Abstract

BRUSCA, JAMES PHILLIPE. Inheritance of tomato late blight resistance from 'Richter's Wild Tomato' and evaluation of late blight resistance gene combinations in adapted fresh market tomato backgrounds. (Under the direction of Randolph G. Gardner.)

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is a destructive disease of tomato worldwide. Originating in the highlands of central Mexico, through migration, mutation, and sexual recombination, this pathogen has proven mobile and highly variable. Three monogenic R genes (*Ph-1, Ph-2, and Ph-3*) for resistance to late blight have been identified in tomato. However, *P. infestans* isolates which overcome each of these individual R genes have been documented. The objectives of this research were to characterize the inheritance of late blight resistance in the home garden tomato 'Richter's Wild Tomato', to determine if this resistance is allelic to any of the three previously identified resistance sources, and to evaluate late blight resistance of R gene F1 hybrid combinations relative to individual R genes. 'Richter's Wild Tomato' was crossed with NC215E-1, a late blight susceptible fresh market breeding line, and evaluation of derived BC1F2, BC2F2, and BC3F1 populations indicated single gene inheritance of late blight resistance. Evaluation of an F2 population derived from the cross 'Richter's Wild Tomato' x 'Legend' (*Ph-2*) indicated that the monogenic resistance

conferred by 'Richter's Wild Tomato' was allelic to *Ph-2*. Combinations of *Ph-2*, either from 'Richter's Wild Tomato' or 'Legend', and a fresh market line possessing *Ph-3* displayed superior resistance versus individual R genes and other R gene combinations in North Carolina field trials under natural inoculation. Combined resistance has the potential to not only provide a superior level of resistance, but to also be more stable over time.

Inheritance of tomato late blight resistance from 'Richter's Wild Tomato' and evaluation of late blight resistance gene combinations in adapted fresh market tomato backgrounds

by

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Biography

I was born May 13, 1974, on the island of Mauritius. At the time, my folks and three older siblings were in their second year of a two year Peace Corps term. Shortly thereafter, my family relocated to Eureka, California, where I was raised.

I attended the University of California at Davis for my undergraduate education. Aside from majoring in Genetics and earning a minor in English, I also played for the school varsity soccer team. During the summer prior to my final year at U.C.D., I had the opportunity to work with Elaine Graham and Joseph Jacobs at Harris Moran Seed Company as a field research assistant. It was this opportunity that stimulated my interest in plant breeding. Upon graduating in 1997, I worked part time for Harris Moran as a breeding technician, and part time for the Tomato Genetics Resource Center (TGRC) under the direction of Roger Chetelat. The TGRC opportunity gave me first hand insight into the value of germplasm diversity. In 1998, I accepted a full time breeding technician position with Harris Moran Seed Company in their processing tomato program. During the next few years, under the direction of Mike Kuehn, I was offered many opportunities to explore plant breeding and to learn about the seed industry in general.

In August of 2001, I moved to Raleigh, North Carolina to pursue a graduate degree at North Carolina State University in Horticultural Science under the direction of Dr. Randy Gardner.

Acknowledgements

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Special thanks go to Elaine Graham and Joseph Jacobs who helped me initially discover the excitement of plant breeding. Also, thanks to Mike Kuehn and Jeff McElroy for there advice and support with regards to pursuing a graduate degree.

I also wish to acknowledge my family for their support and encouragement. Being away from them the past two years was perhaps the most difficult part of this endeavor, but their visits, phone calls, e-mails, and letters were much enjoyed reminders of the strength of family. Finally, I wish to thank my fiancée Christina, for taking a huge risk in joining me out here; a risk that required incredible strength and trust, but has proved a fortuitous roll of the dice for both of us.

Table of Contents

List of Tables

List of Figures

Inheritance of tomato late blight resistance from 'Richter's Wild Tomato' and evaluation of late blight resistance gene combinations in adapted fresh market tomato backgrounds

Introduction

Late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary, is a devastating disease of tomato (*Lycopersicon esculentum* Mill.) and potato (*Solanum tuberosum* L.). Many aspects of the pathogen's biology have hindered efforts to develop stable chemical control, as well as efforts to breed resistant plant cultivars. This introduction reviews the literature regarding pathogen history and biology, the interaction between *P. infestans* and its hosts, and efforts to breed resistant tomato and potato cultivars. This information lays the foundation for research investigating the inheritance of a new source of resistance to late blight in tomato and the potential for combining different resistance sources into adapted tomato germplasm.

History

Late blight is best known as the disease responsible for the Irish potato famine during the 1840s. The disease essentially destroyed the potato crop in Ireland during 1845 and 1846. The resulting famine was responsible for over one million deaths and the emigration of at least 1.5 million Irish citizens (Large, 1940). The Irish potato famine led to intense investigation into the nature of plant disease and resulted in de Bary's confirmation of germ theory (Fry and Goodwin, 1997a).

The use of pathogen free seed tubers, application of fungicides, and elimination of other pathogen harboring sources helped to reduce late blight effects in the middle of the

twentieth century (Fry and Goodwin, 1997a). However, devastating outbreaks of the disease during the 1980s and 1990s throughout the world have led to renewed efforts to understand the nature of the pathogen and to develop novel methods of late blight control (Fry and Goodwin, 1997b). The economic ramifications of late blight remain significant, with current potato losses estimated at one billion dollars per year (Kamoun, 2001).

Pathogen Distribution

Phytophthora infestans appears to have evolved on wild potato relatives (*Solanum* species) in a limited area in the highlands of central Mexico (Reddick, 1939). For *Solanum* species, this region is a secondary center of diversity (Hawkes, 1966). A recent evaluation of late blight diversity documented late blight isolates worldwide (Goodwin et al., 1994b).

Pathogen Host Range

The two economically significant hosts of *P. infestans* are cultivated potato and tomato, both members of the family Solanaceae. Other hosts include all species of *Lycopersicon* and 47 additional *Solanum* species (Erwin and Ribeiro, 1996). Furthermore, when inoculated, many unrelated species, including *Acer* spp., are susceptible to infection by *P. infestans* (Pshedetskaya, 1968). Although infection of these other hosts may not be economically significant, infection does provide opportunities for the pathogen to overwinter.

Symptoms/Signs

Late blight affects all above ground portions of the tomato plant. Leaf symptoms begin as small, water-soaked spots, generally at the tips or edges of lower leaves. These spots can grow quickly into large pale green to brown lesions. Under appropriate environmental conditions, gray to white mycelial growth may occur on the undersides of foliar lesions. Foliage infected with late blight eventually turns brown, shrivels, and dies (Agrios, 1997; Stevenson, 1991).

Symptoms on petioles and stems resemble leaf symptoms. Petiole and stem lesions are typically dark patches or brown spots, leading to brittleness and plant death (Blancard, 1994; Stevenson, 1991).

Tomato fruit symptoms begin as dark, greasy spots on the fruit surface. These spots may increase in size to cover the entire fruit. Under environmental conditions conducive to late blight sporulation, white mycelium may be visible on fruit (Stevenson, 1991). Lesions on tomato fruit may establish a ringed pattern, similar to buckeye rot, *P. parasitica* (Erwin and Ribeiro, 1996). Late blight fruit infection is often followed by soft rot leading to total fruit decay (Watterson, 1986). *Phytophthora infestans* fruit infection can penetrate the seed, resulting in infected tomato seedlings (Vartanian et al., 1985).

Environment

The significance of environment on disease progression is characterized by the disease triangle, which integrates host, pathogen, and environment (Van der Plank, 1968). It has been noted that a key component to the Irish potato famine, aside from the reliance on potato as a staple and the migration of the pathogen into that region of the world, was a period of weather characterized by abnormally low temperatures and increased humidity in 1845 facilitating pathogen establishment (Schumann, 1991). Late blight is highly responsive to weekly or even daily environmental changes (Duniway, 1983). Several components affect the survival, germination, penetration, and sporulation of late blight. Temperatures between 15 and 20 °C and high relative humidity are optimal for late blight initiation (Watterson, 1986). Zoospore germination is most efficient between 12 and 15 °C, with temperatures higher than 15 °C resulting in direct germination via germ tube production (Agrios, 1997). Mycelial growth is optimized at temperatures near 21 °C, with a relative humidity of 100% (Alexopoulos, 1962). Although temperatures of 30 °C or greater halt progression of the disease, they do not kill it and a return to appropriate environmental conditions can re-initiate infection (Agrios, 1997). Late blight lesion expansion is primarily a function of temperature (Rotem and Cohen, 1974). Sporangia can be dispersed by air or by water splash. As a result, the primary factors governing late blight spore dissemination are moisture and air turbulence (Aylor, 1978). Thick-walled oospores of *P. infestans* can survive harsh environmental conditions and are means for overwintering (Carlile and Watkinson, 1994). Oospores have been found to

survive in the soil for five to seven months at temperatures ranging between 0 and 20 °C (Pittis and Shattock, 1994). Furthermore, oospores remain capable of germinating after exposure to temperatures between -20 and 40 °C (Fay and Fry, 1997).

Asexual Reproduction

Late blight overwinters as mycelium associated with cull piles, plant debris, or volunteer hosts. Under environmental conditions conducive to sporulation, mycelium produce branched sporangiophores extending 200-µm from the foliar surface (Carlile and Watkinson, 1994). At the sporangiophore tip, lemon-shaped, papillate sporangia are produced. These sporangia range in size from 29 x 19-µm to 36 x 22-µm (Erwin and Ribeiro, 1996). Sporangia are dispersed via wind or water splash. Each sporangium contains between three and eight zoospores which are released upon bursting of the sporangial wall (Agrios, 1997). Zoospores each have two flagella facilitating locomotion in an aqueous environment (Bartnicki-Garcia and Hemmes, 1976). Upon coming to rest, and in the presence of cool temperatures and adequate water, sporangia germinate by the production of zoospores. If temperatures are warmer, over 20°C, zoospores encyst in the plant tissue, and penetrate the host tissue via a germ tube (Agrios, 1997). An appresorium is produced by the germ tube, from which an infection peg extends and enters into the host epidermal cell (Alexopoulos, 1962). Late blight has the potential to multiply asexually at a rapid rate. *Phytophthora infestans* has been shown to complete its disease cycle in as few as four days (Day, 1973). Late blight lesions have been found

to produce up to 30,000 sporangia per day (Legard et al., 1995). Furthermore, foliar disease has been noted to increase from a level of one to 75 percent in a period of two weeks under favorable environmental condition (Turkensteen, 1973).

Sexual Reproduction

Phytophthora infestans is a heterothallic organism, defined as the requirement of two different mating types for sexual reproduction. The two mating types are termed A1 and A2, and isolate mating type is determined by a single nuclear locus (Judelson et al., 1995). Initial interaction between mating types occurs between diploid mycelium. Upon recognition of the other mating type, haploid antheridia and oogonia are formed (Smart et al., 1998). An antheridium can fertilize an adjacent oogonium, yielding a diploid oospore (Agrios, 1997). Oospores are aplerotic, thick walled, and spheroid, approximately 30-µm in diameter (Erwin and Ribeiro, 1996). Germination of oospores occurs through a germ tube and results in the production of a sporangium (Agrios, 1997). Until recently, the A2 mating type was known only in Mexico, and the A1 was found worldwide. However, surveys over the past 20 years have found the A2 mating type in several other regions of the world.

The presence of both *P. infestans* mating types and the subsequent potential for sexual reproduction raises several concerns with respect to disease variability and survivability. Progeny of crosses between *P. infestans* mating types demonstrate genetic

recombination (Shaw and Shattock, 1991). Sexual recombination provides the potential for new, more adapted or aggressive isolates (Gavino et al., 2000). Research suggests that the isolate US-11, virulent on both potato and tomato and prominent in the Pacific Northwest, originated as a sexual recombinant (Goodwin et al., 1995).

Oospores further confound late blight control because of their potential to over-winter. In regions in Mexico where both mating types exist, oospores in the field can remain infective for over two years (Niederhauser, 1991). Oospores can survive up to four years in sandy soils under natural weather conditions in the Netherlands (Turkensteen et al., 2000).

Taxonomy

The genus *Phytophthora* was first described by Anton de Bary in 1876, with *P. infestans* being the type species (Zentmyer, 1983). *Phytophthora* is a member of the kingdom Chromista, phylum Oomycota, order Peronsporales, and family Pythiaceae (Cavalier-Smith, 1986; Hawksworth et al., 1995). There are 60 described species within *Phytophthora*, including: *P. cactorum*, an important pathogen of apple, *P. capsici* which affects pepper, the citrus pathogen *P. citrophthora*, and *P. cinnamomi*, which affects many woody plants including conifers (Erwin and Ribeiro, 1996; Hawksworth et al., 1995). Although originally classified as a fungus, oomycetes have been re-classified in the kingdom Chromista. Oomycetes are distinguishable from fungi based on metabolism (Pfyffer et al., 1990), cell wall composition (Bartnicki-Garcia and Wang, 1983), rRNA

sequence (Cooke et al., 2000; Förster et al., 1990), and zoospore motility (Zentmyer, 1983).

Traditionally, specific characterization within the genus *Phytophthora* has been based on structure of the sporangium, antheridial form, and on sexual compatibility/incompatibility (Waterhouse, 1973). *Phytophthora infestans* is described as having semipapillate sporangium, amphigynous antheridium, and being heterothallic and sexually incompatible (Cooke et al., 2000).

Isolate Characterization and Intraspecific Diversity

Phytophthora infestans displays a high degree of intraspecific diversity, and the recent resurgence of late blight in tomatoes has corresponded with changes in the characteristics of predominant isolates. Evidence suggests that the central highlands of Mexico, specifically the Toluca Valley, is the center of origin for *P. infestans* (Grünwald et al., 2001). Isolates of *P. infestans* are characterized based on mating type, specific virulence, isoenzyme analysis with glucose-6-phosphate (GPI) and peptidase (PEP), metalaxyl sensitivity, and a nuclear DNA probe, RG57 (Goodwin et al.; 1992, Shattock et al.; 1990, Tooley et al.; 1985).

With respect to North Carolina isolates, a study evaluating collected isolates between 1993 and 1995 revealed four genotypes, US-1, US-7, US-8, and US-18 (Fraser et al., 1999). A more recent study suggests a shift in genotypes has occurred in North Carolina during the past ten years. US-7 was the dominant genotype in 1993 and 1994,

however since that time, US-18 and US-19 have dominated. US-18 and US-19 are characterized as being the A2 mating type and sensitive to metalaxyl, with some variation (Wangsomboondee et al., 2002).

Pathogenic Specialization

Research confirms that late blight infection levels vary with pathogen genotype (isolate) and host plant species (Platt, 1999). With respect to the two economically most important hosts, tomato and potato, Small concluded that isolates taken from potato did not always infect tomato, but that isolates found on tomato always infected potato (Small, 1938). Another study investigating host-pathogen interaction suggested that isolates had differential virulence on tomato, but that all isolates, regardless of origin, were virulent on potato (Kishi, 1962). More recent research confirmed the hypothesis that *P. infestans* pathogenic specialization occured in tomato (Legard et al., 1995).

Migrations

Late blight migration has played a pivotal role in the proliferation of the disease. The first documented global migration occurred in the 1840s. The disease was observed first in the Northeastern United States, then Europe, and then South America, Africa, the Middle East, and Asia (Bourke, 1964; Stevens, 1933). Two paths have been hypothesized for this original migration. The pathogen may have traveled from Mexico to the U.S.A. and then to Europe or directly from Europe to Mexico (Fry et al., 1993). It

is likely that once the disease entered Europe, it spread to other continents via international seed potato trade (Cox et al., 1960). Based on pathogen diversity studies, it is hypothesized that this original migration and dispersal was based on a single clonal lineage (Goodwin et al., 1994b).

Phytophthora infestans populations appeared to remain relatively stable until the late 1970s. In the early 1980s, populations of *P. infestans* significantly different from the historical populations were observed in many areas outside of Mexico. Characteristics of these new populations included a different isozyme profile, and many were of the A2 mating type (Malcolmson, 1985; Mosa et al., 1993). The path of this migration was likely from Mexico to Europe on infected tubers and subsequently to the rest of the world via international potato seed trade (Fry et al., 1993). In Europe, this new population displaced historic late blight populations indicating that new populations were more fit and perhaps more aggressive (Spielman et al., 1991).

Recent changes in *P. infestans* populations have been documented in the United States and Canada (Fry and Goodwin, 1997a; Goodwin et al., 1994a). Increased disease severity in the early 1990s was associated with major genetic changes in late blight populations in this region (Deahl et al., 1991). The re-emergence was associated primarily with two isolates, US-7 and US-8 (Fry et al., 1997b). These new isolates were of the A2 mating type and metalaxyl resistant (Goodwin et al., 1996; Goodwin et al., 1998). In addition, US-7 is virulent on both tomato and potato. Although the mechanism

for spread is not completely known, evidence suggests that spread in seed tubers and spread in infected seed are partially to blame (Goodwin et al., 1995).

A resurgence of late blight was observed in North Carolina in the early 1990's, with consistent disease outbreaks since 1991 (Fraser et al., 1999). The increased frequency of late blight in North Carolina was associated with a change in late blight population structure, with the primary isolates now being US-7, US-8, US-18, and US-19 (Wangsombondee, 2002).

Control

Various means are employed to control late blight. Primary control consists of preventative fungicide application along with appropriate cultural practices, including sanitary field practices, crop rotation, and removal of diseased material and weed hosts (Agrios, 1997; Stevenson, 1991). Potato cull piles often serve as a source of primary inoculum, and elimination of the cull pile helps to prevent infection and reduce early occurrence (Bonde and Schultz, 1943).

Acylalanine fungicides, including metalaxyl, have shown efficacy against the late blight pathogen. However, metalaxyl resistant late blight strains were reported in the late 1970s (Dowley and O'Sullivan, 1981). Subsequent studies have documented the spread of resistant genotyopes and determined that metalaxyl resistance in *P. infestans* is controlled by a single dominant gene (Lee et al., 1999). Furthermore, a recent study has

demonstrated that metalaxyl can induce a switch in mating type and increase the potential for oospore formation (Groves and Ristaino, 2000).

Predictive models and forecasting systems have been developed in which a severity index is calculated based on weather patterns and pathogen presence. Programs such as Tom-Cast and Blightcast aid a grower in timing fungicide application (Gleason, et al. 1995; Krause et al., 1975). Integrated approaches using cultivars with high levels of field resistance along with a forecast system have shown promise in Mexican potato production (Grünwald et al., 2001).

Although there are some commercially available potato cultivars with moderate levels of resistance, there are no commercially accepted tomato cultivars resistant to late blight (Agrios, 1997).

Tomato Biology

Cultivated tomato (*Lycopersicon esculentum* Mill.) is an important Solanaceous crop worldwide. Although a perennial, tomato is cultivated universally as an annual. The center of origin for *Lycopersicon* is in the Andes Mountains of South America. Evidence suggests Mexico is the site of domestication for *L. esculentum* (Taylor, 1986). Tomato is grown worldwide, with primary growing countries being U.S.A., Italy, Brazil, India, and Turkey. Although work to improve existing cultivars began over 200 years ago, it was not until approximately 100 years ago that an effective breeding program was established (Stevens and Rick, 1986).

Lycopersicon esculentum is a diploid species with 12 chromosomal pairs (2n=24) (Winkler, 1909). The species is self-compatible and exclusively inbreeding. *L.ycopersicon esculentum* can intercross with many related wild species, either directly, through bridge species or by utilizing embryo rescue. These wild species have served as a source of genetic diversity and greatly enhance the breeding potential for *L. esculentum* (Kalloo, 1991). Efforts to evaluate, catalogue, and integrate wild germplasm have yielded many successes in terms of the development of disease resistant cultivars. The related wild species *L. pimpinellifolium* has served as a source of several resistance genes, including *Fusarium oxysporum* resistance (*I*, *I-2*, and *I-3*), *Psuedomonas syringae* PV. *tomato* resistance (*Pto*), and several resistance genes to different races of *Cladosporium fulvum* (*Cf-2, Cf-3, Cf-5, Cf-6, Cf-7, Cf-8, Cf-9,* and *Cf-10*) (Lutyanenko, 1991).

Horizontal vs. Vertical Resistance

Differential reaction of a cultivar to different races is termed "vertical" resistance. A cultivar equally resistant to all races of a pathogen is termed "horizontally" resistant (Van der Plank, 1968). *Phytophthora infestans* displays a gene-for-gene interaction between monogenic host resistance (R gene) and corresponding pathogen avirulence genes (Al-Kherb et al., 1995).

Tomato Breeding for Late Blight Resistance

In tomato, three monogenic sources of resistance to *P. infestans* have been identified. A late blight epiphytotic in the United States in 1946 stimulated breeding efforts to control the disease. In the early 1950s, a sceening of many cultivars led to the discovery of a single, dominant resistance gene, *Ph-1*, conferring resistance to late blight race T0 (Bonde and Murphy, 1952; Gallegly, 1952). Subsequent linkage tests indicated that *Ph-1* is located at the distal end of chromosome 7 (Pierce, 1971). *Ph-1* existed originally in two cultivars, 'West Virginia 36' and 'West Virginia 106', and was later integrated into 'Geneva 11', 'Rockingham', and 'New Hampshire Surecrop' (Walter, 1967). A new race of *P. infestans*, T1, which overcame the resistance conferred by *Ph-1* was observed shortly thereafter (Conover and Walter, 1953).

A second tomato resistance gene was found in a late blight resistant wild relative, *L. pimpinellifolium*, accession 'West Virginia 700'. This resistance was originally documented by Gallegly in 1960. However, at the time, the inheritance was unknown (Gallegly, 1960). Subsequent investigations indicated that the resistance in 'West Virginia 700' was controlled by a single, incompletely dominant gene, named *Ph-2* (Laterrot, 1975; Turkensteen, 1973). *Ph-2* maps to the long arm of chromosome 10 (Moreau et al., 1998). This source of resistance is currently available in the large fruited, determinate vined, fresh market cultivar 'Legend', released by Oregon State University in 2001.

Research investigating resistance in *L. pimpinellifolium* accession L3708 indicated that it possessed a single, dominant resistance gene, *Ph-3* (Black et al., 1996). This was confirmed and the locus was mapped to chromosome 9 (Chunwongse et al., 1998). L3708 has been further investigated in virulent California fields, and quantitative trait loci aside from *Ph-3* were identified on chromosomes 6 and 8, although the effect of the QTL on chromosome 6 is hypothesized to be a pleiotropic effect of the *sp* locus (Frary et al., 1998).

Other studies have identified tomato sources of resistance to late blight, however the mode of inheritance of resistance in these sources is unknown. In 1951, Barham and Ellis screened all tomato plant introduction accessions using a seedling greenhouse inoculation, and found 28 of the 909 available lines to possess some resistance (Barham and Ellis, 1951). A collection of heirloom cultivars was screened in a field trial with natural US-11 strain inoculation. 'Matt's Wild Cherry' was identified as possessing resistance relative to the other varieties (Inglis et al., 2000). Black et al. screened Asian Vegetable Research and Development lines with Taiwanese *P. infestans* isolates and found four accessions, L3707, L3708, L3683, and L3684, with some resistance (Black et al., 1996).

Late-blight-resistance plants have also been recovered by the transgenic approach of T-DNA insertion mutagenesis. However, the frequency of recovery was quite low (0.1%), and the transmissibility over generations is unknown (Lutova et al., 2001).

Potato Breeding for Late Blight Resistance

Late blight causes billion dollar losses yearly in potato (Kamoun, 2001). Monogenic (R gene) resistance to late blight in potato was discovered nearly a century ago in *S. demissum*, a wild relative of potato (Müller and Black, 1952). Breeding efforts throughout the last century have focused on *S. demissum* as well as *S. bulbocastanum*, *S. berthaulti*, *S. andigenum* and *S. stoloniferum* as sources of R genes (Ballvora et al., 2002; Ewing et al., 2000; Malcolmson and Black, 1966). At least 15 individual R genes have been identified in potato and several potato cultivars have been released containing single or combined R genes (Umaerus et al., 1994; Van der Plank, 1971).

Despite the identification and introgression of several R genes, monogenic resistance in potato has proved transient (Ross, 1986), and Black and Gallegly recommended strategies breeding exclusively for R gene resistance be abandoned (Black and Gallegly, 1957). Recent breeding efforts have focused on more durable, partial resistance. Partial resistance, often called field resistance, is characterized by polygenic control and lack of race specificity. Many lines and cultivars have shown field resistance. In one study, 22-R-gene free potato cultivars were evaluated over time in the field. The resistance appeared to be more durable than monogenic resistance. However, resistance was also associated with later maturing cultivars (Colon et al., 1995). Other studies have confirmed the correlation between maturity, vigor, and resistance to late blight (Collins et al., 1999). DNA markers have been integrated into breeding programs to aid in selection for R genes and quantitative resistance (Leister et al., 1996; Oberhagemann et al., 1999).

Research Objectives

The open-pollinated tomato ‘Richter’s Wild Tomato’ displayed resistance to late blight in a home garden in Washington State (John Novazio, personal communication). The objective of this study was to characterize the inheritance of late blight resistance in ‘Richter’s Wild Tomato’, to determine its relationship to other previously identified sources of resistance to late blight in tomato, and to examine the potential for pyramiding multiple sources of resistance to late blight in an adapted breeding line having resistance to early blight (*Alternaria solani*).

Materials and Methods

Inheritance Study

The open-pollinated, home garden tomato cultivar 'Richter's Wild Tomato' (produced and distributed by Richter's Seed Company) displayed resistance to late blight in a Washington State home garden (John Novazio, personal communication). Subsequent assays of 'Richter's Wild Tomato' verified its resistance to multiple isolates of late blight, including North Carolina isolates 97-1-1 (US-19), 01MHCRS, 02MHCRS, and to Taiwanese isolates (Peter Hanson, A.V.R.D.C., personal communication).

The cultivar appears to be a derivative of a *Lycopersicon esculentum* x *Lycopersicon pimpinellifolium* hybrid. This conclusion is based on both the plant and fruit characteristics. 'Richter's Wild Tomato' has small round fruit, approximately 1-cm in diameter. The fruit ripen from green to red and possess the *u+* gene, phenotypically expressed as a dark green shoulder of the tomato fruit. Fruit are attached by a jointed (*j+*) pedicel. The 'Richter's Wild Tomato' plant is indeterminate in growth. The foliage is dark green, and the leaves are deeply serrated (Figure 1).

To investigate the mode of inheritance of its late blight resistance, 'Richter's Wild Tomato' was crossed with inbred tomato line NC215E-1 (Figure 2). NC215E-1 was developed by Dr. R.G. Gardner in the North Carolina State University fresh market tomato breeding program at the Mountain Horticultural Crops Research and Extension Center (M.H.C.R.E.C.), and is a determinate, large-fruited line with quantitative

resistance to early blight (*Alternaria solani*), but susceptible in field and detached leaf assays to all isolates of late blight.

The 'Richter's Wild Tomato' x NC215E-1 F1 was evaluated in the field and backcrossed to NC 215E-1. The backcross was grown in the field at the Mountain Horticultural Crops Research Station, Fletcher, NC and the Mountain Research Station, Waynesville, NC in the summer of 2001. A large-fruited, resistant, single-plant selection (139LB-3W) with determinate growth habit was made from the BC1F1 population at Waynesville. Selfed seed of this selection, representing the BC1F2 generation, was planted in the greenhouse at Fletcher. 100 BC1F2 plants were evaluated against *P. infestans* isolate 97-1-1 (US-19) via a detached leaf assay.

In a continued effort to integrate this source of resistance into an adapted background, pollen of the 139LB-3W selection was backcrossed to NC215E-1, yielding the BC2F1. A detached leaf assay was used to screen this population and 21 selected resistant individuals were selfed to make the BC2F2 and backcrossed to NC215E-1 to make the BC3F1. Both the BC2F2 population derived from selfing the 21 selected BC2F1 resistant plants and the BC3F1 populations derived from crosses with each of the 21 resistant BC2F1 plants were evaluated for late blight resistance in the field in 2001 at Fletcher and Waynesville under natural field inoculation.

Progeny derived from selfing resistant BC1F2 individuals were screened in a detached leaf assay with isolates 97-1-1 and 01MHCRS in spring 2002. Two selections, 139LB-3W(2001)-8 and 139LB-3W(2001)-64, which did not segregate for late blight

susceptibility when progeny tested as the BC1F3 generation, were utilized as homozygous resistant lines in subsequent crosses to determine the relationship between 'Richter's Wild Tomato' late blight resistance and other sources of late blight resistance.

The preceding crosses to determine inheritance of 'Richter's Wild Tomato' are depicted in Figure 3.

Chi-square analysis (Steel et al., 1997) was used to test the fit to expected segregation ratios for the BC1F2, BC3F1, and BC2F2 populations and determine the inheritance of late blight resistance in each of these three populations.

Tests for allelism

Three monogenic sources of resistance to late blight have been previously identified. A single, dominant resistant gene, derived from *L. pimpinellifolium*, and located on chromosome 7 was characterized in 1952 and is named *Ph-1* (Gallegly, 1952; Bonde and Murphy, 1952). A second resistance gene from *L. pimpinellifolium* accession West Virginia 700 was characterized and named *Ph-2* (Turkensteen, 1973; Laterrot, 1975). *Ph-2* is a single, partially dominant gene mapping to chromosome 10 (Moreau et al., 1998). A third resistance gene, *Ph-3*, was characterized in *L. pimpinellifolium* accession L3708 and gives dominant resistance (Black et al., 1996). The *Ph-3* locus maps to chromosome 9 (Chunwongse et al., 1998).

To investigate whether the resistance found in 'Richter's Wild Tomato' is allelic to any of these three other sources, hybrids between the 'Richter's Wild Tomato' derived

BC1F3 line 139LB-3W(2001)-8 and homozygous lines containing each of the three R genes were made, and F2 populations were created for segregation analysis.

The source of *Ph-1* was the cultivar 'New Yorker'. 'New Yorker' has determinate plant type, large round fruit, and is early maturing. A cultivar released by Oregon State University, 'Legend', was used as a source of the *Ph-2* gene. 'Legend' is a determinate-vined, round-fruited, parthenocarpic cultivar. Breeding line 96LB-1(2001), developed by Dr. R.G. Gardner, N.C.S.U., contains *Ph-3*. The line was developed from a cross between *L. pimpinellifolium* accession L3708 and breeding line NC215E-1, with subsequent backcrossing to NC215E-1 and selection for resistance. The source of 'Richter's Wild Tomato' resistance used in these crosses was line 139LB-3W(2001)-8, and represents a homozygous selection.

Ninety-six plant F2 populations derived from the hybrids between 139LB-3W(2001)-8 and the *Ph* gene sources were grown in the greenhouse during winter 2003. If 'Richter's Wild Tomato' resistance resides at a different locus than the other resistance source in the hybrid, the F2 population would be expected to segregate at a ratio of 15:1, resistant to susceptible (assuming resistance is dominant) when screened with the late blight pathogen. If the resistance in 'Richter's Wild Tomato' occurred at the same locus as the other source of resistance in the hybrid, the F2 population should not segregate for susceptibility. Based on the binomial distribution with p=1/16, evaluating a population of 96 plants results in power (1-â) of 99.8% to detect at least one susceptible plant if the two sources of resistance occur at different loci.

Combining *Ph* genes in an adapted background

To investigate the potential for combining *Ph* genes in adapted fresh market tomato backgrounds, a trial was grown under natural inoculum pressure at Fletcher, NC and Waynesville, NC during summer 2002. The trial contained original sources of resistance, adapted inbred lines with each of the respective *Ph* genes, and adapted F1 hybrid combinations of *Ph* genes. Six-plant plots were arranged in a randomized complete block design with four replications at each location. Plots were evaluated on a 1-5 disease severity scale. A rating of 1 indicated no visible late blight symptoms. A leaf with few, small, non-expanding lesions and no visible sporulation received a rating of 2. Larger lesions and moderate sporulation was rated a 3. Near completely covered leaves with heavy sporulation received a 4. A rating of 5 was assigned to completely covered leaves with severe necrosis and heavy sporulation.

Disease ratings from the field evaluations were subjected to analysis of variance with PROC GLM of SAS version Windows V8 (SAS Institute, Cary, N.C.) The least squared differences procedure was used to separate cultigen means.

Detached Leaf Assays

Detached leaves were screened for *P. infestans* resistance via a protocol adapted from, "A Technique for screening tomato plants for single gene resistance to race 0, *Phytophthora infestans*" (Pierce 1970). The detached leaf assay allows controlled inoculation with specific isolates, replication over time without destroying the plants, and

the option of making controlled pollinations on plants after resistance results are known. Seeds were planted in a peat-vermiculite medium in a metal tray, watered in thoroughly, and trays were placed in a greenhouse. Upon germination, seedlings were transplanted to plastic, 24 cell trays. At the 4-5- week stage, seedlings were transplanted to a peat-vermiculite-pine bark mix in 1.92-liter pots. When the plants reached a height of 20-30 cm., large single leaves were removed from plants to be assayed. The first leaf beneath the top flower cluster was selected and removed by slicing at the leaf base with a razor blade. Clear plastic 100-mL cups were filled with distilled water and covered with a lid. Each lid had a 1-cm in diameter hole in it, and the leaves were placed one per cup in a fashion such that the petiole was inserted into the hole and into the water. Cups were arranged in a clear plastic box, 12-14 cups per box depending on the screen.

The *P. infestans* isolates were maintained on plants of the late blight susceptible tomato cultivar 'Mountain Pride'. In preparation for inoculating a detached leaf assay, heavily sporulating leaves from appropriate maintenance plants were removed. These leaves were held over a 100-mL plastic beaker and spores were washed off of the leaf into the cup by spraying with distilled water from an Ace brand household spray bottle. Spore concentration was calculated for the spore/water suspension using a hemaecytometer. The spore suspension was diluted to 5000sp/mL. Using a household spray bottle, leaves within each plastic box were inoculated with a mist spray of diluted spore suspension. The boxes were sprayed until each of the 12-14 leaves had a

glistening, moist appearance. In total, each box received between 15 and 20-mL of inoculum at 5000sp/mL.

Several late blight isolates were utilized in the detached leaf assays. These isolates were taken from fields in Western North Carolina and maintained at the Mountain Horticultural Crops Research and Extension Center (M.H.C.R.E.C.) at Fletcher, NC. Isolate 97-1-1 represents a metalaxyl sensitive isolate taken from a Fletcher tomato field and subsequently typed as the US-19 strain. Based on isolate surveys from the mid to late 1990's, US-19 was a prevalent tomato isolate found in Western North Carolina (Wangsomboondee et al., 2002). It is metalaxyl senesitive and of the A2 mating type. 01MHCRS and 02MHCRS represent isolates taken from the field at the M.H.C.R.S. in 2001 and 2002 respectively. 01MHCRS is metalaxyl resistant. Metalaxyl resistant isolates show no inhibition to sporulation at 100 ppm and sensitive isolates show approximately 99% inhibition at 1.0 ppm metalaxyl.

Based on previous detached leaf assays, the interaction between these isolates and the individual R genes has been determined. *Ph-1* is susceptible to all isolates maintained at the M.H.C.R.S. No strains maintained by the M.H.C.R.S. overcome *Ph-2*. Ph-3 shows resistance to 97-1-1, but is overcome by 01MHCRS and 02MHCRS.

Once inoculated, the boxes were covered with a clear plastic top. Boxes were then placed in an incubator. The incubator was on a 12/12 light schedule, with light temperature maintained at 21 °C, and the dark temperature at 16 °C. After six days, the

boxes were removed from the growth chamber for disease reaction evaluation. Individual leaves were evaluated on a 1-5 rating scale.

Field Trials

BC3F1 and BC2F2 populations and the combined resistance trial were evaluated for late blight resistance in the field at two North Carolina locations, Fletcher and Waynesville, in 2002. Seed for these evaluations was started in a peat-vermiculite medium in metal trays in the greenhouse. Upon germination, seedlings were transplanted to the same mix in plastic-celled trays, and kept in the greenhouse for 5-6 weeks. Prior to transplanting to the field, plants were drenched with a 12-48-8 starter solution at a rate of 2lbs. per 100 gallons of water.

Plants at Fletcher, N.C. were set in the field in single rows on 5-foot centers. Within rows, plants were spaced on 1.5-foot centers. The BC2F2 and BC3F1 plants were set in the ground July 2. The resistance combination trial was transplanted August 15. The field was irrigated as necessary by overhead irrigation to facilitate pathogen reproduction. The trial received no fungicide treatment throughout the growing season. Approximately 3 weeks after transplant date, plants were pruned to 3 growing points. When the plants reached 1.5 to 2 feet in height, stakes were placed between every two plants and the plants were trellised with the string-weave system as per standard North Carolina fresh market tomato growing practices (Konsler and Gardner, 1990).

Plants at Waynesville, N.C were set into raised beds covered with black plastic mulch on 5-ft. centers. The BC2F2 and BC3F1 populations were transplanted July 3, and the resistance combination trial was transplanted August 16. Within rows, plants were spaced on 1.5-foot centers. Plants were drip irrigated as necessary. The trial received no fungicide treatment. The BC2F2 and BC3F1 populations were pruned and trellised using the string and weave system. The resistance combination trial at Waynesville was not pruned or staked.

Late blight infection was started in the fields at Fletcher and Waynesville by placing tomato tissue with actively sporulating lesions in the canopy of susceptible check plots grown adjacent to the BC2F2 and BC3F1 populations. The combined resistance trials were not inoculated at either location.

The BC2F2 and BC3F1 populations at Fletcher and Waynesville were evaluated August 30 and August 31 respectively. The trial for combined resistance was evaluated October 11 at Fletcher and October 12 at Waynesville.

Tomato Pollination

Tomato is an obligate self pollinator. Controlled hybridizations were made according to Georgiev (1991). Prior to flower opening and mature pollen release, the corolla and androecium were removed by grasping and pulling with forceps. Pollen was collected by placing a teaspoon beneath the flower and vibrating the anther cone with an electric toothbrush. The flower was vibrated until visible observation of pollen release.

The pollen was then applied to the female by inserting the emasculated flowers pistil into the collected pollen in the teaspoon. Controlled hybridizations were marked by attaching a string tag to the pollinated flower. The tag had the female parent listed on one side, and the pollen donor listed on the opposite side.

Results

'Richter's Wild Tomato' Inheritance Study

In the detached-leaf assay of 100 BC1F2 plants derived from 'Richter's Wild Tomato', three classes of disease reaction were observed, resistant, intermediate, and susceptible. Chi-square analysis of the data confirmed that the pattern of segregation was consistent with a 1:2:1 ratio, indicating incompletely dominant, monogenic Mendelian inheritance (P=0.194) (Table 1).

In the field evaluation at Fletcher, NC and Waynesville, NC of 1025 BC2F2 plants and 726 BC3F1 plants derived from 'Richter's Wild Tomato', only two classes of late blight disease reaction were observed, resistant and susceptible. The BC2F2 population's pattern of segregation was consistent with a 3:1 ratio, resistant to susceptible (P=0.505). The BC3F1 population segregated in a manner consistent with a 1:1 resistant to susceptible ratio (P=0.088). These two populations, under natural field conditions, indicate completely dominant, monogenic inheritance (Table 1) (Raw data in Appendix A.).

Tests for Allelism

In the detached-leaf assay with *P. infestans* isolates 97-1-1 and 02MHCRS on 96 F2 plants derived from the 'Legend' (*Ph-2*) x 139LB-3W(2001)-8 F1, no susceptible plants were detected in either screen. Furthermore, in each screen, check lines reacted as

expected, indicating successful inoculation. This screen was repeated on 96 additional plants from this F2 population to verify results. No segregation for susceptibility was observed in the repeat screen. Lack of segregation indicates the monogenic source of resistance in ‘Richter’s Wild Tomato’ acts at the same locus as the *Ph-2* resistance in 'Legend'. Furthermore, to verify that the population was in fact an F2, and not an aberrant self, the plants were also evaluated for the parthenocarpic trait, present in 'Legend', and controlled by a single, recessive gene. The F2 derived from a 'Richter's Wild Tomato' (non-parthenocarpic) x 'Legend' hybrid would be expected to segregate three plants normal to every one parthenocarpic. Of 93 total plants, 73 were non-parthenocarpic and 20 were parthenocarpic. Chi-square analysis of the data confirm that the pattern of parthenocarpic segregation is consistent with a 3:1 ratio (P=0.5-0.25).

Ph-2 has previously been mapped to chromosome 10, while *Ph-1* and *Ph-3* have been mapped to chromosome’s 7 and 9 respectively. Based on this map information, and the result that the monogenic resistance in ‘Richter’s Wild Tomato’ is allelic to *Ph-2*, ‘Richter’s Wild Tomato’ derived resistance can not be allelic to *Ph-1* or *Ph-3*, and therefore it was not necessary to evaluate F2 populations derived from hybrids between ‘Richter’s Wild Tomato’ and sources of *Ph-1* and *Ph-3* respectively.

Combining *Ph* genes

The genotypes in the combined resistance trial displayed a range of plant disease reactions at both field locations. No genotypes were completely resistant. The observed

range of ratings was 1.5 to 4.5 at both locations (Fig. 4). The susceptible check, NC215E-1, showed severe late blight symptoms at both locations, with a mean rating of 4.5 at Fletcher, and 4.3 at Waynesville. Overall, the effect of location on disease severity was significant (P=0.0043). When ratings were analyzed jointly over both locations, the data suggested a significant genotype by location interaction (P<0.0001) (Fig. 5). As a result of this significant interaction, locations were analyzed individually.

Significant genotypic effects were observed at Fletcher, NC and Waynesville, NC, with P<0.0001 at both locations (ANOVA). At Fletcher, NC, three lines displayed significantly lower levels of disease infection when compared with the other 13 lines (Table 2). The three lines were: 139LB-3W(2001)-8 x 96LB-1(2000), 139LB-3W(2001)-6 x 96LB-1(2000), and 96LB-1(2000) x 'Legend', with disease severity means of 2.0, 1.9, and 2.4 respectively. All three of these entries were F1 hybrids with both *Ph-2* and *Ph-3* in the heterozygous state. A similar result with respect to these three entries was observed at Waynesville, NC. 139LB-3W(2001)-8 x 96LB-1(2000), 139LB-3W(2001)-64 x 96LB-1(2000), and 96LB-1(2000) x 'Legend' displayed significantly lower levels of disease severity with means of 2.1, 1.9, and 2.1 respectively. Furthermore, these three combinations were the only entries which produced mature, marketable fruit. In all other entries, plants either failed to produce fruit, or all fruit that were produced were infected by late blight.

'New Yorker', homozygous for *Ph-1*, and the homozygous *Ph-3* line 96LB-1(2000), did not differ in terms of disease severity from the susceptible check NC215E-1

at either location. At Fletcher, all six homozygous *Ph-2* lines displayed significantly lower levels of infection than ‘New Yorker’ (*Ph-1/Ph-1*) and 96LB(2001)-1 (*Ph-3/Ph-3*). However, at Waynesville, only four of the six homozygous *Ph-2* lines displayed better resistance than ‘New Yorker’ and 96LB(2001)-1.

At both locations, 'Richter's Wild Tomato' received a significantly lower mean disease severity rating than did homozygous *Ph-2* cultivar 'Legend'. Furthermore, at both locations, the two 'Richter's Wild Tomato' derived homozygous lines, 130LB-3W(2001)-8 and 139LB-3W(2001)-64, were rated equal to 'Richter's Wild Tomato' or intermediate between 'Richter's Wild Tomato' and 'Legend' in terms of disease severity. The two lines with homozygous 'Richter's Wild Tomato' derived *Ph-2* integrated into an adapted background (130LB-3W(2001)-8 and 139LB-3W(2001)-64) could not be separated at either location in terms of disease severity.

Least squared difference mean separation did not distinguish between the heterozygous and homozygous states of *Ph-2* at either location. Entries 139LB-3W(2001)-8-BK (*Ph-2/Ph-2*) could not be distinguished from 139LB-3W(2001)-8 x NC215E-1 (*Ph-2*/+), and line 139LB-3(2001)-64-BK (*Ph-2/Ph-2*) could not be separated from 139LB-3W(2001)-64 x NC215E-1 (*Ph-2*/+) based on field evaluations.

The combination of *Ph-1* and *Ph-2*, both in the heterozygous state, showed significantly less disease severity than the homozygous *Ph-1* genotype at Fletcher. At Waynesville, these combinations could not be distinguished from the homozygous *Ph-1* genotype.

Discussion

Ph-2 was originally reported by Turkensteen (1973) as being a single dominant gene conferring partial late blight resistance in field screens. Laterrot (1975) reported *Ph-2* as being monogenic and incompletely dominant based on whole plant growth chamber assays. Our results with 'Richter's Wild Tomato' support single gene resistance, conferred by a gene allelic to the *Ph-2* gene in 'Legend'. This information has important implications for breeding because the majority of commercially grown tomato cultivars in the United States are hybrids. Evaluation of the BC1F2 via a detached leaf assay yielded three reaction classes, indicative of incomplete dominance. However, the results from the BC2F2 field screen in which only two classes were observed, and the lack of significant distinction between homozygous and heterozygous Ph-2 lines in the combined resistance trials, indicate that the resistance under our field conditions was completely dominant. The combination of high spore numbers and more favorable environmental conditions in the detached leaf tests likely provided conditions severe enough to distinguish heterozygous from homozygous resistance. However, under natural field conditions, with lower spore concentrations, the gene acted in a completely dominant fashion.

In the combined resistance trials, no difference was observed between the two adapted genotypes with 'Richter's Wild Tomato' derived *Ph-2*. However, 'Richter's Wild Tomato' by itself shows equal or better resistance than either derived line.

'Richter's Wild Tomato' is indeterminate, whereas both derived lines were selected for determinant growth habit. Previous QTL research has identified the locus controlling indeterminant growth as possessing late blight resistance (Frary et al., 1998). This pleiotropic effect of indeterminant growth is the most likely explanation for the stronger resistance in 'Richter's Wild Tomato' relative to its derived lines at the Waynesville location.

Overall, at Waynesville late blight was more severe than at Fletcher. One possible explanation for this difference is that different, more aggressive isolates may have moved into the field at Waynesville. Cultural differences between the two locations could also explain this difference. Plants at Fletcher were trellised with the string-weave system. The trial at Waynesville was grown on the ground. Trellising opens up the plant canopy, allowing air movement. Ground culture produces a more compact, heavy canopy, which may limit air movement and maintain humidity within the canopy, resulting in an environment more conducive to late blight sporulation.

The results of the combined resistance field screens showed that hybrids with *Ph-2* and *Ph-3* in the heterozygous condition provided an effective level of resistance against late blight. A similar pyramiding approach has been attempted in breeding potatoes resistant to late blight. Late blight R gene combinations in potato have been quickly overcome by the pathogen and have proven largely ineffective. Black and Gallegly (1957) recommended that pyramiding approaches should be abandoned in potato late blight resistance breeding, and that the focus should be placed on developing quantitative

resistance. The question then arises as to whether any greater success could be expected with gene combinations in tomato for resistance to late blight in terms of durability. A fundamental difference between *P. infestans* biology exists with respect to the pathogens life cycle and its interaction with these two species. On potato, the pathogen can overwinter as mycelium associated with tubers. The pathogen is unable to overwinter directly associated with tomato. The opportunity for late blight strains to overwinter and produce many generations under the direct selection pressure of the potato host's resistance increases the likelihood of a novel strain to arise which overcomes the host. In tomato, however, each season relies on new inoculation. Therefore, the particular isolate will not be associated with the host for as many generations as in potato, and the likelihood of novel isolate genotypes overcoming the host's genetic resistance is greatly reduced.

Although the tomato hybrids containing both *Ph-2* and *Ph-3* had significantly lower disease ratings than other resistance sources, they were not completely resistant and late blight lesions still formed on these plants. Future research should evaluate the efficacy of combining resistant cultivars with fungicide sprays. A similar approach has been taken in controlling tomato early blight (*Alternaria solani*). Intermediate, quantitative tomato resistance has been combined with limited chemical treatment to yield an effective integrated strategy to control early blight. There are several benefits to this approach. First, the reduction of chemical sprays saves the grower money and limits the potential environmental impact of the chemicals. Additionally, by using an integrated

approach, the selection pressure on the pathogen is distributed between two forms of control, genetic and chemical, potentially offering a more durable control solution.

Along with the combined *Ph-2* and *Ph-3* lines, these combined trials contained many highly susceptible lines. The presence of highly susceptible tomato lines allows the pathogen to build up and increase the inoculation pressure on this gene combination. Perhaps, in a situation in which this gene combination was the only host present, the disease would not be able to start, or at least would be delayed and much slower in terms of inoculum build-up, resulting in reduced disease pressure.

The ultimate goal of this research is to develop an adapted, late blight resistant tomato hybrid for release from the NCSU fresh market tomato breeding program. The two hybrids with 'Richter's Wild Tomato' derived *Ph-2* and *Ph-3* from 96LB(2000)-1 represent efforts towards this end. Because the two hybrids lack in fruit size and other necessary horticultural traits, additional breeding will be needed to develop commercially acceptable hybrids with combined early and late blight resistance.

Finally, late blight resistance is currently being derived from several other germplasm sources. As these sources are identified and characterized, future research should investigate the potential for even stronger and more durable resistance by combining new genetic sources of resistance with *Ph-2* and *Ph-3*.

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Tables

Table 1. Segregation data for late blight resistance of populations derived from (NC215E-1 x Richter's Wild Tomato) F1.

Population	screen/ isolate	No. eval.	Res.	Interm.	Susc.	expected ratio	$\div^2$	P value
[NC215E-1 x Richter's] BC1F2	detached leaf assay/ 97-1-1	100	32	42	26	1:2:1	3.28	0.194
[NC215E-1 x Richter's] BC2F2	field/ multiple isolates	1025	778	0	247	3:1	0.4452	0.505
[NC215E-1 x Richter's] BC3F1	field/ multiple isolates	726	386	0	340	1:1	2.9146	0.088

Table 2. Late blight field severity on 16 tomato lines with various combinations of resistance genes.[z]

Tomato line	Resistance genes	2002 Fletcher, NC field rating[y]	2002 Waynesville, NC field rating[y]
139LB-3W(2001)-64 x 96LB-1(2000)	*Ph-2*/+, *Ph-3*/+	1.9a	1.9a
139LB-3W(2001)-8 x 96LB-1(2000)	*Ph-2*/+, *Ph-3*/+	2.0ab	2.1a
96LB-1(2000) x Legend	*Ph-3*/+, *Ph-2*/+	2.4b	2.1a
139LB-3W(2001)-8-BK	*Ph-2*/*Ph-2*	2.9c	3.8cd
Richter's Wild Tomato	*Ph-2*/*Ph-2*	2.9c	3.0b
139LB-3W(2001)-64-BK	*Ph-2*/*Ph-2*	3.3cd	3.9cde
139LB-3W(2001)-8 x NC215E-1	*Ph-2*/+	3.3cd	3.8cd
139LB-3W(2001)-8 x Legend	*Ph-2*/*Ph-2*	3.3cd	3.5c
139LB-3W(2001)-64 x NC215E-1	*Ph-2*/+	3.5d	4.1de
139LB-3W(2001)-8 x New Yorker	*Ph-2*/+, *Ph-1*/+	3.5d	3.8cd
Legend	*Ph-2*/*Ph-2*	3.5d	4.1de
139LB-3W(2001)-64 x New Yorker	*Ph-2*/+, *Ph-1*/+	3.5d	4.0de
139LB-3W(2001)-64 x Legend	*Ph-2*/*Ph-2*	3.6d	3.9cde
New Yorker	*Ph-1*/*Ph-1*	4.4e	4.0de
96LB-1(2000)	*Ph-3*/*Ph-3*	4.4e	4.0de
NC215E-1	Susc. check	4.5e	4.3e

[z]Tomato lines evaluated on a 1-5 resistant to susceptible disease severity scale. A rating of 1 indicated no visible late blight symptoms. A rating of 2 indicated leaves had a few, small non-expanding lesions and no visible sporulation. Larger lesions and moderate sporulation received a rating of 3. A rating of 4 indicated leaves nearly covered with lesions and heavy sporulation. Complete lesion coverage of leaves, severe necrosis, and heavy sporulation received a rating of 5.

[y]Mean separation by protected least squared differences multiple range test at P 0.05.

Figures

Figure 1. 'Richter's Wild Tomato'

Figure 2. NC215E-1 x 'Richter's Wild Tomato' F1

Figure 3. Crossing scheme to determine inheritance of late blight resistance in 'Richter's Wild Tomato'.

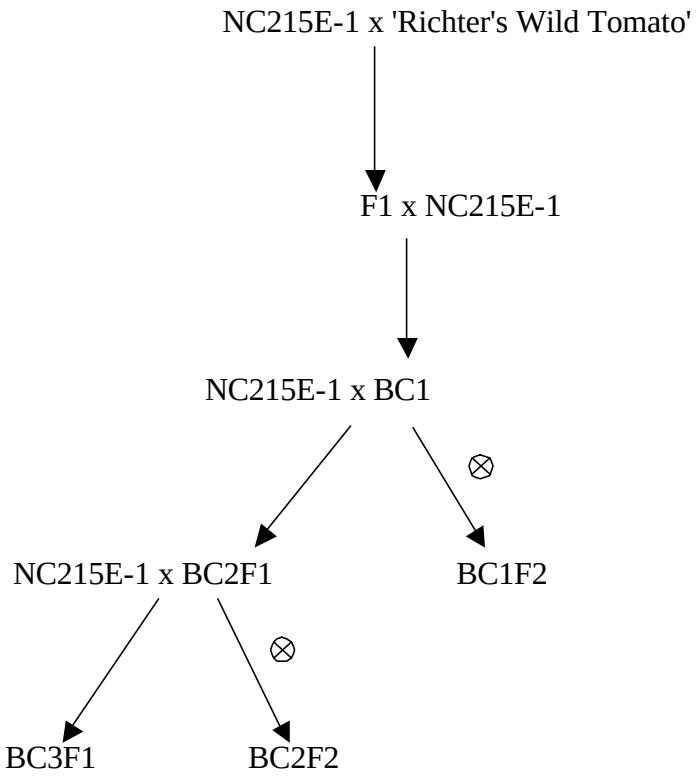


Figure 4. Late blight severity scale

Rating = 2
Small and non-expanding late blight lesions with no sporulation.

Rating = 3
Large lesions with moderate levels of sporulation.

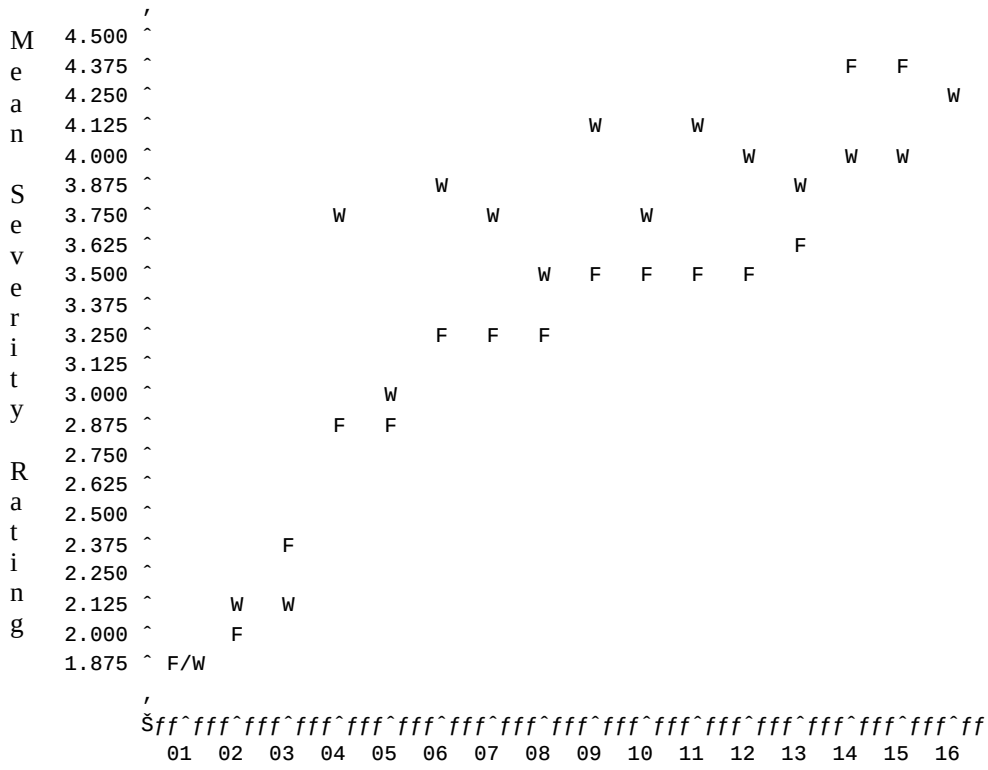
Figure 4. (cont.) Late blight severity scale

Rating = 4
Large portion of leaf area covered with lesions, heavy sporulation .

Rating = 4.5
Leaves completely covered with lesions. severe necrosis, and heavy sporulation.

Figure 5.

Mean Late Blight Disease Severity Rating of Tomato Lines Containing Various Combinations of Resistance Genes at Two Locations



W=Waynesville, NC
F=Fletcher, NC

Key for Tomato Lines from **Figure 5.**

Tomato Line	Pedigree	Resistance genes
1	139LB-3W(2001)-64 x 96LB-1(2000)	*Ph-2*/+, *Ph-3*/+
2	139LB-3W(2001)-8 x 96LB-1(2000)	*Ph-2*/+, *Ph-3*/+
3	96LB-1(2000) x Legend	*Ph-3*/+, *Ph-2*/+
4	139LB-3W(2001)-8-BK	*Ph-2*/*Ph-2*
5	Richter's Wild Tomato	*Ph-2*/*Ph-2*
6	139LB-3W(2001)-64-BK	*Ph-2*/*Ph-2*
7	139LB-3W(2001)-8 x NC215E-1	*Ph-2*/+
8	139LB-3W(2001)-8 x Legend	*Ph-2*/*Ph-2*
9	139LB-3W(2001)-64 x NC215E-1	*Ph-2*/+
10	139LB-3W(2001)-8 x New Yorker	*Ph-2*/+, *Ph-1*/+
11	Legend	*Ph-2*/*Ph-2*
12	139LB-3W(2001)-64 x New Yorker	*Ph-2*/+, *Ph-1*/+
13	139LB-3W(2001)-64 x Legend	*Ph-2*/*Ph-2*
14	New Yorker	*Ph-1*/*Ph-1*
15	96LB-1(2000)	*Ph-3*/*Ph-3*
16	NC215E-1	Susc. check

Appendix Ai. Raw data from Fletcher, NC late blight field screen of NC215E-1 x 'Richter's Wild Tomato' derived BC3F1's.

Trt. #	Pedigree	Generation	Resistant	Susceptible
96	026-5 x NC215E-1	BC3F1	15	9
97	026-14 x NC215E-1	BC3F1	12	12
98	026-18 x NC215E-1	BC3F1	15	10
99	026-23 x NC215E-1	BC3F1	13	11
100	026-27 x NC215E-1	BC3F1	12	13
101	026-40 x NC215E-1	BC3F1	11	14
102	026-42 x NC215E-1	BC3F1	14	11
103	026-45 x NC215E-1	BC3F1	11	14
104	026-51 x NC215E-1	BC3F1	13	11
105	026-73 x NC215E-1	BC3F1	14	10
106	026-79 x NC215E-1	BC3F1	11	14
107	026-115 x NC215E-1	BC3F1	15	9
108	026-2 x NC215E-1	BC3F1	2	2
109	026-20 x NC215E-1	BC3F1	no plants	no plants
110	026-80 x NC215E-1	BC3F1	2	1
111	026-97 x NC215E-1	BC3F1	13	12
112	026-116 x NC215E-1	BC3F1	16	9
TOTAL		BC3F1	189	162

Appendix Aii. Raw data from Waynesville, NC late blight field screen of NC215E-1 x 'Richter's Wild Tomato' derived BC3F1's.

Trt. #	Pedigree	Generation	Resistant	Susceptible
96	026-5 x NC215E-1	BC3F1	12	13
97	026-14 x NC215E-1	BC3F1	12	13
98	026-18 x NC215E-1	BC3F1	5	4
99	026-23 x NC215E-1	BC3F1	no plants	no plants
100	026-27 x NC215E-1	BC3F1	10	15
101	026-40 x NC215E-1	BC3F1	13	12
102	026-42 x NC215E-1	BC3F1	10	15
103	026-45 x NC215E-1	BC3F1	18	7
104	026-51 x NC215E-1	BC3F1	17	8
105	026-73 x NC215E-1	BC3F1	13	12
106	026-79 x NC215E-1	BC3F1	18	7
107	026-115 x NC215E-1	BC3F1	12	13
108	026-2 x NC215E-1	BC3F1	11	13
109	026-20 x NC215E-1	BC3F1	10	8
110	026-80 x NC215E-1	BC3F1	8	17
111	026-97 x NC215E-1	BC3F1	13	11
112	026-116 x NC215E-1	BC3F1	15	10
TOTAL		BC3F1	197	178

Appendix Aiii. Raw data from Fletcher, NC late blight field screen of NC215E-1 x 'Richter's Wild Tomato' derived BC2F2's.

Trt. #	Pedigree	Generation	Resistant	Susceptible
113	026-14	BC2F2	17	7
114	026-18	BC2F2	19	6
115	026-20	BC2F2	20	5
116	026-34	BC2F2	15	8
117	026-37	BC2F2	22	3
118	026-40	BC2F2	18	7
119	026-49	BC2F2	20	5
120	026-57	BC2F2	20	5
121	026-59	BC2F2	18	7
122	026-65	BC2F2	17	7
123	026-72	BC2F2	20	5
124	026-75	BC2F2	no plants	no plants
125	026-79	BC2F2	no plants	no plants
126	026-80	BC2F2	8	7
127	026-93	BC2F2	16	6
128	026-97	BC2F2	20	5
129	026-101	BC2F2	18	6
130	026-103	BC2F2	18	7
131	026-106	BC2F2	21	4
132	026-108	BC2F2	21	4
132	026-114	BC2F2	19	6
TOTAL		BC2F2	347	110

Appendix Aiv. Raw data from Waynesville, NC late blight field screen of NC215E-1 x 'Richter's Wild Tomato' derived BC2F2's.

Trt. #	Pedigree	Generation	Resistant	Susceptible
113	026-14	BC2F2	19	6
114	026-18	BC2F2	19	6
115	026-20	BC2F2	19	6
116	026-34	BC2F2	15	5
117	026-37	BC2F2	21	4
118	026-40	BC2F2	19	6
119	026-49	BC2F2	17	8
120	026-57	BC2F2	19	5
121	026-59	BC2F2	17	8
122	026-65	BC2F2	37	13
123	026-72	BC2F2	23	5
124	026-75	BC2F2	34	12
125	026-79	BC2F2	21	4
126	026-80	BC2F2	20	5
127	026-93	BC2F2	18	7
128	026-97	BC2F2	18	7
129	026-101	BC2F2	17	8
130	026-103	BC2F2	22	3
131	026-106	BC2F2	15	10
132	026-108	BC2F2	23	2
133	026-114	BC2F2	18	7
TOTAL		BC2F2	431	137

Appendix B. Raw data from late blight field screen of various combinations of resistance genes

Fletcher, N.C.

Tomato Line	Rep One	Rep Two	Rep Three	Rep Four
139LB-3W(2001)-8 x Legend	3	3.5	3	3.5
139LB-3W(2001)-8 x New Yorker	3.5	3.5	4	3
139LB-3W(2001)-8 x 96LB-1(2000)	2	2	2	2
139LB-3W(2001)-64 x Legend	4	3.5	3.5	3.5
139LB-3W(2001)-64 x New Yorker	4	3	3.5	3.5
139LB-3W(2001)-64 x 96LB-1(2000)	2	1.5	2	2
139LB-3W(2001)-8-BK	2.5	3	3	3
139LB-3W(2001)-64-BK	3.5	3.5	3	3
139LB-3W(2001)-8 x NC215E-1	3	3.5	3	3.5
139LB-3W(2001)-64 x NC215E-1	3.5	3.5	3.5	3.5
Richter's Wild Tomato	2.5	3	3.5	2.5
NC215E-1	4.5	4.5	4.5	4.5
Legend	3.5	3.5	3.5	3.5
New Yorker	4.5	4.5	4	4.5
96LB-1(2000)	4	4.5	4.5	4.5
96LB-1(2000) x Legend	2	2.5	2.5	2.5

Waynesville, NC

Tomato Line	Rep One	Rep Two	Rep Three	Rep Four
139LB-3W(2001)-8 x Legend	3	3.5	3.5	4
139LB-3W(2001)-8 x New Yorker	3.5	3.5	4	4
139LB-3W(2001)-8 x 96LB-1(2000)	1.5	2.5	2.5	2
139LB-3W(2001)-64 x Legend	4	3.5	4	4
139LB-3W(2001)-64 x New Yorker	4	4	4	4
139LB-3W(2001)-64 x 96LB-1(2000)	1.5	2	2	2
139LB-3W(2001)-8-BK	4	3	4	4
139LB-3W(2001)-64-BK	4	4	4	3.5
139LB-3W(2001)-8 x NC215E-1	4	3.5	4	3.5
139LB-3W(2001)-64 x NC215E-1	4	4	4.5	4
Richter's Wild Tomato	3	3.5	3	2.5
NC215E-1	4	4.5	4.5	4
Legend	4	4.5	4	4
New Yorker	4	4	4	4
96LB-1(2000)	4	4	4	4
96LB-1(2000) x Legend	2	2.5	2	2

Appendix Ci. SAS code and output for late blight field screen of various combinations of resistance genes at Fletcher, NC

Code

```
Title 'gene combos/field 2002/Fletcher';
data a;
input var rep rating;
cards;
1   1 3
1   2 3.5
1   3 3
1   4 3.5
2   1 3.5
2   2 3.5
2   3 4
2   4 3
3   1 2
3   2 2
3   3 2
3   4 2
4   1 4
4   2 3.5
4   3 3.5
4   4 3.5
5   1 4
5   2 3
5   3 3.5
5   4 3.5
6   1 2
6   2 1.5
6   3 2
6   4 2
7   1 2.5
7   2 3
7   3 3
7   4 3
8   1 3.5
8   2 3.5
8   3 3
8   4 3
9   1 3
```

```
9   2 3.5
9   3 3
9   4 3.5
10  1 3.5
10  2 3.5
10  3 3.5
10  4 3.5
11  1 2.5
11  2 3
11  3 3.5
11  4 2.5
12  1 4.5
12  2 4.5
12  3 4.5
12  4 4.5
13  1 3.5
13  2 3.5
13  3 3.5
13  4 3.5
14  1 4.5
14  2 4.5
14  3 4
14  4 4.5
15  1 4
15  2 4.5
15  3 4.5
15  4 4.5
16  1 2
16  2 2.5
16  3 2.5
16  4 2.5
;
proc glm; class var rep;
model rating=var rep;
means var/lsd;
run;
```

Output

The GLM Procedure

Class Level Information

Class	Levels	Values
var	16	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
rep	4	1 2 3 4

Number of observations 64

Dependent Variable: rating

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	18	35.88281250	1.99348958	25.49	<.0001
Error	45	3.51953125	0.07821181		
Corrected Total	63	39.40234375			

R-Square	Coeff Var	Root MSE	rating Mean
0.910677	8.502840	0.279664	3.289063

Source	DF	Type I SS	Mean Square	F Value	Pr > F
var	15	35.83984375	2.38932292	30.55	<.0001
rep	3	0.04296875	0.01432292	0.18	0.9073

Source	DF	Type III SS	Mean Square	F Value	Pr > F
var	15	35.83984375	2.38932292	30.55	<.0001
rep	3	0.04296875	0.01432292	0.18	0.9073

t Tests (LSD) for rating

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	45
Error Mean Square	0.078212
Critical Value of t	2.01410
Least Significant Difference	0.3983

Means with the same letter are not significantly different.

t Grouping	Mean	N	var
A	4.5000	4	12
A	4.3750	4	15
A	4.3750	4	14
B	3.6250	4	4
B	3.5000	4	5
B	3.5000	4	13
B	3.5000	4	2
B	3.5000	4	10
C B	3.2500	4	1
C B	3.2500	4	9
C B	3.2500	4	8
C	2.8750	4	11
C	2.8750	4	7
D	2.3750	4	16
E D	2.0000	4	3
E	1.8750	4	6

Appendix Cii. SAS code and output for late blight field screen of various combinations of resistance genes at Waynesville, NC

Code

```
Title 'gene combos/field 2002/Waynesville';
data a;
input var rep rating;
cards;
1  1 3
1  2 3.5
1  3 3.5
1  4 4
2  1 3.5
2  2 3.5
2  3 4
2  4 4
3  1 1.5
3  2 2.5
3  3 2.5
3  4 2
4  1 4
4  2 3.5
4  3 4
4  4 4
5  1 4
5  2 4
5  3 4
5  4 4
6  1 1.5
6  2 2
6  3 2
6  4 2
7  1 4
7  2 3
7  3 4
7  4 4
8  1 4
8  2 4
8  3 4
8  4 3.5
9  1 4
```

```
9  2 3.5
9  3 4
9  4 3.5
10  1 4
10  2 4
10  3 4.5
10  4 4
11  1 3
11  2 3.5
11  3 3
11  4 2.5
12  1 4
12  2 4.5
12  3 4.5
12  4 4
13  1 4
13  2 4.5
13  3 4
13  4  4
14  1 4
14  2 4
14  3 4
14  4 4
15  1 4
15  2 4
15  3 4
15  4 4
16  1 2
16  2 2.5
16  3 2
16  4 2
;
proc glm; class var rep;
model rating=var rep;
means var/lsd;
run;
```

Output

The GLM Procedure

Class Level Information

Class	Levels	Values
var	16	1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
rep	4	1 2 3 4

Number of observations 64

Dependent Variable: rating

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	18	37.35156250	2.07508681	23.98	<.0001
Error	45	3.89453125	0.08654514		
Corrected Total	63	41.24609375			

R-Square	Coeff Var	Root MSE	rating Mean
0.905578	8.386581	0.294186	3.507813

Source	DF	Type I SS	Mean Square	F Value	Pr > F
var	15	36.93359375	2.46223958	28.45	<.0001
rep	3	0.41796875	0.13932292	1.61	0.2003

Source	DF	Type III SS	Mean Square	F Value	Pr > F
var	15	36.93359375	2.46223958	28.45	<.0001
rep	3	0.41796875	0.13932292	1.61	0.2003

t Tests (LSD) for rating

NOTE: This test controls the Type I comparisonwise error rate, not the experimentwise error rate.

Alpha	0.05
Error Degrees of Freedom	45
Error Mean Square	0.086545
Critical Value of t	2.01410
Least Significant Difference	0.419

Means with the same letter are not significantly different.

t Grouping			Mean	N	var
	A		4.2500	4	12
B	A		4.1250	4	13
B	A		4.1250	4	10
B	A		4.0000	4	15
B	A		4.0000	4	5
B	A		4.0000	4	14
B	A	C	3.8750	4	8
B	A	C	3.8750	4	4
B		C	3.7500	4	7
B		C	3.7500	4	9
B		C	3.7500	4	2
		C	3.5000	4	1
	D		3.0000	4	11
	E		2.1250	4	16
	E		2.1250	4	3
	E		1.8750	4	6