

Race Shift in *Xanthomonas campestris* pv. *vesicatoria* Within a Season in Field-Grown Pepper

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ABSTRACT

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Race shifts in *Xanthomonas campestris* pv. *vesicatoria*, (proposed: *X. axonopodis* pv. *vesicatoria*), the pepper bacterial spot pathogen, were studied in the field during 1993 and 1994. In 1993, Early Calwonder (ECW) pepper plants inoculated with rifampicin-resistant race 1 strain Xcv 33^{rif}, which carries avirulence gene *avrBs3*, were planted in a plot of ECW-30R plants, which carry the *Bs3* gene for resistance to race 1 of the pathogen. Disease was observed on plants adjacent to the inoculum plants 2 weeks after inoculation, and within 10 weeks, all plants were diseased. Rifampicin-resistant single colonies isolated from diseased ECW-30R plants were screened for race reaction on pepper differentials, and all were race 3 because they were compatible on ECW-30R. Total DNA

from 25% of the single colonies hybridized to an *avrBs3* gene probe, and the plasmid carrying *avrBs3* was not detected in the colonies that did not hybridize to *avrBs3*. Similarly, in 1994 the population derived from a strain of race 2 that carries *avrBs1* rapidly shifted to race 3 when inoculated plants were placed in a plot of ECW-10R plants carrying the single resistance gene *Bs1*. Total DNA from all isolated single colonies with the race 3 phenotype hybridized with *avrBs1* and insertion element IS476. Thus, a race 3 pathogen population of *X. campestris* pv. *vesicatoria* rapidly developed from race 1 and 2 populations within a growing season in the field when exposed to a resistant pepper cultivar carrying a single resistance gene. The race shift from race 1 to 3 was due to loss of the plasmid carrying the avirulence gene or to inactivation of *avrBs3*. Shift from race 2 to 3 apparently resulted from insertion element IS476 inactivating avirulence gene *avrBs1*.

Bacterial spot caused by *Xanthomonas campestris* pv. *vesicatoria* (proposed: *X. axonopodis* pv. *vesicatoria*, [32]) is a major limiting factor in pepper production (13). The disease can cause significant fruit loss during warm, moist weather. Single-gene host resistance is inadequate in managing this disease because of host-differentiated races (4,26). To date, seven races (0 to 6) of the pathogen have been detected in diseased peppers (2,4,18,26,27).

A gene-for-gene relationship in the pepper-bacterial spot pathogen system has been defined (23). All pepper strains of races 0 to 3 carry avirulence gene *avrBs2* and elicit a hypersensitive response (HR) on pepper carrying resistance gene *Bs2* (Table 1). In addition, race 1 and 2 strains carry *avrBs3* and *avrBs1* and elicit a HR on plants carrying resistance genes *Bs3* and *Bs1*, respectively. Race 0 strains carry all three avirulence genes and elicit a HR on plants carrying any one of the resistance genes (26). *AvrBs3* and *avrBs1* are located on self-transmissible plasmids (1,23,30,31), whereas *avrBs2* is located on the bacterial chromosome (23). Based on laboratory studies, changes within populations of some of these races can occur at a high frequency (4×10^{-4} mutants per cell division), and the change is from pepper race 2 to race 3 (5). Race change also is thought to nullify the effects of resistance genes in the field (6). Based on laboratory research, several mechanisms can lead to loss of avirulence gene activity, including inactivation by insertion of a transposable element, loss of plasmids

carrying avirulence genes, and various other mutations (23). The current study was conducted with strains with phenotypic markers to determine the ability of *X. campestris* pv. *vesicatoria* to genetically mutate and shift race in the field and build up a virulent population when placed in plantings of resistant genotypes.

MATERIAL AND METHODS

The experiments were conducted at the Sandhills Research Station, Jacksonsprings, NC, in 1993 and 1994. This station is not located in a commercial pepper-growing region of the state. Experiments were conducted at sites where no peppers were grown previously. Each plot was surrounded by at least 9.1 m of rye during both years. General cultivation and tillage practices recommended for pepper production were followed (28). Plants were grown for 10 weeks in polystyrene trays with 200 2.5-cm cells and were transplanted to raised beds during the last week of April. Overhead irrigation (1.6 cm/h) for 4-h periods was applied to the crop as needed to maintain optimum plant growth, normally twice weekly during both years. During each year, a different site separated by 1 km within the research station was used.

Details of the bacterial strains and plasmids used in this study are presented in Table 2. The race 1 (Xcv 33^{rif}) and race 2 (Xcv 43) strains used in this study belong to previously classified *X. campestris* pv. *vesicatoria* group A and did not hydrolyze starch (2,32). Race 1 strain Xcv 33^{rif} did not elicit a HR on tomato cv. Bonny Best and does not carry avirulence gene *avrBsP*, which has homology to *avrBs3* (3). A single colony of race 1 strain Xcv 33 was isolated and selected on nonselective yeast dextrose calcium-car-

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bonate agar (YDC) from diseased pepper samples in 1989. The colony was purified further by isolating a single colony on YDC and utilizing it for isolating rifampicin-resistant mutants. A single rifampicin-resistant race 1 colony (*Xcv* 33^{rif}) was used in the experiments. Similarly, the race 2 strain (*Xcv* 43) was purified three times by single-colony isolations prior to use.

Shift from race 1 to 3. In 1993, a plot of 20 rows × 20 plants per row of pepper Early Calwonder (ECW) isogenic line ECW-

TABLE 1. Reaction of known races of *Xanthomonas campestris* pv. *vesicatoria* isolated from pepper on three Early Calwonder (ECW) pepper differentials^a

Race	ECW-10R (<i>Bs1</i>) ^b	ECW-20R (<i>Bs2</i>) ^b	ECW-30R (<i>Bs3</i>) ^b	Reference
0	HR ^c	HR	HR	26
1	C	R	HR	4, 12, 23
2	HR	HR	C	4, 12, 23
3	C	HR	C	12, 23
4	C	C	HR	18
5	HR	C	C	18
6	C	C	C	27
	<i>avrBs1</i> ^d	<i>avrBs2</i> ^d	<i>avrBs3</i> ^d	

^a Seeds of pepper differentials were provided by R. E. Stall (4,12,23). All pepper races are compatible on ECW

^b Details in parentheses indicate the resistance gene present in the near isogenic lines of pepper.

^c HR = hypersensitive response (resistant reaction); C = compatible response (disease reaction).

^d *avr* indicates the avirulence gene in the pathogen corresponding to the resistance gene in the plants. Extensive details of the avirulence genes have been provided by Minsavage et al. (23).

30R, which carries the single resistance gene *Bs3*, was established. Rows were spaced 97 cm apart, and plants within rows were spaced 36 cm apart. ECW plants, which are susceptible to all known races of the bacterial spot pathogen, inoculated with rifampicin-resistant race 1 strain *Xcv* 33^{rif} and expressing symptoms

TABLE 2. Details of *Xanthomonas campestris* pv. *vesicatoria* strains and plasmids used in this study^a

Strain	Characteristics	Reference
<i>Xcv</i> 33 ^{rif}	Rif ^R , Cu ^S , Sm ^S <i>avrBs1</i> ⁻ , <i>avrBs2</i> ⁺ , <i>avrBs3</i> ⁺ , <i>avrBsP</i> ⁻	This study Isolated from diseased peppers in Sampson County, NC, 4/19/89; selected for Rif ^R in laboratory
<i>Xcv</i> 43	Rif ^S , Cu ^R , Sm ^R , <i>avrBs1</i> ⁺ , <i>avrBs2</i> ⁺ , <i>avrBs3</i> ⁻ , <i>avrBsP</i> ⁻	This study. Isolated from diseased peppers in Sampson County, NC, 7/19/89
pEC83 Eco 7	Contains a 3.3-kb <i>Bam</i> HI fragment with <i>avrBs3</i> activity in pLAFR3	1,23
pXv 2007	Contains a 5.3-kb <i>Bgl</i> III- <i>Pst</i> I fragment with <i>avrBs1</i> activity in pWB5A	23,31
pXv2M109	Contains the 0.35-kb <i>Sal</i> I- <i>Sma</i> I fragment of insertion element IS476 in pUC 118	16

^a Rif = rifampicin, 50µg/ml; Cu = copper, 200 µg/ml; Sm = streptomycin, 100 µg/ml.

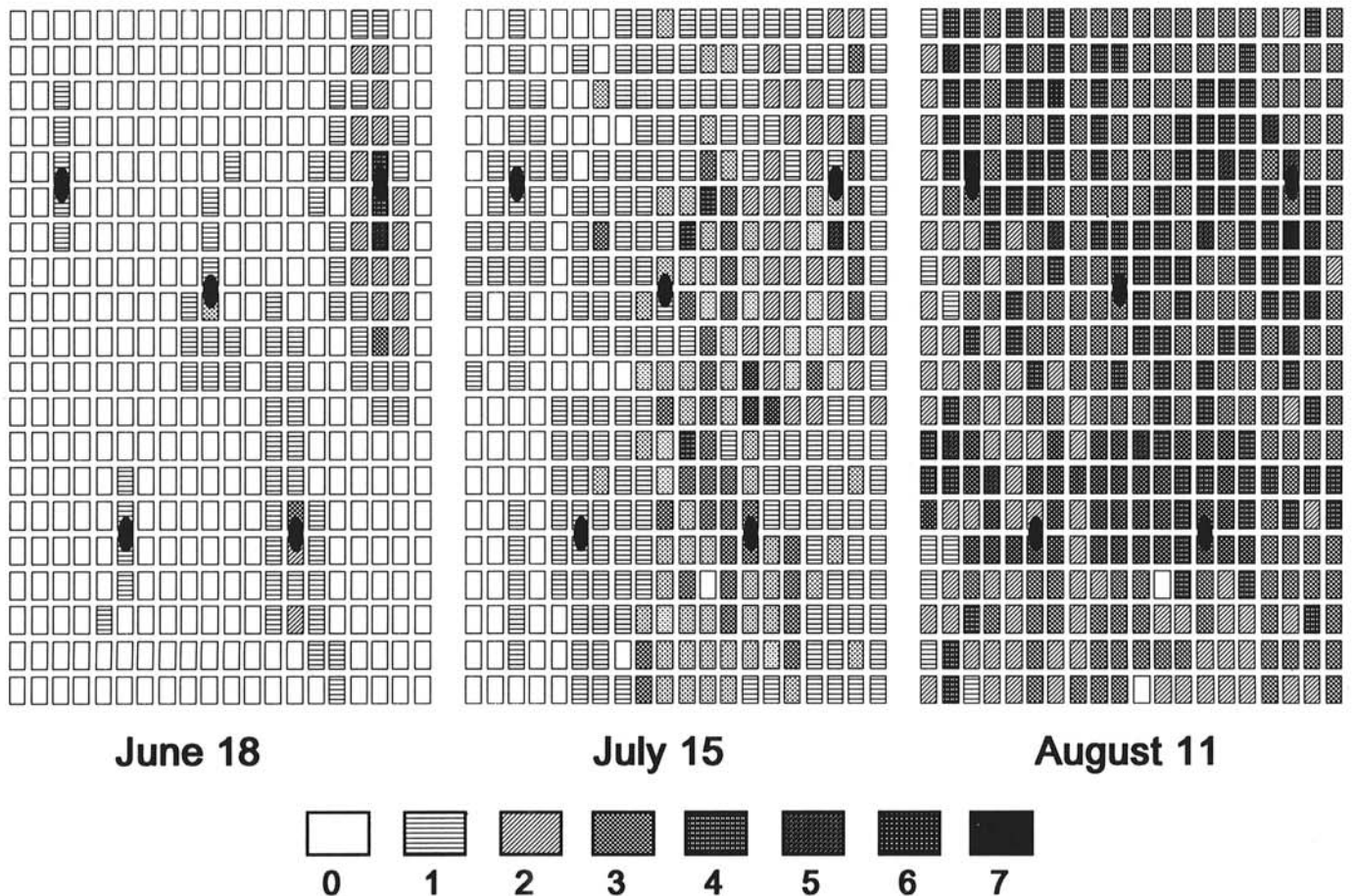


Fig. 1. Spatial and temporal increase of bacterial leaf spot disease of Early Calwonder (ECW) bell pepper plants during 1993 in a plot of ECW-30R, which has resistance gene *Bs3*. Each box in the grid represents a single plant. The dark ovals denote the position where the inoculum plants with a race 1 strain, *Xcv* 33^{rif}, of *Xanthomonas campestris* pv. *vesicatoria* were planted on 20 May. Race 1 strains are not compatible on ECW-30R. Disease ratings were based on a 0 to 9 scale, in which 0 = no diseased leaves and 9 = 100% of leaves diseased, complete defoliation, and plant dying or dead.

were planted as inoculum sources at five locations in the plot on 20 May (Fig. 1). Inoculum plants were prepared as described previously (20,21).

Briefly, a bacterial suspension of *Xcv* 33^{rif} (10^8 CFU/ml) was rubbed onto leaves with a sterile cotton swab, and Carborundum was used as an abrasive. The inoculated plants were maintained in the laboratory, with occasional misting of foliage, until disease symptoms were observed. The plants were kept in isolated locations in the laboratory under plastic covers and away from any other pepper plants or inoculum sources. The presence of *Xcv* 33^{rif} on the inoculum plants was verified by isolation of bacteria on YDC from the ECW plants expressing disease and by determination of race reactions on pepper differentials prior to transferring the plants to the plot as described previously (23).

Resistance to rifampicin was determined on sucrose (10 g/liter) and peptone (5 g/liter) agar (15 g/liter) plates (SPA) containing 50 µg of rifampicin per ml. All race 1 strains elicit a HR on plants of ECW-30R. ECW-30R plants in the field plot were observed weekly for development of disease. Disease ratings were recorded on every plant based on a 0 to 9 scale as described previously (21). Diseased leaf samples were taken from ECW-30R plants periodically, for a total of six sampling dates, and the pathogen was isolated on SPA containing 50 µg of rifampicin per ml. For sampling, the plot was divided into five sections, with each section containing one inoculum location. Diseased leaves were removed randomly from plants throughout each section. One to two disease

lesions from each leaf were excised, and lesions from at least 10 leaves from each section were pooled and crushed in 500 µl of sterile distilled water in an Eppendorf microfuge tube using a pestle. Two loopfuls of this leaf homogenate was streaked for single colonies onto two SPA plates for each sample. Single colonies were selected at random from these plates, and the race of each rifampicin-resistant colony was determined with pepper differentials ECW-10R (contains *Bs1*), ECW-20R (contains *Bs2*), and ECW-30R (contains *Bs3*) as previously described (23,26).

The occurrence of avirulence gene *avrBs3* was monitored with a 3.3-kb *Bam*HI fragment of the gene as a hybridization probe for colony hybridizations (1). Plasmid extractions (8) and restriction digests were performed as previously described (29). The 3.3-kb probe was labeled and used for dot blots and Southern hybridizations by Genius nonradioactive detection methods (Genius kit protocol, Boehringer Mannheim Biochemicals, Indianapolis, IN) with digoxigenin-labeled probes under stringent wash conditions (65°C, 0.5× SSC [1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0], and 0.1% sodium dodecyl sulfate).

Shift from race 2 to 3. In 1994, a plot with 10 rows × 20 plants per row of pepper isogenic line ECW-10R, which carries the single resistance gene *Bs1*, was established. Race 2, strain *Xcv* 43, which is resistant to copper and streptomycin, was used to inoculate susceptible cv. Camelot plants. Diseased Camelot plants were planted as inoculum sources at three locations in the ECW-10R plot on 12 May. Strain *Xcv* 43 carries the plasmid-borne avirulence gene *avrBs1* and elicits a HR on ECW-10R plants. In addition, *Xcv* 43 also consistently carries a 1.8-kb cryptic miniplasmid found primarily in all race 2 strains examined (8), and this plasmid also served as an apparently neutral marker in the experiments. The presence of *Xcv* 43 on Camelot plants was verified prior to transferring the plants to the field plots as described above. A plot with 10 rows × 10 plants per row of susceptible, open-pollinated cv. Jupiter also was established and inoculated with strain *Xcv* 43. Disease ratings were recorded weekly based on a 0 to 9 scale as described previously (21). Diseased leaf samples were taken periodically, for a total of six sampling times during the season.

The pathogen was isolated on YDC, and the race was determined as described above. For sampling, the plot was divided into three sections, with each section containing one inoculum location. Diseased leaves were removed randomly from plants throughout each section, and single colonies were isolated as described

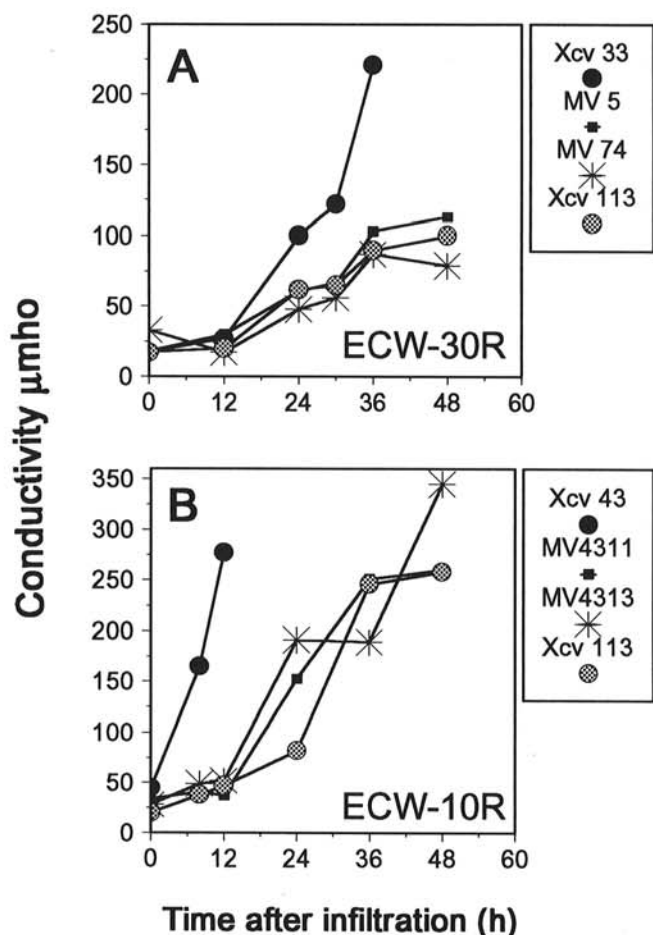


Fig. 2A, Electrolyte leakage from Early Calwonder (ECW) bell pepper leaf tissue of ECW-30R inoculated with bacterial suspensions (10^8 CFU/ml) of a wild-type race 1 strain (*Xcv* 33^{rif}), race-shift mutants (MV 5 and MV 74), and a known race 3 strain (*Xcv* 113) of *Xanthomonas campestris* pv. *vesicatoria*. **B,** Electrolyte leakage from pepper leaf tissue of ECW-10R plants inoculated with bacterial suspensions (10^8 CFU/ml) of a wild-type race 2 strain (*Xcv* 43), race-shift mutants (MV 4311 and MV 4313), and a known race 3 strain (*Xcv* 113) of *X. campestris* pv. *vesicatoria*.

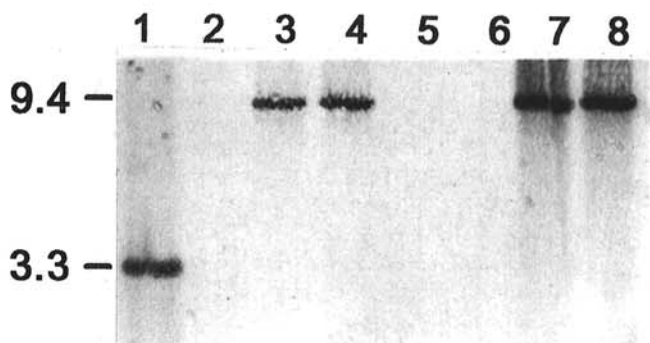


Fig. 3. Southern blot of restriction digests with *Bam*HI of plasmids of a wild-type race 1 strain (*Xcv* 33^{rif}, lane 1) and strains changed to race 3 (lanes 2 through 8) of *Xanthomonas campestris* pv. *vesicatoria* in a field plot of Early Calwonder (ECW) bell pepper ECW-30R (carries resistance gene *Bs3*) plants. All race 1 strains carry *avrBs3* and cause a hypersensitive response on ECW-30R. Strain *Xcv* 33^{rif} was introduced into the plot of ECW-30R, and disease development was seen 2 to 3 weeks later. Rifampicin-resistant race-change mutants (race 3) that are compatible on ECW-30R were obtained throughout the season. The blot was probed with a 3.3-kb *Bam*HI fragment of avirulence gene *avrBs3* (in pEC83 Eco 7), which regulates host specificity for race 1 strains. Strains in lanes 2, 5, and 6 lost the plasmid carrying *avrBs3*. Hybridizing bands that were ~6-kb larger were observed for strains in lanes 3, 4, 7, and 8 compared to the wild-type race 1 strain in lane 1 (*Xcv* 33^{rif}).

above. Resistance to copper and streptomycin were determined as described previously (26). Plasmid extractions (8) and restriction digests were done as previously described (29). The 5.3-kb *BgIII-PstI* fragment of avirulence gene *avrBs1* (16,31) was labeled and used as a probe for detection in Southern and dot blot analyses by the Genius nonradioactive methods as described above. A 350-bp *SalI-SmaI* fragment of insertion element IS476 was used as a probe to determine whether the insertion element (IS) is involved in race change (14,16). Electrolyte leakage patterns of selected strains during both years were determined as described previously (12,23).

RESULTS

The initial appearance of bacterial spot disease in the ECW-30R plot was on plants adjacent to inoculum plants (Fig. 1). Although only a few plants adjacent to inoculum plants exhibited disease 2 to 3 weeks after inoculation, all plants in the plot were diseased within 10 weeks after inoculum plants were placed in the plot. During the growing season from May to August, 425 rifampicin-resistant single colonies isolated from diseased ECW-30R plants were screened for race phenotype, and all were race 3. The race 3 phenotype of representative colonies also was evident in electrolyte leakage patterns (Fig. 2). The original strain, Xcv33^{nif}, caused a HR on ECW-30R plants and greater electrolyte leakage within 24 to 36 h after infiltration, whereas the strains that had changed race and a known race 3 strain did not. Of the 425 single colonies, 25% contained DNA that hybridized with the *avrBs3* probe in colony hybridizations (data not shown). The 41-kb plasmid (1,23) carrying avirulence gene *avrBs3* was not detected in colonies that did not hybridize with the *avrBs3* probe. Southern blot analysis of plasmids digested with *BamHI* and probed with *avrBs3* showed an

~6-kb larger fragment in colonies that hybridized with the *avrBs3* gene probe in dot blot analysis compared to the 3.3-kb fragment in the original strain, Xcv 33^{nif} (Fig. 3). Plasmid DNA also was probed with insertion element IS476, but there was no hybridization with this probe. Similarly, none of the colonies hybridized with the *avrBs1* probe (data not shown).

In 1994, disease was observed within 2 weeks on ECW-10R plants adjacent to inoculum plants. Within 8 weeks after inoculation, all plants in the ECW-10R plot were diseased (Fig. 4). Leaves with pinpoint-sized lesions taken from ECW-10R plants adjacent to inoculum plants 12 days (24 May) after placing the diseased inoculum plants in the plots, indicated the presence of race 2 (Xcv 43). No race 3 strains were detected on ECW-10R during this sampling period. Subsequent samples taken during the season and representing a total of 166 single colonies isolated from diseased ECW-10R plants exhibited a race 3 phenotype. These single colonies also were resistant to copper (200 µg/ml) and streptomycin (100 µg/ml). Diseased samples obtained from cv. Jupiter plants throughout the season (five sampling dates) indicated that 94% of 46 single colonies were race 2, and 6% were race 3. Electrolyte leakage patterns confirmed the race 3 phenotype of the race-changed strains (Fig. 2). The original strain, Xcv 43, caused a HR on ECW-10R plants and greater electrolyte leakage within 8 to 12 h after infiltration, whereas the strains that had changed race and a known race 3 strain did not. Single colonies of strains that had changed race, hybridized with *avrBs1* and IS476 probes in dot blot analysis (data not shown). Plasmid DNA from these strains also hybridized with both probes. Southern analysis of total genomic DNA digested with *BgIII-PstI* or *EcoRV* and probed with the *avrBs1* gene fragment indicated an ~1.2-kb larger band in the race-changed strains compared to the original strain, Xcv 43 (Fig. 5). The race-changed

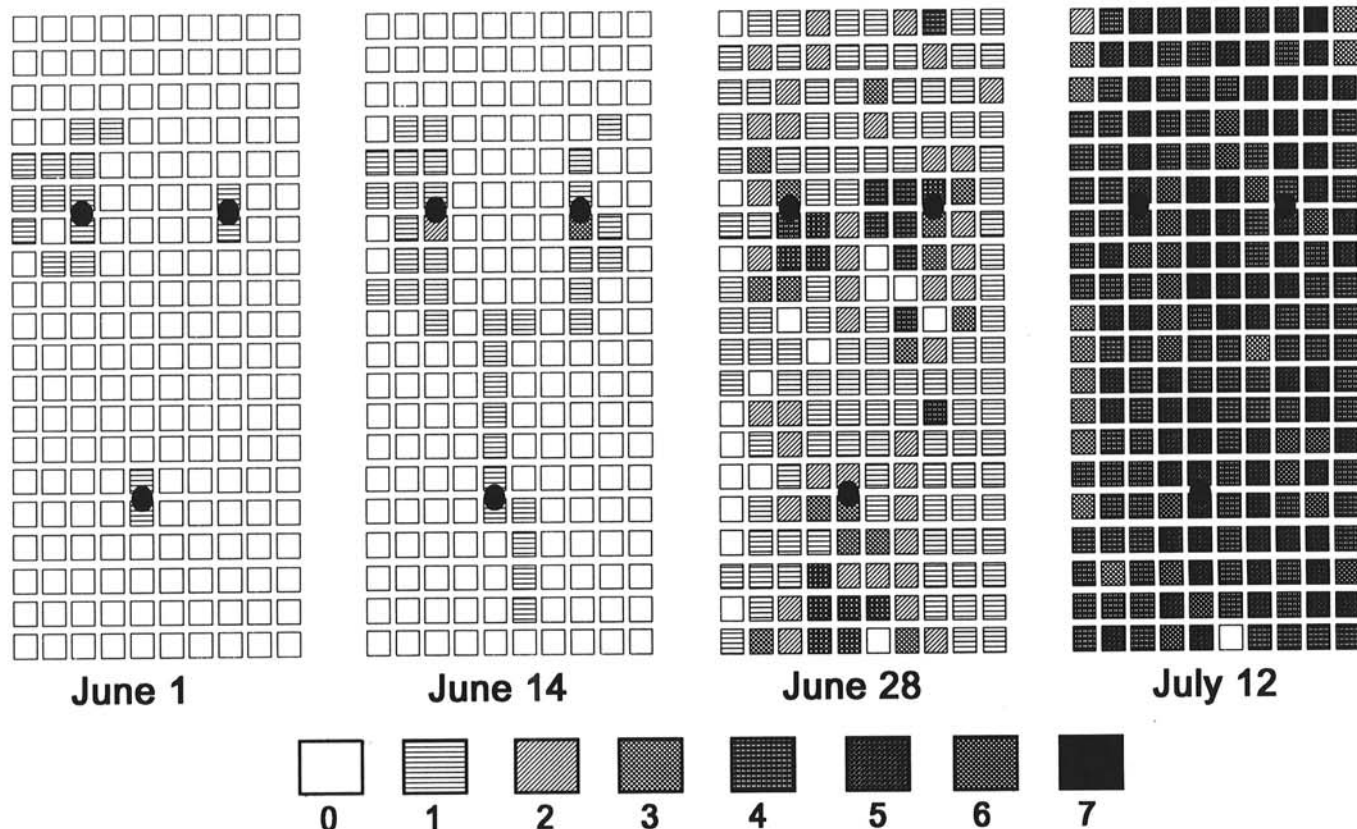


Fig. 4. Spatial and temporal increase of bacterial leaf spot disease during 1994 in an Early Calwonder (ECW) bell pepper plot of ECW-10R plants, which have resistance gene *Bs1*. Each box in the grid represents a single plant. The dark ovals denote the position where the inoculum plants with a race 2 strain (Xcv 43) of *Xanthomonas campestris* pv. *vesicatoria* were planted on 12 May. Race 2 strains are not compatible on ECW-10R. Disease ratings were based on a 0 to 9 scale, in which 0 = no diseased leaves and 9 = 100% of leaves diseased, complete defoliation, and plant dying or dead.

strains also retained the 1.8-kb miniplasmid that was present in the original inoculum strain, Xcv 43 (data not shown).

DISCUSSION

When growing uniform pepper genotypes, there is little deterrent to disease spread once the bacterial pathogen is present and environmental conditions are favorable. Such ideal disease conditions are found in the southeastern United States, where warm, moist conditions typically prevail during the growing season. A sudden increase in the prevalence of race 1 was reported in Florida in 1989 and 1990; race 2 was the predominant strain in Florida prior to 1988 (25). In laboratory studies, Dahlbeck and Stall (5) reported a race change in the pathogen when it was infiltrated into a resistant host. Similar race-change strains were obtained in laboratory studies with *X. campestris* pv. *malvacearum* in a cotton line containing a single resistance gene (7). These reports and the results of our study substantiate the inadequacy of using single-gene host resistance, because of the ability of the pathogen to readily change race and rapidly develop a population that defeats the resistance gene.

During both years, initial disease symptoms on previously resistant plants were observed within 2 weeks after introduction of diseased inoculum plants into the plots, and within 10 weeks, all

plants were diseased. These results indicate that a shift in the race structure of the population of this bacterial pathogen occurs very rapidly when exposed to a resistant host carrying a complementary single gene for resistance. This rapid shift has resulted in defeat of host resistance and severe disease on a previously resistant pepper. Race-change strains (i.e., strains with the race 3 phenotype) were first isolated from diseased leaves from plants previously resistant to the pathogen that were adjacent to the inoculum plants; evidence that the inoculum came from the introduced diseased plants. In both of the experiments, the pathogen strains introduced into the field plots had genetically defined, selectable characteristics, such as resistance to rifampicin or copper and streptomycin. The race-changed strains recovered from diseased plants in these plots retained these characteristics but differed in that they expressed a race 3 phenotype rather than either the race 1 or 2 phenotype of the inoculum strains initially introduced into the plots. We also failed to detect any overwintering during the spring of 1994 on rye or weeds growing in the plot in which the rifampicin-resistant strain of race 1 was used in 1993, suggesting the pathogen does not readily overwinter at this location. Transplants were carefully examined for any symptoms of bacterial spot, and none were observed. These lines of evidence suggest that the pathogen populations that caused disease in these plots in 1993 and 1994 originated from the pathogen strains introduced on diseased inoculum plants.

In the 1994 plot of susceptible cv. Jupiter, 94% of the *X. campestris* pv. *vesicatoria* strains assayed retained the characteristics of the strain used for inoculum. These strains were race 2, were resistant to copper and streptomycin, and harbored the 1.8-kb cryptic miniplasmid. Of the 46 single colonies of bacteria from diseased cv. Jupiter plants, only 3 were race 3. Spontaneous mutations in race 2 strains have been observed in laboratory studies (5). The race 3 strains in the current study could have moved from the ECW-10R plot, which was located 9.1 m from the cv. Jupiter plot. Of 166 single bacterial colonies isolated from diseased leaves in the ECW-10R plot, all exhibited a race 3 phenotype but retained the other characteristics of the race 2 strain used for inoculum.

We attempted, through use of single-colony cultures and assays of the inoculum developed from the single-colony cultures, to establish that we did not have any race-change mutants in the race 1 and 2 bacterial populations prior to their introduction into the field plots. Although we used cultures of bacteria that as a population exhibited the race 1 (Xcv 33^{Rif}) and 2 (Xcv 43) phenotypes, we cannot determine when the actual race change occurred that led to the race 3 populations from each of these strains. Only copper- and streptomycin-resistant race 2 strains were detected on ECW-10R plants adjacent to the inoculum plants when the first leaf samples with pinpoint-sized lesions were taken 12 days after Xcv 43 was introduced into the plot. This line of evidence indicates that the race 2 population had moved from the inoculum plants onto the resistant host. The small lesions could have been due to a HR occurring on the ECW-10R plants in response to race 2. Failure, however, to detect a significant number of race 3 strains or an increasing race 3 population on susceptible cv. Jupiter, while detecting increasingly high frequencies of race-change mutants on ECW-30R and ECW-10R, could be interpreted as evidence that the race shifts occurred in the presence of the resistances genes. These results do indicate that the pressure of resistance genes is important in bringing about a race shift, if not a race change, and the subsequent rapid shift in a pathogen population consisting of a new virulent race.

The avirulence gene *avrBs3* contains 17 repetitive motifs, each consisting of 102 bp. Loss of repetitive motifs can cause changes in race specificity and symptom expression (9). Seventy-five percent of the isolates from diseased ECW-30R lacked the plasmid that carries *avrBs3*. Loss of this plasmid and subsequent loss of *avrBs3* has been demonstrated in the laboratory (1,23). However, 25% of the race-change strains isolated from diseased ECW-30R



Fig. 5. Southern blot of restriction digests with *EcoRV* of genomic DNA, including plasmids of a wild-type race 2 strain (Xcv 43, lane 1) and strains shifted to race 3 (lanes 3 through 8) of *Xanthomonas campestris* pv. *vesicatoria* in a field plot of Early Calwonder (ECW) bell pepper ECW-10R (carries resistance gene *Bs1*) plants. Lane 2, strain Xcv 43 isolated from the plot of susceptible cv. Jupiter, which behaved as race 2. All race 2 strains carry *avrBs1* and cause a hypersensitive reaction on ECW-10R. Strain Xcv 43 was introduced into the plot of ECW-10R, and disease development was seen 2 to 3 weeks later. Race-shift mutants (race 3) that are compatible on ECW-10R and carry the phenotypic markers were obtained throughout the season. The blot was probed with a 5.3-kb *PstI*-*Bgl*III fragment of avirulence gene *avrBs1* (in pXV2007). All the race-shift mutants (race 3) hybridized with the *avrBs1* probe, indicating inactivation of this gene. The inactivation was accompanied by a ~1.2 kb (size of transposable insertion element IS476) increase (13.4-kb band) compared to the wild-type strain (12.2-kb band).

that hybridized with the *avrBs3* probe contained a larger fragment than that which hybridized with the *avrBs3* probe in the original Xcv 33^{rrf} race 1 strain used for inoculum. These race-shift isolates expressed a race 3 phenotype despite hybridizing with the *avrBs3* probe, suggesting that an unidentified IS or some other factor may play a role in causing a race change by inactivation of *avrBs3*. We previously reported that DNA from numerous strains that expressed a race 3 phenotype hybridized with the *avrBs3* probe (17). These strains may contain avirulence gene *avrBsP*, which has a high degree of homology with *avrBs3* and controls the HR in tomato cv. Bonny Best (3); however, many of our race 3 strains failed to elicit a HR in tomato cv. Bonny Best (C. S. Kousik and D. F. Ritchie, unpublished data).

Most strains of race 2 are resistant to copper, and when this characteristic is lost, a race shift occurs (30). However, in our 1994 study, a change from race 2 to 3 was not associated with loss of copper resistance. The plasmid carrying a copper-resistance gene and the *avrBs1* gene was detected in these race-change strains isolated from diseased ECW-10R plants. All strains assayed that had changed from race 2 to 3 on ECW-10R in the field hybridized with probe IS476. IS476 is found in race 2 strains of the bacterial spot pathogen (14,16). This IS element appears to play a significant role in race changes under field conditions in race 2 strains. Southern analysis of *EcoRV* plasmid digests of race-change strains from Xcv 43 were similar to digests previously reported in which the IS element had inserted in the coding region of *avrBs1*, and the function of this avirulence gene was completely lost (14). Such a complete loss of function of an avirulence gene may be necessary for the pathogen to overcome a resistance gene under field conditions.

Pepper cultivars resistant to one or more races of the bacterial spot pathogen currently are available. However, the impact of the absence of resistance to a particular pathogen race, such as race 3, can be devastating despite using several cultivars with resistance to race 2 or a combination of races 1 and 2 (20). Currently, gene *Bs2* confers resistance to the most commonly detected races, 0 through 3; however, it is single-gene resistance. Recently, strains of *X. campestris* pv. *vesicatoria* from tomato and pepper were virulent on pepper carrying gene *Bs2* (2,18,24,27). Strains that overcome the *Bs2* gene in laboratory experiments had reduced fitness, as measured by reduced pathogen multiplication rates in planta (15). In field studies, we found that pepper cultivars with a combination of *Bs1* and *Bs2* resistance genes had significantly less disease and greater fruit yield than cultivars with only gene *Bs2* when grown in the presence of race 4 (19). Resistance genes *Bs1* and *Bs2* are defeated individually by strains of race 4 (18,19).

A combination of resistance genes in the same cultivar may provide some increase in the stability of host resistance; however, this still is not likely to provide long-term disease resistance to a pathogen such as *X. campestris* pv. *vesicatoria*. In a 1995 field experiment in which pepper with resistance genes *Bs1*, *Bs2*, and *Bs3* was grown, we isolated a race 6 that defeats these three resistance genes (C. S. Kousik and D. F. Ritchie, unpublished data). These results and those from other experiments indicate that the current host resistance available in bell pepper cannot be solely relied on for control of bacterial spot. An alternative approach to manage this disease in terms of host resistance would be to identify sources of rate-reducing/quantitative resistance. There have been a few attempts to identify quantitative resistance (10,11,22). Such a rate-reducing resistance may be enhanced further if it is combined with resistance genes *Bs1*, *Bs2*, and *Bs3*. Ultimately, host resistance should be used as a component of an integrated management program that begins with pathogen-free seeds and transplants and continues with the judicious use of chemical sprays (13,21,22).

LITERATURE CITED

- Bonas, U., Stall, R. E., and Staskawicz, B. 1989. Genetic and structural characterization of the avirulence gene *avrBs3* from *Xanthomonas campestris* pv. *vesicatoria*. Mol. Gen. Genet. 218:127-136.
- Bouzar, H., Jones, J. B., Stall, R. E., Hodge, N. C., Minsavage, G. V., Benedict, A. A., and Alvarez, A. M. 1994. Physiological, chemical, serological, and pathogenic analyses of a worldwide collection of *Xanthomonas campestris* pv. *vesicatoria* strains. Phytopathology 84: 663-671.
- Canteros, B., Minsavage, G., Bonas, U., Pring, D., and Stall, R. E. 1991. Gene from *Xanthomonas campestris* pv. *vesicatoria* that determines avirulence in tomato is related to *avrBs3*. Mol. Plant-Microbe Interact. 4:628-632.
- Cook, A. A., and Stall, R. E. 1982. Distribution of races of *Xanthomonas vesicatoria* pathogenic on pepper. Plant Dis. 66:388-389.
- Dahlbeck, D., and Stall, R. E. 1979. Mutations for change of race in cultures of *Xanthomonas vesicatoria*. Phytopathology 69:634-636.
- 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.
- De Feyter, R., and Gabriel, D. W. 1991. At least six avirulence genes are clustered on a 90-kilobase plasmid in *Xanthomonas campestris* pv. *malvacearum*. Mol. Plant-Microbe Interact. 4:423-432.
- Dittapongpich, V., and Ritchie, D. F. 1993. Indigenous miniplasmids in strains of *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 83: 959-964.
- Herbers, K., Conrads-Strauch, J., and Bonas, U. 1992. Race-specificity of plant resistance to bacterial spot disease determined by repetitive motifs in a bacterial avirulence protein. Nature (Lond.) 356:172-174.
- Hibberd, A. M. 1989. Quantitative resistance to bacterial leaf spot of pepper compared in mono and polycyclic disease progress tests. Pages 213-219 in: Tomato and Pepper Production in the Tropics. Asian Vegetable Research and Development Center, Shanhua, Taiwan.
- Hibberd, A. M., and Gillespie, D. 1982. Heritability of field resistance to bacterial leaf spot disease in pepper (*Capsicum annuum* L.). Sci. Hortic. 17:301-309.
- Hibberd, A. M., Stall, R. E., and Bassett, M. J. 1987. Different phenotypes associated with incompatible races and resistance genes in bacterial spot disease of pepper. Plant Dis. 71:1075-1078.
- Johnston, S. A. 1991. Fungicide Benefits Assessment: Vegetables—East. U.S. Department of Agriculture and the States National Agricultural Pesticide Impact Assessment Program (NAPIAP).
- Kearney, B., Ronald, P. C., Dahlbeck, D., and Staskawicz, B. J. 1988. Molecular basis for evasion of plant host defence in bacterial spot disease of pepper. Nature (Lond.) 332:541-543.
- Kearney, B., and Staskawicz, B. J. 1990. Widespread distribution and fitness contribution of *Xanthomonas campestris* avirulence gene *avrBs2*. Nature (Lond.) 346:385-386.
- Kearney, B., and Staskawicz, B. J. 1990. Characterization of IS476 and its role in bacterial spot disease of tomato and pepper. J. Bacteriol. 172: 143-148.
- Kousik, C. S., and Ritchie, D. F. 1993. *AvrBs3* hybridizes to DNA from strains of *Xanthomonas campestris* pv. *vesicatoria* expressing a race 3 phenotype. (Abstr.) Phytopathology 83:1359.
- Kousik, C. S., and Ritchie, D. F. 1995. Isolation of pepper races 4 and 5 of *Xanthomonas campestris* pv. *vesicatoria* from diseased peppers in southeastern U.S. fields. Plant Dis. 79:540.
- Kousik, C. S., and Ritchie, D. F. 1996. Evaluation of bell pepper varieties against races 1-4 of the bacterial spot pathogen, 1995. Biol. Cult. Tests Plant Dis. Control 11:97.
- Kousik, C. S., Sanders, D. C., and Ritchie, D. F. 1994. Yield of bell peppers as impacted by the combination of bacterial spot and a single hail storm: Will copper sprays help? HortTechnology 4:356-358.
- Kousik, C. S., Sanders, D. C., and Ritchie, D. F. 1996. Mixed genotypes combined with copper sprays to manage bacterial spot of bell peppers. Phytopathology 86:502-508.
- McCarter, S. M. 1992. Effects of bactericide treatments on bacterial spot severity and yield of different pepper genotypes and on populations of certain insects. Plant Dis. 76:1042-1045.
- Minsavage, G. V., Dahlbeck, D., Whalen, M. C., Kearney, B., Bonas, U., Staskawicz, B. J., and Stall, R. E. 1990. Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*-pepper interactions. Mol. Plant-Microbe Interact. 3: 41-47.
- O'Garro, L. W., and Tudor, S. 1994. Contribution of four races of *Xanthomonas campestris* pv. *vesicatoria* to bacterial spot in Barbados. Plant Dis. 78:88-90.
- Pohronezny, K., Stall, R. E., Canteros, B. I., Kegley, M., Dantoff, L. E., and Subramanya, R. 1992. Sudden shift in the prevalent race of *Xanthomonas campestris* pv. *vesicatoria* in pepper in southern Florida. Plant Dis. 76:118-120.

26. Ritchie, D. F., and Dittapongpitch, V. 1991. Copper- and streptomycin-resistant strains and host differentiated races of *Xanthomonas campestris* pv. *vesicatoria* in North Carolina. *Plant Dis.* 75:733-736.
27. Sahin, F., and Miller, S. A. 1995. First report of pepper race 6 of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper. *Plant Dis.* 79:1188.
28. Sanders, D. C., Averre, C. W., Sorensen, K. F., Estes, E. A., Beasley, E. O., and Bonanno, A. R. 1988. Commercial pepper production in North Carolina. Page 16 in: N.C. Coop. Ext. Serv. AG-387. N.C. Cooperative Extension Service, Raleigh.
29. Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
30. Stall, R. E., Loschke, D. C., and Jones, J. B. 1986. Linkage of copper resistance and avirulence loci on a self-transmissible plasmid in *Xanthomonas campestris* pv. *vesicatoria*. *Phytopathology* 76:240-243.
31. Swanson, J., Kearney, B., Dahlbeck, D., and Staskawicz, B. J. 1988. Cloned avirulence gene of *Xanthomonas campestris* pv. *vesicatoria* complements spontaneous race change mutants. *Mol. Plant-Microbe Interact.* 1:5-9.
32. Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. *Int. J. Syst. Bacteriol.* 45:472-489.