Association of the Fusarium Wilt Race 3 Resistance Gene, *I-3*, on Chromosome 7 with Increased Susceptibility to Bacterial Spot Race T4 in Tomato

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ABSTRACT. Bacterial spot of tomato (*Solanum lycopersicum*), caused by several *Xanthomonas* species, is one of the most important diseases of the crop in humid production regions of the world. Conventional breeding approaches for resistance to bacterial spot previously identified race-specific resistances, but current efforts also seek to use quantitative trait loci (QTLs) effecting broad-spectrum resistance. Resistance QTLs and candidate QTLs have been reported on several chromosomes, including a major QTL on chromosome 11. Fusarium wilt (*Fusarium oxysporum* f. sp. *lycopersici*) race 3 resistance gene, *I-3*, is associated with smaller fruit size and has been implicated in other associations with negative characteristics. We evaluated four F₂ populations involving the bacterial spot-tolerant breeding lines Fla. 8517, Fla. 8233, and Fla. 8326 across two field seasons to validate and quantify previously identified loci and to test for an effect of *I-3* on bacterial spot sensitivity. The chromosome 11 QTL and the *I-3* locus were each consistently positively and negatively associated with resistance, respectively, and together explained from 44% to 47% of the variation in each population. The chromosome 11 QTL displayed a dominant to incompletely dominant effect, reducing infection by 14% to 25%. This QTL is distinct from the *X. perforans* race T3 hypersensitivity loci, *Rx-4* and *Xv3*. The *I-3* locus contributed to as much as a 20% increase in infection in *I-3/I-3* plants vs. *i-3/i-3* plants, and heterozygosity for *I-3* generally resulted in an intermediate susceptible response. Significant effects for QTLs on chromosomes 3, 5, and 12 were also observed, but these effects were not consistent in all populations or seasons in which they were segregating. Implications of these findings toward breeding strategies are discussed.

Tomato is one of the most economically important vegetable crops in the world. In 2012, production reached nearly 162 million tonnes worldwide, more than a 70% increase in production over 1996 levels (Food and Agriculture Organization of the United Nations, 2014). The United States is among the leading producers of tomatoes in both processing and fresh markets. Approximately 1.38 million tonnes of fresh-market tomatoes were produced in the United States in 2012 and valued at \$864 million [U.S. Department of Agriculture (USDA), 2012]. Fresh-market tomato production is the number one vegetable industry in Florida, where a subtropical climate allows for winter production while also creating a favorable environment for numerous diseases in the crop.

Fusarium wilt, caused by the soilborne fungus *Fusarium oxysporum* f. sp. *lycopersici*, has been a major disease of tomato in Florida and in other warm production regions worldwide. The fungus colonizes the vascular tissue of plants after infecting the roots by direct penetration or through wounds. Host resistance has proven to be an effective control strategy in commercial cultivars and relies primarily on three single dominant genes that have been discovered and introgressed from *Solanum pimpinellifolium* (*I* and *I-2*) or from *Solanum pennellii* (*I-3*) and which confer resistance to three races of the pathogen. Bohn and Tucker (1939) identified a single gene conferring resistance to race 1, which they termed *I* for “immunity.” Race 2 resistance was discovered by Alexander (1959) and subsequently characterized by Stall and Walter (1965), who named the gene *I-2*. Both *I* and *I-2* are located on chromosome 11, and *I-2* has

been positionally cloned (Simons et al., 1998). Resistance to race 3 was reported by McGrath et al. (1987) and by Scott and Jones (1989b), and *I-3* has been fine-mapped on chromosome 7 (Lim et al., 2008). Although cultivars containing *I-3* are quite common and have been commercially available since the early 1990s, there have been some difficulties in developing commercially acceptable race 3-resistant hybrids. Some of the earliest *I-3* parents had a greater susceptibility to blossom-end rot (Scott and Jones, 2004; J.W. Scott, unpublished data). Scott (1999) also showed that homozygosity for *I-3* resulted in reduced fruit size compared with heterozygosity. Moreover, the consensus among breeders is that *I-3* is associated with weaker vines and may also be associated with a greater susceptibility to bacterial spot (J.W. Scott, unpublished data).

Bacterial spot is also a major disease of tomato in Florida and in many regions of the world where the crop is grown in humid environments. The disease is caused by several species of *Xanthomonas*, including *X. euvesicatoria* (race T1), *X. vesicatoria* (race T2), *X. perforans* (races T3 and T4), and *X. gardneri* (Jones et al., 2000, 2005). There is no good control measure commercially available for bacterial spot. Copper compounds have been and are widely used but are largely ineffective as a result of resistance in bacterial populations (Horvath et al., 2012; Marco and Stall, 1983; Quezada-Duval et al., 2003). Chemicals that induce systemic acquired resistance and bacteriophages provide alternative control strategies (Byrne et al., 2005; Louws et al., 2001; Obradovic et al., 2005), but these are not widely used in commercial production.

Bacterial spot symptoms occur on leaves, stems, and fruit of tomato. Infected fruit are unmarketable, whereas leaf and stem infections cause defoliation, which may result in reduced yield

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and quality as a result of cracking, sunscald, or black shoulder (Scott and Jones, 1986). Yield losses resulting from bacterial spot can be severe. Pohronezny and Volin (1983) compared yields of non-inoculated plots with those that were inoculated at one of two different times. Compared with the non-inoculated control, reductions in total yield ranged from 22% in the late-inoculated plots to 52% in the early-inoculated plots; the authors also showed a 42% to 66% reduction in yields of USDA large-sized fruit in the early-inoculated plots. Horvath et al. (2012) reported a very high level of resistance to bacterial spot was achieved in transgenic tomatoes expressing the *Bs2* resistance gene from pepper (*Capsicum annuum*). In repeated trials, *Bs2* transgenic lines and hybrids showed a 1.5-fold or greater yield increase over non-transgenic isogenic lines and hybrids, and yield increases were consistently 2-fold or greater under periods of greater disease pressure (Horvath et al., 2012, 2014).

Much conventional breeding research has been directed toward identification of sources of host resistance to bacterial spot, incorporation of this resistance into cultivated germplasm, and elucidation of the genetic control of resistance. For the most part, these efforts have focused on single pathogen races and have led to the identification and mapping of several single genes regulating resistance. Yang et al. (2005) confirmed a major role for *Rx-3* from Hawaii 7998 (H7998) in field resistance to *X. euvesicatoria* race T1. *Xv3* from Hawaii 7981 (H7981) and *Rx-4* from the *S. pimpinellifolium* accession PI128216 condition hypersensitivity to *X. perforans* race T3 (Robbins et al., 2009; Scott et al., 1996; Wang et al., 2011). *RXopJ4* from *S. penellii* accession LA716 controls hypersensitivity to *X. perforans* races T3 and T4 (Sharlach et al., 2013). These loci, however, provide only partial resistance, and additional QTLs are often necessary for field resistance (Scott et al., 2001; Scott and Jones, 1989a; Somodi et al., 1996; Wang et al., 1994).

In addition to the use of such single, race-specific resistance genes, there is ongoing research to identify and use QTLs conferring a more broad-spectrum, race-nonspecific resistance (Hutton et al., 2010a, 2012; Scott et al., 2003). Hutton et al. (2010b) identified QTLs significantly associated with resistance on chromosomes 3, 11, and 12, as well as candidate QTLs on chromosomes 1, 2, 5, 9, and 10. Of these, the chromosome 11 QTL had the largest effect and was mapped near the location of the race T3 hypersensitivity loci, *Xv3* and *Rx-4* (Robbins et al., 2009; Wang et al., 2011). The objectives of this research were primarily to determine what effect *I-3* has on bacterial spot sensitivity; secondary objectives were to validate and quantify the effect of the QTLs previously identified for bacterial spot resistance on chromosome 11 and at other loci and to determine the location of the chromosome 11 QTL relative to the race T3 hypersensitivity locus.

Materials and Methods

PLANT MATERIALS. Three bacterial spot-tolerant breeding lines developed at the University of Florida, Fla. 8233, Fla. 8517, and Fla. 8326, were each crossed with Fla. 7946, and Fla. 8233 was also crossed with Fla. 8111B. Both Fla. 7946 and Fla. 8111B are highly susceptible to bacterial spot, and Fla. 7946 has resistance to fusarium wilt race 3 (*F. oxysporum*, f. sp. *lycopersici*) conferred by the *I-3* gene on chromosome 7 (Scott, 2004). The resulting F_1 s from these crosses were allowed to self-pollinate, and F_2 populations were evaluated for bacterial spot in the field at Balm, FL, in Fall 2008 and/or Fall 2009.

For all experiments, seed were sown directly into peat-lite soilless media (Speedling, Sun City, FL) in 128-well flats (38 cm³ cell size; Speedling). Transplants were grown in a greenhouse and planted to the field $\approx$ 5 weeks after sowing. Plants were transplanted to field beds that were 20 cm high and 81 cm wide and had been fumigated with a 50:50 mixture of methyl bromide and chloropicrin (55 kg per treated hectare) and covered with white plastic mulch. Plants were spaced 46 cm apart in rows with 152 cm between rows. Plants were staked and tied and were irrigated by drip tape beneath the plastic mulch of each bed. A recommended fertilizer program was followed, and plants were sprayed with pesticides (excluding copper and other bactericides) as needed throughout the season (Olson et al., 2011).

EXPERIMENTAL DESIGN. In Fall 2008, parents and F_2 populations from the crosses (Fla. 8233 $\times$ Fla. 8111B), (Fla. 8517 $\times$ Fla. 7946), and (Fla. 8326 $\times$ Fla. 7946) were sown with their respective parents on 23 July. F_1 s were also sown at this time for the Fla. 8233 and Fla. 8517 populations. Parents, F_1 s and F_2 s, were planted in three separate experiments, each corresponding to one of the three crosses. Transplants were planted to the field on 26 Aug. Parents and F_1 s were planted in a randomized complete block design with three blocks and eight-plant plots (except for the Fla. 8517 population, in which plots were 12- to 13-plant plots). Each F_2 population consisted of 270 plants planted in nine 30-plant plots that were distributed evenly and placed randomly across the three blocks. Experiments were inoculated with *X. perforans* race T4 in the evening on 24 Sept. and again before sunrise on 10 Oct. (because weather conditions were cool and dry at the first inoculation and thus unfavorable for disease development). Plants were rated individually for bacterial spot disease severity between 29 and 31 Oct. Tissue was collected and DNA was extracted from each F_2 plant. On 3 Nov., two cuttings were taken from each F_2 plant in the (Fla. 8517 $\times$ Fla. 7946) F_2 population as well as from 13 or more plants of each of the two parents and the F_1 . Cuttings were rooted in a 1:1 perlite:vermiculite media under mist for 2 weeks and then transplanted to small pots. One cutting from each F_2 plant and the cuttings from the parents and the F_1 were infiltrated with *X. perforans* race T3 on 9 Dec. for evaluation of hypersensitivity to race T3 as described below.

In Fall 2009, seed were sown on 22 July to generate $\approx$ 850 F_2 plants from the Fla. 8517 $\times$ Fla. 7946 cross and $\approx$ 750 F_2 plants from the Fla. 8233 $\times$ Fla. 7946 cross along with the respective parents of these populations. Tissue was collected and DNA extracted from each F_2 plant, and seedlings were genotyped for the chromosome 11 QTL using the marker UF_C2_At4g10050 and for the *I-3* locus using the marker UF_I-3 CAPS (Table 1). Half of the plants heterozygous for the chromosome 11 marker and half of the plants heterozygous at the *I-3* locus were removed from the population to balance the representation from each allelic category. Parents and the F_2 s were planted into two separate experiments according to the two crosses. Transplants were planted to the field on 11 Sept. For each population, a randomized complete block design was used with three blocks and 12-plant plots; each block included one plot for each parent and nine plots for the F_2 . Experiments were inoculated with *X. perforans* race T4 on 28 Sept. Plants were rated individually for bacterial spot disease severity on 3 Nov. In the field, tissue was collected from individual plants for DNA extraction and marker analysis.

DNA EXTRACTION AND MARKER ANALYSES. For all experiments, DNA was extracted using a modified cetyltrimethylammonium

Table 1. Summary of molecular markers used for genotyping segregating populations for candidate quantitative trait loci for bacterial spot race T4 resistance in tomato.

Marker	Marker type ^a	Physical location ^b		Primer sequence (5' → 3')	Annealing temp (°C)	Restriction enzyme	Detection	Tested in populations arising from ^c
		Chromosome	location (Mb)					
LEOH23.3	CAPS	2	33.7	F CTATGCGTTTGTCGGTCTGT R CAAGGTAGTTGAAGGTATGACCA	54	<i>Tsp509I</i>	3% agarose	Fla. 8517
C2_At5g62390	CAPS	3	47.5	F TGCTACTAACTGTGTGATGCCATTGAG R TTGGGGTTCGATAACATCAAGC	55	<i>HinfI</i>	2% agarose	Fla. 8517
SSR111	SSR	3	53.0	F TTCTTCCCTTCCATCAGTTCT R TTGTGCTATATACTGCTGACA	55	n/a	6.5% polyacrylamide	Fla. 8233, Fla. 8326
Rx3-L1	CAPS	5	62.5	F CTCCGAGCGAAGAGTCTAGAGTC R GAAGGCAAAAGGAAAGGAGAAAGGATGG	61	<i>BsrBI</i>	2% agarose	Fla. 8233
UF_I-3 CAPS	CAPS	7	60.1	F TGTGCGTCTCTGTCAACCTTACC	56	<i>BsaJI</i>	2% agarose	Fla. 8233, Fla. 8517, Fla. 8326
SSR383	SSR	9	61.5	R TGCTGTGCGAGAGAATCTGGTGAGT F ATGTGACAAAGACCCGTGGC	50	N/A	6.5% polyacrylamide	Fla. 8233, Fla. 8517
TG403	dCAPS	10	62.8	R GTTGACACACTGGATCAATGC F TTGCTTGGTTCCTTATGCAGC	56	<i>Hpy188I</i>	3% agarose	Fla. 8233
UF_C2_At4g10050	CAPS	11	31.8	R TACGTATTTTGAATAATCCTGTCTTTTCAG F TCCTGTTGAAAGTTCAATCTGTGT	56	<i>BstNI</i>	2% agarose	Fla. 8233, Fla. 8517, Fla. 8326
LEOH 57	CAPS	11	46.6	R CTCCACTCATGTCACAAACCA F TGGTCAACAGATGGTGAAGAA	52	<i>BstUI</i>	2% agarose	Fla. 8233, Fla. 8517, Fla. 8326
SL 10737i	indel	11	47.5	R GGATCCCATGCCAATGAATA F CCACTCCTGGGACTCAAATC	55	N/A	3.5% agarose	Fla. 8233, Fla. 8517, Fla. 8326
C2_At1g30825	CAPS	11	50.3	R TGGACCCACAGGTAATGAGG F ATGTGACCGTCATATTTCTATGAG	56	<i>BseRI</i>	2% agarose	Fla. 8517
UF_C2_At3g54470	dCAPS	11	50.5	R AGGGGGCATTATAAGTCCAGCAG F AAGAGTTCAACTATTGCATCCAA GGAGTAACAT	57	<i>NdeI</i>	3% agarose	Fla. 8517
cLEC-24-C3	dCAPS	11	50.6	R CAAAATGGTCCAGAACCCATA F CAGACTGGAGAGTCAAAGGT	56	<i>HpaI</i>	3% agarose	Fla. 8233, Fla. 8517, Fla. 8326
SSR20	SSR	12	6.8	R CCTTGTGATAATCTGCAAGTTGTAA F GAGGACGACAAACAACGA R GACATGCCACTTAGATCCACA	50	N/A	4% agarose	Fla. 8233, Fla. 8517

^aLocation based on version SL2.40 of the tomato genome assembly available through the Sol Genomics Network (Bombarely et al., 2011).^bCAPS = cleaved amplified polymorphic sequence; SSR = simple sequence repeat; dCAPS = derived CAPS; indel = insertion-deletion.^cNumbers correspond to Florida breeding lines Fla. 8517, Fla. 8233, and Fla. 8326.

N/A = not applicable.

bromide procedure as described by Fulton et al. (1995). Molecular markers, their chromosomal locations, and parameters are described in Table 1. Markers were selected based on candidate QTL identified previously (Hutton et al., 2010b). For each population and within each year, a stepwise analysis of variance was performed using the MIXED procedure of SAS (Version 9.1; SAS Institute, Cary, NC) to determine which loci were significantly associated with resistance and to test for interactions; the GLM procedure of SAS was used to perform Duncan's means separation to quantify the effect of each locus.

INOCULUM PREPARATION, PLANT INOCULATIONS, AND DISEASE EVALUATIONS. *X. perforans* race T3 inoculum was produced by growing the bacteria on Difco nutrient agar (Becton Dickinson and Co., Sparks, MD) for 24 to 36 h at 28 °C. Bacterial cells were removed from the agar plates and suspended in 10 mM MgSO₄·7 H₂O, and the suspensions were standardized to A₆₀₀ = 0.30 [a concentration of ≈2 to 5 × 10⁸ colony-forming units (cfu)/mL]. Leaves of cuttings were infiltrated with the bacterial suspension as described by Hibberd et al. (1987), and hypersensitivity was evaluated as described by Scott et al. (1996) with some modifications. Briefly, infiltrated plants were maintained in a greenhouse that ranged from 13 °C at night to 24 °C during the day. Assessments for hypersensitive reactions were carried out at 12- to 24-h intervals between 24 and 72 h after infiltration. Infiltrated areas exhibiting confluent necrosis within 36 h were scored as hypersensitive.

Field trials were inoculated with *X. perforans* race T4 for two reasons; first because this race is both predominant and endemic in Florida; and second because there is no race-specific resistance in the germplasm used for this study, and race T4 is therefore suitable for testing non-race-specific resistance. Race T4 inoculum was produced as described previously for race T3, and the bacterial suspensions were adjusted to a concentration of ≈2 to 5 × 10⁶ cfu/mL. Inoculum was applied along with an organosilicone surfactant [0.025% v/v (Silwet L77; Setre Chemical Co., Memphis, TN)] by misting the foliage with a backpack sprayer. Plants in the field were rated on each side of the staked row for disease severity using the Horsfall-Barratt (HB) scale (Horsfall and Barratt, 1945), where 1 = 0%, 2 = 0% to 3%, 3 = 3% to 6%, 4 = 6% to 12%, 5 = 12% to 25%, 6 = 25%

to 50%, 7 = 50% to 75%, 8 = 75% to 87%, 9 = 87% to 94%, 10 = 94% to 97%, 11 = 97% to 100%, and 12 = 100% diseased tissue; where there was a discrepancy between the ratings of the two sides of a plant, an average rating was recorded. For presentation purposes, midpoints of the ranges above were used to convert HB ratings to percent disease for individual plants.

Results

Bacterial spot disease pressure in the fall seasons of 2008 and 2009 resulted in severe infections on susceptible parents, from which resistant parents were clearly distinguished in all populations (Table 2). Mean disease severities of F₂ populations were intermediate to the parents, and individual plant ratings of F₂ populations generally fell within the range of the two parents.

Stepwise analysis of variance identified two major loci affecting bacterial spot disease severity, one on chromosome 11 and one at the *I-3* locus on chromosome 7 (Table 3). These loci together explained between 44% and 47% of the variation in each population. The chromosome 11 QTL was significant in every population across both years and was always represented in the model by the marker C2_At4g10050, which consistently explained more variation than nearby chromosome 11 markers located more distal on the chromosome. The effect of this QTL varied between dominance and incomplete dominance. Homozygosity for this QTL lowered disease from 14% in both the Fla. 8233 and Fla. 8326 populations in 2008 to 25% in the 2009 Fla. 8517 population. Heterozygosity for the chromosome 11 QTL had a similar effect to homozygosity in all three populations tested in 2008, reducing disease by 10% to 21%, yet in both populations tested in 2009, the heterozygous effect of this QTL, although quite strong, was slightly less than the homozygous effect.

Resistance to fusarium wilt race 3 was associated with greater susceptibility to bacterial spot in all populations segregating for *I-3*. The most pronounced effect was observed in the Fla. 8326 population, where homozygosity for *I-3* resulted in 20% greater diseased tissue than the absence of this allele (Table 3). In the 2008 Fla. 8517 and the 2009 Fla. 8233 populations,

Table 2. Bacterial spot race T4 disease severity for tomato parents and their derived F₂ generations.

Season							
Fall 2008				Fall 2009			
Line	Plant no.	Disease severity index ^z		Line	Plant no.	Disease severity index	
		Mean	Range			Mean	Range
Fla. 8233	17	3.4 c ^y	3–4	Fla. 8233	34	3.4 c	2–5
Fla. 8111B	23	6.4 a	6–7	Fla. 7946	34	6.9 a	6–7
(Fla. 8233 × Fla. 8111B) F ₂	283	4.5 b	2–7	(Fla. 8233 × Fla. 7946) F ₂	312	5.4 b	3.5–7
(Fla. 8233 × Fla. 8111B) F ₁	19	4.3 b	3–5				
Fla. 8517	39	2.0 c	2	Fla. 8517	36	2.5 c	2–3.5
Fla. 7946	39	6.3 a	6–6.5	Fla. 7946	36	7.0 a	6.5–7
(Fla. 8517 × Fla. 7946) F ₂	256	3.5 b	2–7	(Fla. 8517 × Fla. 7946) F ₂	297	5.5 b	3–7
(Fla. 8517 × Fla. 7946) F ₁	34	3.4 b	3–4				
Fla. 8326	24	4.0 c	3–5				
Fla. 7946	23	6.7 a	6–7				
(Fla. 8326 × Fla. 7946) F ₂	241	5.7 b	3–7				

^zDisease severity index rated on the Horsfall-Barratt (Horsfall and Barratt, 1945) scale, where higher numbers indicate more disease.

^yDifferent superscript letters represent statistically significant differences among lines in a population at *P* < 0.05 based on Duncan's multiple range test.

Table 3. Effect of two loci on bacterial spot race T4 foliar disease severity in tomato across four populations and two seasons.

Population	Season	Genotype ^z	<i>I</i> -3 (UF_I-3 CAPS)			Chromosome 11 QTL (UF_C2_At4g10050)			<i>R</i> ²
			Plant no.	Mean DSI ^y	Dis. (%) ^x	Plant no.	Mean DSI	Dis. (%)	
(Fla. 8233 × Fla. 8111B)	Fall 2008	+/+				70	4.1 b	11	44
		+/-				100	4.2 b	12	
		-/-				61	5.3 a	25	
(Fla. 8233 × Fla. 7946)	Fall 2009	+/+	103	5.7 a	32	98	4.9 c	19	45
		+/-	103	5.4 b	28	104	5.3 b	25	
		-/-	103	5.2 c	25	98	6.1 a	41	
(Fla. 8517 × Fla. 7946)	Fall 2008	+/+	58	4.2 a	16	68	3.1 b	6	47
		+/-	111	3.3 b	9	137	3.1 b	6	
		-/-	62	3.0 c	7	46	5.3 a	27	
(Fla. 8517 × Fla. 7946)	Fall 2009	+/+	99	5.5 a	30	97	4.9 c	20	46
		+/-	105	5.7 a	33	97	5.3 b	26	
		-/-	92	5.4 b	28	98	6.3 a	45	
(Fla. 8326 × Fla. 7946)	Fall 2008	+/+	58	6.2 a	44	46	5.4 b	28	44
		+/-	100	5.8 b	35	125	5.6 b	32	
		-/-	62	5.1 c	24	69	6.1 a	42	

^z+/+ = homozygous for fusarium wilt race 3 resistance or for the resistant allele at the chromosome 11 quantitative trait loci; +/- = heterozygous; -/- = homozygous-susceptible.

^yDisease severity index rated on the Horsfall-Barratt (Horsfall and Barratt, 1945) scale, where higher numbers indicate more disease. Different superscript letters represent statistically significant differences among genotypes in a population and year at *P* < 0.05 based on Duncan's multiple range test.

^xPercentage diseased tissue as converted from Horsfall-Barratt ratings.

homozygosity resulted in 9% and 7% greater disease, respectively. The smallest effect was observed in 2009 in the Fla. 8517 population, where homozygosity for *I*-3 resulted in only a 2% increase in disease over *i*-3/*i*-3 genotypes. In this population, heterozygosity for *I*-3 resulted in greater disease than homozygosity; in all other populations, however, *I*-3 had an additive effect, and heterozygosity at *I*-3 resulted in intermediate disease severity between homozygous-resistant and homozygous-susceptible. Within each of these populations, an intermediate phenotype was observed for plants homozygous for both *I*-3 and the chromosome 11 QTL (data not shown); average disease severities of these plants were approximately equal to the mean F₂ disease severity indices presented in Table 2.

QTL were also detected in some populations on chromosomes 3, 5, and 12, but effects were not consistent across both seasons (data not shown). In 2008, homozygosity for a QTL on chromosome 3 provided a 2% reduction in disease in the Fla. 8517 population and a 5% reduction in the Fla. 8326 population; this QTL was not significant in the Fla. 8517 population in 2009 nor in the Fla. 8233 populations. Homozygosity for a QTL on chromosome 5 significantly lowered disease by 3% in the 2008 Fla. 8233 population, but no effect was observed in 2009. Likewise, a QTL on chromosome 12 lowered disease by 4% in the Fla. 8233 population in 2008 and by 8% in the 2009 Fla. 8517 population, but the same locus had no significant effect in the 2009 Fla. 8233 population or the 2008 Fla. 8517 population. No effect was observed for candidate QTL on chromosomes 2, 9, or 10, and no interactions were detected among any of the loci that significantly affected disease.

Genes controlling *X. perforans* race T3 hypersensitivity are located in the same region as the chromosome 11 QTL confirmed in this study (Robbins et al., 2009; Wang et al., 2011). To determine the relationship between the two traits, race T3 hypersensitivity was measured on individuals of the Fall 2008 Fla. 8517 population. Tests for race T3 hypersensitivity in the greenhouse were clearly distinctive with Fla. 8517 exhibiting

confluent necrosis in 24 h and Fla. 7946 showing a susceptible reaction in 72 h (Table 4). The F₁ between these parents had an intermediate reaction, averaging 33 h to confluent necrosis. Single marker analysis of the F₂ progeny showed that hypersensitivity to race T3 was highly significantly associated with all markers tested on chromosome 11 (Table 5). Among these, the strongest association was with the more distal marker, cLEC-24-C3, and F values of nearby markers progressively decreased as markers were located more proximal to the centromere. The hypersensitivity phenotypes of F₂ plants homozygous, heterozygous, or susceptible for the resistant allele at cLEC-24-C3 very closely resembled the Fla. 8517, F₁, and Fla. 7946 phenotypes, respectively (Table 4). Field resistance to race T4 was also highly significantly associated with all markers tested on chromosome 11 (Table 5). In this case, as previously mentioned and conversely to race T3 hypersensitivity, field resistance to race T4 showed the strongest association with the marker C2_At4g10050, and F values of nearby markers progressively decreased as their distance from C2_At4g10050 increased. These results clearly suggest two separate loci involved in race T3 hypersensitivity and race T4 field resistance with the race T3 hypersensitivity locus located distal to the QTL.

Discussion

Bacterial spot is one of the most important diseases of tomato worldwide and is a major problem for the fresh-market industry in Florida. Conventional breeding for resistance has been ongoing at the University of Florida for more than three decades, yet these efforts have been unsuccessful at producing a resistant cultivar, and this timespan has only been punctuated by the development of tomato lines with race-specific resistance. Recent breeding efforts at the University of Florida have shifted toward identification of and selection for QTLs providing broad-spectrum resistance to multiple races. Hutton et al. (2010b) identified QTLs associated with non-hypersensitive

Table 4. Hypersensitivity as measured by time to confluent necrosis in tomato plants infiltrated with *Xanthomonas perforans* race T3 in 2008.

Line	Genotype at cLEC-24-C3 ^z	Total plants (no.)	Frequency distribution of time to confluent necrosis ^y					Avg time to confluent necrosis (h)
			24 h	36 h	48 h	60 h	72 h	
Fla. 8517	+/+	16	16					24
(Fla. 8517 × Fla. 7946) F ₁	+/-	15	4	11				33
Fla. 7946	-/-	13					13	72
(Fla. 8517 × Fla. 7946) F ₂	+/+	60	54	5			1	26
	+/-	130	26	97	2	3	2	35
	-/-	44	1			10	33	68

^z+/+ = homozygous for *X. perforans* race T3 hypersensitivity resistance allele; +/- = heterozygous; -/- = homozygous-susceptible.

^yPlants were infiltrated with ≈ 2 to 5×10^8 colony-forming units/mL.

Table 5. Relationship between a quantitative trait loci conferring field resistance to *Xanthomonas perforans* race T4 and hypersensitivity to *X. perforans* race T3 on chromosome 11 in tomato.

Marker	Physical position (Mbp) ^z	Race T4 field resistance		Race T3 hypersensitivity	
		F	P	F	P
C2_At4g10050	31.8	95.43	<0.0001	44.81	<0.0001
LEOH 57	46.6	86.68	<0.0001	49.48	<0.0001
SL 10737i	47.5	88.45	<0.0001	52.56	<0.0001
C2_At1g30825	50.3	40.37	<0.0001	157.41	<0.0001
C2_At3g54470	50.5	23.35	<0.0001	143.18	<0.0001
cLEC-24-C3	50.6	29.83	<0.0001	409.76	<0.0001

^zLocation based on version SL2.40 of the tomato genome assembly available through the Sol Genomics Network (Bombarely et al., 2011).

bacterial spot resistance on chromosomes 3, 11, and 12 as well as candidate loci on chromosomes 2, 5, 9, and 10.

The present study found no significant effect for the candidate loci on chromosomes 2, 9, or 10. QTLs on chromosomes 3, 5, and 12 were confirmed, but their effects were neither large nor consistent across all seasons or populations where they segregated. The QTL on chromosome 3 was significant in two populations segregating in 2008, but not in one segregating population in 2009. Its effect in 2008 was rather slight, lowering infection by only 2% to 5%. The QTL on chromosome 12 was significant for one of two populations segregating in 2008 and also for one of two populations segregating in 2009, and it effected a reduction in infection of 4% to 8%. The chromosome 5 QTL segregated in one population in 2008 and in one population in 2009. Its effect was significant only in 2008, where it accounted for a 3% reduction in infection. Whereas these loci may provide some use to breeders seeking to combine multiple QTLs, the relatively small and inconsistent effects present considerable difficulties in selection strategies. Part of this problem, however, could be a lack of tightly linked markers in the present study; thus, the development and use of improved markers may mitigate some of these difficulties.

The QTL on chromosome 11 consistently produced a strong effect, ranging from 14% to 25% reduction in infection, and this QTL may be of particular interest to breeders seeking to develop improved tolerance to bacterial spot race T4. Moreover, this QTL was found to confer broad-spectrum resistance (Francis et al., 2014; Hutton et al., 2010b) and should be useful across a range of production regions. Hutton et al. (2010a) determined that dominance was a major contributor to resistance in the breeding lines Fla. 8233, Fla. 8517, and Fla. 8326. The results of the present study are consistent with this, showing a dominant to incompletely dominant effect for this QTL. Use of this QTL heterozygously in a hybrid should contribute toward

a reduction in bacterial spot disease, but incorporation of it into both parents of a hybrid should generally provide a higher level of tolerance.

The location of the chromosome 11 QTL near the centromere does not contradict previous results (Hutton et al., 2010b), and the location of the *X. perforans* race T3 hypersensitivity locus near the marker cLEC-24-C3 is in complete agreement with recent fine-mapping results (Pei et al., 2012). Recognizing that the two loci are distinct and that there is considerable recombination between them, it will be important to select both loci for environments where race T3 may be a problem. In such situations, it will likely be desirable to use the recombination of the QTL and race T3 hypersensitivity in *cis*. This could be accomplished independently, or breeders may wish to use materials such as Fla. 8233 in which the recombination has already been selected (Francis et al., 2014; Hutton et al., 2010b).

Tomato breeders who work with fusarium wilt race 3 resistance in regions where bacterial spot is prevalent have found that such materials tend to be extra susceptible to bacterial spot. This tendency was confirmed in the present study for every population that segregated for *I-3*. The three major fusarium wilt resistance alleles are regularly used by breeders in developing resistant cultivars, yet although most cultivars contain *I* and *I-2*, not all current cultivars contain *I-3*. There may be several reasons for this. First, race 3 is not a problem in all production regions and thus resistance is not always needed. Along with this, because *I-3* was more recently introgressed into cultivated tomato, it may be that more time is needed for *I-3* to be incorporated into all modern cultivars. However, the findings of this study together with previous reports and observations indicate there are significant negative effects associated with *I-3*, including increased bacterial spot susceptibility, smaller vines, and smaller fruit size (Scott, 1999; Scott and Jones, 1995). It is thus likely that such negative effects significantly

hamper the development of race 3 resistant cultivars, providing further explanation as to why *I-3* is not more prevalent in current cultivars.

The association of *I-3* with bacterial spot susceptibility has important implications for breeders seeking to develop cultivars for regions where both bacterial spot and fusarium wilt race 3 are problems. It is clear that breeding lines carrying *I-3* will generally have greater susceptibility to bacterial spot than those without this resistance gene. In three of four populations evaluated across two seasons, plants heterozygous for *I-3* had less disease than plants homozygous for the gene. On the positive side, because the *I-3* gene is dominant, it is used heterozygously in commercial hybrids. Breeders should understand that homozygous *I-3* plants in segregating populations will likely be more susceptible to bacterial spot as well as having smaller fruit size (Scott, 1999). Otherwise, there will be a tendency for continual selection of plants heterozygous for *I-3*.

It is not clear whether the association between *I-3* and bacterial spot sensitivity is the result of linkage to unfavorable alleles or to pleiotropy. If the former, a directed effort to select for recombination near the *I-3* gene may prove successful in breaking the linkage, and the fine mapping efforts of Lim et al. (2008) should prove helpful in this endeavor. If the problem is pleiotropy, however, strategies might involve efforts to accumulate multiple resistance QTL to offset the negative effect or to identify and incorporate alternative sources of fusarium wilt race 3 resistance that potentially do not have this association. Either approach is long term. Such prospects make alternative strategies such as the transgenic approach using the pepper *Bs2* gene seem more attractive. Among the hybrids presented by Horvath et al. (2014) for which the *Bs2* gene provided high levels of resistance was Fla. 8726, which contains *I-3* and is otherwise highly sensitive to bacterial spot.

Ongoing research efforts at the University of Florida are seeking to address the *I-3* association with bacterial spot through each of the conventional approaches mentioned. Materials with reduced *I-3* introgressions are being developed and will soon be tested to determine if this relationship is the result of linkage and may be broken. Fusarium wilt race 3 resistance was found in all available accessions of *S. pennellii* (Scott and Jones, 1990). These accessions are being screened to determine if their resistance is allelic to *I-3* or perhaps unique. Additionally, considerable effort is aimed at developing *I-3* parents with a trait we call non-blighting, where plants maintain healthy, green foliage despite infection with bacterial spot. Several advanced breeding lines, including Fla. 630, carry this non-blighting trait, which is being advanced into an array of elite parent lines.

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