

Sudden Shift in the Prevalent Race of *Xanthomonas campestris* pv. *vesicatoria* in Pepper Fields in Southern Florida

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ABSTRACT

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In the spring of 1988, 89% of 97 strains of *Xanthomonas campestris* pv. *vesicatoria* isolated from naturally infected pepper plants in commercial fields in southern Florida were race 2 and 11% were race 3. During the 1989-1990 season, however, 84% of 118 strains isolated were race 1, 15% were race 2, and 1% were race 3. Race 1 was predominant again during 1990-1991, with 68% of 100 strains identified as race 1, 13% as race 2, 11% as race 3, and 8% as the tomato race of *X. c. vesicatoria*. Four of 97 and four of 118 strains were found to be resistant to 200 µg/ml of streptomycin in 1988 and 1989-1990, respectively. During 1990-1991, streptomycin resistance was recorded for 26 of the 100 strains collected. Copper tolerance was detected for 114 of the 118 strains during 1989-1990. Possible reasons for these shifts in populations of *X. c. vesicatoria* are discussed.

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doi) Dye, is generally considered to be the most serious disease of pepper (*Capsicum annuum* L.) in southern Florida (15). Control of bacterial spot is difficult when warm, rainy weather persists. Copper tolerance is widespread among strains of *X. c. vesicatoria* (1,14), leading to reduced efficacy of copper sprays in the field. Additionally, the use of the fungicide maneb with copper bactericides for enhanced control is no longer possible (4) because maneb is no longer labeled for pepper.

Single-gene resistances to bacterial spot have been discovered and bred into large-fruited, horticulturally desirable lines of pepper. These resistant lines have never been planted commercially because of the existence of races of the pathogen that could potentially overcome the resistance in each of the cultivars (10). In 1989, one company (Pepper Research, Inc., Belle Glade, FL) released two large-fruited cultivars that had resistance to race 2 of the pathogen. In November 1989, symptoms of bacterial spot were noted in commercial plantings of those

cultivars, and a severe epidemic of bacterial spot developed throughout the growing area on all cultivars.

Strains of race 2, but not race 1, of *X. c. vesicatoria* were widely distributed in Florida, and cultures of the bacterium collected as far back as 1960 were predominantly race 2 (6). Cultures of race 1 seldom were obtained. In the fall of 1989, however, race 1 strains routinely were obtained from symptomatic plants. Objectives of this study were to determine the relative abundance of the races attacking pepper in southern Florida and to compare results with a preliminary survey conducted in the spring of 1988.

MATERIALS AND METHODS

Survey design. Samples of diseased leaves were collected in all three growing seasons in Palm Beach and Collier counties during 1989-1990 and 1990-1991. Additionally, several strains were collected in Lee County during 1990-1991. These counties produce 70% of the state's peppers from November through March (2). Collection sites were commercial farms where bacterial spot was known to occur. Specific fields were selected to ensure that the most commonly grown pepper cultivars were included in the survey. Samples were obtained from three fields in 1988 in southern Palm Beach County (Delray Beach/Boca Raton). During 1989-1990, a total of 14 geographically distinct fields were sampled—nine in southern Palm Beach County, three in Collier County

(Immokalee/Naples), and two in northern Palm Beach County (Jupiter). Thirteen fields were included in the 1990-1991 survey, seven in southern Palm Beach County, four in Collier County, and two in Lee County (North Fort Myers).

At all locations, pepper plants were grown on raised, plastic-mulched beds. Typically, fields were planted as a series of 10-12 beds separated by windbreaks. Rows were on 2-m centers and ranged from 100 to 170 m in length. A random numbers table was used to select a row to be sampled in a bed series. Beginning from one of the ends of the row or from a service path within the field, a random number of strides (one to 99) were taken to determine the sample site. If diseased plants were not found in the immediate vicinity of this site, workers kept walking slowly in the same direction until bacterial spot was found. Each sample consisted of three to four diseased leaves. Six to 12 sites were chosen per field, depending on field size. Preference was given to leaves with recently developed lesions as characterized by abundant water-soaking on abaxial surfaces. Leaves were placed in plastic bags, sealed, and transported to the laboratory in an ice chest. Samples were processed within 24 hr.

Pathogen isolation. The isolation technique used was modified from Goth (7). The circular end of a metal bacterial loop was pulled open with a forceps to form a short right-angle bend. The wire then was pushed through a leaf sample at the junction of healthy and diseased tissue. Without withdrawing the loop, the infested wire was S-streaked across duplicate plates of nutrient agar (8 g of nutrient broth, 1 g of yeast extract, and 17 g of agar per liter of distilled water adjusted to pH 7.0 before autoclaving). In most cases, 5 ml of a 1% alcoholic solution of cycloheximide was added per liter of sterilized nutrient agar cooled to 55 C. Plates were incubated at 28 C for 3 days. Colonies characteristic of *X. c. vesicatoria* were purified by restreaking on nutrient agar.

Pathogen identification. Presumptive cultures of *X. c. vesicatoria* were streaked on yeast extract-glucose-calcium carbonate agar (YGC) (16) and incubated at 28 C for 3 days. Bright yellow, mucoid colonies were saved for further testing.

Bioassay was used to confirm the identification of strains. Cultures were grown on nutrient agar containing 0.5% (w/v) glucose for 3 days at 28 C. Plates were flooded with sterile buffered saline solution (13), and a bent glass rod was used to suspend the bacteria. Subsequent suspensions were adjusted turbidimetrically to about 2×10^8 cfu/ml. Two 10-fold dilutions in sterile saline buffer were used to make suspensions of 10^6 cfu/ml.

A 10-ml tuberculin syringe without the needle was used to infiltrate test suspensions into leaves of greenhouse-grown pepper plants, cv. Jupiter, in the three- to four-leaf stage. The blunt end of the tube was appressed to the abaxial surface and suspensions were forced into the leaf until abundant water-soaking was evident. Two sites were inoculated per strain on each of two leaves. Test plants immediately were covered with plastic bags and placed in an air-conditioned greenhouse with a temperature range of 23–28 C. After 4 days, bags were removed. Strains were scored as pathogenic if water-soaking was still evident at sites of inoculation. Identification of strains as *X. c. vesicatoria* was confirmed by observing plants for development of typical bacterial spot lesions 7–9 days later. Cultures identified as *X. c. vesicatoria* were maintained in sterile 15% aqueous glycerol at –70 C (17). Working cultures were kept up to 1 mo on YGC slants at 4 C.

Race determination. To identify the race of each of the *X. c. vesicatoria* cultures, bacterial suspensions in sterile tap water (3×10^8 cfu/ml) of test strains were infiltrated into leaves of differential cultivars. Cultivars used were Early Calwonder (ECW) and three near-isogenic lines with genes for vertical resistance to specific pathogenic races (9). Disease reactions were as follows: ECW, susceptible to all 3 races; ECW-10R, susceptible to races 1 and 3, hypersensitive (HR) response to race 2; ECW-20R, HR for all three races; and ECW-30R, HR for race 1, susceptible to races 2 and 3.

Sensitivity to streptomycin and copper. Sensitivity of all strains to streptomycin was assayed qualitatively by comparing growth of strains streaked on nutrient agar and nutrient agar amended with 200 μ g/ml of streptomycin (18,20). Pathogen strains from the 1989–1990 survey were tested in vitro for sensitivity to 200 μ g/ml of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ using the methods of Stall et al (19).

RESULTS

In 1988, race 2 of *X. c. vesicatoria* was predominant among strains recovered, accounting for 89% of the 97 strains

recovered. The remainder of the strains were race 3; no race 1 strains were recovered. In contrast, during 1989–1990, 84% of the 118 strains collected were race 1 (Table 1). Similarly, during 1990–1991, 68% of 100 strains were identified as race 1 (Table 2). Race 2 was much less common in the latter two seasons. Only one strain of race 3 was recovered during 1989–1990. Similar rates of recovery (11%) were recorded for race 3 in 1988 and 1990–1991.

In the 1988 survey, no relationship was found between recovery rates for races and the three cultivars represented (Yolo Wonder, a Yolo Wonder hybrid, and Ssupersweet 860). During 1989–1990, race 1 was the prevalent race on all major cultivars grown except Ranger (Table 1). All eight of the strains recovered from Ranger were identified as race 2. These were collected from the same farm in Collier County on the same sampling date.

Some association between races and specific cultivars was noted during 1990–1991 (Table 2). All six strains from Cubanelle and 90% of the 30 strains from Ssupersweet 860 were identified as race 1. The six strains from Capistrano and Orabelle were race 2. As expected, only race 1 and race 3 strains were recovered from race 2-resistant PR 892 and PR 893. Each of the eight strains from Galaxy

were identified as a tomato strain of *X. c. vesicatoria*.

Resistance to streptomycin was generally low in all locations throughout the study. Four of 97 and four of 118 strains were resistant to streptomycin in 1988 and 1989–1990, respectively. Although resistance was still not widespread during 1990–1991, 26 of the 100 were resistant to 200 μ g/ml of streptomycin. This was about a sevenfold increase over the two previous seasons. Tolerance to copper was recorded frequently. Of the 118 strains collected during 1989–1990, 114 were tolerant to 200 μ g/ml of $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ incorporated into nutrient agar.

DISCUSSION

In past surveys, Florida was one of the few major pepper-producing areas of the world where race 2 was more prevalent than race 1 (6). Our data showed that this trend continued through the spring of 1988. The trend, however, was not evident in subsequent seasons. Of the strains collected from 1989 through 1991, a population shift had occurred from race 2 to race 1 of *X. c. vesicatoria* in Florida. Because strains of *X. c. vesicatoria* are endemic in the state (11), race 1 could continue as a threat to production in Florida. We found little change in the relative abundance of race 1 strains in two consecutive cropping seasons.

Table 1. Races of *Xanthomonas campestris* pv. *vesicatoria* recovered from seven cultivars in a survey of commercial pepper farms in southern Florida during the 1989–1990 season^a

Cultivar	Number of strains	Percentage of strains recovered identified as		
		Race 1	Race 2	Race 3
Jupiter	54	92	6	2
Early Calwonder	11	91	9	0
PR 892 and 893 ^b	23	100	0	0
Ssupersweet 860	16	75	25	0
Galaxy	4	100	0	0
Ranger	8	0	100	0
Cubanelle	2	100	0	0
Total	118	84	15	1

^aBacteria recovered from 14 geographically distinct fields in Palm Beach and Collier counties.

^bTwo genotypes with the *Bsl* gene for resistance to *X. c. vesicatoria* race 2.

Table 2. Races of *Xanthomonas campestris* pv. *vesicatoria* recovered from eight cultivars in a survey of commercial pepper farms in southern Florida during the 1990–91 season^a

Cultivar	Number of strains	Percentage of strains recovered identified as			
		Race 1	Race 2	Race 3	Tomato strain
Jupiter	37	70	19	11	0
Early Calwonder	4	100	0	0	0
PR 892 and 893 ^b	9	56	0	44	0
Ssupersweet 860	30	90	0	10	0
Cubanelle	6	100	0	0	0
Capistrano	4	0	100	0	0
Orabelle	2	0	100	0	0
Galaxy	8	0	0	0	100
Total	100	68	13	11	8

^aBacteria recovered from 13 geographically distinct fields in Palm Beach, Collier, and Lee counties.

^bTwo genotypes with the *Bsl* gene for resistance to *X. c. vesicatoria* race 2.

The shift to a predominantly race 1 pathotype has hampered efforts to manage bacterial spot by host-plant resistance. The commercial cultivars PR 892 and PR 893, with hypersensitive resistance to race 2, were heavily damaged in several fields during 1989–1990 and 1990–1991. Cultivars with resistance to all three races of *X. c. vesicatoria* may be necessary for adequate disease management.

It is interesting to consider the possible sources of the race 1 inoculum. It has been suggested that the linkage of an avirulence gene that determined race 2 with a gene cluster for tolerance to copper on a self-transmissible plasmid was a possible reason for a predominance of race 2 in Florida before the 1989–1990 season (19). Genes for copper tolerance are not present on the plasmid containing the avirulence gene that determines race 1 (19). Strains of race 1 collected in Florida before 1989–1990 tended to be copper sensitive (6). Possibly, populations of race 1 remained low because of the judicious use of copper bactericides in pepper fields. Presence of a high incidence of copper tolerance among the strains of race 1, as shown in the 1989–1990 survey, may allow the survival and continued presence of race 1 in the pepper production areas. It is not known if the copper tolerance in these race 1 strains is genetically similar to the copper tolerance in strains of race 2.

The sudden shift of prevalent races in Florida occurred in widely separated geographic areas within the state. Populations of race 1 strains possibly were selectively increased by the race 2-resistant cultivars planted during 1989–1990, and these strains were then spread throughout the pepper-growing areas. However, those cultivars constituted less than 5% of the total hectareage and probably were not a factor in the development of the shift in races. Another possibility is the introduction of copper-tolerant race 1 strains with seed. Hybrid pepper seed used in the United States may be produced in Taiwan. Race 1 strains were recently reported to be the predominant race from pepper in Taiwan (8). The percent breakdown of races in Taiwan was similar to that found in Florida in the past two cropping seasons. Evidence exists that *X. c. vesicatoria* can be transmitted at low frequencies in pepper seed

(3,11). Better methods for detection of the pathogen in seed are needed to further explore this possibility.

The recovery of tomato strains of *X. c. vesicatoria* from Galaxy pepper plants was not expected. Tomato strains are not considered to be pathogens of pepper (5). This particular planting was surrounded by tomato fields with bacterial spot. The appearance of symptoms of bacterial spot on the peppers may represent one type of hypersensitive response (5). In greenhouse tests, tomato strains produced a hypersensitive (nonpathogenic) response in Galaxy pepper (*data not shown*). Therefore, the tomato strains recovered from Galaxy were probably the result of a hypersensitive response from heavy doses of bacteria from adjacent tomato fields.

The majority of strains of each race recovered during 1989–1990 were tolerant to copper, supporting earlier reports of widespread copper tolerance in field populations of *X. c. vesicatoria* (1,14). The majority of strains were sensitive to streptomycin, although a sharp increase in resistance was noted between the 1989–1990 and 1990–1991 seasons. The use of streptomycin has been minimal in the pepper industry (15,18). However, loss of maneb labeling in 1989 has seriously affected growers' ability to control bacterial spot. With few new options available (12), growers may be using streptomycin more frequently. The increase in resistance to streptomycin may be a reflection of increased industry use of the antibiotic and the rapid selection of resistant strains (20).

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