Local Sources of *Clavibacter michiganensis* ssp. *michiganensis* in the Development of Bacterial Canker on Tomatoes

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ABSTRACT

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Seed transmission, overwinter survival, and dispersal of *Clavibacter michiganensis* ssp. *michiganensis* on processing tomatoes (*Lycopersicon esculentum*) and spread of *C. m. michiganensis* on alternative hosts and nonhost plants were evaluated with rifampicin-resistant strains of *C. m. michiganensis* (Rif$^+$ *C. m. michiganensis*) and a selective medium. The bacterium was transmitted at a low rate from seed to transplants by sowing infested seed in the greenhouse and transplanting the seedlings to a production field. Populations of Rif$^+$ *C. m. michiganensis* detected on seed harvested from systemically infected plants ranged from about 10^2 to 10^5 cfu/g seed. Survival of Rif$^+$ *C. m. michiganensis* associated with infested tomato debris was greater for debris on the soil surface than for debris that was buried. Populations of Rif$^+$ *C. m. michiganensis* declined at about the same rate in samples buried 10, 20, and 30 cm. Populations of Rif$^+$ *C. m. michiganensis* on alternative hosts and nonhost plants fluctuated from 0 to about 10^9 cfu/g fresh weight on solanaceous plants (*Capsicum annuum, Datura stramonium, Lycopersicon esculentum, Nicotiana tabacum, Solanum melongena, S. nigrum,* and *S. tuberosum*) and from 0 to about 10^3 cfu/g fresh weight on nonsolanaceous weeds (*Amaranthus retroflexus, Chenopodium album,* and *Xanthium saccharatum*). Leaf surface populations of Rif$^+$ *C. m. michiganensis* on nonhost solanaceous plants and nonsolanaceous plants were significantly lower than on tomatoes. Symptoms of secondary infection were not observed on plants of these species. Susceptible (Heinz 1810) and moderately resistant (Heinz 7417) tomato cultivars supported leaf surface populations of about 10^7 to 10^9 cfu/g fresh weight. Symptoms of secondary infection, marginal scorch of leaflets or bird's-eye spots of fruit, were not observed on tomatoes until populations exceeded 10^6 and 10^7 cfu/g fresh weight for the moderately resistant and susceptible cultivars, respectively. Symptoms of systemic infection were not observed in either cultivar when Rif$^+$ *C. m. michiganensis* spread from foci of infection, but symptoms of secondary infection occurred earlier and were more severe on the susceptible cultivar.

Bacterial canker of tomato (*Lycopersicon esculentum* Mill.), caused by *Clavibacter michiganensis* ssp. *michiganensis* (Smith) Davis et al, is an economically important disease in many areas of the world. The disease causes premature death of plants and reductions in yield. Losses as high as 50–80% have been reported from the United States, Canada, and Kenya (23,26,31).

The processing tomato industry in the midwest United States often uses transplants from Georgia and Florida. Tomato transplants are inspected and certified to be free of bacterial canker before shipping to production areas in the Midwest (11,12). However, an outbreak of bacterial canker throughout the Midwest and several Canadian provinces in 1984 resulted from infected transplants in spite of seed treatment, crop rotation, and certification procedures (12). Bacterial canker occurred sporadically in the Midwest before 1984 and was epidemic in 1969 (9). Local sources of inocula may have caused these sporadic outbreaks (8).

Although *C. m. michiganensis* introduced on tomato transplants can cause epidemics of bacterial canker (5,9), the importance of local sources of inocula is poorly understood. Bacterial canker is transmitted on tomato seed, where the bacteria are capable of surviving for long periods (3,31). Seed transmission rates are generally low (16,36), and seed treatments are generally effective (7,10,27,34). Thus, the incidence of plants in production fields that are initially infected from seed is relatively low (16). Nevertheless, infected or infested seed is believed to be the major source of primary inocula in production fields (3,7,31). Secondary inocula from a few diseased plants grown from infested seed can result in a high level of secondary infection because plants are handled many times during pruning, tying, and harvesting (1,5,31).

In addition to seed transmission, *C. m. michiganensis* survives in tomato debris (8,24), on alternative hosts (32,33,39), on volunteer plants (3,20), and in soil (2,29,35). Inocula from these sources probably are spread to plants by splashing rain, application of chemicals, and irrigation water (30,31), thus allowing *C. m. michiganensis* to grow on leaf surfaces. Recent studies (14,15,28,37) have shown that populations of certain leaf surface bacteria are sources of inocula that can initiate epidemics (18,22).

The objectives of this research were to evaluate the role of local sources of inocula in the spread of *C. m. michiganensis* and the development of bacterial canker. Local sources of inocula evaluated included *C. m. michiganensis* transmitted directly on seed, *C. m. michiganensis* that overwintered in infested tomato debris in the field, *C. m. michiganensis* that survived and grew on leaf surfaces of alternative hosts and on nonhost plants, and *C. m. michiganensis* that spread from point and line sources of inocula in tomato fields. Preliminary results from this study have been reported (4).

MATERIALS AND METHODS

Rifampicin-resistant (Rif$^+$) mutants and preparation of inocula. Ten strains of *C. m. michiganensis* were obtained from diverse geographical locations (5). Each strain was selected for rifampicin resistance as described by Weller and Saettler (40). All Rif$^+$ *C. m. michiganensis* strains were confirmed as pathogenic by producing typical one-sided wilting of tomato plants (cv. Heinz 1810) and a hypersensitive reaction on four-o'clock, *Mirabilis jalapa* L. (11). The strains of Rif$^+$ *C. m. michiganensis* were stored in King's B broth at −80 C and revived before each experiment to avoid repeated subculturing. Inocula were prepared as described previously (5). Bacteria were suspended in sterile phosphate buffer, 0.01 M, pH 7.2, containing 0.85% NaCl (PBS), and adjusted

to approximately 2×10^8 cfu/ml ($A_{590nm} = 0.16$). The inocula were a mixture of equal proportions of the 10 strains of Rif$^+$ *C. m. michiganensis* in order to account for variation in virulence that may have existed among strains.

Seed transmission. Seed transmission of Rif$^+$ *C. m. michiganensis* was evaluated in 1990 by sowing infested seed in the greenhouse and transplanting the resulting seedlings to a production field. Tomato seed (cv. Heinz 1810) were harvested in 1989 from fruits of systemically infected plants that had been inoculated with Rif$^+$ *C. m. michiganensis* by cutting the first true leaf with scissors dipped in inocula as described previously (5). These seed were mixed in various proportions with uninfested seed obtained from D. A. Emmatty (Heinz U.S.A., Bowling Green, OH). Eight treatments were evaluated. Six treatments included mixtures of 0, 1, 5, 10, 50, and 100% seed from systemically infected plants. The seventh treatment was seed from systemically infected plants treated with 1.8% hydrochloric acid solution for 5 min before direct-seeding. The eighth treatment was similar to the seventh treatment except seedlings from these seed were clipped with a cordless grass shearer (Black & Decker, Easton, MD) at heights of 18, 20, 22, and 24 cm. The shearer was flame-sterilized after each replicate and each clipping date. Seedlings were clipped in the greenhouse before transplanting.

Seed were sown on 5 April 1990 in 35 × 50 cm flats that contained a sterilized mixture of equal proportions (by volume) of soil, sand, peat, and vermiculite. There were three replicates with about 9 g of seed for each treatment. Each experimental unit consisted of three flats with 80–90 plants per flat. Treatments were arranged in a randomized complete block design. Before seeding, 1 g of seed from each experimental unit was sampled for Rif$^+$ *C. m. michiganensis* by dilution plating as described below. Germination rates were determined by placing 50 seed from each experimental unit on wet filter paper in petri dishes for 8 days at 25 C. Flats were kept in a greenhouse at 25 C day and 20 C night. Granular potash 5-23-27 (FS Special, Growmark, Inc., Bloomington, IL) was applied every 2 wk. When seedlings reached about 25 cm in height on 30 May 1990, 120 seedlings from each experimental unit were dug and handled individually to minimize contamination before transplanting. Four seedlings from each experimental unit were sampled for Rif$^+$ *C. m. michiganensis* by dilution plating. The remaining seedlings were transplanted in field plots at the University of Illinois Pomology Farm, Urbana, IL. Each plot consisted of four rows, with 29 transplants per row. Transplants were spaced 30 cm apart within rows and 90 cm between rows. Incidence of systemically infected plants was based on visual observations at 1-wk intervals after transplanting. Incidence was converted to a percentage of plants per plot.

Populations of Rif$^+$ *C. m. michiganensis* recovered from seed and seedlings, germination rate, and incidence of systemically infected plants measured at each rating were analyzed by ANOVA. Waller-Duncan Bayesian least significant difference (BLSD) values with K = 100 were used to compare the effects of treatments. A regression analysis was performed using the incidence of systemically infected plants measured 12 wk after transplanting as the dependent variable and the proportion of seed from systemically infected plants as the independent variable. *F* statistics ($P < 0.05$) were used to test the significance of the regression model and independent variables.

Overwinter survival. Survival of Rif$^+$ *C. m. michiganensis* in infested tomato debris and in infested soil was studied during the winters of 1988-89 and 1989-90 in Urbana, IL. Systemically infected tomato plants (cv. Heinz 1810) that had been inoculated with Rif$^+$ *C. m. michiganensis* by methods described previously (5) were collected and air-dried in the field in the autumns of 1988 and 1989. Stems were cut into 3-cm pieces and mixed with field soil at the ratio of 5 g of dry stem to 95 g of soil. The mixture was placed in nylon mesh bags and either placed on the soil surface or buried 10, 20, or 30 cm below the soil surface. Treatments were arranged in a randomized complete block design with four replicates. The experiments were initiated on 27 October 1988 and 29 October 1989. Samples were collected six to seven times at 4- to 6-wk intervals from 25 November 1988 until 12 May 1989 and from 29 November 1989 until 28 May 1990. At each sampling date, four bags were collected per treatment. Tomato debris was separated from soil in each bag, washed in running tap water for 15 min, and air-dried for 12 h. During the winter of 1989-90, survival of Rif$^+$ *C. m. michiganensis* in the soil, which had been mixed with the infested tomato debris in the nylon mesh bags, was also evaluated. One gram of the infested tomato debris or soil mixed with infested tomato debris was taken from each nylon mesh bag. Populations of Rif$^+$ *C. m. michiganensis* in tomato debris or soil were measured by dilution plate methods described below.

Populations of Rif$^+$ *C. m. michiganensis* recovered from infested tomato debris and from soil mixed with the infested tomato debris were analyzed by ANOVA. Populations of Rif$^+$ *C. m. michiganensis* recovered from infested tomato debris and from the soil mixed with the infested tomato debris that were buried at the four depths were regressed on days after burying. *F* statistics ($P < 0.05$) were used to test the significance of the regression model and independent variables.

Leaf surface populations on alternative hosts and nonhost plants. Seven species of solanaceous plants that had been reported as alternative hosts of *C. m. michiganensis* (33) and two species of nonhost, nonsolanaceous weeds were compared in 1988 for their ability to support leaf surface populations of Rif$^+$ *C. m. michiganensis*. These plants included: *Capsicum annuum* L. (pepper), *Datura stramonium* L. (jimsonweed), *Lycopersicon esculentum* Mill. (tomato), *Nicotiana tabacum* L. (tobacco), *Solanum melongena* L. (eggplant), *S. nigrum* L. (black nightshade), *S. tuberosum* L. (potato), *Chenopodium album* L. (goosefoot), and *Xanthium saccharatum* Wallr. (cocklebur). In 1989, five species (*D. stramonium, L. esculentum, S. melongena, Amaranthus retroflexus* L. [pigweed], and *X. saccharatum*) and four cultivars of pepper were evaluated. Seed were planted in the greenhouse on 6 April 1988 and 7 April 1989. Seedlings were transplanted at the Pomology Farm on 26 May 1988 and 18 May 1989. Each plot of three rows contained one row of various solanaceous plants and nonsolanaceous weeds planted between two rows of tomatoes. In the center row, each species or cultivar of solanaceous plants and nonsolanaceous weeds was separated by a tomato plant. Plants were spaced 30 cm apart within rows and 90 cm between rows. Treatments were arranged in a randomized complete block design with four replicates. Tomato plants in the outer rows were inoculated with Rif$^+$ *C. m. michiganensis* by methods described previously (5) on 10 June 1988 and 9 June 1989 and served as the sources of inocula for the center row. In 1989, tomato plants were removed from two of the four replicates 8 wk after inoculation to compare leaf surface populations of Rif$^+$ *C. m. michiganensis* on alternative hosts and nonhost plants in the presence and absence of continued inocula from infected tomato plants.

Three to 10 leaves from each of the alternative and nonhost plants were sampled from each experimental unit at 1-wk intervals after inoculation. Populations of Rif$^+$ *C. m. michiganensis* were assayed from 1 g fresh weight of plant tissue from each experimental unit and determined by the dilution plate method described below.

Populations of Rif$^+$ *C. m. michiganensis* recovered from solanaceous plants and nonsolanaceous weeds were analyzed by ANOVA. Waller-Duncan Bayesian least significant difference (BLSD) values with K = 100 and pairwise *t* tests (LSD) were used to compare the effects of species ($P < 0.05$).

Dispersal. The secondary spread of Rif$^+$ *C. m. michiganensis* from infection foci was studied in field plots of a susceptible (Heinz 1810) and a moderately resistant (Heinz 7417, D. A. Emmatty, *personal communication*) tomato cultivar in 1988 and 1989. The two cultivars were seeded in flats in a greenhouse (25 C day, 20 C night) on 6 April 1988 and 7 April 1989. Seedlings that were about 25 cm were transplanted at the Pomology Farm on 26 May 1988 and 1 June 1989. There were six plots of each cultivar. Each plot was 6.0 × 4.5 m^2 and consisted of five rows, with 20 transplants per row. Transplants were spaced 30 cm apart

within rows and 90 cm between rows. Plots were separated by 4.5 m of soybean to reduce interplot interference. Sprinkler irrigation was applied as needed in 1989.

Two types of infection foci were evaluated for each cultivar. A point source of inocula was established by inoculating one transplant in the center of each plot. A line source of inocula was established by inoculating five transplants in the center row of each plot. In 1988, seedlings were inoculated in the greenhouse on 4 May by cutting the first true leaf with scissors dipped in inocula as described previously (5). On 30 May, the inoculated seedlings were transplanted to each plot, replacing healthy plants. Because of poor survival of inoculated transplants in 1988, transplanted seedlings were inoculated in the field on 10 June 1989. Treatments were arranged in a randomized complete block design with three replicates. Sampling locations were established 30, 90, 180, and 270 cm from the focus in four directions except for the moderately resistant cultivar in 1988, for which two sampling locations were evaluated 90 and 180 cm from a line source of inocula. At each sampling location, a single transplant was tagged for evaluation throughout the season. Three terminal leaflets from each tagged plant were sampled at 1-wk intervals. One gram fresh weight of plant tissue from each sample was selected for evaluation. Populations of Rif$^+$ *C. m. michiganensis* were measured by dilution plating methods.

Data were analyzed by analysis of covariance with cultivars and infection foci as qualitative independent variables and distance from foci and days after transplanting (or days after inoculation) as quantitative independent variables. Populations of Rif$^+$ *C. m. michiganensis* were plotted over time and distance by cultivar, infection foci, and year to compare population dynamics. Data were fit to least-squares linear regression models. Polynomial terms were included in the model based on F statistics ($P < 0.05$) and sums of squares.

Dilution plate methods to detect populations of Rif$^+$ *C. m. michiganensis*. Populations of Rif$^+$ *C. m. michiganensis* from plant tissues were measured by dilution plating. Individual samples from an experimental unit were pooled. One gram of plant tissues selected from each sample was cut into 2.5-cm pieces and submerged in 20 ml of sterile PBS in a 125-ml flask. Chlorothalonil (0.1%; 0.5 g a.i./L, w/v) was added to the buffer to inhibit growth of fungal contaminants (13). Samples were washed in buffer for 1 h at room temperature on a reciprocating shaker. Populations of Rif$^+$ *C. m. michiganensis* from plant tissues, tomato debris, and soil were determined by plating the resulting wash solution in 10-fold serial dilutions on a modified CNS medium (17) (minus lithium chloride and polymycin B sulfate) amended with rifampicin (50 mg/L). For all samples, plates were incubated at 25 C and colonies were counted after 5–7 days.

TABLE 1. Populations of Rif$^+$ *C. m. michiganensis*, germination rates, and incidence of systemically infected plants 84 days after transplanting seedlings grown from seed with various percentages of healthy seed mixed with seed from plants systemically infected with Rif$^+$ *C. m. michiganensis*

Seed from infected plants (%)[t]	Log cfu/g		Germination (%)[v]	Incidence (%)[w]
	Seed	Seedling[u]		
0	0.0 a[x]	0.0	89 a	0.0 a
1	2.1 a	0.0	91 a	0.0 a
5	3.3 a	0.0	89 a	0.0 a
10	3.5 a	0.0	91 a	0.0 a
50	4.7 b	0.0	85 b	0.9 a
100	4.7 b	4.5	83 b	0.6 a
100 + acid[y]	0.0 a	0.0	82 b	0.3 a
100 + clip[z]	4.8 b	0.0	82 b	10.7 b

[t] Tomato seed (cv. Heinz 1810), harvested from fruits of systemically infected plants which had been inoculated with strains of Rif$^+$ *C. m. michiganensis*, are mixed with healthy seed at the percentages shown.
[u] Four seedlings from each experimental unit were samples before transplanting. Cfu were determined by dilution plating.
[v] Germination rates (%) were determined by placing 50 seed from each experimental unit on wet filter paper in petri dishes for 8 days at 25 C.
[w] Incidence of systemic infection was measured 84 days after transplanting.
[x] Values in each column followed by the same letter do not differ significantly according to Waller-Duncan's test ($P < 0.05$).
[y] Seed was soaked in 1.8% hydrochloric acid solution for 5 min.
[z] Seedlings were clipped four times with a grass shearer before transplanting.

RESULTS

Seed transmission. Populations of Rif$^+$ *C. m. michiganensis* detected on seed harvested from systemically infected plants and mixed with seed from healthy plants ranged from 10^2 to 10^5 cfu/g seed and increased as the proportion of infested seed increased (Table 1). Before transplanting, Rif$^+$ *C. m. michiganensis* was recovered only from seedlings grown from seed from systemically infected plants. The germination rate was about 83% for the seed lots with a high mixture (>50%) of seed from systemically infected plants and about 90% for seed lots that had less than 10% seed from infected plants (Table 1).

Rif$^+$ *C. m. michiganensis* was transmitted from seed to transplants. Symptoms of systemic infection on transplants were first observed 6 wk after transplanting (Fig. 1). The mixtures of 50 and 100% seed from systemically infected plants resulted in 0.9 and 0.6% systemically infected plants 12 wk after transplanting (Table 1). Although Rif$^+$ *C. m. michiganensis* was not detected from seed from systemically infected plants that was treated with hydrochloric acid, 0.3% of the transplants grown from this seed were systemically infected 12 wk after transplanting (Fig. 1 and Table 1). For the seedlings grown from nonacid treated seed lots that came from systemically infected plants, incidence of systemically infected plants increased from 0.6 to 10.7% when seedlings were clipped before transplanting (Table 1).

Overwinter survival. Survival of Rif$^+$ *C. m. michiganensis* associated with infested tomato debris was greater for debris on the soil surface than for buried debris (Fig. 2). Populations of Rif$^+$ *C. m. michiganensis* declined at about the same rate in samples buried 10, 20, and 30 cm below the surface. Based on t tests, regression coefficients for these treatments did not differ significantly within years ($P < 0.05$). Approximate 10^6 and 10^4 viable cells of Rif$^+$ *C. m. michiganensis* were detected per gram of tomato debris from buried samples after 196 and 210 days in 1988-89 and 1989-90, respectively. Populations of Rif$^+$ *C. m. michiganensis* recovered from tomato debris on the soil surface were about 10^7 and 10^6 cfu/g tomato debris after 196 and 210 days in 1988-89 and 1989-90, respectively. Populations of Rif$^+$ *C. m. michiganensis* recovered from soil mixed with infested tomato debris were consistently lower than from infested tomato debris placed in the same nylon mesh bag (Fig. 3). Viable cells detected from soil mixed with tomato debris ranged from about 10^2 to 10^4 cfu/g soil after 210 days. Populations of Rif$^+$ *C. m. michiganensis* from the soil mixed with tomato debris were

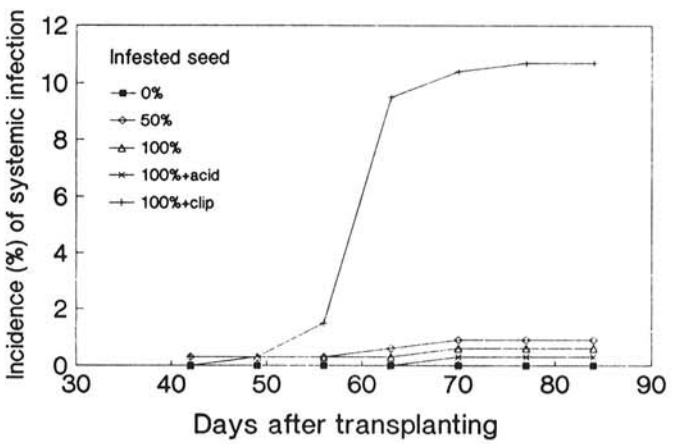

Fig. 1. Progress of bacterial canker of tomato in a field after transplanting seedlings grown from seed with different percentages of infested seed (i.e., seed from systemically infected plants).

higher for bags placed on the soil surface than for bags that were buried (Fig. 3).

Leaf surface populations on alternative hosts and nonhost plants. Leaf surface populations of Rif$^+$ *C. m. michiganensis* on alternative hosts and nonhost plants were first detected 6 and 3 wk after inoculation of tomato species in 1988 and 1989, respectively (Tables 2 and 3). Populations fluctuated during the 12-wk sampling period but approached a maximum at the end of the each season. In 1988, leaf surface populations of Rif$^+$ *C. m. michiganensis* generally were lower on nonsolanaceous weeds than on solanaceous plants except tobacco and jimsonweed.

Populations of Rif$^+$ *C. m. michiganensis* on alternative hosts and nonhost plants were significantly lower than on tomatoes. Populations of Rif$^+$ *C. m. michiganensis* recovered from solanaceous plants other than tomato ranged from 0 to about 10^8 cfu/g fresh weight (Tables 2 and 3). Populations of Rif$^+$ *C. m. michiganensis* recovered from nonsolanaceous weeds ranged from 0 to about 10^3 cfu/g fresh weight (Tables 2 and 3). Populations of Rif$^+$ *C. m. michiganensis* on tomato plants were recovered at least 8 wk after inoculation and were consistently about 10^6 to 10^9 cfu/g fresh weight.

Symptoms of secondary infection were first observed on uninoculated tomato plants 8 and 6 wk after inoculation in 1988 and 1989, respectively. Symptoms of secondary infection were never observed on plants of the other nine species.

When all tomato plants (including inoculated plants) were removed 8 wk after inoculation in 1989, Rif$^+$ *C. m. michiganensis* continued to be detected on solanaceous plants and nonhost plants for an additional 7 wk (Table 3). However, based on *t* tests, leaf surface populations of Rif$^+$ *C. m. michiganensis* recovered from the treatments for which tomatoes were removed were lower in 19 of 24 cases than populations recovered from the treatment for which inoculated tomato plants were not removed (Table 3).

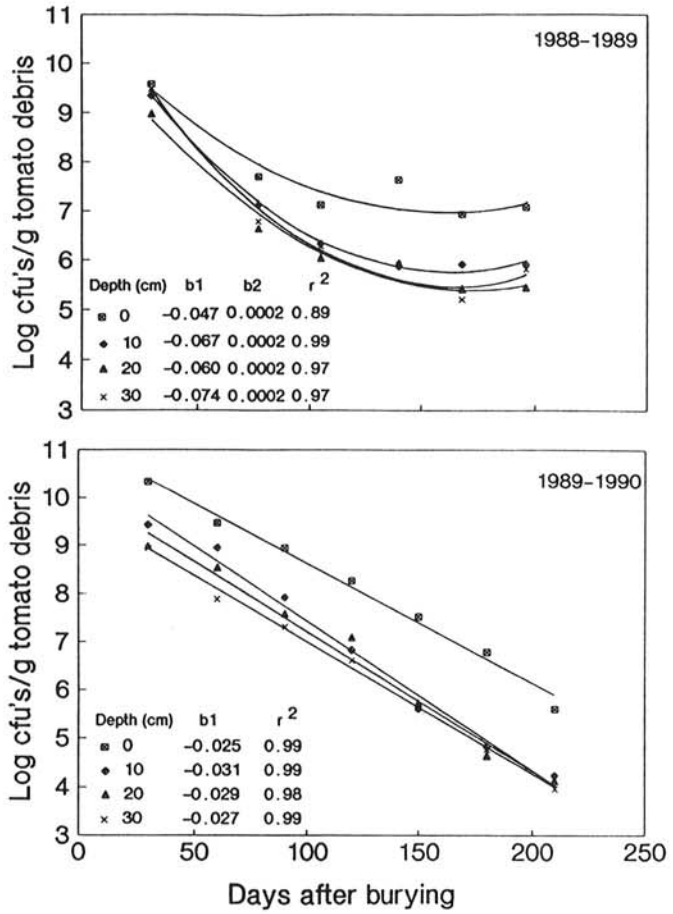

Fig. 2. Recovery of Rif$^+$ *Clavibacter michiganensis* ssp. *michiganensis* from infested tomato debris placed on the soil surface or buried 10, 20, and 30 cm below the surface during 1988-89 and 1989-90.

Dispersal. The susceptible and the moderately resistant cultivars supported leaf surface populations of Rif$^+$ *C. m. michiganensis* at about 10^7 to 10^9 cfu/g fresh weight by the end of the growing season (Figs. 4 and 5). On the susceptible cultivar, the response surface of populations of Rif$^+$ *C. m. michiganensis* by distance and time were similar in both years except from the point sources of inocula in 1988 (Table 4). On the moderately resistant cultivar, the response surface of populations of Rif$^+$ *C. m. michiganensis* from both point and line sources of inocula were similar in 1989; however, the regression models were affected significantly by the type of infection focus in 1988 (Table 4). Cultivar had no significant affect on populations of Rif$^+$ *C. m. michiganensis* recovered from either infection focus in 1988 (Table 4 and Fig. 4). However, in 1989, populations of Rif$^+$ *C. m. michiganensis* recovered from the susceptible cultivar were significantly higher than those from the moderately resistant cultivar (Table 4 and Fig. 5).

Leaf surface populations of Rif$^+$ *C. m. michiganensis* were affected significantly by time (Table 4). In 1988, populations of

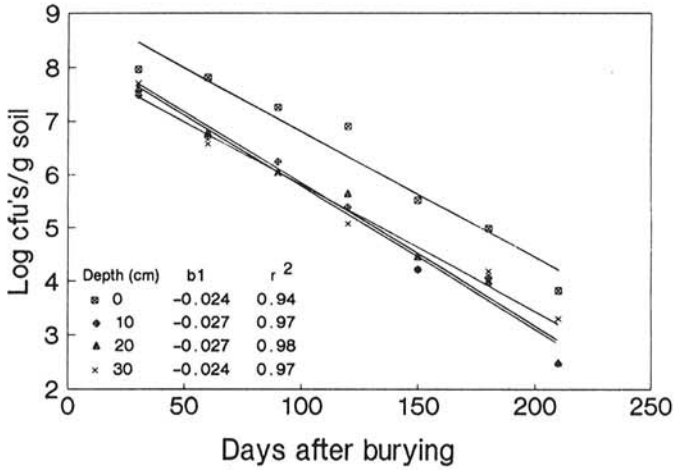

Fig. 3. Recovery of Rif$^+$ *Clavibacter michiganensis* ssp. *michiganensis* from soil mixed with infested tomato debris in nylon mesh bags and placed on soil surface or buried 10, 20, and 30 cm below the surface during 1989-90.

TABLE 2. Leaf surface populations (log cfu/g fresh weight) of Rif$^+$ *C. m. michiganensis* on alternative and nonhost plants in 1988[y]

Alternative and nonhost species	Weeks after inoculation of tomato						
	5	6	7	8	9	10	11
Solanaceous species							
Capsicum annuum (pepper cv. California Wonder)	0.0	0.0	2.2	2.7	2.5	4.4	4.2
Datura stramonium (jimsonweed)	ND[z]	ND	0.0	0.7	0.8	2.0	2.0
Lycopersicon esculentum (tomato cv. Heinz 1810)	2.3	2.7	4.2	6.4	6.8	8.5	8.2
Nicotiana tabacum (tobacco cv. Samsun NN)	0.0	0.0	0.0	1.3	0.9	2.1	1.7
Solanum melongena (eggplant cv. Early Prolific)	0.0	0.0	0.0	1.8	2.0	3.0	3.1
Solanum nigrum (black nightshade)	0.0	2.0	1.9	3.2	2.6	4.7	3.9
Solanum tuberosum (potato cv. Kennebec)	0.0	0.0	0.0	2.1	2.5	4.3	3.9
Nonsolanaceous species							
Chenopodium album (goosefoot)	0.0	0.0	0.0	0.0	1.8	2.3	2.1
Xanthium saccharatum (cocklebur)	ND	ND	ND	0.0	0.0	2.3	2.1
BLSD	1.5	2.0	1.6	1.8	1.6	1.4	1.0

[y]Three to 10 leaves per species were collected and pooled as a sample per experimental unit. Cfu were determined from 1 g of plant tissue per sample by dilution plating.
[z]ND = not determined.

Rif$^+$ *C. m. michiganensis* in plots of the susceptible cultivar were first detected from the line and the point sources of inocula 21 and 42 days after transplanting, respectively. Populations continued to increase throughout the growing season (Fig. 4). In 1989, Rif$^+$ *C. m. michiganensis* was first detected 14 days after inoculating transplants in the field (Fig. 5). Populations in plots with point and line sources of inocula reached a maximum of about 10^9 cfu/g on the susceptible cultivar at 63 and 56 days after inoculation, respectively. Populations increased to about 10^6–10^8 cfu/g on the moderately resistant cultivar until 63 days after inoculation.

Distance from the source of inocula affected populations of Rif$^+$ *C. m. michiganensis* for the entire season in 1988 and for about 42–49 days in 1989 for the susceptible and the moderately resistant cultivar, respectively (Table 4 and Figs. 4 and 5).

Populations of Rif$^+$ *C. m. michiganensis* were affected significantly by the type of infection focus in 1988 (Table 4). Populations recovered from a line source of inocula were higher than from a point source of inocula for both cultivars. The bacteria were distributed evenly over plots with a line source of inocula by the end of the growing season in 1988 (Fig. 4). However, populations recovered from a point source of inocula in plots of the susceptible cultivar ranged from 0 to about 10^6 cfu/g fresh weight by the end of the growing season and were detected only within a distance of 90 cm from the focus. In 1989, the type of infection focus did not significantly affect leaf surface

TABLE 3. Leaf surface populations (log cfu/g fresh weight) of Rif$^+$ *C. m. michiganensis* on alternative and nonhost plants in 1989[w]

Alternative and nonhost species	Weeks after inoculation of tomato							
	3	4	5	6	8	11	13	15
Solanaceous species								
Capsicum annuum (pepper cv. California Wonder)	2.4	4.1	3.8	5.0	3.9	4.2 (2.8)[x]	6.7 (5.2)	7.8 (6.7)
C. annuum (pepper cv. Jalapeno)	1.8	1.2	0.0	3.4	1.4	1.5 (2.6)	5.2 (4.3)	6.9 (5.7)
C. annuum (pepper cv. Super Shepherd)	0.7	2.7	1.7	0.9	2.8	3.2 (2.0)	6.5 (5.2)	7.5 (4.9)
C. annuum (pepper cv. Yellow Hungarian)	2.4	0.0	0.6	2.8	1.0	3.1 (3.6)	5.4 (5.6)	6.9 (4.2)
Datura stramonium (jimsonweed)	0.0	1.3	0.8	1.0	2.1	3.3 (1.2)	3.9 (0.0)	4.7 (2.0)
Lycopersicon esculentum (tomato cv. Heinz 1810)	5.0	6.7	7.9	8.4	8.8	9.0 (ND)[y]	8.5 (ND)	8.4 (ND)
Solanum melongena (eggplant cv. Kurume Long Black)	1.8	1.8	1.6	2.8	2.0	3.4 (0.0)	4.9 (3.6)	5.3 (4.0)
Nonsolanaceous species								
Amaranthus retroflexus (pigweed)	0.0	1.1	0.6	0.7	1.5	1.0 (0.0)	1.2 (1.4)	2.5 (0.0)
Xanthium saccharatum (cocklebur)	0.0	0.5	1.0	0.7	0.0	2.8 (1.8)	1.0 (1.1)	1.4 (0.0)
BLSD/LSD[z]	1.8	2.4	2.2	3.0	2.6	3.1	2.2	2.0

[w]Three to 10 leaves per species or cultivar were collected and pooled as a sample per experimental unit. Cfu were determined from 1 g of plant tissue per sample by dilution plating.
[x]Values in parentheses are determined from the two plots where tomato plants were removed.
[y]Tomato plants were removed 56 days after inoculation of tomato. ND = no data.
[z]LSD was calculated 77 days after inoculation of tomato.

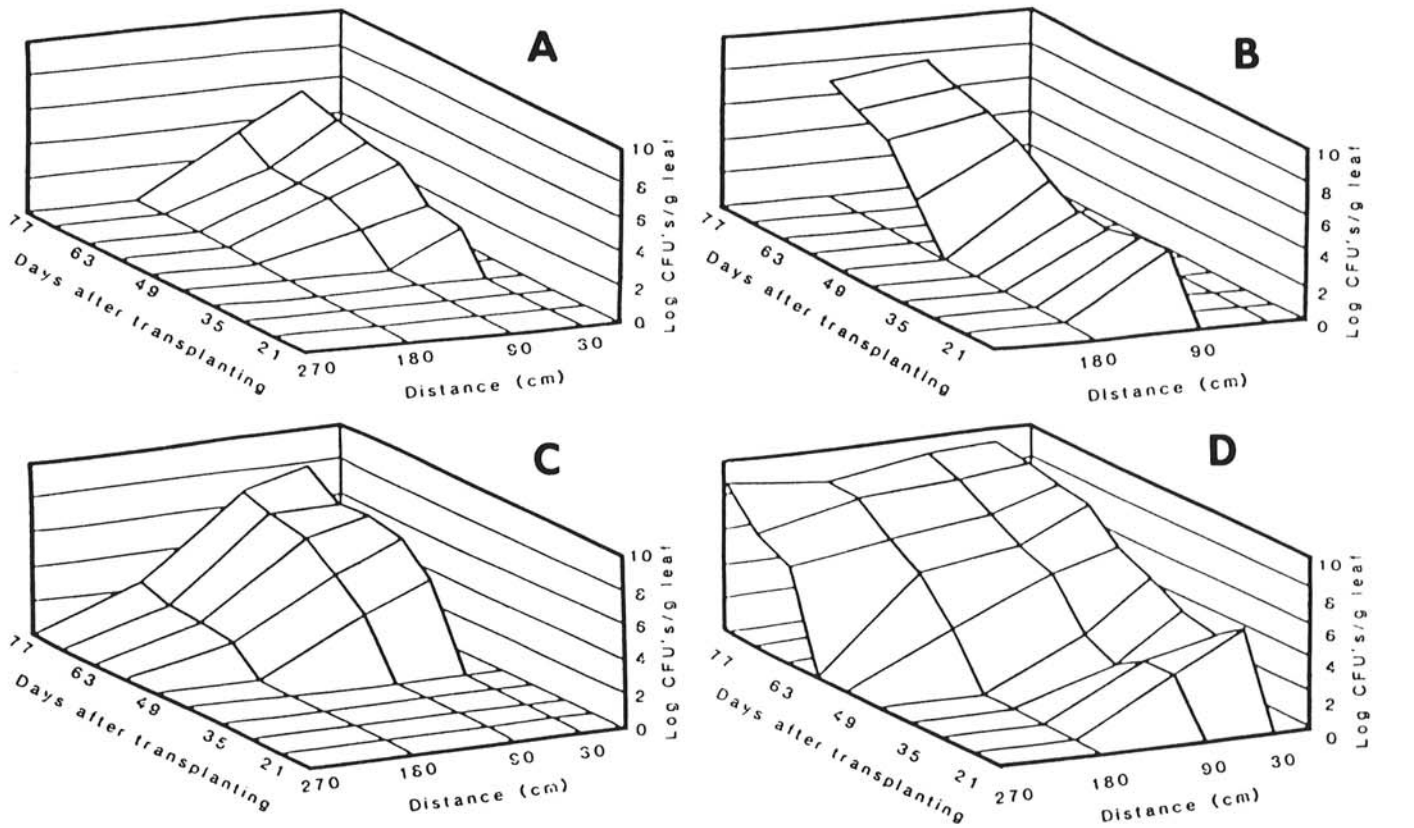

Fig. 4. Secondary dispersal of leaf surface populations of Rif$^+$ *Clavibacter michiganensis ssp. michiganensis* (log cfu/g fresh weight) from point (**A,C**) and line (**B,D**) sources of inocula in plots of a moderately resistant, Heinz 7417 (**A,B**), and a susceptible, Heinz 1810 (**C,D**), tomato cultivar in 1988.

populations of Rif$^+$ *C. m. michiganensis* recovered from either cultivar (Table 4).

Dry weather in 1988 may have affected secondary dispersal of Rif$^+$ *C. m. michiganensis* in production fields. Leaf surface populations of Rif$^+$ *C. m. michiganensis* were not detected from a point source of inocula in plots of the susceptible and the moderately resistant cultivar until 42 and 49 days after transplanting of inoculated seedlings and only after the first rainfall (12 July 1988) after transplanting (Fig. 4). By this time, inoculated seedlings of the susceptible cultivar at the infection focus had died, but inoculated seedlings of the moderately resistant cultivar were still alive and provided secondary inocula.

Symptoms of secondary infection, such as marginal scorch of leaflets, brown to tan spots on peduncles, and bird's-eye spots on fruit, were not observed until leaf surface populations of Rif$^+$ *C. m. michiganensis* exceeded about 10^6 and 10^7 cfu/g fresh weight for the moderately resistant and susceptible cultivar, respectively. Symptoms of systemic infection of transplants were not observed in either cultivar in either year, but symptoms of foliar and/or fruit infection occurred earlier and were more severe on the susceptible cultivar in both years. In 1988, leaf surface colonization was similar between cultivars; however, symptoms of secondary infection were first observed on the susceptible cultivar 56 days after transplanting and occurred only in plots with line sources of inocula. Symptoms of secondary infection did not appear on the moderately resistant cultivar until 70 days after transplanting in 1988. In 1989, symptoms of secondary infection were first observed on the susceptible and moderately resistant cultivars 42 and 49 days after inoculation, respectively.

DISCUSSION

Seed infested with *C. m. michiganensis* that are sown in production fields, infested tomato debris that overwinters in the

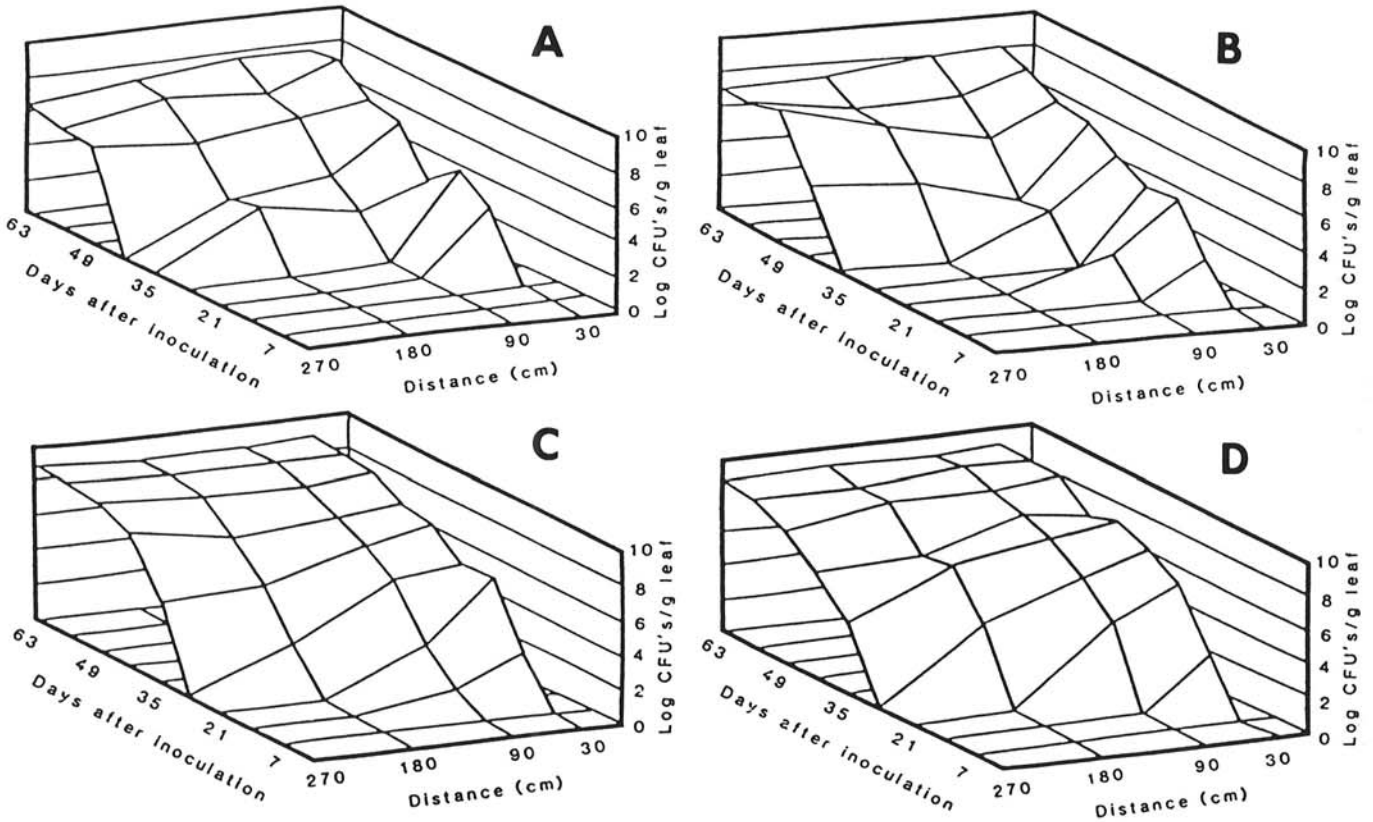

Fig. 5. Secondary dispersal of leaf surface populations of Rif$^+$ *Clavibacter michiganensis ssp. michiganensis* (log cfu/g fresh weight) from point (**A,C**) and line (**B,D**) sources of inocula in plots of a moderately resistant, Heinz 7417 (**A,B**), and a susceptible, Heinz 1810 (**C,D**), tomato cultivar in 1989.

TABLE 4. Multiple regression models describing secondary dispersal of leaf surface populations of Rif$^+$ *C. m. michiganensis* (log cfu/g fresh weight) from point and line sources of inocula in plots of a susceptible (Heinz 1810) and a moderately resistant (Heinz 7417) tomato cultivar in 1988 and 1989

Cultivar	Focus[x]	Year	Model[y]	R^{2z}
H. 1810	P	1988	$-3.89 + 0.15T + 0.031D - 0.000060D^2 - 0.0013TD + 0.0000026TD^2$	0.95
H. 1810	L	1988	$11.47 - 0.16T + 0.016T^2 - 0.00011T^3 + 0.0065D - 0.0015TD + 0.000018T^2D$	0.94
H. 7417	P	1988	$-5.72 + 0.20T + 0.021D - 0.00077TD$	0.89
H.7417	L	1988	$11.46 - 0.22T + 0.0026T^2 - 0.060D + 0.00064TD$	0.95
H. 1810	P	1989	$-4.89 + 0.77T - 0.016T^2 + 0.00011T^3 + 0.035D - 0.0059TD + 0.00019T^2D - 0.0000016T^3D$	0.97
H. 1810	L	1989	$-6.21 + 0.94T - 0.019T^2 + 0.00013T^3 + 0.035D - 0.0059TD + 0.00017T^2D + 0.0000014T^3D$	0.96
H. 7417	P	1989	$0.77 + 0.024T + 0.0017T^2 - 0.0089D$	0.86
H. 7417	L	1989	$1.99 + 0.044T + 0.0015T^2 - 0.037D + 0.000095D^2$	0.86

[x] P = point sources of inocula; L = line sources of inocula.
[y] Model and polynomial terms significant at $P < 0.05$; D = distance from the focus in centimeters, T = time, days after transplanting in 1988 and days after inoculation in 1989.
[z] Coefficient of multiple determination.

field, and leaf surface populations of *C. m. michiganensis* on tomato and alternative hosts can be local sources of inocula for bacterial canker of tomato. Secondary spread of *C. m. michiganensis* from foci of infected plants that may arise from these local sources of inocula can result in relatively high leaf surface populations on susceptible and moderately resistant tomato cultivars. A few (1–5) systemically infected transplants in production fields can result in leaf surface populations of *C. m. michiganensis* of 10^7–10^9 cfu/g fresh weight at least 2.7 m from the focus of infection.

In this study, we observed secondary infection of bacterial canker, which was typified by spotted fruits and scorched leaflets. In another study (25), this type of infection had a relatively minor effect on yield of processing tomatoes. We did not observe symptoms of systemic infection from these local sources of inocula. However, it would be unreasonable to infer from our limited data that systemic infection could not arise from local sources of inocula.

The role of infested seed as a local source of inocula is probably dependent upon secondary spread of *C. m. michiganensis*. Transmission of *C. m. michiganensis* from seed to tomato transplants was demonstrated by sowing seed from systemically infected plants in the greenhouse and transplanting the seedlings grown from this seed. However, the seed transmission rate was only 0–0.9% in this study. Nevertheless, secondary spread of *C. m. michiganensis* from foci created by seed transmission could result in leaf surface populations of about 10^7–10^9 cfu/g if the dispersal of *C. m. michiganensis* in production fields is similar from plants infected by seed transmission and from plants infected by our inoculation procedure.

C. m. michiganensis associated with infested tomato debris was able to overwinter in central Illinois. The soil environment may have influenced survival of Rif$^+$ *C. m. michiganensis*, which, if similar to other phytopathogenic bacteria, is associated with the rate of decomposition of plant debris (6). Tomato debris decomposed more rapidly when buried and was accompanied by a decline in Rif$^+$ *C. m. michiganensis* of about 10^5 cfu/g debris from November to May. Populations of Rif$^+$ *C. m. michiganensis* declined only about 10^3 cfu/g debris when placed on the soil surface during the same period. Gleason et al (15) also reported that *C. m. michiganensis* associated with infested tomato debris overwintered in Iowa and significantly reduced yield in a subsequent crop of processing tomatoes when transplants were planted directly on top of the buried debris. Although systemic and local infections of transplants from overwintered, infested debris probably occurs, crop rotation, plowing and/or disking practices employed before transplanting should reduce the occurrence of such infections.

Populations of Rif$^+$ *C. m. michiganensis* were also detected from soil mixed with infested tomato debris and placed into nylon mesh bags. From these results, we infer that Rif$^+$ *C. m. michiganensis* is released from decomposing tomato debris. Populations of Rif$^+$ *C. m. michiganensis* recovered from the soil were always about 10- to 100-fold lower than those from tomato debris. In another study (R. J. Chang, *unpublished data*), we found that Rif$^+$ *C. m. michiganensis* died quickly in the soil when the bacteria were suspended and released into nonsterile soil. Populations of Rif$^+$ *C. m. michiganensis* could not be recovered from a mixture of nonsterile soil and the bacteria 6 wk after the soil/bacterium mixture was placed in a covered beaker and stored at room temperature. Thus, survival of *C. m. michiganensis* in the field depends on cool dry environments (2) and on the presence of plant tissues to provide nutrients to maintain minimal metabolism for *C. m. michiganensis* (21).

C. m. michiganensis may have a leaf surface phase on alternative hosts in the absence of tomato. Populations of Rif$^+$ *C. m. michiganensis* on solanaceous plants other than tomatoes ranged from 0 to about 10^8 cfu/g fresh weight. When potential sources of inocula (inoculated tomatoes) were removed from plots, populations of Rif$^+$ *C. m. michiganensis* continued to be detected on solanaceous plants. This phase may serve as a continuing source of inocula that can be disseminated to healthy tomatoes. The leaf surface populations of Rif$^+$ *C. m. michiganensis* on alternative hosts did not cause systemically infected plants, but the localized phase of the disease was observed on noninoculated tomato transplants. Symptoms of secondary infection on pepper and black nightshade were not observed in our studies, even though these plants are susceptible to *C. m. michiganensis* (33,39). Leaf surface populations of Rif$^+$ *C. m. michiganensis* recovered from alternative hosts and nonhost plants fluctuated greatly throughout the growing season. Although the highest populations were detected at the end of the growing season, populations on alternative hosts did not increase continually as they did on tomato plants.

A leaf surface phase may be common for *C. m. michiganensis*. Symptoms of secondary infection of bacterial canker were not observed until leaf surface populations exceeded about 10^6–10^7 cfu/g fresh weight. Such leaf surface survival and high foliar populations have been reported for several other bacteria, including phytopathogenic corynebacteria (14,28,37,38). Leaf surface populations might serve as inocula that spread by windsplashed rain and cause secondary, local infections (30,31).

In 1988, leaf surface populations of Rif$^+$ *C. m. michiganensis* recovered from the susceptible cultivar were lower than from the moderately resistant cultivar in plots with a point source of inocula. In 1989, populations were consistently higher on the susceptible than on the moderately resistant cultivar from both the point and line sources of inocula. The population differences between the susceptible and moderately resistant cultivars in 1988 and 1989 probably were due to dry weather in 1988, inherent cultivar differences, and unequal dying rates of inoculated plants. Thus, the susceptible cultivar may support higher leaf surface populations than the moderately resistant cultivar if dry weather does not occur.

Detection of *C. m. michiganensis* in a large population of asymptomatic transplants presents a difficult challenge. Our methods did not detect Rif$^+$ *C. m. michiganensis* from clipped seedlings grown from seed from systemically infected plants, yet this treatment resulted in 10.7% systemically infected plants. Apparently, subsampling four seedlings from each experimental unit of 120 asymptomatic transplants was not adequate to estimate the populations of *C. m. michiganensis*.

A severe foliar blight epidemic of bacterial canker on fresh market tomatoes that occurred during extensive wet weather in western North Carolina was caused by leaf surface populations of *C. m. michiganensis* (30). Systemic infection from leaf surface populations of *C. m. michiganensis* may also occur, especially if wounds are created by removal of axillary leaves, trellising, etc. (15,19). However, such infections of processing tomatoes are not as likely, because plants are not handled extensively after transplanting (25). Thus, environmental conditions and plant physiology (such as plant age and cultivar) may play a significant role in the occurrence of systemic infections from leaf surface populations.

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