Evaluation of Pepper Fruit for Resistance to *Phytophthora capsici* in a Recombinant Inbred Line Population, and the Correlation with Fruit Shape

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Abstract

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Phytophthora capsici causes fruit, root, and foliar blight on pepper (*Capsicum annuum*) in field production. Breeding for disease-resistant commercial pepper cultivars is essential to long-term management of *P. capsici*. In this study, the severity of *Phytophthora* fruit rot was evaluated in an F6 recombinant inbred line population between CM334, a landrace from Mexico, and the commercial 'Early Jalapeño'. The two parents and 67 progeny lines were evaluated for fruit rot resistance at 3 and 5 days post inoculation (dpi) using three *P. capsici* isolates. Fruit shape was also evaluated for each line, and the correlation between

shape and disease symptoms was investigated. Significant differences were detected among lines in lesion area measured 3 and 5 dpi, and in phenotypic traits (fruit length, width, and shape index). Of the fruit phenotypic traits measured, only fruit shape index had a significant, albeit weak ($r = 0.2892$, $P = 0.02$), correlation with lesion area when inoculated, and with only one of the three isolates of *P. capsici* evaluated. These results suggest that breeding for fruit rot resistance in pepper will have minimal linkage with fruit shape in the CM334 background.

Pepper (*Capsicum annuum*) is an important vegetable crop worldwide. In the United States, pepper plants are grown widely across the country (1). Bell pepper fruit, renowned for high levels of vitamin C and sweet flavor, were ranked eighth and chili pepper fruit twentieth in total vegetable production value in the United States in 2011 (1). From 2010 to 2011, the production area of bell and chili pepper increased across the United States, with more than 31,000 ha harvested and a combined production value greater than \$830 million (1).

Phytophthora capsici L., an oomycete, is a devastating pathogen that can cause fruit and root rot and foliar blight in all pepper market classes, and is found in many pepper-growing regions (15,16). The disease is managed through a combination of chemical and cultural control practices but, under conducive conditions, economic reductions in yield still occur (15). Availability of resistant varieties, an important component for most disease management programs, is limited in the pepper industry (10). A few *Phytophthora* root rot-tolerant varieties have been developed but many were susceptible to highly virulent isolates of *P. capsici* and none were resistant to fruit rot (10). Breeding and research programs have continued to focus on *Phytophthora* root rot resistance, with minimal consideration regarding other *P. capsici*-induced diseases such as fruit rot.

To our knowledge, research on the inheritance of resistance to *Phytophthora* fruit rot in pepper has been limited to a single published study (22), which determined that resistance was controlled by a single dominant gene. This differs from the complex inheritance of *P. capsici* root rot and foliar blight found in other studies and suggests that resistance to each *Phytophthora*-induced disease may be inherited independently (3,17,27).

Pepper fruit morphology is important for growers of both sweet bell and chili-type pepper (4). Fruit ideotype, the ideal proportion and shape of the fruit, differs among and within pepper classes. Bell pepper fruit, accounting for the largest portion of the pepper

market (1), are large and blocky with thick, nonpungent, sweet flesh (4). Chili pepper fruit have a larger range in size and shape depending on the subtype (e.g., serrano, jalapeño, poblano, habanero, and so on; 4). A typical chili-type pepper has a slender shape with either a blunt or conical end, thin flesh, and varying degrees of pungency. Fruit ideotype for a market class and disease resistance are important considerations when breeding and choosing a variety.

Previous studies have tested correlations between *Phytophthora* fruit rot susceptibility and pepper fruit age, cuticle thickness, pericarp thickness, and fruit shape (5,26). Cuticle and pericarp thickness were evaluated but only cuticle thickness was significantly correlated with resistance (5). Fruit age, wounding, sugar content, and peroxidase content significantly impacted disease susceptibility in pepper fruit (5). Pepper heat index had no significant correlation with root rot or fruit rot (26). In 1978, Saini and Sharma (22) looked at the inheritance of pepper fruit rot resistance to *P. capsici* and found no correlation with pungency. In each of these studies, many characteristics of fruit morphology and physiology were evaluated. However, a major limitation in these studies was the small number of genotypes evaluated, with undetermined genetic relationships among the genotypes. Finding trait associations using unrelated material is difficult when evaluating small numbers of genotypes (<100) or when there is limited variation in the genotypes (e.g., if all the genotypes are susceptible; 30,31). The objectives of this study were to phenotype fruit shape index, measured as the ratio of fruit length to width, and *Phytophthora* fruit rot resistance in a segregating mapping population, previously characterized for *Phytophthora* root rot, stem rot, and foliar blight resistance (24); and to identify significant correlations between fruit morphology and disease resistance following exposure of the fruit to *P. capsici*.

Materials and Methods

Plant material. A previously published, F2-derived F6 New Mexico State University recombinant inbred line (NMRIL) pepper population ('Early Jalapeño' × 'Criollo de Morelos'; CM334) was developed by Dr. Paul Bosland. CM334 is a primary source of resistance to *Phytophthora* root rot, and this population had previously been shown to segregate for resistance to *P. capsici*-induced root rot and foliar blight resistance (24,25). The population, consisting of 67 individuals, was used for fruit screening (24,25). In all, 10 seeds from each line were planted 1 seed/cell into 72-cell

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flats containing a soil-less potting medium (Sure-mix Michigan Grower Products, Inc.). The seedlings were then grown in a glass-house at Michigan State University in 3.79-liter round pots containing the same soil-less potting medium.

***P. capsici* isolate selection and inoculum preparation.** Three isolates of *P. capsici* were selected from the culture collection of Dr. Mary Hausbeck (Michigan State University). Isolates had previously been characterized by mating type (A1 or A2), mefenoxam sensitivity (S = sensitive or I = insensitive), and host of origin (10,12,20,21). The *P. capsici* isolates used were 12889 (A1, I, and pepper, respectively), OP97 (A1, S, pickling cucumber), and 13709 (A1, S, lima bean). The *P. capsici* isolates varied in virulence on pepper fruit and represented unique genetic clusters (14). Isolates 12889 and OP97 were from Michigan and isolate 13709 was from New Jersey. Isolate virulence was confirmed by inoculating bell pepper fruit and reisolating the pathogen prior to the experiment. *P. capsici* isolates were maintained on unclarified V8 agar (UCV8; 16 g of agar, 30 mM CaCO₃, 160 ml of unfiltered V8 juice, and 840 ml of distilled water) and grown under constant fluorescent light at room temperature (25 ± 2°C).

Fruit screen. Detached immature green pepper fruit (3.8 to 6.4 cm in length, depending on the line) from each line were bulked, taken to the lab, surface-disinfested in a 10% bleach solution (0.62% sodium hypochlorite) for 5 min, and rinsed in distilled water. Nine individual pepper fruit from each line were inoculated with each isolate of *P. capsici*. A single agar plug (6 mm in diameter) from an actively growing *P. capsici* culture on V8 agar was placed, topside down, on the upper, nonwounded surface of a pep-

per fruit. A sterile microcentrifuge cap was then placed on top of each plug and affixed to the fruit using petroleum jelly. Control pepper fruit were inoculated as described above using a sterilized, noncolonized plug (6 mm in diameter) of UCV8 medium. Pepper fruit were placed into disinfested plastic trays that contained wet paper towels, and the trays were covered with clear plastic lids to maintain high relative humidity in each chamber. Fruit were placed into the chambers in a completely randomized design for both lines and isolates. Chambers were incubated at room temperature under constant fluorescent lighting.

At 3 and 5 days post inoculation (dpi), maximum lesion width and length were measured for each individual fruit using a caliper. Isolations were performed on approximately 20% of the pepper fruit displaying symptoms to confirm the presence of the appropriate *P. capsici* isolate. External layers of each fruit were peeled, and three sections (approximately 1 to 3 mm long) of the symptomatic tissue were plated onto ampicillin- and rifampicin-amended V8 agar plates. Cultures were incubated at room temperature under constant fluorescent lighting, and checked microscopically to confirm the presence of *P. capsici* using morphological characteristics described in the key by Waterhouse (29). After morphological confirmation, cultures were tested for mating type and mefenoxam sensitivity to confirm the phenotype matched that of the isolate used for inoculation (14,21).

Trait evaluation. Thirty immature pepper fruit from each line were evaluated for fruit width (maximum width in centimeters), fruit length (distance from pedicel attachment in centimeters), and fruit shape index (the ratio of length to width). Fruit pericarp was

Table 1. Mean values for pepper fruit traits and lab-inoculated fruit disease severity for 67 progeny lines and 2 parent lines ('Criollo de Morelos' 334 [CM] and 'Early Jalapeño' [EJ]) of New Mexico recombinant inbred line (NMRIL) mapping population inoculated with each of the three isolates of *Phytophthora capsici* (13709, 12889, and OP97) in a laboratory assay¹

Line	Lesion area 3 dpi (cm ²)			Lesion area 5 dpi (cm ²)			Shape	Length (cm)	Width (cm)	Color	Peri (cm)
	13709	12889	OP97	13709	12889	OP97					
CM	0.13	0.36	0.41	1.13	3.09	2.33	1.99	2.93	1.49	Green	0.15
EJ	5.30	2.67	3.71	29.23	12.90	38.53	1.96	3.40	1.78	Green	0.24
A	1.35 ^v	0.96 ^z	1.21 ^y	17.23 ^w	9.33 ^z	21.33 ^w	1.66 ^y	2.89 ^z	1.76 ^w	Dark green	0.20 ^v
AA	0.59 ^y	1.76 ^w	1.26 ^y	6.21 ^y	15.89 ^w	14.63 ^w	2.41 ^x	3.93 ^x	1.67 ^w	Light green	0.18 ^y
AAA	1.41 ^v	1.93 ^w	1.91 ^w	18.96 ^w	18.17 ^w	50.00 ^w	2.18 ^z	3.32 ^w	1.54 ^y	Purple	0.23 ^w
AAB	0.23 ^y	1.68 ^z	0.37 ^y	1.32 ^y	4.09 ^y	3.49 ^y	1.50 ^v	2.67 ^z	1.82 ^w	Purple	0.20 ^v
AAC	0.44 ^y	1.13 ^z	0.63 ^y	1.48 ^y	2.92 ^y	3.00 ^y	2.83 ^x	3.52 ^w	1.27	Light green	0.13 ^y
AAD	5.73 ^w	1.35 ^z	1.19 ^y	42.44 ^w	25.74 ^w	24.54 ^w	2.27 ^x	3.55 ^w	1.58 ^y	Green	0.18 ^y
AAE	1.55 ^v	3.61 ^w	1.57 ^z	50.00 ^w	50.00 ^x	39.33 ^w	2.31 ^x	3.47 ^w	1.54 ^y	Green	0.20 ^v
AAF	0.83 ^y	1.41 ^z	1.11 ^y	1.26 ^y	2.84 ^y	3.22 ^y	2.78 ^x	3.99 ^x	1.45 ^y	Light green	0.22 ^w
AAG	0.43 ^y	0.35 ^y	1.19 ^y	1.79 ^y	3.81 ^y	12.75 ^w	1.67 ^y	2.97 ^z	1.79 ^w	Green	0.15 ^y
AAJ	3.57 ^w	1.00 ^z	1.89 ^w	26.65 ^w	8.76 ^z	43.90 ^w	3.60 ^x	4.57 ^x	1.29 ^y	Light green	0.21 ^w
AAK	2.58 ^w	0.83 ^z	1.16 ^y	18.75 ^w	3.63 ^y	9.42 ^y	2.51 ^x	4.06 ^x	1.62 ^y	Green	0.24 ^w
AAM	1.28 ^y	0.81 ^z	1.55 ^z	10.46 ^w	6.54 ^y	18.33 ^w	2.96 ^x	4.26 ^x	1.46 ^y	Light green	0.19 ^v
AAN	1.05 ^y	0.67 ^y	0.13 ^y	2.74 ^y	4.37 ^y	3.12 ^y	2.14 ^z	3.64 ^w	1.70 ^w	Mixed	0.11 ^y
AAO	1.92 ^v	2.96 ^w	1.05 ^y	34.79 ^w	25.21 ^w	24.08 ^w	3.01 ^x	4.20 ^x	1.43 ^y	Green	0.16 ^y
AAP	1.11 ^y	1.16 ^z	1.33 ^z	29.04 ^w	50.00 ^x	33.22 ^w	3.18 ^x	4.50 ^x	1.43 ^y	Light green	0.13 ^y
AAQ	1.78 ^v	3.56 ^w	1.53 ^z	25.38 ^w	45.38 ^w	28.44 ^w	3.52 ^x	4.96 ^x	1.45 ^y	Dark green	0.17 ^y
AAR	0.41 ^y	0.84 ^z	1.10 ^y	1.17 ^y	3.55 ^y	3.41 ^y	2.20 ^y	3.84 ^x	1.76 ^w	Green	0.21 ^w
AAS	1.07 ^y	1.08 ^z	4.80 ^w	7.31 ^y	2.90 ^y	20.24 ^w	2.78 ^x	4.13 ^x	1.53 ^y	Light green	0.21 ^w
AAT	1.06 ^y	3.25 ^w	4.04 ^w	9.13 ^w	35.39 ^w	36.28 ^w	2.37 ^x	4.01 ^x	1.70 ^w	Mixed	0.18 ^v
AAU	0.58 ^y	0.34 ^y	0.30 ^y	5.80 ^y	5.76 ^y	9.25 ^y	2.05 ^z	3.35 ^w	1.65 ^w	Green	0.15 ^y
AB	0.42 ^y	0.85 ^z	0.97 ^y	5.36 ^y	4.10 ^y	44.60 ^w	2.27 ^x	3.62 ^w	1.63 ^y	Light green	0.27 ^w
AC	2.37 ^w	1.43 ^z	2.43 ^w	18.04 ^w	22.95 ^w	4.73 ^y	2.58 ^x	3.76 ^x	1.49 ^y	Green	0.16 ^y
AD	2.32 ^w	2.69 ^w	1.16 ^y	27.64 ^w	13.72 ^w	22.26 ^w	2.72 ^x	3.90 ^x	1.52 ^y	Mixed	0.26 ^w
AE	0.87 ^y	0.73 ^y	0.33 ^y	6.61 ^y	2.03 ^y	4.18 ^y	2.29 ^x	3.23 ^z	1.42 ^y	Dark green	0.13 ^y
AF	3.91 ^w	3.34 ^w	2.78 ^w	31.96 ^w	30.09 ^w	36.27 ^w	2.20 ^y	3.70 ^w	1.69 ^w	Green	0.26 ^w
AG	0.46 ^y	0.26 ^y	0.25 ^y	3.47 ^y	0.90 ^y	7.32 ^y	2.78 ^x	4.46 ^x	1.63 ^y	Mixed	0.13 ^y

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¹ Data show the results pooled across nine individual replicate fruit for each line. Fruit from each NMRIL were evaluated for lesion area (cm²) measured at 3 and 5 days post inoculation (dpi), shape (length/width), length, width, and pericarp thickness (Peri) with *P. capsici*, and fruit color. For fruit color, lines with stripes are denoted by an asterisk (*). Significant differences were identified using the lsmeans statement with the analysis of variance ($P = 0.05$).

^v Pepper lines for which the fruit trait was significantly less than fruit of either CM or EJ.

^w Pepper lines for which the fruit trait was between and significantly different than EJ and CM.

^x Pepper lines for which the fruit trait was not significantly different than fruit of EJ.

^y Pepper lines for which the fruit trait was significantly greater than fruit of either CM or EJ.

^z Pepper lines for which the fruit trait was not significantly different than fruit from CM only.

^z Pepper lines for which the fruit trait was not significantly different from fruit of either CM or EJ.

measured as the thickness of the wall of the fruit in millimeters using a caliper. Fruit color was evaluated on a visual basis as light green, green, dark green, or purple. Only 20 fruit from NMRIL lines D, H, and M were evaluated due to minimal fruit set for each of these lines. Fruit measurements were performed using a caliper.

Evaluations and data analysis. Data for the control fruit, each inoculated with a sterilized V8 plug, were removed prior to data analysis to avoid violating homogeneous variance assumptions. Lines were evaluated for initial resistance to infection measured at 3 dpi and the rate of lesion expansion (difference in lesion size from 3 to 5 dpi) and resistance to prolonged exposure to *P. capsici* measured at 5 dpi, corresponding to inducibility, infectivity, and receptivity, respectively, as described by Pochard et al. (19). Occasionally, symptoms were areas of many tiny lesions, similar to a hypersensitive response. Data were adjusted to account for the percentage of the maximum lesion area that was symptomatic. If the symptoms (browning or watersoaking of the fruit) covered >75% of the lesion area, the fruit was assigned a coverage score of 1; if symptoms covered 50 to 75% of the lesion area, the fruit was assigned a score of 0.75; if area coverage was 25 to 50%, the assigned score was 0.5; and if area coverage was ≤25%, the assigned score was 0.25. Lesion area was multiplied by the coverage score to determine the final lesion area for statistical analysis. When the pepper fruit was completely covered with symptoms, the lesion area was given a value of 50, indicating that the pathogen had encompassed the entire fruit, because this value was greater than partial lesion coverage of any fruit lines evaluated. For fruit disease ratings, lesion and line were fixed effects. Fruit

width, length, shape ratio, and disease ratings were tested for significance among lines using an analysis of variance for each variable with the PROC MIXED procedure of SAS (version 9.3; SAS Institute). Significant interactions between main effects were separated using the least-squared means (LSMEANS) function when applicable for lines and isolates. Correlations among fruit traits and fruit reactions to inoculation were investigated using the PROC CORR procedure of SAS for each isolate of *P. capsici* at 3 and 5 dpi.

Results

Disease resistance. Significant differences in disease response to *P. capsici* were detected among pepper lines at 3 ($P < 0.0001$) and 5 ($P < 0.0001$) dpi. Significant interactions among individual pepper lines and *P. capsici* isolates were also detected at 3 ($P = 0.024$) and 5 ($P < 0.0001$) dpi. Isolate, a main effect, was not significant at 3 dpi ($P = 0.310$) but was significant at 5 dpi ($P = 0.0005$).

At 3 dpi, most lines (43 to 49) were not significantly different in fruit disease severity than the fruit of CM334, depending on the isolate of *P. capsici*. At 5 dpi, however, fruit from many lines (30 to 42) were as or more susceptible than Early Jalapeño, depending on the isolate. The mean of the population at 3 dpi was $2.64 \text{ cm}^2 \pm 2.01$ standard deviations. At 3 dpi, fruit of line D were the most susceptible of the 67 NMRILs, with a lesion area of 7.75 cm^2 , which was not significantly different than the reaction of fruit of Early Jalapeño; in contrast, fruit from line H were the least susceptible, with a lesion area of 0.23 cm^2 , which did not differ from the reaction of fruit from CM334 (Table 1). None of the lines ex-

Table 1. (continued from previous page)

Line	Lesion area 3 dpi (cm ²)			Lesion area 5 dpi (cm ²)			Shape	Length (cm)	Width (cm)	Color	Peri (cm)
	13709	12889	OP97	13709	12889	OP97					
AH	0.46 ^y	1.48 ^y	0.79 ^y	6.67 ^y	5.71 ^y	2.52 ^y	1.86 ^z	3.21 ^z	1.71 ^w	Mixed	0.27 ^w
AI	0.70 ^y	1.38 ^y	0.64 ^y	2.36 ^y	3.58 ^y	15.38 ^w	1.77 ^z	3.30 ^w	1.86 ^w	Mixed	0.24 ^w
AJ	1.95 ^v	1.40 ^z	0.58 ^y	11.64 ^w	17.67 ^w	4.28 ^y	2.23 ^x	4.40 ^x	1.39 ^y	Mixed	0.17 ^y
AK	0.31 ^y	0.70 ^y	1.00 ^y	2.64 ^y	14.63 ^w	12.14 ^w	1.61 ^x	3.92 ^x	1.53 ^y	Light green	0.17 ^y
AL	4.43 ^w	6.44 ^w	2.61 ^w	50.00 ^w	39.74 ^w	30.55 ^w	2.33 ^x	3.88 ^x	1.67 ^w	Light green	0.14 ^y
AM	0.40 ^y	0.28 ^y	0.35 ^y	10.59 ^w	2.58 ^y	5.20 ^y	1.93 ^z	3.94 ^x	2.07 ^x	Light green	0.27 ^w
AN	0.19 ^y	0.86 ^z	0.68 ^y	4.63 ^y	4.73 ^y	3.85 ^y	2.37 ^x	4.14 ^x	1.77 ^w	Green	0.22 ^w
AO	0.28 ^y	1.06 ^z	0.79 ^y	6.95 ^y	13.18 ^w	12.17 ^w	3.17 ^x	4.53 ^x	1.45 ^y	Green	0.28 ^x
AP	2.65 ^w	0.51 ^y	1.64 ^z	37.80 ^w	3.51 ^y	33.44 ^w	1.46 ^y	2.71 ^z	1.87 ^w	Dark green	0.21 ^w
AQ	0.47 ^y	2.24 ^w	3.21 ^w	13.33 ^w	25.56 ^w	29.18 ^w	2.38 ^x	3.35 ^w	1.41 ^y	Purple*	0.14 ^y
AR	0.86 ^y	0.37 ^y	0.63 ^y	16.27 ^w	22.63 ^w	14.19 ^w	1.80 ^z	2.70 ^z	1.50 ^y	Dark green	0.19 ^v
AS	0.15 ^y	0.96 ^z	0.93 ^y	0.64 ^y	2.00 ^y	4.48 ^y	2.38 ^x	3.98 ^x	1.68 ^w	Mixed	0.16 ^y
AT	0.44 ^y	0.28 ^y	0.08 ^y	3.86 ^y	0.62 ^y	0.29 ^y	2.06 ^z	3.59 ^w	1.79 ^w	Light green	0.16 ^y
AU	0.83 ^y	1.86 ^w	0.83 ^y	28.01 ^w	50.00 ^x	50.00 ^w	4.26 ^x	4.59 ^x	1.09 ^y	Green	0.15 ^y
AV	1.14 ^y	0.76 ^y	1.25 ^y	10.87 ^w	13.39 ^w	33.07 ^w	1.84 ^z	3.43 ^w	1.89 ^w	Dark green	0.19 ^v
AW	0.30 ^y	0.69 ^y	0.61 ^y	10.08 ^w	13.74 ^w	17.86 ^w	1.70 ^y	3.00 ^z	1.80 ^w	Green	0.17 ^y
AX	1.31 ^v	0.58 ^y	0.19 ^y	24.39 ^w	10.78 ^z	50.00 ^w	1.95 ^z	3.20 ^z	1.66 ^w	Purple	0.19 ^v
AY	0.29 ^y	0.30 ^y	0.51 ^y	1.00 ^y	1.08 ^y	3.16 ^y	2.58 ^x	3.99 ^x	1.60 ^y	Green	0.13 ^y
AZ	1.10 ^y	1.11 ^z	2.70 ^w	11.70 ^w	5.43 ^y	15.96 ^w	2.20 ^y	3.64 ^w	1.68 ^w	Mixed	0.12 ^y
B	3.44 ^w	2.36 ^w	1.69 ^z	50.00 ^w	46.54 ^x	24.94 ^w	2.53 ^x	4.09 ^x	1.65 ^w	Green	0.16 ^y
C	1.86 ^v	0.49 ^y	0.94 ^y	43.72 ^w	50.00 ^x	7.96 ^y	2.43 ^x	3.78 ^x	1.60 ^y	Green	0.17 ^y
D	2.69 ^w	5.85 ^w	1.32 ^z	26.64 ^w	18.96 ^w	19.02 ^w	5.22 ^x	5.42 ^x	1.07 ^y	Dark green	0.17 ^y
E	4.07 ^w	4.45 ^w	3.88 ^w	41.18 ^w	33.00 ^w	41.82 ^w	1.93 ^z	3.51 ^w	1.85 ^w	Light green	0.22 ^w
F	0.35 ^y	0.17 ^y	0.14 ^y	6.96 ^y	3.87 ^y	0.83 ^y	2.31 ^x	3.69 ^w	1.63 ^y	Green	0.19 ^v
G	1.43 ^v	3.40 ^w	1.24 ^y	50.00 ^w	45.90 ^x	16.22 ^w	2.65 ^x	4.55 ^x	1.77 ^w	Green	0.21 ^w
H	0.29 ^y	0.18 ^y	0.15 ^y	1.52 ^y	0.88 ^y	1.24 ^y	1.85 ^z	2.78 ^z	1.52 ^y	Light green	0.18 ^y
I	1.50 ^v	2.66 ^w	4.21 ^w	5.98 ^y	14.86 ^w	23.79 ^w	2.56 ^x	4.15 ^x	1.66 ^w	Green	0.24 ^w
J	0.85 ^y	1.51 ^z	0.90 ^y	11.71 ^w	12.88 ^w	7.37 ^y	2.45 ^x	3.48 ^w	1.44 ^y	Light green	0.19 ^v
K	0.59 ^y	0.13 ^y	0.14 ^y	5.70 ^y	2.37 ^y	3.50 ^y	1.70 ^y	2.89 ^z	1.72 ^w	Dark green	0.18 ^v
L	1.60 ^v	0.62 ^y	0.55 ^y	5.72 ^y	6.53 ^y	5.24 ^y	1.77 ^z	3.07 ^z	1.76 ^w	Light green	0.24 ^w
M	2.40 ^w	2.00 ^w	1.13 ^y	17.18 ^w	9.10 ^z	24.24 ^w	2.19 ^z	3.03 ^z	1.39 ^y	Purple	0.18 ^y
N	0.96 ^y	0.77 ^y	1.93 ^w	12.22 ^w	8.82 ^z	45.59 ^w	2.99 ^x	3.84 ^x	1.29 ^y	Light green	0.20 ^v
O	0.73 ^y	0.48 ^y	0.37 ^y	9.93 ^w	2.75 ^y	14.00 ^w	3.11 ^x	5.14 ^x	1.69 ^w	Green	0.16 ^y
P	0.03 ^y	0.44 ^y	0.13 ^y	1.44 ^y	2.84 ^y	1.08 ^y	2.62 ^x	3.75 ^x	1.45 ^y	Green*	0.11 ^u
Q	1.32 ^v	1.12 ^z	0.54 ^y	14.69 ^w	6.11 ^y	33.09 ^w	2.54 ^x	3.97 ^x	1.58 ^y	Mixed	0.20 ^v
R	2.64 ^w	3.59 ^w	1.22 ^y	21.90 ^w	23.46 ^w	23.06 ^w	2.23 ^x	3.96 ^x	1.80 ^w	Mixed	0.17 ^y
S	0.72 ^y	1.94 ^w	3.47 ^w	5.95 ^y	8.84 ^z	30.68 ^w	3.33 ^x	4.44 ^x	1.36 ^y	Dark green	0.21 ^w
T	0.02 ^y	0.86 ^z	0.19 ^y	0.22 ^y	19.20 ^w	0.97 ^y	1.97 ^z	4.30 ^x	2.19 ^x	Green	0.21 ^v
V	0.81 ^y	0.45 ^y	2.33 ^w	12.40 ^w	9.44 ^z	18.03 ^w	1.85 ^z	2.99 ^z	1.66 ^w	Dark green	0.23 ^w
X	0.99 ^y	0.59 ^y	1.04 ^y	11.44 ^w	2.65 ^y	19.60 ^w	2.21 ^y	3.44 ^w	1.56 ^y	Purple*	0.18 ^y
Z	0.43 ^y	0.91 ^z	0.40 ^y	3.16 ^y	6.01 ^y	8.07 ^y	1.51 ^v	2.56 ^v	1.72 ^w	Purple	0.16 ^y

hibited complete fruit resistance to *P. capsici*, and variation in resistance was evident within each line.

At 5 dpi, the mean $\pm$ standard deviation of the fruit lesion area for the population was 24.63 ± 11.61 cm². Isolate OP97 was the most virulent at 5 dpi, followed by isolates 13709 and 12889, with no significant difference in virulence between 13709 and 12889 or between 13709 and OP97. At 5 dpi, line AAE was the most susceptible, with a mean lesion area of 47.53 cm², which did not differ statistically from fruit of the cultivated parent Early Jalapeño. Fruit from line AT was the most resistant, with a lesion area of 4.82 cm². At 5 dpi, fruit from CM334 were partially resistant to each isolate of *P. capsici*, with a mean lesion area of 10.36 cm², whereas fruit from EJ varied in disease susceptibility, with a mean lesion area of 32.85 cm² (Fig. 1). The frequency distribution of resistance showed variation among isolates of *P. capsici* (Fig. 2).

Line-isolate interactions were significant. For each *P. capsici* isolate, the mean $\pm$ standard deviation lesion area for the population was 14.49 ± 14.57 , 18.50 ± 14.70 , and 15.19 ± 14.27 cm² for isolates 12889, OP97, and 13709, respectively. The most susceptible pepper line-pathogen isolate combination at 3 dpi was fruit from line AL inoculated with isolate 12889, which had a mean lesion area of 6.44 cm² and was statistically equivalent to the reaction of fruit from Early Jalapeño (Table 1). Fruit from Early Jalapeño had mean lesion areas of 2.67, 3.71, and 5.29 cm² for isolates 12889, OP97, and 13709, respectively. Fruit from CM334 had a mean lesion area of 0.36, 0.41, and 0.13 cm² for isolates 12889, OP97, and 13709, respectively. The most resistant combination at 3 dpi was fruit from line T inoculated with isolate 13709, with a mean fruit lesion area of 0.02 cm². No line was significantly more resistant than CM334 or susceptible than Early Jalapeño at 3 or 5 dpi, with the exception of fruit from lines B, C, G, AU, AAE, and AAP that were more susceptible than fruit from Early Jalapeño when inoculated with isolate 12889 at 5 dpi. At 5 dpi, fruit from several lines had a mean lesion area of approximately 50 cm²: AU, AX, and AAA inoculated with isolate OP97; AL, AAE, G, and B inoculated with isolate 13709; and AU, C, AAE, and AAP inoculated with 12889 (Table 1). The most resistant interaction at 5 dpi between line and isolate was fruit from line T inoculated with 13709, with a mean lesion area of 0.21 cm², but

this was not significantly different than the lesion area of CM334.

The mean $\pm$ standard deviation for the lesion expansion rate of the population was 10.99 ± 0.04 cm²/day. The main effect line ($P < 0.0001$) but not the main effect *P. capsici* isolate ($P = 0.067$) was highly significant when evaluating lesion expansion rate. Lesion expansion rate per day also had a significant line-isolate interaction ($P < 0.0001$). The slowest fruit lesion expansion was on fruit from line H inoculated with isolate 13709, with an average growth of 0.06 cm²/day. The most rapid lesion expansion was seen on fruit from line AX inoculated with isolate OP97 at 24.8 cm²/day (R. P. Naegel, unpublished data).

Fruit characteristics. The pepper parents Early Jalapeño and CM334 showed significant differences in phenotypic characteristics. The main effect line was significant ($P > 0.0001$) for each of the traits evaluated. Fruit shape ranged from 1.46 for line AP to 5.22 for line D, with a population mean $\pm$ standard deviation of 2.41 ± 0.64 (Table 1). Fruit length varied from 2.67 cm for line AAB to 5 cm for line AAQ, with a mean $\pm$ standard deviation of 3.73 ± 0.61 cm for the entire population compared with 2.93 and 3.40 cm for CM334 and EJ, respectively (Table 1). Fruit width for the 67 progeny lines ranged from 1.1 cm for line D to 2.2 cm for line T, with a mean $\pm$ standard deviation value for the population of 1.61 ± 0.20 cm, compared with 1.49 and 1.78 cm for CM334 and Early Jalapeño, respectively. Immature fruit color in the population ranged from light, medium, and dark green to purple. The fruit of most of the lines were green (25) or light green (17). Ten individual lines produced dark-green fruit and seven lines had purple fruit (Table 1). Three lines (P, X, and AQ) had purple fruit with green stripes. Fruit length, width, and color for the 67 NMRILs showed a larger variation than that of the two parent lines, indicating that both parents contained allelic variation for these traits (Table 1). The mean pericarp thickness for the population $\pm$ standard deviation was 0.19 ± 0.04 cm. Fruit from line AO had the thickest pericarp, fruit from line P had the thinnest pericarp, and both were significantly different from the two parents (Table 1). The two parents produced green fruit with length, width, and shape ratios of 2.93 cm, 1.49 cm, and 1.99, respectively, for CM334, and 3.40 cm, 1.78 cm, and 1.33, respectively, for Early Jalapeño.

Correlation analysis. When averaged across all three isolates of *P. capsici*, fruit lesion area at 3 dpi was not significantly correlated with fruit shape index ($r = 0.1818$, $P = 0.1350$) but lesion area at 5 dpi was weakly correlated with fruit shape index ($r = 0.2446$, $P = 0.043$). Lesion size of individual fruit at 3 dpi was significantly correlated with lesion size at 5 dpi ($r = 0.5048$, $P < 0.0001$).

Fig. 1. Disease symptoms on pepper fruit of lines 'Criollo de Morelos' 334 (CM) and 'Early Jalapeño' (EJ) at A and C, 3 days and B and D, 5 days post inoculation with *Phytophthora capsici* isolates A and B, 12889 and C and D, 13709. D is reprinted from Granke et al. (15).

Fig. 2. Frequency distribution of the lesion area (cm²) measured 5 days post inoculation with each of the three isolates of *Phytophthora capsici* of pepper fruit from a New Mexico recombinant inbred line mapping population and the two parent lines, 'Criollo de Morelos' 334 (CM) and 'Early Jalapeño' (EJ). The category of the mean lesion area for CM and EJ for each of the three isolates is indicated by black arrows. Gray bars denote the number of individuals inoculated with isolate 12889, black bars denote isolate 13709, and white bars indicate isolate OP97.

For isolate 12889, fruit shape index was significantly correlated with lesion area at both 3 dpi ($r = 0.3440$, $P = 0.004$) and 5 dpi ($r = 0.2892$, $P = 0.016$). For isolate 13709, there was no significant correlation of lesion area at 3 and 5 dpi with fruit shape index ($r = 0.1354$, $P = 0.27$ and $r = 0.1609$, $P = 0.19$, respectively). Similarly, isolate OP97 had no significant correlation with lesion area at 3 and 5 dpi for fruit shape index ($r = 0.0807$, $P = 0.51$ and $r = 0.1452$, $P = 0.23$, respectively). Fruit width and fruit length were inversely correlated ($r = -0.3292$, $P = 0.006$). Fruit pericarp thickness was not correlated significantly with lesion area at 3 or 5 dpi for any of the isolates evaluated or for any of the fruit phenotypic traits evaluated (R. P. Naegele, unpublished data).

Discussion

Fruit shape and quality are important traits when breeding pepper. Numerous studies have identified major quantitative trait loci (QTLs) controlling fruit shape, thickness, firmness, and color in pepper and have reported one to several QTLs for each trait (2,6–8,18,23,28). With multiple QTLs to incorporate for each trait of interest, linkage is an important consideration when moving traits such as disease resistance from unadapted or wild backgrounds. Identifying significant correlations between fruit phenotypic traits and traits of interest, such as disease resistance, could hasten the selection process for commercial varieties. For example, *Phytophthora* root rot resistance has been incorporated from small-fruited, landrace accessions into several commercial pepper varieties. Many of these lines are susceptible to highly virulent isolates of *P. capsici*, and have no resistance to fruit rot or foliar blight (10). As breeding programs continue to integrate *Phytophthora* resistance into commercial cultivars, attention to resistance to fruit and foliar infections of *P. capsici* is needed in addition to root rot resistance. In this study, *P. capsici* isolate-specific interactions were evident among individual NMRIL progeny lines and isolates of the pathogen, which is consistent with previous studies on root rot and foliar blight in pepper, tomato, and eggplant (11,13,14,17,21).

In this study, CM334, which is a landrace from Mexico and a primary source of resistance to *Phytophthora* root rot and foliar blight (17), was not completely resistant to *Phytophthora* fruit rot. However, compared with the commercial Early Jalapeño, CM334 had less fruit rot caused by each of the three isolates of *P. capsici* at both 3 and 5 dpi (i.e., CM334 was more resistant to fruit rot than Early Jalapeño). Breeders utilizing CM334 as a source of *Phytophthora* root rot resistance in pepper might need to consider using other sources of germplasm to integrate a greater level of fruit rot resistance than detected in CM334. CM334 was not significantly different in fruit rot resistance than any of the partially resistant NMRIL progeny, suggesting that Early Jalapeño is contributing little, if any, resistance to the NMRIL progeny.

The high variability among the inbred lines that were grown in the greenhouse suggests that fruit resistance is influenced by environmental conditions, making breeding for fruit rot resistance more challenging. Unlike the previous study by Saini and Sharma (22), continuous variation was observed among lines of the NMRIL population described in this study. This was different than the 3:1 ratio of inheritance for fruit rot previously described (22). The difference in distribution of fruit reactions could be attributed, in part, to the different sources of resistance—CM334 for this study versus Waxy Globe in the study by Saini and Sharma (22)—or the different *P. capsici* isolates used. In this study, fruit from pepper lines were evaluated in the lab under optimal disease conditions, whereas Saini and Sharma (22) evaluated natural occurrence of infection in a field setting. It is possible that the lines identified in the present study as partially resistant may be more resistant under field conditions if disease conditions are not optimal.

Phenotypic evaluation of the NMRIL population for fruit characteristics revealed continuous variation among the progeny lines for fruit pericarp thickness, length, width, and shape index. Fruit color varied but was grouped into discrete categories. Pericarp thickness and fruit color had no significant correlation with fruit

susceptibility to *P. capsici* at 3 or 5 dpi. Only fruit shape index was significantly correlated with lesion area; however, the correlation was only significant for one of the three *P. capsici* isolates evaluated, and even then was only weakly correlated ($r = 0.3440$, $P = 0.004$ at 3 dpi and $r = 0.2892$ at $P = 0.016$ at 5 dpi). These results suggest that linkage between fruit shape index and fruit susceptibility to *P. capsici* may exist for some isolates of *P. capsici*.

To our knowledge, this is the first published evaluation of the correlation between pepper fruit phenotypic characteristics and *Phytophthora* fruit rot in a mapping population. Most of the fruit traits evaluated had no significant correlation with fruit resistance or susceptibility to *P. capsici* at 3 or 5 dpi, which is in agreement with previous studies evaluating pericarp thickness and other fruit phenotypic traits (5,9,22). However, in this study, a significant correlation between fruit shape index and lesion size for one of the three isolates of *P. capsici* suggests that pepper lines from the CM334 and Early Jalapeño cross with a greater length/width ratio may be more susceptible to *Phytophthora* fruit rot caused by some isolates. Although future studies should evaluate larger populations of pepper and perform genetic analyses to identify potential fruit resistance QTL, these data provide a good first approximation of the relationships between *Phytophthora* fruit rot resistance and pepper fruit phenotypic traits.

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