

Contribution of Four Races of *Xanthomonas campestris* pv. *vesicatoria* to Bacterial Spot in Barbados

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ABSTRACT

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The size of typical lesions of bacterial spot on tomato and pepper fruits was used to assess the extent to which each race of *Xanthomonas campestris* pv. *vesicatoria* contributes to disease in the field. Lesions associated with race 1 strains of the pepper or tomato group only were significantly larger than those from which pepper-tomato group race 2 or race 3 strains were detected. Pepper-tomato group race 3 was isolated from the smallest lesions. Six strains from tomato were virulent on pepper carrying the *Bs2* resistance gene to bacterial spot, and no pepper group strains were detected on tomato. Race 1 strains of the pepper or tomato group were also most abundant and were associated with more lesions on pepper and tomato than pepper-tomato group race 2 or race 3 strains. Mixtures of strains comprising pepper or tomato group race 1 and pepper-tomato group race 2 were obtained from 10 to 15% of lesions on pepper and 11 to 20% of lesions on tomato fruit.

Bacterial spot of pepper (*Capsicum annuum* L.) and tomato (*Lycopersicon esculentum* Mill.), caused by *Xanthomonas campestris* pv. *vesicatoria* (Doiidge) Dye, is widespread and severe in Barbados (15). Three groups of the bacterium, namely the pepper group (XcvP), the tomato group (XcvT), and the pepper-tomato group (XcvPT) have been described (6,8). XcvT is virulent on tomato and avirulent on pepper, whereas XcvP is virulent on pepper but not on tomato. Pepper and tomato are susceptible to XcvPT. Strains belonging to XcvP and XcvPT are further divided into a number of races on the basis of virulence on differential near-isogenic pepper lines (6). Resistance to bacterial spot of pepper is conferred by single dominant genes, *Bs1*, *Bs2*, and *Bs3* (4). Genes *Bs3* and *Bs1* offer resistance to races 1 and 2, respectively, and the *Bs2* gene restricts races 1-3. Races 1 and 2 of XcvT also have been differentiated on tomato cultivars Walter and Hawaii 7998 (15). Walter has no resistance to strains of XcvT, and Hawaii 7998 restricts race 1 but not race 2 of this group (11,15). No known resistant pepper or tomato genotypes are grown in Barbados.

Bacterial spot of pepper and tomato in Barbados is associated with *X. c. vesicatoria* races 1, 2, and 3 (15). Another feature of the disease is the occurrence of strains of the bacterium belonging to the pepper group on tomato and strains of the tomato group on pepper (15). Strains of the tomato group were also isolated from pepper in Florida (7).

These observations are unexpected, since strains of the pepper group and tomato group are avirulent on tomato and pepper, respectively. This study reports on the extent to which each race of *X. c. vesicatoria* contributes to bacterial spot severity in the field in Barbados on tomato and pepper.

MATERIALS AND METHODS

Assessment of virulence. Pepper and tomato fruits were collected at harvest from a random sample of 0.05% of the plants on each farm, and the area of typical lesions of bacterial spot on each fruit was used to assess disease severity. The margin of each lesion was traced onto transparent plastic wrap placed over the fruit surface and lesion area estimated using graph paper. Only lesions at an advanced stage of development, characterized by a dark and rough appearance, were measured. One pepper farm and three tomato farms in different regions (Butcher, Spring Hall, National Hatcheries, and Mount Wilton) of Barbados were selected for study in 1990. The study was repeated in 1991 on two farms each of pepper and tomato. All crops were grown in the rainy season (June to November) of each year of study.

Isolation and identification of the pathogen. Fruits were washed in tap water to remove soil debris and then surface-disinfected by being dipped in ethanol (95%) for 2 sec, rinsed for 10 sec in sodium hypochlorite solution (1.25%), and then rinsed in sterile distilled water. Each lesion was then excised and crushed in 100 μ l of sterile distilled water using an ethanol-sterilized mortar and pestle. Loopfuls of suspension were streaked onto nutrient yeast extract glycerol agar (NYGA) (14) for isolation

of *X. c. vesicatoria*. Three or five colonies of the bacterium from each lesion were randomly selected and stored until required at -20 C in nutrient yeast extract glycerol broth (NYGB) (14) amended with 20% glycerol. A random sample of 31 strains of presumptive *X. c. vesicatoria* obtained from lesions on pepper and tomato fruits was tested for the presence of xanthomonadins (10).

Host and pathogen cultures. The pathogen was cultured routinely on NYGA or in NYGB (14). The tomato cultivars Walter and Hawaii 7998 and the pepper lines Early Calwonder (ECW), ECW10R, ECW20R, and ECW30R were used as potential hosts. ECW carries no genes for resistance to bacterial spot of pepper and ECW10R, ECW20R, and ECW30R contain the *Bs1*, *Bs2*, and *Bs3* resistance genes, respectively (6). Plants were grown in sterilized potting compost under conditions previously described (15).

Host inoculation and race classification. Plants were inoculated when 4-wk old by infiltrating the intercellular spaces of fully expanded leaves with a bacterial suspension (10^8 - 10^9 cells/ml) using a syringe without the needle (13). Strains of *X. c. vesicatoria* were classified into races on the basis of the presence of a hypersensitive or susceptible response on Walter, Hawaii 7998, ECW, ECW10R, ECW20R, and ECW30R as described (6,15). Three or five strains from each lesion were tested, and each test was replicated twice on two occasions over a 2-yr period.

RESULTS

Bacterial spot fruit infection occurred on all tomato and pepper farms. The pepper cultivar Calwonder 300TMR (Ferry-Morse, United States) and the tomato cultivar FA38 (Zeraim, Israel) were the crops grown.

All strains of the presumptive pathogen tested for xanthomonadins produced yellow spots with an average R_f value of 0.46 on thin layer silica gel chromatography plates. The proportion of each race of *X. c. vesicatoria* detected on fruits from each farm in 1990 is shown in Table 1. XcvP race 1 strains from pepper were seven times more prevalent than XcvT race 1 and four and a half times more common than XcvPT race 2 strains. XcvT race 1 strains accounted for the majority (54-66%) of strains detected on tomato from Spring Hall and National Hatcheries, but they were as abundant

as XcvPT race 2 strains from Mount Wilton. XcvPT race 3 strains accounted for less than 3% of the strains from each farm.

No pepper strains were detected on tomato, and six strains from tomato from National Hatcheries were virulent on ECW20R.

XcvP race 1 strains only were detected in more lesions on pepper than XcvPT race 2 or race 3 strains (Table 2). XcvPT race 2 strains together with XcvP or XcvT race 1 strains were isolated from 10 to 15% of the pepper fruit lesions. XcvT race 1 strains only were detected in the majority of lesions from Spring Hall and National Hatcheries and in similar numbers of lesions as XcvPT race 2 strains from Mount Wilton. Mixtures of strains comprised of XcvPT race 2 and XcvT race 1 pathotypes were associated with more tomato fruit lesions than XcvPT race 3 strains.

Lesion areas ranged from 0.5 to 7.0 mm² on pepper and tomato and gave means of 2.1 mm² on pepper and 2.8, 4.3, and 2.2 mm² on tomato from Spring Hall, National Hatcheries, and Mount Wilton, respectively. On three of four

farms, XcvP or XcvT race 1 strains only were isolated from lesions that were significantly more extensive than lesions associated with XcvPT race 2 strains only or XcvPT race 2 strains combined with the race 1 strains (Table 3). The smallest lesions were associated with XcvPT race 3 strains.

The incidence and severity of bacterial spot induced by each race of *X. c. vesicatoria* were generally similar in 1990 and 1991. A notable difference is the isolation of five XcvT race 2 strains from four lesions on tomato from National Hatcheries in 1991 (*data not shown*).

DISCUSSION

Studies on pathogenic variation of *X. c. vesicatoria* on pepper and tomato have addressed the occurrence of physiologic races (1,3,7,9,15) but not the aggressiveness or virulence of the bacterium. In the present study, the area on pepper and tomato fruits covered by typical lesions of bacterial spot and the incidence of each race of the bacterium were used to assess virulence. Bacterial spot fruit infection was selected for study because lesions on fruits were more discernable

than those on leaves. Data presented show that XcvP or XcvT race 1 strains were generally more virulent than XcvPT race 2 and race 3 strains in Barbados (Table 3). XcvPT race 2 strains only or when combined with XcvP or XcvT race 1 strains were also more virulent than XcvPT race 3 strains.

Strains of *X. c. vesicatoria* virulent on ECW20R were isolated on two occasions from diseased tomato. No strains of the bacterium from the field were previously known to overcome the *Bs2* resistance gene in ECW20R.

It is interesting that XcvT strains, which by definition are avirulent on pepper, were isolated along with XcvPT race 2 strains from single lesions on pepper. In contrast, XcvP strains were not recovered from tomato (Table 1). Mutation for race change in *X. c. vesicatoria* has been reported (2,5,6,12), but its frequency is unlikely to account for the magnitude of XcvT race 1 and XcvPT race 2 strains of the bacterium observed in single lesions in the present study. XcvPT race 2 strains probably initiated pepper fruit lesions, which became colonized at a later time by XcvT

Table 1. Classification of *Xanthomonas campestris* pv. *vesicatoria* obtained from diseased pepper and tomato fruits from specific locations

Farm	Fruit type	No. of strains	Percentage of strains				Others
			Tomato group race 1	Pepper group race 1	Pepper-tomato group race 2	Pepper-tomato group race 3	
Butcher	pepper	1,275	10.5	72.2	15.8	1.5	0
Spring Hall	tomato	1,560	66.2	0	31.5	2.3	0
National Hatcheries	tomato	1,116	54.3	0	42.5	2.7	0.5
Mount Wilton	tomato	1,540	48.1	0	49.9	2.0	0

Table 2. Proportion of lesions on pepper and tomato fruits induced by four races of *Xanthomonas campestris* pv. *vesicatoria*

Farm	Fruit type	No. of lesions	Percentage of lesions						Others ²
			Tomato group race 1 only	Pepper group race 2 only	Pepper-tomato group race 2 only	Tomato group race 1 and pepper-tomato group race 2	Pepper group race 1 and pepper-tomato group race 2	Pepper-tomato group race 3	
Butcher	pepper	255	0	42.1	31.3	15.1	10.1	1.4	0
Spring Hall	tomato	312	43.2	0	34.4	20.2	0	2.2	0
National Hatcheries	tomato	372	56.0	0	24.0	12.0	0	7.2	0.8
Mount Wilton	tomato	308	42.0	0	42.0	10.7	0	5.3	0

²Lesions from which strains virulent on ECW20R were isolated.

Table 3. Average sizes of lesions incited by four races of *Xanthomonas campestris* pv. *vesicatoria*

Farm	Fruit type	Average lesion size ¹ (mm ²)					
		Tomato group race 1 only	Pepper group race 1 only	Pepper-tomato group race 2 only	Tomato group race 1 and pepper-tomato group race 2	Pepper group race 1 and pepper-tomato group race 2	Pepper-tomato group race 3
Butcher	pepper	...	2.6 a	1.7 c	1.0 d	2.1 b	0.7 e
Spring Hall	tomato	3.8 a	...	2.1 c	2.3 b	...	0.9 d
National Hatcheries	tomato	4.5 a	...	2.2 b	4.1 a	...	0.7 c
Mount Wilton	tomato	2.2 a	...	2.3 a	2.3 a	...	0.9 b

¹Mean lesion size values in the same row followed by the same letter are not significantly different ($P < 0.05$) based on Tukey's honestly significant difference test.

²No lesions associated with the races indicated were detected.

strains through saprophytic growth.

Similarly, tomato fruit infection was probably incited by compatible strains of the bacterium, but the inability to detect XcvP strains on tomato may reflect poor saprophytic growth of these strains.

The present study provides information on the extent to which each race of *X. c. vesicatoria* causes bacterial spot in Barbados. The possibility that strains of *X. c. vesicatoria* can survive saprophytically on lesions on an incompatible host will reduce the ability of the host to select against these strains. The extent to which each race of *X. c. vesicatoria* is maintained in field populations of the bacterium in the presence of incompatible pepper and tomato cultivars should be investigated further.

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