

Effects of Pruning on Tomato Plants Supporting Epiphytic Populations of *Clavibacter michiganensis* subsp. *michiganensis*

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ABSTRACT

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Infection of pruning wounds by epiphytic populations of the bacterial canker pathogen, *Clavibacter michiganensis* subsp. *michiganensis*, increased the amount of diseased foliage and vascular colonization in field-grown tomato plants in 1991 but not in 1990. Pruning significantly reduced yield from inoculated plants compared with nonpruned inoculated plants in 1991 but not in 1990. Inoculation significantly reduced estimated yield in both years. Pruning had no effect on disease development or yield in noninoculated tomato plants.

Clavibacter michiganensis subsp. *michiganensis* (Smith) Davis et al is a vascular wilt pathogen that can cause serious losses in both fresh-market and processing tomato (*Lycopersicon esculentum* Mill.) production (6-8,16). Recently, it was shown that *C. m. michiganensis* can establish epiphytic populations as high as 10^8 cfu per leaflet (5,7,11). Although it is likely that epiphytic populations of this magnitude play a role in disease development, it is unclear how these bacteria enter the plant.

Pruning of axillary branches is a common cultural practice in fresh-market tomato production. Pruning opens the plant canopy and promotes early ripening of fruits, which often enables producers to participate in early-season markets and achieve a larger net return on investment. Pruning also may provide an infection court for *C. m. michiganensis*. Ark (1) demonstrated that *C. m. michiganensis* could be disseminated by pruning axillary branches with a contaminated knife. Thomas (17) determined that the pathogen was disseminated by workers' hands during pruning and staking operations, and that 50% of the pruned plants eventually developed canker symptoms. He also showed that sap extracted from systemically infected plants induced canker symptoms when applied directly to pruning wounds. Significant yield reductions have been reported in trellised, fresh-market tomatoes that were wound-inoculated at first pruning and first flowering (8). It has not been determined, however, whether epiphytic populations of *C. m. michiganensis* are an important source of inoculum for infection of plants via pruning wounds. The purposes of this study

were to determine whether pruning wounds serve as an infection court for epiphytic populations of *C. m. michiganensis* and to assess the impact of this mode of infection on disease development and yield in fresh-market tomatoes.

MATERIALS AND METHODS

Experimental design. The field experiment was conducted during 1990 and 1991 at Iowa State University's Curtiss Farm. Plots were located several hundred meters from fields in which tomatoes had been grown previously. In both 1990 and 1991, plots were planted in fields where soybeans had been grown previously. The experimental design was a 2×2 factorial (inoculation \times pruning) arranged in a randomized complete block with five replicates. Each plot contained two rows of 13 plants, with 1.2 m between rows and 0.6 m between plants within a row. Plots were separated by 9.1- and 13.7-m buffer zones planted with field corn in 1990 and 1991, respectively. Half of the plots were spray-inoculated with *C. m. michiganensis*, and half remained uninoculated.

Fertilizer, pest control, and tillage. Fields were fall-fertilized with 256 kg/ha of 0-23-46 fertilizer and were top-dressed in the spring with 224 kg/ha of 15-15-15 fertilizer after transplanting. Trifluralin (Treflan) was applied at 1.75 L/ha to control grasses. Plants were sprayed weekly with 3.5 L/ha of chlorothalonil (Bravo 720) and 140 ml/ha esfenvalerate (Asana XL) to control fungi and insects, respectively. Plots were cultivated twice with a rotary tiller before inoculation. Overhead irrigation was applied as needed.

Inoculum production. A 1:1:1 mixture of three naturally occurring strains of rifampicin-resistant *C. m. michiganensis* (Cmm-1-R2, BR4-R1, and DR60-R1) was used as inoculum (11). Stock cultures of *C. m. michiganensis* were stored below

0 C on anhydrous silica gel crystals (14). Inoculum was grown on nutrient-broth yeast extract agar (9) amended with cycloheximide at 40 mg/L and rifampicin at 50 mg/L (Sigma Chemical Co., St. Louis, MO) (NBYCR) and was incubated at 25 C for 5-7 days. Bacterial cells were suspended in sterile 0.02 M phosphate buffer (pH 7.0), and the concentration was adjusted by measuring absorbance on a spectrophotometer (A_{540}).

Inoculation and pruning. Eight-week-old tomato seedlings (cv. Jet Star) were transplanted into field plots on 29 May 1990 and 22 May 1991. On 2 July 1990 and 13 June 1991, plants were enclosed in cylindrical, wire-mesh cages (46 \times 122 cm). On 15 June 1990 and 17 June 1991, plants in the inoculated treatments were sprayed with approximately 23 ml per plant of a suspension of *C. m. michiganensis* in 0.02 M phosphate buffer (pH 7.0) at 5.0×10^4 cfu/ml by using a hand-held sprayer. A portable settling tower was placed around each plant during inoculation to minimize spray drift. Plants in the noninoculated treatments were not sprayed. Plants in the pruned treatments were pruned manually on 29 June 1990 and 27 June 1991 by snapping or tearing off the lowest axillary branches up to but not including the axillary branch immediately below the first flower cluster. Six to eight axillary branches per plant were removed during pruning. To prevent plot-to-plot contamination, plants in the noninoculated treatments were pruned on the same dates but before the inoculated treatments.

Epiphytic populations. Epiphytic populations of *C. m. michiganensis* were estimated periodically from June to September by determining the number of cfu on asymptomatic leaflets. For each sampling period, five asymptomatic, terminal leaflets were excised with a flamed scalpel from each of four randomly selected plants from each plot and were placed immediately in a plastic bag on ice. The 20 leaflets from each plot were bulked and shaken in 250 ml of 0.1 M phosphate buffer (pH 7.0) with 0.1% peptone for 1 hr on a platform shaker at 140 rpm. Tenfold dilutions were performed, and 0.1-ml aliquots were plated on NBYCR. Colonies were counted after incubation at 25 C for 5-7 days.

Disease severity. Disease severity was assessed four times in 1990 beginning 48

days after inoculation, and five times in 1991 beginning 24 days after inoculation by visually estimating the percentage of diseased foliage on each of 18 plants per plot. The percentage of diseased foliage was averaged for each plot, and area under the disease progress curve (AUDPC) was calculated for each treatment (3).

Vascular colonization. To monitor movement of *C. m. michiganensis* in the vascular system over time, a main stem of one randomly selected plant from each plot was excised at the soil line, placed in a plastic bag, and processed in the laboratory. Each main stem was sectioned into 10-cm segments, and each segment was dipped in 95% ethanol and flamed twice. Segments were resectioned with a sterile blade, and the freshly cut ends were touched to NBYCR (10). Plates were incubated at 25 C for 5–7 days. The mean percent stem segments colonized for each treatment was calculated at each sampling period, and area under the curve (AUC) for vascular colonization was calculated for each treatment (3).

Yield. Mature fruits were harvested weekly from the center 10 plants (five plants per row) in each plot. Fruits were weighed and counted.

Statistical analysis. AUDPC for diseased foliage, AUC for vascular colonization, and yield were analyzed by analysis of variance (ANOVA).

RESULTS

Epiphytic populations. Epiphytic populations of *C. m. michiganensis* in spray-inoculated plots increased to 10^8 cfu per leaflet by midsummer (Fig. 1). In non-

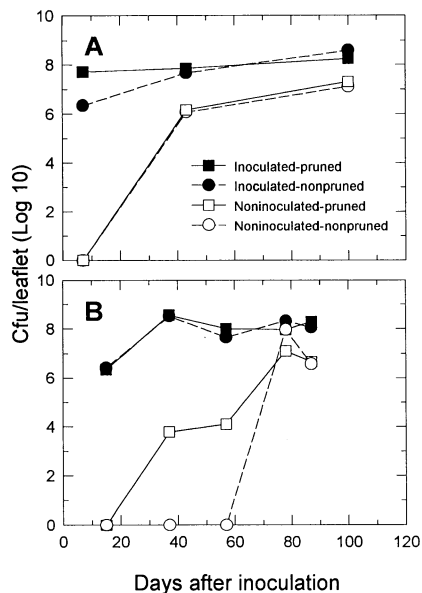


Fig. 1. Mean epiphytic populations of *Clavibacter michiganensis* subsp. *michiganensis* on tomato plants (cv. Jet Star) that were spray-inoculated on (A) 15 June 1990 and (B) 17 June 1991. Each point is the mean of five replicates with 20 leaflets per replicate.

inoculated pruned plots, epiphytic populations of *C. m. michiganensis* were detected 43 and 37 days after inoculation in 1990 and 1991, respectively. In plots containing noninoculated nonpruned plants, epiphytic populations were detected 43 and 58 days after inoculation in 1990 and 1991, respectively.

Foliar disease development. Diseased foliage was observed on plants in inoculated treatments 48 days and 24 days after inoculation in 1990 and 1991, respectively (Fig. 2). Less diseased foliage developed in the noninoculated treatments in both years. Inoculated plants had significantly ($P = 0.01$) more diseased foliage over time (AUDPC) than noninoculated plants in both 1990 and 1991 (Table 1). Pruning further increased significantly ($P = 0.05$) the amount of diseased foliage in 1991 but not in 1990.

Extent of vascular colonization. Vascular colonization over time (AUC) was significantly ($P = 0.05$) greater in the

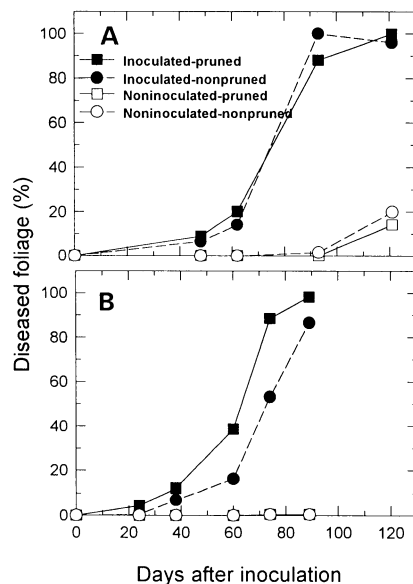


Fig. 2. Effects of pruning and of inoculation with *Clavibacter michiganensis* subsp. *michiganensis* on the development of diseased foliage. Plots were spray-inoculated on (A) 15 June 1990 and (B) 17 June 1991. Each point is the mean of five replicates with 18 plants per replicate.

Table 1. Area under the disease progress curve (AUDPC) for tomato plants either spray-inoculated or not sprayed with *Clavibacter michiganensis* subsp. *michiganensis* and either pruned or nonpruned

Treatment	AUDPC ^{a,b}			
	1990		1991	
	Pruned	Nonpruned	Pruned	Nonpruned
Inoculated	4,478	4,285	2,697	1,840
Noninoculated	461	283	4	4
LSD ^c	366		399	

^a AUDPCs were calculated by visually estimating the percent diseased foliage in five replicates with 18 plants per replicate.

^b Level of significance for the interaction (inoculation \times pruning) effect is $P = 0.9661$ and $P = 0.0377$ for 1990 and 1991, respectively.

^c Means within each column and row were compared within each year with an F test.

inoculated plants than in the noninoculated plants in both years (Fig. 3, Table 2). Pruning significantly ($P = 0.05$) increased AUC for vascular colonization of inoculated plants in 1991 but not in 1990. In 1990, the vascular tissue of all of the plants assayed in both the inoculated pruned and inoculated nonpruned plots was infected 2 wk after pruning. However, only the vascular tissue of plants in the inoculated pruned plots was infected 2 wk after pruning in 1991. By the end of September in both years, the pith and cambium of inoculated pruned plants were completely collapsed 10 cm above the soil surface. The cambial layer of inoculated nonpruned plants was discolored and exhibited collapsed areas. Stems of the noninoculated plants remained asymptomatic.

Yield. Fruits from inoculated plants became shrunken and mottled by early September in 1990 and 1991. Spray-inoculation significantly ($P = 0.05$) re-

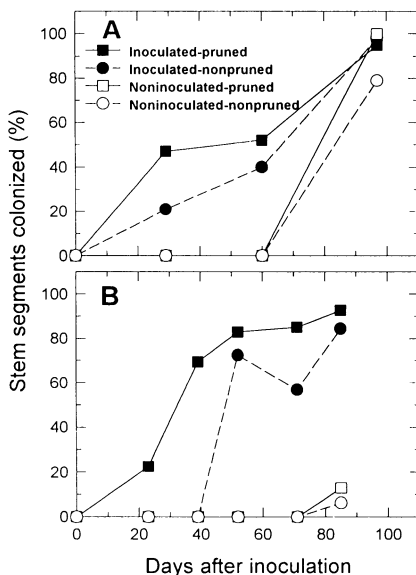


Fig. 3. Percentage of 10-cm stem segments with *Clavibacter michiganensis* subsp. *michiganensis* in the vascular tissue in plants either spray-inoculated with *C. m. michiganensis* or unsprayed, and either pruned or nonpruned, in (A) 1990 and (B) 1991.

duced yield of both the pruned and non-pruned plants in 1990 and 1991 (Table 3). Pruning noninoculated plants did not significantly ($P = 0.05$) affect yield in either year. Pruning inoculated plants further reduced yield in both years; the differences were significant ($P = 0.05$) in 1991 but not in 1990.

DISCUSSION

Our study provides the first direct evidence that pruning, a common cultural practice on fresh-market tomatoes, can facilitate the entry of *C. m. michiganensis* into the vascular system, reducing yield and causing large economic losses. In Australia, Dullahide et al (8) also observed that infection through branch stubs suppressed yield, but these workers deliberately inoculated the pruning wounds with a contaminated knife blade. Our findings also add to the recent evidence (4,11) that the epiphytic phase of *C. m. michiganensis* can play an important role in the canker disease cycle on tomatoes. Although epiphytic populations of *C. m. michiganensis* can reach levels as high as 10^8 cfu per leaflet (5, 7,11), systemic infections can result from the introduction of as few as five pathogen cells into the vascular tissue of tomato seedlings (18). The source of inoculum for stem-wound infection is difficult to determine under field conditions. It is possible that vascular infections developed from latent stem infections; however, the fact that no bacteria were found in the vascular tissue of inoculated non-pruned plants until 40 days after inoc-

ulation in 1991, and that they were then found only below the pruning wounds (W. M. Carlton, *unpublished*), suggests that the inoculum entered through these wounds. Whether bacterial cells were washed or blown from the leaves or stems into the pruning wounds, or were introduced directly into the wounds during pruning, our results indicate that pruning wounds provide suitable infection courts for the pathogen.

In 1990, the pruning treatment did not have as much impact on disease development as in 1991. On 19 June 1990, 4 days after inoculation, a severe thunderstorm with high winds, heavy rain (15 cm), and light hail may have created wounds that facilitated the entry of the pathogen into the inoculated nonpruned plants, masking the effects of pruning that year. The vascular tissue of all plants in the inoculated nonpruned treatments was infected 2 wk after pruning with no observable signs of wounding. This result suggests that extreme weather events may provide pathways for the entry of *C. m. michiganensis* almost as efficiently as pruning.

In addition to pruning wounds, other infection courts exist for epiphytic *C. m. michiganensis*. Even in the absence of severe weather in 1991, the spray-inoculated, nonpruned plants developed canker symptoms and were extensively colonized 8 wk after inoculation. Infections may have occurred during weekly harvesting operations or pesticide applications (16), or through natural openings such as trichomes (12,13), stomates (15),

or hydathodes (4). Basu (2) also reported canker development in non-wounded, spray-inoculated plants.

Because detection of epiphytic populations of *C. m. michiganensis* is impractical in commercial tomato production, producers are generally unaware of epiphytic populations on asymptomatic transplants. Our study has shown that pruning axillary branches from plants colonized with an epiphytic population of *C. m. michiganensis* may inadvertently provide an avenue of entry for the pathogen. Entry of the pathogen into the vascular tissue can cause severe economic losses to producers. Gross income was reduced from \$74,220 to \$50,730 per hectare by spray-inoculating the plants when local wholesale prices (H. Taber, Department of Horticulture, Iowa State University, Ames, *personal communication*) were applied to our 1991 yields. Pruning the spray-inoculated plants further reduced the gross income to \$27,590 per hectare. Cultural practices that minimize epiphytic colonization by *C. m. michiganensis* (e.g., rotation and strict sanitation) and avoidance of pruning are likely to be prudent disease preventative measures in fresh-market tomato production.

Table 2. Area under the curve (AUC) for vascular colonization of tomato plants either spray-inoculated or not sprayed with *Clavibacter michiganensis* subsp. *michiganensis* and either pruned or nonpruned

Treatment	AUC ^{a,b}			
	1990		1991	
	Pruned	Nonpruned	Pruned	Nonpruned
Inoculated	5,078	4,000	4,564	2,820
Noninoculated	1,820	1,550	91	43
LSD ^c	626		211	

^a AUC was calculated as the percentage of 10-cm stem segments colonized. Means were calculated from five replicates with one plant per replicate.

^b Level of significance for the interaction (inoculation \times pruning) effect is $P = 0.1858$ and $P = 0.0001$ for 1990 and 1991, respectively.

^c Means within rows and columns within each year were compared with an F test.

Table 3. Yield (metric tons/ha) of fresh-market tomatoes either spray-inoculated or not sprayed with *Clavibacter michiganensis* subsp. *michiganensis* and either pruned or not pruned

Treatment	Yield ^{a,b}			
	1990		1991	
	Pruned	Nonpruned	Pruned	Nonpruned
Inoculated	55.3	64.9	46.8	82.0
Noninoculated	86.2	82.3	99.3	109.4
LSD ^c	10.6		10.5	

^a Means were calculated from five replicates with 10 plants per replicate.

^b Level of significance of interaction (inoculation \times pruning) was $P = 0.1929$ and $P = 0.0229$ in 1990 and 1991, respectively.

^c Means within rows and columns were compared within each year with an F test.

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