Development of Bacterial Spot on Near-Isogenic Lines of Bell Pepper Carrying Gene Pyramids Composed of Defeated Major Resistance Genes

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ABSTRACT

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Disease severity caused by races 1 through 6 of *Xanthomonas campestris* pv. *vesicatoria* on eight near-isogenic lines (isolines) of Early Calwonder (ECW) with three major resistance genes (*Bs1*, *Bs2*, and *Bs3*) in different combinations was evaluated in the greenhouse and field. Strains representing races 1, 3, 4, and 6 caused similar high levels of disease severity, followed by races 2 and 5 on susceptible ECW. Race 3 caused severe disease on all isolines lacking resistance gene *Bs2*. Race 4, which defeats *Bs1* and *Bs2*, caused less disease on isolate ECW-12R (carries *Bs1* + *Bs2*), than on isolines ECW, ECW-10R (carries *Bs1*), and ECW-20R (carries *Bs2*). Similar results were obtained with race 4 strains in field studies conducted during 1997 and 1998. In greenhouse studies, race 6, which defeats all three major genes, caused less disease on isolate ECW-13R (carries *Bs1* + *Bs3*) and ECW-123R (carries *Bs1* + *Bs2* + *Bs3*) than on isolines ECW, ECW-10R, ECW-20R, and ECW-30R (carries *Bs3*), but not on ECW-23R (carries *Bs2* + *Bs3*). In greenhouse studies with com-

mercial hybrids, strains of races 4 and 6 caused less disease on Boynton Bell (carries *Bs1* + *Bs2*) than on Camelot (carries no known resistance genes), King Arthur (carries *Bs1*), and X3R Camelot (carries *Bs2*). Race 6 caused less disease on hybrid R6015 (carries *Bs1* + *Bs2* + *Bs3*) and Sentinel (carries *Bs1* + *Bs3*) than on Camelot. Residual effects were not as evident in field studies with race 6 strains. Defeated major resistance genes deployed in specific gene combinations (i.e., gene pyramids) were associated with less area under the disease progress curve than when genes were deployed individually in isolines of ECW or commercial hybrids. Successful management of bacterial spot of pepper is achieved incrementally by integrating multiple tactics. Although there is evidence of residual effects from defeated genes, these effects alone likely will not provide acceptable bacterial spot control in commercial production fields. However, when combined with sanitation practices and a judicious spray program, pyramids of defeated resistance genes may aid in reducing the risk of major losses due to bacterial spot.

Additional keywords: *Capsicum annuum*, ghost genes, host-pathogen interactions, residual gene resistance.

Host-plant resistance usually forms the central component of an integrated disease-management program and is considered a preferred and effective means of managing diseases from both ecological and economic perspectives. Effective host resistance can provide significant benefits by reducing disease losses, while minimizing pesticide input. However, development of acceptable disease-resistant cultivars can require a significant investment of resources. Strategies for deploying host-plant resistance and whether use of defeated resistance genes has value have been discussed extensively (2,6,17, 29,30,32,33,35,39). Disease resistance genes have been classified using different terminology, including vertical resistance genes, major or single genes, and race- or genotype-specific genes (32). These terms can be used to describe the R genes used in our experiments.

In 1971, Slesinski and Ellingboe (45) found that an intermediate level of ³⁵S transfer from wheat carrying the powdery mildew, caused by *Blumeria graminis* f. sp. *tritici* (synonym = *Erysiphe graminis* f. sp. *tritici*), R gene *Pm1* to a virulent strain of the pathogen occurred, although the pathogen produced a susceptible reaction. When the virulent strain was placed on susceptible wheat cv. Chancellor, a high level of ³⁵S transfer occurred. Subsequently, Martin and Ellingboe (26), using powdery mildew on wheat lines carrying

defeated R gene *Pm4*, showed that gene-for-gene relationships can contribute to the phenomenon of horizontal resistance. These two studies laid the basis for the hypothesis of residual gene resistance. In a review by Nelson in 1978 (32), he suggested that combining R genes, including "defeated genes," into a genetic pyramid may provide a residual gene effect and greater durability against both virulent and avirulent pathogen races. Several terms, such as residual gene resistance and ghost-gene effects, for apparent residual resistance derived from defeated genes have been used (6,32,35, 39). The use of gene pyramids composed of defeated major genes remains controversial and has been studied most for management of fungal diseases of cereals (4,7,31,32,33,35,39). Recently, using bacterial blight of rice, it was found that resistance genes (including defeated genes) deployed in combinations (i.e., a gene pyramid) had a lower value for area under disease progress curves (AUDPCs) than that for the single most effective gene of each combination deployed individually in pure stands (2).

Bacterial spot of pepper (*Capsicum* spp.), caused by *Xanthomonas campestris* pv. *vesicatoria* (proposed: *X. axonopodis* pv. *vesicatoria* [48]), remains a constant threat in pepper production, especially under warm, moist conditions. Current management strategies for bacterial spot are based on a combination of practices that include starting with presumed pathogen-free seeds and seedlings, use of resistant cultivars, and multiple applications of copper sprays. However, copper sprays are protective and must be applied on a rigorous schedule to be effective under conditions favorable for disease development. Chemical control of bacterial spot is complicated further by pathogen strains tolerant to copper and streptomycin that are found in many pepper-producing regions (34,37,40,

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42). Both qualitative (1,8,9,13,16,28) and quantitative (12,36) forms of resistance to bacterial spot of peppers have been described. However, qualitative resistance has been more widely used in commercial cultivars, primarily because of the ease of selection. Three independent, dominant (major) genes for qualitative resistance to bacterial spot in peppers have been identified and are referred to as *Bs1*, *Bs2*, and *Bs3* (8,9,13,16,28). A fourth resistance gene (proposed designation *Bs4*) recently was identified in *C. pubescens* PI 235047 (43).

The prevalence of host-differentiated races makes bacterial spot difficult to manage solely through host resistance (3,18,34,37,40–42). Furthermore, race shifts within pathogen populations can rapidly negate the effectiveness of major resistance genes (10,19). Currently, 11 *X. campestris* pv. *vesicatoria* pathogenic races (designated 0 to 10) that infect pepper have been identified (3,18,28,34,38, 41–43) based on hypersensitive reactions (HR) in three near-isogenic lines (isolines) of Early Calwonder (ECW) and in PI 235047 (43). The isolines of ECW are ECW, susceptible to all known pepper races; ECW-10R (carries *Bs1*), resistant to races 0, 2, and 5; ECW-20R (carries *Bs2*), resistant to races 0, 1, 2, 3, 7, and 8; and ECW-30R (carries *Bs3*), resistant to races 0, 1, 4, 7, and 9. PI 235047 (carries *Bs4*) is resistant to races 0, 1, 3, 4, and 6. Recently, strains previously designated as race 6 were detected that defeat *Bs4* and were designated race 10 (38). Thus, all currently known major genes for resistance to the pepper bacterial spot pathogen have been defeated by at least one race.

We have been studying the effects of gene deployment on resistance durability and pathogen virulence evolution using bacterial spot of pepper. We reported that commercial bell pepper cultivars

carrying at least two major resistance genes, each individually defeated, sustained less damage from bacterial spot than did cultivars carrying individual defeated genes (22). The genetic background used to develop commercial hybrids is diverse and usually not publicly disclosed. Thus, it is likely that these commercial cultivars may have a source of resistance in addition to that provided by the major gene(s) detected in them. The following experiments were designed to examine the interaction between different races of the pepper bacterial spot pathogen and major resistance genes and gene pyramids in isolines of ECW for residual resistance effects of major individually defeated genes. A preliminary report on the experiments was published (21).

MATERIALS AND METHODS

Plant and pathogen sources. Seeds of isolines ECW, ECW-10R, ECW-20R, ECW-30R, ECW-13R, and ECW-123 were originally provided by R. E. Stall (University of Florida, Gainesville). Seeds of the lines were increased, the presence of resistance gene(s) was verified, and seeds were maintained by the authors. Lines ECW-12R and ECW-23 were developed and verified by the authors. Descriptions and sources of commercial hybrid cultivars were reported previously (22). The presence of different resistance genes was verified with known races of the pathogen as described previously (22). The pathogen strains used were characterized and maintained at –70°C (Table 1). The strains belong to *X. campestris* pv. *vesicatoria* group A (3) and have similar repetitive extragenic palindromic sequence polymerase chain reaction (REP-PCR) profiles (C. S. Kousik, unpublished data).

Greenhouse experiments. Disease severity caused by races 1 through 6 of *X. campestris* pv. *vesicatoria* on the eight isolines of ECW (Table 2) was studied in the greenhouse. A mixture of two strains of each race was used to prepare inoculum. Silwet L-77 was added to bacterial suspensions (10^6 CFU/ml) to produce a 0.04% concentration as described previously (20). Pepper plants of isolines grown in 10-cm clay pots until the 8- to 10-leaf stage were dipped in inoculum suspension for 15 s. Plants were covered with plastic bags for 3 days to maintain high relative humidity and promote disease development (20). The experiment used four single-plant replications for each isolate-race combination in a two-way factorial arrangement. Whole plants were rated for disease severity on a 0 to 9 scale, with 0 = no disease observed and 9 = 100% diseased leaves or defoliation, as previously described (20). Ratings were taken twice weekly until ≈1 month after inoculation. Experiments were done twice.

Field experiments. Field experiments were conducted during the growing seasons of 1997 and 1998. The eight isolines of pepper (Table 2) used in the greenhouse experiments also were evaluated in the field. Pepper plants were grown and transplanted to the field according to the standard practices for commercial production in North Carolina (44). Transplants were grown in the greenhouse for 7 weeks in polystyrene trays with 3.81-cm (1.5-in.) cells and 128 cells per tray. In the field, overhead irrigation (1.6 cm/h) was applied to the crop as needed to maintain optimum moisture for plant growth,

TABLE 1. Strains of *Xanthomonas campestris* pv. *vesicatoria* used in experiments

Strain ^a	Race	Sensitivity ^b	Geographic source	Year isolated	Exp. ^c
Xcv 79	1	Cu ^R , Sm ^S	NC	1991	G
Xcv 40	1	Cu ^R , Sm ^S	NC	1989	G
Xcv 6	2	Cu ^R , Sm ^R	NC	1986	G
Xcv 126	2	Cu ^R , Sm ^R	GA	1992	G
Xcv 110	3	Cu ^R , Sm ^R	VA	1992	G
Xcv 113	3	Cu ^S , Sm ^S	NC	1992	G
Xcv 181	4	Cu ^R , Sm ^S	NC	1994	G
Xcv 183	4	Cu ^R , Sm ^S	NC	1994	G
Xcv 314 ab	4	Cu ^S , Sm ^S	KY	1996	F
Xcv 316 ab	4	Cu ^S , Sm ^S	KY	1996	F
Xcv 293	5	Cu ^R , Sm ^R	NC	1995	G
Xcv 294	5	Cu ^R , Sm ^R	NC	1995	G
Xcv 295 ab	6	Cu ^R , Sm ^S	NC	1995	G, F
Xcv 296 a	6	Cu ^R , Sm ^S	NC	1995	G, F
Xcv 310 b	6	Cu ^S , Sm ^S	OH	1995	F
Xcv 329 b	6	Cu ^R , Sm ^R	FL	1997	F
Xcv 376 b	6	Cu ^S , Sm ^S	NC	1995	F
Xcv 378 b	6	Cu ^R , Sm ^S	NC	1995	F

^a a = Strain used in field experiment in 1997; b = strain used in field experiment in 1998.

^b Cu = Copper; Sm = streptomycin.

^c G = Greenhouse; F = field.

TABLE 2. Response of near-isogenic lines of Early Calwonder (ECW) carrying single major genes or combinations of genes resistant to races 1 through 6 of *Xanthomonas campestris* pv. *vesicatoria*

Race	Near-isogenic line (resistance genes) ^a							
	ECW	ECW-10R (<i>Bs1</i>)	ECW-20R (<i>Bs2</i>)	ECW-30R (<i>Bs3</i>)	ECW-12R (<i>Bs1</i> + <i>Bs2</i>)	ECW-13R (<i>Bs1</i> + <i>Bs3</i>)	ECW-23R (<i>Bs2</i> + <i>Bs3</i>)	ECW-123R (<i>Bs1</i> + <i>Bs2</i> + <i>Bs3</i>)
1	C	C	HR	HR	HR	HR	HR	HR
2	C	HR	HR	C	HR	HR	HR	HR
3	C	C	HR	C	HR	C	HR	HR
4	C	C	C	HR	C	HR	HR	HR
5	C	HR	C	C	HR	HR	C	HR
6	C	C	C	C	C	C	C	C

^a C = compatible response (disease); HR = hypersensitive response (resistance).

usually twice weekly. Each plot consisted of one row on a 112-cm center, with nine plants per row spaced 36 cm apart. Each isolate plot was replicated four times in a randomized complete block design. Experiments were conducted in two separate fields 1 km apart, with race 4 (strains Xcv 314 and Xcv 316) as the initial inoculum in one field and race 6 (strains Xcv 295 and Xcv 296) as the initial inoculum in the second field in 1997. For 1998, plots were designed similarly to those in 1997 but were located at different sites. The same two race 4 strains were used as inoculum. However, the inoculum in race 6 plots in 1998 consisted of a mixture of five strains (Table 1). Corn also was planted around the plots, and in both years, care was taken to prevent the spread of inoculum from one field to another. The central plant in each plot was inoculated by dipping the top of the plant, including the emerging three to five leaves, in an inoculum suspension (10^8 CFU/ml) containing 0.04% Silwet L-77 for 15 s. Data on disease progress were recorded weekly, using a 0 to 9 scale as described previously (22).

Statistical analyses. Disease ratings were used to calculate AUDPCs as described by Campbell and Madden (5). Specific non-orthogonal contrasts to compare the effects of resistance genes individually in ECW, ECW-10R, ECW-20R, and ECW-30R versus the same gene used in pyramids were examined. Comparisons were made only for pyramids with various gene combinations for races that could overcome the specific genes used in a pyramid individually. Treatment means from field experiments were separated by the Waller-Duncan *K* ratio *t* test ($K = 50$ and $\alpha = 0.10$). All statistical analyses were done using SAS software (version 6.10, SAS Institute, Cary, NC).

RESULTS

Greenhouse experiments. On susceptible ECW, strains representing races 1, 3, 4, and 6 caused similar levels of disease severity, whereas disease caused by strains representing races 2 and

TABLE 3. Disease response in the greenhouse of eight near-isogenic lines of Early Calwonder (ECW) containing the three major resistance genes, *Bs1*, *Bs2*, and *Bs3*, individually and in different combinations, to races 1 through 6 of *Xanthomonas campestris* pv. *vesicatoria* (expressed as area under disease progress curves [AUDPC])^a

Line ^b	Resistance gene(s)	AUDPC caused by race ^c					
		1	2	3	4	5	6
Experiment I							
ECW	None	111	105	125	114	100	120
ECW-10R	<i>Bs1</i>	125	42	128	115	31	115
ECW-20R	<i>Bs2</i>	40	43	39	117	98	125
ECW-30R	<i>Bs3</i>	49	115	128	44	117	122
ECW-13R	<i>Bs1</i> + <i>Bs3</i>	49	45	121	43	38	96
ECW-12R	<i>Bs1</i> + <i>Bs2</i>	52	51	49	91	55	111
ECW-23R	<i>Bs2</i> + <i>Bs3</i>	48	52	58	31	114	118
ECW-123R	<i>Bs1</i> + <i>Bs2</i> + <i>Bs3</i>	50	40	84	34	29	101
Experiment II							
ECW	None	107	88	104	108	83	98
ECW-10R	<i>Bs1</i>	112	29	101	95	23	99
ECW-20R	<i>Bs2</i>	29	24	48	100	74	104
ECW-30R	<i>Bs3</i>	44	104	108	42	85	96
ECW-13R	<i>Bs1</i> + <i>Bs3</i>	47	35	110	43	28	71
ECW-12R	<i>Bs1</i> + <i>Bs2</i>	51	26	54	82	28	82
ECW-23R	<i>Bs2</i> + <i>Bs3</i>	61	23	48	60	60	93
ECW-123R	<i>Bs1</i> + <i>Bs2</i> + <i>Bs3</i>	41	26	65	34	29	68

^a AUDPCs were calculated based on seven disease ratings on a scale of 0 to 9 (20) using previously described methods (5).

^b Standard error ± 7.1 and 6.34 for experiments I and II, respectively.

^c Means of at least four plants per race per near-isogenic line for each experiment.

TABLE 4. Linear contrasts of the effects of four races of the pepper bacterial spot pathogen on disease development (area under the disease progress curve [AUDPC])^a in the greenhouse in near-isogenic lines of Early Calwonder (ECW) carrying defeated genes in gene pyramids with the effects of the same genes individually in lines

Race	Contrast ^b	Estimate	Contrast sum of squares	<i>F</i>	<i>Pr</i> > <i>F</i>
Experiment I					
3	ECW-13R vs. ECW + ECW-10R + ECW-30R ^c	6.7	133.3	0.63	0.4282
4	ECW-12R vs. ECW + ECW-10R + ECW-20R	24.4	1,782.4	8.44	0.0043
5	ECW-23R vs. ECW + ECW-20R + ECW-30R	9.3	259.0	1.23	0.2700
6	ECW-12R vs. ECW + ECW-10R + ECW-20R	8.9	238.5	1.13	0.2897
6	ECW-13R vs. ECW + ECW-10R + ECW-30R	23.4	1,639.2	7.76	0.0061
6	ECW-23R vs. ECW + ECW-20R + ECW-30R	4.7	66.5	0.31	0.5756
6	ECW-123R vs. ECW + ECW-10R + ECW-20R + ECW-30R	25.7	1,189.7	5.63	0.0190
Experiment II					
3	ECW-13R vs. ECW + ECW-10R + ECW-30R ^c	6.2	115.6	0.76	0.3846
4	ECW-12R vs. ECW + ECW-10R + ECW-20R	19.3	1,121.3	7.38	0.0074
5	ECW-23R vs. ECW + ECW-20R + ECW-30R	20.4	1,250.5	8.23	0.0048
6	ECW-12R vs. ECW + ECW-10R + ECW-20R	18.5	1,022.1	6.73	0.0105
6	ECW-13R vs. ECW + ECW-10R + ECW-30R	26.8	2,153.6	14.17	0.0002
6	ECW-23R vs. ECW + ECW-20R + ECW-30R	6.9	143.5	0.94	0.3328
6	ECW-123R vs. ECW + ECW-10R + ECW-20R + ECW-30R	41.8	3,143.8	20.68	0.0001

^a AUDPCs were calculated based on seven disease ratings on a scale of 0 to 9 (20) using previously described methods (5).

^b Means of gene pyramids were contrasted with means of ECW and near-isogenic lines of ECW containing individual genes used in pyramids, e.g., the mean AUDPC of ECW-13R was contrasted with the mean AUDPCs of ECW, ECW-10R, and ECW-30R.

^c Neither gene *Bs1* nor *Bs3* originally provided genotype-specific resistance to race 3.

5 was less severe (Table 3). Race 1 caused severe disease on ECW and ECW-10R, whereas race 2 was most severe on ECW and ECW-30R. An HR was observed as small, discrete pinpoint spots with light purple margins on plants carrying the *Bs2* gene that were inoculated with either race 1, 2, or 3. These plants generally were rated, using the same rating scale used to rate susceptible plants, as a 1 or 2 based on the extent of leaf area covered by the HR. Race 3 caused severe disease on all isolines not carrying *Bs2*. Disease was severe on ECW-13R (gene pyramid *Bs1* + *Bs3*), which contained the only gene pyramid in this study that race 3 could overcome, and not different from disease severity on susceptible ECW, ECW-10R, and ECW-30R (Table 3). Race 4 caused less disease on ECW-12R (gene pyramid *Bs1* + *Bs2*) than on ECW-10R or ECW-20R. Race 6, which defeats genes *Bs1*, *Bs2*, and *Bs3*, caused less disease on ECW-13R and ECW-123R (gene pyramid *Bs1* + *Bs2* + *Bs3*) than on ECW, ECW-10R, ECW-20R, and ECW-30R; however, race 6 caused similar disease severity on ECW-23R (gene pyramid *Bs2* + *Bs3*) and ECW (Table 3). Estimated contrasts between the cumulative effects of defeated genes for individual races compared with genes present individually in isolines also produced similar results (Table 4). Gene pyramid *Bs1* + *Bs3* in ECW-13R did not result in significantly less disease than the mean of *Bs1* in ECW-10R, *Bs3* in ECW-30R, and ECW when race 3 was used as inoculum (Table 4). Significantly less disease occurred on ECW-12R (gene pyramid *Bs1* + *Bs2*) compared with the mean of *Bs1* in ECW-10R, *Bs2* in ECW-20R, and ECW when race 4 was used as inoculum (Table 4). Gene pyramid *Bs1* + *Bs2* + *Bs3* in ECW-123R resulted in significantly less disease when race 6 was used as the inoculum in contrast to the mean of ECW carrying *Bs1*, *Bs2*, or *Bs3* individually and ECW (Table 4). Similarly, *Bs1* + *Bs3* in ECW-13R resulted in significantly less disease due to race 6 in contrast to the mean of ECW carrying *Bs1* and *Bs3* individually and ECW. Gene pyramids in isolines such as ECW-12R, ECW-123R, and ECW-13R provided protection during the initial stages of disease development; however, these plants were severely diseased by the last disease-rating date $\approx$ 1 month after inoculation.

In similar greenhouse studies with commercial cultivars (Table 5), races 4 and 6 caused less disease on Boynton Bell (*Bs1* + *Bs2*) than on Camelot (no known resistance genes), King Arthur (*Bs1*), or X3R Camelot (*Bs2*). Race 6 caused less disease on hybrids R6015 (*Bs1* + *Bs2* + *Bs3*) and Sentinel (*Bs1* + *Bs3*) than on Camelot (Table 5).

Field experiments. Disease developed more slowly on ECW-12R in the field compared with ECW, ECW-10R, and ECW-20R (Table 6) when race 4 strains were used as inoculum during 1997

and 1998. Estimated linear contrasts for comparison of the cumulative effects of the gene pyramid in ECW-12R compared with the same genes individually in ECW-10R, ECW-20R, and susceptible genotype ECW indicated that race 4 strains caused significantly less disease on ECW-12R (Table 7). Disease development in the field on ECW-13R inoculated with race 6 was slower compared with ECW-10R and ECW-30R in 1997 (Table 6). Similarly, disease development on ECW-123R inoculated with race 6 strains was slower than on ECW-10R, ECW-20R, and ECW-30R in 1997 (Table 6). Estimated contrasts for comparison of the cumulative effects of gene pyramids in ECW-13R and ECW-123R suggested that the gene pyramids resulted in less disease compared with the same genes deployed individually when race 6 was used as the initial inoculum (Table 7). In 1998, when five strains of race 6 were used as inoculum, the results differed from those obtained in 1997 (Table 6). Estimated contrasts for comparison of the cumulative effects of genes deployed in gene pyramids indicated that ECW-13R did not have less disease compared with lines with the same genes deployed individually (Table 7). However, ECW-123 had less disease compared with lines with the same genes deployed individually.

TABLE 6. Area under disease progress curves (AUDPC)^a for races 4 and 6 of *Xanthomonas campestris* pv. *vesicatoria* and near-isogenic lines of Early Calwonder (ECW) containing the three major resistance genes, *Bs1*, *Bs2*, and *Bs3*, individually and in different combinations in field plots during 1997 and 1998

Line	Resistance gene(s)	AUDPC			
		Race 4		Race 6	
		1997	1998	1997	1998
ECW	None	124	72	112	95
ECW-10R	<i>Bs1</i>	128	87	118	105
ECW-20R	<i>Bs2</i>	141	82	115	83
ECW-30R	<i>Bs3</i>	54	... ^b	130	97
ECW-13R	<i>Bs1</i> + <i>Bs3</i>	63	...	95	88
ECW-12R	<i>Bs1</i> + <i>Bs2</i>	103	54	116	76
ECW-23R	<i>Bs2</i> + <i>Bs3</i>	54	...	103	79
ECW-123R	<i>Bs1</i> + <i>Bs2</i> + <i>Bs3</i>	52	...	99	80
Waller-Duncan LSD ^c (<i>K</i> = 50)		20.3	17.1	17.5	19.9

^a AUDPC were calculated on a scale of 0 to 9 (20) using previously described methods (5). AUDPCs were based on eight disease ratings taken in the field in which race 4 was used as inoculum; seven disease ratings were taken in the field in which race 6 was used as inoculum in 1997; and six disease ratings were taken in the field in which race 6 was used as inoculum in 1998.

^b Near-isogenic line not included in 1998.

^c Least significant difference (LSD) was calculated by the Waller-Duncan *K* ratio *t* test (*K* = 50 and α = 0.10).

TABLE 5. Disease response of greenhouse-grown commercial pepper hybrid cultivars containing different combinations of the three major resistance genes, *Bs1*, *Bs2*, and *Bs3*, to races 1 through 6 of *Xanthomonas campestris* pv. *vesicatoria* expressed as area under disease progress curves (AUDPC)^a

Cultivar ^b	Resistance gene(s) ^c	AUDPC caused by race					
		1	2	3	4	5	6
Experiment I							
Camelot	None	97	81	97	89	75	87
King Arthur	<i>Bs1</i>	90	32	95	90	38	86
X3R Camelot	<i>Bs2</i>	55	32	61	87	84	88
Sentinel	<i>Bs1</i> + <i>Bs3</i>	35	38	90	32	32	61
Boynton Bell	<i>Bs1</i> + <i>Bs2</i>	36	25	38	71	37	60
PR9300-4	<i>Bs1</i> + <i>Bs2</i>	27	21	39	64	28	66
R6015	<i>Bs1</i> + <i>Bs2</i> + <i>Bs3</i>	45	25	45	38	29	59
Experiment II							
Camelot	None	101	86	99	62	84	89
King Arthur	<i>Bs1</i>	92	24	107	55	23	90
X3R Camelot	<i>Bs2</i>	38	49	55	61	82	91
Sentinel	<i>Bs1</i> + <i>Bs3</i>	43	30	104	23	24	72
PR9300-4	<i>Bs1</i> + <i>Bs2</i>	24	28	51	42	23	75
R6015	<i>Bs1</i> + <i>Bs2</i> + <i>Bs3</i>	25	28	70	26	23	60

^a AUDPCs were calculated based on seven disease ratings on a scale of 0 to 9 (20) using previously described methods (5).

^b Standard error $\pm$ 5.0 and 4.6 for experiments I and II, respectively.

^c Major resistance genes in hybrids were determined as described previously (22).

DISCUSSION

Numerous studies have been conducted on the effects of gene pyramids on fungal diseases of cereals (4,7,29,30,31,35,39). In contrast, such studies are limited for bacterial diseases, particularly for vegetable crops. A recent study reported that a combination of genes, including defeated genes, in a rice line was more effective in reducing disease than genes deployed individually in different lines (2). Also, using stem rust of wheat, Brodny et al. (4) found that the combination of defeated genes used significantly affected the number of spores produced per pustule. In our study, we used the three major resistance genes carried in isolines of ECW and *X. campestris* pv. *vesicatoria* (cause of bacterial spot of pepper) to examine the effects of gene pyramiding on disease development when genes have been defeated individually. Pyramiding resistance genes *Bs1* and *Bs2* into a single isolate or cultivar improved protection against strains of race 4 compared with the same genes deployed individually. Pyramiding genes *Bs1*, *Bs2*, and *Bs3* or *Bs1* and *Bs3* in an isolate slowed bacterial spot development due to race 6 strains compared with disease development when the three genes were deployed individually.

Not all pyramids of defeated genes were effective against all pathogen races. Pyramiding *Bs1* and *Bs3* was not effective against race 3 strains. However, this gene combination is composed of two genes that are not known to provide genotype-specific resistance to race 3. Also, in earlier field studies (20,22,24) with a mixture of races, race 3 prevailed on cultivars with no major genes or a combination of genes *Bs1* and *Bs3*. In previous studies with mixtures of races, the effect of race 6 on cultivars with *Bs1* + *Bs3* was not assessed; however, in the current study, in which strains representing individual races were used, the combination of *Bs1* + *Bs3* exhibited residual resistance against race 6. Pyramiding *Bs2* and *Bs3* in the isolate ECW-23R did not consistently provide disease resistance against race 5 compared with the same genes deployed individually. Thus, our study suggests that other unexplained factors need to be identified and accounted for in determining the appropriate gene combinations to use in developing effective gene pyramids.

One advantage of doing controlled studies, such as are reported here, in which strains representing individual races interact with isolines carrying individual resistance genes or gene pyramids is that the effect of each of the races and resistance-gene combinations can be assessed. In previous studies in which we used a mixture of races (22,24), information such as the current results showing reduced disease with pyramiding of *Bs1* + *Bs3* due to race 6 but not race 3 could not have been obtained.

In the current study, a mixture of two strains representing each race was used because data describing variation among strains within

all races of the pepper bacterial spot pathogen were not available. Subsequently, we conducted several experiments to determine variations in levels of aggressiveness among Xcv strains within races 4, 5, and 6. In several greenhouse experiments, some minor variations among strains within races were observed on a susceptible host when inoculated individually. When the same strains were used as an inoculum mixture in the field, one strain, Xcv 376, dominated the pathogen population (C. S. Kousik, unpublished data). Also, we observed a continuum in variation in aggressiveness among race 3 strains in greenhouse studies (40). It is possible that some of the residual gene effects may reflect differences in the aggressiveness of the strains selected to represent each race in these studies, especially strains of race 6. Further studies with multiple strains of individual races may clarify the issue of whether residual gene effects are pathogen strain (versus race) specific. In the current study, the strains representing each race were isolated from commercial pepper fields and research plots and, thus, cannot be considered isogenic. However, we established that the strains belonged to group A and that the strains representing races 4, 5, and 6 had very similar PCR fingerprint profiles, using REP, BOX, and GTG primers (C. S. Kousik, unpublished data). Host-differentiated races of Xcv are determined by the presence or absence of three (*avrBs1*, *avrBs2*, and *avrBs3*) functional avirulence genes (19,28), and it would be possible to develop near-isogenic strains representing each race. Studies using such near-isogenic bacterial spot pathogen races and gene pyramids in isolines of ECW should more definitively elucidate whether residual resistance is a component of gene pyramids.

The mechanism(s) for ghost effects of defeated R genes remains unknown, but based on information on types of mutations that occur in avirulence genes, a possible scenario could be constructed. Avirulent strains of race 2 can become virulent on pepper carrying R gene *Bs1* by loss or inactivation of *avrBs1* (15,46). The insertion sequence *IS476* was discovered in the inactive *avrBs1* locus and associated with race 2 mutants containing different phenotypes (14,15). In fully virulent mutants (i.e., that do not elicit a HR), *IS476* resides in the protein-encoding region of *avrBs1*. In mutants that elicit an intermediate reaction (i.e., delayed HR), *IS476* resides upstream of the *avrBs1* coding region (14). Intermediate reactions also have been observed in strains carrying deletion mutations of *avrBs1* (46). Such mutations presumably cause differential expression of *avrBs1*. A second avirulence gene in the pathosystem is *avrBs3*, which is composed of a nearly identical 34 amino acid repeat unit present in 17.5 copies, and deletions of repeat units give rise to different avirulence specificities (11). Several types of mutations also occur in *avrBs2* (23,47). At present, the possible impact of different mutations in *avrBs2* on the level of avirulence specificity is unknown. Although unproved, the residual or ghost

TABLE 7. Bacterial spot development (area under the disease progress curve [AUDPC])^a in two separate fields in 1997 and 1998 on near-isogenic lines containing gene pyramids, Early Calwonder (ECW)-12R (*Bs1* + *Bs2*), ECW-13R (*Bs1* + *Bs3*), ECW-23R (*Bs2* + *Bs3*), and ECW-123R (*Bs1* + *Bs2* + *Bs3*), contrasted with the effect of the same genes present individually in ECW, ECW-10R (*Bs1*), ECW-20R (*Bs2*), and ECW-30R (*Bs3*)

Race	Contrast ^b	Estimate	Contrast sum of squares	F	Pr > F
1997					
4	ECW-12R vs. ECW + ECW-10R + ECW-20R	28.6	2,447.2	8.01	0.0100
6	ECW-12R vs. ECW + ECW-10R + ECW-20R	0.8	2.1	0.01	0.9093
6	ECW-13R vs. ECW + ECW-10R + ECW-30R	25.5	1,955.5	12.27	0.0021
6	ECW-23R vs. ECW + ECW-20R + ECW-30R	15.8	744.2	4.67	0.0424
6	ECW-123R vs. ECW + ECW-10R + ECW-20R + ECW-30R	19.3	1,197.3	7.51	0.0122
1998					
4	ECW-12R vs. ECW + ECW-10R + ECW-20R	27.2	2,136.8	21.69	0.0016
6	ECW-12R vs. ECW + ECW-10R + ECW-20R	18.7	784.8	7.87	0.0140
6	ECW-13R vs. ECW + ECW-10R + ECW-30R	10.5	248.1	2.49	0.1370
6	ECW-23R vs. ECW + ECW-20R + ECW-30R	12.0	325.9	3.27	0.0922
6	ECW-123R vs. ECW + ECW-10R + ECW-20R + ECW-30R	14.6	509.5	5.11	0.0402

^a AUDPCs were calculated on a scale of 0 to 9 (20) based on seven disease ratings in 1997 and six in 1998 using previously described methods (5).

^b Means of gene pyramids were contrasted with means of ECW and near-isogenic lines containing the individual genes composing the pyramid, e.g., the mean AUDPC of ECW-12R was contrasted with the mean AUDPCs of ECW, ECW-10R, and ECW-20R.

effects of defeated major genes that we observed may be related to less than fully functional avirulence gene products that may elicit a delayed or intermediate HR or other defense mechanisms in a quantitative manner.

Disease reductions in isolines carrying gene pyramids was more apparent in greenhouse experiments than in field experiments. In greenhouse experiments, disease ratings were recorded twice weekly compared with weekly evaluations in field experiments. Disease ratings at greater intervals in the field may have obscured subtle differences that were observed in greenhouse experiments. Furthermore, environmental conditions were more variable in the field experiments, and the continued abundance of inoculum and interplot interference may have masked some of the disease-reducing effects of isolines carrying gene pyramids. In preliminary greenhouse studies with isolines, we did not observe significant differences among isolines when bacterial suspensions greater than 10^7 CFU/ml were used for inoculum. Thus, high inoculum concentrations may negate residual effects of pyramids composed of defeated genes.

Data from the 2 years of field experiments with strains of race 4 as inoculum were very similar. However, variations were observed among the data from 1997 and 1998 for race 6. Some of the race 6 strains used as inoculum in 1998 were different from those used in 1997 (Table 1), which may account for some of the year-to-year variation. One of the strains, Xcv 376, dominated the pathogen population in the 1998 field experiment (C. S. Kousik, *unpublished data*), which may explain some of the difference in the results with race 6 across the 2 years. Using northern leaf blight fungus race 2 strains and maize carrying *Ht* and *ht* versions, Leath and Pedersen (25) observed that *Ht* or closely linked genes conditioned a low level of resistance to some virulent isolates of *Exerohilum turcicum*. Our results with pepper and strains of race 6 may have behaved similarly.

In field studies with commercial hybrid cultivars carrying a combination of *Bs1* + *Bs2* or *Bs1* + *Bs2* + *Bs3*, there was less disease compared with deployment of the same genes individually (22). However, it is possible that some quantitative resistance is present in the commercial cultivars in addition to the residual resistance of defeated major genes. In the current study, the percentage of disease reduction generally was greater in commercial cultivars carrying major gene combinations than in isolines carrying the same gene combinations. In an attempt to compare the isolines with several commercial cultivars, we examined AUDPC per day for the appropriate gene combinations. AUDPC per day for bacterial spot development in isolines of ECW was greater than in commercial hybrids carrying gene pyramids. AUDPC per day for hybrids Sentinel (carries *Bs1* + *Bs3*) and R6015 (carries *Bs1* + *Bs2* + *Bs3*) were 2.8 ± 0.41 and 2.69 ± 0.09 , respectively, compared with those for ECW-13R (3.54 ± 0.40) and ECW-123R (3.74 ± 0.30) when race 6 was the inoculum. When race 4 was the inoculum, a similar trend was observed. ECW-12R had 16% (AUDPC per day = 3.0) less disease compared with ECW-10R and ECW-20R (mean AUDPC per day = 3.6). However, hybrids Boynton Bell and PR9300-4 (both carry *Bs1* + *Bs2*) had 20 and 28% less disease (mean AUDPC per day = 2.7) compared with the mean for King Arthur (carries *Bs1*) and X3R Camelot (carries *Bs2*) (mean AUDPC day = 3.5), respectively. The trend for less disease in commercial cultivars was observed when both races 4 and 6 were used, suggesting that resistance traits other than those associated with major genes may play a role in overall resistance in some commercial hybrid cultivars.

Nelson proposed that "polygenes" for partial resistance are defeated race-specific genes that provide incomplete or residual gene resistance (32). He also suggested the idea of building genetic pyramids by collecting defeated resistance genes, currently undefeated genes, and any newly found genes (32). Our results indicate that genetic pyramids composed of specific genes apparently are needed. The specific genes used to make gene pyramids in rice also affected the severity of bacterial blight of rice (2). More critical studies with strains within races and different R gene combi-

nations are needed to critically determine whether variation in aggressiveness within a race influences the effectiveness of residual gene resistance.

In the current and previous studies (22), we also observed that some cultivars with two-gene pyramids performed similarly to three-gene pyramids. The resources involved in developing a two-gene pyramid versus a three- or four-gene pyramid and the relative protection provided may be a major consideration in developing cultivars containing gene pyramids. Thus, simply combining all available major genes may not be the optimal approach.

Recently, a putatively new major gene in *C. pubescens* (PI 235047) with HR-type resistance to race 6 was reported (43). The gene, however, does not provide resistance to races 2 and 5 or some strains previously designated races 1 and 3 (43). Because some races that overcome the resistance gene in *C. pubescens* are already present, pyramiding it with one or more of the other major resistance genes (i.e., *Bs1*, *Bs2*, or *Bs3*) may be an appropriate method to effectively utilize this gene. If resistance is to be used successfully to manage bacterial spot of pepper, research should continue to identify additional qualitative and quantitative resistance. Development of molecular marker technology (27) and identification of molecular markers could make it relatively easy to pyramid several major resistance genes into a single line that also carries quantitative traits.

Mixed genotypes combined with copper sprays to manage bacterial spot have been examined (24). In the study by Kousik et al. (24), the cultivar mixtures consisted of susceptible cultivars and cultivars carrying only a single major resistance gene. The use of cultivar mixtures composed of gene pyramids combined with chemical sprays for managing bacterial spot was not examined. Thus, it is unknown whether deployment of mixed genotypes composed of cultivars carrying gene pyramids would provide increased resistance-gene durability in pepper. Because it is difficult to predict what race(s) may be present in a given location in a given year, the effect of using genotype mixtures with various gene pyramids may be beneficial. Currently, race 6 overcomes all three commercially used resistance genes (20,38,41,42); however, disease progress may be reduced by mixtures of a cultivar carrying *Bs1* + *Bs3* and a cultivar carrying *Bs1* + *Bs2* + *Bs3* and combining host resistance with chemical sprays for managing bacterial spot. This strategy also may aid in management of race 3 strains that cause disease when *Bs1* + *Bs3* (22,24) is used but not when *Bs1* + *Bs2* + *Bs3* is used.

Race changes in plant-pathogenic bacteria that overcome qualitative host-plant resistance can occur rapidly (10,19). In a previous field study in which races 1 through 4 were used as initial inoculum, we detected race 6 on a cultivar carrying *Bs1* + *Bs2* + *Bs3* (22). Whether such rapid race changes take place in gene pyramids has not been evaluated under controlled conditions. The development of "super races" has been a concern with use of cultivar mixtures and gene pyramids. The probability of mutations occurring in resistance-gene pyramids and leading to multiple virulence and affecting the durability of the pyramids is controversial (17,29,30). Currently, with pepper race 6, which can overcome all three major resistance genes but causes less disease on plants carrying *Bs1* + *Bs3* or *Bs1* + *Bs2* + *Bs3*, one would need to determine whether the race may increase in aggressiveness when cultivars carrying *Bs1* + *Bs2* + *Bs3* are deployed as a monoculture.

Since the 1960s, only four major genes for resistance to bacterial spot of pepper have been identified (8,9,13,16,43). Three of the genes have been used to develop commercially acceptable hybrid cultivars. However, the appearance of new races constantly threatens to nullify the effects of these major genes. In the current and a previous study (22), we observed that defeated major genes provide some residual gene resistance when they are pyramided. Successful management of bacterial spot of pepper is achieved incrementally by integrating multiple tactics. Thus, the residual effects when combined with quantitative resistance and other management tactics, such as use of judicious spray programs, may provide an

additional component for reducing the risk of major losses due to bacterial spot of pepper.

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