**TITLE**
Developing Management Strategies for Bacterial Canker on Tomatoes

**PRINCIPAL INVESTIGATOR**
Helene R. Dillard
Cornell University, NYSAES
Department of Plant Pathology
Geneva, NY 14456

**COOPERATORS**
Carol MacNeil, Extension Vegetable Area Specialist
Abby Seaman, Extension IPM Area Specialist
John Gibbons, CCE
Meg McGrath, Long Island Horticultural Research Lab

**ABSTRACT (non technical)**
The objective of this study was to identify sources of on-farm inoculum of *Clavibacter michiganensis* subsp. *michiganensis* (CMM), the causal agent of bacterial canker of tomatoes. Two hundred ninety seven samples from inanimate (swabbing from walls, floor, wood, hoses, etc.) and animate sources (weeds, other plants, tomatoes) from 3 commercial greenhouses were tested to see if they carried the CMM pathogen. A wide variety of methods were utilized in attempts to conclusively identify the bacteria, including an agglutination kit and a commercial diagnostic testing service that used a quick ELISA test. Any bacteria isolated that were likely to be the pathogen were further tested to see if they could cause disease on tomatoes. The various isolation procedures were cumbersome, slow, expensive, and difficult to interpret since none were diagnostic alone. The disease organism is difficult to isolate, identify conclusively, and even more difficult to prove to cause disease.

The tomato transplants from one of the commercial grower's greenhouses were subsequently planted in 2 locations. In the commercial field where bacterial canker occurred 3 years previously, moderate disease developed on the leaves and fruit starting at fruit load. Disease also occurred in transplants planted on "new" ground, as well as on tomato volunteers from an area with canker in 1999. The transplants appeared to be harboring the disease in the planted fields, but contaminated seed or overwintering debris or weeds or other crops seemed to be the source of inoculum for the volunteers. Weather is obviously a driving factor in disease development, since the disease has varied from severe, moderate, to low over three consecutive years at one location. Bacterial canker did not develop in a sample of the same transplants that were grown in a field in Geneva that did not have a history of bacterial canker. Transplants from the other 2 growers did not develop bacterial canker in the greenhouses or in the fields.

There are no highly effective chemical control measures for bacterial canker. Disease control starts with clean seed and continues with good sanitation practices in the greenhouse. This includes disinfecting surfaces in the seedling greenhouse and sanitizing any reused equipment or supplies. Bacteria may survive as an epiphyte on other plants

present in a greenhouse. Epiphytes do not cause disease on plants, but will grow and multiply on non-host plants, which become an inoculation source for susceptible plants such as tomatoes. The best practice is to dedicate a greenhouse to tomato production and avoid co-mingling tomato transplants with transplants of ornamentals or other crops. Avoid damage to the tomato plants since damage from storms, wind, blowing soil, mechanical damage, handling, etc. followed by water may increase a population above threshold level and cause or promote disease. Careful management practices are key to mitigate the impact of this disease.

**OBJECTIVES**

1. Identify sources of on-farm inoculum of *Clavibacter michiganensis* subsp. *michiganensis* (CMM).
2. Develop greenhouse and field sanitation practices that eliminate inoculum and reduce spread of the pathogen.

**NARRATIVE REPORT**

*Identification of on-farm sources of inoculum of CMM:*

Isolations were made from objects and plants in three different grower's greenhouses. Q-tip cotton swabs were wiped on specific surfaces or plants and streaked four times on Semiselective Clavibacter Media (SCM) (Fatmi and Schaad, 1988, Phytopathology 78:121-126) or on King's B (KB). Weeds were swabbed as above, removed from the greenhouse, and potted in our experiment station greenhouse to facilitate identification. Symptomatic tomato plant tissue was surface disinfected with 10% chlorox containing several drops of 95% ethanol for 3 minutes and rinsed in sterile distilled water (sdw). The sterilized tissue was ground in a mortar and pestle with a minimum amount of water and streaked on SCM. Colonies were grown for 10-14 days at 25C on SCM. Various other media were tested (King's B, CM (modified SCM), YDCP ) that would promote further growth, but there were too many non CMM colonies that also grew on these media to make them useful. Individual gray to black colonies suspected to be CMM were transferred from SCM to Difco bacto potato dextrose agar (PDA) by streaking four different colonies per plate with parallel lines dividing them. The plates were incubated for 2 to 3 days and restreaked several more times, if necessary, to separate single colonies. The color and texture of the bacterial colonies were recorded while on PDA to assist in determining which colonies actually were CMM. For all tests a known culture of CMM (usually 942) was the positive check and sterile distilled water the negative check. The positive check lost virulence over time and had to be replaced. The results were always compared to the appropriate checks when evaluating the results.

The isolates were tested using Adgen Agrifood Diagnostic *Clavibacter michiganensis* subsp. *michiganensis* 50 test 60 second granular agglutination EXPRESS Kit (number 1113-13). The kits were expensive, of questionable results because of the difficulty of evaluating clumping (agglutination), and the kit had a limited shelf life even when stored in the refrigerator.

For some isolates agar cultures and/or plant tissue were sent to the company Agdia for identification using their ELISA test protocol. The results were inconclusive. The

advantages were rapid turn over (about 4 days from the date mailed), but the disadvantages were the high cost and questionable accuracy.

CMM is Gram positive which helps differentiate it from other organisms and pathogens that are Gram negative. The samples were potassium hydroxide (KOH) Gram tested in the lab by adding 2% KOH to a loop of bacteria, and mixing with a sterile toothpick. The toothpick is drawn away from the slide and if there is a mucouslike string (stringing) between the colony and the toothpick, the culture is gram negative (not CMM). All the negative colonies were eliminated from further testing.

However, due to the nature of the test with inherent false negatives and positives, we then used the more labor intensive Becton Dickinson 3-Step Gram Stain Kit-T on the suspect CMM colonies to further eliminate incorrect colonies. In this test the Gram positive bacteria turn from pink to purple. Results were positive or negative.

After the 3 step gram test, the Gram positive bacteria were further examined under the microscope and their phenotypes were compared with a known colony of CMM to further verify their identity (should be round and purple). Any cultures that did not look like CMM were discarded.

We found that these tests were necessary because identification of CMM is difficult with many less than perfect methods to choose from. Identification of CMM is more like gathering forensic evidence. CMM was difficult to identify to genus and species, and also feel confident that the correct subspecies that is pathogenic on tomatoes was isolated. Identification is also complicated by the fact that many 'strains' of CMM are known to exist that have diverse phenotypic characteristics.

Koch's postulates were conducted to test the pathogenicity of the isolates. Two to three day old bacterial colonies were suspended in sterile distilled water. The optical density of the suspension was at least 2.0 absorbance (about $4.3 \times 10^8$ cfu/ml) using a model Spectronic 20+ spectrophotometer. The suspension was injected 0.5 to 1.0 cm into unripe hydroponically grown greenhouse tomato fruit by compressing the plunger of a 26 gauge needle on a 1 cc syringe until a droplet appeared on the tomato fruit surface. Each fruit was inoculated 5 times. The replicated fruit were put on wire racks in plastic boxes with distilled water in the bottom to maintain high humidity. Lids were placed on the boxes and the boxes placed in unsealed plastic bags. The tomatoes were incubated at 25C for 10 to 14 days, and were rated (plus or minus) by cutting through the injection point and looking for discoloration or hollowing. The proportion of the inoculated tissue with disease was recorded.

The same suspension was also injected into the base of the stems (three injections per stem) and the upper leaf axil (one injection) of 3 to 4 week old New Yorker tomato plants grown in Cornell soilless mix in 4 1/2 inch square pots in the greenhouse. After inoculation the plants were placed in a mist chamber and misted for three to four days, removed from the mist chamber, and placed on a greenhouse bench. The stems were longitudinally dissected four to five weeks after inoculation to look for discoloration or hollowing indicative of bacterial canker and were rated for disease severity using a 0-9

scale. The results from each pot were averaged and then the replications were averaged. If the number was greater than the CMM control, the result was considered to be positive.

The above suspension was also injected into fully expanded leaves of approximately four-week-old Four O'clock plants. The leaves were examined daily for up to seven days to see if the bacterial suspension produced a necrotic hypersensitive reaction (dead tan spot) on the leaves. Using a pin or 26 gauge needle, the plants were inoculated introducing the bacteria into each half of a leaf between the veins. The test was scored as positive or negative. If any leaf was positive the test was considered positive.

**Results**

Of the three greenhouses sampled, one grower had bacterial canker develop in the field. The disease developed on leaves at about the time of fruit load, but the disease was not severe. The same area of the field had severe bacterial canker three years previously. A newly acquired field about one quarter mile east was planted later in the season and CMM developed with similar severity in this field at the time of fruit development. In a different field volunteer tomatoes were allowed to grow in an area where bacterial canker was a problem the previous year. The plants developed bacterial canker in a pattern similar to the other areas. This particular grower did not have significant monetary losses due to bacterial canker 2000, had moderate losses in 1999, and heavy losses in 1998.

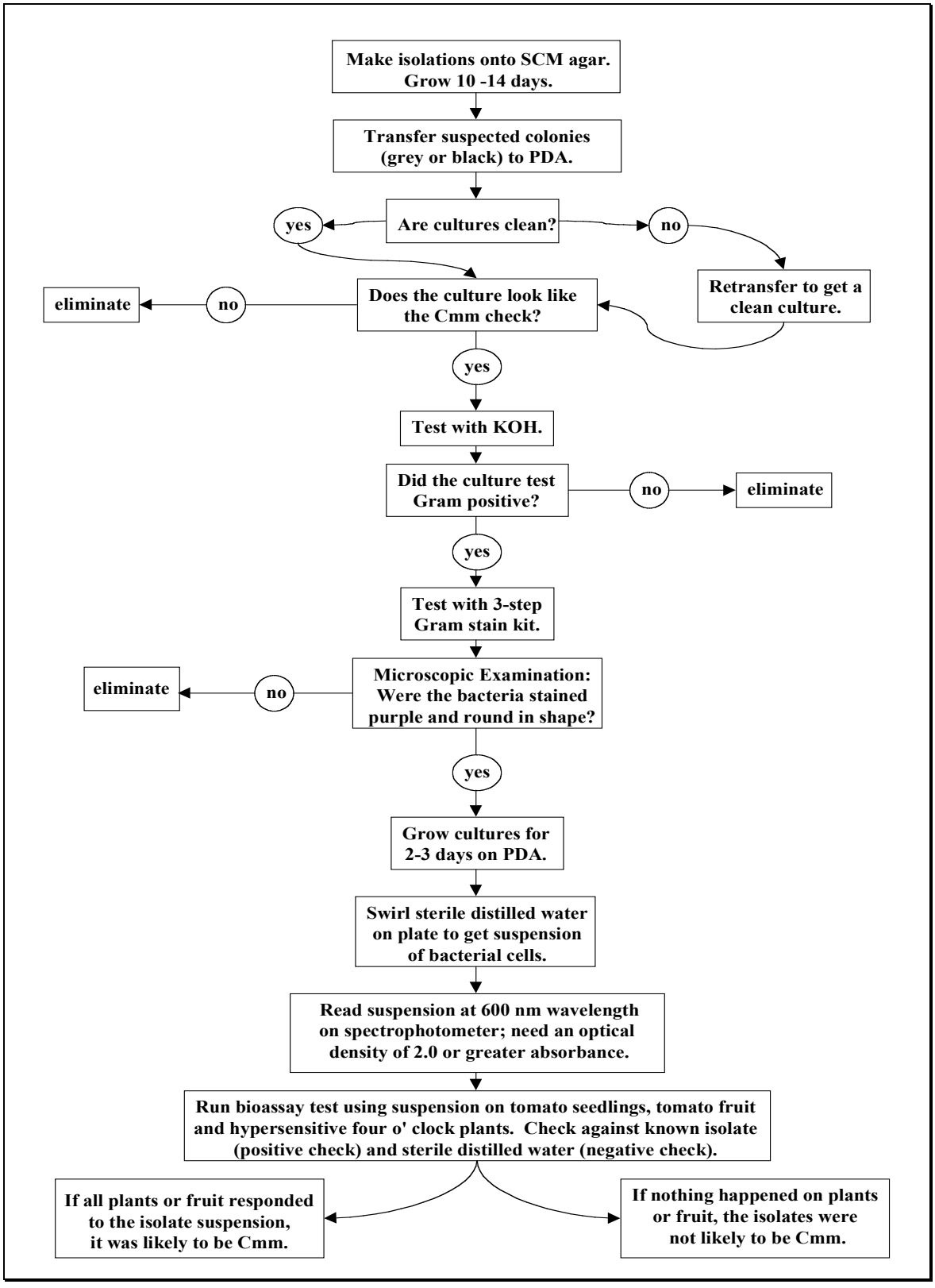
The "weeds" found in the various greenhouses that were subsequently identified included hairy bittercress, curly dock, chickweed, dandelion, bedstraw, chenopodium, oxalis, asparagus fern, potentilla, goldenrod, and various unidentified grasses.

Several isolates were sent to Dr. Dennis Fulbright's laboratory at Michigan State University for additional phenotypic typing. The results were: isolate 29-1 from old transplant trays collected 2000 was not CMM; isolate NY946 was identified as CMM BOX-PCR type C from pepper leaves collected in 1999; and isolate NY945 was identified as CMM BOX-PCR type C from tomato leaves collected in 1999.

**Current and Future Protocol**

Isolate from walls, plastic, flats, weeds, plants, etc. on SCM and grow for 10 to 14 days.
Transfer suspected gray or black colonies individually to PDA.
Re-streak onto PDA as necessary to isolate and clean up individual colonies.
Eliminate colonies that do not look like the CMM check.
Do KOH test and eliminate all Gram negative bacteria.
Do 3-step Gram test and save only Gram positive bacteria.
Microscopic elimination of all cultures that are not CMM.
Run pathogencity tests on isolates and include a positive and negative check (sdw).
Use suspect CMM colonies grown for two to three days on PDA for all subsequent tests.
Prepare a bacterial suspension; achieve an optical density of 2.0 or greater absorbance; read suspension at 600 nm wavelength.
Inject suspect cultures into tomatoes as detailed above and score at 10 to 14 days.
Infuse suspension into Four o'clock plants.
Inject bacterial suspension into New Yorker tomato stems and leaf axil.
Tabulate results and draw conclusions.

Send CMM isolates to other researchers to verify identification and type.


Make isolations onto SCM agar. Grow 10 -14 days.
Transfer suspected colonies (grey or black) to PDA.
Are cultures clean?
yes
no
Retransfer to get a clean culture.
Does the culture look like the Cmm check?
no
eliminate
yes
Test with KOH.
Did the culture test Gram positive?
no
eliminate
yes
Test with 3-step Gram stain kit.
Microscopic Examination: Were the bacteria stained purple and round in shape?
no
eliminate
yes
Grow cultures for 2-3 days on PDA.
Swirl sterile distilled water on plate to get suspension of bacterial cells.
Read suspension at 600 nm wavelength on spectrophotometer; need an optical density of 2.0 or greater absorbance.
Run bioassay test using suspension on tomato seedlings, tomato fruit and hypersensitive four o' clock plants. Check against known isolate (positive check) and sterile distilled water (negative check).
If all plants or fruit responded to the isolate suspension, it was likely to be Cmm.
If nothing happened on plants or fruit, the isolates were not likely to be Cmm.

***Proposed greenhouse and field sanitation practices to eliminate inoculum and reduce disease spread.***

1. Use disease free certified seed.

2. Destroy weeds and any volunteer plants in and around the production greenhouse. The bacteria can function as epiphytes (grow saprophytically on nutrients associated with leaf surfaces) and not produce symptoms on plants. Volunteer plants may be hosts and will provide a potential source of inoculum.

3. Remove supplies from production greenhouses. Supplies should be stored in a clean separate shed.

4. Clean debris out of the greenhouse and disinfect all surfaces, benches, and hoses. Greenshield and other products are available for this purpose.

5. Any flats, pots, or other reusable items should be steam sterilized or disinfected.

6. Dedicate a greenhouse for production of tomato transplants only, to avoid introduction of epiphytic bacteria from other crops that may be harboring tomato pathogens.

7. Minimize handling of transplants to reduce spread of the bacteria.

8. Avoid heavy watering and splashing water onto leaf surfaces, which encourages proliferation of bacteria.

9. Avoid wounding or damaging plant tissue. These areas are prime sites for ingress of the bacteria.

10. Discard any unused transplants. Do not allow them to sit unattended in the greenhouse where they can spread pathogens to younger transplants and greenhouse surfaces.

11. Incorporate diseased tomato residue into the field in the fall as early as possible to facilitate tissue degradation.