Tomato Early Blight Resistance in a Breeding Line Derived from *Lycopersicon hirsutum* PI 126445

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ABSTRACT

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Field studies were conducted in 1983, 1984, and 1985 comparing NC EBR-1, an early blight-resistant breeding line derived from *Lycopersicon hirsutum* PI 126445, with the resistant lines 71B2 and C1943 and with a susceptible check, Flora-Dade. Additional field studies were conducted in 1984 and 1985 comparing NC EBR-1 with the early blight-susceptible lines Piedmont and NC 8233(X)-2(X) and with the F_1 hybrids of Piedmont and NC 8233(X)-2(X) crossed to NC EBR-1. Area under the disease progress curve (AUDPC) was used to measure resistance levels. AUDPC values for C1943, 71B2, and NC EBR-1 did not differ in any of the three seasons and were much lower in all three seasons than values for Flora-Dade. AUDPC values for NC EBR-1 were lower than for Piedmont and NC 8233(X)-2(X) in both years, and the F_1s were intermediate to their respective parents in both years. In three greenhouse studies, lesion diameters resulting from point inoculation of leaflets were measured over time. NC EBR-1 had significantly smaller lesion diameters than NC 8233(X)-2(X) in all experiments. NC EBR-1 had significantly smaller lesion diameters than Piedmont in one experiment, and in two other experiments, there was a trend for smaller lesion diameters for NC EBR-1 than for Piedmont. The F_1s were intermediate to their respective parents, but they were not always significantly different from either or both parents.

Additional key words: *Alternaria solani*, tomato diseases

Early blight of tomato (*Lycopersicon esculentum* Mill.) (incited by the fungus *Alternaria solani* (Ellis & Martin) Jones & Grout [8]) is characterized by dark-colored leaf spots that expand and coalesce to cause defoliation (7). Where frequent rainfall and heavy dew are common, such as in the western North Carolina mountains, disease can be very severe. Recommendations for tomato early blight control in western North Carolina include initiating protectant fungicide applications within 1–2 days of transplanting and continuing on a 5-day schedule thereafter, with additional applications after heavy rains.

Various aspects of early blight resistance have been studied (2,4,6,10, 13,14,16,20). The objective of this study was to compare the tomato early blight resistance in NC EBR-1, an advanced breeding line derived from *L. hirsutum* Humb. & Bonpl. PI 126445 (1,10), with other known resistant and susceptible lines in both the field and greenhouse to determine if the level of resistance would be useful in an applied breeding program.

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MATERIALS AND METHODS

Field studies (1). Three early blight-resistant tomato breeding lines and one susceptible check were used in field tests in 1983, 1984, and 1985. The resistant lines were 71B2, a breeding line released by the USDA-ARS, Beltsville, MD (5); C1943, a breeding line with both foliar (6) and stem (16) resistance and which was released by the Campbell Agricultural Research Institute; and NC EBR-1, a breeding line developed at North Carolina State University (NCSU) that has resistance derived from *L. hirsutum* PI 126445 (1,10). Flora-Dade is a susceptible cultivar released by the Florida Agricultural Experiment Station (19).

In all three seasons, 4- to 6-wk-old seedlings were transplanted to plots with a row spacing of 1.5 m and a plant spacing of 0.6 m. Cultural practices recommended for growing trellised, fresh-market tomatoes in western North Carolina were followed. The 5-day recommended protectant fungicide spray schedule was stopped 15–30 days after transplanting, and a 10-day spray schedule was resumed 15–21 days later. The fungicide spray used was a 378.5-L tank mixture of 454 g mancozeb (Dithane M-45 80WP) and 454 g anilazine (Dyrene 50WP) per acre. In 1983, the plants in the field were artificially inoculated with local isolates of *A. solani* conidia but not in 1984 and 1985. Inoculum preparation and inoculation were carried out similarly to the procedures described by Barksdale (3).

In 1983, six-plant plots were evaluated in four replicates in a randomized complete block design. Disease was estimated as percent defoliation (4–6, 15,20) on each of four dates with a single rating assigned to each plot. In 1984 and 1985, six-plant plots were evaluated in three replicates in a randomized complete block design. Percent defoliation was

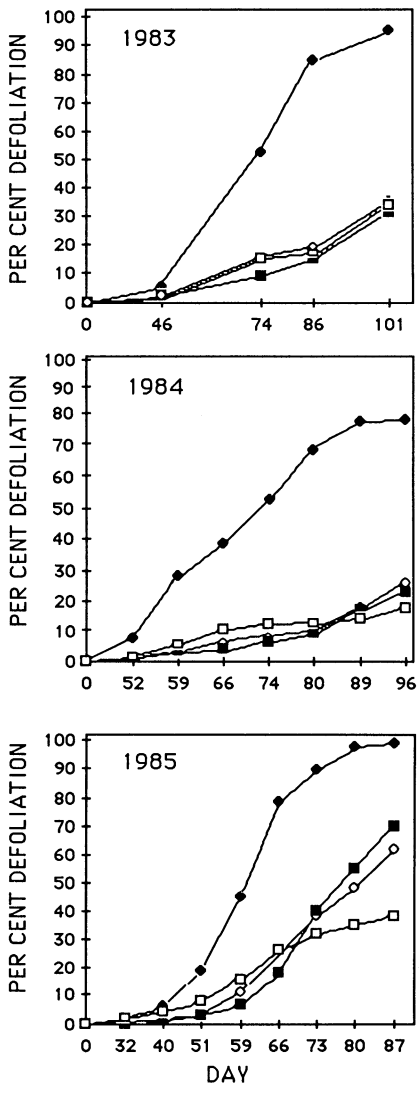

Fig. 1. Estimated percent defoliation for tomato lines artificially (1983) and naturally (1984 and 1985) inoculated with *Alternaria solani* as plotted over time. Protectant fungicides were applied at 5-day intervals for 2–4 wk after transplanting and subsequently at 10-day intervals. Solid diamond = Flora-Dade, open diamond = C1943, solid square = 71B2, and open square = NC EBR-1.

estimated seven times in 1984 and eight times in 1985 as a single rating per plot.

Area under the disease progress curve ($AUDPC = \Sigma_{i=1}^{n} [(R_{i+1} + R_i)/2][t_{i+1} - t_i]$, where R = rating (estimated proportion defoliated tissue) at the i^{th} observation, t_i = time (days) since previous rating at the i^{th} observation, and n = total number of observations) (18) was calculated for all lines over each replicate each year and was used in analysis of variance of AUDPC.

Field studies (2). The early blight resistance of NC EBR-1 was compared with that of two susceptible lines (Piedmont and NC 8233(X)-2(X)). F_1s of NC EBR-1 × Piedmont and NC EBR-1 × NC 8233(X)-2(X) were also evaluated. Piedmont is a midseason cultivar released by NCSU (11) and NC 8233(X)-2(X) is an early-season breeding line developed by NCSU that was derived from the cross Piedmont × Florida-1B.

In 1984, 30-day-old seedlings were transplanted to plots with a spacing of 1.5 × 0.5 m. In 1985, 32-day-old seedlings were set at a spacing of 1.5 × 0.6 m. In 1984, the recommended 5-day fungicide spray schedule was stopped 15 days after transplanting (22 days before artificial inoculation) and a 10-day spray schedule was resumed 15 days after inoculation. Inoculum preparation and inoculation were modified versions of Barksdale's methods (3). In 1985, the 5-day spray schedule was stopped 21 days after transplanting and a 10-day spray schedule was resumed 21 days later (no artificial inoculation was performed). Materials and rates for fungicide applications were the same as mentioned earlier.

In both years, six-plant plots were evaluated in a randomized complete block design with four replicates. To ensure equal inoculum pressure throughout the field in 1984, individual plants of a susceptible check (Flora-Dade) were alternated with individual test plants. In 1985, two susceptible check plants (Flora-Dade) were planted at the beginning and end of each plot for this purpose.

Percent defoliation was estimated using the Horsfall-Barratt rating scheme (12). Ten ratings (about one per week for 10 wk) were made for each plant in the study in both years. Ratings were converted to percent values using Elanco conversion tables (Elanco Products Co., Indianapolis, IN) and AUDPC was calculated for each plant and used in analysis of variance.

Greenhouse studies. Tomato genotypes used in greenhouse studies were the same as those used in field studies 2. NC EBR-1 was used as an early blight-resistant genotype and Piedmont and NC 8233(X)-2(X) were used as susceptible genotypes. In one study, F_1s (NC EBR-1 × Piedmont and NC EBR-1 × NC 8233(X)-2(X)) were also used for disease comparisons. There were eight replicates in fall 1984, six replicates in summer 1985, and nine replicates in fall 1985. Single plants were used as replicates.

Twenty-eight- to 40-day-old plants in 10-cm plastic pots were inoculated by placing a single drop of inoculum (about 0.05 ml containing 1×10^4–1.5×10^4 *A. solani* conidia per milliliter) on terminal leaflets of basal leaves using a Pasteur pipet. Plants were then arranged in a

Table 1. Mean AUDPC[w] for field-grown tomato lines artificially (1983) and naturally (1984 and 1985) inoculated with *Alternaria solani* in three seasons

Line	1983[x]	1984[y]	1985[y]
Flora-Dade	2,980 a[z]	2,287 a	2,789 a
C1943	833 b	430 b	1,128 b
71B2	640 b	382 b	1,126 b
NC EBR-1	804 b	479 b	1,023 b

[w] $AUDPC = \Sigma_{i=1}^{n} [(R_{i+1} + R_i)/2][t_{i+1} - t_i]$, where R = rating (proportion of defoliated tissue) at the i^{th} observation, t_i = time (days) since previous rating at the i^{th} observation, and n = total number of observations.

[x] Each value is the average of a single rating of a plot of six plants over four replicates and four rating dates.

[y] Each value is the average of a single rating of a plot of six plants over three replicates and seven (1984) or eight (1985) rating dates.

[z] Values within a column followed by the same letter do not differ significantly at $P = 0.05$ according to the Waller-Duncan K-ratio t test.

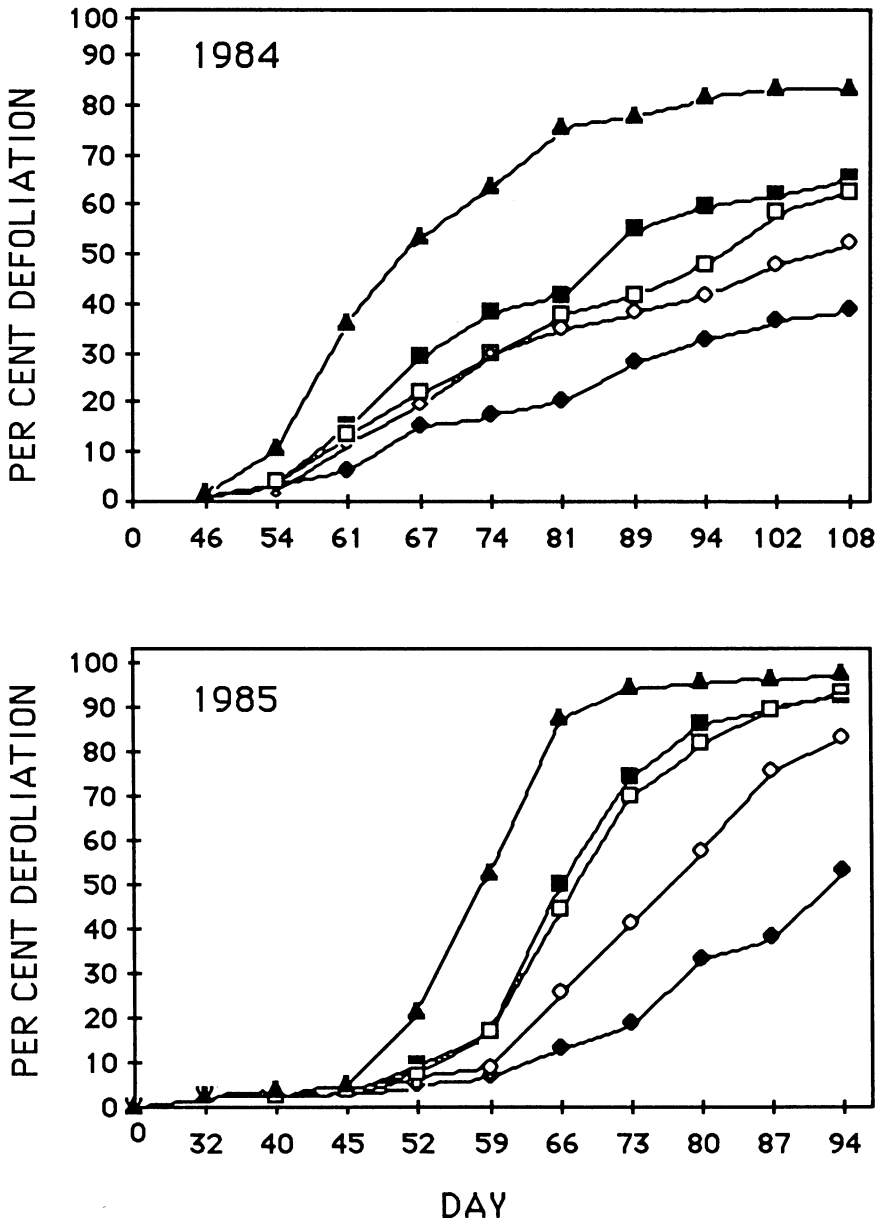

Fig. 2. Estimated percent defoliation of tomato lines artificially (1984) and naturally (1985) inoculated with *Alternaria solani* as plotted over time. Protectant fungicides were applied at 5-day intervals for 2–4 wk after transplanting and subsequently at 10-day intervals. Solid triangle = NC 8233, open square = EBR-1 × 8233, solid square = Piedmont, open diamond = EBR-1 × Piedmont, and solid diamond = NC EBR-1.

randomized complete block design in a dew chamber that consisted of a polyethylene tent attached to a wooden frame over a greenhouse bench. Each evening, the plants were misted, the bench was watered down and the sides of the chamber were secured tightly. A 7.6-L capacity, cool-mist humidifier was operated continuously throughout the night to maintain high humidity necessary for disease development (3,15,17,20). Each morning, the sides of the chamber were opened for ventilation. One lesion per plant was measured to determine lesion diameter. Measurements were begun on day 2 after inoculation in fall 1984 and on day 3 after inoculation in the two 1985 studies. Lesion diameters were recorded until lesion expansion resulted in leaf blighting on the most susceptible plants. An analysis of variance was performed on the data to determine differences in lesion expansion over time.

Table 2. Mean AUDPC[w] for field-grown tomato lines artificially[x] (1984) and naturally (1985) inoculated with *Alternaria solani* in two seasons

Line	1984	1985
NC 8233 (X)-2(X)	3,394 a[y,z]	3,540 a
Piedmont	2,177 b	2,657 b
(NC EBR-1 × NC 8233)	1,887 c	2,541 b
(NC EBR-1 × Piedmont)	1,736 c	1,837 c
NC EBR-1	1,206 d	1,019 d

[w] $AUDPC = \Sigma_{i=1}^{n} [(R_{i+1} + R_i)/2][t_{i+1} - t_i]$, where R = rating (proportion of defoliated tissue) at the i^{th} observation, t_i = time (days) since previous rating at the i^{th} observation, and n = total number of observations.

[x] Inoculum density was measured at about 7,000 spores per milliliter with a hemacytometer.

[y] Each value is the average of six ratings per plot over four replicates and 10 rating dates.

[z] Values within a column followed by the same letter do not differ significantly at $P = 0.05$ according to the Waller-Duncan K-ratio t test.

RESULTS AND DISCUSSION

Field studies (1). Percent defoliation progressed much slower for NC EBR-1, 71B2, and C1943 than for Flora-Dade, which defoliated rapidly after reaching 10% defoliation (Fig. 1). Defoliation curves for NC EBR-1, 71B2, and C1943 were very similar in 1983 and 1984. In 1985, disease development for NC EBR-1 slowed in late season compared with the other two resistant lines. An analysis of variance of percent defoliation for the last rating in each season indicated no significant differences among resistant lines in 1983 and 1984. In 1985, however, NC EBR-1 had significantly less disease than 71B2 but not C1943. AUDPC values for NC EBR-1, 71B2, and C1943 did not differ significantly in any of the three seasons and were much lower than the susceptible check, Flora-Dade (Table 1).

Field studies (2). Disease progress curve comparisons showed that NC EBR-1 consistently had lower percent defoliation ratings than all other lines from the third disease rating through the final disease rating (Fig. 2). Piedmont and NC 8233(X)-2(X) had similar defoliation curves in both years, but throughout both seasons, Piedmont had lower percent defoliation ratings. Defoliation curves for the F_1s in both families in both years were intermediate to their respective parents. NC EBR-1 had much lower AUDPC values than all other lines in both 1984 and 1985 (Table 2). The AUDPC values for the F_1s were intermediate and significantly different from those of their parents in both years.

Greenhouse studies. NC EBR-1 had a smaller average lesion diameter than NC 8233(X)-2(X) (Table 3). In one experiment, NC EBR-1 had a significantly smaller average lesion diameter than Piedmont, whereas in the other two experiments, the difference was not significant. There was a trend for smaller average lesion diameter for NC EBR-1 than for Piedmont. F_1 average lesion diameters were intermediate to those of the parents although not significantly different from either.

Early blight resistance in NC EBR-1 was equivalent to that in C1943 and 71B2 under field conditions at Fletcher, NC. C1943 and 71B2 have foliar resistance conferred by recessive genes (2,5,16), and NC EBR-1 confers resistance intermediate to it and susceptible parents in the F_1 generation (Table 2).

Although vines are excessively vigorous and fruit size is smaller than desired, NC EBR-1 has other desirable vine and fruit characteristics. Gardner (9) reported that when he selected for increased yield, better vine type, and improved fruit quality in successive generations of crosses, resistance levels decreased when PI 126445 was used as the resistant source. Continued stringent testing of material for early blight resistance during successive crossing and selection will be important because of this phenomenon.

To develop resistant cultivars, NC EBR-1 would best be used in a pedigree program. The backcross program that resulted in the selection of NC EBR-1 was successful in that it produced a line with a high level of resistance to tomato early blight. NC EBR-1 should now be crossed to susceptible lines that have good plant and fruit quality, and successive generations should be evaluated for both disease resistance and superior quality.

Ultimately, cultivars derived from this material would best be used in conjunction with a reduced fungicide application program (15); therefore, resistance levels as high as those seen in NC EBR-1 might not be necessary. Before cultivar release, lines should be tested under varied fungicide application regimes to determine the most efficient way to use this resistance in an early blight management program.

Table 3. Average lesion diameters (mm) for tomato lines inoculated with *Alternaria solani*[x] in the greenhouse as measured over time

	Day				
Line	**2**	**4**	**6**	**8**	**10**
Fall 1984					
NC 8233(X)-2(X)	3.0 a[y,z]	6.3 a	9.4 a	12.6 a	15.2 a
Piedmont	3.1 a	6.2 a	8.5 a	11.2 a	13.2 a
NC EBR-1	2.7 a	4.5 b	6.4 b	8.1 b	10.4 b
Summer 1985	**3**	**5**	**7**		**8**
NC 8233(X)-2(X)	5.8 a	9.8 a	13.6 a		15.7 a
(NC EBR-1 × NC 8233(X)-2(X)	5.0 a	8.2 a	12.8 a		14.2 ab
Piedmont	4.1 a	7.7 a	11.5 ab		14.0 ab
(NC EBR-1 × Piedmont)	3.8 a	7.1 a	11.2 ab		12.8 ab
NC EBR-1	3.4 a	6.3 a	9.2 b		10.7 b
Fall 1985	**3**	**5**	**7**		**9**
NC 8233(X)-2(X)	3.2 a	6.4 a	11.7 a		16.0 a
Piedmont	1.8 b	3.7 b	7.2 b		10.1 b
NC EBR-1	1.8 b	3.2 b	6.4 b		8.6 b

[x] Inoculum density was measured at about 12,000 conidia per milliliter in fall 1984 and fall 1985 and 13,000 conidia per milliliter in summer 1985 with a hemacytometer.

[y] Each value is an average based on a single-lesion measurement per plant for eight replicates (fall 1984), six replicates (summer 1985), and nine replicates (fall 1985).

[z] Values within a column followed by the same letter do not differ significantly at $P = 0.05$ according to the Waller-Duncan K-ratio t test.

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