right lighting, the right conditions."

"What disease are you trying to screen for? What conditions make that disease, well, severe enough that you can see that. So we know for certain things by heat. So 14 days after planting, we upped the temperature in the greenhouse and we can start to see will develop usually within 24 to 48 hours once we increase the temperature prior to that, we also treat the seed with apron to prevent put them in the soil from confounding the effects of zero."

"So we're trying to separate other things that might be going on that soil. We know sometimes we get pretty fly larvae and they soil, so we actually drench with an insecticide to avoid the larvae coming out and moving between the pots and killing all the spinach. So we've learned over the years of optimizing this to separate these other things that could confound our ability to quantify preserved risk."

"So it's not as simple as I'm making it out to be. It's pretty complicated, but I think it can certainly be done if you know what disease you're trying to screen for, you know, what conditions favor that disease and how you can create those favorable conditions in your greenhouse. So you said."

"Thanks, Lindsey. And someone named Jeffrey asked about the soil before and after the lime addition."

"Yeah. So when we started this work, Jeffrey, we were in a lot of trials. We had plots that we didn't put any limestone on. We have plots that we had limestone on, have these different rates. The



randomized replicated. We're measuring this before and after. And we basically see if you put on one our most assaults typically on the flies, high fives, mid to high fives, if there's no limestone use at all and one ton per acre, we'll usually shift at about a half unit, two tons per acre will usually shift ourselves in western Washington."

"Again, it's highly variable, so this is generalizing grossly to tonnes per acre will usually shift soil for western Washington soils by about one unit. So I usually recommend to our growers that this is in the high fives. They should be putting on about two tons per acre to get as close to neutral as possible. If this is already in the mid low to mid sixes, I would say maybe one ton would suffice."

"And it's it's a really interesting, Jeff, as I look at 15 years of records, when we started, there were almost no soils, the patch in the sixes. Now we have very few soils that in the fives because of how much growers are aware now of this benefit of limestone application. So it's been remarkable to see this paradigm shift, but it's happened slowly because we have to persuade growers about this value and it's an investment you're making in the soil."

And so it's been remarkable to see the shift towards majority of our sales now coming in already in the sixes.

"Now that's actually a great transition to a question someone else had where there's sometimes this tension between plant breeders and soil scientists where, you know, soil scientists think that management is the most important thing. And and breeders think that having disease resistant varieties is the most important thing. And I think your research maybe shows a little bit of both, but is really focused on the soil management piece."

So maybe you could speak to your perspective on on what are the most important factors. Is it variety or is it soils?

"Well, you know, in the case of finishing growers, they don't get a choice. They get a contract, they never own the crop. It's proprietary genetics. They have no choice in what variety they give them. Now the field reps for those companies have a choice in where they put the contract for specific lines, and that's why we do that parent line screening, because the field reps for the company can say, All right, you know, Jeff's field over here looks pretty high risk."

"We were going to give them a contract for those hybrids, but this one parent and that hybrid is turning out to be really susceptible based on apparent line screening. So we want to avoid that field. If Jeff's going to have that contract or we need to find a different variety for Jeff to grow. If you're a commercial grower that you're dealing with."

"Alex's work, where he screened the Spinach Varieties is a commercial spinach variety, not proceed production, but for commercial production. So if you're a grower spinach for something to eat, you now can say all these which varieties are resistant, which varieties are susceptible, you can make that choice. And that's where varietal resistance and knowledge of that becomes much more important because you have a choice."

"Growers don't have a choice. So you can see where both play a really important role, how much you can control. And I always tell growers

what what we're trying to do is figure out what do you have the budget control, what do you not have The guys you control, and how do you manage risk around that? So see, grower doesn't have a choice on parent lines."

"A grown of a commercial crop doesn't have a choice. If there's resistance in some diseases are much, much harder to develop resistance, especially when you've got numerous racism struggles around that push. We put spinach, we have two races and 90% of the fields we test are predominately race one. And so we really focus on that resistance to race one in selection for these fields."

"Thanks to let's switch tracks a little bit and talk about this kind of making data actionable piece. I was really struck between the figures you showed which demonstrated no relationship between the tests that were commercially available and the work that you're doing in your lab. And I'm wondering well, I'm wondering a lot of things, but maybe we'll start with have you worked with the labs that are offering those tests and is there any move towards not trying to spread that misinformation or try to unify methods?"

"I have presented these kind of results quite frequently. For example, the Pacific Northwest Vegetable Association has a big meeting of about 800 people signed up for that meeting in the Twin Cities every November. And I've been asked to speak about soil borne disease management. And I often will take these data from us work to say this is a really important aspect to think about because we have fields farmers in central Washington who are doing extensive grid sampling."

"They're trying to make the best management decisions. They're trying to only treat where they see disease pressure with grid sampling. But when you have these test results that are really meaningless, and I've asked growers before some of these labs before, you know, that these numbers don't mean anything. And they well, growers want these numbers and they want to make decisions around them."

"So we just we just offer what they want. And to me that's a huge conflict of interest and it's it's to me it's abusive. I can't tell them what to do, but they're relying on the fact that growers don't necessarily understand which data are meaningful and which are not in order to to sell a service. And so you have a real problem with this."

"I presented this at meetings where those folks are in the audience, and I just keep hoping that at some point their conscience will get the better of us."

"Yeah, I guess that opens up sort of a larger discussion about both soil testing and also soil inoculations, which I know a lot of people are really excited about. And there's a lot available on the market, so maybe you could say something about your experience and perspective on on soil inoculates soil, microbial Inoculum Yeah."

"I don't have a lot of work with experience on things like legume, viso, biologicals, but I've done a lot of work with microbial modules for disease suppression and there's a lot of work on mycorrhizal inoculum. And we are hoping that because there's a myriad of products out there and there's an increasing number of products, it is very easy to develop these products."

"It's very easy to get a registered, It's very easy to claim effects on diseases with no data to back it up. And unfortunately I've spent over half a million dollars on research trials trying to evaluate products in an independent way where there isn't this conflict of interest product companies selling them to see we can find things that really work."

"And then the vegetable cropping systems that I've tested these products and I have not yet been able to find good efficacy. And it's a real it's a real shame because not because I'm against this, but I don't want growers to be buying and using products that aren't giving them what they think they're getting, the brochures, the marketing is really slick."

"They throw all kinds of science in there. It's easy to show in a petri dish that something works. I can make tables look like an amazing fungicide. It's a real shame. And it's it's a real frustration. And to me, another one of these conflicts of interest that is this significant problem."

"Okay, we have a number of questions, but only a few minutes left. So I think I'm going to just end on this question. Your research is so kind of integrated into the grower community there where growers are really relying on the work that you do to make decisions. And I'm wondering if you could give any advice for either new extension professionals or farm advisors about how they can increase their grower outreach and ongoing engagement."

"Yeah, you know, Danny, when I was hired in 2000, in this job, I had never seen these crops in my life before. And I was terrified of the fact that as opposed to providing recommendations and diagnosing diseases on crops I'd never seen in my life before, and it terrified me. And one of the first things I did was once I got to know some of the growers and I literally invited growers to breakfast with me, I paid for the breakfast."

"I did this for years. Every fall I would bring a group of growers, see growers together in western Washington that it in central, I should say, please meet with me. I'm going to talk about what I've seen. I want you to tell me what you've seen. I want to discuss research needs. I want to discuss and learn from you so that when I'm thinking about writing a grant proposal, thinking about a research project, I can base it on your real world knowledge of the crops, how they grow on the constraints around these practices."

"I'm considering evaluating what might be real, what might be translatable, what might be ivory towers. And I did that for years and I paid for that on my own pocket because I thought I have to based my ability to do research and to make recommendations on their understanding of the crops. And when growers and field reps for these companies realized I respected their expertise as growers or as field reps, and I brought to the table my expertise as pathologists that together we're going to make good management programs that are going to be usable again, economically viable, but independently."

"I'm never going to be able to do that. It was the best thing I ever did. I did it out of fear. I did it out of realizing how little I knew

and how dangerous that was. And I'd say that's one of the most important things you can do, is acknowledge when you don't know things, seek out expertise, bring those growers to the table and make them feel that they are valued because they now have a vested interest in seeing your work succeed and they will provide you them."

"They'll go to bat for you. And that to me was one of the best things I ever did, even if out of sheer desperation."

"Well, thank you so much for those words of wisdom and for your presentation today. For those of you in the audience, this will be recorded and put up on YouTube so you can access it. Probably as soon as early next week. Molly will respond to everybody who's registered with both the recording and all of the resources that Lindsay has provided in terms of her publications and other resources that may be useful."

"So again, thanks so much and we hope to see you guys next week, Wednesday at noon for talking about soil, physical indicators, specifically soil moisture monitor sensors. Thanks so much Lindsay." For extending wonderful so seriously putting on. Awesome. Thank you.