A Third Tomato Race of *Xanthomonas campestris* pv. *vesicatoria*

J. B. JONES, Gulf Coast Research & Education Center, University of Florida, 5007 60th Street East, Bradenton 34203; R. E. STALL, Department of Plant Pathology, University of Florida, Gainesville 32611; J. W. SCOTT, G. C. SOMODI, and H. BOUZAR, Gulf Coast Research & Education Center, University of Florida, 5007 60th Street East, Bradenton 34203; and N. C. HODGE, Department of Plant Pathology, University of Florida, Gainesville 32611

ABSTRACT

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Strains of *Xanthomonas campestris* pv. *vesicatoria* were isolated from several tomato fields in Florida that differed from tomato race 1 (T1) strains commonly found in Florida. The strains produced a compatible reaction on tomato genotype Hawaii 7998 (H7998), were amylolytic and pectolytic, and were classified originally as T2 strains. However, these strains produced a rapid hypersensitive response when infiltrated into the tomato genotypes Hawaii 7981 (H7981), PI 126932, and PI 128216, whereas T2 strains produced a compatible reaction. In an experiment where electrolyte leakage was determined in leaflets of tomato cv. Bonny Best (compatible with all tomato strains), H7998 (resistant to T1 strains), and the two PIs, a member of these new strains induced a rapid hypersensitive response in the three tomato genotypes, whereas a T2 strain did not. After low concentrations of bacteria were infiltrated into the mesophyll of leaflets of PI 126932, PI 128216, H7998, and cv. Walter (compatible with all tomato strains), internal populations of the amylolytic, pectolytic Florida strain were reduced when tested in the first two genotypes but not in the latter two genotypes. Populations of the T1 strain were reduced only in H7998. These new strains are designated tomato race 3 (T3). When the T3 strains were compared with a representative group of T1 and T2 strains by fatty acid analysis and carbon substrate utilization patterns, the new T3 strains clustered distinctly from representative T1 and T2 strains. The evidence suggests the T3 strain is a recent introduction to Florida. An amylolytic, pectolytic strain isolated from seed grown in Thailand reacted similarly to the T3 strains on the tomato differentials. It also clustered with the T3 strains in the fatty acid and carbon substrate utilization dendrograms.

Bacterial spot of tomato, incited by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye, is a major problem in tomato production areas where high temperature and moist conditions prevail (15). Because the pathogen is endemic to Florida (10) and control with bactericides is difficult to achieve (9), recent emphasis has been placed on developing varieties with high levels of resistance to bacterial spot (19,20). The possibility of success in breeding for resistance to this bacterial pathogen has been strengthened by the availability of a high level of resistance in Hawaii 7998 (H7998) (19,20), the first tomato genotype discovered in which *X. c. vesicatoria* induced a hypersensitive reaction (11).

Prior to 1989, all strains collected in Florida and from other areas of the world induced a hypersensitive reaction on H7998 (11). However, a strain from Brazil was identified that did not induce a hypersensitive reaction on H7998 (22,23). Using the race nomenclature of Bouzar et al (4), this strain was identified as tomato race 2 (T2). Eventually other T2 strains were identified from several locations around the world (4,21). T2 strains were phenotypically different from race 1 (T1) strains (3,4,8). Among other characteristics, T2 strains were amylolytic and pectolytic, whereas T1 strains were not (4,21). No tomato genotypes were identified that induced an incompatible reaction with T2 strains. Thus, all strains that were amylolytic and pectolytic and also produced a compatible reaction on H7998 were identified as T2. T2 strains, although prevalent in South America and found more sporadically in a worldwide collection than T1 strains, have been isolated from several locations outside of Florida in the United States (4).

Since 1991, amylolytic and pectolytic strains of *X. c. vesicatoria* increasingly have been isolated in Florida, and in particular, at the Gulf Coast Research and Education Center, Bradenton, FL, these type of strains have been isolated numerous times. The strains were identified initially as T2 because they produced a compatible reaction on the tomato genotype H7998. In this paper, we present results that identify these strains as a new race, based on their ability, unlike T1 or T2 strains, to induce a hypersensitive reaction on three tomato genotypes. We also present information that these strains form a distinct phenotypic group based on bacterial fatty acid profiles and utilization of carbon substrates.

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MATERIALS AND METHODS

Bacterial strains. Strains used in this study were grown on nutrient agar (NA) or trypticase soy broth agar at 28 C for 24 h prior to analysis. All T1 and T2 strains (Table 1) were representative of the diversity within *X. c. vesicatoria* as reported previously (4).

Plant materials and growth of plants. For electrolyte leakage and population dynamics studies, seed of tomato genotypes H7998, PI 126932, PI 128216, and H7981, and cvs. Bonny Best and Walter were planted in Plug-mix (W. R. Grace & Co., Cambridge, MA), and the emerged seedlings were transferred after 2 wk to Metromix 300 (W. R. Grace) in 10-cm plastic pots. Seedlings were grown in a greenhouse at temperatures ranging from 25 to 35 C (night/day). A soluble 20-20-20 (N-P_2O_5-K_2O) fertilizer (W. R. Grace) was added to the pots at 0.4 g per pot every 2 wk. Plants were transplanted after 2 wk and grown for 4 wk in 15-cm plastic pots, and about 1 wk later, the main stem was removed above the fully expanded sixth true leaf. Approximately 7 days after topping, plants were inoculated and transferred to a growth chamber kept at 24 C with a daily 16-h light period.

Hypersensitive reaction. Three methods were used to evaluate the hypersensitive reaction in the tomato genotypes: 1) determination of the time necessary to reach confluent necrosis (complete tissue collapse in infiltrated area) in leaflets infiltrated with high concentrations of a bacterial suspension; 2) measurement of electrolyte leakage over time in leaflet tissue infiltrated with high concentrations of a bacterial suspension; and 3) measurement of internal bacterial populations in leaf tissue infiltrated initially with low populations of the bacterium. For visual assessment of the development of rapid confluent necrosis (<24 h), leaflets were infiltrated with 10^8 cfu/ml of sterile deionized water using a hypodermic syringe (12). Inoculated plants were incubated in a growth room at 24 C. Plants were evaluated at 24, 48, and 72 h for confluent necrosis.

Electrolyte leakage was determined in leaflets of several tomato genotypes by

Table 1. Strains of *Xanthomonas campestris* pv. *vesicatoria* compared in this study, their tomato race identity, and sensitivity to copper and streptomycin

Strain	Source[a]	Year	Location	Race	Cu[b]	Str[b]
XV1147, XV1149, XV1150, XV1153, XV938, XV1156, XV1157, XV1159, XV1160, XV1162	Authors	1991	GCREC[c]	3	0	0
T12, T13	K. Pernezny	1991	Palm Beach Co., FL	3	0	0
91-103	Authors	1991	Manatee Co., FL	3	0	0
XV1129, XV1132, XV1133, XV1136, XV1137, XV1138, XV1127, XV1129, XV1139, XV1141	Authors	1992	GCREC	3	0	0
XV1119, XV1121, XV1122	Authors	1992	Manatee Co., FL	3	0	0
BV5-3A, BV5-4A, BV5-4B, BV20-3A, BA27-1, BA28-1, 0350, ATCC35937, 81-6, X525-85, 8020, 0350	Bouzar et al (4)	...	...	2	3	4
62, 75-3, 85-16, 86-22, 86-46, 87-13, 87-21, 87-44 87-47, 87-56, 89-10, ECW11, A2, 91-68, 91-77, 91-79, 820, BA26.1, 123	Bouzar et al (4)	...	...	1	8	11
7B-0-1	W. Wiebe	1992	Tomato seed	3	0	0

[a] K. L. Pernezny, University of Florida, EREC, Belle Glade; W. Wiebe, Rogers Seed Co., Woodland, CA.
[b] Gulf Coast Research and Education Center, Bradenton, FL.
[c] Value represents the number of strains out of the total tested that was positive for growth on media containing streptomycin (Str) or copper (Cu).

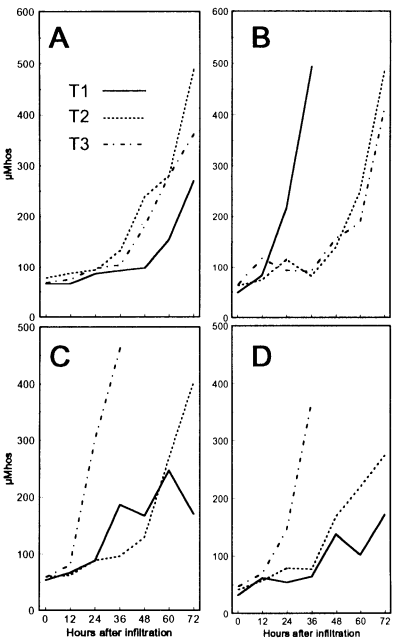

Fig. 1. Effect of infiltrating 10^8 cfu/ml of *Xanthomonas campestris* pv. *vesicatoria* tomato strains T1, T2, or T3 on electrolyte leakage from tomato leaflets of **(A)** Walter, **(B)** Hawaii 7998, **(C)** PI 126932, and **(D)** PI 128216.

infiltrating inoculum, consisting of 5×10^8 cfu/ml of a T1 (75-3), T2 (XV56), or amylolytic, pectolytic Florida strain (XV938), into approximately 5 cm^2 of leaf area as described above. Electrical conductivity of water in baths containing inoculated tissues was determined as previously described (7), with samples taken every 12 h after infiltration. Each treatment was replicated three times.

In bacterial population studies, approximately 2 cm^2 of a leaflet of the most recently developed mature leaf were infiltrated with a bacterial suspension containing 5×10^5 cfu/ml of a T1 strain and an amylolytic, pectolytic Florida strain in tests to determine populations in inoculated leaves. Populations were determined on each of three leaflets five times over a 14-day period by a dilution plate method as described previously (7).

Amylolytic and pectolytic activity. Starch degradation was determined on nutrient agar containing 1% soluble starch. Starch hydrolysis was evidenced by an opaque zone surrounding bacterial growth in the medium. For confirmation, plates were flooded with Lugol's iodine, and clear zones confirmed that the starch had been hydrolyzed.

Pectolytic activity was assayed on modified crystal violet-pectate medium (18) devoid of crystal violet and thallium nitrate (1). After incubation for several days at 28 C, a depression developed in the medium surrounding the growth of each pectolytic strain.

Carbon substrate utilization. The ability of the test strains to utilize 95 compounds as sole carbon sources was determined using the Biolog GN MicroPlate (Biolog, Inc., Hayward, CA). Strains were compared with selected representative *X. c. vesicatoria* strains (Table 1) that were determined in a previous study (4) to have diverse reaction patterns on GN MicroPlates. Processing of the strains and cluster analysis were performed as described previously (2).

Fatty acid analysis. Procedures used to determine fatty acid profiles of bacterial strains have been described previously (2,16,17). The fatty acid profiles of strains were compared with those of selected strains of a representative *X. c. vesicatoria* population. A dendrogram that represented clusters of the profiles was constructed with Microbial Identification System software (version 3.6; MIDI, Newark, DE).

Sensitivity to streptomycin and copper. Sensitivity of all strains to streptomycin and copper was assayed by streaking the strains on NA amended with streptomycin or $CuSO_4 \cdot 5H_2O$ at 200 µg/ml each. Growth on these media was compared with growth on unamended NA.

RESULTS

In 1991 and 1992, 26 amylolytic and pectolytic strains of *X. c. vesicatoria* were collected from tomato in one field on the east coast of Florida and several tomato fields on the west coast of Florida (Table 1). An additional amylolytic, pectolytic strain isolated originally from seed produced in Thailand also was obtained. All 27 strains were sensitive to copper and streptomycin. All strains induced a compatible reaction on H7998 and Walter when infiltrated at 10^8 cfu/ml. One strain, XV938, was selected and infiltrated into a number of tomato genotypes with low levels of resistance to T2 strains. Rapid confluent necrosis was induced in plants of PI 126932 and PI 128216, whereas a T1 strain, 75-3, did not induce rapid confluent necrosis. A third genotype, H7981, was later identified that developed rapid confluent necrosis when infiltrated with high numbers of cells of strain XV938 but not when infiltrated with high numbers of the T1 or T2 strains. When the 27 strains (including the strain from Thailand) were compared with strains 75-3 and XV56 (T2), only the 27 strains induced rapid (<24 h) confluent necrosis on the three tomato genotypes (H7981, PI 126932, and PI 128216). The T1 strain induced rapid confluent necrosis in H7998, whereas none of the strains induced rapid confluent necrosis when infiltrated in the susceptible cultivars, Bonny Best and Walter.

In the electrolyte leakage analysis, the T1 strain produced a sharp increase in electrolyte leakage after 24 h in H7998 (Fig. 1). Strain XV938 (T3) produced a sharp increase in electrolyte leakage after 24 h in PI 126932 and PI 128216, whereas the T1 and T2 strains did not. Maximum internal populations of the T1 strain were 1-2 log units lower in leaflets of H7998 than in the other genotypes (Fig. 2). Strain XV938 reached a concentration greater than 7 log units in leaflets of

H7998 and Walter and 1 log unit lower in PI 128216. PI 126932 had a population level intermediate between PI 128216 and the other two genotypes.

Cluster analysis of carbon substrate utilization patterns revealed two clusters of the amylolytic, pectolytic strains (T3) within six dendrogram distance units (Fig. 3). The strain collected from seed produced in Thailand was within the top large cluster of strains. Only one T3 strain (XV1160) did not cluster with the other T3 strains. There was a small cluster of five T1 strains that was closely related to the two T3 clusters. The remainder of the T1 and T2 strains were interspersed throughout the dendrogram. With fatty acid analysis, the amylolytic strains formed one tight cluster that was distinct from the T1 and T2 strains (Fig. 4). Again, the strain from Thailand was in that large group.

DISCUSSION

In this study, a group of strains of *X. c. vesicatoria* was distinguished based on the differential reactions on tomato genotype H7998 and three other tomato genotypes, H7981, PI 126932, and PI 128216. The fact that the new group, but not the T1 and T2 strains, caused rapid confluent necrosis, as measured by electrolyte leakage and lower internal populations in the latter three genotypes (data not presented for H7981), allows for it to be designated as a new race, T3. This new group of strains causes a strong hypersensitive reaction on all types of pepper (*Capsicum* spp.); therefore, it is a tomato race (13) and is designated as tomato race 3 (T3).

This group of strains can be distinguished by fatty acid profiles and carbon utilization profiles, which probably means that this group has significant genetic differences from the T1 and T2 groups, whereas strains in the T1 and T2 groups only have negligible DNA homology (21) even though both groups cause bacterial spot symptoms on tomato and pepper. The genetic relationship of the T3 group to the T1 and T2 groups has not been determined.

There is evidence that T3 is a recent introduction into Florida. First, in a survey of over 200 strains collected in Florida over 20 yr (5), not one pathogenic, amylolytic, pectolytic strain was isolated. Also, we previously screened a large collection of strains from the Caribbean as well as a worldwide collection and did not find T3. Furthermore, the T3 strains appear to be fairly homogeneous with regard to fatty acid and carbon substrate utilization patterns. Carbon substrate utilization and fatty acid profiles of T1 and T2 strains from diverse geographic regions showed considerable diversity among strains. The uniformity of the T3 group compared to the T1 and T2 strains tends to support the hypothesis that they are a recent introduction from a specific region. The fact that the T3 strain was isolated from seed produced in Thailand and that it clustered with the T3 strains based on fatty acid and carbon substrate utilization patterns tends to suggest that Thailand could be a possible source of this new race. We have very few strains from mainland Asia, although some were obtained from South Korea and Taiwan. Thus, extensive sampling of mainland Asia for the bacterial spot pathogen could be useful for determining the distribution of T3 strains.

The change in races of *X. c. vesicatoria* in Florida without host selection is not new. Cook and Stall (6) determined that the pepper race 2, which is now designated P2, was formerly the predominant race in Florida. Pohronezny et al (14) noted a dramatic shift toward pepper race 1 (P1) in recent years. The shift was not apparently due to a major increase

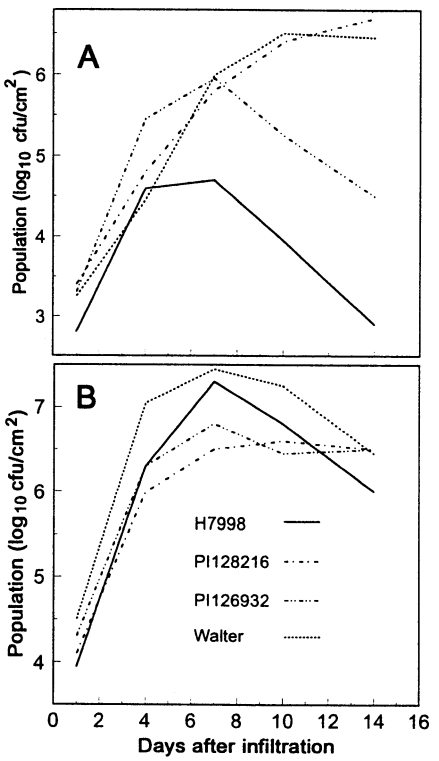

Fig. 2. Population dynamics of *Xanthomonas campestris* pv. *vesicatoria* tomato strains **(A)** T1 and **(B)** T3 when infiltrated at 10^5 cfu/ml into leaflets of four tomato genotypes: Hawaii 7998, PI 128216, PI 126932, and Walter.

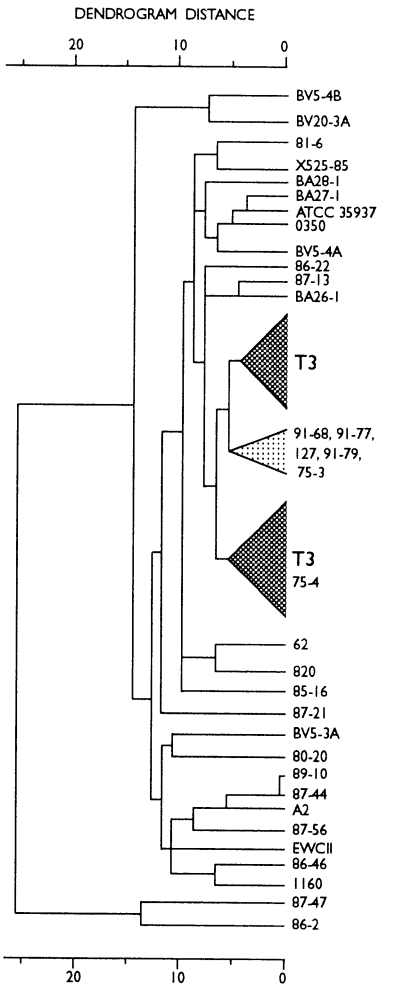

Fig. 3. Cluster analysis of tomato strains T3 of *Xanthomonas campestris* pv. *vesicatoria* and representative T1 and T2 strains based on the differential utilization of the 95 carbon substrates available on the Biolog GN MicroPlate.

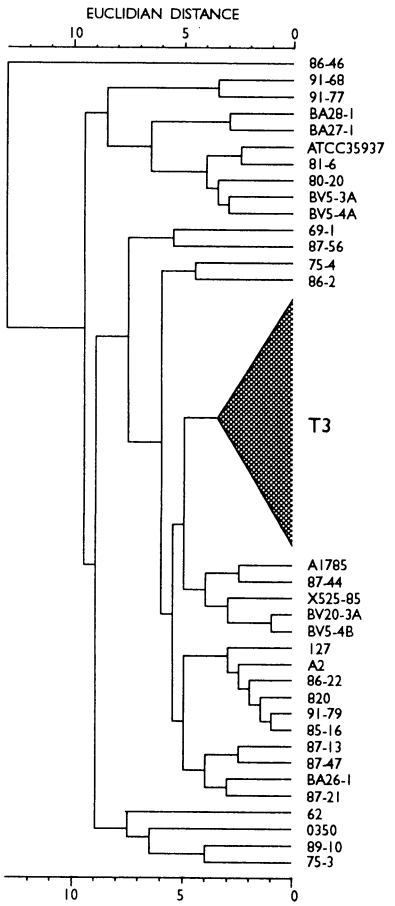

Fig. 4. Cluster analysis of tomato strains T3 of *Xanthomonas campestris* pv. *vesicatoria* and representative T1 and T2 strains based on fatty acids composition data.

of pepper cultivars resistant to P2. They hypothesized that it may have been introduced via infested seed. A similar situation appears to have occurred with the establishment of T3 in Florida.

The designation of this new group of strains is significant, because a high level of resistance has been identified. A breeding program has been initiated to incorporate the gene(s) for resistance to the T3 strains into commercial tomato genotypes. Crosses have been made to determine the inheritance of the hypersensitive reaction to T3 strains. If the inheritance is simple, as expected for such a resistant reaction, a backcross program will be used to transfer the resistance into commercially acceptable cultivars.

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