Evidence for the Preemptive Nature of Tomato Race 3 of *Xanthomonas campestris* pv. *vesicatoria* in Florida

J. B. Jones, H. Bouzar, G. C. Somodi, R. E. Stall, K. Pernezny, G. El-Morsy, and J. W. Scott

First, second, third, and seventh authors: University of Florida, Gulf Coast Research and Education Center, 5007 60th Street E., Bradenton 34203; fourth author: University of Florida, Plant Pathology Department, Gainesville 32611; fifth author: University of Florida, Everglades Research and Education Center, Belle Glade 33430; and sixth author: Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Accepted for publication 8 October 1997.

ABSTRACT

Jones, J. B., Bouzar, H., Somodi, G. C., Stall, R. E., Pernezny, K., El-Morsy, G., and Scott, J. W. 1998. Evidence for the preemptive nature of tomato race 3 of *Xanthomonas campestris* pv. *vesicatoria* in Florida. *Phytopathology* 88:33-38.

Until recently, tomato race 1 (T1) of *Xanthomonas campestris* pv. *vesicatoria* was the only race causing bacterial spot of tomato in Florida. In 1991, tomato race 3 (T3) was first identified in 3 of 13 tomato production fields surveyed. By 1994, T3 was observed in 21 of 28 fields and was the only race identified in 14 fields. In field studies, tomato genotypes with resistance to either T1 or T3 or susceptibility to both were co-inoculated with strains of both races. Lesions on 10 plants in each of three replications for each genotype were sampled three times during the experiment; bacterial isolations were made from each lesion, and tomato race identifications were made for each strain. At the third sampling date,

T3 was isolated from 97% of the lesions on the susceptible genotype Walter and the T1-resistant genotype Hawaii 7998, while T3 was isolated from 23% of the lesions and T1 from the remaining 77% on the T3-resistant genotypes PI 128216 and PI 126932. In surface population studies done in growth rooms, suspensions of T1 and T3 were applied alone and in combination to the leaf surfaces of susceptible and resistant genotypes. T1 populations were reduced more than 10-fold when applied in combination with T3, compared with populations that developed when T1 was applied alone. T3 populations were not affected when applied in combination with a T1 strain. In greenhouse studies with the T3-resistant genotype Hawaii 7981, disease was significantly reduced in plants inoculated with T3 in combination with T1, compared with plants inoculated with T1 alone. These results clearly demonstrate the competitive nature of T3 in the presence of T1 and help explain the emergence of T3 as a prevalent race in Florida.

Bacterial spot of tomato, incited by *Xanthomonas campestris* pv. *vesicatoria*, is an important disease in Florida, causing significant yield losses when weather conditions are optimal (22). Control of this disease is extremely difficult, as chemicals do not work well in wet weather (8,13,14), many strains are resistant to copper compounds (13,17), and the pathogen is endemic in Florida (10). As a result, a program was initiated in the 1980s to identify potential sources of genetic resistance (24). The tomato genotype Hawaii 7998 was found to respond with a hypersensitive reaction when inoculated with typical strains from Florida and was observed to have excellent field resistance to bacterial spot (11). Scott et al. (25,26) initiated a breeding program to incorporate the Hawaii 7998 resistance into commercial genotypes. In 1986, Nagai and Sugimori (20) reported that Hawaii 7998 is susceptible to *X. campestris* pv. *vesicatoria* in Brazil, and in 1988, the strain was determined to be a new race (33). The Florida strains that were incompatible with Hawaii 7998 (11) and the Brazil strain (33) were designated tomato race 1 (T1) and tomato race 2 (T2), respectively (2). Stall et al. (28) determined that the T1 and T2 strains are quite distinct phenotypically and genotypically. Further characterization by Bouzar et al. (1) showed that these two general groups can be differentiated on the basis of protein profiles. T1 strains are in *X. campestris* pv. *vesicatoria* group A, have a 32- to 35-kDa protein band, and exhibit negative or weak amylolytic and pectolytic activity, and T2 strains are in *X. campestris* pv. *vesica-*

toria group B, have a 25- to 27-kDa protein band, and exhibit strong amylolytic and pectolytic activity. Vauterin et al. (32) reclassified the xanthomonads and placed the A and B strains in *X. axonopodis* pv. *vesicatoria* and *X. vesicatoria*, respectively.

T2 strains are found in many countries and at several locations in the United States (3), but T2 was not present in Florida, nor were strains typical of group B found in a collection of *X. campestris* pv. *vesicatoria* strains from Florida isolated over a period of 20 years (4). Thus, the breeding program for field selection of genotypes resistant to T1 strains from crosses made with Hawaii 7998 continued to be successful.

In 1991 an outbreak of bacterial spot in Florida occurred on Hawaii 7998 and genotypes derived from it. Originally thought to be T2, because amylolytic and pectolytic activity placed them in group B, the strains were later identified as a new race (tomato race 3), distinguished by its ability to produce a hypersensitive reaction in several genotypes, including Hawaii 7981 (12). Recently, T3 strains were determined to have more than 70% homology to group A strains and would be considered the same species (J. B. Jones, H. Bouzar, J. Sudbury, and P. M. Strickler, *unpublished data*). T3 strains were originally observed in three fields, including two commercial fields and an experimental field at the Gulf Coast Research and Education Center. T3 strains were found at a very low incidence in the two commercial fields, but at a very high incidence in the experimental field. Efforts to breed for T1 resistance derived from Hawaii 7998 were thwarted by an influx of T3 into the field plots. Furthermore, T3 strains were thought to be more aggressive than T1 in the field, since T3 strains were predominant in plots where the plants had been inoculated with T1 strains only (J. B. Jones, H. Bouzar, J. Sudbury, and P. M. Strickler, *unpublished data*). T3 strains, however, were observed to be anta-

Corresponding author: J. B. Jones; E-mail: jbjones@nersp.nerdc.ufl.edu

Publication no. P-1997-1110-02R

© 1998 The American Phytopathological Society

gonistic to T1 strains in vitro (7) and produce what appears to be a bacteriocin active against T1 strains (30).

This study was undertaken to demonstrate the importance of T3 strains in Florida by surveying fields from 1991 through 1995, comparing T1 and T3 strains in field and greenhouse studies, and assessing the ability of T3 strains to survive in the field on crop refuse.

MATERIALS AND METHODS

Bacterial strains, inoculum production, and inoculation procedure. Bacterial strains used in the experiments were stored long-term at -80°C . Inoculum was produced by growing the bacteria on nutrient agar (NA) for 24 h at 28°C . Bacterial cells were removed from the agar plates and suspended in 10 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and the suspensions were standardized to $A_{600} = 0.15$ (a concentration of approximately 3×10^8 CFU/ml).

Field survey. Plants in tomato production fields were sampled from 1991 to 1995 in the south central, southwest, and southeast tomato production regions of Florida. Leaves with typical bacterial spot lesions were selected at random from the field. Lesions were triturated in sterile distilled water, and the suspension was streaked onto NA. Yellow colonies typical of *X. campestris* pv. *vesicatoria* were selected, and the strains were differentiated by physiological tests (amylolytic and pectolytic activity) as described by Bouzar et al. (3), hypersensitive response (15) on Hawaii 7998 and Hawaii 7981 as described by Jones et al. (12), and indirect enzyme-linked immunosorbent assay (ELISA) (9) reaction to a panel of monoclonal antibodies (MAb) as described by Bouzar et al. (3). This panel included a new monoclonal antibody, designated MAb30, which reacts specifically with T3 strains but not T1 or T2 strains (H. Bouzar and J. B. Jones, unpublished data).

In vitro inhibition of T1 and T3 strains. NA plates were inoculated with one of three T3 strains (XV828, XV938, and XV1220) or one of two T1 strains (XV116 and XV153); 10 μl of a 24-h-old bacterial suspension grown in nutrient broth at 28°C was applied in the center of each plate. The plates were incubated for 48 h at 28°C . Indicator bacteria from 24-h-old cultures grown on NA were suspended in 10 mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, adjusted to 1×10^6 CFU/ml, and streaked from the edge of the plate to within 2 mm of the producer strain, but without contact. The plates were incubated an additional 48 h at 28°C , and then inhibition zones were measured. Each test was replicated three times.

Effect of tomato genotype on incidence of T1 and T3 strains in field studies. The following cultigens were used: Walter (susceptible to T1 and T3), Hawaii 7998 (resistant to T1 and susceptible to T3), and PI 128216 and PI 126932 (susceptible to T1 and resistant to T3). Two days prior to transplanting to the field, the plants were inoculated with a mixture of T1 strains (XV171, XV1113, XV1114, XV1115, XV1116, and XV1117) or a mixture of T3 strains (XV893, XV896, XV1118, XV1130, XV1136, and

XV1141) by misting a suspension on the abaxial and adaxial leaf surfaces. The inoculated plants were placed in clear polyethylene bags and incubated for 36 h at 28°C with 12-h light and 12-h dark periods. Transplants were arranged in a completely randomized block design consisting of three replications. In each block, six T1-inoculated plants and six T3-inoculated plants were alternately planted. One leaflet was collected from the top third of each plant on each sampling date, and individual bacterial spot lesions were triturated in sterile water and plated on NA amended with starch. Amylolytic activity was recorded, and the race was determined by ELISA as previously described (10), using T3-specific MAb30 and T1-specific MAb1. Representative strains were infiltrated into the tomato differentials to determine actual race identity.

A second experiment was conducted, in which the following cultigens were used: Sunny (susceptible to T1 and T3), Campbell 28 (susceptible to T1 and T3), and Hawaii 7998. One panel of a leaflet of each plant was infiltrated with a suspension of a non-amylolytic T1 strain (XV929) by means of a hypodermic syringe. Other leaflets were similarly inoculated with an amylolytic T3 strain (XV824). The inoculated transplants were set in the field in six rows, with 10 plants in each row. The plants were grown in the field approximately 40 days, at which time 50 diseased leaflets from recently infected leaves were randomly selected from each of the three genotypes. Individual bacterial spot lesions from each leaflet were triturated in sterilized water and plated on NA. Amylolytic activity was determined for each strain to determine the number of T1 and T3 strains. Selected strains isolated from each cultigen were tested by ELISA for reaction with MAb30 and tested for race by hypersensitive reaction on differential tomato genotypes (12).

Interaction of T1 and T3 on resistant and susceptible tomato genotypes in growth room studies. Tomato plants of the genotypes Hawaii 7981 (susceptible to T1 and resistant to T3), Hawaii 7998, and Walter were grown in 10-cm pots for about 4 weeks after transplanting. The plants were misted with one of the following treatments: (i) a suspension consisting of a T3 strain

TABLE 2. Inhibition of strains of *Xanthomonas campestris* pv. *vesicatoria* tomato race 1 (T1) by three strains of tomato race 3 (T3) in vitro

Strain	Race	Origin ^y	Inhibition zone reaction to <i>X. campestris</i> pv. <i>vesicatoria</i> group C strain ^z		
			XV828	XV938	XV1220
XV1	T1	Florida	++	++	++
XV12	T1	Florida	+++	+++	+++
XV39	T1	Florida	+	++	+
XV116	T1	Florida	++	++	+
XV127	T1	Florida	+++	+++	++
XV153	T1	Florida	++	++	+++
XV155	T1	Florida	++	++	++
XV225	T1	Guadeloupe	++	++	+
BA26-1	T1	Argentina	++	++	++
XV682	T1	Puerto Rico	++	++	++
XV798	T1	Virgin Islands	+	+	+
XV836	T1	Florida	+	++	++
XV929	T1	Florida	++	++	+
XV1103	T1	Senegal	++	++	+
XV1244	T1	Florida	+	++	+
XV938	T3	Florida	—	—	—
XV828	T3	Florida	—	—	—
XV1220	T3	Thailand	—	—	—

^y XV1103 was provided by N. E. Ahmed, Agricultural Research Cooperation, Gezira Research Station, Wad Medani, Sudan; BA26-1 was provided by B. Canteros, INTA, Bella Vista, Corrientes, Argentina; all other strains were collected by the authors.

^z Nutrient agar was inoculated with the T1 and T3 strains 48 h after application of T3 strains XV828, XV938, and XV1220 to the medium. — = No inhibition; + = inhibition zone less than 5 mm in diameter; ++ = inhibition zone 5 to 10 mm in diameter; +++ = inhibition zone greater than 10 mm in diameter.

TABLE 1. Survey of tomato fields for the prevalence of tomato race 1 (T1) and tomato race 3 (T3) of *Xanthomonas campestris* pv. *vesicatoria*

Year	Number of fields containing T1, T3, or both races ^z			Number (%) of strains isolated	
	T1	T1 + T3	T3	T1	T3
1991	10	3	0	164 (98.8)	2 (1.2)
1992	5	1	3	283 (92.2)	24 (7.8)
1993	4	1	9	77 (45.8)	91 (54.2)
1994	7	7	14	101 (55.2)	82 (44.8)
1995	1	1	5	9 (45.0)	11 (55.0)

^z The races were identified by infiltrating leaflets of tomato genotypes Hawaii 7998, Hawaii 7981, and Walter with bacterial suspensions adjusted to 1×10^8 CFU/ml and determining compatible and incompatible reactions. Strains were also characterized by enzyme-linked immunosorbent assay reactions using monoclonal antibodies that differentiate between T1 and T3 strains.

(XV938) followed by a suspension of a rifampicin-resistant T1 strain (75-3^R); (ii) a suspension of a T1 strain (75-3) followed by a suspension of a rifampicin-resistant T3 strain (XV938^R); (iii) 10 mM MgSO₄·7H₂O followed by a suspension of 75-3^R; or (iv) 10 mM MgSO₄·7H₂O followed by a suspension of XV938^R. The plants were placed in clear polyethylene bags and incubated in a growth chamber at 28°C with 12-h light and 12-h dark periods. Individual leaflets were removed 0, 24, and 48 h after inoculation, placed in 10 ml of sterile 10 mM phosphate buffer in an Erlenmeyer flask, and rotary-shaken for 30 min to dislodge bacteria from the leaf surface. The washings and their 10-fold dilutions were plated on NA amended with rifampicin (75 µg/ml), and the plates were incubated at 28°C. The experiment was repeated three times.

In a separate experiment, foliage of Hawaii 7981 plants (susceptible to T1 and resistant to T3) were misted with 10 mM MgSO₄·7H₂O or a suspension of T3 strain XV938. The buffer-treated plants were then inoculated with a suspension of XV938 or T1 strain 75-3. The plants initially inoculated by misting with XV938 were then sprayed with a suspension of 75-3. They were then placed in clear polyethylene bags, incubated for 36 h in a growth chamber at 28°C, and then unbagged and returned to the growth chamber, in which the relative humidity was greater than 90%. The plants were evaluated for disease by counting the lesions on each leaf, weighing the leaf tissue, and determining the number of lesions per gram of leaf tissue. The experiment was repeated three times.

Survival on crop residue. Sunny tomato transplants were inoculated with XV938^R and placed in a growth room at 28°C with humidity greater than 90% for 36 h. They were then planted in the field at the beginning of the growing season, in early August. Diseased leaves were collected at the end of the season (December 1994), packaged in paper bags, and wrapped in a plastic screen. The packaged leaves were either buried 15 cm below the soil surface or left at the soil surface in a fallow field. The tissue was assayed for the marked strain at the time of placement in the field and 15, 30, 60, 120, 180, and 270 days after placement. The samples were shaken in deionized H₂O on a wrist-action shaker, and serial dilutions of the washings were plated on a selective medium (agar, 15 g/liter; nutrient broth, 2 g/liter; yeast extract, 0.1 g/liter; starch, 4 g/liter; Bravo 720, 15 µl/liter; cycloheximide, 100 mg/liter; and rifampicin, 75 mg/liter). This medium had an efficiency of recovery for the marked strain comparable to that of NA supplemented with rifampicin, and it allowed visual detection of amylolytic activity 2 days after plating. The identity of randomly selected colonies was confirmed by ELISA reaction with MAB30.

In another experiment, Sunny tomato plants infected with XV938^R were incorporated into the soil, and the field was left undisturbed until the following growing season. Weeds and tomato volunteers growing in that field were screened for the presence of XV938^R on the selective medium described above.

RESULTS

Field survey. In the survey of Florida tomato fields, a dramatic shift in the tomato race composition occurred over a 5-year period (Table 1). In 1991, T3 was found only sporadically and at a low frequency (<1%), except in one field at the Gulf Coast Research and Education Center. In 1993 and 1994, the number of T1 and T3 strains was about the same. Few samples were processed in 1995, but the proportion of T3 strains continued to increase.

In vitro inhibition of T1 strains by T3 strains. All T1 strains were inhibited by the three T3 strains (Table 2); however, there was variation in the size of zone of inhibition. T3 strains did not inhibit other T3 strains. When the two T1 strains were spotted in the centers of NA plates, no inhibition zones were observed with any of the 12 T3 and 15 T1 strains tested (data not shown).

Effect of tomato genotype on incidence of T1 and T3 strains in field studies. In Walter tomato coinoculated with T1 and T3,

T3 was the prevalent race in lesions approximately 1 month after transplanting (Table 3). In the two T3-resistant genotypes (PI 128216 and PI 126932), the proportion of T3 lesions was quite high in the first two samplings, but declined to 23% in the final sampling period. In the T1-resistant genotype Hawaii 7998, T3 was isolated from all lesions during the first two sampling periods and from 97% of the lesions during the last sampling period. In the second experiment, T3 strains were isolated from 48 of 50 lesions on the Hawaii 7998 plants, while neither race was isolated from two of the lesions. On Sunny plants, T1 and T3 were isolated from 1 and 49 of 50 lesions, respectively. On Campbell 28 plants, T3 was isolated from 49 of 50 lesions, while the remaining lesion contained neither strain.

Interaction of T1 and T3 strains on resistant and susceptible tomato genotypes in growth room studies. In growth population studies in which Walter tomato plants were inoculated with T1 or T3 individually or in combination, a sharp reduction in surface populations of T1 occurred after 48 h when plants were coinoculated with T1 and T3 (Fig. 1A); however, T3 populations were unaffected by coinoculation with T1. Similar results were observed on Hawaii 7981 plants (Fig. 1B) and Hawaii 7998 plants (Fig. 1C). Similar findings were observed in two other experiments.

In an experiment in which the T3-resistant genotype Hawaii 7981 was inoculated with various combinations of T1 and T3 strains, significantly fewer lesions formed on plants inoculated with T3 followed by T1 than on plants inoculated with MgSO₄ solution and then T1 (Table 4). Plants inoculated with T3 alone had very few lesions. Similar results were found in two additional experiments.

Survival in crop residue. A rifampicin-resistant strain of race T3 (XV938^R) did not survive well in field soil. The population on diseased plant residues placed at the soil surface declined to below the lowest detectable level after 10 months, and the strain could be recovered from similar samples buried in the soil for only up to 2 months after burial (Table 5). Volunteer tomatoes with bacterial spot symptoms were found in the field where tomato plants diseased with strain XV938^R were incorporated in the soil by plowing. Strain XV938^R was recovered from these volunteer plants, but not from weeds growing in the same field.

DISCUSSION

X. campestris pv. *vesicatoria* race T1 has been displaced by race T3 as the prevalent race in many Florida tomato fields. Race shifts of this pathogen in production fields were previously observed in Mexico and Florida (2,21). In 1982, pepper race 2 (P2) was the primary race on pepper in Florida (5), but, by the late 1980s, Pohronezny et al. (21) observed that pepper race 1 (P1) had become dominant in Florida. In 1988, P2 accounted for 89% of the strains, whereas, in 1989, P2 accounted for only 15% of the strains.

Shifts in race populations can occur several ways. Pohronezny et al. (21) explained the sudden shift by the introduction of P1 on seed. They reasoned that since the pepper genotypes grown in

TABLE 3. Recovery of *Xanthomonas campestris* pv. *vesicatoria* tomato race 3 (T3) from lesions on tomato plants inoculated with strains of tomato race 1 (T1) and T3 and transplanted on 17 August

Tomato genotype	Race resistance	Lesions with T3 (%) ^z		
		17 Sep.	13 Oct.	25 Oct.
Walter	None	80	100	97
Hawaii 7998	T1	100	100	97
PI 128216	T3	59	41	23
PI 126932	T3	40	57	23

^z Average percentage recovery of T3 from lesions on plants in three replications, with 10 lesions assayed per replication. The remaining lesions were caused by T1 strains. T1 and T3 strains were identified by starch hydrolysis and by enzyme-linked immunosorbent assay.

Florida at that time were susceptible to all known races, and no selection pressure existed that would result in a race change as observed by Dahlbeck and Stall (6), the new race had probably been introduced on seed. Bouzar et al. (2) speculated that seed contamination was responsible for shifts in *X. campestris* pv. *vesicatoria* races in Mexico. The change in pepper races observed by Pohronezny et al. (21) was dramatic compared with the gradual increase in T3 observed in this study. This may be due to differences in the level of seed transmission of the strains. It is likely that T3 was initially introduced into Florida on seed (12), since it was observed in three different locations in 1991, but was not found among several hundred strains collected over many prior years (4). However, the low incidence of T3 in two of the fields in

1991 (data not shown) suggests that the incidence of T3 on seed was extremely low. Race T1, which has been shown to be endemic in Florida, accounted for most of the disease in the field at that time.

Race shifts also occur when varieties containing new resistance genes are introduced and select for new races that do not contain or have lost the corresponding avirulence gene (18). Kousik and Ritchie (16) noted dramatic race shifts after inoculating resistant pepper genotypes with strains of incompatible races of *X. campestris* pv. *vesicatoria*. In their study, the race changes in P1 strains occurred as a result of the loss of the plasmid carrying *avrBs3* or as a result of insertional inactivation of *avrBs1* by IS476 in a P2 strain. In regard to the shift in tomato races at the Gulf Coast Research and Education Center, where a breeding program for incorporating resistance to T1 was ongoing, T3 strains had a selective advantage over T1 strains in that they lacked *avrRxv*, which reacts with the *Rxv* genes in Hawaii 7998. It is unlikely that T1 mutated to T3, since T3 strains are phenotypically (11) and genotypically (J. B. Jones, H. Bouzar, J. Sudbury, and P. M. Strickler, unpublished data) quite different from T1 strains, and such mutation would require the loss of *avrRxv* (34) from T1 strains and the acquisition of the avirulence gene associated with T3 strains (19). Furthermore, the race shift that occurred throughout the state of Florida could not be the result of the introduction of resistance genes, since all commercial tomato cultivars grown are susceptible to both races.

Some races may also have a competitive advantage over other races in colonizing leaf surfaces. Marco and Stall (17) noted that all P2 strains were copper-resistant, whereas most of the P1 pepper strains were copper-sensitive. Furthermore, Stall et al. (29) determined that *avrBs1* was present in the plasmid carrying copper resistance. With the widespread use of copper in Florida, copper resistance gave P2 strains a competitive advantage in Florida and may have been the reason for the high percentage of P2 strains in Florida (5) prior to 1989. When T3 was initially identified in Florida, strains were sensitive to copper; however, as the organism became more widely distributed in Florida tomato fields, all strains were copper-tolerant (H. Bouzar and J. B. Jones, unpublished data). With most T1 strains in Florida being resistant to copper (17),

Fig. 1. Growth of strains of *Xanthomonas campestris* pv. *vesicatoria* tomato race 1 (T1) and tomato race 3 (T3) when applied alone (after buffer) or in combination to tomato genotypes Walter (susceptible to T1 and T3), Hawaii 7981 (resistant to T3), and Hawaii 7998 (resistant to T1). In dual inoculations, the population of the strain used in the second inoculation is given. The treatments were as follows: T1 inoculum applied immediately before T3 inoculum (○); T3 inoculum applied immediately before T1 inoculum (●); buffer applied immediately before T1 inoculum (■); and buffer applied immediately before T3 inoculum (---). The error bars represent the standard deviations.

TABLE 4. Disease development in tomato genotype Hawaii 7981 treated with $MgSO_4$ or *Xanthomonas campestris* pv. *vesicatoria* tomato race 3 (T3) prior to inoculation with tomato race 1 (T1) in a growth chamber

First treatment ^y	Second treatment	Lesions/g tissue ^z
$MgSO_4$	$MgSO_4$	1.5 a
$MgSO_4$	T3	1.2 a
T3	T1	20.2 b
$MgSO_4$	T1	110.8 c

^y Hawaii 7981 plants (T3-resistant) were misted with 10 mM $MgSO_4$ or a T3 strain suspended in 10 mM $MgSO_4$.

^z Values (averages from one experiment) followed by the same letter are not significantly different according to Duncan's multiple range test, 5% level.

TABLE 5. Recovery of a rifampicin-resistant strain (XV938^R) of *Xanthomonas campestris* pv. *vesicatoria* tomato race 3 (T3) from diseased leaves

Sampling date	T3 population (CFU/ml) recovered from	
	Leaves at the soil surface	Leaves buried in the soil
Day 0	5×10^7	ND ^y
Day 15	$5 \times 10^6 - 1 \times 10^7$	$3 \times 10^5 - 1 \times 10^6$
Day 30	$3 \times 10^6 - 5 \times 10^6$	1×10^3
Day 60	$1 \times 10^5 - 5 \times 10^5$	$<10^2$
Day 120	1×10^3	<10
Day 180	$1 \times 10^3 - 3 \times 10^3$	<10
Day 270 (Sept. 1995)	<10	<10

^y ND = not determined.

^z Below detection level.

acquisition of copper resistance by T3 strains may have been a factor in enabling T3 strains to maintain the competitive advantage over T1 strains in fields where copper bactericides are routinely used. This may help to explain the low incidence of the bacterium in the two commercial fields the first year and the high incidence of T3 strains in the following years.

We demonstrated in this study and previously (7) that T3 strains are antagonistic to T1 in vitro. It appears to be some type of antibiosis rather than an effect on pH, since T1 strains were inhibited when assays were done on phosphate-buffered NA (G. El-Morsy, unpublished data). Tudor et al. (30,31) determined that T3 strains produce a bacteriocin-like substance that has activity against T1 strains. With T3 strains being antagonistic to T1 strains in vitro (7,32) and our in planta studies corroborating the antagonistic properties of T3 strains, the shift in race composition toward T3 strains in Florida may, in part, have resulted from antagonism of T3 strains toward T1 strains. In this study, T3 strains were shown to reduce populations of T1 strains on leaf surfaces of three tomato genotypes coinoculated with strains of both races. In contrast, T1 strains did not significantly affect T3 populations on any of the tomato genotypes. When the T3-resistant genotype was inoculated with a T1 or a T3 strain or coinoculated with strains of both races, it was clear that T3 was inhibitory to T1 from the reduced number of lesions on coinoculated plants, compared with those inoculated with T1 alone. Our field studies also showed that T3 strains are more competitive than T1 strains. When isolations were made from lesions on susceptible plants inoculated with both races, T3 was the predominant strain recovered.

Tudor-Nelson et al. (31) determined that more than one bacteriocin is produced by T3 strains, but their role in the interaction between T1 and T3 strains is not known. Bacteriocin-producing strains may have a competitive advantage over nonproducing strains. Treatment of rice plants with an avirulent, bacteriocinogenic strain of *X. oryzae* pv. *oryzae* reduced bacterial blight incidence and disease severity when plants were inoculated with bacteriocin-sensitive strains (23). Smidt and Vidaver (27) observed a significant reduction in a bacteriocin-sensitive strain of *Pseudomonas syringae* when red kidney bean stems were coinoculated with this strain and a bacteriocin-producing strain.

In the survival studies, the T3 strain was detected approximately 6 months after diseased tissue was placed in the field; however, T3 did not appear to survive long enough to be a problem on the following crop. Although the bacterium was recovered from volunteer tomatoes, it was not detected on weed species present in the field. Because neither T1 nor T3 strains appear to survive from one growing season to the next (10), the increased prevalence of T3 compared with T1 in production areas cannot be explained by different survival capabilities, but it appears to be the result of the competitive nature of T3 in the presence of T1.

ACKNOWLEDGMENTS

Florida Agricultural Experiment Station Journal Series R-05433. We thank R. O. Kelly for his technical assistance. This research was supported by grant 93-34135-8606 from the U.S. Department of Agriculture Special Research Grants, Tropical and Subtropical Agricultural Research agreement.

LITERATURE CITED

- Bouzar, H., Jones, J. B., Minsavage, G. V., Stall, R. E., and Scott, J. W. 1994. Proteins unique to phenotypically distinct groups of *Xanthomonas campestris* pv. *vesicatoria* revealed by silver staining. *Phytopathology* 84:39-44.
- Bouzar, H., Jones, J. B., Somodi, G. C., Stall, R. E., Daouzli, N., Lambe, R. C., Felix-Gastelum, R., Trinidad-Correa, R. 1996. Diversity of *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper fields of Mexico. *Can. J. Plant Pathol.* 18:75-77.
- Bouzar, H., Jones, J. B., Stall, R. E., Hodge, N. C., Minsavage, G. V., Benedict, A. A., and Alvarez, A. M. 1994. Physiological, chemical, serological, and pathogenic analyses of a worldwide collection of *Xanthomonas campestris* pv. *vesicatoria* strains. *Phytopathology* 84:663-671.
- Canteros, B. I. 1990. Diversity of plasmids and plasmid-encoded phenotypic traits in *Xanthomonas campestris* pv. *vesicatoria*. Ph.D. dissertation. University of Florida, Gainesville.
- Cook, A. A., and Stall, R. E. 1982. Distribution of races of *Xanthomonas vesicatoria* pathogenic on pepper. *Plant Dis.* 66:388-389.
- Dahlbeck, D., and Stall, R. E. 1979. Mutations for change of race in cultures of *Xanthomonas vesicatoria*. *Phytopathology* 69:634-636.
- El-Morsy, G. A., Somodi, G. C., Scott, J. W., Stall, R. E., and Jones, J. B. 1994. Aggressiveness of *Xanthomonas campestris* pv. *vesicatoria* tomato race 3 (T3) strains over tomato race 1 (T1) strains: Evidence for antagonism. (Abstr.) *Phytopathology* 84:1094.
- Jones, J. B., and Jones, J. P. 1985. The effect of bactericides, tank mixing time and spray schedule on bacterial leaf spot of tomato. *Proc. Fla. State Hortic. Soc.* 98:244-247.
- Jones, J. B., Minsavage, G. V., Stall, R. E., Kelly, R. O., and Bouzar, H. 1993. Genetic analysis of a DNA region involved in expression of two epitopes associated with lipopolysaccharide in *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* 83:551-556.
- Jones, J. B., Pohronezny, K. L., Stall, R. E., and Jones, J. P. 1986. Survival of *Xanthomonas campestris* pv. *vesicatoria* in Florida on tomato crop residue, weeds, seeds, and volunteer tomato plants. *Phytopathology* 76:430-434.
- Jones, J. B., and Scott, J. W. 1986. Hypersensitive response in tomato to *Xanthomonas campestris* pv. *vesicatoria*. *Plant Dis.* 70:337-339.
- Jones, J. B., Stall, R. E., Scott, J. W., Somodi, G. C., Bouzar, H., and Hodge, N. C. 1995. A third tomato race of *Xanthomonas campestris* pv. *vesicatoria*. *Plant Dis.* 79:395-398.
- Jones, J. B., Woltz, S. S., Jones, J. P., and Portier, K. L. 1991. Population dynamics of *Xanthomonas campestris* pv. *vesicatoria* on tomato leaflets treated with copper bactericides. *Phytopathology* 81:714-719.
- Jones, J. B., Woltz, S. S., Kelly, R. O., and Harris, G. 1991. The role of ionic copper, total copper, and select bactericides on control of bacterial spot of tomato. *Proc. Fla. State Hortic. Soc.* 104:257-259.
- Klement, Z. 1982. Hypersensitivity. Pages 149-177 in: *Phytopathogenic Prokaryotes*. Vol. 2. M. S. Mount and G. H. Lacy, eds. Academic Press, New York.
- Kousik, C. S., and Ritchie, D. F. 1996. Race shift in *Xanthomonas campestris* pv. *vesicatoria* within a season in field-grown pepper. *Phytopathology* 86:952-958.
- Marco, G. M., and Stall, R. E. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. *Plant Dis.* 67:779-781.
- Mew, T. W., Vera Cruz, C. M., and Medalla, E. S. 1992. Changes in race frequency of *Xanthomonas oryzae* pv. *oryzae* in response to rice cultivars planted in the Philippines. *Plant Dis.* 76:1029-1032.
- Minsavage, G. V., Jones, J. B., and R. E. Stall. 1996. Cloning and sequencing of an avirulence gene (*avrRxv3*) isolated from *Xanthomonas campestris* pv. *vesicatoria* tomato race 3. (Abstr.) *Phytopathology* (Suppl.) 86:S15.
- Nagai, H., and Sugimori, M. H. 1986. Suscetibilidade dos tomateiros 'H 7998' e 'C-28' a pústula bacteriana (*Xanthomonas campestris* pv. *vesicatoria*) em São Paulo. (Abstr.) *Hortic. Bras.* 4:62.
- Pohronezny, K., Stall, R. E., Canteros, B. I., Kegley, M., Datnoff, L. E., and Subramanya, R. 1992. Sudden shift in the prevalent race of *Xanthomonas campestris* pv. *vesicatoria* in pepper fields in southern Florida. *Plant Dis.* 76:118-120.
- Pohronezny, K., and Volin, R. B. 1983. The effect of bacterial spot on yield and quality of fresh market tomatoes. *HortScience* 18:69-70.
- Sakthivel, N., and Mew, T. W. 1991. Efficacy of bacteriocinogenic strains of *Xanthomonas campestris* pv. *oryzae* on the incidence of bacterial blight disease of rice (*Oryza sativa* L.). *Can. J. Microbiol.* 37:764-768.
- Scott, J. W., and Jones, J. B. 1986. Sources of resistance to bacterial spot [*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye] in tomato. *HortScience* 21:304-306.
- Scott, J. W., Jones, J. B., and Somodi, G. C. 1991. Disease severity of tomato hybrids heterozygous or homozygous for resistance to bacterial spot: commercial outlook. *Proc. Fla. State Hortic. Soc.* 104:259-262.
- Scott, J. W., Somodi, G. C., and Jones, J. B. 1989. Genetic resistance to bacterial spot in tomato. Pages 200-207 in: *Proc. Tomato and Pepper Production in the Tropics*. S. K. Green, ed. AVRDC, Shanhuai, Tainan, Taiwan.
- Smidt, M. L., and Vidaver, A. K. 1982. Bacteriocin production by *Pseudomonas syringae* PS W-1 in plant tissue. *Can. J. Microbiol.* 28:600-604.
- Stall, R. E., Beaulieu, C., Egel, D., Hodge, N. C., Leite, R. P., Minsavage, G. V., Bouzar, H., Jones, J. B., Alvarez, A. M., and Benedict, A. A. 1994. Two genetically diverse groups of strains are included in a pathovar of *Xanthomonas campestris*. *Int. J. Syst. Bacteriol.* 44:47-53.

29. Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* 76:240-243.
30. Tudor, S. M. 1995. An analysis of antagonism in tomato race three strains of *Xanthomonas campestris* pv. *vesicatoria*. M.Sc. thesis. University of Florida, Gainesville.
31. Tudor-Nelson, S. M., Jones, J. B., Minsavage, G. V., and Stall, R. E. 1995. Characterization of antagonism of tomato race 3 strains of *Xanthomonas campestris* pv. *vesicatoria* to other strains of the same bacterium. (Abstr.) *Phytopathology* 85:1148.
32. Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. *Int. J. Syst. Bacteriol.* 45:472-489.
33. Wang, J. F., Jones, J. B., Scott, J. W., and Stall, R. E. 1990. A new race of the tomato group of strains of *Xanthomonas campestris* pv. *vesicatoria*. (Abstr.) *Phytopathology* 80:1070.
34. Whalen, M. C., Wang, J. F., Carland, F. M., Heiskell, M. E., Dahlbeck, D., Minsavage, G. V., Jones, J. B., Scott, J. W., Stall, R. E., and Staskiewicz, B. J. 1993. Avirulence gene *avrRxv* from *Xanthomonas campestris* pv. *vesicatoria* specifies resistance on tomato line Hawaii 7998. *Mol. Plant-Microbe Interact.* 6:616-627.