Detection of *Clavibacter michiganensis* subsp. *michiganensis* in Symptomless Tomato Transplants

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ABSTRACT

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Clavibacter michiganensis subsp. *michiganensis*, the cause of bacterial canker, was isolated from symptomless tomato (*Lycopersicon esculentum*) seedlings that were surface-disinfested with 100% ethanol, sectioned, and pressed onto the surface of a semiselective medium (mCNS). In greenhouse tests, movement of bacteria down stems was variable 1–3 days after inoculation of the apical end of the stem, but by 7 days, *C. m. michiganensis* was isolated from sections at least 10 cm below the inoculation site of all plants. However, symptoms were not observed for 17 days. Inoculated plants were infectious within 2 days because the pathogen was transmitted when the tops of plants were clipped with a razor blade. *C. m. michiganensis* was isolated on mCNS from only three of 24,000 transplants sampled from 24 commercial fields in southern Georgia. Color changes in ELISA wells that had been stamped with tomato stem pieces excised from symptomless transplants provided presumptive evidence for bacterial presence when compared with positive controls. The presumption of *C. m. michiganensis* was confirmed when the bacterium was recovered on mCNS and identified. Bacterial fatty acids of *C. m. michiganensis* were detected in extracts from stem tissues by gas-liquid chromatography. A minimum threshold of 1×10^8 colony-forming units (cfu) of bacteria per milliliter of saponification solution was necessary for identification of bacteria in plant tissues. The mean population of *C. m. michiganensis* in symptomless transplants by 7 days after inoculation was 1.7×10^8 cfu/cm of stem. In comparison, populations of *Pseudomonas syringae* pv. *syringae* and *P. s.* pv. *tomato* in lesions were determined to be 9.5×10^5 and 8.1×10^6 cfu/cm^2 of leaf disk, respectively, and were not detected by gas-liquid chromatography. In addition, several fatty acids common to all three bacteria are constituents of healthy tomato tissue, thus complicating the use of gas-liquid chromatography for routine detection of phytopathogenic bacteria in host tissues.

Additional keywords: bacterial speck, latent infections, syringae leaf spot

Southern-grown tomato transplants (*Lycopersicon esculentum* Mill.) are produced under a plant certification program governed by the Georgia Department of Agriculture (GDA). Although significant advances have been made in the diagnosis of bacterial pathogens commonly associated with tomato transplants (7,10–12), a major concern still exists with the length of time required for confirmation of bacterial identity by laboratory tests.

Latent infections or epiphytic residents are constant and difficult problems faced by GDA plant inspectors because all plant inspections are conducted in the field on a visual basis. Latent infections would escape detection and have been speculated as being important in the epidemiology of bacterial canker. A severe outbreak of bacterial canker of tomato in the midwestern United States and Ontario in 1984 was traced to tomato transplants produced in Georgia. However, all transplants connected with the 1984 epidemic were certified as "disease free" based on their appearance at the time of inspection. The incubation period for bacterial canker can range from 7 to more than 20 days, and the expression of symptoms depends on the age of the host, environmental conditions, inoculum density, and plant nutrition (17,19). In the past, discernible symptoms of the disease have not developed on southern field-grown transplants. Methods are needed so that large samples of symptomless plants can be routinely screened for *Clavibacter michiganensis* subsp. *michiganensis* Davis et al (1).

The use of cross sections of cut tomato stems pressed (printed) onto either semiselective agar media or into ELISA wells is discussed in this paper as a possible method to detect latent infections of bacterial canker. Also, the detection of fatty acid methyl esters (FAMES) of bacterial origin directly in plant tissues was evaluated as a method to reduce the time required to detect not only *C. m. michiganensis* but also other bacterial pathogens of tomato transplants. Portions of this research have been published previously as abstracts (5,8,18).

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MATERIALS AND METHODS

Stem printing technique. Tomato plants (cv. Heinz-722) were grown in a commercial potting mix (Pro-Mix, Premier Brands Inc., New Rochelle, NY) in 15-cm-diameter plastic pots. Temperatures in the greenhouse ranged from 20 to 38 C. The experimental design was a randomized complete block with three to five replications depending on the test. Plants were inoculated by clipping the apical end of stems of 14- to 21-day-old tomato seedlings with a razor blade, the edge of which had been moved through a colony of strain CM-O (obtained from D. Emmatty, Heinz USA, Bowling Green, OH) on mCNS agar (CNS agar medium [9] modified by the omission of lithium chloride [14]). Control plants were clipped with a sterile razor blade. Samples were taken immediately after inoculation and daily for the next 6–14 days. Exterior portions of the stems were surface-disinfested with 100% ethanol applied as a mist with a chromatography sprayer. After the ethanol evaporated, the top 1 cm of the stem was excised with a sterile razor blade. The excised stem piece was held between the fingers and gently squeezed as the freshly cut surface was pressed firmly (printed) onto the surface of mCNS agar. Plates were incubated for 7–10 days at 30 C. Yellow, fluidal colonies, typical of *C. m. michiganensis*, were tested for a hypersensitive reaction in four-o'clock (*Mirabilis jalapa* L.) (4), and cellular fatty acids were characterized to confirm bacterial identity (6).

Bacterial population dynamics. Populations of *C. m. michiganensis* in stems of greenhouse-grown tomatoes were determined by triturating 1-cm stem sections in 1 ml of PBS (0.05 M $KH_2PO_4 \cdot K_2HPO_4$, 0.85% NaCl, pH 7.4). Samples were serially diluted and 0.1-ml aliquots were spread on mCNS agar. Colonies characteristic of *Clavibacter* were counted after 7–10 days at 30 C. All treatments were replicated three times and the test was repeated twice. Populations of bacteria in stem prints were estimated from 1-cm stem sections that were printed into sterile test tubes to which 1 ml of sterile PBS was immediately added. Serial dilutions, incubations, and bacterial identifications were made as described earlier.

Bacterial suspensions of *C. m. michiganensis* were prepared in sterile PBS at concentrations of approximately 1×10^3,

1×10^6, and 1×10^9 colony-forming units (cfu) per milliliter as described previously (4). A 20-μl aliquot from each suspension was pipetted onto the wound of freshly clipped stems of greenhouse-grown tomato seedlings. Sterile PBS was used as a control. After 3, 6, and 9 days of incubation in the greenhouse, stem sections were printed onto mCNS agar.

Downward movement of *C. m. michiganensis* was monitored in greenhouse-grown tomato seedlings inoculated with strain CM 8704A (obtained from transplants in Tift County, GA). Stem sections 1 cm in length were sampled serially from the apical end of the stem to the soil line. The abaxial end of each serial section was printed onto mCNS agar at 1, 3, 7, 9, 11, 13, and 21 days after inoculation. Twenty plants were used for each sampling date. The test was repeated twice.

Latent period. The latent period, defined as the time needed for generation of the pathogen for secondary spread, was studied in the greenhouse. The experimental design was a randomized complete block with four plants per pot and three replications. The test was repeated twice. The top 1 cm of stems of symptomless plants (source plants) that had been clip-inoculated the same day or 1–6 days earlier was cut off with a razor blade, which was then used to clip additional seedlings. For the controls, the source plant was not inoculated. After 7 days, stem sections were excised from test plants and printed onto mCNS agar as described earlier.

Detection of *C. m. michiganensis* in field-grown transplants. Field experiments were conducted at the Blackshank Farm of the Coastal Plain Experiment Station near Tifton, GA. Plots were established on a Tifton loamy sand soil. Rye was seeded as a cover crop, and rye strips were maintained as windbreaks between plots. The plots were plowed with a moldboard plow and harrowed to create a fine seed bed. The preplant pesticides napropamide (Devrinol at 3.4 kg/ha), fenamiphos (Nemacur at 8.99 kg/ha), and metalaxyl (Ridomil at 2.34 L/ha) were incorporated into the soil with a Rototiller for control of weeds, nematodes, and damping-off, respectively. Fertilizer (1,343 kg/ha, 6-12-6) was broadcast and incorporated into the soil.

Coated tomato seeds of cv. Heinz-722 were direct-seeded on 18 March 1988 and of cv. Heinz-7135 on 20 February, 13 March, and 3 April 1989 in raised beds with four rows spaced 35 cm apart. Tomato seeds were spaced 1 cm apart within the row. A side dressing of fertilizer (448 kg/ha) was applied 4 wk after planting. All plots were sprayed with carbaryl (Sevin 80%, 2.2 kg/ha) and chlorothalonil (Bravo 720, 2.63 kg/ha) on a 7-day schedule for insect and fungal leaf spot control, respectively. Bactericides were not applied. The apical portions of the plants were clipped on a 3- to 4-day schedule to promote uniform growth. Irrigation was applied as needed by solid-set overhead sprinklers. In 1988, the experimental design was a randomized complete block with four replications.

At 58 days after planting, the seedlings were clip-inoculated with a razor blade, the edge of which had been contaminated with *C. m. michiganensis*. Plants clipped with a sterile blade served as a control. Two hundred plants per treatment were pulled 7 days after inoculation, transported to the laboratory, and surface-disinfested with ethanol. After the ethanol evaporated, stem sections were excised and 20 stem pieces were printed per mCNS agar plate. In 1989, the stem print method was used to detect movement of inoculum within plots. The experimental design was a randomized complete block with eight replications.

A line source of inoculum was established at the front edge of plots for the three planting dates when the petiole of the first true leaf of 21-day-old tomato seedlings was clipped with a razor blade that had been contaminated with a colony of strain CM 14-4 (obtained from A. Vidaver, University of Nebraska, Lincoln) as described earlier. A total of 12 plants per plot (three plants at the front of each row) were inoculated. Bacteria were disseminated from the line source by natural means or when plants were clipped with a PTO-driven, rotary, transplant clipper (Talley Made, Tifton, GA). At 65 days after planting, 40 transplants per treatment (10 per row) were pulled from areas 1–3 m down the row from the edge of the line source. Stem sections were printed on to mCNS agar as described earlier. Control (uninoculated) plants were clipped with a sanitized clipper and were pulled from uninfested areas.

Field survey of commercial transplants. In 1988, 24 commercial tomato transplant fields in southern Georgia were surveyed for *C. m. michiganensis*. The survey was initiated on 4 April and terminated on 20 May. All fields had a continuous cropping history of tomato or pepper transplants for a minimum of 5 yr. Samples were collected in an X pattern across the field; 50 plants were sampled at 20 subsites randomly selected along the path and bulked for a total of 1,000 plants per field. Samples were pulled 1–5 days before harvest, stored separately in plastic bags to avoid contact between samples, and analyzed for populations of *C. m. michiganensis*. Contaminated field plots were established so that 100 inoculated plants could be assayed as a control. A remnant supply of seed was obtained from one grower whose field-grown plants were found contaminated with *C. m. michiganensis*. The claylike coating material was removed, fungicides were rinsed off until the red color no longer bled into the rinse water, and seeds were tested for the presence of *C. m. michiganensis* on SCM and mCNS agar by the methods of Fatmi and Schaad (3).

ELISA. ELISA kits (Agdia Inc., Mishawaka, IN) prepared with polyclonal antibodies specific for *C. m. michiganensis* were used for all serological tests. Symptomless tomato transplants were assayed for internal populations of *C. m. michiganensis* as outlined earlier except that the cut end of the tomato stem was printed directly into an ELISA well. Printed wells were covered with 100 μl of sample extract buffer (2 ml of Tween 20; 2 g of egg albumin; 2 g of polyvinylpyrrolidone, MW 10-40,000; 0.13 g of sodium sulfite; and 100 ml of phosphate-buffered saline with Tween 20) (per liter of distilled water, add 8 g of NaCl, 1.15 g of Na_2HPO_4, 0.2 g of KH_2PO_4, 0.2 g of KCl, and 0.5 g of Tween 20, final pH 7.4). ELISA plates were incubated in a moist chamber at room temperature for 2 hr. Wells were then processed with the antibody-enzyme conjugate as recommended by the manufacturer. Results were recorded visually as strong, moderate, weak, or no reaction based on color changes in the ELISA well, compared with positive control wells prepared with a pure culture of *C. m. michiganensis*.

Forty 21-day-old tomato seedlings were clip-inoculated with strain CM-O as described earlier. After 7 days, the apical 1 cm of the stems was excised, gently squeezed, and pressed into ELISA wells and onto mCNS agar. Ten plants with symptoms of bacterial spot (caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye), bacterial speck (caused by *Pseudomonas syringae* pv. *tomato* van Hall), and 20 healthy plants, for a total of 40 greenhouse-grown transplants, were used as negative controls. Pure cultures of CM-O and CM 14-4 of *C. m. michiganensis* were processed according to manufacturer's instructions in four wells each as positive controls. ELISA plates were processed and read as described earlier.

Field plots of tomato transplants, cv. Heinz 7135, were established as described earlier in 1989. Treatments consisted of an uninoculated control and a line source of inoculum established at the front edge of the plot. For the line source, the petiole of the first true leaf was clipped with a blade contaminated with a colony of CM 14-4. Bacteria were disseminated by natural means and clipping with the transplant clipper. Forty plants were sampled for each treatment and processed as described earlier. ELISA plates were processed and read, CNS plates were incubated for 10 days at 30 C, and suspect colonies were identified as described earlier.

FAMES analysis. Previously reported

methods for preparation and analysis of FAMES from cellular fatty acids were used (6). Strains of *C. m. michiganensis* were grown at 30 C for 48–72 hr on mCNS agar, and strains of *P. s. tomato* and *P. s.* pv. *syringae* (Okabe) Young et al were grown at 27 C for 48 hr on King's medium B (KMB). A loopful of bacteria was harvested and saponified in 1 ml of 1.2 N NaOH in 50% aqueous methanol for 30 min. After cooling, samples were sonicated and then acidified with 6 M HCl (final pH 2). Samples were methylated with 12% BCl_3 in methanol and incubated at 100 C for 4 min. FAMES were extracted with a hexane and diethyl ether mixture (1:1). After separation of phases, the lower phase was discarded and the upper phase was washed with 0.3 N NaOH. Upon separation of phases, the upper phase was removed for analysis. FAMES were analyzed by gas-liquid chromatography with a Hewlett-Packard model 5710 gas chromatograph equipped with a 30 m × 0.25 mm phenyl methyl silicone-fused silica capillary column. The gas chromatograph was calibrated with a commercial FAMES mix (Supelco, Bellefonte, PA). The column temperature was programmed to increase from 145 to 280 C at a rate of 4 C/min. The carrier flow of helium was 0.8 ml/min. FAMES were identified by comparing their retention times with the standard using the Hewlett-Packard 3350A Laboratory Automation System (Hewlett-Packard, Palo Alto, CA).

Single lesions or a mixture of 10 lesions from leaves with either syringae leaf spot or bacterial speck symptoms were used as diseased tissue samples for the detection and identification of the fluorescent pseudomonads by direct FAMES analysis of diseased tissues. Stem sections 1 cm in length sampled 1 cm below the apical end of clipped tomato transplants were used for the detection of *C. m. michiganensis* in symptomless plants. Plants were clip-inoculated with *C. m. michiganensis* and were sampled 1, 3, 5, 7, and 9 days after inoculation. Each sampling period was replicated six times. Syringae leaf spot lesions, bacterial speck lesions, stems containing populations of *C. m. michiganensis*, and healthy tomato leaves and stem sections were crushed in 1 ml of 1.2 N methanolic NaOH and analyzed as described earlier. In addition, a series of suspensions of *C. m. michiganensis*, *P. s. tomato*, and *P. s. syringae* were prepared in the saponification solution and adjusted at concentrations of 1×10^2 to 1×10^9 cfu/ml as described previously (4). The various suspensions then were analyzed by gas-liquid chromatography as outlined earlier. Chromatograms of FAMES were prepared for different population levels. All population levels from diseased tissues and in suspensions were confirmed by dilution plate analysis from similarly prepared inocula adjusted in PBS.

RESULTS

Stem printing on semiselective medium. The apical 1-cm stem sections of 78% (25 of 32) of greenhouse-grown plants contained internal populations of *C. m. michiganensis* within 2 days after seedlings had been clip-inoculated. By day 4, the pathogen was isolated from every plant, whereas initial symptoms appeared on day 15. Saprophytic bacteria were not recovered. Each presumptive culture of *C. m. michiganensis* induced a hypersensitive reaction in four-o'clock and contained FAMES typical of that organism.

C. m. michiganensis was recovered almost as readily from field-grown transplants as from those grown in the greenhouse, except that a few to a moderate number of saprophytic bacteria also developed on the plates. In 1988, each of the 200 inoculated transplants contained internal populations of *C. m. michiganensis*. In 1989, the pathogen was found in prints from 87.5% of the transplants ($n = 960$) within 3 m of the line source of inoculum. In contrast, *C. m. michiganensis* was detected in only three of 24,000 commercial transplants sampled from 24 different fields. There were no symptoms in any of the plants sampled from either field plots or commercial plantings. The three plants identified with latent infections were traced to two different seed lots. *C. m. michiganensis* was recovered on both media from seed washes of remnant seed of one of the lots that was provided by the grower. Identification of the bacterium was confirmed as described earlier.

Bacterial population dynamics. Bacterial populations increased rapidly in stems over a relatively short time without producing symptoms. Populations recovered from triturated stems exceeded 5×10^6 cfu/cm of stem within 2 days after clip-inoculation and increased to 1×10^9 cfu/cm of stem by 14 days (Fig. 1). In contrast, populations recovered from stem prints in dry test tubes were approximately 100-fold less than populations recovered from triturated stem sections (Fig. 1).

Early detection of *C. m. michiganensis* in plants by the stem print method was affected by inoculum concentration. Populations of *C. m. michiganensis* were isolated from each of 12 plants inoculated with 2×10^4 or 2×10^7 cfu/ml within 3 days of inoculation. However, the pathogen was detected in only 50% of the plants inoculated with 20 cfu/ml after 3 days but was found in all plants after 6 days. None of the uninoculated controls tested positive.

The rate of bacterial movement within stems varied within the plant population (Fig. 2). After 1 and 3 days of incubation, *C. m. michiganensis* was detected in 50% of the plants at 4 and 10 cm, respectively, below the inoculation site. By 7 days, bacteria were detected 10 cm below the inoculation site in more than 90% of the plants.

Latent period. Bacterial multiplication (Fig. 1) and movement (Fig. 2) within plant stems was such that 38 and 50% of the upper 1 cm of the plants proved infectious 2 and 3 days, respectively, after clip-inoculation and the remainder by 4 days. Symptoms appeared 11–13 days later.

Detection by ELISA. ELISA and stem print assays had similar detection capabilities. When stamped into sensitized wells, all 40 of the stems from inoculated, greenhouse-grown plants reacted with a color change equivalent to those produced by pure cultures of bacteria. Presence of *C. m. michiganensis* was con-

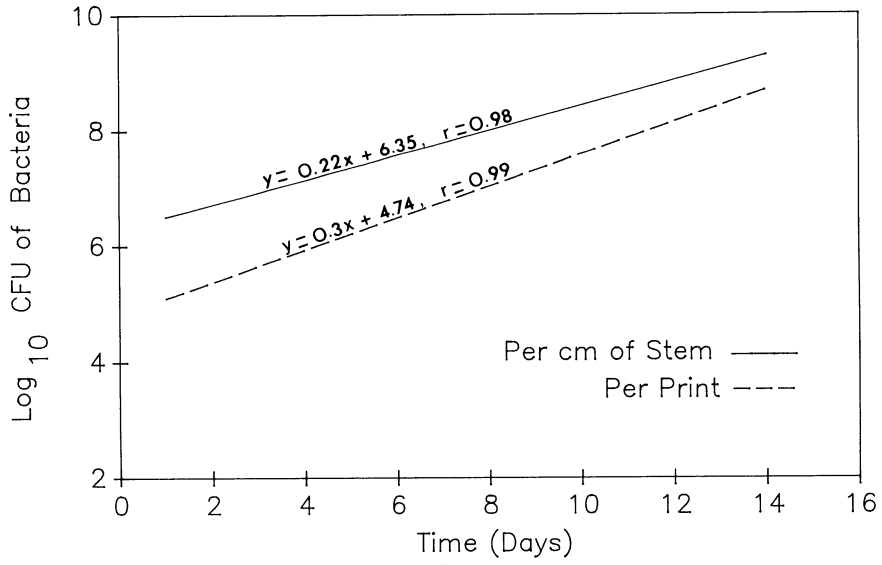

Fig. 1. Populations (colony-forming units [cfu] per centimeter of stem) of *Clavibacter michiganensis* subsp. *michiganensis* in the top 1 cm of tomato stems over time and numbers of bacteria (cfu/ml of buffer) in prints made in the bottom of dry, sterile test tubes which then were covered with 1 ml of phosphate-buffered saline.

firmed in the same stems by the recovery of colonies on mCNS agar. All bacteria from mCNS agar were identified as described earlier. Negative controls did not react and failed to yield bacteria when printed onto mCNS agar. Among field-grown transplants sampled 3 m from a line source of inoculum, 85% of the stem prints produced a color change that corresponded with the recovery of *C. m. michiganensis* from 86% of the stems printed onto mCNS agar.

Detection of FAMES. Based on gas-liquid chromatography analysis of dilutions of cultures of *P. s. syringae*, *P. s. tomato*, and *C. m. michiganensis* in methanolic NaOH, a minimum of 1×10^8 cfu/ml was required to produce a typical FAMES profile. Populations of *P. s. syringae* and *P. s. tomato* per 5-mm-diameter diseased leaf tissue were 9.5×10^5 and 8.1×10^6 cfu/ml, respectively, which was approximately 10–100 times below the minimum necessary for routine detection. On the other hand, the systemic pathogen, *C. m. michiganensis*, multiplied to much higher populations in stems (Fig. 1).

The FAMES present in healthy tomato leaves and stems included lauric acid (12:0), palmitoleic acid (c16:1^9), palmitic acid (16:0), linoleic acid (c18:2^9,12), and oleic acid (c18:1^9). The FAMES content of tomato tissues basically was unaltered by the presence of any of the bacterial pathogens. FAMES in *C. m. michiganensis* that were different from those in healthy tomato included the branched-chain FAMES of isopentadecanoic acid (i15:0), anteisopentadecanoic acid (a15:0), anteisopentadecenoic acid (a15:1), isopalmitic acid (i16:0), and anteisoheptadecanoic acid (a17:0). Other FAMES in the bacterium found in healthy tomato were those of lauric and palmitic acids.

Although bacterial populations in stems should have been large enough to produce a normal FAMES profile for an equivalent population in pure culture, only an incomplete profile of the canker bacterium could be perceived when a mixture of either 1×10^8 or 1×10^9 cfu/ml was added with tomato tissues prepared from a 1-cm section of stem. However, the detection of branched-chain fatty acids in tomato tissues, in particular a15:0, a15:1, and a17:0, were highly diagnostic for *C. m. michiganensis*; their presence was assumed to be of bacterial origin and used for tentative identification of latent infections in tomato stems. In contrast, FAMES of either *P. s. syringae* or *P. s. tomato* were not detected from analysis of single lesions. However, when 10 lesions were combined, elaidic acid (t18:1^9) was detected and presumed to be of bacterial origin because it was present in the normal FAMES profile of both pseudomonads. The peak most diagnostic for *P. s. syringae*, c-9,10 delta 17:0, which is necessary for its separation from *P. s. tomato*, was never detected in samples prepared from 10 lesions of syringae leaf spot.

DISCUSSION

The most effective method for the detection of *C. m. michiganensis* in symptomless transplants was the recovery of the pathogen from stem prints on mCNS agar. The high populations of the pathogen and the relatively low populations of saprophytic bacteria in apparently undamaged stem tissue partly explain the success of this method. When used in conjunction with a semiselective medium, stem printing has been extremely useful in research studies and could be of some value in the plant certification program. It is inexpensive, easy to perform for a large sample size (as illustrated by the 24,000 plant survey), and has the advantage of producing a living culture for further analysis, experimentation, or storage. Disadvantages are the time necessary for growth of the organism on the plate, space required for incubation of the plates, and further confirmation of bacterial identity.

FAMES analysis was the least effective method of detecting latent infections of bacterial canker. Although it has the advantage of providing results within 4–6 hr for an individual sample, the time required for a large number of samples would make this technique prohibitive for routine regulatory testing. The technique still may have application in small research studies when a rapid diagnosis is required, but like ELISA, it has the disadvantage of not providing a living culture for further experimentation or confirmation of identity. In addition, the FAMES profile was never as sensitive as the other two techniques. It required high populations for the threshold of detection and a complete bacterial profile was never obtained with extracts from infected tissues.

ELISA may be the most practical of the three methods reviewed for screening a large number of samples in the shortest amount of time. Stephens et al (15) estimated that 1×10^4 cfu of *C. m. michiganensis* per milliliter of extraction buffer was necessary for a positive ELISA reaction. This represented a sensitivity of 1×10^3 cfu per ELISA well. Stevens and Tsiantos (16) reported similar results with pure cultures of bacteria but observed weaker reactions when bacteria were extracted from infected leaves apparently attributable to nonspecific inhibitory reactions by plant extracts. Such inhibitions were reduced by the addition of polyvinylpyrrolidone to the extraction buffer. In our tests, the numbers of bacteria recovered from a tomato stem print far exceeded these detection thresholds. The advantages of the ELISA technique are that results can be obtained within 4–6 hr, ready-made plates are commercially available, thus reducing time and labor for a large-scale operation such as the transplant certification program, and 96 samples can be run per plate, thus taking up only a small amount of incubator space. The disadvantages are that a living culture is not obtained for confirmation and new serotypes could render the test obsolete.

The population dynamics of *C. m. michiganensis* in tomato stems reported here provide evidence that commercial cultural practices can disseminate the pathogen throughout a transplant field before symptoms appear. Farley and Miller (2) established that clipping, a standard practice for field-grown transplants, could disseminate *C. m. michiganensis*. We demonstrated that plants are fully infectious within 2 days of inoculation. Potentially, five to seven secondary cycles attributable solely to clip-

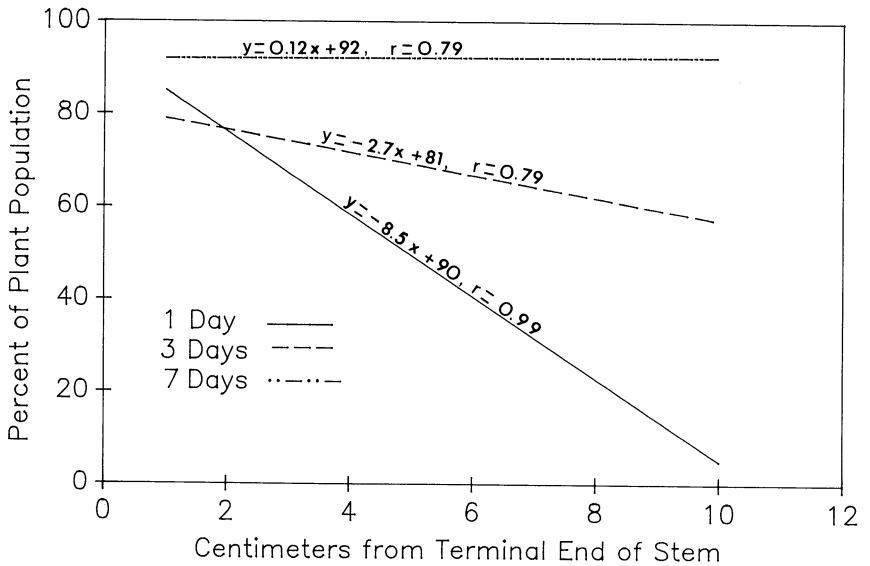

Fig. 2. Recovery of *Clavibacter michiganensis* subsp. *michiganensis* over time from sequential 1 cm sections of tomato stems printed onto semiselective media.

ping could result before the onset of symptoms. One infested seed in 10,000, if capable of growing into an infected plant, could result in 74–124 foci/ha. The field practice of clipping the plant top could disseminate the bacteria and result in thousands of infected plants. Furthermore, the plants would be symptomless and certified as disease free.

Because bacterial populations became detectable in plant stems rapidly after inoculation, plants that are free of this pathogen at harvest are most likely to be free of latent infections. Although variability within a population of plants and levels of inoculum could lead to escapes, the risk may be minimized with the use of large sample sizes and appropriate sample dates. We successfully detected *C. m. michiganensis* in 100% of the plants 4–7 days after inoculation with as few as 20 cfu/ml. Based on these numbers, the rapid colonization of stems by the pathogen over time, and the systemic movement of populations in stems, sampling 7 days after inoculation was selected as a conservative time frame for the detection of most *C. m. michiganensis* populations. The selection of 7 days allows room for error. Pine et al (13) observed that bacteria moved in tomatoes at a rate of 2.45 cm in 1 day and only 3.25 cm by 5 days. However, they were observing mostly upward movement into plant stems after leaf petioles had been inoculated. We observed downward movement (10 cm within 7 days) of bacteria from clipped surfaces. Thus, stem sections sampled by plant inspectors at 1 cm below the apical clipped end would most likely contain detectable populations even if the bacteria moved at a slower rate.

The detection of *C. m. michiganensis* in commercial transplants led to a more thorough examination of the seed used for that particular planting. The seed lot was found to be contaminated with the pathogen when approximately 30,000 seeds were assayed by the methods of Fatmi and Schaad (2). Earlier, the same seed lot had passed the GDA assay of 200 seeds plated onto nutrient agar and was awarded certification. In addition, the seed had been treated commercially with hydrochloric acid for control of seedborne bacteria. The recovery of *C. m. michiganensis* from the seed and plants in the field would indicate that escapes from seed treatments and seed assays may be more common than previously realized.

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