

## Mixed Genotypes Combined with Copper Sprays to Manage Bacterial Spot of Bell Peppers

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### ABSTRACT

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Effects of genotype mixtures combined with three copper + maneb spray schedules on bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) of bell peppers were tested from 1993 to 1995. Planting patterns used for mixing the resistant and susceptible genotypes were either rows of resistant plants interplanted in rows of susceptible plants or a checkerboard pattern. In 1993, three races (1, 2, and 3) of the pathogen were used as inoculum, and two races (1 and 2) were used in 1994 and 1995. Interplanting of resistant and susceptible peppers and application of copper sprays resulted in less disease and greater yields. In 1994, the genotype mixtures had 6 to 45% less disease than pure stands of the

susceptible genotype with three spray schedules. In 1995, the mixtures had 15 to 44% less disease compared with pure stands of the susceptible genotype without the copper + maneb sprays. Significantly less disease was observed for the susceptible genotype in the checkerboard pattern compared with pure stands of the susceptible genotype during 1994 and 1995. For several of the mixtures, no significant differences in yield between the weekly (7-day) and the biweekly (14-day) spray schedules were observed. There was a tendency for one race of the pathogen to predominate, depending on the host resistance gene(s) present. The checkerboard pattern of mixing genotypes was more effective in reducing disease in the susceptible genotype compared with row patterns. The use of genotype mixtures could be viewed as added insurance against the bacterial spot pathogen, in addition to copper spray programs to prolong the durability of genotypes.

Bacterial spot of bell peppers, incited by *Xanthomonas campestris* pv. *vesicatoria* (proposed: *Xanthomonas axonopodis* pv. *vesicatoria* [34]), is one of most destructive diseases of peppers. It is for control of this disease that most bactericide/fungicide sprays on mature green bell peppers are used (15). The disease can cause severe damage during warm, rainy weather and inoculum is spread by several means, such as seed-borne inoculum, water splash, and wind (27,32). Current disease management strategies rely mainly on the use of presumed pathogen-free seed/transplants and the routine application of chemicals such as copper. Once transplanted to production fields, plants may be sprayed with copper on a 5- to 10-day schedule to prevent bacterial spot. These disease management strategies have become complicated because of the occurrence of pathogenic strains resistant to copper and streptomycin (21,29). Management of the copper-resistant pathogen strains can be achieved by the addition of the fungicide maneb (21). Despite such additions and tight spray schedules, spray programs are not totally effective once disease begins to develop and environmental conditions remain favorable.

The gene-for-gene system in the pepper-bacterial spot pathogen combination has been well-defined and characterized (22). Three independently segregating and simply inherited genes for resistance to the bacterial spot pathogen have been described and are designated *Bs1*, *Bs2*, and *Bs3* (12,22). The corresponding aviru-

lence genes in the pathogen are designated *avrBs1*, *avrBs2*, and *avrBs3*, respectively (22). Seven races of the bacterial spot pathogen capable of causing disease on pepper have been described and are designated 0, 1, 2, 3, 4, 5, and 6 (3,7,11,18,29,30). Several of these races are apparently widespread, although races 1 and 2 are the most commonly detected (3,7,11,29).

Host-plant resistance to six (0 to 5) races is available. However, most resistance is based on one or two genes. In studies on the effect of vertical and horizontal resistance, it was observed that a combination of vertical and horizontal resistance in a genotype delayed the disease development by 2 weeks, compared with 1 week by vertical resistance alone (9). The ability of *X. campestris* pv. *vesicatoria* to overcome a single gene for resistance by race changes has been observed in laboratory studies (8,22). Recently, we documented in a field experiment that a race 1 pathogen population can rapidly change race and cause extensive disease on a previously resistant genotype (19). Because of the current use of monoculture and uniform genotypes within pepper fields, the disease spreads rapidly once the pathogen is present and environmental conditions remain favorable. Such ideal conditions are found in the southeastern United States, where hot, humid, and rainy conditions prevail during the growing season. The use of single-gene, host-plant resistance in such situations is inadequate because of the presence of host-differentiated races.

The use of variety mixtures is a relatively old concept (14) and its probable effects on pathogens populations were recognized very early (5,16). Variety mixtures are believed to present pathogens with an evolutionary dilemma, and the outcome would depend upon the genetics of the pathogen and the host mixtures (1).

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Mixed genotypes of crops can affect disease development in several ways, such as presenting the pathogen with a diversity of susceptible and resistant plants, providing a barrier effect between susceptible plants, and limiting production of secondary inoculum (36). Such disease management strategies have been studied most extensively in cereals and other fiber crops for fungal pathogens (4,13,23,24,36), but have received essentially no attention in vegetable crops, particularly with bacterial pathogens. There are probably several reasons for this, such as the importance of uniformity in vegetables for the ease of growing, harvesting, handling, and marketing. The disease management strategy we presented was based on the use of controlled crop heterogeneity using mixed genotypes combined with copper sprays. Preliminary results of this work have been reported previously (20).

### MATERIALS AND METHODS

Experiments were conducted from 1993 to 1995 at the Sandhills Research Station, Jackson Springs, North Carolina. Cultural and tillage practices recommended for pepper production were followed (31). Various planting patterns of bell peppers (Figs. 1 and 2) were used during these years to mix the resistant and susceptible genotypes.

Three pepper genotypes were used in 1993: the susceptible genotype Jupiter (J); and two hybrids, King Arthur (KA), resistant to race 2, and Rebell (R), resistant to races 1 and 2. Each treatment consisted of nine rows on 97-cm centers with nine plants/row spaced 36 cm apart with three replications per treatment. Three planting patterns, one row, two rows, and the DI (delay of initial inoculum by placing the inoculum plants in rows of resistant plants), as shown in Figure 1, were used in addition to pure stands of the resistant and susceptible genotypes. Strains of three races of the pathogen were used as primary inoculum, with each race carrying a specific phenotypic marker: race 1, copper resistance (Cu<sup>r</sup>); race 2, copper + streptomycin resistance (Cu<sup>r</sup> + Sm<sup>r</sup>); or race 3, copper and streptomycin sensitive (Cu<sup>s</sup> + Sm<sup>s</sup>). All races and strains used in these experiments did not hydrolyze starch and belonged to type A (proposed: *X. axonopodis* pv. *vesicatoria* [34]) as described previously (3). After the first spray application, three inoculated plants, each with a different race of the pathogen, were planted in the center of each plot. These plants had been inoculated with a mixture of two strains of each race. For inoculation, a bacterial suspension (10<sup>8</sup> CFU/ml) of the above mentioned races was rubbed onto leaves of susceptible J plants using a sterile cotton swab and carborundum as abrasive. Groups of plants inoculated with each race were maintained sepa-

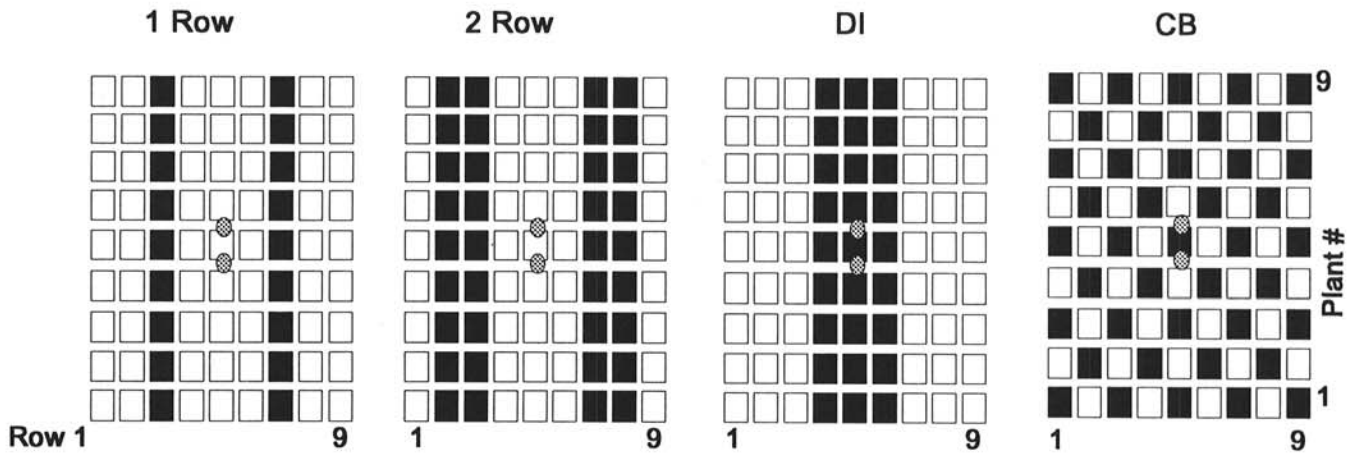


Fig. 1. Different pepper planting patterns used in 1993 and 1994. Each block represents a single plant. Dark boxes represent resistant plants and open boxes represent susceptible plants. CB indicates checkerboard pattern. DI indicates a delay of initial infection by placing three rows of resistant plants in the middle of the plot in which initial inoculum plants were placed. Shaded ovals represent position of initial inoculum plants, planted in the fifth row on either side of the fifth plant.

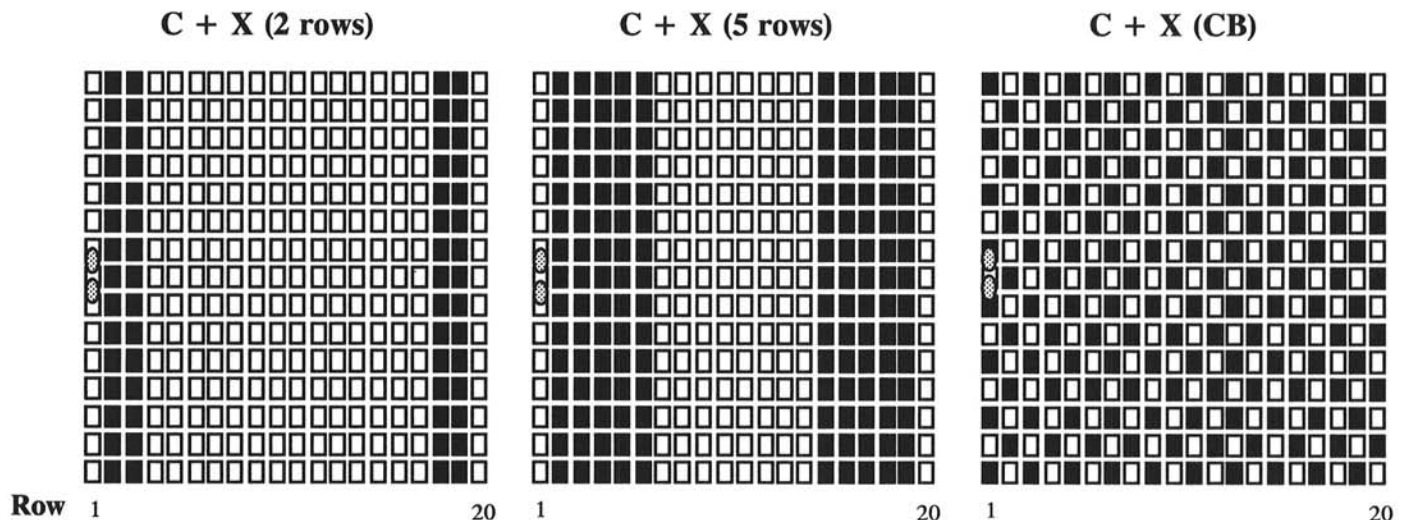


Fig. 2. Different pepper planting patterns used in 1995. Each block represents a single plant. Dark boxes represent resistant plants of X3R Camelot and open boxes represent susceptible plants of Camelot. CB indicates checkerboard pattern. Shaded ovals represent position of initial inoculum plants planted in the first row on either side of the eighth plant.

rately in isolated locations in the laboratory under plastic covers with occasional misting of the foliage until disease symptoms were observed.

In 1994, in addition to KA, the susceptible genotype Camelot (C) and X3R Camelot (X), resistant to races 1, 2, and 3 (as a result of resistance gene *Bs2*), were used. Races 1 and 2 were used as inoculum and plants were inoculated as described for 1993. In 1994, the two-row and the checkerboard (CB) patterns as shown in Figure 1 were tested.

During 1993 and 1994, a no-spray treatment and two spray schedules of copper hydroxide (Kocide DF, 2.84 kg/ha) + maneb (maneb plus zinc F4, 3.6 liter/ha) were used: weekly (7-day schedule) and biweekly sprays (14-day schedule). The chemicals were applied as previously described (17) using a mist blower calibrated to deliver 468 liter/ha. Chemical rates were calculated on a per hectare basis with each row being 0.6 m wide and each plot being 3.2 m long. Experimental plots during these 2 years were planted in a randomized block design with replications as blocks and chemical treatments as blocks within replications to facilitate spraying. Chemical treatment blocks were randomized within each replication. The genotype mixture plots were randomized within each chemical treatment block.

In 1995, the genotypes C and X were used. Races 1 and 2 were used as inoculum and plants were inoculated as described for 1993. Larger plots were used to determine the effects of genotype mixtures (Fig. 2) without the use of copper sprays. Each treatment consisted of 20 rows on 97-cm centers with 15 plants/row spaced 36 cm apart with two replications per treatment. Three planting patterns, two rows, five rows, and CB, as shown in Figure 2, were used in addition to pure stands of resistant and susceptible genotypes.

During all years, isolations of the pathogen were made from inoculum plants to verify the presence of desired strains before planting them in the field. Isolations were also made from plants in the field during the season to monitor the prevalence of individual strains. Resistance of strains to copper and streptomycin (29) and race determination using pepper differentials were done as described previously (22,29).

Mature green bell peppers were harvested three times in 1993 and twice in 1994 and 1995. Overhead irrigation (1.6 cm/ha/h) was applied to the crop during these years as needed to maintain optimum plant growth, normally twice weekly.

Bacterial spot severity ratings on each plant were taken during the growing season at weekly intervals using a 0 to 9 scale, in which 0 = no diseased leaves observed; 1 = trace, <1% leaf area diseased; 2 = 1 to 10% leaf area diseased; 3 = 11 to 20% leaf area

diseased or defoliated; 4 = 21 to 35% leaf area diseased or defoliated; 5 = 36 to 50% leaf area diseased or defoliated; 6 = 51 to 65% leaf area diseased and 51 to 65% defoliation; 7 = 66 to 80% leaf area diseased and 66 to 80% defoliation; 8 = 81 to 99% leaf area diseased and very few leaves (one to three) remaining on plant; and 9 = 100% complete defoliation and plant dying or dead. During 1993, a total of 12 ratings were taken, and nine ratings were taken in 1994 and 1995.

Area under the disease progress curves (AUDPC) were calculated for each experimental unit (6). The appropriateness of logistic, monomolecular, or Gompertz models for disease progress in different genotype mixtures and spray treatments in 1994 was examined by using linear regression analysis of respective transformed values through time (6). Since the ratings were based on a 0 to 9 scale, individual ratings were converted to a scale of 0.0 to 1.0 and then values were transformed for regression analysis. For conversion, each rating was divided by 9 and 0.001 was added to this value prior to transformation. The appropriate model was selected using the criteria described by Campbell and Madden (6). Based on examination of these models, the logistic model ( $r^2$  values ranged from 0.72 to 0.99) was determined to be the most appropriate and used for further analysis of infection rates. The data from 1995 were analyzed and the Gompertz model ( $r^2$  values ranged from 0.85 to 0.94) was deemed to be the most appropriate for description of disease progress and was used for comparison of infection rates among plots. Data from 1993 were not used for this analysis, since the disease progress followed a bimodal pattern through time. Analysis of variance was conducted on AUDPC and rate parameter estimates using the general linear models (GLM) procedure of SAS (SAS Institute Inc., Cary, NC) and means were compared using least-squares means (LSMEANS) procedure.

## RESULTS

Disease progress in 1993 followed a bimodal pattern with an initial peak in disease severity during mid-June, which developed approximately 2 weeks after a hail storm. This was followed by a gradual decline in disease severity. A second peak in disease severity developed during early August, followed by a decline. The pure stands of susceptible genotype J generally had larger AUDPC and lower yields compared with the mixtures of J + KA (Table 1), but were not significantly different, except for J + KA (DI) in no-spray plots. Yields of the mixtures of J + KA were significantly greater (22 to 30%) than pure stands of J in the weekly sprayed plots. The AUDPC in the biweekly sprayed plots

TABLE 1. Area under disease (bacterial spot) progress curve (AUDPC<sup>v</sup>) and yield<sup>w</sup> for genotype mixtures<sup>x</sup> of bell peppers combined with copper + maneb sprays in 1993

Genotype/mixture	No-spray		Biweekly sprays		Weekly sprays	
	AUDPC	Yield (kg/plot)	AUDPC	Yield (kg/plot)	AUDPC	Yield (kg/plot)
Jupiter (J)	325 a <sup>y</sup>	4.99 Ba	219 ab	14.06 Bc	151 a	31.30 Ad
King Arthur (KA)	259 d	9.98 Ca	170 c	30.84 Ba	129 b	51.26 Aa
Rebell (R)	304 bc	6.80 Ca	209 ab	16.33 Bbc	138 ab	49.44 Aab
J + KA (one row)	309 abc	5.90 Ca	199 b	23.59 Bbc	139 ab	44.45 Aabc
J + KA (two rows)	309 abc	4.99 Ca	198 b	28.58 ABab	132 ab	40.37 Aabc
J + KA (DI <sup>z</sup> )	289 c	8.16 Ca	200 b	22.23 Bbc	138 ab	41.28 Aabc
J + R (one row)	321 ab	4.99 Ba	218 ab	14.52 Bbc	142 ab	35.83 Abc
J + R (two rows)	317 ab	5.44 Ca	223 a	19.51 Bbc	151 ab	34.93 Abc
J + R (DI)	301 abc	5.90 Ba	226 a	14.97 Bbc	148 ab	35.83 Abc

<sup>v</sup> AUDPC were calculated as previously described (6).

<sup>w</sup> Plot yields represent the total yield obtained from three harvests.

<sup>x</sup> Susceptible and resistant genotypes were planted as described in Materials and Methods: Jupiter (susceptible to all races), King Arthur (resistant to race 2), and Rebell (resistant to races 1 and 2).

<sup>y</sup> Means followed by the same capital letters across rows were not significantly different and means followed by the same lowercase letters down columns were not significantly different ( $\alpha = 0.05$ ). AUDPC for the no-spray treatment were significantly greater than biweekly sprayed, followed by weekly sprayed, treatments.

<sup>z</sup> DI = delay of initial infection by placing the inoculum plants in rows of resistant plants.

of mixtures of J + KA (one row), J + KA (two rows), and J + KA (DI) were different from the pure stands of J ( $P = 0.07, 0.06,$  and  $0.09,$  respectively). AUDPC in mixtures of J + R were not significantly different from pure stands of J in any of the spray treatments. No significant reduction in disease was observed compared with the pure stands when the susceptible component (J) of the mixtures was analyzed separately. The absence of resistance to race 3 in 1993 was devastating. Isolations of the pathogen made during the season from the various plots across treatments indicated that race 3, despite being sensitive to copper, was the predominant strain (96%), followed by race 1 (4%).

In 1994, the plots with mixtures of susceptible and resistant peppers had significantly less disease compared with pure stands of susceptible genotype C across all spray treatments, except for C + KA in the weekly sprayed treatment (Table 2). Approximately 40 days after inoculation, significantly more disease was observed in pure stands of C compared with mixtures in the no-spray plots, and this difference continued until the end of the season. In the no-spray plots, the mixtures of C + X in the row or the CB pattern had significantly smaller AUDPC than pure stands of C. A similar trend was observed across all the treatments. Yields in the mixtures tended to be higher than that of pure stands of susceptible genotype, but were not significantly different. There were no significant differences in the yield between the weekly and the biweekly sprayed plots. However, no-spray treatments had significantly lower yields, except for the X genotype.

Analysis of AUDPC for the individual components in the CB pattern mixture indicated that the susceptible component (C) had 15 to 25% less disease across spray treatments compared with pure stands (Table 3). The genotype KA had less disease in a mixture compared with pure stands of K. However, the genotype X had more disease when in mixtures compared with pure stands of X.

Significantly slower rates of disease progress occurred in mixtures of C + X (CB) compared with pure stands of C, for the no-spray and biweekly spray treatments (Table 4). No significant differences were observed between the infection rates for the same mixture between the weekly and the biweekly sprayed treatments. Of a total of 426 strains examined in 1994, race 1 was the most predominant strain (97%), followed by race 2 (3%), throughout the growing season. Three race 4 (0.007%) strains were also detected a month after the final harvest.

During 1995, bacterial spot progressed rapidly in the susceptible genotype (C) plots with the first symptoms appearing 21 days after inoculation. All mixtures had significantly less disease compared with pure stands of C (Table 5). Analysis of individual components in the mixtures indicated less disease and greater yield for susceptible genotype C in the CB pattern compared with pure stands. Comparison of apparent infection rates indicated a slower rate of disease progress in the mixtures compared with pure stands of susceptible (C) genotype. The resistant genotype (X) had the smallest AUDPC, apparent infection rate, and highest yield. Yield for X component did not differ among mixtures and pure stands. Race 1 (73%) was the predominant strain in 1995, followed by race 2 (27%), until the final harvest. Strains of races 4 and 5 were detected a month after the final harvest in these plots.

## DISCUSSION

Using mixed genotypes is a strategy for not only reducing the risk of crop loss to bacterial spot, but a means for rapidly utilizing host-plant resistance. This strategy utilizes host-plant resistance, but does not require the incorporation of resistance genes into all genotypes. Interplanting of bacterial-spot resistant and susceptible genotypes may have an advantageous effect on fruit yield,

TABLE 2. Area under disease (bacterial spot) progress curve (AUDPC<sup>v</sup>) and yield<sup>w</sup> for genotype mixtures<sup>x</sup> of bell peppers combined with copper + maneb sprays in 1994

Genotype/mixture	No-spray		Biweekly sprays		Weekly sprays	
	AUDPC	Yield (kg/plot)	AUDPC	Yield (kg/plot)	AUDPC	Yield (kg/plot)
Camelot (C)	121 Aa <sup>y</sup>	23.7 Bb	74 Ba	57.6 Ab	49 Ca	69.3 Aa
King Arthur (KA)	103 Ab	41.2 Bb	54 Bc	76.7 Aab	35 Cb	81.2 Aa
X3R Camelot (X)	32 Ae	72.5 Aa	21 ABe	88.2 Aa	14 Ce	79.1 Aa
C + KA (two rows)	105 Ab	34.7 Bb	60 Bb	75.3 Aab	46 Ca	77.7 Aa
C + X (two rows)	82 Ac	36.9 Bb	55 Bbc	66.8 Aab	32 Cc	74.7 Aa
C + X (CB) <sup>z</sup>	71 Ad	40.6 Bb	46 Bd	68.4 Aab	27 Cd	74.0 Aa

<sup>v</sup> AUDPC were calculated as previously described (6).

<sup>w</sup> Plot yields represent the total yield obtained from two harvests.

<sup>x</sup> Susceptible and resistant genotypes were planted as described in Materials and Methods: Camelot (susceptible to all races), King Arthur (resistant to race 2), and X3R Camelot (resistant to races 1, 2, and 3).

<sup>y</sup> Means followed by the same capital letters across rows were not significantly different and means followed by the same lowercase letters down columns were not significantly different ( $\alpha = 0.05$ ).

<sup>z</sup> Camelot and X3R Camelot were planted (1:1) in a checkerboard (CB) pattern.

TABLE 3. Area under disease (bacterial spot) progress curve (AUDPC<sup>v</sup>) for individual components, Camelot (C), X3R Camelot (X), and King Arthur (K) in genotype mixtures<sup>x</sup> of bell peppers in 1994 for a no-spray and two copper + maneb spray treatments

Genotype/mixture	No-spray			Biweekly sprays			Weekly sprays		
	C	K	X	C	K	X	C	K	X
Camelot (C)	121 A <sup>y</sup>	—	—	74 B	—	—	49 B	—	—
X3R Camelot (X)	—	—	32 B	—	—	21 B	—	—	14 B
King Arthur (K)	—	103 A	—	—	54 A	—	—	35 A	—
K + C (two rows)	124 A	81 B	—	73 B	43 B	—	57 A	33 A	—
X + C (two rows)	121 A	—	31 B	81 A	—	22 B	47 B	—	13 B
C + X (CB) <sup>z</sup>	96 B	—	46 A	63 C	—	30 A	37 C	—	18 A

<sup>v</sup> AUDPC were calculated as previously described (6), based on nine weekly observations using a 0 to 9 disease severity scale.

<sup>x</sup> Susceptible and resistant genotypes were planted as described in Materials and Methods: Camelot (susceptible to all races), King Arthur (resistant to race 2), and X3R Camelot (resistant to races 1, 2, and 3).

<sup>y</sup> Means followed by the same capital letters down columns were not significantly different ( $\alpha = 0.05$ ).

<sup>z</sup> Camelot and X3R Camelot were planted (1:1) in a checkerboard (CB) pattern.

while reducing disease pressure and loss due to bacterial spot. The benefits of host mixtures have been well documented for foliar diseases of cereals (23,36), in which the host plants are small and the chance of inoculum exchange among host genotypes is good. However, Vanderplank (33) hypothesized that host mixtures may not be very effective for crops with large plants. Larger host genotype areas in mixtures of oats were found to be less effective compared with completely random mixtures of plants in reducing rust development compared with pure lines (24). Compared with a cereal plant such as wheat or oats, a pepper plant occupies a larger unit area and this may affect disease control via genotype mixtures.

During 1994 and 1995, the susceptible component in mixtures had smaller AUDPC in the CB pattern, which suggested that interplanting resistant and susceptible plants delayed or reduced inoculum reaching the susceptible component. During 1994, the AUDPC for row 1 (only susceptible genotype C) before final harvest in mixtures was significantly smaller compared with row 1 in pure stands of C (data not shown). These were the first rows in the plot adjacent to the resistant rows. Similarly in 1995, the AUDPC in row 20 (C) in the C + R (two rows) and C + R (five rows) plots were significantly ( $P = 0.001$ ) smaller than AUDPC in pure stands of C (data not shown). The beneficial effects of mixtures on the susceptible component compared with its pure stands was evident in all the rows in the CB pattern, except the first row in which the inoculum was placed in 1995. A similar effect was also noticed in 1994 in the CB pattern. Despite the fact that in 1995 the ratio (1:1) of susceptible plants to resistant plants in the

CB pattern and the C + X (five rows) pattern were the same, the CB pattern was more effective in reducing the disease on the susceptible component compared with row mixtures. With crown rust of oats, Mundt and Leonard (24) observed lower disease levels in completely random mixtures compared with aggregated (block) mixtures. During 1993 and 1994, in several of the mixtures, the AUDPC and yields were approximately the same as the mean of the individual components in pure stands, hence such differences could just have been because of an averaging effect of the two genotypes. Such effects have been observed previously in cereal crops (23,36).

The use of genotype mixtures can be viewed as added insurance against the bacterial spot pathogen in addition to the copper + maneb spray programs to prolong the durability of the resistant and susceptible genotypes. Because of the ability of the pathogen to change races rapidly and cause disease (8,9,19), incorporation of copper + maneb sprays into the management program remains necessary. Copper + maneb sprays are also necessary to maintain acceptable disease control to meet the market requirement of high quality fruits. The yields were not significantly reduced in several genotype mixtures such as J + KA (2 rows), despite using half the number of sprays. Although the total fruit yields were not significantly different between the weekly and biweekly sprayed plots, the fruit quality in the weekly sprayed plots was much better. The spray treatments used in this study were mainly protective. Based on our observations, a weekly spray schedule with reduced dosage of the chemicals may be more effective. This would keep the plants covered and also reduce the total amount of chemical being applied.

Several cultivars with resistance to one or more races of the pathogen are currently available. However, the impact of the absence of resistance to a particular race (e.g., race 3) of the pathogen in the 1993 field experiment was devastating despite using several cultivars with resistance to race 2 or races 1 and 2. This indicated the need for genotypes with greater horizontal or quantitative type of resistance. The *Bs2* gene confers resistance to races 0, 1, 2, and 3 via a hypersensitive reaction. However, this is still single-gene resistance and large scale use of this gene may place pressure on the pathogen to change. Several genotypes carrying the *Bs2* resistance gene have been released. Recently, several strains of *X. campestris* pv. *vesicatoria* isolated from tomato (3,26) and peppers (18,30) were reported to be virulent on pepper carrying the *Bs2* gene for resistance and were classified as pepper races 4, 5, and 6 (18,30). In 1994, we isolated strains of race 4 after the end of the growing season. Similarly, in 1995, races 4 and 5 were isolated a month after the final harvest. No race 4 or 5 strains were isolated from the samples taken during the fruit production season. The plots had been maintained by regular overhead irrigation even after the final harvest to determine if race

TABLE 4. Comparison of infection rates<sup>w</sup> of *Xanthomonas campestris* pv. *vesicatoria* in mixed genotypes<sup>x</sup> of bell peppers and a no-spray treatment and two copper + maneb spray schedules in 1994

Genotype/mixture	No-spray	Biweekly sprays	Weekly sprays
Camelot (C)	0.145 Aa <sup>y</sup>	0.132 Ba	0.115 Ca
King Arthur (KA)	0.140 Aa	0.123 Ba	0.117 Ca
X3R Camelot (X)	0.114 Ac	0.105 Ab	0.089 Bb
C + KA (two rows)	0.140 Aa	0.124 Ba	0.118 Ca
C + X (two rows)	0.133 Aa	0.122 Aa	0.106 Ba
C + X (CB) <sup>z</sup>	0.129 Ab	0.116 ABb	0.106 Ba

<sup>w</sup> Apparent infection rates were calculated using logistic models for disease progress (6).

<sup>x</sup> Susceptible and resistant genotypes were planted as described in Materials and Methods: Camelot (susceptible to all races), King Arthur (resistant to race 2), and X3R Camelot (resistant to races 1, 2, and 3).

<sup>y</sup> Means followed by the same capital letters across rows were not significantly different and means followed by the same lowercase letters down columns were not significantly different ( $\alpha = 0.05$ ).

<sup>z</sup> Camelot and X3R Camelot were planted (1:1) in a checkerboard (CB) pattern.

TABLE 5. Area under disease (bacterial spot) progress curve (AUDPC<sup>w</sup>), infection rates ( $r_g$ ), and yield for genotype mixtures<sup>v</sup> and individual components in mixtures of bell peppers in 1995

Plot	Overall AUDPC	AUDPC for individual components in mixtures		$r_g$	Plot yield (kg) <sup>w</sup>	Yield/plant for individual <sup>x</sup> components in mixtures	
		Camelot	X3R Camelot			Camelot (kg)	X3R Camelot (kg)
Camelot	184 A <sup>y</sup>	184 B	—	0.064 a	52 C	0.17 B	—
X3R Camelot	42 E	—	42 C	0.040 d	329 A	—	1.1 A
C + X (two rows)	157 B	182 B	56 B	0.056 b	98 C	0.17 B	1.0 A
C + X (five rows)	124 C	189 A	58 AB	0.049 c	182 B	0.13 B	1.1 A
C + X (CB) <sup>z</sup>	103 D	144 C	61 A	0.049 c	206 B	0.28 A	1.1 A

<sup>w</sup> AUDPC and infection rates (based on Gompertz model) were calculated as previously described (6), based on nine weekly observations using a 0 to 9 disease severity scale.

<sup>v</sup> Planting pattern for genotype mixtures is as presented in Figure 2 and Materials and Methods.

<sup>w</sup> Plot yields represent the total yield obtained from two harvests.

<sup>x</sup> Yield/plant was used for comparison of individual components of mixtures because of unequal number of plants.

<sup>y</sup> Means followed by the same letter down columns were not significantly different ( $\alpha = 0.05$ ).

<sup>z</sup> Camelot and X3R Camelot were planted (1:1) in a checkerboard (CB) pattern.

changes might take place in the pathogen population. The race 4 strains were Cu<sup>r</sup> and Sm<sup>r</sup>, and may have evolved from race 1 strains. The race 5 strains were Cu<sup>r</sup> and Sm<sup>r</sup>, and may have evolved from race 2 strains. The bacterial spot pathogen is introduced on seeds or transplants (29,32) and is not known to commonly overwinter in North Carolina. Thus, the delayed appearance of race 4 and 5 strains may not pose a problem for the current or next season crop in North Carolina when the *Bs2* gene is deployed. However, it would be advisable to disk in the pepper plants soon after the final harvest.

Mixtures of susceptible and resistant soybean cultivars were reported to delay race shifts of soybean cyst nematodes and prolong the effectiveness of resistant cultivars (35). Epidemiologically, the pepper-bacterial spot pathogen system is very different from a soil-borne pathogen system such as plant pathogenic nematodes. The wind blown, water splashed, and aerosol mode of dispersal of the bacterial spot pathogen also affects using mixtures as a management strategy. During 1994 and 1995, a hypersensitive response (HR) was observed on the resistant genotype X and a severe HR may affect the yield significantly. We observed HR on the margins of the leaves of genotype X and such leaves dropped earlier than in the chemically treated plots of genotype X. Such a premature leaf drop could affect yield. In addition, plots with pure stands of the susceptible genotype (C) had severe disease resulting in abundance of inoculum in the area that would come in contact with the resistant genotype because of the mode of dispersal of the pathogen.

The major concern of using genotype mixtures is the development of super races. Thus, this management strategy is controversial (2,10,36). However, based on available data, it was concluded that the rapid emergence of such races is unlikely (36). Selection of complex pathotypes of *Erysiphe graminis* f. sp. *hordei* was much less intense in row mixtures of barley than in completely random mixtures in which the seeds of different lines are mixed prior to planting in field trials (13). In preliminary experiments using a mixture of races of the bacterial spot pathogen as inoculum on a single genotype, a tendency for a single race to predominate the population was observed (28). Similarly, race 1 predominated the pathogen population, followed by race 2 and race 3 in pepper fields in Barbados (26). In our experiments, there was a tendency for one race of the pathogen to predominate throughout the growing season. In 1993, race 3 was the most predominant; there was no resistance to race 3 among the genotypes used. In 1994 and 1995, race 1 was the most predominant. The prevalence of a particular race appeared to be directed by the host-plant resistance. In 1994, there were two genes for resistance to race 2 (KA and X) and one for race 1. Similarly, in 1993, there was no resistance to race 3, followed by some resistance to race 1 (R), and greater resistance to race 2 (KA and R). In 1995, race 1 predominated the pathogen population; however, race 2 was also present in significant numbers. The amount of plants resistant and susceptible to race 1 and 2 was equal during this year. It is also possible that race 1 strains may have better epidemiological fitness than race 2 strains in North Carolina. Studies to determine variations in virulence of different races and intraracial variation in the pathogen populations may also be useful in deploying mixed genotypes in field situations.

This was the first report on the use of mixed genotypes in vegetable crops to manage a bacterial disease. Recently, the reduced development of anthracnose, caused by the fungus *Colletotrichum lindemuthianum*, in mixtures of bean was reported (25). Some of the reasons for reluctance to use such systems is probably because of the lack of uniformity in produce, and problems in harvesting and handling of vegetable crops. In 1994, during both harvests we measured the dimensions of fruits from weekly sprayed plots. Our results indicated no significant differences between the dimensions of fruits of X and C. However, fruits of KA were larger and heavier (data not shown). This also suggested

that successful mixtures of seeds of genotypes such as C and X can be made for commercial use. Sometimes it can become difficult for commercial growers to obtain sufficient quantities of resistant hybrid seeds, and, in such situations, mixing the seeds of resistant and susceptible varieties could prove to be beneficial. Mixtures with a combination of different resistant genes or gene pyramids also need to be tested to manage bacterial spot. The planting patterns used in the current experiments were designed with the commercial grower in mind. Although the plots in this study were relatively small, their design simulated that of larger plantings.

#### LITERATURE CITED

- Barrett, J. A. 1980. Pathogen evolution in multilines and variety mixtures. *Z. Pflanzenkr. Pflanzenschutz* 87:383-396.
- Barrett, J. A., and Wolfe, M. S. 1978. Multilines and super-races—A reply. *Phytopathology* 68:1535-1537.
- Bouzar, H., Jones, J. B., Stall, R. E., Hodge, N. C., Minsavage, G. V., Benedict, A. A., and Alvarez, A. M. 1994. Physiological, chemical, serological, and pathogenic analyses of a worldwide collection of *Xanthomonas campestris* pv. *vesicatoria* strains. *Phytopathology* 84:663-671.
- Browning, D. A., and Frey, K. J. 1969. Multiline cultivars as a means of disease control. *Annu. Rev. Phytopathol.* 7:355-382.
- Caffrey, M. 1948. Breeding for disease resistance. *J. Dep. Agric. (Irel.)* 45:38-44.
- Campbell, C. L., and Madden, L. V. 1990. Introduction to plant disease epidemiology. John Wiley & Sons, Inc., New York.
- Cook, A. A., and Stall, R. E. 1982. Distribution of races of *Xanthomonas vesicatoria* pathogenic on pepper. *Plant Dis.* 66:388-389.
- Dahlbeck, D., and Stall, R. E. 1979. Mutations for change of race in cultures of *Xanthomonas vesicatoria*. *Phytopathology* 69:634-636.
- Dahlbeck, D., Stall, R. E., and Jones, J. P. 1979. The effect of vertical and horizontal resistance on development of bacterial spot of pepper. *Plant Dis. Rep.* 63:332-335.
- Groth, J. V. 1976. Multilines and "super races": A simple model. *Phytopathology* 66:937-939.
- Hartman, G. L., and Yang, C. H. 1990. Occurrence of three races of *Xanthomonas campestris* pv. *vesicatoria* on pepper and tomato in Taiwan. *Plant Dis.* 74:252.
- Hibberd, A. M., Stall, R. E., and Bassett, M. J. 1987. Different phenotypes associated with incompatible races and resistance genes in bacterial spot disease of pepper. *Plant Dis.* 71:1075-1078.
- Huang, R., Kranz, J., and Welz, H. G. 1994. Selection of pathotypes of *Erysiphe graminis* sp. *hordei* in pure and mixed stands of spring barley. *Plant Pathol.* 43:458-470.
- Jensen, N. F. 1952. Intra-varietal diversification in oat breeding. *Agron. J.* 44:30-34.
- Johnston, S. A. 1991. Fungicide benefits assessment: Vegetables—East. USDA/States National Agricultural Pesticide Impact Assessment Program (NAPIAP).
- Klages, K. H. 1936. Changes in proportions of the components of seeded and harvested cereal mixtures in abnormal seasons. *J. Am. Soc. Agron.* 28:935-940.
- Kousik, C. S., Bennett, M. H., and Ritchie, D. F. 1994. Impact of a single hail storm and copper + maneb sprays on disease and yield of bell pepper, 1993. Page 123 in: *Fungic. Nematic. Tests* 49.
- Kousik, C. S., and Ritchie, D. F. 1995. Isolation of pepper races 4 and 5 of *Xanthomonas campestris* pv. *vesicatoria* from diseased peppers in southeastern U.S. fields. *Plant Dis.* 79:540.
- Kousik, C. S., Sanders, D. C., and Ritchie, D. F. 1994. Race shift in *Xanthomonas campestris* pv. *vesicatoria* within a season in the field. (Abstr.) *Phytopathology* 84:1094.
- Kousik, C. S., Sanders, D. C., and Ritchie, D. F. 1994. Use of mixed genotypes combined with copper sprays to manage bacterial spot of bell peppers. In: *12th National Pepper Conference Proceedings*. New Mexico State University, Las Cruces, NM.
- Marco, G. M., and Stall, R. E. 1983. Control of bacterial spot of pepper initiated by strains of *Xanthomonas campestris* pv. *vesicatoria* that differ in sensitivity to copper. *Plant Dis.* 67:779-781.
- Minsavage, G. V., Dahlbeck, D., Whalen, M. C., Kearney, B., Bonas, U., Staskawicz, B. J., and Stall, R. E. 1990. Gene-for-gene relationships specifying disease resistance in *Xanthomonas campestris* pv. *vesicatoria*-pepper interactions. *Mol. Plant-Microbe Interact.* 3:41-47.
- Mundt, C. C., Hayes, P. M., and Schon, C. C. 1994. Influence of barley variety mixtures on severity of scald and net blotch and on yield. *Plant Pathol.* 43:356-361.

24. Mundt, C. C., and Leonard, K. J. 1985. Effect of host genotype unit area on epidemic development of crown rust following focal and general inoculations of mixtures of immune and susceptible oat plants. *Phytopathology* 75:1141-1145.
25. Ntahimpera, N., Dillard, H. R., Cobb, A. C., and Seem, R. C. 1994. Development of anthracnose in mixtures of dry bean cultivars. (Abstr.) *Phytopathology* 84:1071.
26. O'Garro, L. W., and Tudor, S. 1994. Contribution of four races of *Xanthomonas campestris* pv. *vesicatoria* to bacterial spot in Barbados. *Plant Dis.* 78:88-90.
27. Pohronezny, K., Hewitt, M., Infante, J., and Datnoff, L. 1992. Wind and wind-generated sand injury as factors in infection of pepper by *Xanthomonas campestris* pv. *vesicatoria*. *Plant Dis.* 76:1036-1039.
28. Ritchie, D. F., and Bennett, M. H. 1993. Impact of copper + maneb pre-harvest interval on disease and yield of pepper in the presence of copper-resistant pathogen, 1992. Page 145 in: *Fungic. Nematic. Tests* 48.
29. Ritchie, D. F., and Dittapongpitch, V. 1991. Copper- and streptomycin-resistant strains and host differentiated races of *Xanthomonas campestris* pv. *vesicatoria* in North Carolina. *Plant Dis.* 75:733-736.
30. Sahin, F., and Miller, S. A. 1995. First report of pepper race 6 of *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial spot of pepper. *Plant Dis.* 79:1188.
31. Sanders, D. C., Averre, C. W., Sorensen, K. F., Estes, E. A., Beasley, Z. O., and Bonano, A. R. 1988. Commercial pepper production in North Carolina. Page 16 in: *N.C. Coop. Ext. Serv. AG-387*.
32. Tegegn, T. 1985. A review of bacterial leaf spot of peppers (*Capsicum annum* L.) caused by *Xanthomonas campestris* pv. *vesicatoria* (Doidge) Dye and some methods of its control. *Acta Hort.* 158:369-376.
33. Vanderplank, J. E. 1968. *Disease resistance in plants*. Academic Press, Inc., New York.
34. Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. *Int. J. Syst. Bacteriol.* 45:472-489.
35. Wallace, M. K., Orf, J. H., and Stienstra, W. C. 1995. Field population dynamics of soybean cyst nematode on resistant and susceptible soybeans and their blends. *Crop Sci.* 35:703-707.
36. Wolfe, M. S. 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annu. Rev. Phytopathol.* 23:251-273.