Bacterial Spot of Pepper and Tomato in Barbados

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ABSTRACT

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Bacterial spot of pepper and tomato, caused by *Xanthomonas campestris* pv. *vesicatoria*, is widespread and severe in Barbados. The bacterium was isolated from infected plants and seeds of six of 24 commercial pepper and tomato cultivars. When the bacterial strains were tested for pathogenicity, races 1–3 were detected, with race 1 strains two to nine times more abundant than races 2 and 3. Strains from seeds were commonly race 1 or nonpathogenic, and some tomato strains were virulent on the tomato cultivar Hawaii 7998. Of the strains tested for sensitivity to bactericides, 61, 64 and 47% were resistant to copper, zinc, and streptomycin, respectively.

Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is an important disease of pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.). Three groups of the bacterium, namely, the tomato group, the pepper group, and the pepper-tomato group, are usually associated with the disease (12,13). The tomato group is virulent only on tomato, the pepper group is virulent only on pepper, and both pepper and tomato are susceptible to the pepper-tomato group. Within the pepper and pepper-tomato groups, races 1, 2, and 3 are distinguished on the basis of virulence on differential pepper lines. Single dominant genes, *Bs1*, *Bs2*, and *Bs3* from pepper lines PI 163192, PI 260435, and PI 271322, respectively, confer disease resistance (9). Genes *Bs3* and *Bs1* confer resistance to race 1 and race 2, respectively, whereas gene *Bs2* restricts races 1, 2, and 3. Varying levels of resistance to bacterial spot have also been reported in the tomato cultivars Campbell 28, Ohio 4013-3, Ohio 4014-4, Heinz 1568-F3, and Hawaii 7998 (16). The latter, in particular, has shown high levels of resistance to strains of *X. c. vesicatoria* from Florida.

Chemical control of bacterial spot in Barbados and elsewhere has relied on sprays of copper bactericides, often used in conjunction with maneb, zineb, mancozeb, or streptomycin (1,4,11,14, 17,18). The bactericides have given inadequate control, and resistance in the pathogen to zinc (1), copper (1,10,14), and streptomycin (11,14) has been reported.

The present study reports on the severity of bacterial spot in Barbados and on variations in the pathogen population with respect to pathogenicity and sensitivity to bactericides.

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MATERIALS AND METHODS

Field survey. Seventy-two pepper and tomato farms in Barbados were surveyed for incidence and severity of bacterial spot. Disease severity was assessed as the proportion of the area of four matured leaves of the upper third of the foliage of each plant with symptoms of bacterial spot in a random sample of 0.05–0.10% of the plants of each farm. The survey was started when the plants were 4–6 wk old and continued thereafter at 14-day intervals during the dry season (December to May) and rainy season (June to November) of 1988–1989. Data were pooled to obtain the mean disease severity for each season.

Isolation and identification of the pathogen. Leaves and fruits of tomato and pepper with typical symptoms were surface-sterilized by dipping in 95% ethanol for 2 sec, followed by a 10-sec rinse in 1.25% sodium hypochlorite. Lesions were then excised and crushed in 200 µl of sterile distilled water, and the suspension was streaked onto nutrient yeast extract glycerol agar (NYGA) for isolation of *X. c. vesicatoria*. Isolations from imported pepper and tomato seeds were made by suspending 1 g of seeds in 100 ml of sterile distilled water for 16 hr, shaking the suspension at 90 oscillations per minute for 15 min, then plating 100-µl aliquots of the suspension liquid onto NYGA (20) and incubating them at 25–28 C.

Several standard determinative tests for *Xanthomonas* were used to characterize the bacteria, including cell morphology, Gram reaction, flagellation, catalase, oxidase, nitrate reductase, acid production from sugars, and utilization of asparagine as a sole source of carbon and nitrogen (15).

Pathogen and host culture. NYGA was used routinely for culture of the pathogen. The tomato cultivars Walter and Hawaii 7998 and the pepper lines Early Calwonder (ECW), ECW 10R, ECW 20R, and ECW 30R were used as potential hosts. Seeds of Hawaii 7998 and the pepper hosts were gifts from J. B. Jones (Gulf Coast Research and Education Center, University of Florida, Bradenton). Plants were grown in sterilized potting compost placed in a PGW-108 plant growth chamber set at 25–28 C, 85–95% relative humidity, and a 12-hr photoperiod provided by fluorescent lamps of total light intensity of 510 $\mu E \cdot s^{-1} \cdot m^{-2}$.

Host inoculation and race classification. A differential set of host plants was used to classify *X. c. vesicatoria* into groups and races as described by Minsavage et al (12). Walter tomato and ECW pepper were used to distinguish groups, and ECW 10R, ECW 20R, and ECW 30R were used to differentiate races. A random sample of 10 tomato strains of the bacterium from Barbados was also tested for virulence on Hawaii 7998. All plants were inoculated when 4 wk old by infiltrating the intercellular spaces of fully expanded leaves completely with a bacterial suspension (10^8 to 10^9 cells per milliliter) using a syringe without the needle (19). All treated plants were maintained in a humid chamber under the host culture conditions described above and observed for symptoms. Resistance in pepper and tomato to *X. c. vesicatoria* was associated with the hypersensitive response, indicated by leaf collapse 18–36 hr after inoculation, and the susceptible response, indicated by water-soaked lesions after 3–5 days.

Sensitivity of pathogen to bactericides. Two hundred forty strains of *X. c. vesicatoria* were streaked onto NYGA amended with streptomycin sulfate (250 µg/ml), $CuSO_4 \cdot 5H_2O$ (200 µg/ml), or $ZnSO_4 \cdot 7H_2O$ (100 µg/ml). Bacteria with confluent growth on the respective amended media after 3–5 days of incubation at 25–28 C were considered resistant to streptomycin, copper, or zinc and those that failed to grow were considered sensitive to the bactericides.

RESULTS

Field survey. Bacterial spot was present on all farms surveyed and varied in severity on pepper from 0.01 to 0.08 in the wet season and from 0.01 to 0.03 in the dry season. On tomato, severity ranged from 0.01 to 0.08 in the wet season and from 0.01 to 0.02 in the dry season. Generally, pepper showed more severe symptoms than tomato.

Isolation and identification of the pathogen. *X. c. vesicatoria* was isolated from all diseased plants sampled, but only seeds of two of nine pepper cultivars and four of 15 tomato cultivars tested positive for the presence of *Xanthomonas* (Table 1). The highest titers of *Xanthomonas* were from seeds of pepper cv. Calwonder 300 TMR and tomato cv. Calypso.

Bacteria identified as *X. c. vesicatoria* showed many typical characteristics of xanthomonads. The bacteria were yellow, rod-shaped, obligately aerobic, gram negative, oxidase negative, and catalase positive and utilized glucose, arabinose, mannose, trehalose, and cellobiose for acid production. The strains bore a polar flagellum, but none utilized asparagine as a sole source of carbon and nitrogen. In addition, the strains failed to reduce nitrates but were pathogenic on pepper and tomato.

Pathogenicity studies. Bacteria representing the tomato, pepper, and pepper-tomato groups and races 1–3 of *X. c. vesicatoria* were isolated from diseased plants, but race 1 strains (tomato race 1 and pepper race 1) accounted for 73 and 51% of the xanthomonads from diseased pepper and tomato, respectively (Table 2). In contrast, only races 1 and 3 were obtained from commercial seed samples. All strains from seeds of pepper cv. Calwonder 300 TMR and tomato cv. Indian River were race 1, and only tomato cvs. Floridel and Moneymaker yielded race 3 strains. Four of the 10 tomato strains tested for virulence on Hawaii 7998 induced the hypersensitive response; the remainder were virulent.

Many of the *Xanthomonas* strains failed to induce visible symptoms on the host lines tested and therefore could not be classified into races or groups of *X. c. vesicatoria* (Table 2). All strains from seeds of pepper cv. Yolo Wonder and tomato cv. Calypso were nonpathogenic.

Bactericide sensitivity studies. Of 240 strains of *X. c. vesicatoria*, 61, 64, and 47% were resistant to copper, zinc, and streptomycin, respectively. Moreover, 30% of the strains were resistant to all three bactericides and 28% were resistant to copper and zinc combined. In contrast, only 3–5% of the strains were resistant to streptomycin combined with copper or zinc.

DISCUSSION

The method of assessing the severity of bacterial spot in the field was intense enough to estimate the importance of this disease in Barbados. The survey showed bacterial spot to be widespread and generally severe, particularly during the rainy season.

Several phytopathogenic bacteria are known to maintain inocula in seeds (2,3,7). The seed stocks tested for the presence of *X. c. vesicatoria* in this study included those used on large farms and in home gardens in Barbados. Because the bacterium was not detected in seeds used in large-scale agriculture, imported pepper and tomato seeds are probably not significant sources of *X. c. vesicatoria* in Barbados.

The overall picture of the race classification study indicates the predominance of race 1 strains of *X. c. vesicatoria*. Frequencies of races 2 and 3 are considerably lower, but generally race 2 strains are more common. The spectrum of races observed is probably a natural feature of the pathogen population, since the *Bs1, Bs2*, and *Bs3* disease resistance genes, which can cause race-specific selection, are not known to be used in large-scale agriculture in Barbados.

It has been suggested that race 2 strains of *X. c. vesicatoria* are endemic to Florida and that race 1 strains predominate in other areas of the world (5). The results of the present study support this view, and in a recent study from Taiwan (8) race 1 and race 3 strains were common.

It is interesting that the tomato strain of *X. c. vesicatoria*, which by definition is virulent to tomato but not to pepper, was isolated from diseased pepper. Similarly, the pepper race 1 strain, which is virulent to pepper but not to tomato, was frequently isolated from tomato. The extent to which each group or race of the bacterium contributes to the severity of bacterial spot in the field should be investigated.

Varying proportions of the bacteria from seeds and typical lesions were indistinguishable from *X. c. vesicatoria* on

Table 1. Recovery of *Xanthomonas campestris* pv. *vesicatoria* from seeds of commercial pepper and tomato cultivars in Barbados[a]

Cultivar	Source	Number of xanthomonads (10^3 cfu g^{-1} seed)
Pepper		
Calwonder 300 TMR	Ferry-Morse, United States	4.3
Yolo Wonder	Royal Sluis, Netherlands	0.2
Anaheim Chili	Northrup King, United States	0.0
Belle Star	Ferry-Morse, United States	0.0
Cayenne Long Slim	Nickerson-Zwaan, Netherlands	0.0
Gator Bell	Royal Sluis, Netherlands	0.0
Jalapeno Chili	Northrup King, United States	0.0
Keystone Giant	Royal Sluis, Netherlands	0.0
Resistant Giant	Royal Sluis, Netherlands	0.0
Tomato		
Calypso	Sunblest, United States	3.6
Floridel	Sunblest, United States	2.8
Indian River	Sunblest, United States	2.9
Moneymaker	Ferry-Morse, United States	2.0
Beefsteak	Ferry-Morse, United States	0.0
Burpee's Big Boy	Ferry-Morse, United States	0.0
Chandelier	Takii, Japan	0.0
FA38	Zeraim, Israel	0.0
Floramerica	Ferry-Morse, United States	0.0
Homestead Elite	Ferry-Morse, United States	0.0
Improved Pearson	Neuman Seed Co., United States	0.0
Ravid	Zeraim, Israel	0.0
Sweet 100	Northrup King, United States	0.0
UNH52	Takii, Japan	0.0
Walter	Royal Sluis, Netherlands	0.0

[a]Seed samples were suspended in sterile distilled water for 16 hr and shaken at 90 oscillations per minute for 15 min, after which 100-µl aliquots of the diluted suspension were plated on NYGA in triplicate.

Table 2. Classification of *Xanthomonas campestris* pv. *vesicatoria* obtained from diseased plants and commercial pepper and tomato seeds in Barbados

Origin of strains	No. of strains tested	Percentage of strains				
		Tomato race 1	Pepper race 1	Pepper-tomato race 2	Pepper-tomato race 3	Unknown
Diseased pepper	150	36	37	11	8	8
Diseased tomato	90	38	13	22	21	6
Pepper seed						
cv. Calwonder 300 TMR	100	0	100	0	0	0
cv. Yolo Wonder	100	0	0	0	0	100
Tomato seed						
cv. Calypso	100	0	0	0	0	100
cv. Floridel	100	50	0	0	50	0
cv. Indian River	100	100	0	0	0	0
cv. Moneymaker	100	66	0	0	17	17

the basis of many diagnostic tests but were nonpathogenic toward pepper and tomato. These nonpathogenic strains are probably opportunistic xanthomonads (6).

Recently, the tomato cultivar Hawaii 7998 was reported to show high levels of resistance to tomato strains of *X. c. vesicatoria* from Florida (16), and we assessed this resistance against tomato strains of the bacterium from Barbados. The cultivar failed to restrict all strains, indicating the existence of at least two races of the tomato group of *X. c. vesicatoria* in Barbados.

Chemicals commonly used over the last 20–25 yr to control bacterial spot in Barbados contain copper and/or zinc as the main antimicrobial agent. These bactericides offer little or no control because of the high frequency of resistance to copper and zinc in the pathogen population. A significant amount of streptomycin-resistant strains of the pathogen was also detected. On the basis of these findings, the introduction of host genotypes with suitable resistance gene(s) holds the promise for the control of bacterial spot in Barbados.

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