Disease Potential of Pepper Bacterial Spot Pathogen Races That Overcome the *Bs2* Gene for Resistance

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ABSTRACT

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Bacterial spot, caused by *Xanthomonas campestris* pv. *vesicatoria*, is a major limiting disease in bell pepper production. Gene *Bs2* in pepper confers hypersensitive resistance to the three most common races (1, 2, and 3) and to race 0. Races 4, 5, and 6, which cause disease on pepper plants with the *Bs2* resistance gene, recently were isolated from diseased pepper. Multiplication of races 1 to 6 did not significantly differ in 0.8% nutrient broth (NB), but strains of races 4 and 6 multiplied slower in 0.4 NB than did races 1, 2, and 3 after 32 h. Multiplication of races 4 to 6 did not differ from that of races 1 to 3 in susceptible cv. Early Calwonder (ECW). Races 4 and 6 multiplied similarly to race 1 in near-isogenic line ECW-10R (contains resistance gene *Bs1*). The severity of disease caused by the races that overcome the *Bs2* gene compared to the races that do not was not significantly different on three susceptible pepper genotypes. Races 4 to 6 caused significantly greater disease on plants carrying the *Bs2* gene for resistance than did races 0 to 3, which caused a hypersensitive response. During 1995 when races 1 to 4 were used as inoculum in the field, both ECW and ECW-20R plants became severely diseased and had 83 and 74% lower yields, respectively, compared to noninoculated checks. Race 4 predominated on ECW-20R, whereas race 3 predominated on ECW. During 1996 when races 4, 5, and 6 were used as inoculum in the field, both cv. Camelot and cv. X3R Camelot (contains resistance gene *Bs2*) plants became severely diseased. Race 6 predominated on both Camelot and X3R Camelot. Avirulence gene *avrBs2* was detected by hybridization in all the races without observed polymorphism. Our data indicate that races that overcome the *Bs2* gene for resistance have the potential to cause severe disease and need to be taken into account in disease management strategies.

Host-plant resistance is a preferred and effective means of managing plant diseases. However, development of host-plant resistance can take years before cultivars are released for commercial cultivation. Bacterial diseases of vegetable crops can be difficult to manage once they are present and when environmental conditions remain favorable. Bacterial spot of bell peppers, caused by *Xanthomonas campestris* pv. *vesicatoria* (proposed: *X. axonopodis* pv. *vesicatoria* [27]), is a major limiting factor in bell pepper production (9). The prevalence of host-differentiated races makes it difficult to manage this disease based on host-plant resistance alone. Currently, seven races of the pathogen virulent on peppers have been described and designated 0 to 6 (2,4,12,21–23). The races have been classified on the basis of a hypersensitive reaction (HR) on three near-isogenic lines of Early Calwonder (ECW) pepper, each having a single gene for resistance (Table 1). These near-isogenic lines are ECW-10R (contains resistance gene *Bs1*), ECW-20R (contains resistance gene *Bs2*), and ECW-30R (contains resistance gene *Bs3*) (18). The *Bs2* gene confers resistance against the most commonly detected races, 1, 2, and 3, and race 0. Prior to 1994, races that overcame *Bs2* were not detected in commercial pepper fields, but races 4, 5, and 6, which overcome *Bs2*, have been described recently (2,12,20,22,23). The prevalence of races 4, 5, and 6 is unknown.

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The gene-for-gene relationship is well described in the pepper-bacterial spot pathogen system (18). For each resistance gene in the host, the pathogen strains carry a corresponding avirulence gene. For example, race 1 and 4 strains carry *avrBs3* and elicit a HR on ECW-30R. Races 0, 1, 2, and 3 carry *avrBs2* and elicit a HR on plants carrying *Bs2* (ECW-20R). Thus, resistance gene *Bs2* currently provides the widest range of resistance against bacterial spot pathogen races.

Avirulence gene *avrBs2* and its homologs have been detected in several plant-pathogenic xanthomonads and was reported to be necessary for fitness of *X. campestris* pv. *vesicatoria* strains. Because of this, the *Bs2* gene might provide more long-lasting resistance (11). However, the *Bs2* gene has been overcome by races 4, 5, and 6 (12,22), and inactivation of a homolog of *avrBs2* in the bacterial pathogen of rice (*X. oryzae* pv. *oryzae*) by transposon mutagenesis did not affect fitness (17). The current study was conducted to determine the potential of races 4, 5, and 6 to cause disease on pepper plants and to determine whether *avrBs2* is present in these races.

MATERIAL AND METHODS

Multiplication in media. The characteristics of the bacterial strains used in this study are presented in Table 1. The ability of races to multiply in different concentrations of nutrient broth (NB, Difco Laboratories, Detroit) was determined. Suspensions of all races were adjusted to optical density (OD) readings of 0.26 at 600 nm (10^8 CFU/ml) with a Spectronic 20 spectrophotometer

(Bausch and Lomb, Rochester, NY). Aliquots of bacterial suspensions (20 µl) were added to 4 ml of NB (0.8%, standard concentration, and 0.4%, reduced concentration), and the tubes were incubated in a shaking water bath at 28°C. The multiplication rate was determined by measuring absorbance at 600 nm with a spectrophotometer. The experiment had two replications and was repeated twice for each race and time period.

Multiplication in planta. Pepper plants were grown on greenhouse benches in 10-cm pots filled with Metromix 220 (W.R. Grace & Co., Memphis, TN). Plants were kept under normal (22 to 26°C) day/night conditions. Infiltration of plants with bacteria and estimation of the number of CFU per 0.5-cm^2 disk were completed as described previously (8). Briefly, sections of the top five to seven fully expanded leaves were infiltrated with a syringe and an inoculum suspension of 10^5 CFU/ml. One strain representing each race was assayed. Leaf samples were excised from these plants at 24-h intervals, and numbers of CFU per 0.5-cm^2 disk were determined. Each treatment had three replications, and the experiment was repeated twice.

Disease development on plants in the greenhouse. To determine the ability of the races to cause disease on susceptible and resistant plants, the Silwet L-77 (OSi Specialties, Inc., Danbury, CT) method of inoculation was used. This method also has been used for inoculation of tomato and *Arabidopsis* leaves with bacterial pathogens (19) and mimics the natural mode of entry of the pathogen through stomata. The following strains were used in these experiments: V19, V79, V126, V110, V181, V205, and V199, representing races 0 to 6, respectively. Inoculum suspensions were adjusted to OD readings of 0.26 (10^8 CFU/ml) at 600 nm. Silwet L-77 was added to inoculum suspensions to give a 0.04% concentration. Plants at the 6- to 8-leaf stage were dipped in inoculum suspension for 15 s. These plants were covered with plastic bags for 3 days to maintain high humidity (7) and promote disease development. Whole plants were rated for disease severity with a 0 to 9 scale: 0 = no lesions; 1 ≤ 10% leaf area covered with lesions, but no defoliation; 2 = 11 to 20% leaf area covered with lesions, but no defoliation; 3 = 21 to 30% area covered with lesions, one to two leaves defoliated; 4 = 31 to 40% leaf area covered with lesions; 7 = 61 to 70% leaf area covered with lesions, few leaves (four to six) remaining on the plant; and 9 = 91 to 100% of plant covered with lesions, plant dying or dead, complete defoliation. Pepper near-isogenic lines ECW and ECW-20R and cvs. Camelot, X3R Camelot (contains resistance gene *Bs2*), and Jupiter were tested in this manner. Each experiment consisted of six single-plant replications per race, and the experiments were repeated at least twice.

Field experiments. Plots of susceptible line ECW and resistant line ECW-20R peppers were established at Sandhills Research Station, Jackson Springs, NC, in 1995. Plots had 10 rows spaced 96 cm apart, with 20 plants per row spaced 36 cm apart. Inoculum plants, each inoculated with a mixture of two strains of one of the four races, 1 (V33 and V12), 2 (V6 and V72), 3 (V103 and V113), or 4 (V181 and V182), were developed as described previously (15,16). Strains of these races were selected based on phenotypic markers (Table 1). The presence of desired races and phenotypic markers of strains on inoculum plants was confirmed prior to transplanting these plants into the plots. Four inoculum plants, each with one race, were planted at three locations in each plot. Data on disease development for each plant, based on a 0 to 9 scale, were recorded at weekly intervals (15). Leaf samples were excised from ECW and ECW-20R plants at weekly intervals. Isolations were made on a nonselective yeast dextrose calcium carbonate agar, and the race of single colonies was determined with pepper differentials (18) and growth on plates amended with streptomycin (100 ppm), rifampicin (50 µg/ml), or copper sulfate (200 ppm) as described previously (21). A noninoculated check plot at a site (approximately 300 m from inoculated plot) also was established with a single row of 30 plants for each line. Yield data on groups of several of these plants was recorded, and data are presented as yield per plant.

Similarly, in 1996 plots of cvs. Camelot and X3R Camelot peppers were established at Sandhills Research Station. Plots had eight rows spaced 96 cm apart, with nine plants per row spaced 36 cm apart. Races 4 (V181 and V182), 5 (V293 and V294), and 6 (V295 and V296) were used as inoculum. Three inoculum plants, each with one race, were planted at two locations in each plot. Data on disease severity were recorded at weekly intervals. Leaf samples were excised once every 2 weeks. Bacterial isolations and race determination were done as described above.

To evaluate the ability of strains used in the field experiment to cause disease on plants in the greenhouse, inoculum suspensions

TABLE 1. Details of *Xanthomonas campestris* pv. *vesicatoria* strains[a] used in this study

Strain	Race	Growth on[b]			Plant reaction[c]			Isolation date (mo/yr)	Location
		Rif	Cu	Sm	ECW10R	ECW-20R	ECW-30R		
V19	0	–	+	–	HR	HR	HR	6/87	NC
V79	1	–	+	–	C	HR	HR	5/91	NC
V33	1	+	–	–	C	HR	HR	4/89	NC
V12	1	+	–	–	C	HR	HR	5/87	NC
V6	2	–	+	+	HR	HR	C	9/86	NC
V72	2	–	+	+	HR	HR	C	7/90	NC
V126	2	–	+	+	HR	HR	C	9/92	GA
V110	3	–	+	–	C	HR	C	6/92	VA
V103	3	–	–	–	C	HR	C	6/92	NC
V113	3	–	–	–	C	HR	C	6/92	NC
V181	4	–	+	–	C	C	HR	8/94	NC
V182	4	–	+	–	C	C	HR	8/94	NC
V205	5	–	+	–	HR	C	C	1/95	FL
V293	5	–	+	+	HR	C	C	8/95	NC
V294	5	–	+	+	HR	C	C	8/95	NC
V199	6	–	+	–	C	C	C	12/94	NC
V295	6	–	+	–	C	C	C	8/95	NC
V296	6	–	+	–	C	C	C	8/95	NC

[a] All strains used in this study were isolated from diseased pepper plants, except strain V199, which was isolated by first inoculating race 4 (10^4 CFU/ml) into Early Calwonder (ECW) line ECW-30R and reisolating after 2 weeks as described previously (6).

[b] Rif = resistance to rifampicin at 50 µg/ml; Cu = resistance to copper sulfate at 200 ppm; and Sm = resistance to streptomycin at 100 ppm. + = ability to grow on media containing antibiotics or copper; – = no growth.

[c] Reactions were determined by infiltration of bacterial suspension (10^8 CFU/ml) in ECW pepper near-isogenic lines as described previously (18,21). C = compatible or disease reaction; HR = hypersensitive reaction. All strains were compatible on ECW. Strains causing a HR on the near-isogenic lines carry a corresponding avirulence gene (e.g., race 1 strains carry *avrBs2* and *avrBs3* and cause a HR on ECW-20R and ECW-30R).

(10^8 CFU/ml) with a mixture of two strains per race for races 1 to 4 were used to inoculate ECW, ECW-20R, Camelot, and X3R Camelot plants by the Silwet L-77 method. Disease development was measured on the plants as described above for the greenhouse experiments. Each experiment had six single-plant replications and was repeated three times.

Statistical analyses. Area under disease progress curves (AUDPC) for greenhouse and field experiments was calculated as previously described (3). One-way analysis of variance was performed to compare the means of different races in the greenhouse experiments. Estimated contrasts for *avrBs2* gene effects were tested by comparing the mean for races 0 to 3 (Bs2HR) with the mean for races 4 to 6 (Bs2C) by a *t* test. The least significant difference is provided in Table 2 for comparison of individual means of the races. All statistical analyses were carried out by the SAS statistical package (SAS Institute, Inc., Cary, NC).

Detection of *avrBs2* in different races. Plasmid p81538, which contains a 2.35-kb *Sph*I fragment with *avrBs2* activity (18), was provided by B. J. Staskawicz (University of California, Berkeley). A 2.3-kb *Bam*HI fragment was isolated from p81538 and used as a hybridization probe on Southern blots after being labeled with digoxigenin, using the Genius kit (Boehringer Mannheim Biochemicals, Indianapolis, IN). Total genomic DNA was isolated by the method of Ausubel et al. (1). Total genomic DNA was digested with *Sph*I, *Bam*HI, *Eco*RI, *Hind*III, and *Eco*RV essentially as described previously (1). DNA transfer and hybridizations were completed with stringent wash conditions following the Genius kit protocol (65°C, 0.5× SSC [1× SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate, pH 7.0], 0.1% sodium dodecyl sulfate).

RESULTS

Multiplication in media. Estimated contrasts for the mean absorbance of strains compatible (Bs2C) and incompatible (Bs2HR) on ECW-20R exhibited no significant differences in multiplication in 0.8% NB. The strain of race 1 used in these experiments multiplied rapidly in NB. Strains of races 4 and 6 multiplied slower in 0.4% NB than did strains of races 1, 2, and 3 after 32 h (Fig. 1).

Multiplication in planta. The multiplication curves for races 4 to 6 were similar to curves for races 1 to 3 in susceptible ECW, and most were within 0.5 log units. Some variations among the races were observed over time. However, these variations were not consistent with compatibility or incompatibility of strains on ECW-20R. Estimated contrasts for the mean multiplication levels of strains compatible (Bs2C) and incompatible on ECW-20R (Bs2HR) exhibited no significant differences in multiplication in ECW (Fig. 2A and B). Races 4 and 6 multiplied to levels similar to race 1 in ECW-10R. Race 2, which is incompatible on ECW-10R, multiplied to lower levels compared to races 1, 4, and 6 (Fig. 2C). The

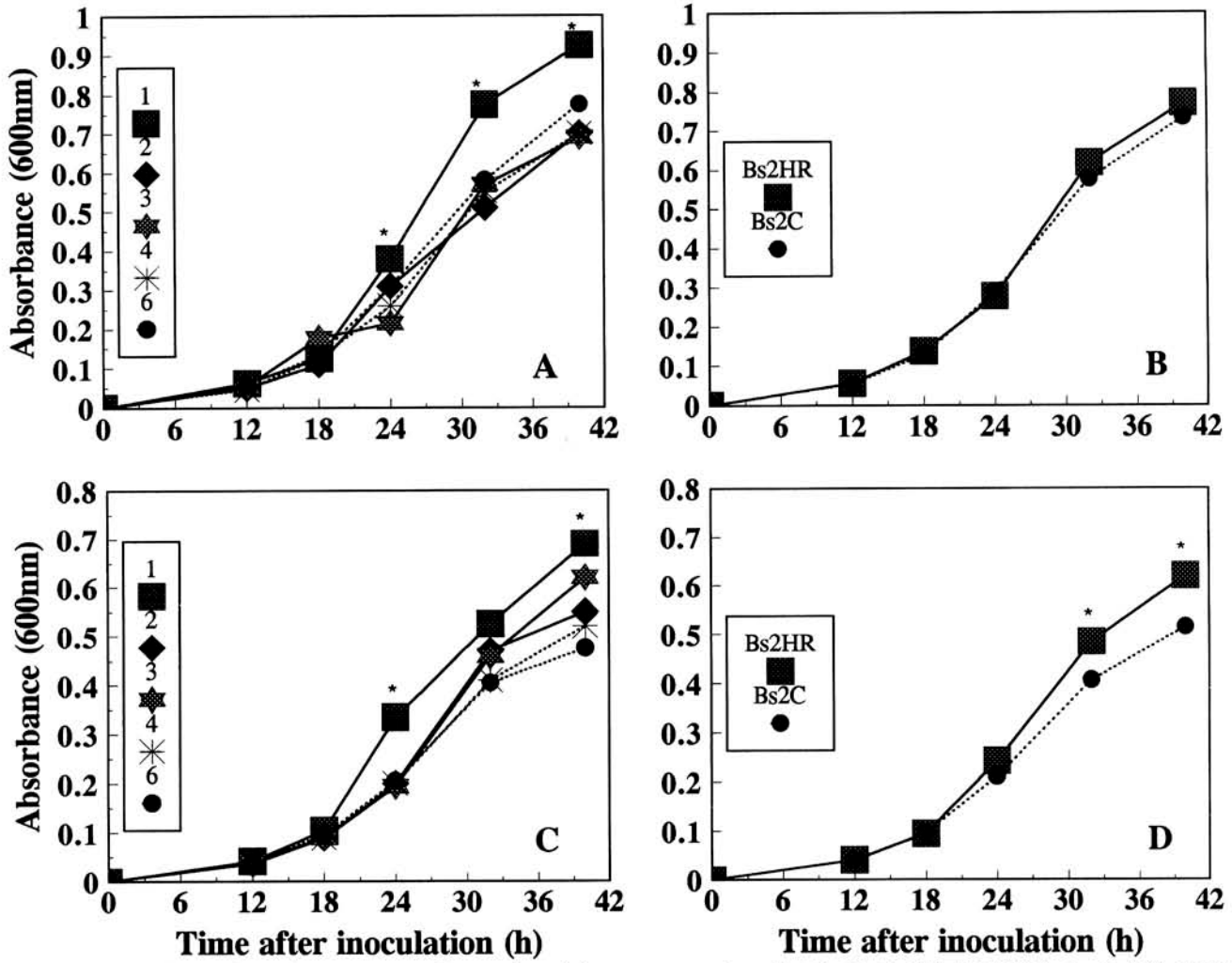

Fig. 1. Multiplication of races of *Xanthomonas campestris* pv. *vesicatoria* in two concentrations of nutrient broth (NB). **A,** Multiplication in 0.8% NB (standard concentration). **B,** Mean levels of multiplication in 0.8% NB of strains compatible (races 4 and 6) (Bs2C) and those (races 1 to 3) eliciting a hypersensitive reaction (HR) (Bs2HR) on ECW-20R. Means were contrasted by a *t* test, and no significant differences were observed. **C,** Multiplication in 0.4% NB. **D,** Mean levels of multiplication in 0.4% NB of strains compatible (races 4 and 6) (Bs2C) and those (races 1 to 3) eliciting a HR (Bs2HR) on ECW-20R. Means were contrasted by a *t* test. * indicates a significant difference ($P = 0.01$).

experiments with each race and genotype combination followed a similar trend, and hence, data from one experiment are presented.

Disease development on plants in the greenhouse. Some variation in the ability of races to cause disease on the susceptible cultivars, ECW, Camelot, and Jupiter, was observed (Table 2). Estimated contrasts for the mean disease severity of strains compatible (Bs2C) and incompatible (Bs2HR) on ECW-20R indicated no significant differences in AUDPC and disease development on ECW, Camelot, or Jupiter (Table 2; Fig. 3A and B). Races 2 (V126) and 5 (V205) caused significantly less disease compared to strains of races 0 (V19), 1 (V79), 3 (V110), 4 (V181), and 6 (V199) on Camelot and ECW (Table 2). Races 4, 5, and 6 caused severe disease on X3R Camelot and ECW-20R compared to races 0 to 3 (Table 2). Race 5 generally caused less disease than races 4 and 6. Races 0 to 3 caused a HR on X3R Camelot and ECW-20R, which resulted in defoliation of these plants. The plants inoculated with races 0 to 3 recovered over the course of the experiment, which was 25 days after inoculation (Fig. 3C and D). The loss of *avrBs2* avirulence was not correlated with the loss of the ability to cause disease on susceptible pepper plants. In addition, races 4 to 6 (lacking *avrBs2* avirulence) caused severe disease on plants with the *Bs2* gene.

Disease progress was very similar on ECW (Fig. 4A and B) and cv. Camelot (data not shown) when a mixture of two strains per race for races 1 (V 33 and V12), 2 (V6 and V72), 3 (V103 and V113), or 4 (V181 and V182) was used. These were the same strains that were used in the 1995 field experiment described below. Races 3 and 4 caused significantly greater disease on ECW compared to race 2. Race 4 caused severe disease on ECW-20R (Fig. 4C and D) and cv. X3R Camelot (data not shown) compared to races 1 to 3.

Disease development in the field. Disease progressed similarly during 1995 on ECW and ECW-20R (Table 3) in the field when epidemics were initiated with races 1 to 4; both plots were severely diseased. Disease levels on ECW-20R, however, were generally less than on ECW (Table 3). Race 3 was predominant on ECW, followed by races 2 and 4, respectively; on ECW-20R, race 4 was predominant (Table 4). There was a fourfold reduction in the yield per plant of ECW-20R plants and a sixfold reduction in ECW plants in the inoculated plot compared to the noninoculated checks (Table 4). Disease progressed very similarly during 1996 on cvs. Camelot and X3R Camelot (Table 5) in the field when epidemics were initiated with races 4, 5, and 6; both plots were severely diseased. Race 6 was predominant on both cultivars (Table 6).

TABLE 2. Area under disease progress curves (AUDPC)[a] for susceptible pepper cultivars and lines (Jupiter, Camelot, and Early Calwonder [ECW]) and cultivars and lines with the *Bs2* gene for resistance (ECW-20R and X3R Camelot)

	AUDPC				
	Susceptible cultivars			Cultivars with *Bs2*	
Race	Jupiter	Camelot	ECW	ECW-20R	X3R Camelot
0	112.2	134.4	122.8	45.8	66.1
1	122.1	137.0	124.3	58.0	87.0
2	104.4	93.1	111.9	53.9	68.3
3	126.1	135.6	134.8	56.8	75.3
4	127.3	129.3	124.3	107.2	133.0
5	103.7	111.8	108.9	107.0	113.3
6	128.8	134.1	127.0	112.3	132.5
$LSD_{0.05}$	9.7	10.1	9.2	16.3	13.2
Bs2HR[b]	116.2	125.0	123.5	53.6	74.2
Bs2C	119.6	125.1	120.1	108.8	126.3
Contrasts Bs2C vs. Bs2HR	0.187[c]	0.982	0.160	0.0001	0.0001

[a] AUDPC was calculated as described previously (3).

[b] BS2HR indicates mean AUDPC caused by races incompatible on ECW-20R (races 0 to 3) and BS2C indicates mean AUDPC caused by races compatible on ECW-20R (races 4 to 6).

[c] *P* value for comparisons of mean AUDPC for Bs2HR and Bs2C. The means were contrasted by a *t* test.

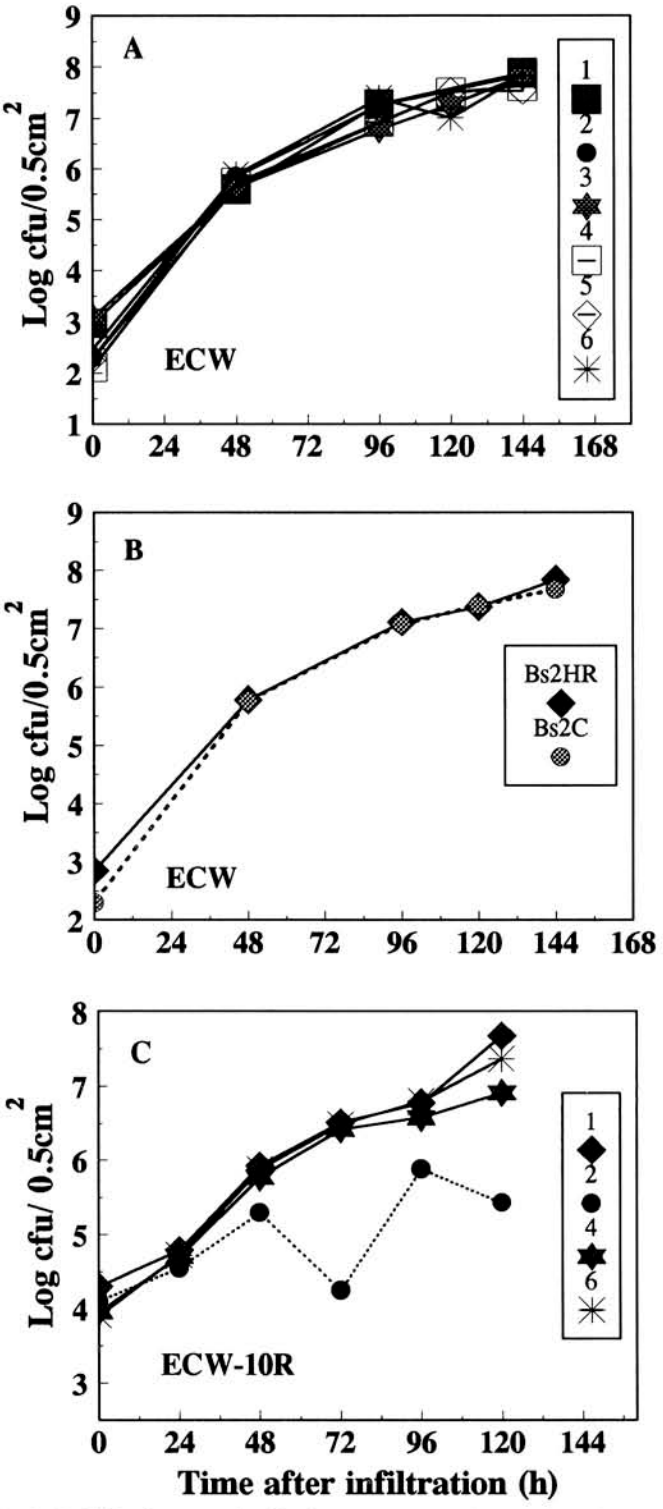

Fig. 2. Multiplication curves for *Xanthomonas campestris* pv. *vesicatoria* races 1 to 6 in Early Calwonder (ECW) and ECW-10R. **A,** Multiplication curves for races 1 to 6 in ECW (susceptible to all known pepper races of *X. campestris* pv. *vesicatoria*). **B,** Comparison of mean multiplication levels in ECW of strains compatible (races 4, 5, and 6) (Bs2C) and those (races 1, 2, and 3) eliciting a hypersensitive reaction (HR) (Bs2HR) on ECW-20R. **C,** Multiplication curves for races 1, 2, 4, and 6 in ECW-10R (susceptible to races 1, 4, and 6). Race 2 elicits a HR on ECW-10R.

Presence of *avrBs2* in different races. A 2.3-kb *Sph*I fragment hybridizing to the *avrBs2* probe was detected in races 0 to 6 (Fig. 5). Restriction digests with different endonucleases (*Sph*I, *Bam*HI, *Eco*RI, *Hind*III, or *Eco*RV) did not reveal polymorphisms with respect to the *avrBs2* region (data not shown).

DISCUSSION

Resistance gene *Bs2* was identified in *Capsicum chacoense* and described in 1984 (5); the corresponding avirulence gene, *avrBs2*, in the bacterial spot pathogen was described in 1990 (11,18). Since 1993, many commercial pepper growers have begun using hybrid pepper cultivars (*C. annuum*) that carry the *Bs2* gene. The *Bs2* gene was believed to be durable and effective in controlling bacterial spot on peppers. This durability was related to the fact that all strains of the pathogen that had been examined contained a functional copy of the *avrBs2* gene, and the loss of the *avrBs2* gene resulted in the pathogen suffering a severe fitness penalty (11). These observations were based on spontaneous *avrBs2* mutants obtained in the laboratory (11). Prior to 1994, races that could overcome this gene had not been isolated from commercial pepper fields (12,22), and hence, the ability of field isolates to cause disease had not been examined.

In the current study, we observed that races 4 to 6 retained their ability to multiply in planta and cause severe disease on susceptible cultivars such as ECW and Camelot in laboratory and greenhouse experiments. Furthermore, in the field experiment in 1995, race 4 was predominant on ECW-20R and caused a severe epidemic. Race 3 was predominant on ECW in the field experiment; races 1, 2, and 4 also were isolated from the ECW plot, but at a lower frequency. The predominance of race 4 on ECW-20R followed expectations, because it was the only race used in the field that could overcome resistance gene *Bs2*. Race 4 strains also can cause significant yield reductions in plants carrying only resis-

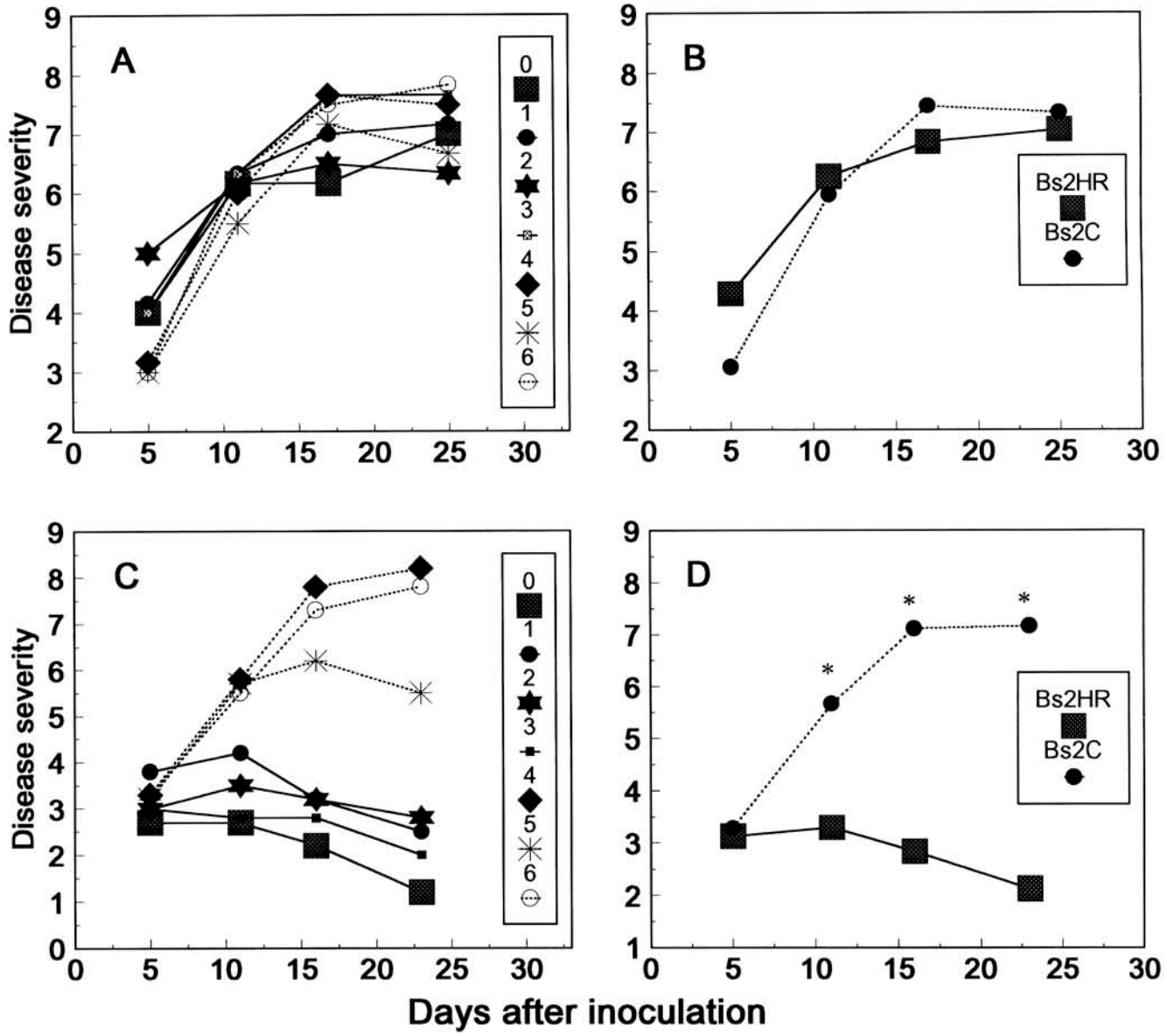

Fig. 3. Disease development on pepper cvs. Camelot (susceptible to all known *Xanthomonas campestris* pv. *vesicatoria* races) and X3R Camelot (contains resistance gene *Bs2*). A description of the disease severity scale is given in text. **A,** Disease progress on Camelot for races 0 (V19), 1 (V79), 2 (V126), 3 (V110), 4 (V181), 5 (V205), and 6 (V199) when plants were inoculated at the 6- to 8-leaf stage by the Silwet L-77 method. **B,** Contrasts of mean disease severity over time on Camelot for races compatible (races 4, 5, and 6) (Bs2C) and those eliciting a hypersensitive reaction (HR) (races 0 to 3) on ECW-20R. The means were contrasted by a *t* test, and no significant differences were observed ($\alpha = 0.05$). **C,** Disease progress on X3R Camelot for races 0 to 6. **D,** Contrasts of mean disease severity on X3R Camelot for races compatible (races 4, 5, and 6) (Bs2C) and those eliciting a HR (races 0 to 3) on ECW-20R. Bs2C was significantly (indicated by *) greater than Bs2HR ($P = 0.0001$).

tance gene *Bs2* (13). Races 5 and 6 were not tested in the field during 1995, because the race 5 isolate was obtained from Florida and the race 6 isolate was a laboratory-derived strain. During 1996 we tested the ability of races 4, 5, and 6 to cause disease on commercial cultivars in the field because these strains were isolated from our fields in 1995. The results of the 1996 field trial confirmed the ability of races that overcome the *Bs2* gene for resistance to cause severe disease. Recently, a strain of race 6 was isolated from commercial pepper fields in Ohio and was reported to be highly aggressive on susceptible pepper cv. Marengo (22, 23). These observations suggest that races that overcome resistance gene *Bs2* retain the ability to cause severe disease on bell pepper.

Strains of the bacterial spot pathogen have been shown to shift races within a season in the field when genotypes with a single gene for resistance have been deployed (14). Such race changes also were observed in laboratory experiments as early as 1979 by Dahlbeck and Stall (6). Various mechanisms were involved in race changes in laboratory experiments, such as inactivation of the avirulence gene by an insertion element (10), loss of the plasmid carrying the avirulence gene, or simple base-pair mutations (18). It also was shown that race shifts in the field were due either to a loss of a plasmid carrying an avirulence gene or inactivation of the avirulence gene (14). In the current study, avirulence gene *avrBs2* was detected in all seven races (0 to 6) in Southern hybridizations. However, races 4, 5, and 6 did not elicit a HR on ECW-20R. No restriction fragment length polymorphisms were observed in the *avrBs2* region among the different races with the restriction enzymes *Sph*I, *Bam*HI, *Eco*RI, *Hind*III, or *Eco*RV. Other enzymes or sequencing the *avrBs2* region may reveal true differences.

Recent studies (26) in which the *avrBs2* region was sequenced indicated two classes of *Xanthomonas* pathogens capable of evading *Bs2* host resistance that were mutated in *avrBs2*. One of these classes had a 5-bp insertion in the *avrBs2* region, whereas the other was distinguished by a divergent 3′ region of *avrBs2* (26). In our experiments, we did not observe any of the polymorphisms observed for strains isolated from the field in Barbados with a divergent 3′ region of *avrBs2* (26). It is possible that the strains of races 4, 5, and 6 used in our experiment may contain the 5-bp insertion in the *avrBs2* region; however, the methods we used would not detect such differences. Alternatively, it also is possible that different mechanisms for overcoming resistance gene *Bs2* and caus-

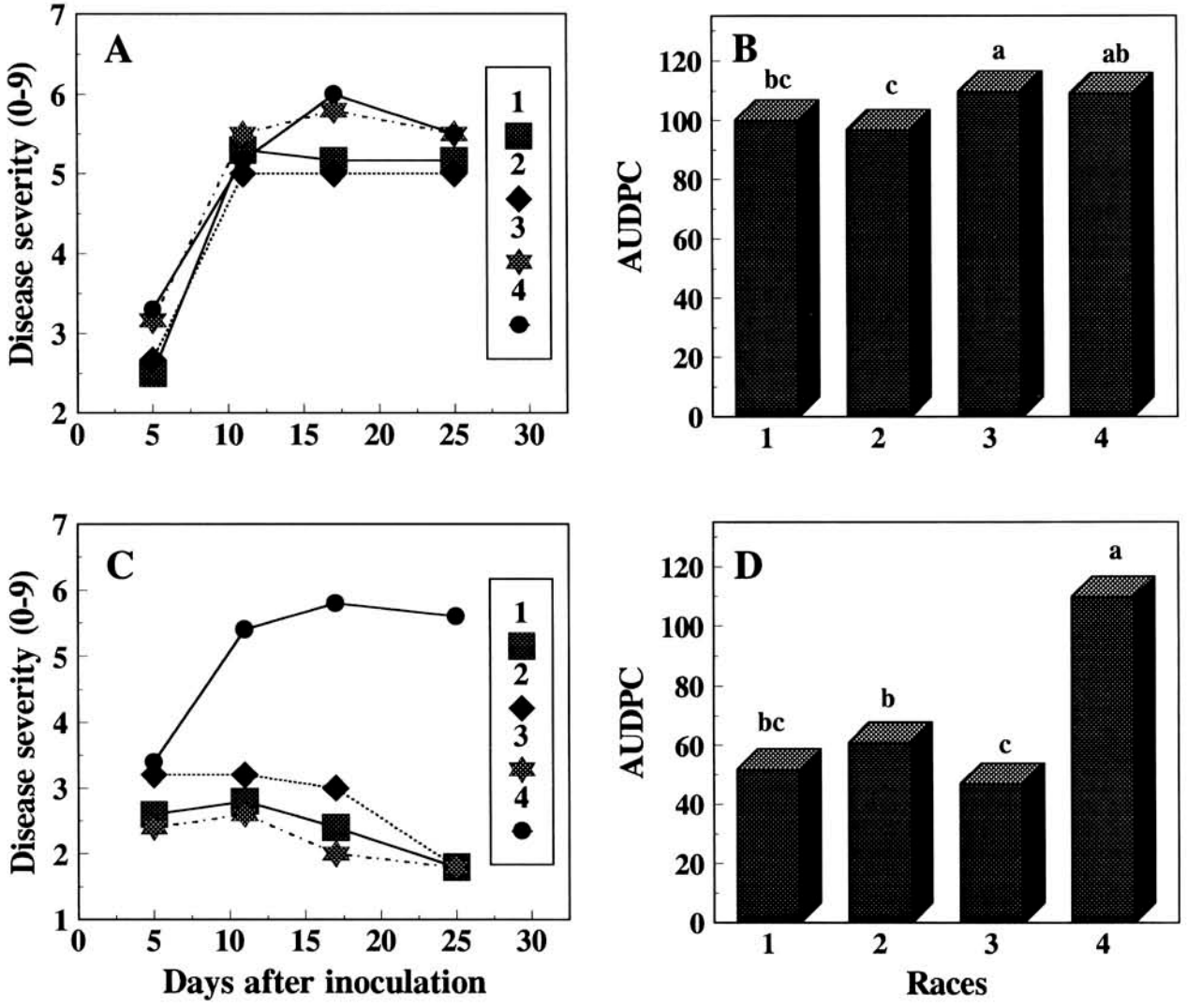

Fig. 4. Disease development on Early Calwonder (ECW) and ECW-20R in the greenhouse when pepper plants were inoculated at the 6- to 8-leaf stage with a mixture of two *Xanthomonas campestris* pv. *vesicatoria* strains per race for race 1 (V33 and V12), 2 (V6 and V72), 3 (V103 and V113), or 4 (V181 and V182) by the Silwet L-77 method. These were the same strains that were used as initial inoculum in the 1995 field experiments. **A,** Disease progress on ECW for races 1 to 4. **B,** Area under disease progress curves (AUDPC) on ECW for races 1 to 4. Means were compared by the LSD procedure. Bars with the same letters are not significantly different ($\alpha = 0.05$). **C,** Disease progress on ECW-20R inoculated with races 1 to 4. Disease severity on ECW-20R plants inoculated with race 4 were significantly greater ($P = 0.0001$) than for those inoculated with races 1 to 3 during the last three observations. **D,** AUDPC on ECW-20R for races 1 to 4. Bars with the same letters are not significantly different ($\alpha = 0.05$).

TABLE 3. Disease development measured as disease severity[a] during the growing season and area under disease progress curves (AUDPC)[b] on Early Calwonder (ECW) and ECW-20R plots inoculated with bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*) races 1 to 4

Near-isogenic line	Mean disease severity (0 to 9) recorded after inoculation (days)[c]								AUDPC
	21	28	34	42	49	56	62	70	
ECW	0.09	0.44	1.33	3.15	4.57	5.03	5.49	5.80	161.07
SD[d]	0.29	0.65	1.13	1.25	1.05	0.96	0.92	0.62	34.25
ECW-20R	0.07	0.27	1.18	3.05	4.41	4.64	5.23	5.68	151.77
SD	0.25	0.53	0.98	1.08	1.05	1.16	1.03	0.62	34.63

[a] Disease severity was measured using a 0 to 9 scale as described previously (16).
[b] AUDPC was calculated as described previously (3).
[c] Plots were inoculated on 4 May 1995.
[d] Standard deviation (SD) of 200 plants within each plot. Disease severity was spatially correlated within the field.

TABLE 4. Comparison of yield[a] and race distribution[b] on near-isogenic line Early Calwonder (ECW) and ECW-20R plants grown in the field during 1995 and inoculated with bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*) races 1 to 3

Near-isogenic line	Yield/plant (g)	Check yield/plant (g)[c]	Race distribution (%)				No. of colonies tested
			1	2	3	4	
ECW	199.5 ± 54.7[d]	1,181 ± 254	1.4	19.1	70.9	8.5	141
ECW-20R	234.4 ± 36.0	895 ± 187	0.8	0.0	0.0	99.2	121

[a] Yield per plant is based on one harvest of six groups of plants, with five plants per group for each near-isogenic line.
[b] Race distribution is based on five sampling dates from 25 May to 13 July. Race determination was based on infiltration of each of the colonies on the pepper differentials as described previously (18,21).
[c] Check plants were not inoculated and were located at a site ~300 m from the inoculated site.
[d] Standard deviation.

TABLE 5. Disease development measured as disease severity[a] during the growing season and area under disease progress curves (AUDPC)[b] on pepper cvs. Camelot and X3R Camelot plots inoculated with bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*) races 4, 5, and 6

Cultivar	Mean disease severity (0 to 9) recorded after inoculation (days)[c]									AUDPC
	19	26	33	40	47	54	68	78	85	
Camelot	0.21	0.57	1.17	2.29	2.47	3.10	5.92	6.01	6.49	224.4
SD[d]	0.41	0.57	0.38	0.90	0.82	0.63	0.28	0.27	0.63	19.6
X3R Camelot	0.15	0.45	1.17	2.41	2.75	3.12	5.88	6.04	6.45	226.2
SD	0.36	0.50	0.51	0.96	0.91	0.80	0.37	0.21	0.53	24.8

[a] Disease severity was measured by a 0 to 9 scale as described previously (16).
[b] AUDPC was calculated as described previously (3).
[c] Inoculum plants were planted in the plots on 9 May 1996.
[d] Standard deviation (SD) of 72 plants within each plot. Disease severity was spatially correlated within the field.

ing disease in the field may be present. Kearney and Staskawicz (11) also suggested the possibility of *Bs2* resistance being overcome in the field due to secondary mutations that restore virulence to the *avrBs2* mutants.

Sequences homologous to *avrBs2* have been identified in 11 *X. campestris* pathovars. Of these, seven pathovars elicited a HR on ECW-20R, and a 2.35-kb *Sph*I fragment was conserved among these pathovars (11). Mutations in the *avrBs2* region reduced the ability of *X. campestris* pv. *vesicatoria* to multiply in host tissue, and a *X. campestris* pv. *alfalfae* mutant carrying a marker-exchange mutation in *avrBs2* multiplied at a slower rate in alfalfa compared to a wild-type (11). The ability of these mutants to multiply normally was restored when complemented with a plasmid-borne copy of *avrBs2* (11). In the current study, strains that overcome *Bs2* multiplied at a slower rate in half-strength NB after 32 h of incubation; however, in normal-strength NB, there were no differences. The strains that overcome *Bs2*, however, retain the ability to cause severe disease on pepper plants in greenhouse and field experiments. The ability of strains that overcome gene *Bs2* to cause disease and yield loss needs to be taken into account in breeding for disease resistance and other disease management strategies.

In a recent cultivar trial, we observed that X3R Camelot, which carries the *Bs2* gene for resistance, was severely diseased, and the predominant strain present was race 4 (13). Significant yield reductions (sixfold compared to controls) also were observed for this cultivar. However, cultivars and lines such as Boynton Bell and PR300-4 that carry two genes for resistance (*Bs1* and *Bs2*) had less disease. Thus, despite the ability of race 4 strains to individually overcome *Bs1* and *Bs2*, pyramiding these two genes offered better disease protection (13). In similar experiments, we also have observed that pyramiding of *Bs1* and *Bs3* genes does not provide effective control against race 3 strains (13,15). This suggests that the *Bs2* gene may still confer protection against the bacterial spot pathogen races when pyramided. The combination of three resistance genes *Bs1*, *Bs2*, and *Bs3* provided better protection compared to only two genes, *Bs1* and *Bs2* (13).

Crop value and use dictate the type of resistance needed to provide acceptable disease control (28). Major-gene resistance is almost a necessity for managing diseases in vegetable crops that usually are consumed directly, unlike cereal crops, the products of which may not be consumed directly. Most consumer markets do not tolerate blemished fresh fruit or vegetables. Acceptable quality in fruits and vegetables is often achieved only by combining host resistance and chemical pesticides. Because of the bacterial spot pathogen's ability to rapidly overcome major genes, breeding for bacterial spot resistance may need to incorporate quantitative resistance and pyramiding of major-gene resistance in commercial cultivars. Recent developments in cloning major genes for resistance to bacterial pathogens (25) and progress in cloning of *Bs2* (24) may enable breeders to shuttle such single genes into genotypes with quantitative resistance. This form of resistance also could be supplemented with chemical sprays for adequate protection.

TABLE 6. Race distribution[a] on pepper cvs. Camelot and X3R Camelot grown in the field during 1996 and inoculated with bacterial spot pathogen (*Xanthomonas campestris* pv. *vesicatoria*) races 4, 5, and 6

Cultivar	Race distribution (%)			No. of colonies tested
	4	5	6	
Camelot	15.4	9.6	75.0	52
X3R Camelot	10.9	18.5	70.8	65

[a] Race distribution is based on three sampling dates during the season. Race determination was based on infiltration of each of the colonies in the pepper differentials as described previously (18,21).

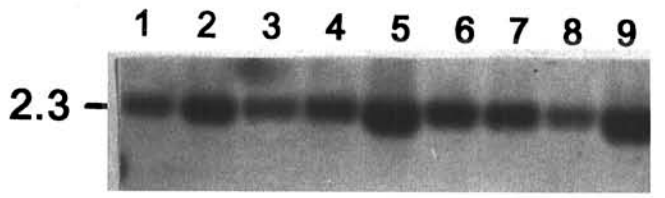

Fig. 5. Southern blot of *Sph*I restriction digests of total genomic DNA from all known races of *Xanthomonas campestris* pv. *vesicatoria* probed with a 2.3-kb *avrBs2* fragment. Lanes 1 through 7, races 0 to 6, respectively. Lanes 8 and 9, races 5 and 6, respectively, obtained from our research plots in 1995. Races 0 to 3 carry a functional *avrBs2* and cause a hypersensitive reaction on plants carrying resistance gene *Bs2*. Races 4, 5, and 6 are compatible on pepper plants with *Bs2*.

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