

Epiphytic Populations of *Xanthomonas campestris* pv. *vesicatoria* on Tomato Cultigens Resistant and Susceptible to Bacterial Spot

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ABSTRACT

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Populations of *Xanthomonas campestris* pv. *vesicatoria* were monitored over four seasons on leaves of two tomato cultivars and five genotypes selected for various levels of resistance to the pathogen. Populations on all five genotypes were consistently below those on the very susceptible Lyconorma, but only those on Hawaii 7998 were significantly below numbers of the susceptible cultivar Walter in every test. Rankings of cultigens based on support of epiphytic populations of *X. c.* pv. *vesicatoria* mirrored those based on defoliation over two seasons. Significant interactions in repeated measures analyses of variance between cultigen and sampling day indicated differences in population and disease development over time. By analyzing the slopes of regression equations, which compared epiphytic populations or defoliation with time, population and disease development were confirmed to be slowest on leaves of the genotypes Campbell 28, Ohio 4013, and Hawaii 7998.

Xanthomonas campestris pv. *vesicatoria* (Doidge) Dye, which causes bacterial spot of tomato (*Lycopersicon esculentum* Mill.) and bacterial leaf spot of bell pepper (*Capsicum annuum* L.), is one of the most economically important pathogens in Florida (17). Some measure of control is available through the use of antimicrobial compounds and mixtures containing copper, but during periods of high disease pressure, the control is frequently inadequate (2). Streptomycin has also proven ineffective in Florida because of the development of resistance in the pathogen (22). Improved plant nutrition can significantly delay the development of *X. c.* pv. *vesicatoria* and the appearance of disease symptoms, but its beneficial effects can be negated by heavy rains (15).

Because chemical control of *X. c.* pv. *vesicatoria* on tomato is often inadequate, breeding programs must incorporate resistance to the pathogen. Based on symptom development measures, sources of resistance have already been identified in a few tomato cultigens (an inclusive term for released cultivars and unreleased breeding lines or genotypes)

(20). Measurements of symptom development, such as percentage of spotting or defoliation, have often been used in making field comparisons of disease severity. In some cases (12,15), these measurements have been related to the numbers of the pathogen living epiphytically. Crosse (4) first established that bacterial populations may possess an epiphytic stage of growth without producing symptoms of disease. During this epiphytic phase, populations may increase to such a level that they overcome some threshold number required to initiate disease (13); they also may establish a reservoir for their subsequent dissemination (4,11). Populations of a few pathovars of *Pseudomonas syringae* van Hall and *X. campestris* (Pammel) Dowson have been reported to be lower on resistant plant genotypes than on susceptible plants (5,8).

The objective of this research was to evaluate seven tomato cultigens for their ability to support epiphytic populations of *X. c.* pv. *vesicatoria* and to determine if there is a relationship between these populations and field resistance to bacterial spot. This information may aid in assessing the breeding value of the resistance.

MATERIALS AND METHODS

Plot development. From previous trials (20), five tomato genotypes with various levels of resistance to foliar bacterial spot were selected for comparison with susceptible cv. Walter and highly susceptible cv. Lyconorma. Of four experiments, three were conducted on a sandy soil in west central Florida. Seeds of the two cultivars, the partially

resistant genotypes Campbell 28, Florida 3316, Heinz 2990, and Ohio 4013, and the resistant genotype Hawaii 7998 were sown in trays containing sand; individual seedlings then were transferred to planter flats. After 4 wk in the greenhouse, the seedlings were transplanted into raised beds 15 cm high \times 16 cm wide spaced 137 cm apart. Beds had previously been fumigated with 67% methyl bromide/33% chloropicrin at 385 kg/ha and dressed with 18-0-25-2 (N-P₂O₅-K₂O-MgO) at 369 kg/ha and with superphosphate (0-8-7-0 plus micronutrients at 40 g/kg) at 671 kg/ha. Before planting, 2,079 kg/ha of 18-0-25-2 was distributed in two bands 46 cm apart. Beds were covered with black polyethylene mulch, which was sprayed with white paint, and were furrow irrigated. A fourth experiment was conducted on the marl soil of southern Florida. Seeds were placed directly into the raised beds that had been fumigated with methyl bromide at 270 kg/ha and fertilized with 2,246 kg/ha of 8-16-16. Beds were covered with black polyethylene mulch and sprinkler irrigated weekly. The experimental design was a randomized block with three or four replications, each containing 10 plants of each cultigen.

Plant inoculation. In each of the growing seasons (fall 1984, spring 1985, fall 1989, and winter 1990), 6-wk-old plants were inoculated with a mixture of strains of *X. c.* pv. *vesicatoria*. Strains 81-18, 83-44, and 84-1, originally isolated from field plantings of tomato and pepper (16) and maintained in sterile tap water, were initially streaked on plates of NYDA (16). Cells were subsequently suspended in 20 L of 0.01 M MgSO₄ at a concentration of 10⁶ colony-forming units (cfu) per milliliter. Plants were inoculated in the early morning with a backpack sprayer while leaves were still wet with dew.

Sampling techniques. Foliar populations were monitored weekly on a semiselective Tween culture medium (16). Sampling was conducted in early morning in one of two ways. The first two seasons, numbers of *Xanthomonas* were estimated by collecting one leaflet without symptoms of disease from the upper part of the canopy of each of the 10 plants of a cultigen replicate. The 10 leaflets were then weighed and washed together for 30 min in 100 ml of a 0.1 M phosphate buffer (pH 7.0) containing,

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Table 1. Univariate analysis of variance (repeated measures design) of epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* on seven tomato cultivars over four seasons

Source of variation	Season ^x											
	Fall 1984			Spring 1985			Fall 1989			Winter 1990		
	df	MS ^y	F	df	MS	F	df	MS	F	df	MS	F
Between plots effects												
Cultigen	5	16.88	7.88***	6	88.13	42.55***	5	56.25	14.03***	5	38.76	32.20***
Replication	3	4.23	NS	3	7.67	3.70*	2	0.41	NS	2	2.26	NS
Cult × Rep	NT	NT	NT	NT	NT	NT	10	2.66	NS	10	2.29	NS
Error	15	2.14	...	18	2.07	...	72	4.01	...	18	1.20	...
Within plot effects ^z												
Day	7	32.22	17.26***	11	21.08	19.92***	4	16.52	5.18***	7	13.48	22.62***
Day × Cult	35	1.97	NS	66	2.55	2.41***	20	4.02	NS	35	1.80	1.96**
Day × Rep	21	1.71	NS	33	1.31	NS	8	1.86	NS	14	0.91	NS
Error (Day)	105	1.87	...	198	1.06	...	288	3.19	...	126	0.92	...

^xNT = not tested, NS = not significant. * = Significant at $P = 0.05$, ** = significant at $P = 0.01$, and *** = significant at $P = 0.001$.

^yMean square.

^zBased on an adjusted F value proposed by Greenhouse and Geisser for repeated measures analyses (19).

per liter, 5.3 g of KH_2PO_4 , 8.61 g of Na_2HPO_4 , and 1 g of Bacto peptone. The last two seasons, one leaflet was collected from four or five plants in each group of 10, and each leaflet was individually weighed and washed for 30 min in 10 ml of the phosphate buffer. One-tenth of a milliliter from serial dilutions of each wash of grouped or single leaflets was spread over the surface of Tween plates, which were subsequently incubated at 28 C for 96 hr, and the number of colonies that developed was related to the fresh weight of the leaf sample.

Throughout the course of the last two seasons, three of 10 plants in each group were rated for development of bacterial spot. The percentage of defoliation, based on a visual acuity rating scale developed by Horsfall and Barratt (9), was measured on three or four dates approximately 2 wk apart.

Statistical methods. Because experiments involved the quantification of bacteria or defoliation over several weeks, the data were analyzed seasonally to determine the effects of time as well as cultivar differences with a repeated measures analysis of variance (14) available through SAS (19). Bacterial populations were analyzed as the \log_{10} of the colony-forming units per gram of leaflet. Horsfall-Barratt values were analyzed without transformation and converted to percentage of defoliation. Cultigens were differentiated each season with respect to mean epiphytic population and defoliation with a means separation test (REGWF) in SAS after a split-plot analysis in time (analyzing day). Subsequently, regression equations were generated for individual cultivar replicates relating epiphytic population (\log_{10} cfu/g) or percentage of defoliation and time after inoculation for each season. The slopes of these equations were analyzed first by individual season and then as the sum of seasons, again with a split-plot analysis in time (in this case, analyzing years). From this analysis, a mean separation of cultivars was

Table 2. Epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* on seven tomato cultivars over four seasons

Cultigen	Seasonal mean population (\log_{10} cfu/g fresh weight) ^z			
	Fall 1984	Spring 1985	Fall 1989	Winter 1990
Lyonorma	6.14 a	6.07 a	2.35 a	4.93 a
Walter	6.06 a	3.00 b	2.46 a	4.58 ab
Florida 3316	5.68 ab	3.01 b	NT	NT
Heinz 2990	NT	2.64 bc	1.37 b	4.13 ab
Campbell 28	4.71 bc	2.52 bc	1.49 b	3.86 bc
Ohio 4013	6.14 a	2.86 b	0.99 b	2.99 cd
Hawaii 7998	4.57 c	1.84 c	0.15 c	2.62 d

^zWithin seasons, means followed by the same letter are not significantly different by the Ryan-Einot-Gabriel-Welsch multiple F test ($P = 0.05$). NT = not tested.

Table 3. Univariate analysis of variance (repeated measures design) of defoliation on six tomato cultivars inoculated with *Xanthomonas campestris* pv. *vesicatoria* over two seasons

Source of variation	Season ^x					
	Fall 1989			Winter 1990		
	df	MS ^y	F	df	MS	F
Between plots effects						
Cultigen	5	78.54	270.73***	5	62.50	78.94***
Replication	2	1.85	6.38**	2	11.92	15.06***
Cult × Rep	10	0.63	2.17*	10	1.83	2.31*
Error	36	0.29	...	36	0.79	...
Within plot effects ^z						
Day	2	36.13	137.72***	3	34.04	29.77***
Day × Cult	10	3.09	11.76***	15	5.69	4.97***
Day × Rep	4	1.26	4.80**	6	3.92	3.43**
Error (Day)	72	0.26	...	108	1.14	...

^x* = Significant at $P = 0.05$, ** = significant at $P = 0.01$, *** = significant at $P = 0.001$.

^yMean square.

^zBased on an adjusted F value proposed by Greenhouse and Geisser for repeated measures analyses (19).

also generated based on the slopes, which represented rates of population development or defoliation.

RESULTS

An analysis of variance (repeated measures design) of the \log_{10} colony-forming units of epiphytic populations disclosed significant differences among cultivars and sampling days ($P = 0.001$) (Table 1). An interaction between these two variables was also significant in two of the four experiments. Lyonorma, a cultivar very susceptible to foliar bacte-

rial spot, developed populations of epiphytic *X. c.* pv. *vesicatoria* that were consistently high (Table 2). Walter, also susceptible to foliar disease, could not, in three of four experiments, be significantly differentiated from Lyonorma in the seasonal mean populations of epiphytic bacteria it supported.

Of the five tomato genotypes that were compared with the two susceptible cultivars, Florida 3316, Heinz 2990, Campbell 28, and Ohio 4013 were intermediate in their support of the pathogen. However, in two of the four experiments,

Table 4. Percentage of defoliation on six tomato cultigens inoculated with *Xanthomonas campestris* pv. *vesicatoria* over two seasons

Cultigen	Percentage of defoliation ^x	
	Fall 1989 ^y	Winter 1990 ^z
Lycanorma	50.32 a	44.58 a
Walter	26.89 b	26.14 b
Heinz 2990	12.22 c	14.03 c
Campbell 28	8.76 d	7.83 c
Ohio 4013	5.48 d	6.92 c
Hawaii 7998	1.57 e	5.69 c

^x Within seasons, means followed by the same letter are not significantly different by the Ryan-Einot-Gabriel-Welsch multiple *F* test ($P = 0.05$).

^y Means of three sampling dates.

^z Means of four sampling dates.

Campbell 28 and Ohio 4013 supported significantly lower populations when compared with both Lycanorma and Walter. Hawaii 7998 consistently developed the lowest populations of epiphytic *X. c.* pv. *vesicatoria*. In every case, populations on Hawaii 7998 were significantly lower than levels on the two susceptible cultivars, and, in many instances, they were significantly different from numbers on the other genotypes as well.

For comparison with epiphytic populations, the percentage of defoliation was measured three and four times throughout the 1989 and 1990 seasons, respectively. Differences between cultigens and sampling days were significant at $P = 0.001$, as was the interaction between these two variables (Table 3). Significant differences between replications and the interactions with replication suggested an additional source of inoculum outside the experimental plot. Defoliation was highest on Lycanorma which was followed by, but was significantly greater than, Walter (Table 4). All genotypes tested averaged significantly less defoliation than Lycanorma and Walter.

Significant interactions between cultigens and sampling days suggested differences in the rates of development of epiphytic populations and defoliation. The rates for individual cultigen replicates were computed as the slopes of regression equations relating populations (\log_{10} cfu) or percentage of defoliation and sampling days during each season. An analysis of the slopes with a separation of means demonstrated that population development was significantly slower on leaves of Hawaii 7998 when compared with Lycanorma and Walter (Table 5). Populations on day 0 (equation intercepts) were not significantly different. (Heinz 2990 was not included in this analysis because it was absent from the fall 1984 test). Rates of defoliation were greatest for Lycanorma, with Walter being intermediate (Table 5). Although defoliation rates for Campbell 28, Hawaii 7998, and Ohio 4013 could not be statistically separated from each

Table 5. Slopes and intercepts of regression equations relating, over time, epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* (\log_{10} cfu/g fresh weight) or percentage of defoliation on five tomato cultigens

Cultigen	Population (\log_{10}) ^z		Percentage of defoliation	
	Slope	Intercept	Slope	Intercept
Lycanorma	0.0587 a	2.59	1.2967 a	-2.59
Walter	0.0538 a	1.72	0.6814 b	0.56
Campbell 28	0.0483 a	1.21	0.1938 c	0.94
Ohio 4013	0.0378 ab	1.67	0.0097 c	6.16
Hawaii 7998	0.0160 b	1.73	0.0834 c	0.25

^z Mean of four seasons (population) or two seasons (defoliation). Within columns, means followed by the same letter are not significantly different by the Ryan-Einot-Gabriel-Welsch multiple *F* test ($P = 0.05$). Intercepts for population and defoliation did not differ significantly.

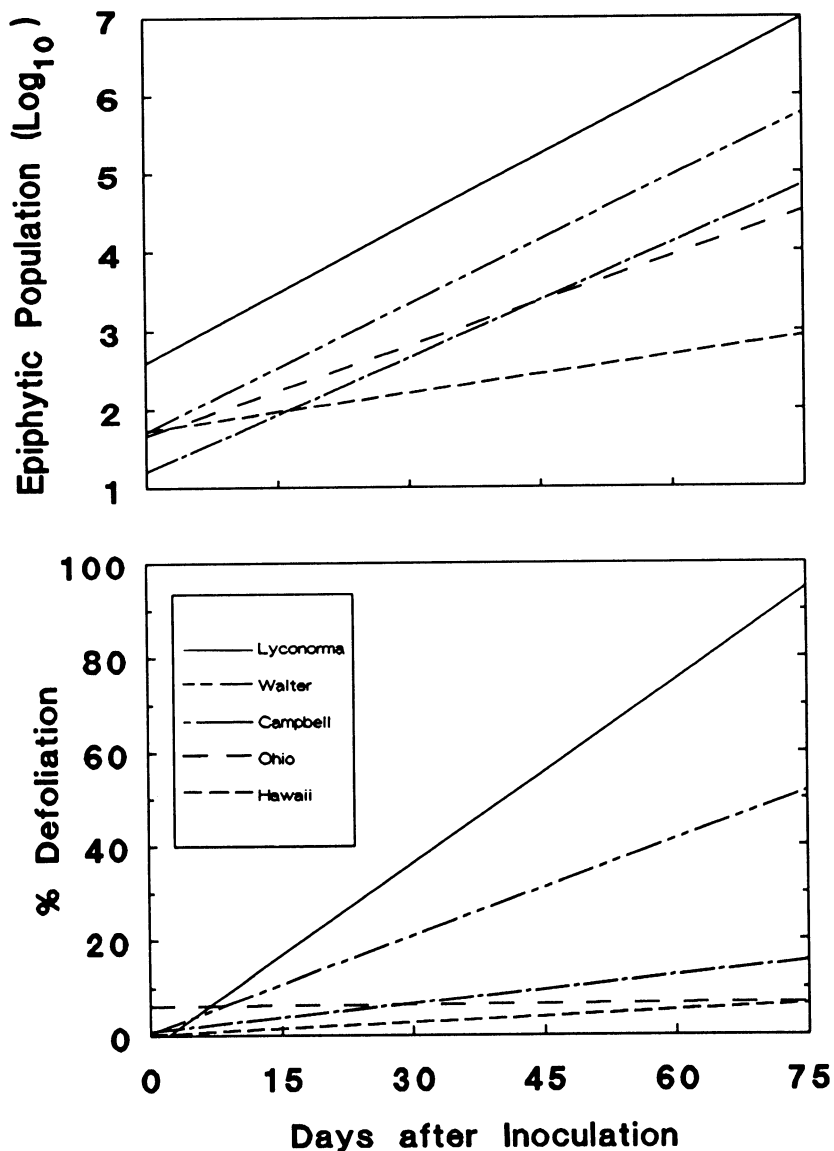


Fig. 1. Plots over time of epiphytic populations of *Xanthomonas campestris* pv. *vesicatoria* (\log_{10} cfu/g of fresh leaflet) (top) and percentage of defoliation (bottom) on five tomato cultigens. Data are means of four and two seasons, respectively.

other, all three were significantly slower than the rates for Lycanorma and Walter. The regression equations are graphed in Figure 1.

DISCUSSION

The five tomato genotypes selected for this survey were chosen after an eval-

uation of 284 genotypes for susceptibility of the leaves and fruit to bacterial spot (20). Among the five, Campbell 28 had been previously reported to have partial resistance to bacterial spot (3), Heinz 2990 had resistance to bacterial canker (1), and Hawaii 7998 was resistant to bacterial wilt (20). Hawaii 7998 also was shown to be the first with a high level

of resistance to *X. c. pv. vesicatoria* after demonstration of a hypersensitive response (8). Populations of the pathogen inoculated at 10^5 cfu/ml into leaflets of Hawaii 7998 remained depressed over 2 wk, whereas populations within leaves of the partially resistant Campbell 28 were similar to those in the susceptible cv. Walter. Subsequently, the resistance of Hawaii 7998 was found to be multigenic, but the genetic control of hypersensitivity remained elusive (21). More recently, the nature of this resistance was confirmed by the discovery of a race of *X. c. pv. vesicatoria* virulent on Hawaii 7998 (23).

Host resistance may be overcome, when incomplete, by an increase in the level of inoculum of the pathogen. Conversely, the level of inoculum and its rate of increase may be influenced by some forms of host resistance with a subsequent impact on the rate of spread of disease through a crop. Because epiphytic bacteria provide a reservoir of inoculum (4,11), one mechanism of host resistance may be to reduce this population or its rate of development.

This study attempted to determine how various types of resistance affected populations of *X. c. pv. vesicatoria* on tomato leaves. Bulk sampling of leaves was initially used in these trials, although such a procedure produces an arithmetic mean for a population that is often lognormally distributed (7). This procedure was considered adequate for an estimate of population differences, although it may overestimate the true population mean. Two final experiments compared populations on individual leaflets, and the statistical separation of cultigens was similar for both procedures.

One form of partial resistance by a crop plant may take the form of an increased threshold beneath which a pathogen population may be theoretically unable to produce disease. This is not to imply a model of cooperative action among the pathogen population but rather that the very small probability of one individual initiating an infection has been further reduced. Such a threshold has been hypothesized in work with brown spot of bean (13), where a "minimum effective dose" of the epiphytic pathogen at a specific growth stage of the host was highly predictive of disease incidence 1 wk later. Alternatively, an apparent infection threshold may operate within the leaf. Such a system was demonstrated with *X. c. pv. glycines* (Nakano) Dye in resistant and susceptible cultivars of soybean (6).

Although pathogen populations increased at similar rates in leaves of the two cultivars, six times as many cells were required to produce a leaf pustule in resistant relative to susceptible plants.

Partial resistance based on an apparent infection threshold could explain differences in disease severity between the susceptible cv. Walter and the genotypes Ohio 4013 and Campbell 28 which, in general, appear to support similar epiphytic populations of *X. c. pv. vesicatoria*. Regression analyses reveal that differences among Campbell 28, Ohio 4013, and Walter probably do not exist in the rates of population development that they support. Yet there remain clear differences between these two genotypes and Walter in the expression of disease symptoms, confirming earlier work (20). Together, these observations might suggest a decreased ability of the pathogen to enter the leaves of Campbell 28. An external, morphological resistance, namely fewer stomata on leaf surfaces, has been correlated with the number of lesions in this disease (18). Once the bacteria are inside the leaves of Campbell 28, however, disease development would appear to proceed at the usual speed (10). In contrast, disease development in Ohio 4013 appears exceptionally slow. Ohio 4013 is unusual in that the plant is short and slow growing, and the leaves are very small. These characteristics make Ohio 4013 difficult to rate for defoliation and to compare with the other genotypes.

Whether through an inability of the pathogen to enter the tomato leaf, an inability for it to multiply once inside, or an inability for it to induce a host response, all five genotypes examined in this survey delay or suppress the development of bacterial spot. However, of these tomato genotypes, only on leaves of the most resistant, Hawaii 7998, is the development of epiphytic populations of *X. c. pv. vesicatoria* reduced below rates on the susceptible cultivars.

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