Avirulence Gene *avrRxv* from *Xanthomonas campestris* pv. *vesicatoria* Specifies Resistance on Tomato Line Hawaii 7998

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The molecular and genetic control of the interaction between tomato races of *Xanthomonas campestris* pv. *vesicatoria* (XcvT) and tomato was studied. Based on inoculation phenotype and analysis of *in planta* bacterial growth, tomato line Hawaii 7998 is resistant to XcvT race 1 75-3 but not to XcvT race 2 89-1. Two cosmid clones from a genomic library of XcvT race 1 75-3 converted the normally virulent race 2 89-1 to avirulence on Hawaii 7998. The two clones contained the previously isolated, nonhost avirulence gene *avrRxv*, and their activity was localized to a 2.1-kbp subclone of *avrRxv*. *avrRxv* inhibits growth of race 2 89-1 in the resistant line Hawaii 7998 and an insertional mutation in *avrRxv* prevents this inhibition. In addition, a dramatic increase in electrolyte leakage of leaves of Hawaii 7998 occurred after 12-hr postinfiltration with race 2 89-1 carrying *avrRxv*. The nucleotide sequence of *avrRxv* revealed one major open reading frame (ORF) that accords well with activity analysis of nested deletions. ORF 2-2 encodes a putative protein of 374 amino acids with a molecular weight of 42.1 kDa and a pI of 10.7. Inheritance of the *avrRxv*-specific resistance in Hawaii 7998 was studied in a total of 587 F_2 individuals from crosses between Hawaii 7998 and susceptible lines. The inheritance of *avrRxv*-specific resistance in Hawaii 7998 appears to be governed by more than one locus.

The spectrum of phenotypic variation within pathogen and host species has allowed studies of the genetics of disease resistance in plants. These studies have shown that in many cases the outcome of an interaction between a pathogen and a host is controlled by the presence or absence of specific avirulence genes and resistance genes (reviewed in Keen 1990). Resistance occurs when the pathogen has an avirulence gene that specifically corresponds to a resistance gene in the host (Flor 1971). Use of genetically engineered, near-isogenic pathogen strains along with near-isogenic host lines has provided extensive evidence in support of the gene-for-gene model for disease resistance (Keen and Staskawicz 1988).

Historically, a distinction has been made between disease resistance found in host and nonhost species (Crute 1985). Host resistance pertains to the resistance of a particular cultivar within a plant species that as a whole is normally susceptible to the pathogen, whereas nonhost resistance is expressed by all members of a plant species to a particular pathogen. Before pathogens could be manipulated using molecular genetic techniques, it was believed that nonhost resistance differed fundamentally from host resistance (reviewed in Crute 1985). However, several recent studies of interactions between nonhosts and bacterial pathogens have shown that these interactions may also be controlled according to the gene-for-gene model. Avirulence genes that induce resistance in a genotype-specific manner on nonhost species have been isolated from pathovars of *Xanthomonas* and *Pseudomonas* (Whalen *et al.* 1988; Kobayashi *et al.* 1989; Whalen *et al.* 1991; Fillingham *et al.* 1992). These genes will be referred to as nonhost avirulence genes. In these studies, the inheritance of avirulence gene-specific, nonhost resistance has been shown to be governed by single plant loci (Whalen *et al.* 1988; Kobayashi *et al.* 1989; Keen and Buzzell 1990; Whalen *et al.* 1991; Fillingham *et al.* 1992). Moreover, in three of these cases a nonhost avirulence gene has been shown to be identical or nearly identical in nucleotide sequence to a host avirulence gene (Kobayashi *et al.* 1989; Bent *et al.* 1992; Dangl *et al.* 1992). These similarities between host and nonhost avirulence genes suggest that nonhost resistance, like host resistance, may be conditioned by gene-for-gene interactions.

Nucleotide sequences have been submitted to GenBank as accesssion number L20423.

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We previously studied nonhost resistance to *X. campestris* pv. *vesicatoria* tomato race (XcvT) race 1 and discovered that the avirulence gene *avrRxv* when carried by normally virulent strains governed induction of resistance in plants diverse as bean, soybean, cowpea, alfalfa, cotton, and corn (Whalen *et al.* 1988). In addition, the *avrRxv*-associated resistance was inherited as a single, incompletely dominant locus in bean. This suggests that the interaction is controlled, at least in part, in a gene-for-gene manner.

Although genetic resistance to XcvT race 1 appears to be widespread in nonhost species, genetic resistance in the natural host tomato is rare. After screening hundreds of tomato accessions, a source of resistance was found in a tomato line called Hawaii 7998 (Jones and Scott 1986; Scott and Jones 1986). Field tests were conducted to gain an understanding of the inheritance of resistance to wild-type XcvT (Scott and Jones 1989). In these tests, resistance segregated quantitatively with additive dominance from more than three genes. Further analysis of wild-type XcvT race 1 resistance through isozyme linkage studies also indicated a complex mode of inheritance (Wang 1992; J.F.W., R. E. S., and C. E. Vallejos, unpublished data).

For a full mechanistic understanding of the XcvT–tomato interaction, knowledge of the genetic basis for the observed phenotypic differences in both virulence and resistance is crucial. We tested the hypothesis that the gene-for-gene model of resistance described the interaction between XcvT race 1 and tomato line Hawaii 7998. To learn whether one or more avirulence genes were present in XcvT race 1, cosmid clones from a genomic library were conjugated into a normally virulent strain, and the resulting transconjugants were screened for resistance-inducing activity on Hawaii 7998. Interestingly, we found that two cosmid clones containing the previously isolated, nonhost avirulence gene *avrRxv* (Whalen *et al.* 1988) induced resistance in Hawaii 7998 and that the activity was localized to a 2.1-kbp subclone of *avrRxv*. We therefore continued our analysis of *avrRxv* and herein report the nucleotide sequence. We demonstrate that the inheritance of *avrRxv*-specific resistance in Hawaii 7998 appears to be governed by more than one locus.

RESULTS

Resistance of Hawaii 7998 to XcvT.

Hawaii 7998 is a tomato line previously found to have a high level of resistance to the pathogen XcvT (Jones and Scott 1986; Scott and Jones 1986). To begin our molecular genetic characterization of this resistance, we examined the response of leaves of the resistant tomato line Hawaii 7998 and susceptible lines to inoculation with XcvT races. XcvT race 1 75-3 (Table 1) induced a resistant response in Hawaii 7998 and a susceptible response in Walter (Table 2). To examine growth of XcvT

Table 1. Bacterial strains, vectors, and plasmid constructions

Strain, vector, or plasmid	Relevant characteristics	Source or reference
Xanthomonas campestris pv. *vesicatoria* tomato races		
75-3	Race 1, Rif^r, *avrRxv*	Whalen *et al.* 1988
75-3 *avrRxv*::Ω	Race 1, Rif^r, Sp^r, *avrRxv*::Ω	Whalen *et al.* 1988
89-1	Race 2, Rif^r	R.E.S.
90-14 and 92-14	Race 1, Rif^r, *avrRxv*	R.E.S.
87-21, 0245, 27-1, 0350, 0026	Race 1 and race 2	R.E.S.
Vectors		
pLAFR3	pLAFR1 containing *Hae*II fragment of pUC8, Tc^r, Tra$^-$ Mob$^+$, RK2 replicon	Staskawicz *et al.* 1987
pL6	pLAFR3 deleted for Plac with *trp* terminators flanking the polylinker	Huynh *et al.* 1989
pDSK519	RSF1010-based with pUC9 polylinker, Km^r	Keen *et al.* 1988
pUC118, pUC119	IG region of M13 in pUC18, ColE1 replicon	Vieira and Messing 1987
pUR278, 288, 289	LacZ fusion vectors	Ruther and Müller-Hill 1983
Plasmids		
pRK2013	Km^r, Tra$^+$ Mob$^+$, ColE1 replicon	Figurski and Helinski 1979
pXV9006	pL6 with 2.1-kbp *Pst*I fragment containing the *avrRxv* gene cloned from *X. campestris* pv. *vesicatoria* tomato race 1 75-3	Whalen *et al.* 1988
pXV9009	Same as pXV9006 except in pLAFR3	Whalen *et al.* 1988
pXV9009::Ω	pXV9009 with Ω fragment inserted into *Xho*I site within *avrRxv*	Whalen *et al.* 1988
pXVSC910	2.1-kbp *Pst*I fragment from pXV9006 cloned into pDSK519	This study
pXV9200	pUR289 containing 1.45-kbp insert from pUC118RXV33	This study
pUC118RXV3 and pUC118RXV1	2.1-kbp *Pst*I fragment from pXV9009 cloned into pUC118 in both orientations	This study
pUC118RXV3X and pUC118RX33	Deletions of pUC118RXV3 beginning at nucleotide 601 and 704, respectively	This study
pRXV31, pRXV36a, pRXV3X, pRXV33, pRXV34, pRXV35, pRXV36, pRXV37, pRXV38, and pRXV38N	Inserts from pUC118RXV3 deletion series cloned into pL6 beginning at nucleotide 240, 532, 601, 704, 931, 1074, 1144, 1295, 1514 and 1643, respectively	This study
pRXV11, pRXV11Z, and pRXV14	Inserts from pUC118RXV1 deletions series cloned into pL6 beginning at nucleotide 1808, 1697, and 1484, respectively.	This study
pRXV3PlacX, pRXVPlac33, and pRXVPlac34	Constructs from pRXV3 series (pL6) with pUC119 cloned in orientation to allow β-galactosidase promoter drive expression	This study

strains in tomato leaves, strains were infiltrated into tomato shoots, and then bacterial populations were sampled during the next 5 days. In leaves of Hawaii 7998, the population size of race 1 75-3 increased about 100-fold by 2 days postinfiltration but afterwards remained nearly static (Fig. 1A). In leaves of Walter, the population size of race 1 75-3 increased about 100,000-fold over a 5-day period after infiltration (Fig. 1B). In contrast to race 1 75-3, race 2 89-1 produced susceptible responses in both Hawaii 7998 and Walter (Table 2). When infiltrated into tomato leaves, the final population size of race 2 89-1 was indistinguishable from that of race 1 75-3 in Walter (Fig. 1B). In leaves of Hawaii 7998 the final population size of race 2 89-1 was about 1,000-fold greater than that of race 1 75-3 (Fig. 1A). Both races induced resistance on the pepper cultivar Early CalWonder (Table 2).

Table 2. Response of tomato and pepper lines to *Xanthomonas campestris* pv. *vesicatoria* tomato races[a]

Race	Strain	Tomato		Pepper
		Walter	Hawaii 7998	ECW[b]
1	75-3	S	R	R
2	89-1	S	S	R

[a] S, susceptible response; R, resistant response.
[b] Early Calwonder cultivar.

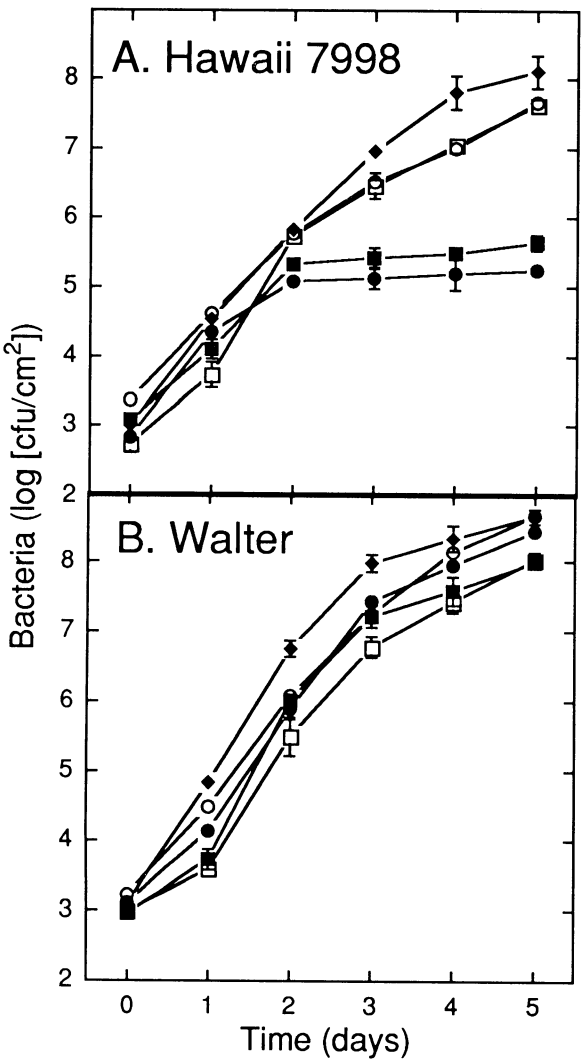

Fig. 1. Time course of growth of *Xanthomonas campestris* pv. *vesicatoria* tomato races and transconjugants in tomato cultivars (**A**) Hawaii 7998 and (**B**) Walter. Leaves were infiltrated with bacterial suspensions of 10^5 colony-forming units per milliliter. Bacterial populations in leaves were sampled during 5 days following infiltration; ●, race 1 75-3; ○, race 1 75-3 *avrRxv*::Ω; ◆, race 2 89-1; ■, race 2 89-1 (pXV9009); □, race 2 89-1 (pXV9009::Ω). Values are means from three repetitions and vertical bars represent ± 1 S.E. Essentially identical results were obtained in three independent experiments on each strain.

Cloning of an avirulence gene associated with resistance in Hawaii 7998.

The resistance of Hawaii 7998 to race 1 75-3, along with its susceptibility to race 2 89-1, allowed us to test whether an avirulence gene was responsible for induction of resistance on Hawaii 7998. We screened a library of DNA from race 1 75-3 for resistance-inducing activity on Hawaii 7998. One thousand clones from the library were conjugated into the normally virulent race 2 89-1 and the resulting transconjugants were inoculated onto leaves of Hawaii 7998. Two cosmid clones, pEC116 and pEC137, carried by race 2 89-1 induced resistance on Hawaii 7998 (Table 3). Race 2 89-1 carrying pEC116 or pEC137 did not induce a resistance response on the susceptible tomato lines Walter, Bonny Best, or Florida 7060, and remained virulent. Surprisingly, the cosmid clone pEC116 carried the previously described nonhost avirulence gene *avrRxv* (Whalen *et al.* 1988). We therefore tested two subclones

Table 3. Response of tomato lines to strains and transconjugants of *Xanthomonas campestris* pv. *vesicatoria* tomato races[a]

Strain or transconjugant	Tomato lines	
	Hawaii 7998	Bonny Best
Race 1 75-3	R	S
Race 2 89-1	S	S
Race 2 89-1 (pEC116)	R	S
Race 2 89-1 (pEC137)	R	S
Race 2 89-1 (pXV9009)	R	S
Race 2 89-1 (pXV9006)	R	S
Race 2 89-1 (pXV9009::Ω)	S	S
Race 1 75-3 avrRXV::Ω	I	S

[a] R, resistant response; S, susceptible response; I, intermediate response.

Table 4. Presence of homologous sequences to *avrRxv* in genomic DNAs from strain of *Xanthomonas campestris* pv. *vesicatoria* tomato races and response of tomato lines

Strain	*avrRxv* Homology[a]	Tomato lines	
		Hawaii 7998	Bonny Best
75-3	+	R[b]	S
90-14	+	R	S
92-14	+	R	S
87-21	+	R	S
0245	+	R	S
89-1	−	S	S
27-1	−	S	S
0350	−	S	S
0226	−	S	S

[a] +, presence of hybridizing sequences in genomic DNA from designated strain; −, absence of hybridizing sequences in genomic DNA from designated strain.
[b] R, resistant response: S, susceptible response.

of *avrRxv*, pXV9009 and pXV9006 (Table 1), and found that they each induced resistance on Hawaii 7998 (Table 3). Using Southern blot analysis, we found that cosmid clone pEC137 contains similar restriction enzyme fragments as pEC116 (data not shown). The inserts of both cosmids contain an active 8-kbp *Bam*HI fragment that in turn contains an active 4.3-kbp *Pst*I fragment (Whalen *et al.* 1988).

DNAs from various XcvT strains were digested, blotted, and probed with the 2.1-kbp *Pst*I fragment from pXV9006 to determine if XcvT strains other than race 1 75-3 had *avrRxv*. We found that when a strain induced resistance on Hawaii 7998 and not on susceptible tomato lines, it contained sequences that hybridized to *avrRxv* (Table 4). To further analyze the hybridizing sequences from the race 1 strains 75-3, 90-14, and 92-14, genomic DNAs were digested with four restriction enzymes alone and in combination (*Pst*I, *Bam*HI, *Xho*I, and *Cla*I) prior to blotting. When these blotted DNAs were probed with the 2.1-kbp *Pst*I fragment from pXV9006, we found that these strains had DNA that hybridized at the same molecular weight as that of race 1 75-3 (data not shown). This suggests that these strains have a homologous copy of *avrRxv*.

In leaves of Hawaii 7998, the final population size of race 2 89-1 carrying pXV9009 was about 300-fold less compared to that of race 2 89-1 alone (Fig. 1A). In contrast, in leaves of the susceptible line Walter, the final population size of race 2 89-1 carrying pXV9009 was indistinguishable from that of race 2 89-1 (Fig. 1B). Race 2 89-1 transconjugants carrying the insertionally mutagenized *avrRxv* construct, pXV9009::Ω (Whalen *et al.* 1988) (Table 1), failed to induce resistance on Hawaii 7998 (Table 3). In addition, the growth of race 2 89-1 (pXV9009::Ω) was no different from that of race 2 89-1 in leaves of both Hawaii 7998 and Walter (Fig. 1). These data suggest that *avrRxv* has a negative effect on growth of race 2 89-1 in the resistant line Hawaii 7998 but not in a susceptible line, and that an insertional mutation in *avrRxv* prevents this negative effect. When the genomic copy of *avrRxv* is insertionally mutated (race 1 75-3 *avrRxv*::Ω), the mutant strain induced an intermediate response on Hawaii 7998 (mean response score = 3.6, S.E. = 0.6, n = 17; Table 3). However, analysis of growth in Hawaii 7998 demonstrated that the final population size of race 1 75-3 *avrRxv*::Ω was at a level normally associated with susceptible responses. In leaves of Hawaii 7998, the population size of race 1 75-3 *avrRxv*::Ω was about 1,000-fold greater than that of wild-type race 1 75-3 (Fig. 1A). In leaves of Walter, the mutation had no effect on growth of race 1 75-3 (Fig. 1B). In addition, in leaves of Hawaii 7998, the final population size of race 2 89-1 (pXV9009) was equivalent to that of wild-type race 1 75-3 from which *avrRxv* was cloned (Fig. 1A). Thus, *in planta* bacterial growth analysis suggested that *avrRxv* plays a crucial role in the ability of race 1 75-3 to induce full resistance on Hawaii 7998.

Another characteristic trait of plants that are resistant to various strains of *X. campestris* pv. *vesicatoria* is rapid electrolyte leakage from infiltrated leaves (Minsavage *et al.* 1990). We analyzed electrolyte leakage from leaves of Hawaii 7998 and the susceptible line Bonny Best in response to infiltration with race 2 89-1 transconjugants (Fig. 2). After 12 hr, electrolyte leakage in response to infiltration with the transconjugant race 2 89-1 (pXV9006) occurred at a dramatically more rapid rate in leaves of Hawaii than in leaves of Bonny Best. Infiltration with race 2 89-1 (pXV9009::Ω) induced similar levels of electrolyte leakage in both Hawaii 7998 and Bonny Best, and these levels were indistinguishable from that of race 2 89-1 (pXV9006) in Bonny Best. Electrolyte leakage thus correlated with macroscopic resistant responses and reduced bacterial growth.

Deletion analysis of *avrRxv*.

A 2.1-kbp DNA fragment contained full *avrRxv* activity on bean (Whalen *et al.* 1988). To define the minimal region of DNA necessary for activity on tomato and bean, we constructed and assayed a set of nested deletions. Exonuclease III deletions generated from pUC118RXV3 and pUC118RXV1 were cloned into pL6 and pLAFR3 (Table 1) and conjugated into race 2 89-1. To test for activity, these race 2 89-1 transconjugants were inoculated onto Hawaii 7998 and the susceptible tomato line Bonny Best. As expected, inoculation with transconjugants carrying any of the deletions produced susceptible responses on Bonny Best (data not shown). The responses of Hawaii 7998 are shown in Figure 3 as resistance (R) or susceptible (S). Full *avrRxv* activity in pL6 was observed in race 2 89-1 transconjugants up to the deletion subclone

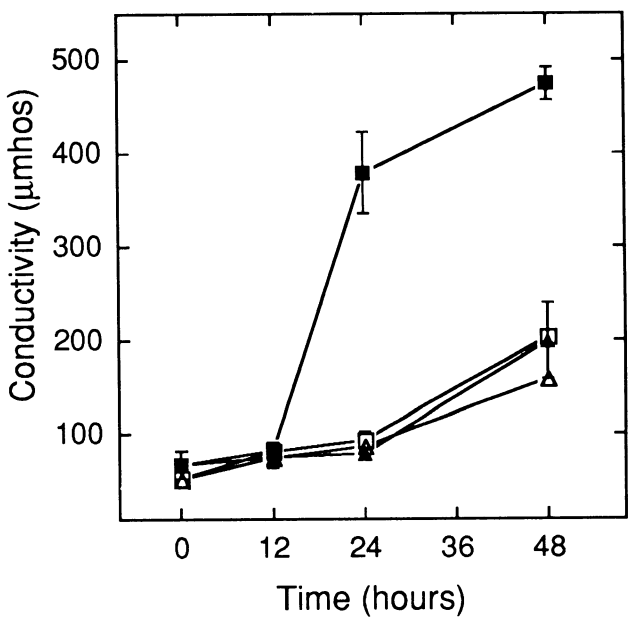

Fig. 2. Time course of electrolyte leakage from leaves of tomato plants grown at 24° C infiltrated with transconjugants of *Xanthomonas campestris* pv. *vesicatoria* tomato race 2 89-1 at a concentration of 5×10^8 colony-forming units per milliliter. Phenotypes of the reactions are given in Table 3. ■, Hawaii 7998 infiltrated with race 2 89-1 (pXV9006); □, Hawaii 7998 inoculated with race 2 89-1 (ppXV9009::Ω; ▲, Bonny Best inoculated with race 2 89-1 (pXV9006); △, Bonny Best inoculated with race 2 89-1 (pXV9009::Ω). Each point represents the mean of three replicate measurements. Vertical lines represent ± 1 S. E.

pRXV36a that begins at bp 532 and extends 3′ to bp 2154, and up to deletion subclone pXRV11Z that begins at bp 1697 and extends 5′ to bp 1. Thus, avirulence activity was delimited to a 1,165-bp region. This region contains an open reading frame called ORF 2-2.

To test whether the loss of activity of *avrRxv* deletions in pL6 could be overcome by fusion to a strong promoter, constructs were produced in which the β-galactosidase promoter of pLAFR3 or pUC119 was positioned to drive the expression of several deletions (Table 1). Race 2 89-1 transconjugants carrying pRXVPlac33, pRXVPlac34, and pRXV3PlacX produced a susceptible response on Hawaii 7998 (Fig. 3). The addition of the *lac* promoter did not compensate for the loss of upstream sequences in these constructs.

Sequence analysis.

The 2,151-bp insert from subclone pXV9009 was sequenced (Fig. 4) in hopes of learning about the molecular basis of the avirulence activity encoded in *avrRxv*. Sequence analysis revealed one major open reading frame (ORF) and two minor ORFs that could not be eliminated by deletion analysis. The longest ORF, ORF 2-2 (Fig. 3), extends from nucleotides 533 to 1654, encoding a putative protein of 374 amino acids with a molecular weight of 42.1 kDa. ORF 2-2 is shown conceptually translated in Figure 4. The endpoints of activity delimited by deletion analysis fit well with the region within ORF 2-2 (Fig. 3). Two extremely short ORFs in frames different than ORF 2-2 were also found, ORF 3-7 from nucleotides 1449 to 1517 encoding a putative protein with 23 amino acids and a molecular weight of 2.5 kDa, and ORF 4-4 from nucleotides 569 to 480 encoding a putative protein of 30 amino acids with a molecular weight of 3.3 kDa. Since these two ORFs are so minor, and we would have to hypothesize unusually extensive promoter regions to accommodate results from endpoint activity analysis, they were eliminated from further consideration.

To verify the absence of stop codons in the majority of the ORF 2-2 nucleotide sequence, we constructed the β-galactosidase expression plasmid pXV9200 that has the 1,452-bp insert from pRXV33 (Table 1). Induction with IPTG of *E. coli* cultures carrying pXV9200 resulted in overexpression of the expected size fusion protein as predicted by the DNA sequence. A fusion protein approximately 35 kDa greater than β-galactosidase was observed when the cell lysate was run on SDS-PAGE (data not shown). The production of a fusion protein in *E. coli* with the predicted molecular weight verifies the absence of stop codons in the ORF 2-2 sequence from bp 704 to

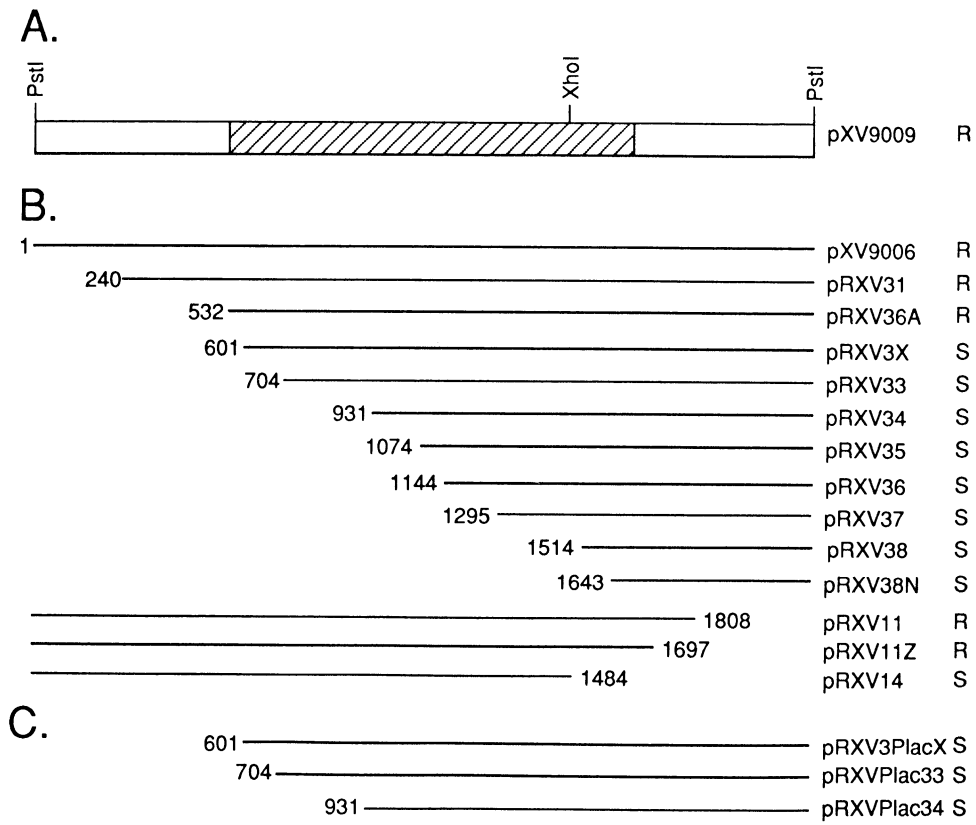

Fig. 3. Open reading frame and activity endpoint deletion analysis of *avrRxv* on the fully active 2.1-kbp subclone pXV9009. **A,** Shaded area represents position of open reading frame ORF 2-2 (bp 533-1654). Restriction enzyme sites are indicated above the line. **B,** Position of exonuclease III deletions and their resistance-inducing activity in pL6. Numbering starts with position 1 and corresponds to the sequence in Figure 4. The name of the plasmid construct is indicated to the right of each line. Response of tomato line Hawaii 7998 to *Xanthomonas campestris* pv. *vesicatoria* tomato race 2 89-1 carrying each DNA fragment in pL6 is designated to the far right by R, resistance or S, susceptible. **C,** Position of deletions and response of Hawaii 7998 to *X. campestris* pv. *vesicatoria* tomato race 2 89-1 carrying designated deletions with the *lacZ* promoter positioned to drive their expression.

1654.

We analyzed the nucleotide sequence to locate possible promoters. Three possible –10 promoter sequences are clustered upstream of the first ATG codon in ORF 2-2 (Fig. 4). There is no sequence that resembles typical –35 promoter sequences upstream of these three putative –10 sequences, but there is a possible –35 sequence starting at bp 488. Sequences resembling the 5′ core of the promoter sequence found upstream of avirulence genes in *P. syringae* (Fellay *et al.* 1991; Jenner *et al.* 1991; Innes *et al.* 1993a; Salmeron and Staskawicz 1993) are present upstream of the first putative initiator codon at bp 533. No typical purine-rich region suggestive of a Shine-Dalgarno sequence is present just upstream of the initiator ATG codons at bp 533, 575, or 587. Interestingly, the deletion fragment in pRXV36a started at bp 532, one nucleotide upstream of the first ATG in ORF 2-2. One of the downstream initiator ATG codons at nucleotide 575 or 587 may

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CGCAGCAGCGGCAAGGCATACGCCAGGGTCTTGCCCGAGCCGGTCTGCGCGGTGGCGATCAGGTCGCGTGCGGCCAGCATCGGCGGAATCATCGCGACCT 100

GGATCGGCGTGAGCGGCATGCACACCGGCCCTGGCGAGCGCGGACTCGAATACGGGCTGTAGCTGTAGCGACAGCAAATCTGCTTTCAGTGACATCGCGC 200

GATTATCCGCGCATTGTCGACGGCGTGCATGCCGAAACGAAGTGCAGGTGCGCTGGCCGCGCATGGCGCTCCATGGCGCCGCCAAGCGGCAGTGGCAACA 300

ACCGGTGTGTCGACCCGAGGTGTGCTGCGTCCAATGCGACTAATCCACGGCCGTGGCCCGGCTTTTTTGGGGTCGGTTAGTCACCGAACATCAGTTGCGC 400

AGATCACTCTTCAGCGTGAATTACTGTAGCTTACTGTAGTGCTTCGTTCAGGGTTTTTCAGCTTTTCGCATTATTGCCTAGATCTTCGCGCAACGAAACC 500

GAGTTCTTCGATTAGCGCGATCTAATAAATAT ATG TGC GAC TCC ATA AGA GTG CAA TTC AGA TCC ATA CAA AAA ATG GTG GTA 583
                                 MET Cys Asp Ser Ile Arg Val Gln Phe Arg Ser Ile Gln Lys MET Val Val

AAA ATG AAG AAA TTT TTC AGA TCA TTA GGA GTG GGC GGC TCA AGC AGC AGT CGT TTT CAA CAT CAT ATT CCG GAG 658
Lys MET Lys Lys Phe Phe Arg Ser Leu Gly Val Gly Gly Ser Ser Ser Ser Arg Phe Gln His His Ile Pro Glu

GCT GAC TCA GCA CCC AGT AGT AAG GCG TCT ACG CCT CCG GCC TCT CCG CCG CCG GAT TCC CCG CCC AGT AAC TCT 733
Ala Asp Ser Ala Pro Ser Ser Lys Ala Ser Thr Pro Pro Ala Ser Pro Pro Pro Asp Ser Pro Pro Ser Asn Ser

GCT TTT TCC GCT CTC CCG ACA AGG CCT CGC AAG AAG GCC GAG GCC TTG TCG GAT GCG GTG GAG TCG CGC GGA CAT 808
Ala Phe Ser Ala Leu Pro Thr Arg Pro Arg Lys Lys Ala Glu Ala Leu Ser Asp Ala Val Glu Ser Arg Gly His

TTA GCC CCG CCA AGC CTG GTC TCC TAT GCC AAC GCA ACC CTT GAT CAA CTG AGG CGA AAT GAA CCC ATC AGC GAG 883
Leu Ala Pro Pro Ser Leu Val Ser Tyr Ala Asn Ala Thr Leu Asp Gln Leu Arg Arg Asn Glu Pro Ile Ser Glu

TCA CTT CGG CTG ATG GAC ATT GAA AAT CTC CCC CAT CTG GTC CGC TCC TAC GAC AAT AGA TTG AAT AAT CTA AAC 949
Ser Leu Arg Leu MET Asp Ile Glu Asn Leu Pro His Leu Val Arg Ser Tyr Asp Asn Arg Leu Asn Asn Leu Asn

CTG CGC AGC TTC GAC ACT CCG GGG CAG TTT TTA CAT GAC CTG AGT CGC TGG CAT AAA ACA GGA TTG CCA TTA AGA 1033
Leu Arg Ser Phe Asp Thr Pro Gly Gln Phe Leu His Asp Leu Ser Arg Trp His Lys Thr Gly Leu Pro Leu Arg

GCG GTA GTG CGG CTG GAT GAA GAC CCT AGG AGA TGG CAT CGC GTC GCG TTC GAC GTG CGC AAC CAC GAG AGT GGA 1108
Ala Val Val Arg Leu Asp Glu Asp Pro Arg Arg Trp His Arg Val Ala Phe Asp Val Arg Asn His Glu Ser Gly

CAC ACG ACG ATT ATC GCA TTG GAG CCT GCG TCT GCT TAC AAT CCG GAC CAT ATG CCT GGT TTC GTG AAA ATG AGA 1183
His Thr Thr Ile Ile Ala Leu Glu Pro Ala Ser Ala Tyr Asn Pro Asp His MET Pro Gly Phe Val Lys MET Arg

GAA AAT CTC ACG TCT CAG TTC GGT AGG AAA ATT TCG TTT GCT GTG ATT GAG GCG GAA GCA CTT AAG TCA ATC GGT 1255
Glu Asn Leu Thr Ser Gln Phe Gly Arg Lys Ile Ser Phe Ala Val Ile Glu Ala Glu Ala Leu Lys Ser Ile Gly

GGG TGT GTC ATA TTT TCT CTT GAT TAT GCC CTG GCG GCA TAC CAG GAA AGA AGC ACC TTT GAC CAA TGG CAT AAA 1333
Gly Cys Val Ile Phe Ser Leu Asp Tyr Ala Leu Ala Ala Tyr Gln Glu Arg Ser Thr Phe Asp Gln Trp His Lys

GAT CTT CGA AAG AAA GGA AAT ATC AAG GGG ATG ACT CCC GAA AGT CAG CAC CTT AAC GAG CTT GGC GTC TAT TTG 1408
Asp Leu Arg Lys Lys Gly Asn Ile Lys Gly MET Thr Pro Glu Ser Gln His Leu Asn Glu Leu Gly Val Tyr Leu

CTT AAA GGA ACC AGG TTG CTG CCG GCA AAC TTC TAC AAG CAT GCG CAT TCC AGG CGC ACC ATC GAC GAG CTC GAG 1492
Leu Lys Gly Thr Arg Leu Leu Pro Ala Asn Phe Tyr Lys His Ala His Ser Arg Arg Thr Ile Asp Glu Leu Glu

GCA GAT CAG CCT GGC GCG TCG GGT ACC GAC GTG AGG TCA GGC AGA GCC GCT GTC TAC AAG GAG TCG CTG AGC CGT 1558
Ala Asp Gln Pro Gly Ala Ser Gly Thr Asp Val Arg Ser Gly Arg Ala Ala Val Tyr Lys Glu Ser Leu Ser Arg

AGA CTG GAG GAG TTC CAG GTC CAG CGC GAT AAG ACC TAC AGC ATG TCA ATC GAA GCA TCC AGA GCT CGA AAG ATC 1633
Arg Leu Glu Glu Phe Gln Val Gln Arg Asp Lys Thr Tyr Ser MET Ser Ile Glu Ala Ser Arg Ala Arg Lys Ile

CGT CAC GCC TTA GAA TCC TGA GACAATTACCAAATATTATTTACTTTCCTTACCTTCACAGGCCCGCTTCCCAGCGGCTTTTTTTCGGTAT  1724
Arg His Ala Leu Glu Ser  .

GTAATAAGGCGACCCTGATGGAAAGCGCAACGGCAGGCTTGTCGAATGAGGTTTGCTGTGTCTGAAGGGCTTGTCGCTACGCCAGGCAGGATCATCCAGT 1824

GGCAGCGGCTGCCGGCGCATCGTGACAGATGCGAGAGCGCAAGCGACGTCAGCACTGGCGCCGTGTTCTTGTCCATGCTGCGAATCTGAACTTGGTGCAA 1924

CCAACCTGGCGCTTGATCGAGGTTCCTTAGCACACCGCAACATTGCGCGGCTGCGGGCGCCGCTGACGTTGGAAATCATCCGCTGCATTGCGCTCGTCCA 2024

CCTGGCACACGCGCGCATCTGCGTGTACCCGCGTTCGCGGTTGCTGCCGGGGCGTTTTCCAGCCTGCGCGGCGAGCCGGCAAGCCGCCGCCAAGTGCTCA 2124

GTTGGCCAACGCCAGATCCGTCAACCG 2151
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Fig. 4. Nucleotide sequence of a 2,151-bp segment of DNA and putative amino acid translation of ORF 2-2. Putative promoter sequences are underlined and three possible initiator ATG codons are indicated in boldface. Stop codon is designate by a period (.).

have been used as the translation start site in race 2 transconjugants carrying pRXV36a and in fact, may be the ATG used for translation initiation in XcvT (Fig. 4).

According to both the Kyte and Doolittle (1982) and the Hopp and Woods (1981) algorithms for determining the hydropathicity of an amino acid sequence, the putative amino acid sequence of ORF 2-2 is primarily hydrophilic (data not shown). Although there are a few hydrophobic regions (Hopp and Woods algorithm: 12 and 14 amino acids in length at amino acids 93–104 and 242–255, respectively, and Kyte and Doolittle algorithm: 17 amino acids in length at 239–255) these regions are not long enough to suggest function in membrane spanning or transport, as membrane spanning regions are usually at least 20 residues long and free of positively charged residues (Doolittle 1987). The putative ORF 2-2 protein has a pI of 10.7 with a high percentage of the basic amino acids arginine (9.4% of residues) and histidine (4.0% of residues) and the amino acid serine (11.2% of residues).

FastDB (Brutlag *et al.* 1990) was used to search GenBank, EMBL, PIR, and Swiss-Protein databases to find other sequences with similar nucleotides or amino acids. When the amino acid sequence of ORF 2-2 was compared in its entirety to the PIR and Swiss-Protein amino acid sequence databases, no strong similarities were revealed. No significant similarities to sequences of other avirulence genes were observed.

To find localized regions of high similarity, the Blastp (Altschul *et al.* 1990) program was used to compare ORF 2-2 to the protein databases (PIR, Swiss-Protein, and Genpept). A region of low compositional complexity in ORF 2-2, amino acids 40–75 high in serine and proline, was statistically similar to regions in many proteins with similar low complexity sequences; however, the biological significance of these similarities is difficult to interpret because of the low complexity. The Block program (Henikoff and Henikoff 1991) was also used to compare ORF 2-2 to the Prosite protein subsequence database. A region in ORF 2-2 (amino acids 81–109) was 31% identical and 62% similar to a region within a subunit of eukaryotic RNA polymerases (RPB3_Hum; Kolodziej and Yound 1989; Pati and Weissman 1990) and 21–24% identical and 48–59% similar to other members of the subsequence family. The functional significance of similarities is currently unknown and will be tested with mutagenesis studies.

Inheritance of *avrRxv*-associated resistance.

Crosses were made between Hawaii 7998 and susceptible lines to determine the mode of inheritance of *avrRxv*-specific resistance in tomato. Leaves of tomato plants were inoculated with race 2 89-1 carrying *avrRxv* and the responses were analyzed as described in Materials and Methods. The average response of F_1 individuals from crosses between Hawaii 7998 and susceptible lines was intermediate. For crosses between Hawaii 7998 and Ailsa Craig, the mean response score of F_1's was 3.5 (S.E. = 1.2; n = 5) in comparison to parental mean scores for the resistant parent Hawaii 7998 of 7.3 (S.E. = 0.2, n = 41) and for the susceptible parent Ailsa Craig of 1.0 (S.E. = 0.0, n = 11). For crosses between Florida 7060 and Hawaii 7998, the mean response score of F_1's was 0.7 (S.E. = 0.2; n = 15) in comparison to parental mean scores for Hawaii 7998 of 5.0 (S.E. = 0.0, n = 9) and for the susceptible line Florida 7060 of 0.1 (S.E. = 0.1, n = 9).

Analysis of growth of race 2 89-1 carrying *avrRxv* in F_1 individuals from a cross between the susceptible line Walter × Hawaii 7998 showed levels intermediate to those in Walter or Hawaii 7998. Over a 5-day period, the change in population size of race 2 89-1 (pXV9009) was 20-fold greater in these F_1's than in the resistant parent Hawaii 7998 and conversely, was 15-fold less in these F_1's than in the susceptible parent Walter (data not shown; Fig. 2).

Four hundred and fifty-eight F_2 individuals from reciprocal crosses between Hawaii 7998 and the susceptible parent Ailsa Craig were scored for their response to race 2 89-1 carrying *avrRxv* and numbers of individuals with resistant, intermediate, and susceptible responses were statistically analyzed (Table 5). The ratio of 1:7:8 for resistant:intermediate:susceptible fit the F_2 data set. This phenotypic ratio is consistent with a model whereby two additive loci govern the *avrRxv*-specific resistance in Hawaii 7998. By this model full resistance was seen only when both resistance loci were present in the homozygous state. However, it is difficult to assign genotypes to the intermediate and susceptible phenotypic classes. Other phenotypic ratios such as 1:6:9 and 1:3 (pooling intermediates and susceptibles) were dismissed based on χ^2 analysis.

In addition, 129 individuals from an F_2 population from a cross between Hawaii 7998 and the susceptible line Florida 7060 were examined for their response to race 2 89-1 carrying *avrRxv* (Table 5). Consistent with the data

Table 5. Analysis of *avrRxv*-specific resistance in F_2 populations derived from crosses between Hawaii 7998 and susceptible tomato lines[a]

	Crosses	
	Ailsa Craig × Hawaii 7998[b]	**Florida 7060 × Hawaii 7998[c]**
Response group	**Number of F2 individuals**	
Resistant	36	9
Intermediate	194	50
Susceptible	228	70
Total	458	129
	Statistical analysis	
χ^2 Value for 1:7:8 Phenotypic Ratio of Resistant: intermediate: susceptible	1.915	1.327
P level[d]	0.30	0.50

[a] Several leaves on individual plants from designated populations were inoculated with XcvT race 2 89-1 carrying *avrRxv* on a plasmid. The responses of both F_1 populations were intermediate.

[b] Responses were scored 72 hr postinoculation. Results from reciprocal crosses were combined. Individuals classified as resistant had 61–100% necrosis in the infiltrated area; as intermediate, 1–60% necrosis; and as susceptible, no necrosis and watersoaking.

[c] Responses were scored 56 hr postinoculation. Individuals classified as resistant had 61–100% necrosis in the infiltrated area; as intermediate, 1–60% necrosis; and as susceptible, no necrosis.

[d] Probability greater than designated level.

from Hawaii 7998 and Ailsa Craig F_2 populations, the ratio of 1:7:8 for resistant:intermediate:susceptible fit the data from this F_2 population. A 1:6:9 phenotypic ratio also fit (χ^2 = 0.256, P = 0.88), suggesting that two additive loci control *avrRxv*-specific resistance in Hawaii 7998.

DISCUSSION

Bacterial spot disease caused by tomato races of *X. campestris* pv. *vesicatoria* (XcvT) is a serious problem in many areas of the world where tomatoes are grown. Although resistance to XcvT is rare in tomato, it is widespread in nonhost species. Our study of nonhost resistance to XcvT race 1 resulted in identification of the avirulence gene *avrRxv* (Whalen *et al.* 1988). When carried by normally virulent pathovars, *avrRxv* induced cultivar/line-specific resistant responses in six nonhost plant species, bean, soybean, cowpea, alfalfa, cotton, and corn (Whalen *et al.* 1988). Studies of host resistance to XcvT were impossible until a line of tomato resistant to XcvT (Jones and Scott 1986; Scott and Jones 1986) and virulent strains of XcvT were found (race 2 89-1; Table 2). Once this material became available, we were able to begin studying the molecular genetic basis of resistance in tomato to XcvT race 1. We tested the hypothesis that the gene-for-gene model of resistance described the interaction between XcvT race 1 and the resistant tomato line Hawaii 7998. We screened a library of DNA from XcvT race 1 for resistance-inducing activity on Hawaii 7998 and to our surprise we independently cloned the previously described avirulence gene *avrRxv* (Table 3, Figs. 1 and 2). Our results indicate that *avrRxv* is involved in host as well as nonhost resistance to XcvT. In studies with *P. syringae* pathovars, observations similar to ours were made, suggesting that the same avirulence gene is involved in induction of specific resistance in both host and nonhost species. The avirulence gene *avrPpi2* from *P. syringae* pv. *pisi,* when carried by a normally virulent strain, induces cultivar-specific resistance in its natural host, pea, and in the nonhost species, bean (Fillingham *et al.* 1992). In addition, *avrPpi1A*, another avirulence gene from *P. syringae* pv. *pisi*, and *avrPma1* (*avrRpm1*) from *P. syringae* pv. *maculicola* are 97% similar (Dangl *et al.* 1992). Both avirulence genes induce resistance on hosts and nonhosts. Furthermore, *avrB* from *P. syringae* pv. *glycinea* has also been shown to induce resistance in a genotype-specific manner on both its natural host, soybean, and nonhost species, *Arabidopsis thaliana* (Bent *et al.* 1992; Innes *et al.* 1993b). These molecular genetic studies have shown that single avirulence genes contribute to restriction of host range at both the species and genus level by inducing resistant responses in host and nonhost species (Keen and Staskawicz 1988).

As part of our effort to understand the avirulence function encoded in *avrRxv*, the gene was sequenced (Fig. 4). Sequence analysis revealed a major open reading frame, ORF 2-2, encoding a putative protein of 374 amino acids with a molecular weight of 42.1 kDa. Future studies with specific antibodies will allow us to verify the production of a protein in XcvT with the predicted molecular weight. Analysis of the sequence of *avrRxv* revealed possible −10 and −35 promoter sequences but no sequences that resemble Shine-Dalgarno sequences proposed for *E. coli* or *Xanthomonas* genes (Fig. 4) (Ronald and Staskawicz 1988; Bonas *et al.* 1989; Parker *et al.* 1993). Comparison of promoter regions of *Xanthomonas hrp* and avirulence genes to those of *P. syringae* pathovars revealed the presence of core sequences from the putative *P. syringae* avirulence gene promoter with similarities to the *P. syringae hrp* box (Fellay *et al.* 1991; Jenner *et al.* 1991; Innes *et al.* 1993a); in *avrRxv* (GAAACGGA), in *avrBs3* (Bonas *et al.* 1989), *avrBsP* (Canteros *et al.* 1991) and *avr10* (Hopkins *et al.* 1992) (GGAAGCGAN$_8$CCACN$_2$A) but, not in either ORF of *avrBs1* (Ronald and Staskawicz 1988) or two *X. campestris* pv. *glycines hrp* genes (Hwang *et al.* 1992). Parker *et al.* (1993) recently reported a sequence resembling the *P. syringae hrp* box sequence in *avrXca* from *X. campestris* pv. *raphani*. When they become available, comparisons among more *Xanthomonas* avirulence and *hrp* gene sequences may allow description of consensus regulatory sequences in *Xanthomonas* and comparison to those in *P. syringae*.

The putative amino acid sequence of ORF 2-2 is hydrophilic with no obvious hydrophobic membrane spanning regions or transport-associated amino acids. Localization studies will be pursued in the future with ORF 2-2-specific antibodies. A possible function of the putative ORF 2-2 protein was found in similarity searches of amino acid databases. Using a localized similarity search of a protein subsequence database, we found that a region in RNA polymerase subunits has a high degree of similarity. This similarity along with the predicted, highly basic pI of 10.7 for ORF 2-2 are consistent with a possible function in DNA binding.

To understand the inheritance of *avrRxv*-specific resistance in tomato line Hawaii 7998, the response of about 580 F_2 individuals from crosses between Hawaii 7998 and two susceptible tomato lines were analyzed under controlled environmental conditions. The phenotypic ratio of 1:7:8 for resistant:intermediate:susceptible fit F_2 data with a statistically acceptable level of significance (Table 5). This ratio suggests that two additive loci may govern *avrRxv*-specific resistance in Hawaii 7998 and that resistance is only observed when both loci are present in the homozygous state. Since the assignment of genotypes to the intermediate and susceptible phenotypic classes is difficult, further work is necessary for a full understanding of the genetics. Analysis of spot inoculations and *in planta* growth demonstrates that F_1 individuals exhibit an intermediate level of resistance and supports our model of additive inheritance. The observable phenotype corresponds to halving the gene dosage suggests that the products of these alleles are limiting factors in determining the outcome of the interaction between XcvT and tomato (Kacser and Burns 1981). At the most simple level in accordance with the elicitor-receptor model (Gabriel and Rolfe 1990), our results may suggest that the two resistance gene products dimerize to produce a functional receptor for the

avirulence gene product or its elicitors. Several alternative explanations are also possible.

Our results indicating that two additive loci may govern the *avrRxv*-specific resistance in Hawaii 7998 differ from our earlier results in bean with respect to number of loci. In bean a single, incompletely dominant locus appeared to condition resistance (Whalen *et al.* 1988). Although in both bean and tomato the dosage of the resistance allele(s) appears to be important, genetic control of the resistance appears to operate differently in the two species. The differences in the genetic control of *avrRxv*-specific resistance in the nonhost species, bean, and in the host species, tomato, do not support our hypothesis that nonhost and host resistance are fundamentally similar (Whalen *et al.* 1988). Our findings that one locus is required in bean for specific resistance and two in tomato merit attention considering their implications for the concept of using cloned resistance genes from heterologous species to increase resistant germplasm. It is obvious that more genetic and molecular studies are necessary before drawing conclusions regarding the universality of avirulence gene specific-resistance mechanisms.

Our model of two genes in Hawaii 7998 governing resistance to a XcvT-derived avirulence gene is supported by results from related studies on resistance to wild-type XcvT race 1. Field studies of the segregation of resistance in an F_2 population from a cross between Hawaii 7998 and the susceptible line Walter demonstrated that more than three genes conditioned the resistance to wild-type race 1 (Scott and Jones 1989). Data from field studies, however, must be cautiously interpreted because of variable environmental conditions and inoculum recruitment from surrounding fields. Temperature in field studies presents an especially important consideration because we find that resistance associated with *avrRxv* is temperature sensitive; race 1 75-3 in Hawaii 7998 grew 40 times more at 30° C than at 24° C (M.C.W., M.E.H., F.M.C., J.F.W., unpublished data). In another set of studies, co-segregation of resistance to wild-type race 1 with various isozyme markers (J.F.W., R.E.S., and C. E. Vallejos, unpublished data) and RFLP markers (Yu, Z.H., J.F.W., R.E.S., and C. E. Vallejos, unpublished data) was studied in F_1 backcross progeny from a cross between *Lycopersicon pennelli* and Hawaii 7998. Results from these studies suggested that more than one unlinked factor from the resistant parent is important for expression of resistance to wild-type race 1. In addition, modifying factors contributed by the susceptible parent were shown to be involved. In the study presented here, we specifically analyzed resistance associated with *avrRxv* but not with wild-type race 1. Differences in the number of factors thought to be required for resistance may be attributed to other avirulence factors present in race 1 strains. Although our screen of the race 1 library on plants of Hawaii 7998 resulted in the isolation of only one avirulence gene, race 1 may contain more avirulence genes that were not detected in our screen. This is also suggested by our results from inoculations of race 1 75-3 *avrRxv*::Ω in which *avrRxv* is insertionally mutated (Table 3) and from inoculations of race 1 90-14 Δ*avrRxv,* in which *avrRxv* is deleted (M.C.W., and S. M. Conover, unpublished data). Insertional mutation or deletion of *avrRxv* in race 1 strains produced an intermediate response on Hawaii 7998 (Table 3; M.C.W. and S. M. Conover, unpublished data). It is interesting to note that growth of race 1 75-3 *avrRxv*::Ω in Hawaii 7998 was indistinguishable from that of virulent strains (Fig. 1A), which suggests that *avrRxv* may be the only avirulence gene restricting growth of race 1 75-3. Results on the response of Hawaii 7998 based on inoculations with race 1 75-3 *avrRxv*::Ω at 5×10^8 cfu/ml may differ from those based on analysis of *in planta* bacterial growth with starting inoculum concentration of 1×10^5 cfu/ml because of the different concentration of inoculum used in the two types of analyses.

The gene-for-gene model of resistance does not formally describe a specific interaction between two resistance loci and one avirulence gene. Our work provides a description of this type of interaction from environmentally controlled studies in which artifactual effects from the genetic background of the pathogen are eliminated by using pathogen strains that are isogenic except for a cloned avirulence gene. Reports of this general type of deviation from the gene-for-gene model, however, are common (reviewed by Crute 1985; Sidhu and Webster 1977; Fillingham *et al.* 1992). In deviant interactions in which specific resistance is governed by additive genes, the resistance appears to be phenotypically similar to that governed by single genes. It is possible that the two genes are identical but lie in unlinked locations in the genome. Further molecular analysis is necessary before drawing conclusions regarding the similarities and differences in the mechanisms governing resistance specified by a single gene or multiple genes. The complexity of the *avrRxv*-specific resistance has important implications for ease of use in plant breeding schemes and for molecular isolation strategies.

MATERIALS AND METHODS

Bacterial strains, media, and plasmids.

Bacterial strains, vectors, and plasmid constructions are described in Table 1. All pathovars of *X. campestris* were provided by R. E. S. *Escherichia coli* HB101 (Boyer and Roulland-Dussoix 1969) and DH5α (Bethesda Research Laboratories) were routinely used. Strains of XcvT were subcultured at 30° C on nutrient yeast glycerol medium (Daniels *et al.* 1984) or nutrient broth (Difco) and *E. coli* strains at 37° C on Luria medium (Miller 1972). Bacto agar (Difco) at 1.5% (w/v) was added to media for plate cultures. Antibiotics (Sigma) were used for selection at the following concentrations (in μg/ml): tetracycline (Tc), 10; rifampicin (Rif), 100; spectinomycin (Sp), 100; ampicillin (Ap), 50; kanamycin (Km), 25.

Triparental matings using the helper plasmid pRK2013 (Table 1) were used to mobilize clones of DNA from *E. coli* into XcvT. The identity of recombinant plasmids from all XcvT transconjugants was verified by isolating plasmids from XcvT transconjugants, transforming *E. coli* DH5α, and reisolating the plasmids and analyzing them using restriction enzymes and agarose gel electrophoresis.

Growth of plants, plant inoculations, *in planta* bacterial growth curves, and electrolyte leakage studies.

Plants were grown from seed in greenhouses or in growth chambers in plastic or clay pots with standard potting soil. Growth chambers and growth rooms were set with 16-hr photoperiods at 24° C. Plants grown in greenhouses were incubated in growth chambers or rooms beginning at least 24 hr before experimental use.

Reactions of plants to infection with XcvT strains were determined by infiltrating ~10 μl of a bacterial suspension (10^7–10^8 colony-forming-units [cfu]/ml) into leaflets as described (Swanson *et al.* 1988). Leaflets on the fourth through eighth leaves were inoculated with several replicates per strain. The responses of plants to inoculation, scored by visual inspection, were classified as resistant, intermediate, or susceptible. The responses were grouped into these classes based on comparison with the response of the resistant line Hawaii 7998, of the particular susceptible line and F_1. In addition, these classifications were based on analysis of *in planta* bacterial growth (see Results). The responses differed depending on whether plants were incubated in growth chambers or growth rooms. Plants in growth chambers showed an extended range of phenotypes including watersoaking in susceptible tomato lines. Unless otherwise specified, plant responses were scored under these conditions.

Responses of plants incubated in growth chambers classified as susceptible (response score = 1) were typified by spreading watersoaking. Responses classified as intermediate and resistant were typified by necrosis in the infiltrated area. Some responses classified as intermediate had infiltrated areas with watersoaking in the center and necrosis at the periphery (response score = 2). Others classified as intermediate showed no watersoaking and up to 60% necrosis and were scored as follows: 0% necrosis (response score = 3), 1–20% necrosis (response score = 4), 21–40% necrosis (response score = 5); 41–60% necrosis (response score = 6). Responses classified as resistant had greater than 60% necrosis in the infiltrated area and were scored as follows: 61–80% necrosis (response score = 7) or 81–100% necrosis (response score = 8). For plants incubated in growth chambers, susceptible and resistant responses were most clearly differentiated at 72 hr after inoculation.

F_1 and F_2 progeny derived from crosses with the susceptible line Florida 7060 were incubated in growth rooms after inoculation. Responses of plants incubated under these conditions were classified solely by the percentage of necrosis in the infiltrated area. Responses classified as susceptible had no necrosis (response score = 0). Responses classified as intermediate had up to 60% necrosis and were scored as follows: 1–20% necrosis (response score = 1); 21–40% necrosis (response score = 2); 41–60% necrosis (response score = 3). Plants classified as resistant had greater than 60% necrosis in the infiltrated area and were scored as follows: 61–80% necrosis (response score = 4); 81–100% necrosis (response score = 5). For plants incubated in growth rooms, susceptible and resistant responses were most clearly differentiated at 56 hr after inoculation.

To determine levels of bacterial growth *in planta*, leaves of plants were vacuum infiltrated with bacterial suspensions of 10^5 cfu/ml and sampled as described (Whalen *et al.* 1988). To study electrolyte leakage from leaf tissue in response to infiltration with various strains, bacterial cells at 5×10^8 cfu/ml were infiltrated into a 5-cm^2 area of leaf tissue. Conductivity of water in baths containing inoculated tissues was measured every 12 hr after infiltration according to published procedures (Hibberd *et al.* 1987).

Recombinant DNA techniques.

Standard techniques were used for subcloning, plasmid preparations, gel blot hybridizations, and agarose gel electrophoresis (Ausubel *et al.* 1987; Maniatis *et al.* 1989). Individual clones from a library of DNA from XcvT race 1 75-3 (Whalen *et al.* 1988) in the wide host range cosmid pLAFR3 (Table 1) were mobilized into XcvT race 2 89-1. The resulting transconjugants were inoculated onto resistant and susceptible tomato lines as described above. Genomic DNA used in DNA blot analysis was isolated from XcvT strains as described (Staskawicz *et al.* 1984). DNA restriction endonuclease fragments were ^{32}P-labeled with random primers (Feinberg and Vogelstein 1983).

Exonuclease III deletion analysis.

Plasmid constructions are listed in Table 1. To create deletions of *avrRxv*, the 2.1-kbp *Pst*I fragment was isolated from pXV9009 (Whalen *et al.* 1988), termini were filled in with T4 DNA polymerase, and then subcloned into the *Sma*I site of pUC118 in both orientations, producing pUC118RXV1 and pUC118RXV3. To make a set of deletions going from the *Bam*HI site of pUC118 into the *avrRxv* sequence, these plasmids were serially digested with *Bam*HI, *Pst*I, and then exonuclease III as described (Henikoff 1984). Klenow enzyme was used to create blunt ends for religation. The deletion called pUC118RXV3X was created by digesting pUC118RXV3 with *Eco*RI and *Xmn*I, isolating the 1.54-kbp *Eco*RI/*Xmn*I fragment, and cloning it into pUC118 digested with *Eco*RI and *Sma*I. All of the pUC118 deletion plasmids were transformed into *E. coli* MV1193 (Maniatis *et al.* 1989) to allow production of single-stranded DNA by superinfection with the helper phage M13KO7.

To analyze activity of deleted inserts in XcvT, deleted fragments were cloned from pUC118 into pL6 (Table 1). The pUC118 deletion plasmids and pL6 were digested with *Hin*dIII, ligated, and transformed into *E. coli* C2110 (Maniatis *et al.* 1989); the pUC118 sequences from miniprepped DNA were then removed using an *Eco*RI digest, followed by religation. Following transformation into DH5α, the pL6-deletion constructs (pRXV3 and pRXV1 series; Table 1) were conjugated into XcvT race 2 89-1 and the resulting transconjugants were tested on tomato (Fig. 3).

To test whether the loss of activity of the deletion could be overcome using transcriptional fusions to a strong promoter, constructs were produced in which the β-galactosi-

dase promoter was positioned to drive expression of insert DNA. The *lacZ* promoter-driven activity of inserts from some deletions was tested by cloning pUC119 in the appropriate orientation into the pL6 constructs containing these deletions, producing pRXV3PlacX, pRXVPlac33, and pRXVPlac34 (Table 1). These constructs were conjugated into XcvT race 1 89-1 and the resulting transconjugants were tested on tomato (Fig. 3).

Nucleotide sequencing and analysis.

Single-stranded templates were prepared from the deletion constructs of pUC118RXV1 and pUC118RXV3 as described (Vieira and Messing 1987). The dideoxy chain-termination sequencing method was used (Sanger *et al.* 1977) to sequence both strands of a 2.1-kbp fragment that had full avirulence activity.

The DNA sequence and the predicted translation product of *avrRxv* were compared to the NIH Genbank and EMBL nucleotide sequence databanks and the PIR and Swiss-Protein amino acid databanks using the FastDB (Brutlag *et al.* 1990) and IFIND programs (Intelligenetics) and the Blastp (NCBI; Altschul *et al.* 1990) program. The Intelligenetics program called PEP was used to analyze the predicted amino acid sequence and predict secondary structure. The algorithms of Hopp and Woods (1981) and Kyte and Doolittle (1982) were used to produce the hydropathy patterns from the deduced protein. To determine percentage of similarity, PAM 250 matrix substitutions with scores ≥1 were considered conservative amino acid substitutions.

To determine if the predicted translation product of *avrRxv* was produced in *E. coli*, three pUR vectors containing cloning sites at the carboxy terminus of the β-galactosidase gene were used (Table 1). The deletion construct pUC118RXV33 was digested with *Hin*dIII, termini were filled in with T4 DNA polymerase, and then religated with *Bam*HI linkers. This new plasmid, in which the *Hin*dIII site was eliminated and a *Bam*HI site was introduced, was then digested with *Eco*RI, blunt-ended with T4 DNA polymerase and religated with *Hin*dIII linkers. The insert from pUC118RXV33 was isolated using a *Hin*dIII and *Bam*HI digestion and then cloned into *Bam*HI and *Hin*dIII digested pUR278, 288, and 289 (Table 1). The pUR series carrying the insert from pUC118RXV33 were transformed into *E. coli* TG-1 (Gibson 1984). Clones were identified with colony hybridization and verified with restriction enzyme analysis. To analyze production of fusion proteins, TG-1 cells carrying the pUR fusions were grown in LB with ampicillin. One-milliliter cultures induced with 1 mM isopropylthiogalactoside (IPTG) were grown 2 hr following induction. Bacteria were harvested, resuspended in Laemmli buffer (Laemmli 1970), and boiled. Samples were run on a 10% polyacrylamide stacking gel and stained with Coomassie blue R-250.

Genetic analysis of inheritance of resistance in tomato.

To determine the inheritance of *avrRxv*-specific resistance in tomato, crosses were made in the greenhouse between the resistant cultivar Hawaii 7998 and the susceptible cultivars, Ailsa Craig and Florida 7060. Individuals from populations of the resulting F_1 and F_2 progeny were screened by inoculating just-expanded leaves with race 2 89-1 (pXV9006) or race 2 89-1 (pXVSC910) and results analyzed as described earlier.

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