Transfer to Processing Tomato and Characterization of Late Blight Resistance Derived from *Solanum pimpinellifolium* L. L3708

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ABSTRACT. Late blight [caused by *Phytophthora infestans* (Mont.) de Bary] causes severe loss of tomato [*Solanum lycopersicum* L. (formerly *Lycopersicon esculentum* Mill.)] production in environments favorable to the pathogen. Researchers at the Asia Vegetable Research Development Center (AVRDC) identified resistance to late blight in an accession of *S. pimpinellifolium* [formerly *L. pimpinellifolium* (L.) Mill.] that they named accession L3708. This resistance has now been transferred to processing tomato lines, which are resistant to multiple *P. infestans* isolates. Lab trials, inoculated field trials in New York, and naturally infested field trials in Mexico all indicate that these processing tomato lines are fixed for late blight resistance. Segregation data obtained for resistance in the breeding populations were dependent on the pathogen isolate used for the disease screen. Segregation data do not support the hypothesis of single gene control of the full resistance trait, but instead suggest that more than one gene is involved, and that these genes interact in an epistatic manner.

Late blight results in severe loss of tomato production when environment is favorable to the pathogen and disease development. As a result, late blight is ranked as one of the most important diseases in tomato (Garelik, 2002; Judelson, 1997; Umaerus and Umaerus, 1994). Within the United States, late blight ranked as the eighth most important tomato disease, based on a weighted average of loss per unit area (Davis et al., 1998). Late blight is currently controlled in tomato by the use of fungicidal sprays; the timing of these sprays in some regions is guided by blight forecasts based upon weather conditions (Davis et al., 1996, 1998; Raposo et al., 1993). However, despite the use of monitoring and controlling chemicals, losses in tomato due to late blight can be substantial. Furthermore, the use of sprays for control of this disease is increasingly difficult due to changes in pathogen virulence and increased chemical resistance of pathogen (Fry and Goodwin, 1997a, 1997b; Goodwin et al., 1998; Kato et al., 1997).

Attempts to breed late blight resistant tomato lines started ~50 years ago (Richards and Barratt, 1946), ultimately resulting in the identification of two tomato resistance genes, *Ph-1* and *Ph-2* (Gallegly, 1960; Peirce, 1970, 1971). *Ph-1* is a single dominant race-specific resistance gene derived from *S. pimpinellifolium*, which protects against race-O of the pathogen (Bonde and Murphy, 1952). The resistance of *Ph-1* was rapidly overcome due to the race specificity of this resistance gene (Conover and Walter, 1952, 1953; Gallegly, 1952). *Ph-1* is included in the old fresh-

market tomato cultivar, 'New Yorker', and in the old processing tomato cultivar, 'Nova'.

Ph-2 is a single incompletely dominant gene also derived from *S. pimpinellifolium*. *Ph-2* does not affect a number of *P. infestans* isolates, and provides incomplete resistance against the *P. infestans* isolates it does affect (Goodwin et al., 1995). The degree of disease suppression by *Ph-2* is affected by additional factors (Laterrot, 1975; Turkensteen, 1973). Reported to contain *Ph-2* are the cultivars West Virginia 63, Legend, Centennial, Caline, Macline, Pieraline, Heline, Fline, Piline, Pieralbo, Heinz 1706, Campbell 28, Flora Dade, Earlymech, and Europeel (Gallegly, 1960; Laterrot, 1994). Therefore, there were no tomato cultivars with usable resistance to recent isolates of late blight.

Researchers at the AVRDC identified a new source of resistance in an *S. pimpinellifolium* accession L3708 (Asian Vegetable Research and Development Center, 1994), which they generously provided to other breeding programs. In later work with this resistance, they observed segregation for a single gene, *Ph-3*, which they mapped to bottom of chromosome 9 (Chunwongse et al., 2002). The goal of the work reported here was 2-fold: to rapidly transfer the resistance in L3708 to commercial quality processing tomato line, and, in the process, collect additional information regarding the genetic control and nature of this resistance to facilitate future use of this resistance in elite tomato lines and hybrid cultivars.

Materials and Methods

SOURCE OF LATE BLIGHT RESISTANCE. The ultimate source of the late blight resistance used was *S. pimpinellifolium* accession L3708 (also known as LA1269, C.M. Rick Tomato Genetics Resource Center, Davis, Calif.; USDA accession NSL116890, and PI365957). However, R. Gardner of North Carolina State Univ. had already started the transfer of late blight resistance from L3708 to fresh-market tomato, and provided us with cuttings of five blight-resistant selections from a BC₁F₂ population [NC215E x (NC215E x *S. pimpinellifolium* accession L3708)]. These cuttings were rooted, and the resulting clonally produced plants were used as pollen donors to pollinate processing to-

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matatoes. This approach saved the time required to create and to screen the initial F_1 , BC_1F_1 , and BC_1F_2 generations for transfer of late blight resistance.

PROCESSING TOMATO BACKCROSS PARENTS USED. Multiple female parents with different characteristics were used to provide genetic variability for horticultural characteristics in the breeding program. The processing tomatoes used for the first backcross to processor were 'Heinz 8892' (Heinzseed, Stockton Calif.), 'Rogers 1108' (ROGERS Brand Vegetable Seeds, Boise, Idaho), and 'Orsetti 3155' (Orsetti Seed Co., Hollister, Calif.), which are commercial hybrids that are *VFFMi* [possessing the resistance genes against verticillium (*Verticillium dahliae* Kleb) race 1, fusarium [*Fusarium oxysporum* Schlecht. emend. f.sp. *lycopersici* (Sacc.) Snyder and Hans.] race 1 and 2, and root knot nematode [*Meloidogyne incognita* (Kofoid & White) Chitwood], respectively]. The second backcross females were either 'Hypeel 303' or 'Hypeel 45' (Seminis, Oxnard, Calif.), which were also commercial hybrids and are *VFFMiPto* [possessing the resistance genes against the diseases listed above plus bacterial speck (*Pseudomonas syringae* van Hall pv. *tomato* Okabe)].

DESIGNATION OF BACKCROSS GENERATIONS IN TOMATO POPULATIONS. Due to the use of fresh-market BC_1F_2 selections as the source of resistance, a method was needed for designating the backcross generations in the processing tomato populations subsequently produced in this project. To avoid confusion, backcross numbers used below to describe the processing tomato populations produced refer only to additional backcrosses made to processing tomato. Therefore, the first backcross to processing tomato was described as a BC_1F_1 , not BC_2F_1 , even though the population has a total of two backcrosses to *S. lycopersicum*.

PHYTOPHTHORA INFESTANS ISOLATES USED. The *P. infestans* isolates US-17 (Fry Lab No. 970001) and US-7 (Fry Lab No. 940486 and 940330) were obtained from W. Fry (Dept. of Plant Pathology, Cornell Univ.). These two isolates were chosen due to the high levels of disease they caused on tomato in prior field and lab assays, and since we knew that these isolates overcome the resistance of *Ph-2*. US-7 was a dominant isolate throughout the United States, but has largely been replaced by newer isolates. US-17 is a more recent isolate that is found in the southeastern United States up the east coast to New Jersey and New York (Fry and Goodwin, 1997).

The original US-7 isolate (Fry Lab No. 940486) was used through 1998, but had lost pathogenicity by 1999. Therefore, another US-7 isolate (Fry Lab No. 940330) was used starting in 1999. These two isolates were both classified as being US-7 based upon RG57 fingerprinting. However, our work indicates that the initial pathogenicity of the 940330 isolate of US-7 is different than that of the 940486 isolate. Therefore, these two isolates are treated as unique, rather than the same isolate.

PHYTOPHTHORA INFESTANS CULTURE, MAINTENANCE, AND INOCULUM PREPARATION. *Phytophthora infestans* isolates were subcultured every month on ryeB (Caten and Jinks, 1968) and pea media (Goodwin et al., 1994) for maintenance. Sporangia were isolated from culture plates with ddH₂O and concentrated with brief centrifugation, and used to inoculate leaflets of the susceptible tomato genotype 'VFT Vendor' to increase sporangia numbers and maintain pathogenicity, which might weaken during long-term culture. The 'VFT Vendor' plants used for this purpose were grown in a clean greenhouse without the use of any chemical controls. The leaflets were placed adaxial surface down on the lids of water agar plates, inoculated with sporangia suspension, and then covered by water agar containing petri dish bottoms.

The plates were incubated upside down at 18 °C and 16-h light condition for 6–7 d. Production of sporangia was confirmed by examining leaflets under microscope (100×).

This method was also used to increase sporangia for preparing inoculum for the disease screens by substantially increasing the number of 'VFT Vendor' leaflets inoculated and cultured. Sporangia produced on 'VFT Vendor' tomato leaflets were adjusted to the desired concentrations in the final inoculum suspension (sporangia per milliliter).

SCREENING METHODS USED TO TEST FOR LATE BLIGHT RESISTANCE. For all of the field trials, plants were grown to ~5 weeks of age then transplanted. In the Ithaca field trials, each plant was spray-inoculated at ~12 weeks of age with 20 mL/plant of a 2000 sporangia/mL solution. The Mexico field trials used natural infestation by local isolates. The plants were then surveyed on a periodic basis for percentage defoliation due to late blight using a modified Horsfall–Barratt scale (Horsfall and Barratt, 1945) and data were converted to percent defoliation using Elanco conversion tables (Redman et al., 1969).

The detached leaflet droplet test (Legard et al., 1995) was used in laboratory assays for resistance in plants grown either in the field or in greenhouses with modifications as follows. All leaflet samples were taken at least 6 weeks after germination. Tomato leaflets were collected from the 3–4th leaf from the vine apex to synchronize leaflet age. Each leaflet was placed adaxial surface down on the lids of water agar plates. The abaxial surface of each leaflet was inoculated with two 20- μ L drops of 40,000 sporangia/mL, and then covered by water-agar containing petri dish bottom to keep relative humidity near 100%. The plates were incubated upside down at 18 °C and 16 h light for 6–7 d. The droplet tests were repeated at least three times. The inoculated leaflets were visually evaluated for the hyphal growth, and then counts of sporangia produced were taken as follows. Leaflets were placed into 5 mL of sodium acetate and copper sulfate preservation solution followed by 10 s. mixing using a Vortex (Mizubuti and Fry, 1998). Leaflets were removed and sporangia suspensions were held at 4 °C until sporangia numbers were counted using a 0.2-mm-deep hemocytometer (Hausser Scientific, Horsham, Pa.), and sporangia per leaflet were calculated. The droplet test requires more time/effort to perform than the dipping method (below), but has the advantage of allowing collection of sporangia number data as well as lesion development.

A detached-leaflet dipping test was also developed by modification of the droplet test method. Leaflets were collected for the dipping test in the same manner as for the droplet test. The distal tip half of each leaflet was dipped into 40,000 sporangia/mL sporangia solution then the leaflets were incubated in the same manner as the detached droplet test method. Only visual evaluation data were taken of the leaflets in the dipping test, since sporangia applied per leaflet is not as tightly controlled as in the droplet test. The advantage of dipping method is that it is more rapid to set up than the droplet method and produces results quickly with extremely low rates of escapes.

DATA ANALYSIS. Where the population structure permitted valid use of the statistical tests, segregation data was analyzed using chi-square test with SAS (version 10; SAS Institute, Cary, N.C.).

Results and Discussion

BC_1F_1 TO PROCESSOR TOMATO POPULATIONS AND THEIR PARENTAL BC_1F_2 FRESH-MARKET ROOTED CUTTINGS. The crosses of the rooted cuttings of R. Gardner's five late blight-resistant [NC215E

x (NC215E x L3708)] F₂ selections were used as pollen donors to the three processing hybrid tomato cultivars Heinz 8892, Rogers 1108, and Orsetti 3155, resulting in production of 114 BC₁F₁ seeds.

The resulting BC₁F₁ plants were grown in the greenhouse and tested against the *P. infestans* isolate US-17 by the detached leaflet dipping test to examine segregation of the resistance and to identify the most resistant and susceptible plants (Table 1). The plants maintained from the five original cuttings were tested as resistant controls and the three female parental genotypes and NC215E were used as susceptible controls. The dipping test results suggested that two of the five original rooted cuttings (97A45 and 97A47) had lower levels of resistance than the other three rooted cuttings. A subsequent test using the leaflet dipping assay showed that the average sporangia numbers obtained from 97A45 and 97A47 were higher than those for the other three rooted cuttings. The BC₁F₁ populations derived from 97A45 and 97A47 also included very few potentially resistant plants. Therefore, the BC₁F₁ plants derived from 97A45 and 97A47 were excluded from future work. Plants derived from 97A49 were also eliminated due to greater difficulties in producing seed using this selection as the male parent. Therefore, the subsequent work is all based upon the 34 and 30 BC₁F₁ plants from crosses for which 97A46 or 97A48, respectively, was the male parent. Of these 64 BC₁F₁ plants, 26 were classified as resistant by the leaflet dipping assay.

Although the sizes of the remaining processor BC₁F₁ populations were too small for a conclusive test of the genetic control of late blight resistance, some observations could be made. Since the processor BC₁F₁ populations derived using either 97A46 or 97A48 as the male parent all segregated for resistance, neither 97A46 nor 97A48 was homozygous for the resistance. The processor BC₁F₁ population derived from 97A48 fits a 1:1 segregation ratio expected for control by a single gene; however, the processor BC₁F₁ population derived from 97A46 does not fit a 1:1 segregation ratio, due to a preponderance of susceptible plants (Table 1).

The controls and the 26 BC₁F₁ plants that were resistant in the leaflet dipping assay were retested using the *P. infestans* isolate US-17 and the detached leaflet droplet assay to provide an opportunity to collect sporangia number data. The average sporangia numbers of susceptible controls were 275,667 ± 55,467 sporangia/leaflet, in contrast to average sporangial numbers of 6750 and 1500 sporangia/leaflet for the resistant parents 97A46 and 97A48, respectively. The average sporangial numbers obtained from these 26 putative resistant BC₁F₁ plants ranged from 1833 to 67,750 sporangia/leaflet, which are all considerably less than that of the susceptible controls. We ultimately selected six of these resistant processor BC₁F₁s, with average sporangial numbers of 1833 to 8833 sporangia/leaflet (Table 1), which are similar to those of the resistant parents 97A46 and 97A48. The six selected BC₁F₁ plants were self pollinated to create BC₁F₂ populations, and were also backcrossed to 'Hypeel 45' and 'Hypeel 303' to create BC₂F₁ plants. Self-pollination of the BC₁F₁ produced copious seed, and the crosses resulted in a total of 1212 BC₂F₁ seeds.

PROCESSOR BC₁F₂ POPULATIONS. The six BC₁F₂ populations generated from the six BC₁F₁ selections were field grown and lab and field tested for late blight response in Ithaca 1998. Each population included 40 plants, for a total of 120 processor BC₁F₂ plants each derived from 97A46 and 97A48. Leaflets from each plant were collected and tested in the lab against US-17 (970001) with the detached leaflet droplet method and sporangia numbers were counted. After the leaflets were collected for the lab assay,

the field was inoculated with US-7 (940486) for a field trial.

The disease response in the Ithaca field test was very severe and very uniform across susceptible controls and spreader rows. All plants of the three processing tomato susceptible controls were more than 90% defoliated, and NC215E was 58% to 84% defoliated at 15 to 18 d postinoculation (DPI). The reasons that defoliation of NC215E plants was slower than that of the processing tomatoes might include its larger vine type, later maturity, and/or some other background trait of fresh market tomato that are different from those of processing tomatoes. Processing tomato have far greater concentration of fruit set and greater fruit load per plant size than fresh market lines such as NC215E, and the stress that these traits impose on the processing tomato plants may increase the rate of defoliation of a diseased plant.

The processor BC₁F₂ populations in the field trial clearly segregated for resistance, dividing the populations into plants that were resistant or susceptible to US-7, with the segregation for resistance : susceptibility fitting a 3:1 ratio (Table 2). A very different segregation pattern was obtained when the same plants from these processor BC₁F₂ populations were tested using the detached leaf droplet test with US-17. In this lab assay, the plants were either fully resistant to US-17 or they are not fully resistant. The plants that were not fully resistant could have low levels of resistance or be fully susceptible; it was not possible to reliably assign plants to separate classes of low level resistant vs. fully susceptible. The fully resistant plants were the minority, rather than the majority of the population, and the data do not fit either the 3:1 or the 1:3 ratio expected for control by one gene. Therefore, the results obtained from the same plants by the two tests using different isolates (US-17 vs. US-7) are clearly different. The US-17 data from processor BC₁F₂ populations fit either the 3:13 or 1:15 ratios segregation of resistant to susceptible plants expected for control by two epistatic genes (Table 2); however, data from larger populations would be required for a more reliable test of these hypotheses.

Since the same plants were used for the Ithaca field and lab tests, it is possible to simultaneously consider the data from both tests, revealing a relationship between the plants reactions to US-7 and US-17, and separating the plants into three classes (Table 2). The plants fully resistant to US-17 are also fully resistant to US-7, and plants fully susceptible to US-7 are all fully susceptible to US-17. The remaining plants, which are all fully resistant to US-7, were the plants that were not resistant to US-17, but had reduced disease expression compared to their fully susceptible siblings. It appears that plants cannot be resistant to US-17 without also being resistant to US-7, that full susceptibility to US-7 is related to full susceptibility to US-17, and that resistance to US-7 slightly reduces the level of disease response to US-17.

Considering the relative sizes of the three phenotypic classes observed in the BC₁F₂ populations (Table 2), this pattern clearly does not fit the expectation of control by a single incompletely dominant gene, as suggested by Chunwongse et al. (1998, 2002). But rather it suggests that the resistance to US-7 and US-17 involves more than one gene, and that at least some of the genes interact epistatically.

The two gene ratio with the closest fit to the BC₁F₂ data is the 3:9:4 ratio resulting from an epistatic system in which the dominant allele at the epistatic locus conditions resistance to US-7, regardless of the genotype at the hypostatic locus, and in which the homozygous recessive phenotype at the hypostatic locus gives resistance to US-17 only in the presence of the dominant allele at the epistatic locus. Testing the data to this two-gene ratio

Table 1. Response to *P. infestans* pathogen isolate US-17 (970001) of susceptible tomato controls and processor tomato populations derived from 97A46 and 97A48 after one backcross and two backcrosses to processor parents.

Parent or cutting name	Type of parent or cutting ^y	BC ₁ F ₁ selection name (pedigree)	BC ₁ F ₁ test ^z (avg no. sporangia/leaflet)	BC ₂ F ₁ selection name (pedigree)	BC ₂ F ₁ test ^z (avg no. sporangia/leaflet)
'NC215E'	S		197,667		399,167
'Rogers 1108'	S		775,000		
'Heinze 8892'	S		315,000		
'Orsetti 3155'	S		162,500		
'Hypeel 45'	S				998,333
'Hypeel 303'	S				261,667
97A46	R		6,750		
		971071-3 ('Rogers 1108' x 97A46)	8,833		
		971083-7 ('Orsetti 3155' x 97A46)	2,167		
				982114-2 ('Hypeel 45' x 971083-7)	14,844
				982114-4 ('Hypeel 45' x 971083-7)	7,031
97A48	R		1,500		
		971079-1 ('Heinz 8892' x 97A48)	6,167		
		971079-10 ('Heinz 8892' x 97A48)	2,250		
		971085-3 ('Orsetti 3155' x 97A48)	2,000		
				982128-39 ('Hypeel 303' x 971079-10)	17,188

^zData obtained using detached leaflet droplet method and the pathogen isolate US-17 (970001).

^yS = susceptible control/parent; R = resistant donor selection.

Table 2. Segregation for resistance to *P. infestans* isolate US-17 and US-7 in BC₁F₂ tomato populations, and tests of the segregation data from the test using each isolate to one-gene (3:1) or two-gene (3:13 and 1:15) ratios, and of combined data from both tests to two-gene (9:4:3) and three-gene (36:16:9) ratios.

Population ^y	No. of plants S US-17, R US-7 ^x	No. of plants S US-17, S US-7 ^x	No. of plants R US-17, R US-7 ^x	No. of plants R US-17, S US-7 ^x	<i>P</i> ^z 3R:1S for US-7	<i>P</i> ^z 1R:3S for US-17	<i>P</i> ^z 3R:13S for US-17	<i>P</i> ^z 1R:15S for US-17	<i>P</i> ^z 9:4:3 for S US-17, R US-7: S US-17, S US-7: R US-17, R US-7	<i>P</i> ^z 39:16:9 for S US-17, R US-7: S US-17, S US-7: R US-17, R US-7
97A46-derived processor BC ₁ F ₂	72	37	11	0	0.140	0.000	0.007	0.187	0.020	0.158
97A48-derived processor BC ₁ F ₂	69	35	16	0	0.292	0.003	0.128	0.001	0.254	0.574
Pooled					0.074	0.000	0.003	0.001	0.007	0.138
Homogeneity					0.766	0.456	0.408	0.182	0.709	0.647

^zProbability of the chi-square test of this ratio.

^yBC₁F₂ populations derived either from resistance parent 97A46 or 97A48, as described in materials.

^xData generated by testing the same field-grown plants by both a detached-leaflet droplet test using isolate US-17 (970001) followed by field inoculation of plants with isolate US-7 (940486). For the field test, R US-7 indicates resistant plant, without blight symptoms, S US-7 indicates susceptible plants, with significant defoliation. For the detached leaflet droplet test data: R US-17 = resistant, detected by sporangia numbers less than log₁₀ (avg. sporangia) = 4.50, which is equivalent to 31,623 sporangia/leaflet. S US-17 = susceptible, detected by sporangia numbers greater than log₁₀ (avg. sporangia) = 4.50. The average number of sporangia for the susceptible control plants were log₁₀ (avg. sporangia) = 5.83, which is equivalent to 680,724 sporangia/leaflet.

(3:9:4) gives a poor overall fit to the model since only one of the populations fits this ratio (Table 2). Therefore, a three-gene system was considered.

The closest three gene ratio is the (9:39:16) in which two genes for resistance are dominant and one resistance gene is recessive. In this model, 9/64 of the population would have the genotype $A_bbC_$ and the phenotype R to US-7, full R to US-17, 16/64 of the population would have the genotype $aa_ _ _$ and the phenotype S to US-7, S to US-17, and 39/64 of the population would have the genotype A_bbcc or $A_B_ _ _$ and the phenotype R to US-7 with variable degrees of S to US-17. The data from the BC_1F_2 populations have good fit to this three-gene ratio (Table 2) indicating that the hypothesis might be correct, but this cannot be proved solely by these BC_1F_2 data sets.

PROCESSOR BC_2F_1 POPULATIONS. The BC_2F_1 populations derived from three BC_1F_1 selections (971083-7, 971079-1, and 971079-10) were grown in the greenhouse for late blight lab screens. A total of 173 BC_2F_1 plants were tested against US-17 with detached-leaflet dipping method. Susceptible BC_2F_1 plants were identified through four replicates of the dipping test. Again the majority of the plants were susceptible, rather than resistant to US-17 in the lab test. The data sets are homogeneous, but clearly do not fit the 1:1 ratio expected under a one-gene model, or the 1:3 backcross ratio expected for control by two-genes (Table 3). However, the populations, grouped by male parent, fit the 1:7 ratio expected for genetic control of resistance by the interaction of three genes (Table 3).

Eighteen putative resistant plants selected out of 173 processor BC_2F_1 plants used in the dipping test were retested with detached leaflet droplet method to compare degree of disease expression with that of the resistant parents. The droplet test was replicated three times and sporangia per leaflet were calculated. Three BC_2F_1 s were ultimately selected, two of which were derived from 97A46 and one derived from 97A48 (Table 1). The average sporangia numbers

of these three BC_2F_1 s were $13,021 \pm 3,070$ sporangia/leaflet and the average sporangia numbers of the susceptible controls were $553,056 \pm 118,255$ sporangia/leaflet. These three BC_2F_1 s were self-pollinated to create BC_2F_2 populations.

PROCESSOR BC_1F_3 AND BC_2F_2 GENERATIONS. BC_1 and BC_2 derived populations were lab-tested with US-17 using the dipping test and field-tested with US-7 (940330), along with controls in 1999. The susceptible controls, 'Hypeel 45' and 'Hypeel 303', and the *Ph-1* control, 'New Yorker', were all highly and uniformly susceptible in the field test. The *Ph-2* control 'West Virginia 63' initially showed a delay in symptom development but was also, ultimately, very susceptible. The response of the susceptible controls demonstrated that the field trial had excellent disease pressure. All 20 BC_1F_3 populations derived from BC_1F_2 plants that were highly susceptible to US-17 in lab tests and to US-7 (940486) in field tests in 1998 were uniformly susceptible in the 1999 field test. Six BC_1F_3 populations derived from BC_1F_2 plants that were resistant to US-17 in lab tests and to US-7 (940486) in field tests in 1998 were uniformly resistant in the 1999 field test. Two of these resistant BC_1F_3 populations still segregated for indeterminate growth habit, so there were no relationships between indeterminate trait controlled by *Sp* (on the lower arm of chromosome 6) and late blight resistance. Even though pathogenicity of US-7 isolate 940330 was different than the original US-7 isolate used (940486), populations fixed for resistance to US-17 were also fixed for resistant against the 940330 US-7 isolate.

RETESTING PROCESSOR BC_1F_2 AND BC_1F_3 GENERATIONS IN 2000 TO DETERMINE EFFECTS OF ISOLATE VS. TEST METHOD ON DISEASE SCREEN RESULTS. The question regarding severity of tests used in the lab vs. those in the field test arose from the results of the 1998 and 1999 trials. If the lab test was a more severe screen than the field test, different results might be obtained due to the test used as well as from the differences in the isolate used. Therefore, populations from remnant seed of various generations

Table 3. Segregation in BC_2F_1 tomato populations for late blight response using detached leaflet dipping test with the isolate US-17 (970001), and tests of these data to two-gene (1:3), and three-gene (1:7) backcross ratios.

BC_2F_1 population ^y	R US-17 ^x	S US-17 ^x	Total	df	P^z 1R:3S	P^z 1R:7S
Derived from 97A46						
'Hypeel 45' x 971083-7	2	32	34	1	0.010	0.243
'Hypeel 303' x 971083-7	1	31	32	1	0.004	0.109
Pooled	3	63	66	1	0.000	0.051
Homogeneity				1	0.796	0.735
Derived from 97A48						
'Hypeel 303' x 971079-1	4	31	35	1	0.064	0.848
'Hypeel 45' x 971079-10	4	29	33	1	0.088	0.948
'Hypeel 303' x 971079-10	6	33	39	1	0.166	0.586
Pooled ^w	14	93	107	1	0.004	0.855
Homogeneity				2	0.915	0.859
Combined population						
Pooled	17	156	173	1	0.000	0.288
Homogeneity				4	0.767	0.535

^zProbability of the chi-square test of this ratio.

^yEach of the five BC_2F_1 populations were derived from heterozygous BC_1F_1 resistant selections.

^xFor the detached leaflet droplet test data: R US-17 = resistant, detected by sporangia numbers $< \log_{10}(\text{avg. sporangia}) = 4.50$, which is equivalent to 31,623 sporangia/leaflet. S US-17 = susceptible, detected by sporangia numbers greater than $\log_{10}(\text{avg. sporangia}) = 4.50$. The average number of sporangia for the susceptible control plants was $\log_{10}(\text{avg. sporangia}) = 5.83$, which is equivalent to 680,724 sporangia/leaflet.

^wThe minimum population sizes to distinguish between 1:3 vs. 1:7 are 143 and 246, at $P = 0.05$ and $P = 0.01$, respectively (Hanson, 1959).

were field-tested with US-17 and tested by the dipping test with US-7 (940330), testing the same plant populations in a test that was the reverse of the testing in 1998. As controls, eight BC₁F₃ populations that were fixed for resistance to US-7 and US-17 and 12 BC₁F₃ populations that were susceptible to both isolates in the 1999 trials were included. The 2000 results on these 20 populations exactly matched the 1999 results, regardless of switching which of the isolates was used in the field vs. the lab assays. Two of the processor BC₁F₂ populations lab-tested in 1998 with US-17 were retested in 2000 field using the same isolate. The results of the tests of these two populations were also similar to those observed in 1998. The segregation of the 1998 lab data fit either a 3:13 or a 1:15 ratio (Table 2); the segregation of the 2000 field data fit either a 3:13 or a 1:3 ratio (Table 4). Therefore, the method used (lab vs. field), does not appear to have a major impact on the results obtained with the same isolate. Consequently the differences observed in segregation ratios, such as those in the 1998 Ithaca field and lab assays, are more likely due to differences in the isolate used in the tests, not differences in the method used (lab vs. field test). These results support the reliability of the lab test method to predict the performance of resistant plants/lines in field tests against the same isolates. This has important practical ramifications, since, to prevent the release of new isolates, breeders cannot use pathogen isolates not already present in the regions of their field tests. Use of the lab test could be advantageous, facilitating the use of multiple isolates in the disease screens for breeding further resistant lines.

A change in the US-7 isolate used was required when the original stock used (940486) was found to have lost pathogenicity. To test for differences in the pathogenicity of the original and new stock of US-7 isolates, two of the BC₁F₂ populations field-tested in 1998 with US-7 (940486) were retested by detached leaflet dip test in using US-7 (940330). The segregation of these populations tested with US-7 in 1998 fit a 3R:1S ratio, but the 2000 results clearly did not fit this ratio, since the resistant class was smaller than the susceptible class. The 2000 segregation data did not fit 1R:3S or 1R:15S ratios, but did fit a 3R:13S ratio (Table 4), which is similar to the results obtained from US-17 results of same populations in 1998 (Table 2). Therefore, these results suggest that the pathogenicity of 940330 isolate is different from that of 940486 isolate of US-7.

FRESH-MARKET BC₁F₂ AND PROCESSOR BC₂F₃ GENERATIONS TESTED IN 2000. Dr. Gardner kindly provided us with remnant seeds of the two fresh-market BC₁F₂ populations from which 97A46 and 97A48 had been derived (00A02, 00A03, respectively). The results of field-testing these BC₁F₂ populations with US-17 in 2000 support the hypothesis that they would segregate in the same manner as the processor BC₁F₂ and BC₂F₂ populations derived from 97A46 and 97A48 (Table 4). The segregation ratio of the two fresh-market BC₁F₂ populations did not fit the ratio expected for control by single dominant gene, but rather fit the expectation under either a one recessive gene model (1:3) or an epistatic two-gene model (3:13) (Table 4).

This agreed with the results of our processor BC₁F₂ populations (Table 4). These results, and the lack of fit of the processor BC₂F₁ population segregation ratio to the 1:1 ratio for control by a single gene in a backcross F₁ population (Table 3), clearly indicate that the resistance against US-17 is not controlled by single gene. When these fresh-market BC₁F₂ populations were tested with US-7 (940330) using the leaflet-dipping test, the results indicated a lack of fit to either a 1:3 or 3:13 ratios but fit 1:15 ratio (Table 4). Therefore, the results with isolate US-7 (940330) also

failed to support the expectations for control by one gene in this population. This also supports the observation of differences in pathogenicity between US-7 (940486) and (940330).

BC₂F₃ seeds collected from 21 BC₂F₂ plants selected in 1999 were also grown in 2000, and the resulting 21 BC₂F₃ populations were field tested with US-17 and lab tested with US-7 (940330). The results indicated that the BC₂F₃ progeny from 20 of the BC₂F₂ selections were fixed for resistance against both US-17 and US-7 (940330) isolate, and the one remaining BC₂F₃ population segregated for the resistance. Again, making selections based upon both lab and field trials chiefly resulted in selection for plants that were not only resistant to both isolates, but were fixed for the resistance to both isolates. This agrees with the 1998 selections tested in 1999.

LATE BLIGHT TRIALS UNDER NATURAL INFESTATION IN MEXICO. A winter/spring late blight trial was performed in 2001 in Los Mochis Mexico under the supervision of I. Stein of Campbell Soup Co. (Camden, N.J.). The trial included 23 late blight-resistant lines (5 BC₁F₃ and 18 BC₂F₃ lines), which were supposed to be fixed for late blight resistance as well as six susceptible genotypes as controls. The test rows alternated with susceptible spreader rows, so that all test rows were flanked with spreader rows that provide even disease pressure throughout the entire field. Disease was evenly spread through the spreader rows across the entire field. By mid-Mar. 2001, the plants of the susceptible controls and the alternating spreader rows were 50% to 70% defoliated; however, the resistant lines were all 0% defoliated, except for one BC₂F₃ population that still segregated for the resistance. This population had also segregated in 2000 Ithaca experiment. Disease symptom expression of the susceptible segregants in this BC₂F₃ population was less severe than that of the susceptible processing tomato lines used as a control. This parallels the reduced susceptibility seen in the susceptible segregates in the 1998 field trial in Ithaca. Since natural infestation was used in the Mexico field trial, we do not know the identity of the pathogen isolate(s) present in this trial. However, the results show that the lines fixed for late blight resistance withstand heavy pathogen pressure under conditions very conducive to severe disease.

SYNTHESIS. L3708-derived late blight resistance, which controls disease caused by a number of *P. infestans* isolates, was transferred to processing tomato lines after two generations of backcrossing. In the process of this transfer, segregation for the resistance shows several points of importance for the further transfer and use of this resistance. The breeding populations supported the importance of the *Ph-3* gene in the resistance from L3708, and all of our breeding populations fixed for the full resistance are homozygous for markers linked to the *Ph-3* gene, as located by Chunwongse et al. (1998). However, segregation for full resistance in our backcross generations does not support the hypothesis that the full resistance is controlled by a single dominant or incomplete dominant gene controlling resistance. Rather, the data indicate that more than one gene is involved in control of this resistance, with the genes interacting in an epistatic manner. The number of genes is not certain, but is likely to be one or hypostatic genes in addition to the epistatic gene, *Ph-3*. It is possible that segregation distortion found in early generation interspecific population may affect segregation data and so affect estimates of gene number. In populations derived from cross of tomato and the distant relative, *S. pennellii* Correll [formerly *L. pennellii* (Correll) D'Arcy] 80% and 21% of markers in F₂ 21% of BC₁F₁ populations show skewed segregation (de Vincente and Tanksley, 1991, 1993), with the skewed regions located in 16 segments in the 12 chro-

Table 4. Segregation for late blight response in BC₁F₂ processor tomato populations and BC₁F₂ fresh-market tomato populations^a against isolate US-17 (970001) in a field trial, and isolate US-7 (940330) in a detached leaflet dipping test.

				P^y 1R:3S US-17	P^y 3R:13S US-17			P^y 1R:3S US-7	P^y 3R:13S US-7	P^y 1R:15S US-7
	df	R US-17 ^x	S US-17 ^x			R US-7 ^x	S US-7 ^x			
97A46-derived processor BC ₁ F ₂										
971083-7 self	1	36	124	0.465	0.224	22	138	0.001	0.105	0.000
971071-3 self	1	33	127	0.201	0.543	21	139	0.001	0.068	0.000
Pooled	1	69	251	0.156	0.167	43	277	0.000	0.015	0.000
Homogeneity	1			0.699	0.671			0.897	0.886	0.817
Fresh market BC ₁ F ₂										
00A02	1	33	107	0.696	0.143	13	127	0.000	0.004	0.138
00A03	1	35	125	0.361	0.311	11	149	0.000	0.000	0.744
Pooled	1	68	232	0.351	0.082	24	276	0.000	0.000	0.210
Homogeneity	1			0.735	0.708			0.630	0.594	0.389
Combined populations										
Pooled	1	137	483	0.095	0.033	67	553	0.000	0.000	0.000
Homogeneity	3			0.947	0.930			0.346	0.442	0.035

^aBC₁F₂ populations 00A02 and 00A03 were grown from remnant seed of seed lots which also grew the BC₁F₂ populations from which plants 97A46 and 97A48 were selected. Remnant seed was provided by R. Gardner.

^yProbability of the chi-square test of this ratio.

^xFor the field test, R US-17 indicates resistant plant, without blight symptoms, S US-17 indicates susceptible plants, with significant defoliation. For the detached leaflet dipping test, R US-7 indicates resistant leaflet, without fungal growth and sporulation, S US-7 indicates susceptible leaflet, with visible fungal growth and sporulation.

^wThe minimum population sizes to reliably distinguish between 1:3 vs. 3:13 are 667 and 1152, between 1:3 vs. 1:15 are 50 and 86, between 3:13 vs. 1:15 are 98 and 170 at $P = 0.05$ and $P = 0.01$ respectively (Hanson, 1959).

mosomes. However, much less segregation distortion is observed in populations derived from close relatives, such as tomato and *S. pimpinellifolium*. A survey of a BC₁F₁ population derived from tomato and *S. pimpinellifolium*, and using 120 markers, revealed skewing for only 10 markers (8%), five of which were clustered on chromosome 11 (Grandillo and Tanksley, 1996). The four regions of these 10 skewed markers account for slightly less than 4% of map distance of the genome. In agreement with that report, no skewing was observed for segregation for known monogenic traits in our breeding populations, such as visually observed segregation for self-pruning (*Sp*), and segregation for resistance genes (*Ve*, *I2*, *Mi*, and *Pto*) using linked PCR markers. Therefore, it is more likely that the deviation from a monogenic ratio observed is due to the action of additional genes, rather than due to segregation distortion.

The segregation results have important implications for breeding efforts to transfer this resistance to additional tomato lines. Use of larger populations and strategies for transfer of more than one gene will be required for further transfer of the full resistance trait. It is also important to note that the segregation ratios observed depended on the pathogen isolate used for the screen. Therefore, to insure that full resistance is transferred to new lines, breeding populations should be tested with a range of isolates, a process facilitated by use of a laboratory based assay. The isolates chosen should represent the full breadth of isolates. Since the segregation data indicates that full resistance across pathogen isolates is not dominant, it is strongly recommended that resistant lines and hybrids be homozygous for the resistance genes.

Since more than one gene is implicated in the resistance, it is not clear whether or not all of the genes involved in the full resistance transferred to our processing tomato lines were derived from L3708. The *Ph-3* gene itself is clearly derived from L3708, since it has been mapped to an *S. pimpinellifolium* introgression (Chunwongse et al., 1998). It is possible that the other genes in the late blight-resistance complex are also from L3708 or that

they were derived from the initial tomato parent, NC215E, since some fully late blight-resistant lines have been produced from pedigrees involving only NC215E and L3708. Examination of our stock of L3708 does not answer this question, since the stock itself was not uniform for resistance. Seven of the eight plants of L3708 tested by leaflet dipping method using US-7(940330) and US-17 (970001) were resistant to US-17, and five of those plants also had resistance to US-7. However, the degree of this resistance to US-7 varied among the plants resistant to both US-17 and US-7. If the NC215E contributed some of the resistance genes transferred to the processing tomato lines, the ultimate source of these additional genes is unclear. All relatively modern tomato lines contain introgressions from wild species as the result of their possessing standard resistance genes (such as *Ve* or *I2*), and it is possible that the introgressions carrying those genes, or other regions unknowingly introgressed in the process, may contain some of the hypostatic alleles involved in the full late blight-resistance trait transferred. It may be possible to determine the source of these hypostatic genes as their locations are mapped.

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