Characterization of Ohio Strains of *Xanthomonas campestris* pv. *vesicatoria*, Causal Agent of Bacterial Spot of Pepper

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ABSTRACT

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In 1993 and 1994, 105 samples from pepper (*Capsicum annuum*) plants exhibiting symptoms of bacterial spot were collected from commercial fields and home gardens throughout Ohio and neighboring states. Eighty-nine presumptive *Xanthomonas campestris* pv. *vesicatoria* strains were isolated on a semiselective medium and identified by a combination of biochemical tests and gas chromatographic analysis of fatty acid methyl esters (FAMEs). All 89 strains caused a hypersensitive response (HR) on tobacco (*Nicotiana tabacum* cv. Samsun), and 76 strains were virulent on the susceptible pepper cultivar Marengo. Most (89%) of these strains were also virulent on the susceptible processing tomato cultivar Ohio 8245. The majority of the strains (80%) were identified as pepper race 3. Among the remainder, 2.6, 12, and 4% were pepper races 0, 1, and 2, respectively. One strain was identified as pepper race 6. Of the 68 strains pathogenic on tomato, 96% (65 strains) were tomato race 1 and three strains were tomato race 2. Fifty-two of the 76 strains were nonamylolytic (Amy^-) and nonpectolytic (Pec^-); the remaining 24 strains, including two pectolytic (Pec^+) and 22 Pec^- strains, were amylolytic (Amy^+). Although the composition of fatty acids in amylolytic and nonamylolytic strains was similar, it differed between pathogenic *X. campestris* pv. *vesicatoria* strains and nonpathogenic *X. campestris* strains isolated from pepper. Six serogroups were identified among *X. campestris* pv. *vesicatoria* strains using monoclonal antibodies (MAbs) in an enzyme-linked immunosorbent assay. However, 39.5% of the strains did not react with any of the MAbs and could not be assigned to a serovar. Streptomycin-resistant strains (49%) were more prevalent than copper-resistant strains (13%).

Bacterial spot disease, caused by *Xanthomonas campestris* pv. *vesicatoria*, poses a threat to pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Miller) production in Ohio, especially under conditions of high temperature and abundant rainfall. The disease reduces plant growth, fruit yield, and quality.

Management of bacterial spot depends on a combination of practices including the use of "pathogen-free" seed and seedlings, sanitation, crop rotation, resistant cultivars, and chemical applications. Fixed copper compounds are commonly used to help manage the disease, although they are not highly effective under environmental conditions optimal for disease development or when high inoculum levels are present. Use of streptomycin on transplants is allowed in seedbeds, but usually not in greenhouses. Resistance in *X. campestris* pv. *vesicatoria* strains to both chemicals has been reported (2,23,31,32). This limits the efficacy of these compounds.

Three groups identified within *X. campestris* pv. *vesicatoria* are the tomato group (XcvT), only pathogenic on tomato; the pepper group (XcvP), only pathogenic on pepper; and the tomato-pepper group (XcvTP), pathogenic on both tomato and pepper (22). Different physiologic races are present in each group including three races (1, 2, and 3) within XcvT (designated T1, T2, and T3, respectively) and seven races (0, 1, 2, 3, 4, 5, and 6) within XcvP (designated P0, P1, P2, P3, P4, P5, and P6, respectively) (6,17,22,23,24). No commercial tomato cultivars with resistance to XcvT races have been developed up to now. Although a few pepper cultivars with single-gene resistance to one or more races of *X. campestris* pv. *vesicatoria* have been developed for commercial use, they are not widely grown in Ohio. A better understanding of the distribution of races of *X. campestris* pv. *vesicatoria* in Ohio and neighboring states and their resistance to copper and streptomycin would allow more effective bacterial spot management in pepper production.

X. campestris pv. *vesicatoria* strains have been divided into two different groups (A and B) based on a number of criteria including DNA homology, amylolytic and pectolytic activity, fatty acid (FA) composition, and serology (3,4,5,6,13,30). Recently, Vauterin et al. (36) proposed a new classification for *Xanthomonas* species to distinguish different groups among the pathovars of *X. campestris* on the basis of DNA-DNA hybridization and carbon utilization patterns. They suggested that the two previously described *X. campestris* pv. *vesicatoria* groups be separated into two different species: *X. axonopodis* pv. *vesicatoria* (group A strains) and *X. vesicatoria* (group B strains). These two groups were also recognized as distinct based on rep-polymerase chain reaction (PCR) fingerprint patterns, although two additional groups within *X. campestris* pv. *vesicatoria* were also identified (19).

The first objective of this study was to evaluate the variability among *X. campestris* pv. *vesicatoria* strains isolated in Ohio and neighboring states for taxonomic characteristics. The second was to determine the distribution of races of *X. campestris* pv. *vesicatoria* isolated in this region and to assess their sensitivity to copper and streptomycin. Strains of *X. campestris* pv. *vesicatoria* isolated from pepper in this work were identified and characterized according to race, sensitivity to copper and streptomycin, serology, amylolytic and pectolytic activity, and FA composition.

MATERIALS AND METHODS

Isolation of *X. campestris* pv. *vesicatoria* strains. During 1993 and 1994, 105 pepper samples exhibiting characteristic symptoms of bacterial spot were collected from commercial fields and home gardens primarily in Ohio, but also from Michigan, Illinois, and West Virginia. Bacteria were isolated from surface-disinfested diseased leaves by cutting small pieces of leaf tissue from the margins of spots with a sterilized razor blade. The leaf tissues were soaked in a test tube containing 2 ml of sterile water for 5 min. Loopfuls of the suspension were streaked onto CKTM semiselective medium (28,29) and yeast dextrose carbonate (YDC) medium (18). Plates were incubated at 28°C for 2 to 5 days. Representative round, convex, mucoid, yellow colonies on YDC, or small, yellow colonies surrounded by a clear ring on CKTM, were selected and purified by repeated restreaking on YDC. These presumptive *X. campestris* pv. *vesicatoria* cultures were preserved in sterile, distilled water at 15°C for short-term storage and in 30% glycerol at –80°C for long-term storage.

Identification of strains. Bacteria were identified by morphological observation, growth on CKTM, and biochemical and nutritional tests including the gram (KOH) reaction, catalase and oxidase activities,

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motility, oxygen requirement, and carbon utilization (9,27,34,35). Strains were grown on YDC for 24 h before testing and each test was repeated. All strains were suspended in sterile, distilled water after incubation on YDC and adjusted to a concentration of approximately 10^8 CFU/ml. The resulting suspension was infiltrated by inserting a syringe with a 27-gauge needle into the intercostal area of tobacco leaves (*Nicotiana tabacum* L. cv. Samsun) (16). The inoculated plants were incubated for 24 to 48 h under greenhouse conditions, and the results were recorded. All tests had three replications and were repeated once.

Strains causing a hypersensitive response (HR) in tobacco were tested for pathogenicity by atomizing healthy 4- to 5-week-old pepper (Marengo) and tomato (Ohio 8245) plants with bacterial suspensions (10^8 CFU/ml) in sterile, distilled water (12). Plants were incubated in polyethylene bags at 20 to 28°C for 3 days in a greenhouse, after which the bags were removed. Plants were observed 2 to 7 days later for the development of typical bacterial spot symptoms. Pathogenicity tests were repeated twice.

Race determination. Identification of races of *X. campestris* pv. *vesicatoria* strains was based on their interaction with differential pepper and tomato genotypes (6,7,8,14,15,16,17,22,23,24). *X. campestris* pv. *vesicatoria* strains were suspended in sterile, distilled water, adjusted to a concentration of approximately 10^8 CFU/ml, and infiltrated into fully expanded leaves of the pepper lines ECW, ECW-10R, ECW-20R, and ECW-30R, the tomato cultivars Ohio 8245 and Hawaii 7998, and *Lycopersicum pimpinnellifolium* (PI 128216). Plants were incubated for 48 h under greenhouse conditions as described above. HR was recorded within 24 to 48 h. Each strain was tested three times.

Physiological characterization. Starch hydrolysis and pectolytic activity were determined as described by Bouzar et al. (5). Sensitivity of *X. campestris* pv. *vesicatoria* strains to streptomycin sulfate (20, 100, 150, and 200 µg/ml) and copper sulfate (30, 100, and 150 µg/ml) was assayed as described (23). The tests were repeated at least twice with three replications per test.

Serotyping. Eight *X. campestris* pv. *vesicatoria*-specific monoclonal antibodies (MAbs) were used to serotype strains in indirect enzyme-linked immunosorbent assays (ELISAs) (6). MAbs Xv5, Xv6, Xv8, Xv10, and Xv30 were developed by Bouzar et al. (6), and Xv1, Xv15, and Xv21 were provided by A. Alvarez, Department of Plant Pathology, University of Hawaii, Hilo. Positive control strains obtained from H. Bouzar (Gulf Coast Research Center, University of Florida) and a carbonate buffer (negative control) were included in all tests. Absorbance was measured at 405 nm with a microplate El 309 autoreader (Bio-tek Instruments, Inc., Winooski, VT). Serovars were determined by grouping positive ($A_{405} \geq 2 \times$ the absorbance value of the negative control) and negative reactions with the eight MAbs. The test was repeated once.

Analysis of FAMEs. FAME analysis was performed according to the procedure previously described (21,25,26,33,38). Whole cell FAMEs were separated by gas chromatography (HP5898A; Hewlett-Packard Co., Palo Alto, CA). FAME profiles were identified using the Tripticase Soy Broth Agar (TSBA) database in the Microbial Identification System software package (MIS version 3.8, Microbial ID, Inc., Newark, DE). Pairwise comparison between mean percentages of selected FAs in strain groups were made using *t* tests.

RESULTS

Field survey and identification of the pathogen. Eighty-nine *X. campestris* strains were isolated from 105 pepper samples collected in 12 different counties in Ohio and in Michigan, Illinois, and West Virginia (Table 1). All test strains grew on CKTM medium and produced round, convex, mucoid, yellow colonies typical of *X. campestris* pv. *vesicatoria* on YDC. All 89 strains were gram negative, aerobic, motile, catalase positive, and oxidase negative and grew at 35°C on YDC. They all produced acid from arabinose, glucose, and mannose. All 89 strains were confirmed as *X. campestris* by FAME analysis. Similarity indices ranged from 0.454 to 0.747. All strains induced a HR on tobacco plants after 24 h. Seventy-six of the 89 strains were identified as *X. campestris* pv. *vesicatoria* by pathogenicity tests and caused typical bacterial spot symptoms on pepper seedlings 5 to 10 days after inoculation. Sixty-eight of these strains were also pathogenic on tomato. Thirteen strains did not produce typical leaf spots on either pepper or tomato.

Race determination. In 1993, *X. campestris* pv. *vesicatoria* P0, P1, P2, and P3 were detected in gardens and commercial fields in Ohio (Table 1). P3 was isolated from commercial fields in Michigan and Illinois. In Ohio, P3 was the predominant race. Of the 43 *X. campestris* pv. *vesicatoria* strains tested, two (5%) were P0, five (12%) were P1, two (5%) were P2, and 33 (77%) were P3. One strain was P6, a new race not previously described (22). Forty of 43 strains caused typical symptoms of

Table 1. Distribution of races of *Xanthomonas campestris* pv. *vesicatoria* isolated from pepper plants in Ohio and neighboring states in 1993 and 1994

Year isolated	Location	Total no. of strains	Race[a]								
			P1	P3	T1P0	T1P1	T1P2	T1P3	T1P6	T2P1	T2P3
	Ohio County										
1993	Coshocton	1	...	...	...	...	...	2	...	...	...
	Erie	2	...	...	...	...	...	...	...	1	...
	Franklin	26	...	2	2	...	1	20	...	...	1
	Henry	1	...	...	...	1	...	...	...	...	...
	Holmes	2	...	...	...	...	...	2	...	...	...
	Huron	2	...	...	...	...	...	1	1	...	...
	Muskingham	4	...	...	...	2	...	2	...	...	...
	Summit	1	...	...	...	...	1	...	...	...	...
1994	Coshocton	8	...	...	...	...	...	7	...	...	1
	Meigs	12	...	1	...	...	...	11	...	...	...
	Muskingham	1	...	1	...	...	...	...	...	...	...
	Sandusky	1	...	...	...	...	1	...	...	...	...
	Seneca	1	...	...	...	1	...	...	...	...	...
	Summit	4	...	...	...	3	...	1	...	...	...
	Wayne	3	...	...	...	...	...	3	...	...	...
	Other states										
1993	Michigan	1	...	...	...	...	...	1	...	...	...
	Illinois	3	1	...	...	...	...	2	...	...	...
1994	West Virginia	3	...	3	...	...	...	...	...	...	...
Total		76	1	7	2	7	3	52	1	1	2

[a] Number of strains of each *X. campestris* pv. *vesicatoria* race isolated from each location.

bacterial spot on Ohio 8245 tomato seedlings. Thirty-eight of these strains were T1 and two were T2 (Table 1). During 1994, 33 *X. campestris* pv. *vesicatoria* strains were isolated primarily from commercial fields in Ohio (Table 1). As in 1993, the majority (85%) of the strains were P3. Four strains (12%) were P1 and only one strain (3%) was P2. Most (85%) were pathogenic on tomato. The majority (96%) of these strains were T1 and only one strain was identified as T2.

Starch hydrolysis and pectate degradation. Fifty-two of the 76 pathogenic *X. campestris* pv. *vesicatoria* strains isolated were nonamylolytic (Amy^-), and all of these strains were also nonpectolytic (Pec^-). Twenty-four strains were amylolytic (Amy^+), of which two were also pectolytic (Pec^+) (Table 2). All of the nonpathogenic strains were Amy^+ and most were also Pec^+.

Serotyping. The *X. campestris* pv. *vesicatoria* pepper strains were grouped into six serovars based on reactivity with the MAbs Xv1, Xv5, Xv6, Xv10, Xv15, and Xv21 (Table 2). None of the strains reacted with MAb Xv30, which is specific for *X. campestris* pv. *vesicatoria* T3 strains (J. B. Jones, *personal communication*), or with MAb Xv8. Serotype was not correlated with amylolytic activity or race. The majority (58%) of the 52 Amy^- *X. campestris* pv. *vesicatoria* pepper strains did not react with any of the MAbs and could not be assigned to a serovar. The remaining 22 Amy^- and all 24 Amy^+ strains reacted with at least one of the eight MAbs. Most of these strains were assigned to serovars A1 or A2 based on positive reactions with MAbs Xv1, Xv5, Xv6, Xv10, and Xv21 (serovar A1), or Xv21 only (serovar A2). Reactivity among A1 serovar strains with Xv21 was variable as observed elsewhere (J. B. Jones, *personal communication*). Therefore, strains that reacted positively with MAbs Xv1, Xv5, Xv6, and Xv10 only were also assigned to serovar A1.

The 26 strains assigned to the A1 serovar comprised seven different races and two pathogenicity groups (P_ and T_P_; Table 2). All nine strains in serovar A2 were race T1P3. Only one *X. campestris* pv. *vesicatoria* strain was placed in each of the serovars A3 (T1P2) and B2 (T1P3). One T1P3 strain reacted with MAbs Xv1, Xv5, Xv6, Xv10, Xv15, and Xv21, and one T2P3 strain reacted with MAbs Xv1, Xv10, and Xv15. These were identified as new serovars designated serovar A4 and A5, respectively. None of the nonpathogenic *X. campestris* strains reacted with any of the MAbs tested in this study. Reactivity of MAb Xv6, not previously reported, was identical with that of MAb Xv10 for all *X. campestris* pv. *vesicatoria* strains tested, except for four T1P3 and one T2P3 strains.

FA composition. All test strains were identified by FAME analysis to species level as *X. campestris* or to pathovar level as *X. campestris* pv. *vesicatoria* based on the presence and concentration of at least 15 different FAs that included saturated, unsaturated, methyl-branched, hydroxy, and branched-chain hydroxy FAs. A set of 11 FAs comprised those most common for *X. campestris* pv. *vesicatoria* pepper strains, appearing in all strains with more than 2% of the total named peak area (Table 3). In general, the FA 15:0 iso was present in the highest concentration (21%) followed by 16:1 ω7c (20%), 15:0 anteiso (14%), iso 17:1 ω9c (7%), 17:0 iso (6%), 16:0 (4%), and 11:0 iso (4%). There were no differences between *X. campestris* pv. *vesicatoria* strains and nonpathogenic *X. campestris* strains isolated from pepper in the amount of most FAs present (Table 3). However, there were significantly (P = 0.05) higher levels of the FAs iso 17:1 ω9c and 17:0 iso in *X. campestris* pv. *vesicatoria* strains than in nonpathogenic *X. campestris* strains. In *X. campestris* pv. *vesicatoria* strains, the ratio of 15:0 anteiso to (iso 17:1 ω9c + 17:1 iso) was near 1, while the ratio of 15:0 iso to 15:0 anteiso was 1.5. Within nonpathogenic *X. campestris* strains, the latter ratio was more than 2.0. Significant differences in FA concentrations were not observed when strains were grouped according to race or amylolytic activity.

Sensitivity to streptomycin and copper. Resistance to streptomycin was widespread among strains of *X. campestris* pv. *vesicatoria* isolated and identified in our study (Table 4). Of the 43 *X. campestris* pv. *vesicatoria* strains isolated in 1993, 27 (63%) grew on sucrose peptone agar (SPA) amended with 20 µg/ml of streptomycin sulfate, 42% were resistant to 100 µg/ml of streptomycin sulfate, and 12% were resistant to 150 µg/ml of streptomycin sulfate. No strains were resistant to 200 µg/ml of streptomycin sulfate. All strains were sensitive to copper sulfate at a concentration of 150 µg/ml. Only eight strains (19%) were moderately resistant, growing on SPA amended with 100 µg/ml of copper sulfate. One strain was weakly resistant, growing on a low concentration of copper sulfate (30 µg/ml). Six strains were both copper- and streptomycin-resistant (Table 4).

In 1994, 97% of the 33 *X. campestris* pv. *vesicatoria* strains collected were sensitive to copper sulfate. One strain, which was also highly resistant to streptomycin sulfate, was moderately resistant to copper sulfate. Fifteen percent of the strains were weakly resistant, 9% were moderately resistant, and 6% were highly resistant to streptomycin sulfate (Table 4).

All strains of P2 were resistant to copper and streptomycin. Forty-four percent of the P1 and 58% of the P3 strains were sensitive to copper, streptomycin sulfate, or both (Table 4).

DISCUSSION

Although T1P3 was the most common race detected among *X. campestris* pv. *vesicatoria* strains isolated in Ohio, races T2, P0, P1, and P2 were found as well. Only pepper race 3 (T1P3 and P3) strains were isolated from samples collected in Michigan, Illinois, and West Virginia. However, all of the strains from these three states were isolated from commercial pepper cultivars with resistance to *X. campestris* pv. *vesicatoria* race P1 and P2. Pepper cultivars resistant to any of the *X. campes-*

Table 2. Grouping of *Xanthomonas campestris* pv. *vesicatoria* strains isolated from pepper based on amylolytic activity and serological reaction

No. of strains	Race	Amylolytic activity[a] –	Amylolytic activity[a] +	Serological group[b]
1	P1	1	...	A1
1	P3	...	1	A1
1	T1P2	...	1	A1
17	T1P3	5	12	A1
1	T2P1	...	1	A1
1	T1P0	...	1	A1
4	T1P3	...	4	A1
1	T1P3	...	1	A2
8	T1P3	5	3	A2
1	T1P2	1	...	A3
1	T1P3	1	...	B2
1	T1P3	1	...	A4
1	T2P3	1	...	A5
6	P3	6	...	*[c]
1	T1P0	1	...	*
21	T1P3	21	...	*
1	T1P6	1	...	*
1	T2P3	1	...	*
76		52	24	

[a] Grouping based on amylolytic activity; – = nonamylolytic and + = amylolytic strains.
[b] Serological groups based on indirect enzyme-linked immunosorbent assay with the monoclonal antibodies (MAbs) Xv1, Xv5, Xv6, Xv8, Xv10, Xv15, Xv21, and Xv30.
[c] * = strains that did not react with any MAbs.

Table 3. Composition of fatty acids in 76 strains of *Xanthomonas campestris* pv. *vesicatoria* and 13 nonpathogenic *X. campestris* strains isolated from pepper

Fatty acids	Fatty acid concentration (%) Pathogenic strains	Fatty acid concentration (%) Nonpathogenic strains
11:0 iso	4.10 ± 0.91	5.32 ± 1.40
12:0 3-OH	3.04 ± 0.61	4.30 ± 1.03
13:0 iso 3-OH	3.86 ± 0.77	3.81 ± 0.82
15:0 iso	21.0 ± 2.48	21.5 ± 2.37
15:0 anteiso	13.9 ± 1.58	13.7 ± 1.17
16:0 iso	2.76 ± 0.81	2.34 ± 0.50
16:1 *w*9c	2.28 ± 0.45	3.10 ± 0.49
16:1 *w*7c	20.4 ± 2.4	17.5 ± 2.11
16:0	4.30 ± 0.96	4.39 ± 1.01
iso 17:1 *w*9c	6.61 ± 0.67*[a]	3.18 ± 0.69*
17:0 iso	5.71 ± 1.04*	3.60 ± 0.73*

[a] * = a statistically significant difference (P = 0.05) between pathogenic and nonpathogenic strains for each fatty acid (t test).

tris pv. *vesicatoria* races have not been grown widely in Ohio, and none of the pepper cultivars sampled contained *X. campestris* pv. *vesicatoria* resistance genes. Therefore, no particular race would be expected to predominate, yet P3 was clearly the most common race in both 1993 and 1994. In Ohio, pepper and tomato transplants are grown locally in greenhouses and also imported from southern states. However, the seed is imported from many regions of the world, principally from Asia. The prevalence of P3 in Ohio may be the result of distribution of seed or transplants contaminated mostly with P3. In addition, P3 may be better adapted for survival on pepper and tomato than other *X. campestris* pv. *vesicatoria* races or may survive better as an epiphyte or pathogen on weed hosts.

Nearly all (90%) *X. campestris* pv. *vesicatoria* strains pathogenic on pepper were also pathogenic on tomato. Race T1 was identified more often than T2, which is not surprising since T1 was the only tomato race known to exist in North America until 1990. T2 was reported to be prevalent in South America (6,10,37), but has apparently become more widely distributed recently. Race T3, identified recently in Florida (10,15), was found for the first time in Ohio on processing tomatoes in 1995 (F. Sahin and S. A. Miller, *unpublished data*).

One of the pepper strains isolated in this study from a commercial field in Huron County, OH, in 1993, did not produce HR on any of the pepper *X. campestris* pv. *vesicatoria* race-differential lines. It was highly aggressive on the susceptible pepper cultivar Marengo, was pathogenic on tomato, and produced HR on cultivar Hawaii 7998, characteristic of T1. This strain (*X. campestris* pv. *vesicatoria* 17b) represents a new, previously undescribed race that has been designated T1P6 (24).

The previously reported correlation between race and amylolytic activity was not observed among the pepper strains isolated in this study. In their study of a worldwide collection of *X. campestris* pv. *vesicatoria* strains from pepper and tomato, Bouzar et al. (5,6) found that T2 and T2P3 strains were almost always Amy^+, whereas the other tomato and pepper races were Amy^-. In addition, all of the *X. campestris* pv. *vesicatoria* T2 strains isolated in Ohio from processing tomatoes in 1994 and 1995 were also Amy^+ (F. Sahin and S. A. Miller, *unpublished data*). However, in this study of *X. campestris* pv. *vesicatoria* pepper strains, both of the T2P3 strains were Amy^-, whereas 66% of the 65 race T1P_ strains were Amy^+ (Table 1). The lack of correlation between the ability to degrade starch and pathogenicity of *X. campestris* pv. *vesicatoria* strains may be related to the source of the strains. In their study, most of the amylolytic *X. campestris* pv. *vesicatoria* strains were recovered from tomatoes and were not pathogenic on pepper. However, in this work, all of the *X. campestris* pv. *vesicatoria* strains were isolated from pepper, and most of them were pathogenic on both hosts. Of the eight strains isolated that were pathogenic on pepper only, seven were Amy^-. These data confirm the observation (6) that most of the strains pathogenic on pepper, not tomato, are Amy^-. Other tests such as rep-PCR (19), DNA-DNA hybridization (30,36), or silver staining of protein profiles (5) are needed to determine to which group (A or B) these Amy^+ T1 strains belong.

The *X. campestris* pv. *vesicatoria* strains that reacted positively in indirect ELISA with at least one of the eight MAbs tested were divided into six serological groups (Table 2). Bouzar et al. (6) had previously identified six serovars in their collection of *X. campestris* pv. *vesicatoria* strains by using six MAbs: Xv1 and Xv5, which reacted only with group A strains; Xv8, which reacted with group B strains as well as a few strains in other taxa; Xv15, which reacted only with some group B strains; Xv21, which reacted primarily with group A strains; and Xv10, which reacted with some but not all strains in both groups as well as other taxa. In our study, six serological groups, of which A4 and A5 were newly constructed, defined the reactivity of nearly half of our strains with these and two additional MAbs, Xv6 and Xv30. Three of the 52 Amy^- strains reacted positively with MAb Xv15, and all of the 24 Amy^+ strains reacted positively with MAbs Xv1, Xv21, or both. There were no strains in our collection that reacted with MAb Xv8. All Amy^+ strains could be assigned to a serovar, but 50 of the 52 Amy^- *X. campestris* pv. *vesicatoria* strains did not react with any of the MAbs tested. Clearly, additional MAbs are needed to detect all Amy^- *X. campestris* pv. *vesicatoria* strains and to distinguish *X. campestris* pv. *vesicatoria* strains from other pathovars of *X. campestris*. None of the strains reacted with MAb Xv30, which is specific to *X. campestris* pv. *vesicatoria* T3. This result was confirmed by race determination tests on PI 128216, in which none of the *X. campestris* pv. *vesicatoria* strains induced a HR.

FAME analysis was an easy, accurate method for identification of *X. campestris* strains. FAME profiles consistently identified *Xanthomonas* strains to the species level, but only identified them to the pathovar level 50% of the time. Yang et al. (38) suggested that many strains in the genus *Xanthomonas* may not be correctly identified by the commercial TSBA database, which was constructed based on the FA composition of 24-h bacterial cultures. Many *Xanthomonas* strains grew too slowly on Tripticase soy agar (TSA) medium to be analyzed after a 24-h incubation period and, generally, FAME composition in *Xanthomonas* was found to be more stable at 48 h than at 24 h. They constructed a new database based on the FA composition of 48-h cultures. In our study, 48-h-old cultures were used for FAME analysis because of the slow growth of *X. campestris* pv. *vesicatoria* strains, but identification of the strains was performed using the commercial TSBA database since the reconstructed database for *Xanthomonas* was not available.

Analysis of FAMEs was very useful for distinguishing *X. campestris* pv. *vesicatoria* strains from nonpathogenic *X. campestris* strains isolated from pepper. This work confirmed the results of previous studies (6,12,25,26,38) that indicated that the FAs 15:0 iso (21%), 16:1 ω7c (20%), and 15:0 anteiso (14%) predominate in *Xanthomonas* strains. The ratio of 15:0 anteiso to (iso 17:1 ω9c + 17:0 iso) in pathogenic *X. campestris* pv. *vesicatoria* strains was 1:1, whereas in nonpathogenic *X. campestris* strains, the ratio was more than 2:1 (Table 3).

During this 2-year survey, none of the *X. campestris* pv. *vesicatoria* strains isolated were resistant to a high concentration of

Table 4. Sensitivity to copper and streptomycin sulfate among *Xanthomonas campestris* pv. *vesicatoria* strains determined by growth on amended sucrose-peptone agar medium

			Number of *X. campestris* pv. *vesicatoria* strains resistant to						
			Copper sulfate (μg/ml)			Streptomycin sulfate (μg/ml)			
Year	Race	Total no. of strains	30	100	150	20	100	150	200
1993	P1	1	1	1	...	...	...	...	...
	P3	2	...	...	...	1	...	...	...
	T1P0	2	...	...	...	...	...	...	...
	T1P1	3	2	2	...	2	2	1	...
	T1P2	2	2	1	...	2	2	...	...
	T1P3	30	4	4	...	20	12	3	...
	T1P6	1	...	...	...	1	1	...	...
	T2P1	1	...	...	...	1	1	1	...
	T2P3	1	...	...	...	...	...	...	...
1994	P3	5	...	...	...	2	...	...	...
	T1P1	4	...	...	...	1	1	...	...
	T1P2	1	1	1	...	1	1	1	1
	T1P3	22	...	...	...	6	3	1	...
	T2P3	1	...	...	...	...	...	...	...
		76	10	9	...	37	23	7	1

copper sulfate, and only 12% of the strains were moderately resistant. Also, far fewer copper-resistant strains were isolated in 1994 (one strain) than in 1993 (nine strains). However, resistance in *X. campestris* pv. *vesicatoria* strains to streptomycin was prevalent in Ohio. Forty-nine percent of the *X. campestris* pv. *vesicatoria* strains were resistant to streptomycin sulfate. The source of streptomycin resistance may be from commercial pepper transplants grown in greenhouses or seedbeds in which streptomycin is applied to prevent occurrence of bacterial spot. Copper resistance among the *X. campestris* pv. *vesicatoria* strains in Ohio is less common than in other pepper growing areas including Georgia, Florida, North Carolina, and Mexico (1,11,20,23). Pepper growers in Ohio probably apply less copper sulfate than growers in other states since environmental conditions, although often favorable for bacterial spot development, are not as conducive to disease as they are in the other climates. These data also confirmed the linkage between P2 and copper resistance reported by Stall et al. (31) and others (7,20). In this study, only three P2 strains were isolated, all of which were resistant to copper. Slightly less than half (48%) of the 61 P3 strains isolated in 1993 and 1994 were streptomycin resistant and only four strains (7%) were copper resistant (Table 4). This was in contrast to reports by Ritchie and Dittapongpitch (23) and Buonaurio et al. (7) in which no P3 strains were resistant to copper and streptomycin sulfate.

The most convenient method for control of bacterial spot in pepper and tomato is the use of *X. campestris* pv. *vesicatoria*-resistant cultivars. However, there are no commercial pepper cultivars with resistance to all seven *X. campestris* pv. *vesicatoria* pepper races (P0 through P6), especially the newly designated P6, which would be compatible with any cultivar containing currently known resistance genes. However, *X. campestris* pv. *vesicatoria* P6 is not yet widely distributed. Strategic use of cultivars containing available resistance genes, especially *Bs2* (providing resistance to P0, P1, P2, and P3), should be useful as part of a disease management program. The low frequency of isolation of copper-resistant *X. campestris* pv. *vesicatoria* strains in Ohio supports the use of fixed copper materials as an alternative control method for bacterial spot. However, the application of such materials should not be the major or only control method used. Overuse of copper in Ohio would be likely to result in an increase in the population of copper-resistant *X. campestris* pv. *vesicatoria* strains. Thus, the use of a multi-faceted program that consists of copper application, use of pathogen-free seed and seedlings, resistant cultivars, and crop rotation is necessary for optimal disease management.

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