

Hypersensitive Response in Tomato to *Xanthomonas campestris* pv. *vesicatoria*

J. B. JONES and J. W. SCOTT, Gulf Coast Research and Education Center, University of Florida, 5007 60th Street East, Bradenton 34203

ABSTRACT

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A genotype of *Lycopersicon esculentum*, Hawaii 7998, produced a hypersensitive reaction when leaflets were infiltrated with cells of *Xanthomonas campestris* pv. *vesicatoria* (*X. c. vesicatoria*). Electrolyte leakage increased in Hawaii 7998 12 hr after infiltration with 10^8 cfu/ml, whereas no increase was observed in Walter (known susceptible) after 24 hr. When leaflets of Hawaii 7998 were infiltrated with 10^8 , 10^7 , 10^6 , or 10^5 cfu/ml, electrolyte leakage greatly increased in leaflets 24 hr after infiltration with the first two inoculum levels but only slightly with 10^6 . Two tolerant tomato genotypes (Campbell 28 and Ohio 4013) reacted similarly to Walter in electrolyte leakage. Population levels of *X. c. vesicatoria* in Hawaii 7998 leaflets were 50–100 times lower than in leaflets of Walter or any of the tolerant genotypes when inoculated with 10^5 cfu/ml. Leaflets of the same resistant and susceptible genotypes infiltrated with 10^8 cfu/ml of the bacterium did not differ greatly in bacterial populations after infiltration.

Bacterial spot of tomato (*Lycopersicon esculentum*) Mill. incited by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye (*X. c. vesicatoria*) is one of the most destructive diseases in Florida. Under conditions of high temperature and frequent rain, yield losses can be significant (17), and bactericides provide marginal control. With many crops, resistant genes have been detected that may be useful for controlling bacterial pathogens (1,7,10). In previous studies, a degree of resistance to bacterial spot and bacterial speck of tomato was observed (3,4,13–15,20), but incorporation of this polygenic resistance into commercial tomato cultivars has been difficult (4,24). Therefore, *L. esculentum* cultivars currently grown have no appreciable level of resistance to bacterial spot (18).

Recently, Scott and Jones (19) tested about 300 *Lycopersicon* accessions with reported resistance to bacterial pathogens (2–4,13,20). Several lines significantly reduced disease severity, and one line, Hawaii 7998, apparently had absolute (vertical) resistance to bacterial spot.

Vanderplank (23) categorized plant resistance into two types: vertical resistance, defined as resistance to some races of a given pathogen, and horizontal resistance, defined as equal resistance to all races of a pathogen. The hypersensitive reaction has been viewed as a monogenically controlled resistance mechanism (6,7,11,12) and as vertical resistance (6). Thus

a method useful for screening for vertical resistance is detection of a hypersensitive reaction (6).

Three characteristics of hypersensitivity are commonly used for substantiating that a particular plant produces a hypersensitive reaction to a bacterial pathogen: rapid development of confluent necrosis in the resistant line (11,12), rapid increase in electrolyte leakage in resistant leaves compared with susceptible leaves shortly after infiltration, and lower bacterial populations in resistant leaves compared with susceptible leaves.

The purpose of this study was to determine if the resistance of Hawaii 7998 was associated with a hypersensitive response and to compare this resistance with sources of horizontal resistance previously reported (19).

MATERIALS AND METHODS

Inoculum was prepared by culturing the *X. c. vesicatoria* strains on nutrient-yeast-dextrose agar (8) for 48 hr at 28 C. Bacterial cells were washed from plates and suspended in 0.01 M $MgSO_4 \cdot 7H_2O$. The suspension was adjusted to 10^8

cfu/ml as determined via turbidimetric readings on a spectrophotometer. Other concentrations of bacteria used in this study were obtained by dilution of the 10^8 -cfu/ml suspension with the $MgSO_4$ solution.

Leaflets of Hawaii 7998 and Walter, a known susceptible cultivar (19), were infiltrated with a suspension of *X. c. vesicatoria* using a hypodermic syringe (11) without the needle with 10^8 , 10^7 , 10^6 , or 10^5 cfu/ml. Inoculated plants were kept in a growth room at 28 C and at 600–800 ft-c; 6, 12, and 24 hr after infiltration, three leaflets per inoculum concentration were collected. Three 7-mm-diameter disks were removed from each leaflet and suspended in 3 ml of deionized water. Electrolyte leakage from the disks was determined as described previously (22), except electrolyte leakage was measured only 1 hr after shaking. All values reported represent the means of three replicates.

Electrolyte leakage from Hawaii 7998 also was compared with that of Walter and two tomato genotypes, Campbell 28 and Ohio 4013, reportedly tolerant to bacterial spot (19). The four genotypes were infiltrated with 10^8 cfu/ml by vacuum-infiltration (9). Plants were placed in a growth room at 28 C. After 3, 6, 12, 24, and 48 hr, leaflets were removed and checked for electrolyte leakage. All values represent the means of three replicates.

Populations of bacteria were determined in leaflets of Hawaii 7998 and in leaflets of tomato lines varying in degree of susceptibility to bacterial leaf spot (Table 1). Leaflets were inoculated by infiltration of 10^5 or 10^8 cfu/ml of an *X. c. vesicatoria* mutant resistant to rifampicin and streptomycin. After inoculation, the

Table 1. Populations of *Xanthomonas campestris* pv. *vesicatoria* in leaflets of resistant and susceptible tomato lines when infiltrated with 10^5 cfu/ml of the pathovar

Line	Disease severity ^a	Days after infiltration				
		0	2	4	7	15
Lycorma	7.00	6.8×10^{2b}	3.7×10^5	6.8×10^6	1.5×10^7	6.7×10^7
Walter	6.00	4.9×10^2	4.0×10^5	3.2×10^6	5.7×10^6	7.5×10^7
Monense	4.38	7.3×10^2	4.5×10^5	6.6×10^6	1.2×10^7	7.5×10^7
Hawaii 2990	4.38	5.0×10^2	3.3×10^5	2.8×10^6	1.6×10^7	2.7×10^7
PI 127813	3.88	5.5×10^2	3.8×10^5	5.9×10^6	6.7×10^6	6.9×10^7
([SAD × MH1] × H603)F ₅	3.50	3.9×10^2	3.4×10^5	3.6×10^6	1.9×10^7	7.6×10^7
Campbell 28	3.18	4.9×10^2	4.0×10^5	4.4×10^6	8.9×10^6	5.8×10^7
Heinz 1568	3.13	3.2×10^2	3.6×10^5	7.1×10^6	1.6×10^7	1.0×10^8
Hawaii 7998	2.00	3.2×10^2	8.8×10^4	2.4×10^5	1.2×10^5	1.7×10^5

^aRatings were from field plots in fall 1983.

^bInternal populations in cfu/cm².

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plants were placed in a 12-hr-day/12-hr-night growth room at 28 C. Inoculated leaflets were collected periodically. Two 1.54-cm² disks from each leaflet were macerated in 2 ml of phosphate buffer, pH 6.8. Tenfold serial dilutions were made, and 0.1 ml of the appropriate dilution was plated on nutrient-yeast-dextrose agar amended with rifampicin, streptomycin, and cycloheximide at 50, 50, and 100 µg/ml, respectively.

Forty-eight-hour cultures of 42 strains of *X. c. vesicatoria* isolated from pepper and tomato were individually injected at 10⁸ cfu/ml into leaflets of H7998 and Walter. Plants were placed in the growth room at 28 C and checked for a hypersensitive reaction (confluent necrosis) after 16 and 24 hr.

RESULTS

All 42 strains of *X. c. vesicatoria* produced hypersensitive reactions on Hawaii 7998 16 hr after infiltration.

An increase in electrolyte leakage occurred within 12 hr in leaflets of Hawaii 7998 infiltrated with 10⁸ cfu/ml (Table 2). At 10⁷, there was an increase in electrolyte leakage within 24 hr in Hawaii 7998. Within 24 hr, a slight increase in electrolyte leakage in Hawaii 7998 was observed in leaflets injected with 10⁶ cfu/ml. Electrolyte leakage did not increase within the same time frame in Walter leaflets at any concentration. In comparative studies on electrolyte leakage with leaflets of Walter, Hawaii 7998, and the two moderately resistant genotypes, electrolyte leakage increased only in Hawaii 7998 within 24 hr (Table 3). Electrolyte leakage was unaffected in Walter, Campbell 28, and Ohio 4013.

Population levels of *X. c. vesicatoria* in leaflets of Hawaii 7998 infiltrated with 10⁵ cfu/ml were about 100-fold lower than in Walter leaflets from day 4 through day 13. In several experiments, popula-

tions in Hawaii 7998 remained fairly constant with a slight or no increase 4 days after inoculation. In experiments where leaflets were infiltrated with 10⁸ cfu/ml, populations in Hawaii 7998 leaflets were only about two to six times lower than in Walter leaflets 7 days after incubation.

Population levels of *X. c. vesicatoria* in leaflets of Hawaii 7998 were considerably lower than in Walter or in any of the tolerant genotypes (19) (Table 1). Bacterial populations in Campbell 28, PI 127183, and Heinz 1568, which all had some field resistance, were no lower than in the susceptible genotypes Walter or Lyconorma.

DISCUSSION

The genotype Hawaii 7998, previously reported highly resistant to bacterial spot (19), was confirmed by the hypersensitive reaction to be the first tomato genotype with absolute or vertical resistance. This hypersensitive response was confirmed by a rapid breakdown in tissue (confluent necrosis), by a rapid increase in electrolyte leakage, and by low populations of *X. c. vesicatoria* compared with susceptible or tolerant genotypes. The hypersensitive reaction in Hawaii 7998 was slow, with electrolyte leakage not evident until between 12 and 24 hr. This relatively slow reaction is unusual compared with the rapid hypersensitive reaction reported elsewhere (1,21,22). However, there have been other reports of a slow hypersensitive reaction (7).

Population levels were considerably lower in Hawaii 7998 than in susceptible Walter or in any of the other genotypes tested. Relatively low populations of the bacterium in the resistant line compared with the susceptible lines is a characteristic common in resistant reactions (1,7,12,21). One interesting difference from other studies on internal populations (7,12,22)

was that differences were not observed in leaflets of the resistant and susceptible lines 7 days after infiltration with 10⁸ cfu/ml. Only with a lower infiltration concentration (10⁵ cfu/ml) was there a dramatic difference between the resistant and the susceptible line. One explanation for this is that the infiltrated tissue of the susceptible line inoculated at 10⁸ cfu/ml that became necrotic within 48 hr also became desiccated, resulting in a drastic reduction in bacterial populations.

The tomato genotypes Campbell 28, Heinz 1568, and PI 127813, which have moderate levels of field tolerance, had internal bacterial populations considerably higher than Hawaii 7998 and no lower than the susceptible lines. This would point to some type of external resistance that might be responsible for the field resistance or the production of a toxic compound by the bacterium or a defensive mechanism in the host to the bacterium to which the susceptible lines appear more sensitive. Increased field resistance correlated with smaller lesion size but not with lesion density (J. B. Jones and J. W. Scott, *unpublished*) on plants inoculated under greenhouse conditions.

Vanderplank (23) suggested that it would be unrealistic to incorporate vertical resistance into a breeding program where a pathogen would multiply and spread quickly. Mutants might arise, readily creating new races, so a single resistant gene may be ineffective (5). Instances where single dominant genes have not proven completely effective and where considerable manipulation of the resistance genes was necessary for adequate control are numerous (6,16,23). However, in pepper, although vertical resistance was overcome by *X. c. vesicatoria*, the resistance was shown to significantly reduce the rate of disease development (6). An example where resistance has held up extremely well for nearly 40 yr is gray leaf spot of tomato (*Stemphylium solani* Weber) (25). However, the mechanism of gray leaf spot resistance appears to be one of a more complex nature. Other instances where a single recessive gene(s) has proven useful is with bacterial pustule of bean (10) and where a single dominant gene has proven useful is against bacterial blight in cowpea (R. D. Gitaitis, Coastal Plain Experiment Station, University of Georgia, *personal communication*).

This new source of resistance, incorporated into lines with or without horizontal (polygenic) resistance, may prove effective for reducing losses resulting from bacterial spot. The discovery of this source of absolute resistance revives the hope that more genes for absolute resistance will be found in *Lycopersicon* lines. Genetic control of this resistance is being studied.

LITERATURE CITED

1. Cook, A. A., and Stall, R. E. 1968. Effect of

Table 2. Effect of inoculum concentration of *Xanthomonas campestris* pv. *vesicatoria* on electrolyte leakage of resistant (H7998) and susceptible (Walter) lines of tomato

Hours after infiltration	Inoculum concentration (cfu/ml)							
	10 ⁸		10 ⁷		10 ⁶		10 ⁵	
	R ^a	S	R	S	R	S	R	S
6	44 ^b	49	44	43	50	38	41	32
12	83	43	38	36	40	33	39	30
24	97	47	107	39	73	38	39	40

^aR = resistant and S = susceptible line.

^bValues are expressed in µMhos.

Table 3. The effect of electrolyte leakage by infiltrating tomato leaves of susceptible (Walter), resistant (H7998), and moderately tolerant (Campbell 28 and 4013) tomato lines with 10⁸ cfu/ml of *Xanthomonas campestris* pv. *vesicatoria*

Line	Hours after infiltration				
	3	6	12	24	48
4013	25 ^a	22	21	40	46
Campbell 28	23	21	30	41	70
H7998	32	25	38	232	158
Walter	31	25	35	40	73

^aValues are expressed in µMhos.

- Xanthomonas vesicatoria* on loss of electrolytes from leaves of *Capsicum annum*. *Phytopathology* 58:617-619.
2. Courtney, W. H., Banfield, G. E., and Gray, V. T. 1983. Tomato cultivar trials 1983. *Ont. Hortic. Res. Inst. Res. Rep.* 6.
 3. Coyne, D. P., and Schuster, M. L. 1967. A source of tolerance and reaction of tomato species and varieties to bacterial spot pathogen. *Plant Dis. Rep.* 51:25-28.
 4. Crill, P., Jones, J. P., and Burgis, D. S. 1972. Relative susceptibility of some tomato genotypes to bacterial spot. *Plant Dis. Rep.* 56:504-507.
 5. Dahlbeck, D., and Stall, R. E. 1979. Mutations for change of race in cultures of *Xanthomonas vesicatoria*. *Phytopathology* 69:634-636.
 6. Dahlbeck, D., Stall, R. E., and Jones, J. P. 1979. The effect of vertical and horizontal resistance on development of bacterial spot of pepper. *Plant Dis. Rep.* 63:332-335.
 7. Gitaitis, R. D. 1983. Two resistant responses in cowpea induced by different strains of *Xanthomonas campestris* pv. *vignicola*. *Plant Dis.* 67:1025-1028.
 8. Jones, J. B., McCarter, S. M., and Gitaitis, R. D. 1981. Association of *Pseudomonas syringae* pv. *syringae* with a leaf spot disease of tomato transplants in southern Georgia. *Phytopathology* 71:1281-1285.
 9. Jones, J. B., McCarter, S. M., and Smitley, D. R. 1981. A vacuum infiltration inoculation technique for detecting *Pseudomonas tomato* in soil and plant tissue. *Phytopathology* 71:1187-1190.
 10. Kennedy, B. W., and Tachibana, H. 1973. Bacterial diseases. Pages 491-504 in: *Soybeans: Improvement, Production, and Uses*. B. E. Caldwell, ed. American Society of Agronomy, Madison, WI.
 11. Klement, Z. 1963. Methods for the rapid detection of the pathogenicity of phytopathogenic pseudomonads. *Nature* 188:479-480.
 12. Klement, Z., Farkas, G. L., and Lovrekovich, L. 1964. Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. *Phytopathology* 54:474-477.
 13. Knzeva, Z. V., and Kunnukh, G. S. 1979. Breeding tomato for resistance to *Xanthomonas vesicatoria*. *Ref. Zh.* 6.65.238:141-149.
 14. Lawson, V. F., and Summers, W. L. 1984. Disease reaction of diverse sources of *Lycopersicon* to *Xanthomonas campestris* pv. *vesicatoria* pepper strain race 2. *Plant Dis.* 68:117-119.
 15. Lawson, V. F., and Summers, W. L. 1984. Resistance to *Pseudomonas syringae* pv. *tomato* in wild *Lycopersicon* species. *Plant Dis.* 68:139-141.
 16. Loegering, W. Q., Johnston, C. O., and Hendrix, J. W. Wheat rusts. Pages 307-335 in: *Wheat and Wheat Improvement*. K. F. Quisenberry, ed. American Society of Agronomy, Madison, WI.
 17. Pohrozezny, K., and Volin, R. B. 1983. The effect of bacterial spot on yield and quantity of fresh market tomatoes. *HortScience* 18:69-70.
 18. Scott, J. W., and Jones, J. B. 1984. Severity of bacterial spot (*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye on leaves and fruit of Florida grown tomato cultivars. *Proc. Fla. State Hortic. Soc.* 97:157-159.
 19. Scott, J. W., and Jones, J. B. 1986. Sources of resistance to bacterial spot (*Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye) in tomato. *HortScience*. 21:In press.
 20. Sotirova, V., and Beleva, L. 1975. Resistance of tomato wild species, varieties and cultivars to *Xanthomonas vesicatoria* (Doidge) Dye. *C.R. Acad. Agric. G. Dimitrov* 8:43-47.
 21. Stall, R. E., and Cook, A. A. 1966. Multiplication of *Xanthomonas vesicatoria* and lesion development in resistant and susceptible pepper. *Phytopathology* 56:1152-1154.
 22. Stall, R. E., Bartz, J. A., and Cook, A. A. 1974. Decreased hypersensitivity to xanthomonads in pepper after inoculations with virulent cells of *Xanthomonas vesicatoria*. *Phytopathology* 64:731-735.
 23. Vanderplank, J. E. 1963. *Plant Diseases: Epidemics and Control*. Academic Press, New York. 349 pp.
 24. Volin, R. B. 1979. Selecting fresh-market tomatoes in Florida for resistance to bacterial leafspot, and inheritance studies in improved root development. In: *First International Symposium on Tropical Tomato*. R. Cowell, ed. AVRDC, Shanhuah, Taiwan.
 25. Walter, J. M. 1967. Hereditary resistance to disease in tomato. *Annu. Rev. Phytopathol.* 5:131-162.