Response of Bell Pepper Cultivars to Bacterial Spot Pathogen Races that Individually Overcome Major Resistance Genes

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ABSTRACT

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The effect of major resistance genes (*Bs1*, *Bs2*, and *Bs3*) or gene combinations for resistance to bacterial spot of bell peppers (*Xanthomonas campestris* pv. *vesicatoria*) in 15 commercial cultivars on disease reduction and yield were studied during 1995 and 1996. Reaction of cultivars to specific races (races 1, 2, or 3) of the pathogen corresponded with seed company claims for resistance against these races. Races 1 to 4 were used as initial inoculum in 1995, and races 1 to 6 in 1996 field experiments. Cultivars with no known resistance genes to bacterial spot (e.g., Camelot, Jupiter, and Valiant), a single resistance gene (X3R Camelot, King Arthur), or a combination of *Bs1* and *Bs3* genes (Guardian, Sentinel, and Admiral) were severely diseased. Yields were reduced in all inoculated cultivars compared to non-inoculated cultivars used as controls. Although races 4 and 6 caused significant disease in cultivars with only *Bs1* (King Arthur) or *Bs2* (X3R Camelot) genes, cultivars with a combination of *Bs1* and *Bs2* (Boynton Bell, PR9300-8) had much lower levels of bacterial spot. Roger 4178, a hybrid with a combination of *Bs1*, *Bs2*, and *Bs3* genes, had the lowest disease ratings. Overall, race 3 was predominant during 1995, while races 3 and 6 were recovered most frequently in 1996.

Managing bacterial diseases of vegetable crops is difficult once they are present. Bacterial spot of bell peppers caused by *Xanthomonas campestris* pv. *vesicatoria* (proposed: *X. axonopodis* pv. *vesicatoria*; 28) is a major limiting factor in bell pepper production. Host-plant resistance is an important component of an integrated management program for this disease. Cultivars with host resistance and good acceptable horticultural characteristics generally take years to develop. There are 3 known independent dominant genes (*Bs1*, *Bs2*, and *Bs3*) for qualitative resistance to specific races of *X. campestris* pv. *vesicatoria* (Xcv) in peppers (4,5,9,12). The prevalence of host-differentiated races makes it difficult to manage this disease based only on host resistance (1,6,13,20-23). Furthermore, race shifts can nullify the effectiveness of resistance genes (major genes) when deployed in monoculture (7,14).

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To date, 7 races (designated 0 to 6) of the pathogen have been characterized on the basis of hypersensitive reaction on three near-isogenic lines of Early Calwonder (ECW), each with a single gene for resistance (1,6,13,18,20,22,23). These near-isogenic lines are ECW-10R (contains resistance gene *Bs1*), resistant to races 0, 2, and 5; ECW-20R (*Bs2*), resistant to races 0 to 3; and ECW-30R (*Bs3*), resistant to races 0, 1, and 4 (13,14,18,20). Thus, the currently known genes for resistance to bacterial spot can be overcome by at least one race (13,14,15,18,20), and race 6 can overcome all 3 genes (22,23). The use of pyramids made up of several resistance genes to manage fungal diseases in cereals has been well described (19). In the current study, we determined the presence of major (dominant) resistance genes or gene combinations by phenotypic reactions of commercial hybrids to seven *X. campestris* pv. *vesicatoria* races. Furthermore, we determined how such genes or gene combinations perform when multiple pathogen races are present in the field.

MATERIAL AND METHODS

Determining the presence or absence of major resistance genes in cultivars. During 1995 and 1996, 15 commercial cultivars were evaluated, 14 of which were hybrids. Jupiter, a highly susceptible standard, was the only open-pollinated cultivar tested. The presence or absence of the major (dominant) resistance genes *Bs1*, *Bs2*, *Bs3*, or combinations of them in representative plants of 15 individual cultivars was

determined by infiltrating leaves of 3 8-week-old greenhouse-grown plants with inoculum suspension (10^8 CFU/ml) of races 0 (Xcv 19), race 1 (Xcv 79), race 2 (Xcv 126), race 3 (Xcv 110), race 4 (Xcv 181), race 5 (Xcv 206), and race 6 (Xcv 199) using a needleless syringe in the laboratory. Details of the strains used in these experiments have been described previously (15). This test mainly helps in determining the presence of the 3 major genes for resistance. No attempt was made to assess the cultivars for other quantitative resistance that might occur independent of the major genes. Reaction of the cultivars to the races was observed periodically during a 48-h period, and the presence or absence of a hypersensitive response (HR) or a compatible (C) response was recorded (Table 1). The phenotype and the time required for the HR to occur in response to the different races was also considered in determining the resistance genes (*data not presented*), as this reaction varies among the interactions of the resistance gene in the plant and corresponding avirulence genes present in the pathogen (18). Reactions of races to the four near-isogenic lines of Early Calwonder were also tested. These experiments were conducted during 1995 and 1996.

Field experiments. The field experiments were conducted at Sandhills Research Station, Jackson Springs, North Carolina, during 1995 and 1996. The same 15 commercial cultivars presented in Table 1 were evaluated in this experiment. Pepper plants were grown and transplanted to the fields according to the standard practices for commercial North Carolina production (24). Transplants were grown in greenhouses for 7 weeks in Styrofoam trays with 2.54-cm (1-inch) cells and 200 cells per tray. Overhead irrigation (1.6 cm/h) was applied to the crop as needed to maintain optimum plant growth.

In 1995, transplants were set on 27 April on bare ground ridges. Each plot consisted of one row on a 97-cm center, with 7 plants of a cultivar per row, spaced 36 cm apart. Each plot was replicated four times in a randomized complete block design. On 4 May, 4 plants, 1 each inoculated in the greenhouse with race 1, 2, 3, or 4, were transplanted to the center of each plot. Races had the following phenotypic marker: race 1, rifampicin-resistant (Rif^R, 50 µg/ml); race 2, copper (Cu^R, 200 µg/ml)- and streptomycin (Sm^R, 200

µg/ml)-resistant; race 3, Cu^S and Sm^S; race 4, Cu^R (200 µg/ml) and Sm^S. These inoculum plants (susceptible cv. Camelot) were inoculated with two strains per race (15,17). The details of the strains used in these studies have been reported previously (15). Groups of inoculated plants (grouped by race) were isolated from each other in the greenhouse by maintaining them in different rooms, and misted occasionally until symptom appearance, prior to transferring to the field. The presence of a specific race on the inoculated plants was verified prior to transferring these plants to the field (14,15,17). Data on disease development on plants in the field were recorded weekly using a 0 to 9 scale described previously (14,17). Diseased leaf samples were collected from each cultivar at 2-week intervals, and isolations of the pathogen were made on non-selective yeast dextrose calcium carbonate agar (YDC) to assess the predominant race on each cultivar (20). For bacterial isolations, leaf samples from all four replications for each cultivar were pooled. Single lesions from each of 8 to 10 leaves were excised and macerated in 500 µl of sterile distilled water. The resulting suspension was streaked on 2 plates of YDC. Ten isolated single colonies were randomly picked from the YDC plates for each cultivar. Resistance to antibiotics or copper and the race of each of these single colonies was determined as described previously (20). Sampling was done three times during 1995. Mature green peppers were harvested

twice, and number and weight of fruit recorded.

In addition, a similar experiment with two replications and 5 plants per cultivar per plot was conducted approximately 300 m from the inoculated site at a site which was not inoculated (check).

During 1996, a similar trial with the same 15 cultivars was conducted at a site where peppers had not been grown for at least 2 years. Plants were transplanted on 1 May. Plots were single rows on ridges on a 112-cm center, containing 9 plants spaced 36 cm apart within each row. Cultivars

Fig 1. Disease development in bell pepper cultivar experiments conducted at Sandhills Research Station, Jackson Springs, North Carolina, during 1995 and 1996. Races 1 to 4 were used as inoculum in 1995 and races 1 to 6 in 1996. Field plots were inoculated on 4 May 1995 and 14 May 1996. Cultivar Camelot is susceptible to all known races. X3R Camelot has resistance gene *Bs2* and is resistant to races 0 to 3. Boynton Bell has resistance genes *Bs1* and *Bs2* and is resistant to races 0 to 3 and 5. Rogers 4178 has resistance genes *Bs1*, *Bs2*, and *Bs3*, and is resistant to races 0 to 5. Race 6 causes disease on all of these cultivars.

Table 1. Reaction of bell pepper cultivars^a and near-isolines to races 0 to 6 of *Xanthomonas campestris* pv. *vesicatoria* and the presence of different major resistance genes

Cultivar/isolines	Reaction to races							Putative major resistance genes ^y
	0	1	2	3	4	5	6	
ECW	C	C	C	C	C	C	C	... ^z
ECW-10R	HR	C	HR	C	C	HR	C	<i>Bs1</i>
ECW-20R	HR	HR	HR	HR	C	C	C	<i>Bs2</i>
ECW-30R	HR	HR	C	C	HR	C	C	<i>Bs3</i>
Baron	C	C	C	C	C	C	C	...
Valiant	C	C	C	C	C	C	C	...
Camelot	C	C	C	C	C	C	C	...
Jupiter	C	C	C	C	C	C	C	...
King Arthur	HR	C	HR	C	C	HR	C	<i>Bs1</i>
X3R Camelot	HR	HR	HR	HR	C	C	C	<i>Bs2</i>
Admiral	HR	HR	HR	C	HR	HR	C	<i>Bs1</i> , <i>Bs3</i>
Sentinel	HR	HR	HR	C	HR	HR	C	<i>Bs1</i> , <i>Bs3</i>
Guardian	HR	HR	HR	C	HR	HR	C	<i>Bs1</i> , <i>Bs3</i>
Boynton Bell	HR	HR	HR	HR	C	HR	C	<i>Bs1</i> , <i>Bs2</i>
PR300-4	HR	HR	HR	HR	C	HR	C	<i>Bs1</i> , <i>Bs2</i>
PR300-6	HR	HR	HR	HR	C	HR	C	<i>Bs1</i> , <i>Bs2</i>
PR9300-5	HR	HR	HR	HR	C	HR	C	<i>Bs1</i> , <i>Bs2</i>
PR9300-8	HR	HR	HR	HR	C	HR	C	<i>Bs1</i> , <i>Bs2</i>
Rogers 4178	HR	HR	HR	HR	HR	HR	C	<i>Bs1</i> , <i>Bs2</i> , <i>Bs3</i>
Resistance genes that elicit	<i>Bs1</i>		<i>Bs1</i>			<i>Bs1</i>		
HR to the above races	<i>Bs2</i>	<i>Bs2</i>	<i>Bs2</i>	<i>Bs2</i>			...	
	<i>Bs3</i>	<i>Bs3</i>			<i>Bs3</i>			

^x Leaves of each cv. (8 weeks old) were infiltrated with inoculum suspension (10⁸ CFU/ml) of races 0 to 6 and observations for compatible (C, disease reaction) or hypersensitive reaction (HR, resistant reaction) were recorded during a 48-h period. The phenotype and the time required for the HR to occur in response to the different races was also considered in determining the resistance genes (*data not presented*). This varies among the interactions of the resistance gene in the plant and corresponding avirulence genes present in the pathogen (18).

^y The presence of the major resistance genes was determined based on the phenotypic reaction of the cultivars to races 0 to 6. However, this technique will not detect the presence of other varying levels of quantitative resistance traits.

^z ... = no major resistance gene detected.

were replicated four times in a randomized complete block design. On 14 May, inoculum plants infected with races 1 to 6 were planted in the center of each plot. Inoculum plants were prepared as described above. Disease assessment and race determination were carried out as in 1995. Samples of diseased leaves were collected three times. The non-inoculated check plot did not develop properly due to unknown reasons, and hence no

comparisons between inoculated and disease-free plots could be made in 1996.

Statistical analyses. Areas under the disease progress curves (AUDPC) were calculated as described by Campbell and Madden (2). For each year, one-way analysis of variance was performed on the AUDPC values. Means were separated using the Waller-Duncan *k*-ratio *t* tests. In addition, specific non-orthogonal contrasts

were estimated to compare the effects of individual genes and gene combinations in different cultivars. The cumulative effects of *Bs1* and *Bs2* in five pepper hybrids were contrasted with the effects of *Bs1* in King Arthur and *Bs2* in X3R Camelot and the cultivars without any major genes. All statistical analyses were performed using the SAS statistical package (Ver 6.10, SAS Institute, Cary, NC).

Table 2. Resistance genes present in bell pepper cultivars, area under the disease progress curve (AUDPC), yield/plant for inoculated plots, control yield/plant for non-inoculated plots, and race distribution in a cultivar experiment inoculated with races 1 to 4 of *Xanthomonas campestris* pv. *vesicatoria* in 1995

Cultivar	Putative major resistance genes ^w	AUDPC ^x	Yield/plant (g) ^y	Control yield/plant (g)	Race distribution (%) ^v				Number of colonies tested
					1	2	3	4	
Camelot	...	193 a ^z	28 d	556 d	0	18	77	7	22
Jupiter	...	193 a	20 d	812 bcd	0	0	95	5	21
Baron	...	192 ab	49 d	...	0	0	100	0	25
Guardian	<i>Bs1</i> , <i>Bs3</i>	182 ab	28 d	817 abcd	0	0	100	0	20
King Arthur	<i>Bs1</i>	179 ab	65 d	...	0	0	100	0	25
Admiral	<i>Bs1</i> , <i>Bs3</i>	178 ab	81 d	763 cd	0	0	100	0	24
Sentinel	<i>Bs1</i> , <i>Bs3</i>	174 ab	73 d	1114 a	0	0	100	0	21
Valiant	...	156 c	235 c	741 cd	0	27	68	5	22
X3R Camelot	<i>Bs2</i>	153 c	142 cd	873 abc	0	10	0	90	29
PR9300-5	<i>Bs1</i> , <i>Bs2</i>	104 d	255 c	924 abc	0	0	13	88	24
PR9300-8	<i>Bs1</i> , <i>Bs2</i>	91 de	429 b	1069 ab	0	0	78	22	23
Boynton Bell	<i>Bs1</i> , <i>Bs2</i>	85 e	446 b	1077 ab	0	3	83	14	29
PR300-6	<i>Bs1</i> , <i>Bs2</i>	85 e	437 b	919 abc	0	0	33	67	18
PR300-4	<i>Bs1</i> , <i>Bs2</i>	80 e	425 b	1101 ab	0	0	11	89	18
Rogers 4178	<i>Bs1</i> , <i>Bs2</i> , <i>Bs3</i>	61 f	731 a	910 a	0	0	83	17	24
Total distribution of races					0	4	71	25	370

^v Predominance of individual races was determined by isolating bacteria from diseased leaf samples and testing their reactions on pepper differentials ECW, ECW-10R, ECW-20R, and ECW-30R.

^w Presence of major resistance genes was determined by infiltrating with races 0 to 6 and observing for hypersensitive response (HR) as described in Table 1. ... = no major resistance genes detected.

^x AUDPC was calculated using eight weekly observations using a 0 to 9 disease rating scale, where 0 = no diseased leaves, 4 = 21 to 35% diseased leave, 7 = 65 to 80% diseased leaves, and 9 = 100% diseased leaves, plant dying or dead.

^y Yield/plant was calculated for the control plots (disease-free) for comparison with yield/plant in inoculated plot.

^z Means followed by the same letter within columns are not significantly different based on the Waller-Duncan *k*-ratio (*k* = 100) *t* tests (α = 0.05).

Table 3. Resistance genes present in bell pepper cultivars, area under the disease progress curve (AUDPC), yield/plant, and race distribution in a cultivar experiment inoculated with races 1 to 6 of *Xanthomonas campestris* pv. *vesicatoria* in 1996

Cultivar	Putative major resistance genes ^w	AUDPC ^x	Yield/plant (g) ^y	Race distribution (%) ^v						Number of colonies tested
				1	2	3	4	5	6	
Camelot	...	241 a ^z	385 ef	23	12	62	4	0	0	26
Baron	...	230 ab	229 f	29	0	14	7	0	50	28
Jupiter	...	220 ab	465 def	22	11	48	0	15	4	27
Admiral	<i>Bs1</i> , <i>Bs3</i>	217 ab	394 ef	0	0	80	0	0	20	20
Guardian	<i>Bs1</i> , <i>Bs3</i>	213 ab	392 ef	0	0	94	0	0	6	17
Valiant	...	207 bc	527 de	21	16	47	5	0	11	19
King Arthur	<i>Bs1</i>	204 bc	456 def	18	0	61	0	0	21	28
Sentinel	<i>Bs1</i> , <i>Bs3</i>	200 bc	513 de	0	0	100	0	0	0	25
X3R Camelot	<i>Bs2</i>	176 cd	608 cde	0	9	0	18	46	27	22
PR9300-8	<i>Bs1</i> , <i>Bs2</i>	158 de	697 bcd	9	9	17	26	0	39	23
PR9300-5	<i>Bs1</i> , <i>Bs2</i>	135 ef	848 abc	5	0	20	5	0	70	20
PR300-6	<i>Bs1</i> , <i>Bs2</i>	122 fg	847 abc	0	0	4	4	0	93	29
Boynton Bell	<i>Bs1</i> , <i>Bs2</i>	118 fg	997 a	0	0	27	50	0	23	22
PR300-4	<i>Bs1</i> , <i>Bs2</i>	117 fg	925 ab	4	0	4	26	0	67	27
Rogers 4178	<i>Bs1</i> , <i>Bs2</i> , <i>Bs3</i>	100 g	1,027 a	0	42	4	0	0	54	26
Total distribution of races (%)				9	7	38	9	4	34	389

^v Predominance of individual races was determined by isolating bacteria from diseased leaf samples and testing their reactions on pepper differentials ECW, ECW-10R, ECW-20R, and ECW-30R.

^w Presence of resistance genes was determined by infiltrating leaves with races 0 to 6 and observing for hypersensitive response (HR) during a 48-h period as described in Table 1. ... = no major resistance gene detected.

^x AUDPC was calculated from 10 weekly observations using a 0 to 9 disease rating scale, where 0 = no diseased leaves, 4 = 21 to 35% diseased leave, 7 = 65 to 80% diseased leaves, and 9 = 100% diseased leaves, plant dying or dead.

^y Yield/plant is based on three harvests.

^z Means followed by the same letter within columns are not significantly different, based on the Waller-Duncan *k*-ratio (*k* = 100) *t* tests (α = 0.05).

RESULTS

The current tests confirmed the presence of specific resistance genes or gene combinations in commercial hybrid pepper cultivars (Table 1). During 1995, bacterial spot progressed rapidly and susceptible cultivars (e.g., Camelot and Jupiter) were severely diseased (Fig. 1). Plants with single major resistance gene *Bs1* (King Arthur) or *Bs2* (X3R Camelot) also were severely affected. Similarly, cultivars with *Bs1* and *Bs3* genes (Guardian) were also heavily damaged (Table 2). In contrast, plants with *Bs1* and *Bs2* (e.g., Boynton Bell or PR9300-6) or *Bs1*, *Bs2*, and *Bs3* (e.g., Rogers 4178) genes in combination performed well under heavy disease pressure. Rogers 4178 had the lowest disease ratings and the largest yields. Isolations revealed that race 3 was predominant during the 1995 season on most cultivars. Race 4 was recovered most frequently from X3R Camelot and PR9300-4.

In 1996, bacterial spot development was slow during the early part of the season. However, by late June all susceptible cultivars were severely diseased. X3R Camelot (*Bs2*) had significantly less disease and greater yield than Camelot, but significantly more disease than cultivars with *Bs1* and *Bs2* (Table 3). Rogers 4178 (*Bs1*, *Bs2*, and *Bs3*) had significantly less disease than cultivars with *Bs1* and *Bs3* (Guardian), *Bs1* (King Arthur), and *Bs2* (X3R Camelot). However, the disease level on Rogers 4178 was not significantly different from some of the cultivars with a combination of *Bs1* and *Bs2* (e.g., Boynton Bell). Overall, race 3 and race 6 were predominant in this test. Race 3 prevailed on cultivars which had no specific resistance gene against it. These included Camelot, King Arthur (*Bs1*), and Sentinel (*Bs1* and *Bs3*). Infections on PR9300-6 (*Bs1* and *Bs2*) were mostly due

to race 6. Races 4, 5, and 6 were found on X3R Camelot.

Combining *Bs1* and *Bs2* resistance genes provided significantly greater protection than that provided by *Bs2* gene alone during both years (Table 4). *Bs2* alone was better than having no resistance genes. The effects of combining *Bs1* and *Bs2* appeared to be cumulative. Combination of 3 genes offered improved protection against race 6 compared with a combination of 2 genes (Table 4).

During both years, the cultivar Camelot had the lowest yield and Rogers 4178 had the highest yield in the inoculated plots. In 1995, cultivar Sentinel and 8 other cultivars had higher yields in the non-inoculated plots among the 15 cultivars tested. Significant correlations ($P = 0.0001$) between AUDPC and yield-per-plant were observed in these experiments during 1995 ($R^2 = 0.81$) and 1996 ($R^2 = 0.79$).

DISCUSSION

The presence of resistance to bacterial spot of peppers was described by Horsfall in 1939 (10). Resistance gene *Bs1* was reported in 1963 (5), *Bs2* in 1984 (4), and *Bs3* in 1985 (12). Resistance to bacterial spot in commercial peppers has been derived from 1 or more of these 3 genes for more than 30 years. This suggests that finding and incorporating resistance into bell pepper while maintaining good horticultural characteristics is difficult, and it would be advantageous to use these 3 genes and any newly discovered ones in the most durable manner. Although the *Bs1* gene for resistance to race 2 was reported in 1963 (5), cultivars containing *Bs1* were not released for commercial use at that time because of fear that race 1 epidemics would negate the control by the *Bs1* gene (6,9). Few, if any, commercial hybrids with

only *Bs3* were developed, mainly because of the threat from race 2. Hybrids with only *Bs3* are not available, and therefore were not used in this study. Each of the 3 major genes can be overcome by at least one naturally occurring race of the bacterial spot pathogen (13,14,15,18,20,22,23). In the present study, we looked at the deployment of the major genes individually and in pyramids in commercial cultivars. Interestingly, a cultivar with resistance genes *Bs1* and *Bs2* was described in 1984 (3); however, at that time the effect of the combination of these 2 genes on a race such as 4 or 6 was not known, since these races have been described only very recently (1,13,22,23).

Sixfold yield reductions compared to disease free controls were observed for inoculated X3R Camelot plots in 1995 (Table 2). The gene *Bs2* by itself thus provided little value for the management of bacterial spot. Races that overcome gene *Bs2* for resistance and cause disease and yield loss need to be taken into account in breeding for disease resistance and use of other disease management strategies (15). Despite the ability of race 4 and 6 strains to individually overcome resistance genes *Bs1* and *Bs2*, pyramiding these 2 genes offered improved bacterial spot control; still, twofold yield reductions were observed for those cultivars with *Bs1* and *Bs2* genes. Race 3 strains severely damaged cultivars with *Bs1* and *Bs3* genes. Combination of the 3 resistance genes *Bs1*, *Bs2*, and *Bs3* provided slightly better protection compared to only the 2 genes *Bs1* and *Bs2* against race 6. The cost and time involved in the development of commercially acceptable cultivars with 2 resistance genes compared to cultivars with 3 genes, and the amount of relative protection they offer, should be considered in breeding new va-

Table 4. Estimated contrasts for comparison of areas under the disease progress curves (AUDPC^y) on bell pepper cultivars with a combination of different major resistance^z genes to *Xanthomonas campestris* pv. *vesicatoria*

Contrast	Estimate	Contrast SS	F value	Pr > F
1995				
<i>Bs1</i> vs. None	4.3 ± 8.6	58.9	0.25	0.6187
<i>Bs2</i> vs. None	30.9 ± 8.6	3059.4	13.05	0.0008
<i>Bs1</i> and <i>Bs2</i> vs. None	94.7 ± 5.1	79702.3	339.91	0.0001
<i>Bs1</i> and <i>Bs3</i> vs. None	5.2 ± 5.9	184.4	0.79	0.3800
<i>Bs1</i> , <i>Bs2</i> , and <i>Bs3</i> vs. None	122.6 ± 8.6	48097.5	205.12	0.0001
<i>Bs2</i> vs. <i>Bs1</i> and <i>Bs2</i>	63.8 ± 8.4	13556.0	57.81	0.0001
<i>Bs1</i> and <i>Bs2</i> vs. <i>Bs1</i> , <i>Bs2</i> , and <i>Bs3</i>	27.9 ± 8.4	2596.0	11.07	0.0018
Cumulative effects of <i>Bs1</i> and <i>Bs2</i>	59.5 ± 12.0	5776.4	24.63	0.0001
1996				
<i>Bs1</i> vs. None	20.0 ± 13.4	1275.3	2.21	0.1444
<i>Bs2</i> vs. None	48.3 ± 13.4	7449.2	12.10	0.0008
<i>Bs1</i> and <i>Bs2</i> vs. None	94.4 ± 8.1	79286.5	137.28	0.0001
<i>Bs1</i> and <i>Bs3</i> vs. None	14.4 ± 9.2	1416.6	2.45	0.1245
<i>Bs1</i> , <i>Bs2</i> , and <i>Bs3</i> vs. None	129.9 ± 15.2	42028.8	72.77	0.0001
<i>Bs2</i> vs. <i>Bs1</i> and <i>Bs2</i>	46.2 ± 13.2	7113.7	12.32	0.0010
<i>Bs1</i> and <i>Bs2</i> vs. <i>Bs1</i> , <i>Bs2</i> , and <i>Bs3</i>	35.4 ± 15.0	3229.7	5.59	0.0225
Cumulative effects of <i>Bs1</i> and <i>Bs2</i>	26.2 ± 18.8	1123.6	1.95	0.1701

^y AUDPC was calculated as described previously (Campbell and Madden, 1990). AUDPCs are based on 8 weekly observations in 1995 and 10 in 1996 using a 0 to 9 disease rating scale, where 0 = no diseased leaves, 4 = 21 to 35% diseased leaves, 7 = 65 to 80% diseased leaves, and 9 = 100% diseased leaves, plant dying or dead.

^z The major resistance genes in the cultivars were determined as described in Table 1.

rieties. In recent controlled greenhouse studies with near-isolines of ECW inoculated with race 6; ECW-13R (*Bs1* and *Bs3*) and ECW-123R (*Bs1*, *Bs2*, and *Bs3*) had significantly less disease compared to ECW-10R, ECW-20R, and ECW-30R (16). In the same study, ECW-12R (*Bs1* and *Bs2*) had significantly less disease compared to ECW, ECW-10R, and ECW-20R when inoculated with race 4 (16). Pyramiding may very well be the best option for pepper growers in the southeast who face a number of races in a given season. Though gene pyramids seem to provide more protection than single genes, chemical sprays likely will continue to be needed to provide adequate protection to maximize yield.

The commercial cultivars used in this study were mainly hybrids and not isolines. There may be some level of quantitative resistance (horizontal resistance) present in specific cultivars, and such resistance may play a role in providing some of the observed resistance attributed to gene pyramids. The infiltration technique used to detect the presence of the major resistance genes *Bs1*, *Bs2*, and *Bs3* would not detect other quantitative resistance traits present in the commercial cultivars which may also play a role in overall resistance. However, based on distribution of the races on individual commercial hybrids in the current experiments, it appears that the major genes account for a major portion of the resistance currently available. Variations in levels of quantitative resistance in some cultivars without any of the 3 major genes has been described (8). Among the 4 cultivars without any major genes for resistance in the present study, few differences were observed in susceptibility. Similarly small differences in disease severity among cultivars has been reported previously (11). However, cultivar Valiant, which had no detectable major resistance gene, had significantly lower AUDPC values than the other similar cultivars without major resistance genes (e.g., Camelot). Although Valiant had lower AUDPC values than Camelot or Baron, it failed to provide adequate disease protection, and significant yield loss compared to the non-inoculated plots was recorded.

In these field experiments, the disease pressure was very high because the plots were artificially inoculated and conditions were conducive for development of bacterial spot. The abundance of inoculum generated on susceptible cultivars possibly also resulted in interplot interference effects. The plots were completely randomized within each block, and we had inoculated each of the plots similarly with all the races to reduce the risk of interplot interference. It is possible that the abundance of inoculum in the area could also have masked the benefits of resistance in some cultivars, particularly those with different gene combinations.

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 were deployed (14). Such race changes have also been observed in laboratory experiments (7). During the fruit production part of the season in 1995, only races 2, 3, or 4 were isolated from the commercial hybrids. However, we isolated strains of race 6 from Rogers 4178 near the end of the fruit production period. It may be that race changes/shifts occurred during the course of the experiments in this cultivar with the 3 genes. Because of the pathogens' ability to rapidly overcome major genes (7,14), breeding for bacterial spot resistance may ultimately dictate incorporation of quantitative resistance and major resistance gene pyramids in commercial cultivars.

The predominance of races on the cultivars was to an extent related to the resistance genes present in them. During 1996, races that overcome the *Bs2* gene were predominant on cultivars with the *Bs2* gene. Race 3 was generally more prevalent on cultivars with no resistance genes, or cultivars with *Bs1*, or a combination of *Bs1* and *Bs3*. Race 6 also was isolated from 14 of the 16 cultivars in 1996. During 1995 and 1996, race 3 was isolated from 15 of the 16 cultivars. Race 3 also was common on Rogers 4178 and Boynton Bell in 1995. These two cultivars should have been resistant to race 3, due to the presence of the *Bs2* gene. During the past few years, we have observed the presence of race 3 in diseased samples from commercial pepper fields where cultivars with resistance gene *Bs2* were grown (21). The reason for this is unclear. The effects of high temperature (21) and water soaking (25) have been reported to inhibit the HR in plants containing single major resistance genes. Overall, race 3 appears to be highly aggressive. In other studies, race 3 was observed to emerge as the dominant race in a mixture of 1, 2, and 3 (17), while race 6 did so in a mixture of 4, 5, and 6 (15). In greenhouse experiments, strains of races 1, 3, 4, and 6 were found to cause similar levels of disease on susceptible cultivars (15). Further controlled studies are needed to determine the interaction between individual races and the resistance genes and gene pyramids.

The demand for high quality produce dictates that an integrated program for bacterial spot control, including host-plant resistance and judicious use of chemical sprays, be used. The recent developments in cloning major genes for resistance to bacterial pathogens (27) and progress in cloning of the *Bs2* gene (26) may enable breeders to move major genes for resistance to specific races into genotypes with quantitative resistance. Combined with timely chemical applications, bacterial spot might be managed effectively and consistently.

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