

VERSION: 1.1	DATE: 2017
PATHOGEN: Acidovorax citrulli (syn: Acidovorax avenae ssp. citrulli)	
HOST: All Cucurbits	
COMMON NAME: bacterial fruit blotch (BFB)	
METHOD: Cb 1.1 Seedling Grow-out	
METHOD CLASS: STANDARD (A)	
SAMPLE: 10,000 - 30,000 seeds	

REVISION HISTORY: Version 1.1: Host changed to include “All Cucurbits” 07.12.2017

PROCEDURE:

I. Greenhouse Preparation

- A. Thoroughly clean greenhouse floors, walls, benches and equipment and sanitize with a known bactericide before each test is initiated in the greenhouse.

II. Planting seeds

- A. Plant seed into any common, sanitized greenhouse potting mixture and maintain under conditions optimal for seed germination until seedling emergence. Sample size should be a minimum of 10,000 seeds up to 30,000 seeds per lot.
 - 1. Seedling density should be such to allow unrestricted seedling development for a period of three weeks.
- B. Plant a known A. avenae ssp. citrulli-infested seed sample as a check on the test conditions and subsequent disease development. Maintain the positive check in an isolated area of the greenhouse.

III. Greenhouse conditions

- A. Maintain relative humidity at 70% or higher, from the time seedlings emerge to final reading. Relative humidity should not be lower than 50% for more than 12 h.
- B. Maintain greenhouse temperatures between 24°C (74°F) and 35°C (95°F) from seedling emergence to final inspection. Temperatures should not be out of this range for more than 12 h. The preferred temperature is 29°C (85°F).
- C. Record temperature and relative humidity of the greenhouses, preferably above plant canopy for the duration of the test.

D. Supplement light to 12 hours per day if necessary.

IV. Evaluation Procedures

A. Initiate seedling inspection once the cotyledons begin expanding and continue evaluations on a daily basis until final evaluation. This inspection should include the positive controls. Inspect the positive controls last and do not handle them. If positive controls do not show symptoms of BFB then the grow-out test is not valid. See below for pictures of typical BFB symptoms on seedlings.

1. Avoid contact with the seedlings until the final inspection.
2. Remove symptomatic seedlings as soon as possible.
 - a. Conduct a diagnostic test as described under section IV.B.2 and confirm the identity of the causal agent.

B. At 18 days, or when the cotyledons are fully expanded and first true leaves are expanding, conduct a thorough final inspection of all of the seedlings. Seedling inspection should also be conducted immediately upon finding symptomatic seedlings and confirming the identity of the causal agent.

1. Inspect the seedlings individually.
 - a. Only technicians trained in BFB detection should conduct inspections. Additionally, color photographs of BFB symptoms on seedlings should be provided as a reference.
 - b. Seedlings should be inspected under natural light in a well-lit area.
 - c. Gloves should be worn when handling seedlings and changed between each seedlot.
2. Symptomatic seedlings can be tested initially by field test strips, ELISA, or PCR testing that is specific for *A. avenae* ssp. *citrulli*. Final confirmation of suspect plants should be made by isolating the bacterium onto YDC, or semi-selective media such as King's Medium B (King *et al.*, 1954. *J. Lab. Clin. Med.* 44:301) and Modified Tween Media. Colony morphology on the various media are as follows:
 - Modified Tween:** Aac colonies appear grayish, flat, round to oval, with a crystallized zone surrounding the colonies;
 - YDC:** Aac colonies appear tannish, domed and round. Colonies range in size from 1 mm to 3 mm after 2 – 3 days incubation at 28 °C.
 - Kings B:** Aac colonies are non-fluorescent, cream colored and slightly domed. Colonies remain constricted up to 5 days.
3. Suspect bacteria isolated from selective medium should be transferred to YDC or some other non-selective media. Each isolate should be inoculated onto watermelon or melon seedlings at the expanding cotyledon stage. Inoculations can be made by inserting a sterile toothpick into the suspect colony and inserting the toothpick into the growing point of the plant. Several single colonies of each suspect isolate should be inoculated. Plants should be kept under high humidity for 48 to 96 hours. Water soaked lesions will be visible within 24 to 96 hours.

V. Minimizing the risk of cross-contamination

A. Maintain adequate spacing (60 cm) between different seedlots placed in the same

greenhouse.

1. Barriers that can be disinfested or easily removed and disposed can also give adequate protection. The barriers should be 60 cm to 90 cm tall.
- B. Strict rules and effective chemical treatments should be enforced to keep insects, spiders, rodents and any other known and potential vectors of *A. avenae* ssp. *citrulli* out of the greenhouse.
- C. Avoid manual manipulation of the seedlings during the grow-out except as necessary for inspecting individual seedlings during final inspection.
 1. The only exception to this is the contact necessary to remove the symptomatic seedlings for tissue testing.
- D. Minimize splashing and run-off during watering, especially between different seedlots in the same greenhouse.
- E. Remove all *A. avenae* ssp. *citrulli* infested samples from greenhouse and immediately dispose of tissue appropriately.

RECIPES:

MODIFIED TWEEN MEDIUM:

Peptone 5.0 g
CaCl₂•2H₂O Dihydrate
0.25 g Tween 80 10.0 ml
Agar 15.0 g
Berberine (200 PPM = 200 µg/ml) (hemisulfate salt) 0.2
g Methyl Violet B (crystal violet) 1.0 ml (of a 1% Stock)

1. Use 970 ml of H₂O initially.
2. Be certain to use Tween 80 not Tween 20. Expel, wait, expel, wait, and do not rinse the pipette. Stir quickly to get materials dissolved.
3. Adjust pH = 7.3 1 N
NaOH↑
4. Autoclave for 25 minutes; then
add:
Cycloheximide 50 mg*stock = 50 mg/ml (use 1.0 ml)
Carbenicillin 50 mg*stock = 50 mg/ml (use 1.0 ml)

Stock solutions are sterilized by passing through 0.2 µm filter and aseptically transferred to 1 ml aliquots and stored at -20°C.

This medium was originally described by R. Gitaitis, University of GA and modified by the laboratories at Seminis Vegetable Seeds, Inc.

DIAGNOSTIC PHOTOS:

These photographs show typical symptoms of BFB at the seedling stage.



