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Inheritance of resistance to blackmold (*Alternaria alternata* (Fr.) Keissler) in two interspecific crosses of tomato (*Lycopersicon esculentum* × *L. cheesmanii* f. *typicum*)

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Abstract Inheritance of resistance to blackmold, a disease of ripe tomato fruit caused by *Alternaria alternata*, was studied in two interspecific crosses. The parents, F_1 and F_2 generations of a cross between the susceptible *Lycopersicon esculentum* Mill. cultivar 'Hunt 100' and the resistant *L. cheesmanii* f. *typicum* Riley accession LA 422, and the parents, F_1, F_2, F_3, and BC_1 P_2 generations of a cross between the susceptible *L. esculentum* cv. 'VF 145B-7879' and LA 422 were evaluated. The following disease evaluation traits were used: symptom rating (a symptom severity rating based on visual evaluation of lesions), diseased fruit (the number of diseased fruits divided by the total number of fruit scored), and lesion size (a function derived from the actual lesion diameter). Generation means analysis was used to determine gene action. The data of the 'Hunt 100' × LA 422 cross fit an additive-dominance model for all three traits. The 'VF 145B-7879' × LA 422 cross data best fit a model that included the additive × additive and additive × dominance interaction components for the trait diseased fruit, whereas higher-order epistatic models would have to be invoked to fit the data for the traits symptom rating and lesion size. A minimum of one gene segregated for all three traits. Broad-sense heritability estimates ranged from 0.09 to 0.16 for all three traits, indicating that selection for improved resistance to blackmold will require selection on a family performance basis.

Key words *Alternaria alternata* · Blackmold · Generation means analysis · Tomato disease resistance · Inheritance of resistance

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Introduction

Blackmold is a ripe tomato fruit rot (Miyao et al. 1986) caused by *Alternaria alternata* (Fr.) Keissl. (= *A. tenuis* Nees) (Simmons 1967). The disease can cause severe losses in the field and lead to cannery rejection of harvested fruit (Pearson and Hall 1975). The disease on the ripe fruit is characterized by lesions ranging from small brown flecks to large, black, circular sunken areas that may extend into the carpel wall and the seed locule (Koepsell 1968; Pearson and Hall 1975). In advanced stages, a black, velvety layer of spores may be produced on the surface of the sunken lesions (Hall et al. 1980). Blackmold is especially severe in tomato fields after rain (Butler 1959), but dew also can promote disease (Pearson and Hall 1975).

Blackmold has been considered erroneously to be a synonym of another tomato disease known as stem canker (Paulus 1991). Two pathotypes for *A. alternata* exist: a saprophytic form that infects only ripe tomato fruits (Grogan et al. 1975) and a relatively new pathotype, *A. alternata* f. sp. *lycopersici*, capable of infecting leaves, stems, and green fruits of susceptible tomato cultivars and causing the stem canker disease (Grogan et al. 1975). Unlike blackmold, stem canker can be controlled by the presence of a single incompletely dominant gene, *Asc*, which confers complete resistance to *A. alternata* f. sp. *lycopersici* (Gilchrist and Grogan 1976). *Asc* has no reported effect on *A. alternata*.

Blackmold has been controlled with fungicides (Miyao et al. 1986; Davis et al. 1990), but fungicide use has come under increasing scrutiny. The use of genetic resistance is environmentally safe and may be the most reliable and economical approach to control. To date there is no evidence in the literature of screening either cultivated tomato (*L. esculentum*) or wild *Lycopersicon* species for resistance to blackmold. Rick (1986) presented many successful examples of improving tomato disease resistance by the introgression of wild germplasm into cultivated tomato, thus we surmised that

wild species may represent an untapped source of blackmold resistance. A preliminary investigation in our lab determined that some accessions of *L. cheesmanii* appeared to be resistant to *A. alternaria* (Blaker and St. Clair, unpublished data).

The purpose of the study presented here was to estimate the heritability of genes segregating for blackmold resistance from a previously identified resistant *L. cheesmanii* accession, to determine the gene action associated with the resistance genes, and to determine the number of resistance genes in order to understand the genetic basis of blackmold resistance. Determination of the mode of resistance will assist decisions on how best to transfer and utilize this genetic resistance in cultivated tomato.

Materials and methods

Plant material

Two *L. esculentum* processing tomato cultivars, 'Hunt 100' and 'VF 145B-7879' (referred to as '7879') were chosen as the blackmold susceptible parents, and accession LA 422 of *L. cheesmanii* f. *typicum* (referred to also as LA 422) as the resistant parent. The latter accession was obtained from the Tomato Genetics Resource Center, Department of Vegetable Crops, University of California, Davis. Identification of blackmold-resistant LA 422 resulted from preliminary work that indicated it exhibited a level of resistance higher than any *L. esculentum* line and most *L. cheesmanii* accessions tested (Blaker and St. Clair, unpublished results). This species was of primary interest as a source of genetic resistance to blackmold because it is a close relative that belongs to the same intra-crossable group as *L. esculentum* (Rick 1979; Miller and Tanksley 1990). Moreover, *L. cheesmanii* is known for its high soluble solids content (Garvey and Hewitt 1984), a desirable trait used to maximize paste yield from processing tomatoes. The parent lines were highly inbred and presumed to be homozygous.

The susceptible cultivars served as female parents and each was crossed with LA 422 to produce two F_1 progenies ('Hunt 100' × LA 422 and '7879' × LA 422). The following parental designations were used in this study: P_1 = LA 422, P_2 = '7879', P_3 = 'Hunt 100'. F_2 progenies for each cross were obtained by selfing F_1 plants. F_3 families from the cross '7879' × LA 422 were obtained by selfing F_2 plants and harvesting the F_3 seed from each individual F_2 plant. F_1 plants from the cross '7879' × LA 422 were also backcrossed to '7879' as the female parent to produce the first backcross generation (BC_1P_2). Limited greenhouse space and severe tobacco mosaic virus incidence precluded collecting F_3 and BC_1P_3 data for the cross 'Hunt 100' × LA 422.

All plants for this study were grown in a UC Davis greenhouse using standard horticultural practices. Plants were assigned randomly to bench space in the greenhouse. Greenhouse temperature was maintained at 24°–27 °C day and 18°–21 °C night with a 12- to 14-h photoperiod. Only ripe tomato fruits were harvested for evaluation of blackmold resistance. Care was taken to ensure that all fruits were ripe at harvest for subsequent *A. alternata* inoculation. Plants for all evaluations were grown from June 1991 to June 1992.

Inoculum source and preparation

Alternaria alternata was isolated from ripe tomato fruits of 'Hunt100' and '7879' from a field in Davis that exhibited typical blackmold symptoms. Isolates were obtained at four different sample dates in August 1991, and each was maintained separately. The four isolates were used collectively in all inoculations. None of the four isolates was single-spore derived, but instead each isolate was a population of *A. alternata* spores that represented what exists naturally in ripe fruit field infestations.

The fungus was grown on V-8 juice agar medium (Koepsell 1968) consisting of 200 ml V-8 juice (Campbell Soup Co, Camden, N.J.), 2 g precipitated $CaCO_3$, and 17 g bacto-agar in 1 l of water. The medium was autoclaved, then gently shaken as it was poured into petri plates to disperse the $CaCO_3$ evenly. After inoculation, plates were placed under cool white light with a 12-h photoperiod at 25 °C ± 1 °C to promote sporulation. Subculturing was performed at 10- to 20-day intervals, and isolate virulence was monitored at least once a month by inoculation of susceptible fruits ('Hunt 100' and/or '7879').

Spore suspensions of each of these isolates were prepared by flooding a 10- to 20-day-old *A. alternata* plate with 20 ml of autoclaved double-distilled water, agitating with a glass rod, and pouring off the resulting spore suspension. All the isolates exhibited abundant sporulation at the time of fruit inoculation. Spore suspensions of the four isolates were then mixed together to create a diverse inoculum population. The spore concentration was measured using a hemacytometer and adjusted to the desired concentration of 1.2×10^6 spores/ml. This concentration was determined to be optimal for disease development in a preliminary study.

Inoculation and evaluation of fruits

After harvesting, fruits were stored overnight at 12 °C. The following day, fruits were surface-sterilized with 0.8% sodium hypochlorite for 10 min and then rinsed three times with autoclaved distilled water. Humidity chambers consisted of clear plastic boxes (35 cm × 25 cm × 15 cm) with lids, which were sterilized in 0.8% sodium hypochlorite for 30 min, then rinsed with distilled water. Immediately before use, the chambers were sprayed with 70% ethanol and dried. Surface-sterilized fruits were placed on autoclaved mesh wire screens bent to hold the fruit approximately 2 cm above the bottom of the chamber. Autoclaved distilled water was added to the bottom of each chamber to maintain high humidity. Fruits were inoculated by placing a 20-μl drop of a 1.2×10^6 spore/ml suspension on or near the blossom end of the fruit. The stem scar was avoided because it provides an entry point for the pathogen. Fruits exhibiting growth cracks prior to inoculation were discarded. Inoculated susceptible fruit ('Hunt 100' or '7879') was included in each chamber as a positive control. Chambers were sealed with clear plastic wrap and lids immediately after inoculation. This seal did not prevent air exchange but did help maintain high relative humidity and prevent contamination by other aerial pathogens. Chambers were kept in a growth room maintained at 26 °C ± 1 °C with a 12-h photoperiod. The fruits were evaluated for the presence of lesions 8 days after inoculation. The 8-day period was chosen based on preliminary studies showing that symptoms of blackmold infection rarely developed beyond this time.

A completely randomized design was employed for fruit testing. Forty plants of each parental line (LA 422, 'Hunt 100', '7879') and 30 plants of each F_1 population were used in this study. Since the parental and F_1 generations were genetically uniform, no attempt was made to obtain replicated data per individual plant. Instead, each replication for these generations consisted of 4–10 fruits bulked from more than 1 plant of each genotype. The number of replications ranged from 15 to 30 per parental and F_1 generations because the experiments were conducted during the course of a year, and parental fruits were used as checks throughout all progeny testing. Limited greenhouse space precluded testing all generations simultaneously. Fruits of the following generations were tested for their disease reaction to *A. alternata*: 60 F_2 plants from 'Hunt 100' × LA 422; 114 F_2 plants and 93 F_3 families from '7879' × LA 422; and 43 BC_1P_2 plants. Data consisted of 2–6 replications of 4–10 fruits per individual plant (F_2 or BC_1P_2) or family (fruits bulked from 2–4 plants per F_3 family). Fruits were evaluated according to their availability since there were differences among plants for time to fruit ripening, so replications per plant or family were done in both space and time as fruit became available. The number of harvests per plant (parental, F_1, F_2, or BC_1P_2) or family (F_3) per replication varied because of differences among plants for fruit production and space limitations in the humidity chambers. During disease testing, a total of 6–8 genotypes were assigned randomly to each chamber.

Disease evaluation

Three traits were chosen to evaluate disease development. The first trait used to assess resistance was a rating of symptom severity, referred to hereafter as "symptom rating", with values of 0–3. Ratings were based on visual evaluation of lesions on inoculated fruits (0 = no symptoms; 1 = shallow discrete flecks; 2 = shallow lesions, expanding laterally into epidermal and subepidermal tissue; and 3 = deep or sunken lesions, extending into the carpel walls or beyond).

The second trait was the proportion of diseased fruits with blackmold, referred to hereafter as "diseased fruit". This parameter was calculated by dividing the observed number of diseased fruits by the total number of fruits evaluated. Any fruit receiving a symptom rating of one or greater was considered a diseased fruit for calculating this parameter.

The third trait was a calculated function based on the diameter of the lesion, referred to hereafter as "lesion size". Lesion size was estimated by dividing the square of the lesion diameter (in mm) by the sum of the square of the lesion diameter plus a constant equal to 36. This constant equals the square of half of the largest fruit diameter (12 mm) of the resistant parent LA 422. This function of lesion diameter was used to account for the large difference in fruit size between the parents (7–12 mm for LA 422, 24–64 mm for '7879' and 'Hunt 100') and the effect of fruit size on the ultimate lesion size. The large-fruited genotypes could have lesion diameters greater than that of the fruit diameter of the smaller-fruited genotypes. The function of the lesion diameter that was utilized in this study weighted lesion diameters appropriately so that very large and very small values would not be overly emphasized by forming an essentially linear relationship for small lesions and approaching an asymptote as the lesion size increases. This function de-emphasizes the large lesions but keeps smaller lesions in a linear relationship. If parental and progeny genotypes were approximately equal in fruit size, criteria such as the simple ratio between lesion and fruit diameters, or lesion diameter by itself, would be appropriate for the present genetic study.

Genetic analyses

The variance component method (formula 1) was used to calculate broad-sense heritability (H) values (Wright 1968; Strausbaugh and Murray 1989).

$$H = \{V_{F2} - [(V_{Ps} + V_{Pr} + 2V_{F1})/4]\} \quad (1)$$

In formula 1, V_{F2} = the variance of the F_2 generation, V_{Ps} = the variance of the susceptible parent, V_{Pr} = the variance of the resistant parent, and V_{F1} = the variance of the F_1 generation. A second broad-sense heritability estimate was obtained using parent-offspring regression (Lush 1940; Simmonds 1979). Only F_2 plants and F_3 families for which data had been obtained for both the parental F_2 and its corresponding F_3 family were included in this analysis. The F_2 plants and their corresponding F_3 families were grown under the same environmental conditions in the greenhouse. The regression of the F_3 family means on the F_2 plant values was computed and multiplied by the correction factor of 2/3 to adjust for inbreeding in the F_2 parents (Smith and Kinman 1965).

The gene action of blackmold resistance in the two crosses for each of the three disease evaluation traits was determined through generation means analysis using the joint scaling tests described by Mather and Jinks (1977, 1982). In order to obtain the best estimates for the genetic components, generation means (Table 1) and their expectations (Table 2) (Mather and Jinks 1977, 1982; M. Foolad, personal communication) were weighted using the reciprocal of the variances of the means ($1/V_{\bar{x}}$) (Mather and Jinks 1977). The number of genetic components involved in the model to be tested depended on the number of generations available. For the cross '7879' × LA 422, the parental, F_1, F_2, F_3, and the first backcross to the susceptible parent (i.e., BC_1P_2) data were available, providing six generations that allowed a genetic model with up to five components to be tested. The data from each of the three traits for this cross were tested against the following models: m[d][h], m[d][h][i], m[d][h][j], m[d][h][l], m[d][h][i][j], m[d][h][i][l], and m[d][h][j][l], where m = midparent, [d] = additive, [h] = dominance, [i] = additive × additive, [j] = additive × dominance, and [l] = dominance × dominance. For the cross 'Hunt 100' × LA 422, only the parental, F_1, and F_2 data were available, providing four generations that allowed a genetic model with three components to be tested. The data from each of three traits for the cross 'Hunt 100' × LA 422 were tested against the m[d][h] genetic model. For both crosses analyzed, the simplest model that fit the data was accepted provided that the chi-square test probability exceeded 5% ($P > 0.05$), even though a more complex model may have had a lower chi-square value and/or a higher probability (Mather and Jinks 1982). Components within models that fit the data were evaluated for significance with F values.

The number of genes (n) segregating for blackmold resistance in the F_2 generation was estimated using the following formula (Wright 1968):

$$n = \frac{(P_s - P_r)^2 [1.5 - 2h(1 - h)]}{8[V_{F2} - (V_{Ps} + V_{Pr} + 2V_{F1})/4]} \quad (2)$$

In formula 2, P_s = the mean of the susceptible parent, P_r = the mean of the resistant parent, and $h = (F_1 - P_r)/(P_S - P_r)$. This formula estimates gene number by dividing the square of the genotypic range (i.e., the difference between parental means) by the genotypic variance, and accounts for dominance.

Dunnett's t-test for comparing treatment means to a standard was used to compare individual F_3 family means to the mean of the resistant parent LA 422 and to the mean of the susceptible parent '7879' for each of the three traits in order to identify the families most

Table 1 Generation means for two crosses 'Hunt 100' × *L. cheesmanii* LA 422 and 'VF 145B-7879' × LA 422. Lower values indicate greater resistance

Generation	Disease evaluation trait						
	n[a]	Symptom rating	± SD	Diseased fruit	± SD	Lesion size	± SD
Hunt 100 × *L. cheesmanii* LA 422							
P_1[b]	40	1.33	1.30	0.58	0.49	37.20	46.29
P_3[b]	40	1.71	1.07	0.86	0.34	420.77	597.06
F_1	30	1.22	1.26	0.56	0.50	76.15	128.39
F_2	60	1.28	1.30	0.58	0.49	134.38	212.30
VF 145B-7879 × *L. cheesmanii* LA 422							
P_1[b]	40	1.33	1.30	0.58	0.49	37.20	46.29
P_2[b]	40	1.65	0.88	0.90	0.29	208.02	353.83
F_1	30	1.05	1.34	0.43	0.50	101.01	158.18
F_2	114	1.52	1.34	0.62	0.48	148.36	200.02
F_3	93	1.32	1.05	0.73	0.44	88.49	154.45
BC_1P_2[b]	43	0.66	0.83	0.46	0.50	41.11	151.67

[a] n, Number of plants evaluated per generation

[b] P_1, LA 422; P_2, 'VF 145B-7879'; P_3, 'Hunt 100'; BC_1P_2, 'VF 145B-7879' ('VF 145B-7879' × LA 422)

Table 2 Coefficients of gene effects in an analysis of generation means with six generations

Generation	Mean phenotype[a]					
	m	[d]	[h]	[i]	[j]	[l]
P_1^b	1	1	0	1	0	0
P_2^b	1	−1	0	1	0	0
F_1	1	0	1	0	0	1
F_2	1	0	1/2	0	0	1/4
F_3	1	0	1/4	0	0	1/16
$BC_1P_2^b$	1	−1/2	1/2	1/4	−1/4	1/4

[a] m, Midparent; [d], additive; [h], dominance; [i], additive × additive; [j], additive × dominance; [l], dominance × dominance

[b] P_1, Resistant parent; P_2, susceptible parent; BC_1P_2, first backcross to the susceptible parent

Table 3 Estimates of broad-sense heritability for resistance to *Alternaria alternata* in two interspecific tomato populations

Cross	Method	Disease evaluation trait		
		Symptom rating	Diseased fruit	Lesion size
Hunt 100 × LA 422	Variance component[a]	0.11	0.12	−1.17
7879 × LA 422	Variance component	0.16	0.13	−0.11
7879 × LA 422	Parent-off-spring regression[b]	0.11	0.09	0.09

[a] Broad-sense heritability = $\{V_{F2} - [(V_{Ps} + V_{Pr} + 2V_{F1})/4]\}$, where V_{F2} = the variance of the F_2 generation, V_{Ps} = the variance of the susceptible parent, V_{Pr} = the variance of the resistant parent, V_{F1} = the variance of the F_1 generation

[b] Parent-offspring regression performed with F_2 plants and their respective F_3 families using standard regression techniques

resistant and least resistant to blackmold, respectively. Means were tested for significance at the 5% level of probability.

Results and discussion

Heritability

The broad-sense heritability (H) estimates obtained in this study for all three traits were low (Table 3). The highest heritabilities obtained were 12% for the 'Hunt 100' × LA 422 cross and 16% for the '7879' × LA 422 cross. Because of these low values, selection for improved resistance will be difficult when individual F_2 plants are the selection units in the segregating populations used in this study. The number and type of generations evaluated and the mating design employed precluded estimating the additive genetic variance required to calculate narrow-sense heritability.

When the variance component method was used, negative estimates of heritability were obtained for lesion size in both crosses (Table 3). As mentioned previously, the environment appeared to have a larger effect on this trait since negative values resulted from an environmental variance that was larger than the variance of the F_2 generations of both crosses. The mathematical properties of lesion size, as a function of the lesion diameter, may have also contributed to the estimation of values different from those obtained for the traits symptom rating and diseased fruits. The effect of the function on the variances of lesion size and the resulting negative heritability estimates may be a result of the following considerations. The variance component method provides the greatest flexibility for predicting the effectiveness of alternative selection procedures (Fehr 1987), but it suffers from statistical weakness since it is based on variances, which are affected by departure from normality (Gilbert 1973) and sample size. The partition of variance causes small differences between inaccurate numbers to become extremely inaccurate and, as a result, heritability is poorly estimated and can also be negative (Simmonds 1979).

The use of parent-offspring regression to estimate the heritability of a trait is based on five assumptions: (1) normal diploid Mendelian inheritance, (2) random mating population, (3) no linkage among loci controlling the character or linkage equilibrium, (4) non-inbred parents, and (5) no environmental correlations between the performance of the parents and offspring (Vogel et al. 1980). Assumption 1 was likely met, but assumption 3 might have been violated. Even though the chromosomes of the two parental species are homosequential, the progeny of interspecific crosses can exhibit reduced recombination and linkage drag during gene introgression (Rick 1979; Rick and Yoder 1988), and linkage equilibrium is unlikely in early generations (Mather 1949; Gamble 1962). Failure to meet assumption 3 would bias the heritability estimates obtained (Gardner and Lonnquist 1959). Linkage disequilibrium can bias estimates of H because the reference population for the heritability estimates obtained here is the F_2 population from two inbred parents, and ancestral linkage relationships will not be eliminated with a single generation of crossing. The highly inbred, self-pollinated parents were not randomly mated, so an adjustment factor for the mating system and for inbreeding in the F_2 was applied in order to meet assumptions 2 and 4 (Smith and Kinman 1965). Environmental correlations between the performance of relatives were presumed to be absent and assumption 5 satisfied because the parental F_2 plants and their offspring F_3 families were randomized and grown within the same greenhouse (environmental) conditions. Although G × E could bias the estimate of heritability when parents and their progeny are evaluated in identical environments (Casler 1982), differential environmental expression on parental and progeny generations can be a significant source of bias as well (Frey and Horner 1957). In the present case, little G × E is expected under greenhouse conditions, but the correspondence between greenhouse and field estimates remains to be determined.

The parent-offspring regression method has been advocated as a more conservative approach to estimate heritability than the variance component method (Gilbert 1973; Simmonds 1979). However, since both methods require similar assumptions (Shaw 1989) but each method may reflect different genetic components and relationships, we obtained the results from both methods for comparison. Heritability estimates obtained for lesion size were negative for the variance component method and positive for the parent-offspring regression method, as well as being close in magnitude (0.09) to the estimates for the traits symptom rating (0.11) and diseased fruits (0.09) (Table 3). Thus, the parent-offspring regression method appeared to be a more robust method to estimate heritability in our study.

Gene action

The data for all three traits (Table 1) from the cross 'Hunt 100' × LA 422 best fit a simple additive-dominance model (m[d][h]) (Table 4). The additive component [d] mean square was the largest for all three traits, although neither the d or h gene effects were significant (Table 5). Non-significance of the genetic parameters may be due to sampling error. This cross only had four generations available for analysis, precluding the testing of more complex models. The effects of linkage are not detected in a simple additive-dominance model, since linkage affects only the epistatic terms in the generation means analysis (Hayman 1958). Linkage may or may not be important for resistance to blackmold in the 'Hunt 100' × LA 422 cross, but the failure to detect linkage does not imply that it does not exist. Estimates for the additive and dominance gene effects were negative for all three traits, indicating that they were in the direction of susceptibility (Table 5). However, inferences cannot be drawn since the presence of linkage is unknown and models invoking epistasis could not be tested for the reasons previously discussed.

In the '7879' × LA 422 cross, the data for the trait diseased fruit (Table 1) fit the model m[d][h][i][j] (Table 4). Mean square estimates showed that the dominance component ([h]) accounted for the largest portion of the observed variability, followed by the additive × dominance interaction component ([j]) (Table 6). There is no satisfactory explanation in the literature for the non-significance of the additive × additive gene effect (i), since this interaction component accounted for a larger portion of the variation than the significant additive gene effect (d) according to the mean square estimates.

The significance of digenic interaction effects indicates that resistance to blackmold is complexly inherited in this cross. Comparison of the signs of the significant additive (d) and dominance (h) gene effects (which were both negative) to that of the significant additive × dominance interaction (j) (which was positive) suggested duplicate types of gene interactions (as the signs were opposite), confirming the importance of dominance gene effects (Grewal 1988). In the presence of epistasis, it is not possible to obtain unbiased estimates of additive and dominance effects because the distribution of posi-

Table 4 Gene action for resistance to *Alternaria alternata* in two crosses, 'Hunt 100' × *L. cheesmanii* LA 422 and 'VF 145B-7879' × LA 422

Disease evaluation trait	Model tested[a]	χ^2	P	Component reduced χ^{2b}
Hunt 100 × LA 422				
Symptom rating	m[d][h]	0.737	0.390	
Diseased fruit	m[d][h]	2.332	0.126	
Lesion size	m[d][h]	1.278	0.258	
7879 × LA 422				
Symptom rating	m[d][h]	269.253	0	
	m[d][h][i]	257.743	0	[i]
	m[d][h][j]	31.071	0	[j]
	m[d][h][l]	266.980	0	
	m[d][h][i][j]	21.245	0	[i][j]
	m[d][h][i][l]	234.417	0	
	m[d][h][j][l]	30.793	0	
Diseased fruit	m[d][h]	94.967	0	
	m[d][h][i]	61.319	0	[i]
	m[d][h][j]	11.302	0.003	[j]
	m[d][h][l]	93.565	0	
	m[d][h][i][j]	0.102	0.748	[i][j]
	m[d][h][i][l]	45.361	0	[i][l]
	m[d][h][j][l]	5.642	0.017	[j][l]
Lesion size	m[d][h]	218.482	0	
	m[d][h][i]	145.698	0	[i]
	m[d][h][j]	58.394	0	[j]
	m[d][h][l]	176.338	0	
	m[d][h][i][j]	45.998	0	[i][j]
	m[d][h][i][l]	140.954	0	[i][l]
	m[d][h][j][l]	47.838	0	[j][l]

[a] m, Midparent; [d], additive; [h], dominance; [i], additive × additive; [j], additive × dominance; [l] dominance × dominance

[b] Genetic component that significantly reduced the chi-square value when added to the model tested. Significant chi-square reduction was based on $\chi^2_{1df} = 3.841$ at $P \leq 0.05$

Table 5 Mean square estimates, chi-squares (χ^2), and estimates of gene effects for the best fit model (m[d][h]) of the cross 'Hunt 100' × *L. cheesmanii* LA 422

Generation	Disease evaluation trait						
	df	Symptom rating	±SE	Diseased fruit	±SE	Lesion size	±SE
Model							
[d]	1	10.77		60.78		189.84	
[h]	1	9.97		25.15		27.02	
Residual error		0.74		2.33		1.28	
χ^2		0.74		2.33		1.28	
Effects							
m		1.50[a],*	±0.049	0.71*	±0.031	210.48[NS]	±18.19
d		−0.19[NS]	±0.052	−0.14[NS]	±0.032	−173.45[NS]	±18.36
h		−0.41[NS]	±0.111	−0.24[NS]	±0.073	−144.86[NS]	±31.51

[a] H_0, parameter = 0
t-test probability is significant at $*P \leq 0.05$ and $**P \leq 0.01$, or non-significant at $P > 0.05$[NS]

Table 6 Mean square estimates, chi-squares (χ^2), and estimates of gene effects for the best fit model (m[d][h][i][j]) of the cross 'VF 145B-7879' × *L. cheesmanii* LA 422

Source	*df*	Disease evaluation trait	
		Diseased fruit	±SE
Model			
[d]	1	15.82	
[h]	1	137.88	
[i]	1	33.65	
[j]	1	61.22	
Residual error		0.10	
χ^2		0.10	
Effects			
m		0.84[a],**	±0.006
d		−0.16*	±0.007
h		−0.43*	±0.016
i		−0.09[NS]	±0.009
j		0.87*	±0.036

[a] H_0, parameter = 0
t-test probability is significant at $*P \leq 0.05$ and $**P \leq 0.01$, or non-significant at $P > 0.05$[NS]

tive and negative gene effects in the parents may result in different degrees of cancellation of gene effects in expression of the means (Thompson et al. 1963). Linkage among genes that confer resistance to blackmold is possible, for reasons discussed previously. Linkage may cause serious bias in the estimates, and when epistasis is present, bias due to linkage relations would exist in the estimates of gene effects, especially in the additive × additive (i) and dominance × dominance (l) effects. Trigenic or higher-order epistasis could cause the appearance of linkage (Gamble 1962).

The data for symptom rating and lesion size (Table 1) failed to fit the non-epistatic model (m[d][h]), indicating the presence of epistasis (Table 4). Digenic epistatic models, including the interaction components [i], [j], and [l], did not explain the data either, and this may indicate that either trigenic epistasis, or linkage, or both, are important (Hayman 1958). Although the data did not fit any of the models tested, the models including the additive × additive ([i]) and the additive × dominance ([j]) interaction components resulted in the lowest chi-square for both traits (Table 4). These results are consistent with the results obtained for the trait diseased fruit in this cross. When the three interaction components ([i], [j], and [l]) were compared, the additive × dominance interaction component ([j]) was the component that had the greatest impact in reducing the chi-square estimate when added to the simplest model (m[d][h]) for all three traits. Inferences may be imprecise since the models tested did not fit two of the three traits used to evaluate blackmold resistance, but the results suggested a greater importance of the additive × dominance epistasis in the 'VF 145B-7879' × LA 422 cross.

Number of genes

Estimates of the number of genes segregating for resistance to *A. alternata* based on symptom rating, diseased fruit, and lesion size in the F_2 populations were 0.2, 0.5, and −0.5 for the 'Hunt 100' × LA 422 cross, and 0.2, 1.2, and −0.8 for the '7879' × LA 422 cross, respectively. Wright's (1968) method used to estimate the minimum number of genes is calculated from the phenotypic means and variances in the two parental populations and their F_1 and F_2 progeny. The main assumptions of Wright's method are that the parents are at opposite phenotypic extremes, linkage is absent, gene action is additive, and allelic effects are equal at all loci. When any of these assumptions is violated, the true number of loci is substantially underestimated by this method (Zeng et al. 1990). On the basis of these considerations, the fact that only one gene was found to be associated with resistance in LA 422 does not exclude the possibilities that there is only one gene governing resistance, this gene is actually a linkage group that may involve two or more genes, or that several unlinked genes are involved.

When the environmental variance estimate is larger than the segregating population (e.g., F_2) variance, the formulas used to estimate the number of genes will lead to negative values. This was true for the two observed

negative values. The environmental variance estimates for both crosses were considered reliable since all three parents were homozygous and, therefore, the F_1 hybrids were presumed to be genetically uniform. The environmental variance observed in this study may be due to the fact that disease screening was done over a relatively long period of time because of the divergent parental material that gave rise to the interspecific progeny generations. All the generations were grown under similar greenhouse conditions, but the greenhouse environment does experience some limited influence from the seasons, especially daylength changes.

The negative estimates obtained for lesion size in both crosses may be related to the nature of this trait. The three traits used in this study may measure the interplay between the host-pathogen interaction and the environment in different ways. The larger environmental variances estimated for lesion size suggest that this trait was more susceptible to environmental fluctuations than the other two traits, symptom rating and diseased fruit (data not shown). Moreover, lesion size is a function derived from the direct measurement of lesion diameter, and values assumed by this trait could have caused the estimation of the number of genes to deviate from an expected value by violating the assumption of additive gene action.

Mean comparisons

Dunnett's *t*-test was used to compare the means of the F_3 families with means of the parents LA 422 and '7879' for each of the three traits, symptom rating, diseased fruit, and lesion size (Table 7) in order to identify superior progeny. In all three traits, F_3 means less than or equal to those of LA 422 indicate resistance, while F_3 means greater than or equal to those of '7879' indicate susceptibility. Transgressive segregation was observed towards both resistance and susceptibility. Dunnett's *t*-test was able to detect transgressive F_3 families towards resistance using the trait symptom rating, and those families will be used for future breeding efforts.

Table 7 Dunnett's *t*-test for mean comparisons of F_3 families in the cross 'VF 145B-7879' × *L. cheesmanii* LA 422

Number of F_3 families	Disease evaluation trait		
	Severity rating	Diseased fruit	Lesion size
More resistant than LA 422[a]	6	0	0
As resistant as LA 422[b]	77	75	81
Less resistant than LA 422[a]	10	18	12
More resistant than 7879[a]	19	18	49
As resistant as 7879[b]	71	75	43
Less resistant than 7879[a]	3	0	1

Numbers in table indicate the number of F_3 families (out of 93 total) that were significantly different[a] and not significantly different[b] from the respective parent at $P \leq 0.05$

In summary, although the minimum number of genes estimated for both crosses was one, the possibility that more than one gene is controlling resistance to blackmold cannot be eliminated. This reasoning was supported by evidence of epistasis as detected in the '7879' × LA 422 cross for all three traits. The results obtained from the generation means analysis also indicate, however, that 'Hunt 100' and '7879' have different genetic backgrounds that appear to affect resistance gene(s) from LA 422 differently and that the alleles for susceptibility most likely differ between them. Heritability is a useful parameter for breeders, but it is a property of a specific population in a specific environment (Simmonds 1979). Although heritability was low for both crosses in this study, the observed transgressive segregation towards resistance suggests that genetic progress could be made if a proper breeding strategy was chosen, based on family selection using replicated testing. In addition, most of the data suggested a relatively low number of genes controlling the disease response and that broad-sense heritability may be low due to nongenetic causes. The environmental variation might be reduced further with additional modifications of the disease screen, thus improving the detection of resistant plants.

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