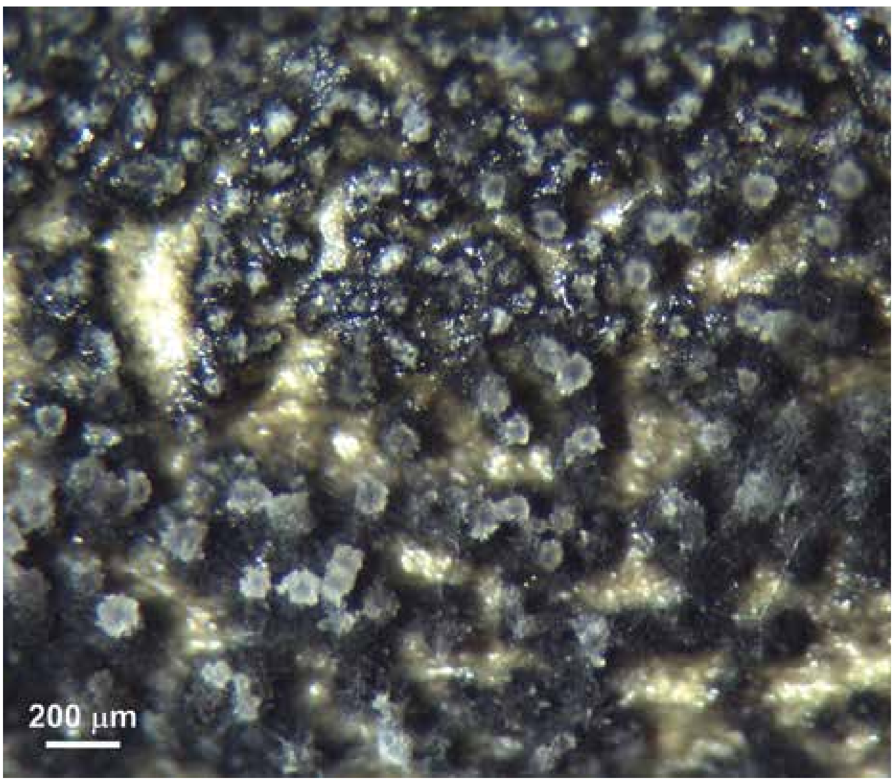
New knowledge on citrus black spot

By Megan Dewdney, Jeff Rollins, Nan-Yi Wang and Ke Zhang

Citrus black spot (CBS) has become established in the groves of Collier and Hendry counties. In other citrus-growing regions of the world where CBS is present, the sexual fruiting bodies of the fungus, known as pseudothecia, form in decomposing leaf litter. Approximately 50 to 180 days following leaf drop, the fruiting bodies mature and eject ascospores (sexual spores). Intermittent wetting and drying appears to be essential for the maturation of these fungal structures, and rain, irrigation or even heavy dew are effective sources of wetting.

Occasionally, if winter temperatures are warm with sufficient rainfall, leaves can decompose before the fungal fruiting bodies mature. Otherwise, the ascospores are ejected from fruiting structures up to 0.5 inch above the litter surface, where they are dispersed by air to susceptible leaves, fruit and twigs.



A citrus twig inoculated with *Guignardia citricarpa* after five weeks at 75°F and 100 percent relative humidity. The black round objects are the pycnidia, and the clear crust on the top is a matrix containing the conidia.

Optimal infection conditions for the pathogen, *Guignardia citricarpa (syn. Phyllosticta citricarpa)*, are temperatures between 70°F and 90°F and leaf wetness for 24 to 48 hours. In regions where black spot is well established, ascospores are reported to be the primary inoculum.

Conidia (asexual spores), produced in pycnidia (asexual spore-forming structures), are produced on mature fruit, leaf litter and dead twigs. The conidia are dispersed over short distances by splashing water from one fruit to the next, water running off dead twigs, or from the leaf litter to low-hanging fruit. Infections on fruit from both spore types have a 4- to 6-month latent period before symptoms develop.

In South Africa and Australia, where much of the black spot disease cycle studies have been done previously, ascospores from leaf litter are almost exclusively responsible for new infections; so there is effectively only one disease cycle per year. Conidia are not of primary importance in South Africa and Australia because of the citrus production characteristics in these regions: 1) uniform flowering and fruiting are common due to the cool temperatures during flower initiation and the use of drip irrigation, and 2) major rainfall events are limited to three months of the year. In Brazil, in addition to ascospores, conidia are also important for disease spread as they can infect young fruit on cultivars such as Valencia oranges that have multiple ages of fruit on a single tree, and there is frequent summer rainfall.

FLORIDA DISEASE CYCLE IS DIFFERENT

We have recently discovered that the disease cycle described above is not what currently occurs in Florida. In our studies on *G. citricarpa*, we showed that to form ascospores, two individuals of opposite mating types are needed. This is not the case for all fungi.

For example, we knew that the common citrus-associated fungus, *G. mangiferae*, can form ascospores without contact with another isolate of this species. It is considered self-fertile. *G. citricarpa*, on the other hand, is self-sterile, as it never produces ascospores when there is only one isolate of the species present.

There are other variations in fertility among fungi, including some fungi like *Alternaria alternate*, the fungus that causes Alternaria brown spot, which never produces sexual spores. These species are considered sexually sterile.

Given this variation, it is important to understand the mode of fertility (mating-type system) used by *G. citricarpa*. We were able to identify the genes responsible for mating-type regulation in these two species of *Guignardia*. The organization of the genes, both (two) mating-type genes present in a single isolate of *G. mangiferae* and only one of the two mating-type genes present in isolates of *G. citricarpa*, confirmed our observations of self-fertility and self-sterility, respectively.

However, when we began looking for both mating-type genes in our collection from 2010 onward of *G. citricarpa* isolates obtained from many Florida groves, we were unable to find

Finding Answers to Bactericide Questions

By Harold Browning

As I wrote a few months ago, the crisis declaration allowing growers to use three bactericide products provided a dose of good news and optimism to the Florida citrus industry. Now the hard work begins: gathering and interpreting data on how the compounds are working.

In order to learn from the grower use of these products, the Citrus Research and Development Foundation (CRDF) is directly overseeing or monitoring more than 70 field trials across all of Florida's growing regions, where growers have agreed to provide a matching untreated block or sector to compare with their treatment program for the season. The trials involve our major varieties, but are skewed toward Valencias. We are trying to cast as broad a net as possible during this first application period. Data will be collected and compared across all of the tests at the end of the season.

Stephanie Slinski and the CRDF field trial team will be working with the cooperator growers to collect data on: reduction in bacterial titer (PCR); disease index rating to measure changes in overall tree health; pre-harvest fruit drop; and yield at harvest. Many growers are directly participating in this process by collecting the data themselves.

Following harvest in the fall/winter, the data can be assembled to highlight results of the season-long program that are evident after one season across the varying treatment programs (how many treatments and which materials), regions of the state, varieties and even tree-age classes. Young blocks receiving treatments with bactericides also are being monitored to determine if the program will delay infection or onset of symptoms. We've tried to make the process as simple as possible for participating growers. You can find the instructions at http://bit.ly/1PQEPGT for grower field trials.

Results from this first season of use, along with data continuing to come from research field trials, will provide more information to guide grower use in the next season. Right now, we clearly have more questions than answers, and the first use of these materials has been almost overwhelming to growers. But we will get there.

Important questions are being asked, such as:

- What are the most effective adjuvants?
- How are the bactericides absorbed by the plant, through the surface or stomata?
- How long does the material remain active on the leaf surface?
- Do oxytetracycline and streptomycin move systemically once absorbed into leaves?
- What can and cannot be tank-mixed with oxytetracycline and streptomycin?
- What is the most effective rate and volume to apply?
- What are the effects of bactericide treatment on the psyllid?

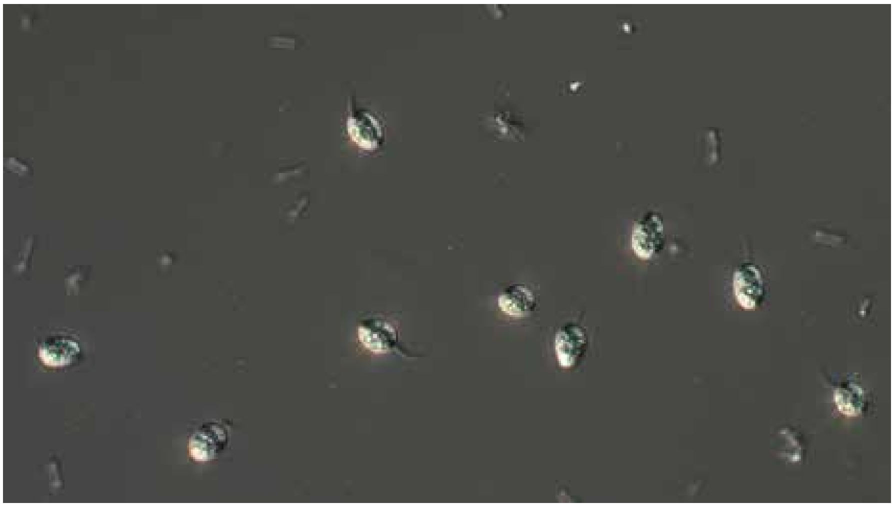
We have precious little information at this point, and I can understand grower frustration at not having this information. CRDF is working to provide answers to these questions.

Growers need to keep in mind that while we wait on the final Section 18 decision by EPA, it is essential to carefully follow the guidelines spelled out in Commissioner Putnam's declaration letter when using the bactericides. Go to http://files.ctctcdn.com/168a5f59201/e7bd14cf-0a3a-4f5c-9d3d-66ac12574bbc.pdf to view the letter.

Harold Browning is Chief Operations Officer of CRDF. The foundation is charged with funding citrus research and getting the results of that research to use in the grove.

CRDF Grower Dollars at work for you

Column sponsored by the Citrus Research and Development Foundation



The conidia (the ovoid spores with one flat end) are asexual spores that can infect citrus trees, and the spermatia (the rods) are asexual spores that are thought to be involved in sexual reproduction. On the rounded end of the conidia, an appendage is present.

the second mating-type gene. All 113 isolates had the same gene. This was a very unexpected result and implied that there could be no ascospore production in Florida. As this is an extreme claim, we needed more evidence that we had not missed the other mating-type gene through poor sampling.

Through a partnership with our Australian collaborators, we were able to test DNA of isolates from Queensland where we knew ascospores were formed. We found that there was nearly a 50:50 ratio of the two mating-type genes in a sample of only 26 isolates and that it was very unlikely that we had missed the other mating-type gene in Florida by chance. This single mating-type result needed to be verified with other evidence. To do this, we tested to see how many genetically unique individuals there were in the Floridian and in the Australian population.

As would be expected, if there was no ability to form sexual spores, there was only one genetic individual in the 70 isolates tested from Florida. In Australia, where sexual reproduction was common, there were 11 unique genetic individuals in 24 isolates tested. In summary, we determined that two mating-type genes were needed to produce *G. citricarpa* ascospores. One of these mating type genes was not found in Florida, and there was only one genetic population in Florida.

What does all of this mean? It means that the disease cycle in Florida is not the same as what happens in the rest of the world. The ascospore inoculum is not present in Florida; therefore, only the splash-dispersed conidia are involved in the disease cycle. This discovery is unprecedented and can help to understand the role conidia play in the disease cycle. Unfortunately, very little is known about the role of conidia in the disease cycle because the majority of research on CBS has been conducted in areas where these spores play a limited role.

It remains very important to clean equipment of all plant debris when traveling between grove sites, especially from infected to uninfected groves.

From our research and research conducted in Brazil, it is known that leaf litter, dead twigs and symptomatic fruit in the tree produce conidial inoculum. Our work has shown that conidia are produced at some level in the leaf litter nearly all year, but we do not know how they get into the canopy in large enough numbers to cause high levels of disease. There is no information on how long twigs and fruit within the tree remain sources of inoculum, but it has been shown that most disease appeared below these sources of inoculum within 32 inches of the source.

At this stage, it remains unclear how the disease has become so widespread in Collier and Hendry counties. The splashed-dispersed spores do not travel large distances by themselves. Since plant debris is a major source of these spores, it is thought that debris like twigs and leaves moved from location to location are the most probable sources for longer-distance movement of inoculum. Debris movement occurs most often on tractors, trucks, picking equipment and other equipment that comes into direct contact with trees. It remains very important to clean equipment of all plant debris when traveling between grove sites, especially from infected to uninfected groves.

HOW DID IT GET HERE?

An often-asked question is: How did *G. citricarpa* get to Florida? This is still a mystery that will likely remain unresolved. It seems even more mysterious when only one individual appears to be the founder of an entire population. However, this does not imply that only a single isolate was introduced. We should consider that even in a very favorable environment, like Florida for *G. citricarpa*, it is difficult for an invasive organism to become established.

It is highly probable that any tissue infected by *G. citricarpa* in another country contained more than one individual. Since there is about a 50 percent chance that any individual is a particular mating type, it is likely that whatever citrus tissue introduced the fungus to Florida contained both mating types. During establishment in Florida, it is speculated that only one individual survived, leaving the one mating type seen today.

The positive side of having only one mating type is that the more mobile ascospores are not present. This scenario of a single mating type slows disease spread and reduces disease intensity. With both spore forms, CBS could be nearly unmanageable in Florida. Hence, it is important to keep the other mating type from entering and becoming established in the future. This is a tough challenge for regulatory authorities, since we do not know how the pathogen was originally introduced.

DISEASE MANAGEMENT

To manage the disease in Florida, it is recommended that unnecessary movement of plant materials be avoided to limit long-distance spread. To manage CBS, continue to apply fungicide treatments to protect the fruit (see the CBS chapter in the Florida Pest Management Guide for recommendations and timing). Remove severely declining trees because they usually have the highest disease severity and produce the most inoculum. If possible, remove as much dead wood from the canopy as is economical.

Finally, in badly affected groves, avoid overlapping fruit ages as much as possible. CBS can be managed currently so that the damage is minimal, but it takes multiple strategies to accomplish this goal.

Megan Dewdney is an associate professor of plant pathology at the Citrus Research and Education Center (CREC) in Lake Alfred. Jeff Rollins is associate professor of plant pathology at the University of Florida in Gainesville. Nan-Yi Wang is a post-doctoral associate at CREC. Ke Zhang is a graduate research associate at CREC.