November 2016

Q&A

Mike Flynn (Bio Specific) Mary Zischke (Lettuce Research Board)

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Can you determine the DM race from the spore in a spore trap?

Not yet. The only way to do it now is to try to remove spores and bulk them up on a universal susceptible and then inoculate the differential series.

If so, than.....

- 1. How wide of an area does a spore trap sample represent (i.e. a 1000 sf, an acre,?) Hard to say because we are talking about airborne spores that will travel as a function of wind. We think they can travel 30-40 miles if conditions are right.
- 2. Has there been any geographic surveys done to see what DM races are in the what areas of CA? Yes, I have attached the last report we have.
- 3. Can a grower set up their own spore trap survey system? Yes, but we think a regional system with a daily or weekly report would be more helpful.

on DM in general.....

- 1. Is there any relationship between spinach leaf surface and DM race? I don't think so. The presence of resistance genes is the big factor.
- 2. Is there any relationship between spinach cultivars and DM race? Yes, based on resistance genes.
- 3.. Is there any seasonality between DM race and time of year?
 Since there is no such thing as a normal year anymore, I would say no. The PCA's say there is a potential for DM every day of the year.
- 4. Is there any difference in level of virulence/infectivity between DM races? Yes, see the report.
- 5. Is there any pairing or bunching of DM races (i.e. where you find one race you find another race)? There has been some evidence of mixed races in the same sample. That's when you really need to worry that the sexual phase is happening.
- 6. Does all spinach have a sub-clinical level of DM on the leaf surface all of the time with the proper environmental conditions causing an outbreak ?

The spores are around most of the time, so yes. Subclinical probably isn't quite the right term because you may have spores on the surface without infection.

on DM in other crops.....

1. What can be learned by looking at DM in other crops (i.e. grapes, millet)? We have researchers on this who have worked on millet earlier in their careers so they are aware. We had one of the grape guys help with the spore trapping project. It's so much easier in

grapes because the final product isn't the foliage!

There is good evidence that the faster a diseased field gets disked, the better, because it destroys inoculum (asexual spores) that would otherwise become airborne and act as a source of infection in other fields. Those spores are relatively delicate and would be largely destroyed by ground work, dry soil conditions and long-term exposure to UV light. We don't think the baby leaf growing cycle is long enough for oospores to develop. Oospores, the result of the sexual phase, have the durable shell that makes them long lived in soil and on seed. Bunched spinach could be another story. With its longer growing cycle, there is time for oospores to develop. As far as I know, there are limited reports of oospores forming in spinach tissue in California. It takes both mating types and no researcher I know of has investigated what D.M. races are what mating type. From our work on lettuce D. M., we know that we only found one mating type the vast majority of the time for over thirty years. Now, they are routinely picking up both mating types for lettuce D. M. and that is part of the reason we are seeing it become more of a problem in organic lettuce production.

There has been work done by the USDA that shows that mildew-infected lettuce tissue is more conducive to E. coli setting up shop. The same could be said for many bacteria because the mildew results in "leaky cells" in the host that provide a source of nourishment for bacteria. The experiments were done using very high inoculum levels of the E. coli, so the results were not surprising. I'm sure they could show the same thing on spinach and with additional species of bacteria that are known foodborne pathogens. I like to point out that if spinach D. M. and E. coli were truly linked, we would see more frequent outbreaks, given that D. M. is so prevalent in spinach production.

Regarding other plant pathogens, since D. M. is an obligate parasite, it tends to attack healthy tissue. It is the first to colonize and other pathogens tend to be secondary colonizers in its presence.

I don't know of any work done on the interaction of Cd and D. M. Is there any anecdotal info that says the mildew is worse or better on high Cd ground? Almost all of the ground in Salinas has some Cd, except on the east side, and I think we see mildew on all types of ground.

Mike Flynn (Bio Ag)

- another idea that can be linked with reducing leaf wetness to reduce DM negative effect:
- shorten irrigation cycle time
- make plants more drought tolerant
- improve soil moisture retention
- make irrigation water more like rain water than well water by lowering the water's surface tension (i.e. making water wetter)

A few ideas that can be for grower's consideration:

1. Reducing the wetness on the spinach leaf.......

Reducing the wetness on the spinach leaf by reducing the length of the irrigation cycle while

at the same time looking to improve the drought tolerance of the spinach and improving the moisture retention capacity of the soil.

2. Spinach DM Trials......

We did a spinach DM trial in Watsonville at one of Dobler's ranches this summer in cooperation with a plant pathology PhD student out of UC Davis. The student's dissertation was on Spinach Downy Mildew, if memory serves.

The trial results showed that the organically certifiable formulation that we (BioSpecific) provided resulted in an overall %DM of 2% while the untreated control spinach was at 23% DM. These results appear hopeful.

We offer this formulation to other organic spinach growers to trial if they are interested. The trial is at no expense to the grower.

Growers can contact us at: mikethewormguy@aol.com

Keep in mind, we have been applying this same formulation, over the past year, on a weekly basis, to our aquaponically grown lettuce which we sell locally to restaurants. We interview our customers, on an ongoing basis, regarding how their customers liked the lettuce they are served.

3. Measuring the BRIX level in spinach......

BRIX is a measure of solids (minerals) in a leaf. High BRIX crops are supposed to be pest resistant. Improving the BRIX level in spinach may be one way to reduce DM. We measure BRIX levels in our lettuce. We see less Powdery Mildew in the winter for our greenhouse lettuce if the BRIX is high.

High BRIX plants can be more drought tolerant.

Our sense is that the long term solution to DM will be based on stringing together a range of partial solutions that involve seed genetics, plant health, soil health, cultural practices, inputs, and the shadow of the grower.

Questions that need consideration:

How does DM resistance manifest itself in spinach? In other words, how is a resistant spinach plant physically and / or chemically different than a non-resistant spinach plant?

How is a Viroflay spinach variety w/o resistance different from a spinach variety that has DM 1-11 or DM 5-9 resistance?

For example,

1. Does a resistant plant have a bigger root structure?

- 2. Is a resistant plant better at taking up minerals?
- 3. Does a resistant plant have a more sensitive plant defense alarm system?
- 4. Does a resistant plant have a different leaf surface structure?
- 5. Does the plant manifest resistance differently depending on the DM race? (i.e. DM race 5 resistance triggers bigger roots while DM race 11 resistance triggers a more sensitive plant defense response.)

In short, what plant processes do the genes turn on or off that make the plant resistant and what does this gene action look like in the plant?

My sense is that having a better understanding into what does the need to do to be resistant can assist us in helping it do it better.

Mike Flynn BioSpecific LLC

Spore Traps

This is the response I got back from Dr. Smart in regard to where she purchased her spore sampler.......

I purchased the traps from Walt Mahaffee (USDA ARS at Oregon State). His email address is mahaffew@science.oregonstate.edu
Best of luck,
Chris

Christine Smart
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----Original Message----

From: Ramy Colfer < Ramy.Colfer@whitewave.com>
To: mikethewormguy < mikethewormguy@aol.com>

Cc: ericschwartz.oms <ericschwartz.oms@comcast.net>; paul <paul@flemingpic.com>;

maryz <maryz@calgreens.org>; jroberts <jroberts@taylorfarms.com>

Sent: Tue, Dec 6, 2016 5:03 pm Subject: Re: Nov 28 Mildew Notes

Hey gentlemen,

I believe Steve Klosterman and Krishna Subbarao at USDA Salinas are using same techniques for lettuce and spinach downy mildew spore monitoring in Salinas valley. Ramy

Listened in on a webinar regarding DM in cucumbers today.

The research discussed using a "Roto-Rod" type spore sampler that we solar powered with a battery. A small weather station was placed with the spore sampler.

Silicone grease was used to catch the spores, as well as, everything else, in the air from the spinning rods.

The sample was placed right near the plant canopy. The rods were checked either daily or weekly depending on the experimental design.

She got her spore sampler from USDA. The sampler is 1980's tech. She used a DNA/PCR test to check for DM DNA.....

The researcher's name was Dr. Christine Smart, a plant pathologist, from Cornell University (cds14@cornell.edu).

It maybe worthwhile to look how other folks are spore sampling in the field.......

Mike

BioSpecific LLC

Mike and Eric.

Klosterman is doing this in spinach. Same technology.

MZ

From Mike.

Does it make sense of doing weekly DM spore trapping and use the dataset produced to forecast low, medium, and high pressure DM times atmospherically in a local area?

Can the level of DM pressure be inferred from weather data and time of growing season?

Do current growing practices create a situation where the DM pressure should be assumed to be high once May comes up north?

It looks like a modified bug or insect like we talked about on the phone would be the way to go especially for organics.

I am not a bug expert, but these guys are using modified bugs to develop therapeutics (https://www.perlara.com/blog/new-insights-targets-niemann-pick-c-therapies-drosophila-genetics/).

Seems like you could work out how to create a 'strain' of mildew eating bugs that could be managed in response to a stimulus that didn't affect the spinach (e.g., UV light).

The idea being the bugs live with the seeds and spinach keeping them clean then when

harvest comes around you just hit the spinach with a black light and you got mildew- and bug-free spinach :)!

I got some friends from Indie Bio I can try to get some contacts and insight from then you just put in a 1 or 2 pages summary to the granting agency to see if they think a full proposal is warranted.

https://nifa.usda.gov/funding-opportunity/organic-agriculture-research-and-extension-initiative

- 1. It would be interesting to test various dilutions of pyroligneous acid (PLA)to inhibit the germination rates of spores associated with contaminated seeds. PLA has been reported in peer reviewed scientific literature to have antifungal, antibacterial and strong antioxidant capacity. PLA and smoke treatments have also been reported to aid germination rates.
- 2. Biochars have been shown to enhance the systemic resistance of plants to pathogens. This works because biochar enhances beneficial microbial populations which suppress pathogen populations. The key term here is biodominance as pathogens are opportunistic. Our strategy will be to inoculate biochars with beneficial microbes & nutrients prior to amending into soils. One farmer we trailed with reported complete elimination of botrytis & powdery mildew on his organic strawberries.
- 3. Our PLA has been approved by CCOF for use on organic farms, but we haven't applied for EPA or DPR registration. (Must get funded first.) Other commercial biochars are approved organic.

I can send you $3^{\rm rd}$ party content if you like. Let me know. MR

Seed Treatment - UV Light

Jason,

Background

I am part of a group seeking a solution to downy mildew in organic baby leaf spinach. Research has shown that the seed may be contaminated with DM oospores, and that we are perpetuating the infection every time we plant.

Under the National Organic Program, it appears that UV may be approved, as long as it is not "ionizing irradiation".

Questions

Is your process ionized irradiation?

Have you applied for NOP approval through OMRI or WSDA?

Is your process be approved?

Would your process lower or eliminated DM on spinach seed?

What would be the negative results to seed germination and viability if exposed to UV?

Thank you.

BioLumic

Paul.

Thanks for your email. To date, we have carried out some in-house pilot work with DM-spinach as regards our technology. This work was done in seedlings, not seeds – our work with seed treatments is not focused on spinach at present.

To answer your questions, UV represents non-ionizing radiation, and because we have not developed a treatment program for organics so far, no organic program approvals have yet been sought in the US. There is no reason to believe that approvals would not be possible, due to the sustainable nature of our technology. If anything, we have seen benefits to germination with BioLumic-treated seed to date, not losses.

At the moment, our company resources are very much focused on our current programs in lettuce and some other non-veg crops. We may establish more work with vegetable seed treatments in the future, as this is of interest to us. To focus part of our future bandwidth on DM-spinach-seeds would require industry support, both financial at the development/trialing stage, and strong partnerships with end-users/growers/produce companies.

Happy to hear your thoughts on the above.

Regards, Jason Jason@biolumic.com www.biolumic.com T - 1 6421 1368 531