

## Inheritance of Resistance in Cabbage to Black Rot

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Supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, and by the National Kraut Packers Association. Project No. 118.

The authors thank S. A. Vicen for his assistance with the photographs.

Accepted for publication 8 September 1971.

### ABSTRACT

The Japanese cabbage cultivar, Early Fuji, known to have a high level of black rot resistance, was used as the source of resistance. Resistant lines were crossed to different susceptible inbred lines, and  $F_1$ ,  $F_2$ , and backcross populations were screened during artificial black rot epidemics in the field. The only symptoms on resistant plants were necrotic hydatodes or necrotic marginal interveinal lesions, whereas the lesions on susceptible plants were v-shaped and surrounded by an expanding chlorotic zone. Black rot resistance was found to be controlled by one major gene, *f*, the expression of which in the heterozygous condition was influenced by one recessive and one dominant modifier gene.

Of 300 cultivars and inbred cabbage lines screened for

*Additional key words:* genetics, *Brassica oleracea*, resistance screening.

resistance to *Xanthomonas campestris*, none contained the major *f* gene for resistance found in Early Fuji. Among the 300 lines, reactions to *X. campestris* ranged from a highly tolerant lesion type with small, slowly spreading chlorotic margins, to those with highly susceptible systemic necrosis. Black rot reactions were classified from 1 to 5 in order of disease severity. All  $F_1$  hybrid cultivars produced in the USA were highly susceptible to *X. campestris*; however, a number of Japanese hybrids were tolerant. Among a number of open-pollinated cultivars grown in the USA, particularly the ballhead types, individual plants showed high tolerance to black rot.

Phytopathology 62:247-252.

Black rot on cruciferous crops has been a disease of world-wide importance, and repeated searches have been made for resistance in *Brassica oleracea* L. to *Xanthomonas campestris* (Pammel) Dows. In 1952, a high level of resistance was found in the cabbage cultivars Early Fuji and Hugenot (1), and the heritable nature of resistance was clearly documented by Bain in 1955 (2). Although Bain reported that resistance was controlled by one or more dominant genes, no black rot-resistant cultivars have been developed from the sources of resistance identified 19 years ago.

In a breeding program designed to incorporate black rot resistance from Early Fuji into existing multiple, disease-resistant breeding lines (7), we observed significant deviations from the expected segregation ratios, which suggested a more complex inheritance than the one suggested by Bain (2). During studies on the genetics of black rot resistance, certain lines which were resistant to black rot produced tolerant or resistant  $F_1$  progenies when crossed to resistant Early Fuji. For the purpose of identifying germplasm suitable for combining with black rot resistance, a number of cabbage cultivars and breeding lines were screened for black rot reaction.

**MATERIALS AND METHODS.**—Black rot-resistant Early Fuji inbreds (EF) in their second inbred generation were crossed to susceptible Badger Inbreds (BI) 3, 5, 8, and 13 (7). Bud pollination (6) was used for all self and cross pollinations. Plants utilized for breeding were selected in the mature stage in the field, grown in pots in the greenhouse, vernalized for 10 weeks, and brought to flower in late winter (5). The combining ability for black rot

resistance was evaluated in cabbage lines, ranging from highly tolerant to highly susceptible (Table 5), by crossing them to EF and screening the  $F_1$  progenies for black rot resistance. To identify further sources of black rot resistance, 300 open-pollinated and  $F_1$  hybrid cultivars were obtained from the major seed firms in the USA and Japan. In addition, several cabbage breeders sent inbred breeding lines for evaluation against black rot.

For field-screening the parental lines,  $F_1$ ,  $F_2$ , and reciprocal backcross populations, as well as the various cultivars and inbreds, were planted at 61 cm in rows 107 cm apart. As a susceptible control, the cultivar Sanibel was planted in every fifth row. Artificial black rot epidemics were initiated by inoculating the plants 4 times at weekly intervals starting 6 weeks after transplanting.

For field inoculations, bacteria were grown 48 hr at 28 C in 800 ml of the medium of Husain & Kelman (4) in 2-liter Fernbach flasks on a rotary shaker. Cultures were diluted to ca.  $5 \times 10^8$  cells/ml with tap water and atomized over the rows of cabbage plants using a Solo power-driven back-pack air blast sprayer (Solo Kleinmotoren GMBH, 7034 Maichingen, West Germany). Inoculation was carried out early in the morning of days with heavy dew when guttation droplets had formed at the hydathodes of the leaves. Bacteria were misted over the plants in such a way as not to dislodge the guttation droplets. Field inoculation represented a more natural means of introducing bacteria into the plant; it proved to be more reliable than seed soaking or vein inoculation (1, 2), and was used exclusively in this study.

Rigid insect control was maintained to avoid transmission of bacteria through wounds caused by

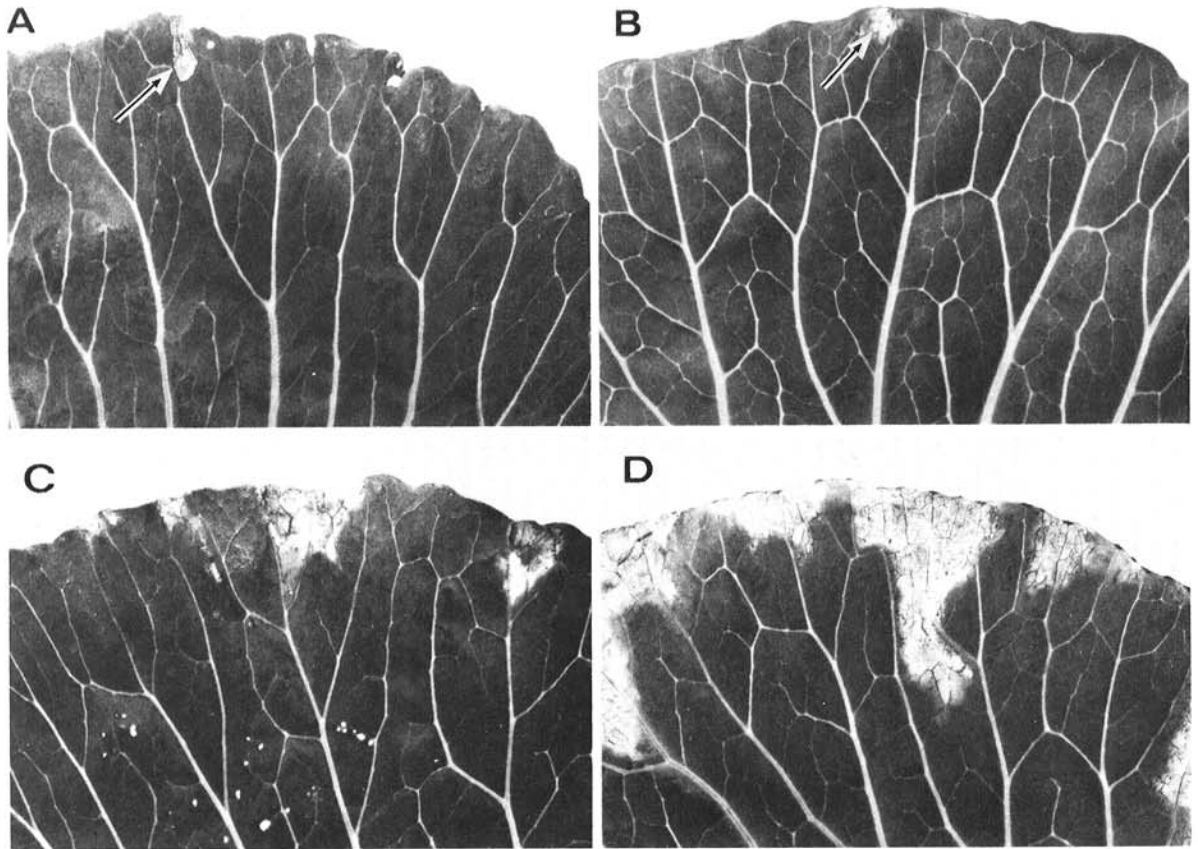
chewing. It was often observed that chlorotic black rot lesions developed adjacent to insect or hail injuries on resistant plants. Counting such plants as susceptible could lead to errors in scoring plants for resistance.

**RESULTS.**—When plants were inoculated in the field, bacteria apparently entered the hydathodes of the leaves as guttation droplets evaporated. Symptoms began to appear 4 to 7 days after inoculation.

On the various cabbage lines, a continuum of symptoms could be observed ranging from systemic symptoms on highly susceptible plants to small necrotic lesions on resistant plants. To facilitate the evaluation of disease severity, the infected plants were assigned to five classes according to the nature and size of the black rot lesions formed. Plants were rated as follows: 1 (resistant) = no symptoms or minute necrotic zones (1 to 3 mm diam) at the hydathodes, or characteristic light brown panels at the leaf margin (0.5 to 1 X 1 to 4 cm in diam) surrounded by a discrete dark rim; there was no chlorosis around panel or hydathode (Fig. 1-A); 2 (highly tolerant) = few small (0.5 to 2 cm in diam)

lesions at the leaf edge showing a diffuse chlorotic margin, or a necrotic panel as in 1, but with a surrounding chlorotic zone where bacteria had moved beyond the lesion rim (Fig. 1-B, 2-A); 3 (tolerant) showed several medium (1 to 5 cm in diam) v-shaped lesions with distinct marginal chlorosis and blackened veins within the lesion (Fig. 1-C, 2-B); 4 (susceptible) showed many large, spreading, v-shaped lesions which often coalesced to produce a dried leaf rim; often there was evidence of systemic invasion in the head (Fig. 1-D, 2-C). On plants of severity category 5 (highly susceptible), leaves were badly scorched by coalescing and rapidly expanding lesions. Many leaves dropped; the heads were systemically invaded. Plants were severely stunted or dying (Fig. 2-D). Although the assignment of plants to a given disease class required careful observation, plants could be rated rapidly with practice.

Although lesions of reaction type 1 were often seen on highly susceptible plants, they were interspersed with the typical v-shaped chlorotic lesions of types 2 to 5. Frequently, in susceptible plants, bacteria would spread beyond the necrotic panels and develop chlorotic lesions. Regardless of



**Fig. 1.** Cabbage leaves showing black rot (*Xanthomonas campestris*) lesions characteristic for the black rot ratings 1 to 4. A) Black rot rating (BR) = 1 (resistant); B) BR = 2 (highly tolerant); C) BR = 3 (tolerant); D) BR = 4 (susceptible). Note the discrete dark margin around necrotic lesion on the resistant leaf (A, arrow) and the diffuse margin around the chlorotic lesion on the highly tolerant leaf (B, arrow).

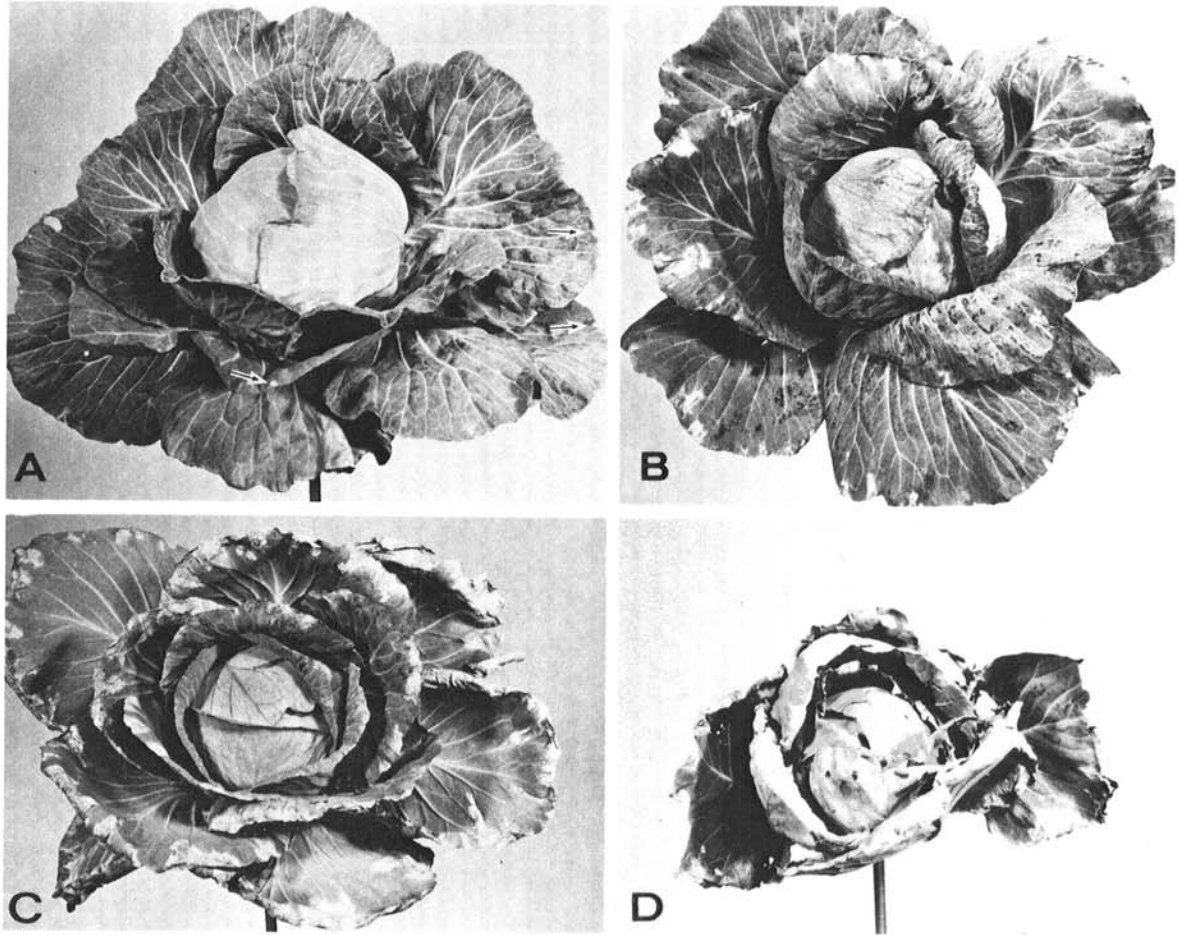


Fig. 2. Cabbage plants showing black rot (*Xanthomonas campestris*) symptoms characteristic for the black rot ratings 2 to 5. A) Black rot rating (BR) = 2 (highly tolerant); note the small chlorotic lesions (arrows); B) BR = 3 (tolerant); C) BR = 4 (susceptible); D) BR = 5 (highly susceptible); note the heavy scorching of the leaves.

the size of the lesions, the plants were rated as susceptible for the genetic study when they were surrounded by diffuse chlorotic zones rather than by discrete dark margins. Plants were rated resistant if they showed only lesion type 1 and were completely free of the lesion types 2 to 5.

All EF plants produced completely resistant progenies upon selfing. In support of the observations by Bain (2), we found that the  $F_1$  plants from the cross BI-8 X EF were either resistant or highly tolerant (Table 5). However,  $F_1$  plants from the crosses of EF with BI-3, BI-5, and BI-13 were not resistant, but only slightly less susceptible than the susceptible parents.

When  $F_2$  and backcross progenies were screened in the field, distinct segregation into resistant (rating 1) and susceptible (ratings 2 to 5) plants occurred. Three distinct segregation patterns were observed among the four selfed  $F_1$  progenies (Table 1). In the cross EF X BI-8, the  $F_2$  progenies segregated three resistant:one susceptible with the backcross to the susceptible parent segregating one resistant:one susceptible. All

plants of the backcross to the resistant parent were resistant.

The  $F_2$  progenies from EF X BI-5 cross segregated one resistant:three susceptible, with the backcross to the resistant parent segregating one resistant:one susceptible and the backcross to the susceptible BI-5 being all susceptible. From these data, it was postulated that resistance could be controlled by two genes, one a major gene for resistance, *f*, the dominance of which was controlled by a modifying gene *a*.  $F_2$  and backcross ratios conformed to the expected ratios (Table 1). The differences in  $F_1$  responses,  $F_2$ , and backcross segregations between EF X BI-8 and EF X BI-5 could be accounted for by differences in the *a* locus of both parents in each cross.

When the  $F_2$  and backcross progenies from EF X BI-3 and BI-13 were rated for black rot resistance, the observed ratios conformed more closely to the trigenic model (Tables 1 and 2), in which plants heterozygous for *f* were resistant only if the modifiers were in the condition *aa B*. Both homozygous classes

TABLE 1. Observed and expected segregation of black rot resistance in  $F_2$  and backcross populations from crosses between resistant cabbage lines derived from Early Fuji and four susceptible Badger Inbred lines

Cross	$F_2$						Backcross to resistant parent						Backcross to susceptible parent					
	Expected for 2 genes			Expected for 3 genes			Observed ratio			Expected for 2 or 3 genes			Observed ratio			Expected for 2 or 3 genes		
	Ratio	$X^2$ <sup>b</sup>	$P$	Ratio	$X^2$	$P^c$	Ratio	$X^2$	$P$	Ratio	$X^2$	$P$	Ratio	$X^2$	$P$	Ratio	$X^2$	$P$
Early Fuji X BI-8	101:34	0.0026	.95	3:1	0.0026	.95	56:0	1:0	1:0	59:67	0.174	.80	1:1	0.508	.45	1:1	0.508	.45
Early Fuji X BI-5	32:93	0.024	.80	1:3	0.024	.80	71:68	1:1	1:1	0.064	.80	.80	1:1	0.064	.80	1:1	0.064	.80
Early Fuji X BI-13	45:92	1.27	.20	6:10	0.213	.60	61:65	1:1	1:1	0.127	.70	.70	1:1	0.127	.70	1:1	0.127	.70
Early Fuji X BI-3	75:142	0.96	.30	6:10	0.0051	.90	55:54	1:1	1:1	0.0094	.90	.90	1:1	0.0094	.90	1:1	0.0094	.90

<sup>a</sup> R:S = Resistant to susceptible ratio.

<sup>b</sup>  $X^2$  = Chi-square.

<sup>c</sup>  $P$  = Probability value.

$FF$ , or  $ff$ , were not affected by the modifiers. Observed  $F_2$  segregations fit most closely the 22 resistant:42 susceptible ratio expected (Table 1) from the trigenic model (Table 2). Backcross ratios to the resistant parent in both EF X BI-3 and BI-13 yielded one resistant:one susceptible, whereas the backcross to the susceptible parents were all susceptible. These ratios indicated that the genotype of the resistant plants used for the backcross differed from that of the original parent at the  $a$  locus. This difference could not be recognized phenotypically if the major genes were in the  $ff$  condition.

Of the 300 cultivars and lines, 248 were either  $F_1$  hybrids or inbred lines. Among the 20 individuals of each line observed, the ratings were very consistent. Although none of the commercial hybrids was rated 1, several hybrids produced by Japanese breeders had uniform ratings of either 2 or 3 (Table 3). Generally, hybrids produced by USA seedsmen were more susceptible than those produced by Japanese firms.

Most of the open-pollinated cabbage cultivars were very susceptible. However, certain lines and cultivars showed heterogeneity in their disease reaction (Table 4). Most striking among these were certain ballhead cultivars which consistently yielded a few plants with disease ratings of 2 and 3. The Japanese cultivar Early Fuji was the only cultivar in which some plants were rated 1 (Table 4).

When a number of susceptible inbreds ranging in reaction type from 2 to 5 were crossed with resistant inbreds homozygous for the  $f$  gene, but of unknown constitution with respect to their modifier genotype, the  $F_1$  progenies were either the same or more resistant than their susceptible parents (Table 5). Badger Inbred-8 and USDA-66 combined with EF to produce resistant progenies. However, when the same BI-8 plant was crossed with two different inbreds containing the homozygous  $f$  gene, disease ratings were 2 and 1, respectively. None of the other susceptible lines combined to produce resistant  $F_1$  progenies.

**DISCUSSION.**—Only by strict adherence to the selection of plants showing reaction type 1 exclusively was meaningful genetic data obtained on black rot resistance in cabbage. Although in the segregating populations, many plants superficially appeared resistant, either because they had very few or very small lesions, the plants were rated as susceptible when these lesions showed any chlorosis around their margins. The necrotic lesions with a discrete dark border (Fig. 1-A) appeared to result from bacterial multiplication in the leaf parenchyma, whereas the chlorotic lesions (Fig. 1-B, C, D) resulted from multiplication of *X. campestris* in the veins (8).

The apparent difficulty encountered by Bain (2) in determining the precise inheritance of black rot resistance from Early Fuji may have arisen from the interaction of the modifier genes  $a$  and  $B$  with the major gene  $f$ . In the proposed trigenic model, a segregation of three resistant:one susceptible, indicating dominance of resistance, would be the exception and not the rule. Only occasionally would one find both the resistant and susceptible plants of a

TABLE 2. Proposed genotypes and expected phenotypic ratios in the trigenic model for resistance in cabbage to *Xanthomonas campestris*

Parents and progenies	Proposed genotype			Genotypic frequency	Expected phenotype	Phenotypic ratio <sup>a</sup>	
						R	S
Early Fuji inbred	ff	aa	BB		R <sup>a</sup>		
Badger Inbred 3	FF	AA	bb		S		
Badger Inbred 13	FF	AA	bb		S		
F <sub>1</sub>	Ff	Aa	Bb		S		
F <sub>2</sub>	ff	—	—	16/64	R		
	Ff	aa	B—	6/64	R		
	Ff	A—	—	24/64	S	22	42
	FF	—	—		S		
BC <sub>R</sub> <sup>b</sup>	Ff	aa	bb	16/64	S		
	ff	—	—	2/64	S		
	Ff	A—	—	1/2	R	1	1
	FF	—	—	1/2	S		
BC <sub>S</sub>	Ff	A—	—	1/2	S	0	1
				1/2	S		

<sup>a</sup> R = resistant; S = susceptible.

<sup>b</sup> Resistant backcross (BC) parent presumed to have genotype *ff AA BB*.

TABLE 3. Black rot ratings of various F<sub>1</sub> hybrid cultivars of cabbage inoculated with *Xanthomonas campestris* in the field

Black rot rating	F <sub>1</sub> hybrid cultivars and origin	
1 <sup>a</sup>	None	
2	Japan:	Hybrid No. 24, Tokyo Pride, R-I Cross
3	Japan:	Atlas, C-M Cross, Green Acre, Green Boy, Hybrid No. 34, Hybrid No. 45, K-K Cross, M-Y Cross, Rio Verde, Savoy King, Sentinel, Titan
4	Japan:	A-S Cross, Harvester Queen, Pack Rite, Perfection Cross, Princess, Saf-Gard, S-D Cross, Spring Cross
	USA:	Badger Hybrid 15, Blue Jacket, Jet Pak, King Cole, Little Rock, Market Dawn, Market Prize, Market Topper, Round-up, Sanibel
5	Japan:	Stonehead
	USA:	Dutchman, Headmaster, Resistant Danish, Superette

<sup>a</sup> Ratings based on 20 plants/line: 1 = resistant; 2 = highly tolerant; 3 = tolerant; 4 = susceptible; 5 = highly susceptible.

cross with the modifiers in the *aa BB* condition necessary to produce a resistant F<sub>1</sub> progeny.

Each of the susceptible BI lines used in this study had been inbred for nine generations, and it is likely that in each the modifiers were homozygous and homogeneous. The resistant EF lines used in the crosses had been selfed for only 2-3 times without selection for modifiers. One can see, therefore, that different resistant plants most probably contained different modifier genes. It also is obvious that, besides the three segregation patterns observed (Table 1), still different segregation ratios can be expected from crosses between resistant and susceptible plants differing at either only the *a* or the *B* locus.

TABLE 4. Black rot ratings of various open-pollinated cabbage cultivars from the United States<sup>a</sup> inoculated with *Xanthomonas campestris* in the field

Black rot rating	Segregation <sup>b</sup>	Open-pollinated cultivars
1 <sup>c</sup>		None
2		None
3	1-4	Early Fuji
	2-3	Stein's Flat Dutch
	2-4	Red Danish
	3-4	Eastern Ballhead
	None	Wisconsin All Season
4	2-4	Bonanza, Chieftan Savoy, Green Acre, Mammoth Red Rock, Red Acre, Red Hollander, Round Red Dutch, Seidel Ballhead, Short Stem Hollander, Wisconsin Hollander No. 8
	None	Badger Ballhead, Badger Market, Bayswater Large Red, Charleston Wakefield, Copenhagen Market, Early Glory, Evergreen, Globelle, Glory of Enkhuizen, Golden Acre, Greenback, Hollander, Late Flat Dutch, Marion Market, Mid-season Market, Oakview Ballhead, Succession, TBR Globe Round Dutch
5	4-5	Jersey Queen, Jersey Wakefield
	None	

<sup>a</sup> With exception of Early Fuji, which was obtained from Japan.

<sup>b</sup> Indicates the range of black rot rating among the 20 plants tested per cultivar.

<sup>c</sup> Indicates the predominant black rot rating: 1 = resistant, 2 = highly tolerant, 3 = tolerant, 4 = susceptible, 5 = highly susceptible.

The range of disease ratings in the different F<sub>1</sub> lines derived from crosses between EF and susceptible plants with varying tolerance (Table 5) confirmed the presence in susceptible plants of genes capable of modifying the *f* gene for black rot resistance. The

TABLE 5. Black rot ratings of various susceptible and tolerant inbred cabbage lines and their F<sub>1</sub> progenies from crosses with various resistant Early Fuji inbreds inoculated with *Xanthomonas campestris* in the field

Inbred	Susceptible or tolerant parent	F <sub>1</sub> progeny	
	Black rot rating	From cross	Black rot rating
USDA-66	2 <sup>a</sup>	USDA-66 X EF-6 <sup>b</sup>	1
BI-8 <sup>c</sup>	3	BI-8 X EF-58	1
BI-8	3	BI-8 X EF-4	2
BI-3	3	BI-3 X EF-4	3
BI-13	3	BI-13 X EF-21	3
BI-5	4	BI-5 X EF-20	3
BI-10	4	BI-10 X EF-6	4
BI-11	4	BI-11 X EF-26	4
CR-132 <sup>d</sup>	5	CR-132 X EF-20	4

<sup>a</sup> Rating based on 20 plants/line: 1 = resistant; 2 = highly tolerant; 3 = tolerant; 4 = susceptible; 5 = highly susceptible.

<sup>b</sup> EF = inbred lines derived from Early Fuji.

<sup>c</sup> BI = Badger Inbred.

<sup>d</sup> CR = club root-resistant inbred.

production of resistant or highly tolerant F<sub>1</sub> plants when EF is combined with tolerant lines (BI-8 and USDA-66) suggests that the modifiers of the *f* gene might confer some tolerance to black rot in plants homozygous (*FF*) for susceptibility at the *f* locus. In the development of inbred lines for production of black rot-resistant F<sub>1</sub> hybrid cabbage, plant breeders may find it useful, therefore, to accumulate modifiers by selecting for black rot tolerant types in germplasm lacking the major gene *f*. A similar influence of modifiers from susceptible plants on the expression of resistance in F<sub>1</sub> plants has been reported for the resistance in cotton to *X. malvacearum* (3).

The results of our black rot screening trials largely confirm the observations by Bain (1) of a range in susceptibility among various cabbage cultivars, and several of the cultivars which he described as resistant also had low black rot ratings in our tests. These

cultivars might be sources for modifier genes, which could possibly be accumulated by selection for tolerance. USDA-66 (Table 5), for instance, had been developed by rigorous selection for black rot tolerance at the United States Department of Agriculture laboratory in Charleston, S. C., and it combined with EF to produce resistant F<sub>1</sub> plants.

Although the inheritance is relatively complex, the incorporation of black rot resistance into F<sub>1</sub> hybrid cabbage appears to be straightforward. Two paths could be followed in breeding programs. By stabilizing the major *f* gene in several inbreds, the breeder may combine two of these to produce a desired hybrid, homozygous for black rot resistance. By selecting a series of highly tolerant inbreds derived from what appears to be an ample germ pool within existing open-pollinated and hybrid cultivars, the breeder may combine these with a single resistant inbred to produce resistant hybrids.

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