BFB Questions & Answers with the Experts

Bacterial Fruit Blotch

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Background

There are many diseases that can affect watermelon and melons. Why does BFB receive so much attention?

DH: There are several reasons.

First, it is a relatively new disease that became a problem in watermelon in the late 1980s. It was first observed in U.S. commercial watermelon fields in 1989.

Second, BFB can be devastating for growers with fruit losses reaching 80-100%. The severe losses are especially disturbing because symptoms of disease may not be obvious to the grower until two weeks prior to harvest. The crop has been raised, looks good, and then the fruit become unmarketable because of fruit blotch.

Third, bacterial fruit blotch is a high-profile disease due to numerous litigations that have occurred.

Fourth, the fact that all aspects of the industry – growers, transplant producers, and seed producers - suffer losses from BFB increases the attention over most other diseases.

There has been a lot of research conducted and money spent on BFB to understand the disease over the past 10-15 years. Why does BFB continue to be such a problem?

DH: One reason is that because the bacteria can spread rapidly in a field and transplant house, there has to be zero tolerance for seed contamination and transplant infection. One infected seed or transplant can result in considerable loss if conditions are favorable for disease, and this can spread to healthy seed lots in a transplant house. The fact that BFB can be seed transmitted on more than one cucurbit type and spread from one cucurbit to another makes the control even more difficult. The control also requires a global effort by the entire cucurbit industry.

RW: Much of the attention given to BFB has been in the areas of chemical disease management and seed health testing. Currently, chemical management options are limited to copper-based compounds that generally work when environmental conditions are favorable. In the case of seed testing, it is unlikely that 100% extraction efficiency, detection accuracy or precision can be achieved with any assay. Hence, while it is possible to detect heavily infested seedlots, lots with low levels of infestation will be difficult to consistently detect. Seed health assays alone cannot guarantee *A. avenae* subsp. *citrulli*-free seedlots.

Healthy watermelon transplants.

Seed production: Since the most significant source of inoculum for *A. avenae* subsp. *citrulli* is seed, an effective strategy would be to manage BFB in seed production fields. Effective management requires an accurate understanding of BFB epidemiology in the seed production field. Unfortunately, there has been little research done on BFB in the seed production environment and as a result there are no guaranteed strategies for preventing seed infestation.

For effective management, it is critical to identify inoculum sources, and mechanisms of dissemination and seed infestation. By understanding these epidemiological factors, strategies aimed at avoiding, or eliminating inoculum can be developed.

Producing *A. avenae* subsp. *citrulli*-free seeds would significantly reduce the frequency of BFB outbreaks in commercial transplant houses and fields. Without fully understanding how seedlots become contaminated or understanding how the bacterium survives on seed, there is little chance that we could effectively limit BFB outbreaks.

Transplant houses: Other possible explanations for the failure to control BFB include less than adequate levels of diligence on the part of transplant and commercial fruit producers. The transplant house has long been recognized as an aspect of commercial watermelon production that significantly influences the incidence and severity of BFB outbreaks.

Because of high plant populations, high levels of humidity and overhead irrigation, low levels of seed infestation could result in high proportions of infected seedlings leaving the transplant houses. Failure to implement preventative measures in the transplant house contribute to BFB incidence in the field.

Unfortunately, there is no cohesive body through which accepted BFB management guidelines can be disseminated to ensure that all transplant house operators are adhering to the best production strategies.

Commercial growers: Efforts to mitigate BFB in seed production may be negated by the shortcomings of seedling producers. Along these same lines, commercial growers also have a part to play. This includes:

- Using seed that has been tested for *A. avenae* subsp. *citrulli*;
- Insisting that the seedlings are inspected;
- Employing crop rotation and preventative copper sprays on a routine basis.

Only when all aspects of the industry work in concert, will we be able to permanently control BFB.

What are some of the things that we still do not know about this organism and disease?

RW: We still know little about the epidemiology of *A. avenae* subsp. *citrulli* in seed production environments. Most importantly, these include:

1) What are the primary sources of inoculum in seed fields (stock seed, weeds);

2) How the bacterium spreads e.g. rain splash or insects;

3) If and how the bacterium overwinters (e.g. weeds).

We also do not know what role the alternative inoculum sources like weeds, fruit and foliage debris play in commercial BFB outbreaks.

Additionally, we do not know how seeds become infested; where the bacterium survives in or on the seed and how seed processing techniques e.g. fermentation, drying, storage, etc., affect the survivability of the pathogen.

This knowledge would allow us to modify seed production practices to mitigate the risks of BFB. Finally, we know very little about the genetics of *A. avenae* subsp. *citrulli* with regards to pathogenicity.

DH: We do not fully understand the mechanisms involved in seed transmission of BFB. Are the bacteria for seed transmission on the seed surface or internal? We do not fully understand how seed contamination can occur without any apparent symptoms in the seed production field. Where are the bacteria coming from in some cases? We need effective seed treatments for wet seed and dry seed that will eliminate the BFB bacterium from infested seed. We need good greenhouse controls that eliminate spread and better field controls. We still do not know what the alternate hosts are for the bacterium.

For effective management, it is critical to identify inoculum sources, and mechanisms of dissemination and seed infestation.

Diagnosing

What are some of the diagnostic symptoms of BFB on seedlings?

DH: Symptoms alone cannot be used to diagnose BFB on seedlings. Symptoms can indicate that BFB may be present and that further testing is needed.

A first symptom of BFB in seedlings is a dark water-soaking on the lower surface of the cotyledon. These water-soaked lesions become necrotic, frequently with chlorotic halos. These lesions often are elongated along a vein of the cotyledon. Lesions can occur in the hypocotyls as seedling emerge, resulting in collapse and death of the seedling.

What are the diagnostic symptoms on leaves and fruit in the field?

DH: BFB is very difficult to diagnose based on leaf symptoms in the field. Often leaf symptoms may be inconspicuous or confused with other diseases that are more prevalent on the foliage. When they do occur, leaf lesions are light brown to reddish-brown and spread along the midrib and main veins.

On watermelon fruit, symptoms begin as small, greasy-appearing, water-soaked areas of less than an inch in diameter and enlarge to several inches in diameter with irregular margins. Initially, these lesions do not extend into the flesh of the watermelon, but later the lesions turn brown and crack. A white, foamy ooze often can be seen on the fruit surface. Fruit decay often follows.

Watersoaking on the lower surface of watermelon cotyledons.

BFB symptoms on mature watermelon leaves.

Dark brown to black leaf lesions on hardened-off watermelon transplants.

Fruit Infection

When and how can fruit infection occur, and does the fruit differ in susceptibility by age?

DH: The BFB bacterium enters the fruit through stomata, natural openings that occur on leaves and on fruit surfaces. Fruit infection occurs primarily during the first 3 – 4 weeks after fruit set. The last 7 – 10 days before maturity of the fruit the stomata become blocked by wax formation on the surface of mature watermelons, preventing entry of bacteria into most stomata. Actually, the fruit becomes more susceptible to the bacterium (symptom development) as the fruit matures and most of the symptom development is in the last 2 weeks prior to maturity. So infection occurs most commonly during early fruit development (1 – 3 weeks after fruit set) and symptoms usually occur most frequently during the last 2 weeks of fruit development.

Why do the symptoms seem to 'suddenly appear' on the fruit about 2 weeks before market maturity?

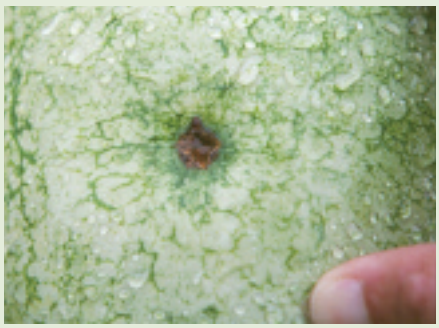
DH: The fruit appears to become more susceptible to multiplication of the BFB bacterium about 2–3 weeks after fruit set (anthesis). While the bacteria may invade the stomata of very young fruit, the resultant lesion may be small and restricted in size until the fruit becomes 2 – 3 weeks old, when typical lesions develop.

Will watermelon or melon fruit become infected after harvest, or show symptoms if they did not show them before harvest?

DH: I do not believe that fruit become infected after harvest. However, in fruit that are infected prior to harvest, symptoms may continue to develop and symptoms may progress from a surface lesions to collapse of the fruit. This can be a problem if it occurs in trucks in transit to market.

Why do some fruit that are infected with BFB crack open and foam?

DH: While BFB lesions are on the surface of the rind in the beginning, lesions progress into the fruit over time. Not only does the BFB bacteria grow in the rotting fruit but other fungi and bacteria may enter through the lesions. Some of these bacteria produce gas in the growing process inside the fruit. The foam is this gas escaping and can lead to the fruit cracking open.



Hosts

Seed can be one possible source of BFB infections. Have you encountered others, such as survival on alternate hosts or volunteer crops?

DH: In research plots, I have seen young, volunteer seedlings of citron and watermelon infected in the spring after citron or watermelon had BFB symptoms the previous year. I think this was the result of survival on seed of infected fruit that were left in the plots or field. I also know of at least one watermelon grower in Florida, who observed that his outbreak of BFB resulted from watermelon volunteers from the previous season when he also had the disease. I have not seen but have heard of volunteer citron infected with BFB in watermelon production areas.

Do you see much difference in susceptibility of different types of watermelon to the disease?

DH: No, I do not. We did some research several years ago that indicated that fruit with a dark-colored rind surface had less fruit symptoms than light-green rinds. However, this slight difference is not very significant. There may be differences in BFB incidence among the different types, such as triploids, hybrids, and open pollinated varieties, but this may be the result of differences in production practices rather than differences in susceptibility.

How susceptible are other cucurbits such as cantaloupe and are there differences in the susceptibility of the different types of melons?

DH: Cantaloupe and honeydew melons are very susceptible to BFB. The foliage of these cucurbits is as susceptible as watermelon foliage. Honeydew fruit are also very susceptible.

Cantaloupe symptoms may not be as prevalent and are often not as obvious on the surface of the fruit. I do not know the relative susceptibility of other types of melons. There have been some severe losses in pumpkin, so it is susceptible to some strains, at least.

While the foliage of other cucurbits, such as summer and winter squash and cucumbers, may harbour the bacterium, fruit of these cucurbits usually do not express symptoms. These crops have not been affected by BFB.

RW: The genetic diversity of *A. avenae* subsp. *citrulli* is greater than originally thought. Initially, it was believed that *A. avenae* subsp. *citrulli* was problematic only on watermelon and that the population was relatively homogenous. Since 1996 however, BFB has been observed on many cucurbits including melons, gourds, pumpkins, and cucumbers.

While it is possible that the pathogen may have evolved, a more likely explanation is that non-indigenous strains have been introduced from different regions of the world. Offshore seed production and worldwide movement of germplasm could have contributed to this phenomenon.

BFB lesions on cantaloupe fruit. Infection may penetrate into the rind.

BFB lesions on cantaloupe leaves. Note the tan to white appearance.

The most dramatic example of this has been the explosive increase in BFB occurrence in melons worldwide. Prior to 1996 there were very few if any reports of BFB on melon. Now, it appears that melons are as much at risk to BFB as watermelon. Severe BFB outbreaks have occurred on melons in the past 5 years in China, Australia, Costa Rica, Brazil and the USA. The melon strains are different from those associated with BFB of watermelon in the US in the early 1990's.

Symptoms on squash leaves inoculated in greenhouse studies.

Do you see other crops at risk in the future, such as squash or cucumbers? Why or why not?

RW: BFB may become a problem on cucumber and squash in the future. Even though both hosts are susceptible to attack in artificial greenhouse inoculations, there have been no reports of BFB in commercial squash or cucumber in the US. A possible reason for this is that the strains that are prevalent in the US are not aggressive on these hosts.

In greenhouse pathogenicity experiments, squash seedlings appeared to be highly resistant to most group II strains of *A. avenae* subsp. *citrulli* (from watermelon). Unfortunately, several group I strains (haplotypes F, I and N) caused disease on squash and pumpkin seedlings. While we have not recovered strains of haplotype I from natural BFB outbreaks, strains of haplotype N were associated with BFB in pumpkin in Georgia in 1998.

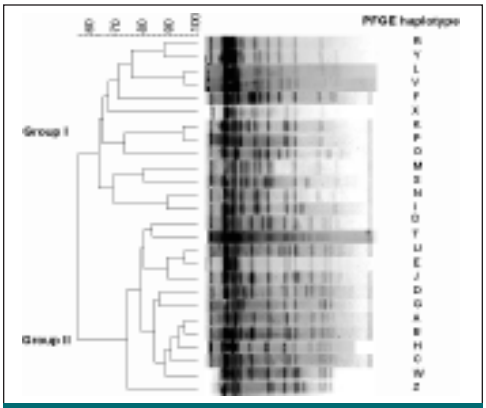
Interestingly, in 2001, strains of haplotypes F and N were associated with BFB outbreaks in melon in Brazil. Additionally, Dr. Petcharat Siriwong from Kohn Kaen University in Thailand has observed the natural occurrence of BFB on squash in northeastern Thailand (Personal communication). If *A. avenae* subsp. *citrulli* strains of haplotypes F, I and N (and other group I strains) are introduced into regions of the US with environmental conditions conducive to BFB, it is possible that the disease could become a significant problem on squash, cucumber and other cucurbits. The likelihood of BFB becoming a problem on other cucurbits will also increase where these crops are produced through greenhouse-grown transplants.

Dark tan BFB lesions on cantaloupe seedlings.

You have done a considerable amount of work profiling *A. avenae* subsp. *citrulli* strains based on their DNA. What are some of the techniques you are using to profile the strains, and how do you interpret the information from these tests ?

RW: To characterize *A. avenae* subsp. *citrulli* strains, we employ a DNA fingerprinting technique that involves digesting bacterial DNA with an enzyme (Spe I) and separating the fragments with pulse field gel electrophoresis. Additionally, we employ a polymerase chain reaction (PCR)-based technique called repetitive extragenic pallindromic (REP)-PCR to generate unique DNA fingerprints for each strain.

Strains with identical DNA fingerprints are considered to be the same haplotype; however, it is not necessarily true that identical haplotypes have the same origin/source. The differences in the



Pulse field gel electrophoresis of *A. avenae* ssp. *citrulli* strains representing the different haplotypes observed in a global collection of strains.

Hosts, continued

numbers and sizes of the DNA fragments between two strains are considered polymorphisms. Hence, strains with no polymorphisms are considered to be completely homogenous or clonal. Strains that differ by 1 – 3 polymorphisms are considered related since they may have developed from a single mutation. On the other hand, strains with 6 or more polymorphisms are considered unrelated.

Are there different strains of the bacterium?

RW: To date we have observed 26 PFGE haplotypes (DNA fingerprints) among *A. avenae* subsp. *citrulli* strains collected from the USA, China, South and Central America, Israel, Thailand, Canada, Mexico, Taiwan and Australia. The data suggest that there are at least two distinct groups among these strains. Group I strains were recovered from a wide range of cucurbit hosts including watermelon, melon, and pumpkin, while group II strains were recovered predominantly from watermelon. While there is no evidence of host specificity, the group II strains appear to be more aggressive on watermelon fruits and seedlings than group I. In seedling inoculation experiments, squash was the most resistant host; however, it was attacked by several group I strains (haplotypes F, I and N).

What does this mean? In general, populations that are more diverse tend to be more successful. A diverse population of *A. avenae* subsp. *citrulli* may be more difficult to manage in the long-term because the population will be able to adapt to selective environmental pressures including harsh environments, the introduction of resistant cultivars or use of chemical controls. Currently, there have been no reports of copper or antibiotic resistance in *A. avenae* subsp. *citrulli* populations, but bacteria are notorious for developing or acquiring such resistance with prolonged chemical exposure. Another troubling aspect is that within a diverse population, there may be nonindigenous strains that have unique characteristics e.g. the ability to attack a wide range of hosts. Once introduced to a region, genes that confer beneficial phenotypes may be shared contributing to a more diverse population that may be more difficult to manage.

How much is known about how *A. avenae* subsp. *citrulli* survives in the environment, including possible hosts other than cucurbits?

RW: Most of the information available on the epidemiology of BFB was generated in commercial fruit production environments, characterized by high temperatures and relative humidity. There are no reports of epidemiological studies conducted in dry cool conditions that persist in most seed production regions. Additionally, while there have been reports of the roles of plant debris, soil, volunteer plants and weeds in providing inoculum for BFB outbreaks, no replicated experiments have been reported indicating the significance of these inoculum sources.

Recently, *A. avenae* subsp. *citrulli* was reported to be detected in tomato seed in Israel. While it is tempting to speculate about the relevance of this report, the epidemiological significance of *A. avenae* subsp. *citrulli* on tomato or eggplant remains to be determined. Within the cucurbits; however, it is clear that many species can be hosts of *A. avenae* subsp. *citrulli* and that seed transmission is possible for most.

What type of additional research needs to be conducted to help clarify how the organism survives in the environment?

RW: To determine the role played by environmental sources of inoculum other than cucurbit seed, statistically sound ecological surveys must be conducted in seed and commercial fruit production regions. These surveys must include all possible sources of inoculum including soil, weeds, plant debris, irrigation sources, equipment, and volunteer seedlings. As one can imagine, this type of research will require considerable effort and resources and should be conducted in multiple locations representing different environmental conditions. Unfortunately, this is considered "high-risk research" because, there is a possibility that the data collected may not lead to conclusive determinations about the epidemiological significance of the inoculum sources. However, once possible inoculum sources are identified subsequent experiments should be conducted to test their epidemiological significance.

Seed Production

What improvements are seed companies making in their testing and seed handling procedures?

DH: There have been many changes in procedures for seed production, handling and testing. More attention has been given to producing seed in locations that have climates (hot and dry) that are less favorable for bacterial fruit blotch development.

Efforts are made to use BFB-free stock seed and transplants, and to isolate fields from other cucurbits. Fields are inspected for BFB symptoms on foliage and, especially on the fruit at harvest.

Only unblemished fruit are utilized in seed harvesting. Seed harvested from an isolated field, or from two or more small fields in close proximity generally constitute one lot. Different fields or harvests will constitute different lots when disease evaluations differ between the fields or harvests.

When possible, except for triploid watermelon varieties, fermentation of seed in residual pulp and juices for 24 hours is used to reduce the risk of seed transmission of BFB. Some companies are using disinfectant treatment after washing with materials such as peroxyacetic acid and hydrochloric acid.

The most important improvement seed companies have made however, is the testing of individual seed lots for presence of BFB. Through this testing infected seed lots are discarded and never planted. The procedures have evolved over the years to become more sensitive and more effective.

In early BFB seed health tests, 10,000 seeds per seed lot were assayed. Currently, 30,000 to 50,000 seeds per seedlot are assayed for BFB. This greatly increases chances of detecting a very low level of seed contamination.

Seed testing methods have also improved since the mid-1990's. The 'grow-out methods' in the greenhouse and in plastic chambers have been defined so that optimum conditions for BFB symptom development are known and these are often combined with molecular methods to positively identify the bacterium.

What are some of the most critical factors in the seed production process that can be used to help minimize the risk of seed-borne BFB?

DH: Start with clean stock seed and use BFB-free transplants.

Produce seed in areas that have not had prior BFB problems.

Use professionals that are familiar with BFB symptoms and will carefully inspect the transplant house and field for symptoms.

Inspect at least twice in the transplant house, within 2 – 3 weeks of transplanting in the field, after rainy, humid periods of weather, and as the fruit nears maturity.

Collect seed only from fields that have no confirmed BFB symptoms.

Run seed health assays on every seedlot.

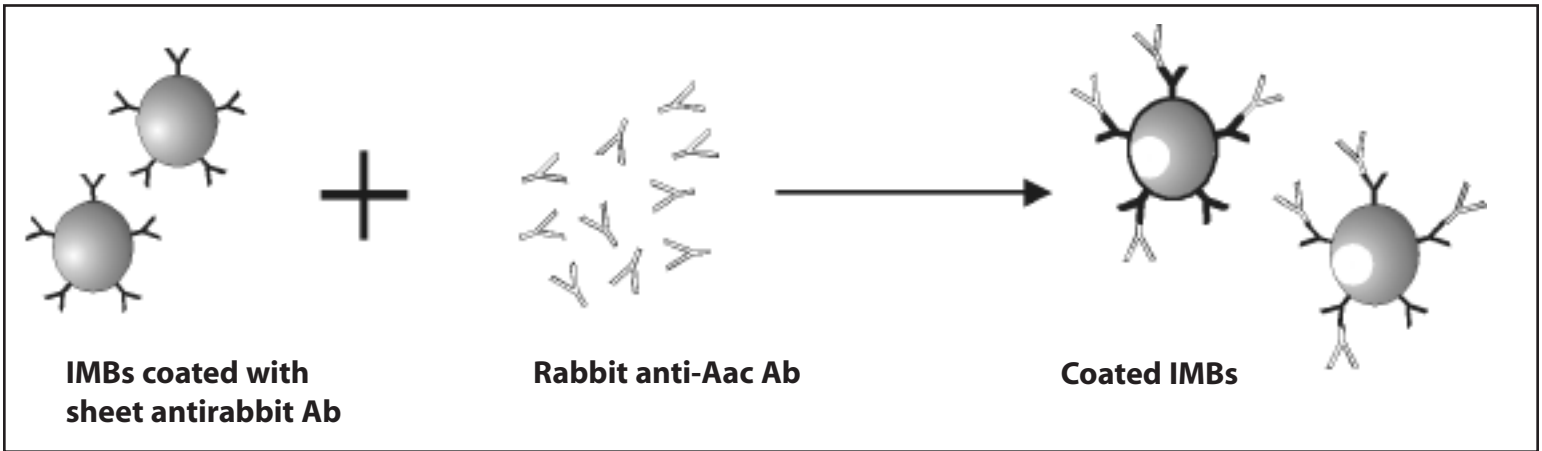
The seed industry is currently using several different *A. avenae* subsp. *citrulli* detection techniques that for the most part are based on growing seedlings from seed in conditions of high temperature and humidity, and waiting for seed transmission. These tests can be expensive and time consuming. You are currently working on a laboratory seed assay that could possibly be more sensitive, faster and less expensive. Would you please explain this research?

RW: The polymerase chain reaction (PCR) is a rapid (completed in 1 day), highly efficient and sensitive in vitro assay for amplifying and thereby detecting specific DNA sequences. As a result, it has many characteristics that are ideal for seed health testing. Unfortunately, cucurbit seeds are relatively large, making them difficult to process. Additionally, cucurbit seed extracts can inhibit PCR leading to the potential for "false-negative" results. Hence, while PCR is an excellent detection assay, there is a need to modify this technique for use in seed detection. Part of my research focuses on developing a PCR-based seed test that can detect one *A. avenae* subsp. *citrulli*-infested seed in 10,000 health seeds. With this objective, we rely on immunomagnetic separation (IMS) to enhance the recovery of *A. avenae* subsp. *citrulli* cells from seed wash. With IMS, *A. avenae* subsp. *citrulli*-specific antibodies are attached to microscopic magnetic beads

When dispersed into seed wash the antibodies allow the magnetic beads to specifically bind to *A. avenae* subsp. *citrulli* cells. Using a magnet, the immunomagnetic beads, carrying the captured bacteria, are collected, while nontarget bacteria and inhibitory compounds are washed away. The captured cells are then lysed to release DNA that can be used for PCR. To enhance the sensitivity of IMS-PCR we have improved immunocapture and incorporated an enrichment step using a semiselective medium. We are also developing a system by which *A. avenae* subsp. *citrulli* cells captured from seed wash can be delivered to two-week-old seedlings as part of a bioassay. The target organism could then be readily recovered from infected seedling tissue to confirm positive PCR results. Finally, we are trying to improve the process by which *A. avenae* subsp. *citrulli* cells are extracted from cucurbit seeds. While it is common to crush seed to extract bacteria, large seed samples create a significant processing problem. We are evaluating the efficiency of vacuum extraction to recover the pathogen from large seedlots (n=10,000 seed). Once the technique has been evaluated for sensitivity, specificity and applicability, we will evaluate its precision and accuracy of detecting the pathogen in naturally infested cucurbit seedlots. The end result should be a highly reproducible and sensitive laboratory seed assay that can be completed within 2 – 3 days.

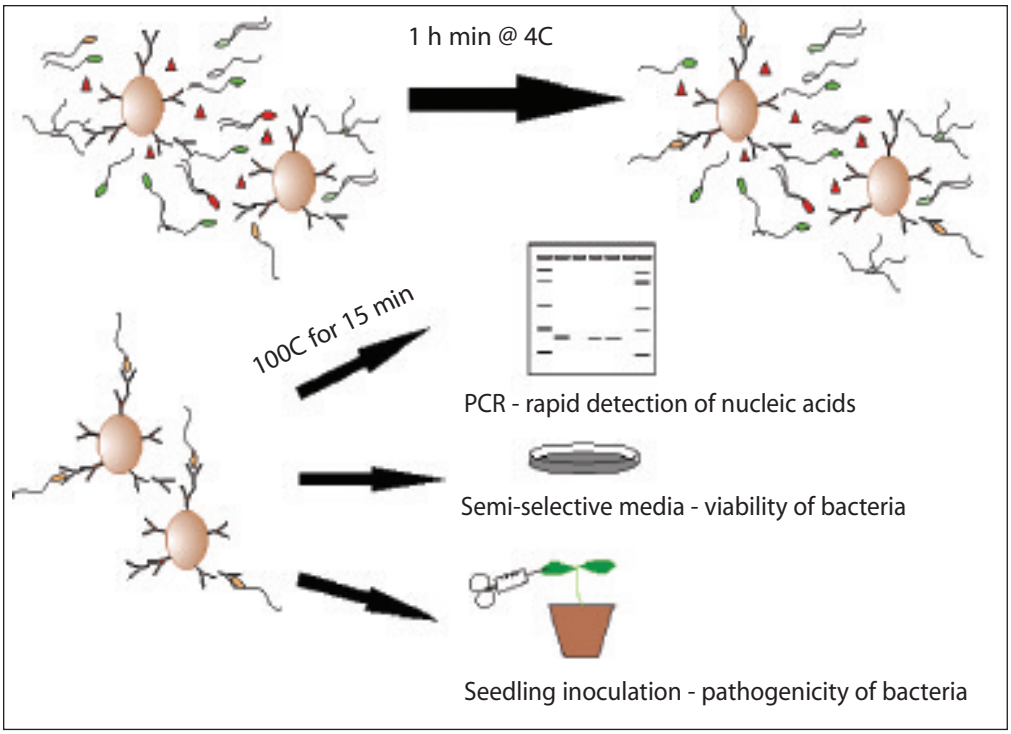
How does BFB cause infections on fruit without showing recognizable symptoms. What are potential implications for seed producers?

RW: As with many bacterial pathogens, environmental conditions play an important role in disease symptom development.



Schematic diagram indicating how immunmagnetic beads are coated with antibodies specific for *Acidovorax avenae* subsp. *citrulli* in the immunomagnetic separation and polymerase chain reaction-based seed test.

Seed Production, continued



Schematic diagram indicating how immunomagnetic beans coated with anti-*A. avenae* subsp. *citrulli* antibodies are used to capture cells for subsequent detection of PCR, plating or inoculation onto watermelon seedlings.

It is possible that typical BFB symptoms do not develop, and therefore are not observed during visual seed field inspections. Even in the absence of BFB symptoms *A. avenae* subsp. *citrulli*-infested seedlots can be produced.

One possible explanation for this phenomenon is the colonization of female blossoms leading to the occurrence of *A. avenae* subsp. *citrulli* in asymptomatic fruit.

In greenhouse experiments, female watermelon blossoms inoculated with *A. avenae* subsp. *citrulli* at anthesis developed into symptomless fruits. However, seed within these fruits were infested with *A. avenae* subsp. *citrulli* and resulted in BFB infected seedlings.

The role of blossoms and pollinating insects in seed infection is currently being investigated to determine their epidemiological significance.

Implications of these findings for seed producers are significant. The absence of typical symptoms on infected watermelons in seed production fields means that visual inspections may be incapable of ensuring *A. avenae* subsp. *citrulli*-free seed. Seed from fields that pass visual inspection may be contaminated with low levels of *A. avenae* subsp. *citrulli* that are not detectable by conventional seed assays.

One positive implication of this finding is that female blossoms could be protected from *A. avenae* subsp. *citrulli* infection by chemical or biological controls.

Unfortunately, the epidemiological significance of blossom colonization in seed production is currently unknown. However, if proven significant, BFB strategies directed at protecting watermelon blossoms could be developed.

Are there any potential implications of symptomless infection for commercial crop growers?

RW: The implication of asymptomatic fruit infection for commercial growers is not as significant as for seed producers; however, it may still be important. If it is proven that pollinating insects can serve as vectors for *A. avenae* subsp. *citrulli*, the movement of bees from infected to noninfected fields may represent a possible inoculum source for BFB outbreaks.

Greenhouse

Early detection is a key to minimizing BFB spread and damage in transplant greenhouses because the disease can spread so rapidly in warm, humid conditions.

What can be done to help minimize the risk of BFB development and spread in greenhouses?

DH: Early detection and discarding infected seedlings can prevent infection of the entire greenhouse. The best way to reduce spread in the greenhouse is to use ebb and flow irrigation. With this method, even if there are infected seedlings, BFB will spread only to a few seedlings surrounding the infected ones.

When overhead irrigation must be used, it is much more difficult to minimize spread. Copper applications, either through the irrigation water or as a separate spray, can reduce spread. Watering only when the foliage is dry may be beneficial.

Every transplant greenhouse must have a sanitation program to help prevent the development, or carry-over of BFB and other diseases. This program would include using clean planting trays and medium, disinfecting all surfaces such as railings and the floor between plantings, and disinfecting any equipment that might be used for seed sowing. Any weeds in or near the transplant house should be removed, and personnel should clean their hands and shoes upon entering and exiting the greenhouse. Personnel should also avoid contact with plants in the greenhouse.

What are some BFB control measures for the greenhouse and do any of them look especially promising?

DH: Several different treatments are being evaluated of which they are primarily applied through the irrigation water. Products that include hydrogen peroxide as part of the active ingredient such as peroxyacetic acid and hydrogen dioxide have looked promising. Copper is continuing to be evaluated both as a spray and ionized copper in the irrigation water. The ionized copper looks promising because it has given good control at very low concentrations of copper. Ozone and the plant defense activator, Actigard, are also being studied.

Production of watermelon transplants.

Combinations of treatments may be most effective.

Are physical barriers such as walls or partitions, or even empty spaces between groups of trays, effective for limiting the spread of BFB?

DH: Barriers and empty space can be effective in limiting spread of BFB by splashing. However, it is difficult to prevent the chance of spread occurring, even from one end of the greenhouse to the other. This may be rare but can occur. A travelling boom used in irrigating greenhouses can create a fog-like condition in some cases and water droplets or aerosols can collect on the boom and drip as it travels down the greenhouse. These aerosols and drops can contain the BFB bacterium. Overall, barriers and open space are good ways to reduce the risk of BFB moving from seedlings of an infected seed lot to BFB-free seedlings.

What is recommended for greenhouse inspections, including when they should begin and how frequently they should be conducted?

DH: Water-soaking symptoms on seedlings can usually be observed first on the 2nd to 3rd day after emergence and necrosis can be seen a couple of days later. Inspections should begin 4 – 5 days after emergence and be conducted as frequently as possible, at least weekly.

If BFB occurs in the greenhouse, what should be done before a new cucurbit crop is planted? Is there a 'safe' amount of time before a new crop can be planted?

DH: The transplant house should be thoroughly decontaminated with a disinfectant, such as bleach, hydrogen dioxide, or 70% ethanol.

There is no documented 'safe' time before cucurbits can be planted in the transplant house. However, in the absence of plant material or organic debris, the bacteria do not survive more than a couple of weeks on inert surfaces in a greenhouse. A wait of 2 – 3 weeks, or longer, is recommended before planting cucurbits in a commercial transplant production greenhouse in which BFB has occurred.

Do techniques such as solarization work for BFB, and if so how long should the greenhouse be empty?

DH: I do not know whether solarization works or not. However, the bacterium does not survive very long on clean plastic trays or benches in the greenhouse in the absence of organic matter.

One of the most difficult questions to answer if BFB occurs in a greenhouse is how much has the disease spread. There will be plants with clear symptoms, but also infected plants that do not show symptoms at any given time. Do you have any suggestions for making decisions about whether or not to try to save plants, or if a whole greenhouse is at risk?

DH: With overhead irrigation, a whole greenhouse may be at risk; however, the spread is normally more localized than that. It certainly may be possible to save plants in the greenhouse if the symptoms are localized in one area in the greenhouse. The problem comes from the fact that once the BFB bacterium is splashed onto a watermelon plant, it normally takes about 3 days for symptoms to develop. Thus, spread that occurred over the previous 3 days can not be seen.

The difficult question is how far beyond the symptomatic plants do you discard? This can not be accurately estimated. My best suggestion would be to select a distance from symptomatic plants, say 20 feet, that you are going to discard. Remove these plants, but place the trays that are 15 – 20 feet from symptoms in an isolated, warm, humid location and observe them for 4–5 days for symptom development. If no symptoms develop, you probably have discarded the infected zone. If symptoms develop in these plants, you may not have discarded them all and will have to decide what to do with the remaining plants in the transplant house.

Dark brown to olive green BFB lesions on watermelon seedlings.

Field Prevention Tips

There are measures that a commercial crop producer can take to minimize the chance of BFB in their production field. Crop rotation, and using disease free transplants are just a few. Would you name some others?

DH: • Direct seed only seed from seed lots that tested negative for the BFB bacterium or use BFB-free transplants.

- Do not introduce BFB into the field on seeds or transplants!
- Be sure that any cucurbit transplants in nearby fields are also free of BFB.
- Eliminate wild cucurbits, such as citron, and volunteer watermelons, cantaloupes, and melons near production fields.
- If BFB has occurred in your field or a neighbor's field the previous season, plant your crop as far as possible from that contaminated field and spray your current field regularly with copper.

Even if transplants or seed (direct seeded crops) is infected, it does not necessarily mean that the disease will develop in the field. Is this true, and if so, what environmental conditions will be necessary for BFB to occur in the field?

DH: It is possible for the BFB bacterium to be introduced into a field and for the disease does not develop. BFB is promoted by hot and wet conditions.

Disease development and spread is most rapid in the summer when weather is hot and sunny with frequent afternoon thundershowers, which includes blowing rains. Disease development may not occur or will be slow if it is cool, dry, or both.

How likely is it that the BFB bacterium could be spread from an infected to a healthy field, and how might this occur?

DH: The BFB bacterium can very easily be spread from and infected to a healthy field. The bacteria can be moved on people's hands, shoes or clothes, when they work in or visit a field that has BFB. The bacterium can also move on any equipment, such as tractors, irrigation supplies, truck tires, cultivation equipment, and hoes, that might be shared between fields and be moved from an infected field to a healthy field.

Harvesting of watermelon crop.

Control & Treatment

What would you recommend for a field BFB detection and control program?

DH: Field BFB detection should begin as soon as transplants are in the field or seedlings have emerged. Seedlings are more susceptible than leaves on older plants, so early in the season is a good time to scout the field for symptoms. Careful observation of the field should be continuous. If symptoms are observed and a diagnosis of BFB is made, begin applications of copper fungicide immediately. In this case, applications should be made weekly at the full rate of copper.

If symptoms are not observed but you want to make protective applications, biweekly applications at the full rate of copper or weekly application at half rate will be protective. Any time symptoms occur switch to the schedule described above. Protective applications should begin at first flower, or earlier, and continue until all fruit are mature. When fruit symptoms occur, it is too late to control BFB.

Are all copper spray formulations created equally in terms of effectiveness against BFB?

DH: There may be small differences in effectiveness of different formulations, but I have not noticed any drastic difference. The amount of soluble copper available in the formulation may be important.

Are there other materials that can be added to copper sprays to enhance its activity?

DH: With BFB as well as other bacterial diseases, there may be a benefit of adding mancozeb. We have done some research with plant defense activators, such as Actigard, and they may be beneficial on young plants if these materials become available.

Do you know of any new products that might become available for use against BFB in the field?

DH: No, unless an effective plant defense activator becomes available.

What will be required in terms of an integrated effort between seed suppliers, transplant producers and commercial crop growers to consistently avoid problems that could be caused by BFB?

DH: An integrated management approach between seed suppliers, transplant producers, and commercial growers will be required to successfully control BFB in cucurbits.

The seed industry must use every tool available to produce pathogen-free seed for the transplant growers and producers.

The transplant industry must make every effort to prevent the introduction and development of BFB in the transplants, including inspections, diagnostic testing, and control procedures to prevent spread if BFB does get into the transplant house.

The growers must be diligent in working to prevent the introduction of BFB into their fields and to control it when it does occur.

Each of these parts of the cucurbit industry is also dependent on the other two parts for success, so they must integrate their efforts.

How can the industry work together to deal with BFB more effectively?

RW: BFB shares many characteristics of other seedborne bacterial diseases. The situation is complicated by the fact that current watermelon and melon production practices require the input of seed, transplant and commercial growers. At each of these stages, there is the potential for *A. avenae* subsp. *citrulli* to be introduced into the system.

Effective BFB management will require cooperation and coordination of activities of these three groups and so far this has not consistently occurred.

Additionally because of the litigious nature of BFB, there is stigma associated with the disease, which embellishes its damage potential. One way to change this view is to launch an educational program directed at providing accurate information to seed, transplant and fruit producers. An adequate information dissemination vehicle (websites, brochures etc.) should be used to inform all aspects of the cucurbit industry about the actual (rather than perceived) BFB threat and what is actively being done to mitigate it. It should also be used to provide useful information on the appropriate steps that should be taken when BFB does develop. Creating a transparent and cooperative atmosphere may help to reduce the litigious atmosphere surrounding BFB.

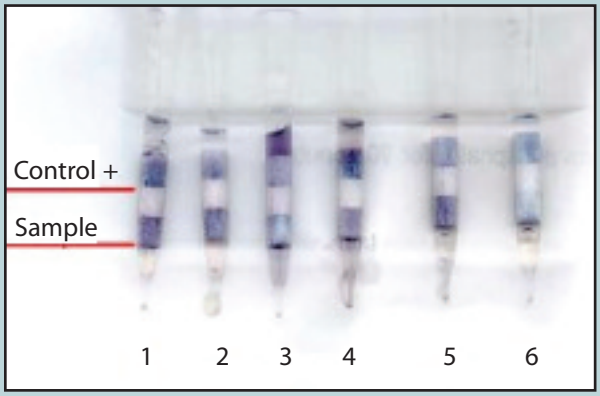
What to Do...

If a grower suspects BFB, what services (including public or private) or rapid testing kits, are available to help determine the cause of the symptoms?

DH: There needs to be a rapid diagnosis if a grower suspects that BFB symptoms are present. The local extension service is a good place to take a sample of the symptoms. The state university diagnostic laboratory should be able to give a rapid, accurate diagnosis. There also are private diagnostic laboratories that can run the tests. There are rapid diagnostic kits available that are easy to use for those that may have a frequent need, such as transplant growers and growers with large acreages. Most growers would be better served to go through their state university.

I have heard you say on occasion that a grower should not panic if they detect BFB in their field. Why is that, and what can be done?

DH: I am primarily referring to early detection when I make that comment. If BFB is detected early in the field (anytime prior to fruit set), losses from the disease can be minimized with copper fungicide applications as described above. The environmental conditions also can limit the losses to the disease. However, if the disease is not detected until symptoms appear on the fruit and environmental conditions are favorable for disease, there is not much to do but salvage healthy fruit.



Bacterial Fruit Blotch detection with rapid diagnostic test.

Bacterial Fruit Blotch (BFB) is caused by the bacterium *Acidovorax avenae* subsp. *citrulli* (Schaad et al.) Willems et al.

There are many sources of additional information on Bacterial Fruit Blotch, including:

Compendium of Cucurbit Diseases, by The American Phytopathological Society, 3340 Pilot Knob Road, St. Paul Minnesota, 55121-2997.

2001 Guidelines for Managing Bacterial Fruit Blotch Disease, by the National Watermelon Association, Inc., P.O. Box. 38, Morven Georgia, 31638.

Watermelons, Characteristics, Production and Marketing. ASHS Press. 113 South West Street, Suite 200, Alexandria, VA. 22314

The sponsors wish to extend their appreciation to Dr. Donald Hopkins of the University of Florida, and Dr. Ronald Walcott of the University of Georgia for their contribution to this bulletin.

International Research

Are researchers outside of the U.S. working on BFB, and if so, what are some of their projects?

RW: Dr. Petcharat Siriwong: Kohn Kaen University, Kohn Kaen, Thailand: Epidemiology, management and genetic diversity of *A. avenae* subsp. *citrulli.*

Dr. Rosa Mariano – Universidade Federal Rural de Pernambuco, Recife Brazil: BFB epidemiology, disease management (biological seed treatments, chemical - copper and antibiotics), alternative hosts.

Dr. Mark Fegan University of Queensland Australia - Development of rapid diagnostic assays for *A. avenae* subsp. *citrulli.*

Heidi Martin: Gatton Research Station, Gatton, Queensland Australia - Investigation of antibiotic and copper resistance among local *A. avenae* subsp. *citrulli* populations.

Biographies

DON HOPKINS, PhD

Dr. Don Hopkins is a professor of plant pathology at the University of Florida's Mid-Florida Research and Education Center in Apopka. He received his B.S. degree in agriculture and chemistry from Western Kentucky University in 1965 and his Ph.D. in plant pathology from the University of Kentucky in 1968. He spent a 15-month post doctorate in plant pathology at the University of Wisconsin. He has been employed by the University of Florida since 1969, with research responsibilities for studies on the etiology, epidemiology, and control of important diseases of fruit crops and cucurbits. He has conducted research on bacterial fruit blotch (BFB) since it first appeared in the U.S.A. in 1989. His research has focused on epidemiology and control of the disease and has included seed treatments, seed health testing, resistance screening, environmental effects on disease development and spread in the greenhouse and field, and chemical control. Current research emphases are on the development of new seed treatments, control of BFB in the transplant house, and incorporation of resistance into watermelon.

RON WALCOTT, PhD

Dr. Ronald R. Walcott is an Assistant Professor of Plant Pathology at the University of Georgia in Athens, GA. He received his B.S. and M.S. degrees in plant pathology from Iowa State University in 1993 and 1995 respectively, and his Ph.D. in plant pathology from the University of Georgia in 1999. He spent part of 1999 as a post doctorate in plant pathology at the Coastal Plain Experiment Station in Tifton, GA. He has been employed at the University of Georgia in his present position since 1999. Ron was the recipient of numerous academic awards during his undergraduate and graduate careers and was a 2001-2002 UGA Lily Teaching Fellow recipient as an Assistant Professor. He first began working on BFB as a Ph.D. student and his research focused on the genetic diversity of *A. avenae* ssp. *citrulli*, and developing an effective seed detection assay. His current main area of responsibility is seed pathology, and approximately 80% of his time and resources are committed to studying the seedborne aspects of BFB. Some of his research interests are development of an effective PCR-based seed detection assay, understanding the epidemiology of BFB in seed production fields and developing effective strategies for managing seedborne aspects of BFB.

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