Identifying novel sources of resistance to *Phytophthora* root rot in backcross American-Chinese hybrid chestnuts: A report to the American Chestnut Foundation External Grants Committee

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Abstract

This final report is in fulfillment of our grant obligations to the American Chestnut Foundation for a grant titled "Identifying novel sources of resistance to *Phytophthora* root rot and assessment of a quantitative trait locus for resistance in interspecific *Castanea* hybrids." We will submit a manuscript based on this report for publication in *Chestnut*.

Previous work by researchers from Clemson University and TACF's Carolinas Chapter has resulted in American-Asian hybrid chestnuts with resistance to *Phytophthora cinnamomi*. While the Carolinas Chapter's efforts to breed chestnuts for *P. cinnamomi* resistance have met with success, there exists a need to develop *P. cinnamomi*-resistant lines for American chestnut restoration efforts in other parts of the range. As a first step toward introgressing *P. cinnamomi* resistance into the Tennessee Chapter's breeding population, we screened for resistance fifteen backcross families derived from local *C. dentata* individuals and a diverse panel of *C. mollissima* cultivars. Backcross families were planted in the nursery using a randomized complete block design and inoculated with a locally isolated strain of *P. cinnamomi.* Roots were assessed for symptom severity during the following winter. Plants with the fewest symptoms were transplanted to a *P. cinnamomi*-positive orchard and evaluated for survival mid-way through the following growing season. In the nursery experiment, the majority of backcross families were significantly less symptomatic than *C. dentata* controls. While nearly all backcross families were significantly more symptomatic than *C. mollissima* and *C. henryi* controls, two first-backcross families, derived from the same Chinese-American F_1 mother, were not significantly different from the Asian chestnut control groups. In the *P. cinnamomi*-positive orchard, the pre-screened backcross families had a higher proportion of survivors than families that had not been pre-screened for *P. cinnamomi* resistance. The results of this study suggest that introgression of new sources of *P. cinnamomi* resistance into Tennessee Chapter lines is a realistic objective. We propose a few modifications to future screening efforts; for example, screening plants in individual containers in the greenhouse, rather than large tubs in the nursery, may allow for greater control of experimental conditions. While a cost-benefit analysis was not performed, these results do raise the possibility that greenhouse/nursery pre-screening for *P. cinnamomi* resistance could be an effective means of reducing resource input at later stages of the TACF breeding program.

Introduction

Phytophthora root rot (PRR), also known as ink disease, is a major obstacle to American chestnut (*Castanea dentata* (Marsh.) Borkh.) restoration in the southern United States. Although the North American *Castanea* species are highly susceptible to PRR, the Asian species possess some resistance to the pathogen (Crandall et al. 1945)—a fact that has allowed the American Chestnut Foundation (TACF) to begin to incorporate PRR resistance into its breeding program (Zhebentyayeva et al. 2014). Incorporating PRR resistance into American chestnut restoration efforts relies on first identifying breeding families with high levels of PRR resistance.

In the nineteenth century, PRR, caused by the oomycete *Phytophthora cinnamomi* Rands, resulted in widespread mortality of American chestnut and Allegheny chinquapin (*C. pumila* (L.) Mill.) in the Southeast (Crandall et al. 1945). More recently, PRR has caused losses in TACF's hybrid chestnut plantings before trees could be screened for resistance to chestnut blight (Jeffers et al. 2009) and PRR has even destroyed entire plantings in poorly-drained sites (Sisco 2009). Plantings of B_3F_3 chestnuts in National Forests have also been compromised by PRR (Clark et al. 2014). Thus, there exists a need to better understand resistance to *P. cinnamomi* and to incorporate sources of PRR resistance into restoration breeding programs.

Initial work to understand and employ PRR resistance for American chestnut breeding began in the 1940s (Crandall et al. 1945). More recently, a collaboration between Clemson University, the American Chestnut Foundation, and Chestnut Return Farm (Oconee Co., SC) has also focused on understanding PRR resistance for American chestnut breeding (James 2011b). Research objectives of this collaboration are to identify interspecific hybrid families with high levels of resistance and establish a population of resistant trees for future breeding efforts (Jeffers et al. 2009; James 2011a, 2011b). In PRR resistance screening trials at Chestnut Return Farm, hybrid families are planted in 568 liter plastic tubs in April, inoculated with *P. cinnamomi* 12 to 14 weeks later, and assessed for survival and disease symptoms during the following winter (Jeffers et al. 2009). First results of these trials indicated that PRR resistance could be introgressed into TACF breeding populations from Chinese chestnut (James 2011a, 2011b).

Results of early work also suggested that resistance to *P. cinnamomi* is incompletely dominant, resistance may be controlled by more than one gene, and the genes for resistance to *C. parasitica* and *P. cinnamomi* do not appear to be linked (Jeffers et al. 2009). However, in later work by the same group, the pattern of inheritance observed for PRR resistance suggested that this trait is controlled by a single gene (Jeffers et al. 2012). In contrast, Olukolu et al. (2012) reported two major effect quantitative trait loci (QTL) on linkage group E that explained 34.6 ± 11% and 40.4 ± 10.9% of the total phenotypic variance of PRR resistance in a 48-individual mapping population. Santos et al. (2015, 2016) also identified two QTL associated with PRR resistance, but the authors do not specify whether or not both of these loci are on linkage group E. A recent study of the genomic regions associated with PRR resistance confirmed the existence of a QTL on linkage group E, however, this locus only explained 40% and 34% of the phenotypic variance in two different American-Asian hybrid families (Zhebentyayeva et al. 2014). The number and location of genomic regions that control PRR resistance is of particular

importance, since this will determine the ease with which high levels of PRR resistance can be bred into TACF populations.

While much progress has been made by the South Carolina group in understanding and implementing PRR resistance in their breeding program, two important questions continue to be addressed by researchers: (i) which Asian cultivars and Asian-American hybrid families display high levels of PRR resistance? and (ii) which genetic loci control PRR resistance? While the goal of our present report is to address the first question, the hybrid families screened for resistance here may eventually be used in efforts to answer the second question.

To identify PRR-resistant germplasm for use in chestnut breeding programs, we created first-backcross families derived from PRR-resistant Chinese-American F_1s and wild *C. dentata* from Tennessee and Alabama. We inoculated progeny of these crosses in the nursery with *P. cinnamomi* and measured PRR symptom severity during their first growing season. We out-planted the least symptomatic plants into an orchard where *P. cinnamomi* was already present, and then assessed the survival of the pre-screened plants mid-way through the second growing season. In the nursery experiment, the percentage of asymptomatic individuals per family ranged from 43.5% to 85.2%. Seven of 11 backcross families were significantly less symptomatic than the *C. dentata* control group, while nine of 11 backcross families were significantly more symptomatic than the *C. mollissima* and *C. henryi* controls. Interestingly, two first-backcross families, derived from the same Chinese-American F_1 mother, were not significantly less symptomatic than the Asian chestnut control groups. In the orchard planting, survival of the pre-screened backcross progeny was 70%, compared to 14% survival in the backcross progeny that were not pre-screened for PRR resistance. Most of the losses in the orchard planting were accompanied by PRR symptoms, however, we did not attempt to isolate *P. cinnamomi* from the necrotic tissue. Results of the nursery experiment suggest that some methodological improvements are warranted—for example, conducting screening in individual containers in the greenhouse. In the *P. cinnamomi*-positive orchard, surviving backcross progeny derived from the following *C. mollissima* cultivars and selections are available for future breeding: 'Amy', 'Byron', 'Gideon', 'Lindstrom 99', 'Nanking', 'Neel 3-262', and 'Payne'. This is the first study to screen backcross derivatives of the above *C. mollissima* cultivars, with the exception of 'Nanking' (see Zhebentyayeva et al. (2014) for a discussion of PRR resistance in 'Nanking'). Although a cost-benefit analysis has yet to be performed, these results do raise the possibility that nursery pre-screening for PRR resistance could be an effective means of reducing resource input at later stages of the TACF breeding program.

Materials and Methods

Plant Materials

Two crossing strategies were used to generate most of the backcross progeny for PRR resistance screening. In the first strategy, F_1 progeny of crosses between *C. dentata* and *C.*

mollissima were backcrossed to wild *C. dentata* from either Tennessee or Alabama, yielding 10 first-backcross (BC_1) families (Table 1). In the second strategy, F_1 progeny of crosses between *C. dentata* and *C. mollissima* were open pollinated by Tennessee Chapter advanced backcross trees that had previously been selected for blight resistance, yielding four "better backcross" families (BB) (P.H. Sisco, unpublished protocol, pers. comm.) (Table 1). The second crossing strategy is justified because the BB progeny could inherit blight resistance from two sources: from the TN Chapter's 'Clapper' line parent and from the Chinese chestnut cultivar used in our recent F_1 cross. Moreover, there is currently no substantial evidence that 'Clapper' derived progeny possess PRR resistance (P.H. Sisco, pers. comm.). In addition to the above strategies, we included one small fourth-backcross family; UTC9 was generated by backcrossing one 'Clapper' derived TN Chapter BC_3 tree (TNSM1) to a wild *C. dentata* (TNMac1).

Seedlings of *C. dentata* and *C. mollissima* were used as susceptible and resistant controls, respectively. In addition, *C. henryi* was used as a resistant control (Crandall et al. 1945).

Nursery Trials

In May 2015, seeds were planted in five 229 liter plastic nursery containers in a randomized complete block design. Nursery containers were filled with Sun Gro Metro-Mix 360 soilless container mix. An equal portion of individuals from each family and control group were planted in each of the five containers. Thus, each container represented one replicate.

In March 2015, soil samples were collected from the Tennessee Chapter's McInturff Farm (N 35.628151°, W -84.104069°) and Bendabout Farm (N 35.097757°, W -84.953744°) backcross orchards. In these orchards, we had previously observed plant death accompanied by PRR symptoms. Soils were baited for *Phytophthora* species in the laboratory of Dr. S. N. Jeffers, using the bioassay of Ferguson and Jeffers (1999). *P. cinnamomi* was isolated from three soil samples from the McInturff Farm orchard, and an unknown *Phytophthora* sp. was isolated from the Bendabout Farm orchard. Using the *P. cinnamomi* isolates from McInturff Farm, a V8-vermiculite inoculum was prepared following a protocol provided by Dr. S.N. Jeffers and S. Sharpe (Clemson University, pers. comm.).

Seedlings were inoculated with *P. cinnamomi* following the methods of Jeffers et al. (2009). Inoculations were performed approximately 12 weeks after planting. To inoculate plants, a thin layer of inoculum was evenly distributed in 1- to 3-cm-deep furrows between rows of seedlings. The furrows were covered and the nursery containers were watered thoroughly. Nursery containers were watered as needed throughout the growing season. The nursery containers were placed within plastic kiddie pools to retain effluent and prevent the spread of *P. cinnamomi* throughout the nursery and greenhouse (see Figure 1). Chlorine tablets were added to the containment pools to kill *P. cinnamomi* in the effluent.

Seedlings were assessed for PRR symptom severity using the rating system developed by Jeffers et al. (2012). In January, each plant was removed from the container mix, while taking care to preserve the root system. Each plant was given a symptom severity rating, which

corresponded to one of four classes: 0 = healthy, no lesions observed on roots; 1 = lesions observed on at least one lateral root; 2 = lesions observed on the tap root; 3 = severe root rot, plant dead. Examples of the symptom severity classes are shown in Figure 2.

Orchard Planting and Survival

In the spring of 2016, the least symptomatic plants (plants rated as "0") from the nursery trials were planted in TACF Georgia Chapter's Lake Allatoona Orchard (N 34.187138°, W -84.706437°), which was previously documented as *P. cinnamomi*-positive (M. Cipollini, pers. comm.). Survival of the UTC backcross families, Georgia Chapter families, and control groups was assessed in August.

Data Analysis

To evaluate the strength of resistance in each family, survival quotients (SQ) were calculated using the following method, described by Jeffers et al. (2009):

$$SQ = ([(1 \times n0) + (0.5 \times n1) + (0.25 \times n2)] / \text{total number of seedlings}) \times 100$$

where n0, n1, and n2 = the number of seedlings rated 0, 1, and 2, respectively. Due to low numbers of progeny obtained from some crosses, four of the fifteen families (UTC7, UTC10, UTC11, and UTC15) were not included in the SQ calculations.

Chi-square tests of independence and Fisher's exact tests of independence were used to determine whether the proportions of symptomatic individuals in the backcross families differed significantly from the *C. dentata*, *C. mollissima*, and *C. henryi* control groups. Fisher's exact test of independence was used in cases where the minimum expected number of plants in a category was less than five. Due to low numbers of progeny obtained from some crosses, four of the fifteen families (UTC7, UTC10, UTC11, and UTC15) were not included in the tests of independence. In the chi-square and Fisher's exact tests, the variable "PRR symptoms" consisted of two categories, "asymptomatic" (plants rated as "0") and "symptomatic" (plants rated "1", "2", and "3"). Microsoft Excel was used to perform both statistical tests, and McDonald's (2014) Excel spreadsheet was used for the chi-squared test.

Analysis of Genetic Loci Associated with PRR Resistance

Leaf tissue samples were collected from surviving trees at the Allatoona Orchard when we assessed the pre-screened and un-screened families for survival. Tissue samples were sent to the Clemson University Genomics Institute and DNA was extracted using a modification of the CTAB protocol described by Kubisiak et al. (2013) (T. Zhebentyayeva, unpublished protocol). Genotyping of the major effect locus for PRR resistance on linkage group E (Zhebentyeyava et al. 2014) will be performed in 2017.

Results

Mortality and PRR symptoms were observed in every backcross family and the *C. dentata* control group, while no mortality or PRR symptoms were observed in the *C. mollissima* and *C. henryi* control groups. Eleven of the *C. dentata* controls survived the nursery screening with various levels of symptom severity, and three of those had no PRR symptoms. The percentage of asymptomatic individuals per family (i.e., plants rated as "0") ranged from 43.5% to 85.2% (Table 2, Figure 3).

Five families (UTC2, UTC3, and UTC4) had an SQ value between 56.5% and 70%. Six families (UTC1, UTC5, UTC6, UTC12, UTC13, UTC14) had an SQ value above 70%. SQ values for every family are provided in Table 3.

Pairwise chi-square tests and Fisher's exact tests of the differences between the backcross families and controls showed that seven of 11 backcross families differed significantly from the American chestnut controls in regard to the number of symptomatic plants per family. Nine of 11 families differed significantly from the Chinese chestnut controls, and the same nine of 11 families differed significantly from *C. henryi* controls. In every case where the number of expected individuals per category was less than five, the results of Fisher's exact tests agreed with those of the chi-square tests. The two families that did not differ significantly from the Asian chestnut control groups were UTC1 (TTU A4 × ALA Frames 4) and UTC12 (TTU A4 × ALA Frames 5). Results of the chi-square and Fisher's exact tests of independence are provided in Table 4.

Discussion

One of the major goals of American chestnut restoration efforts is to produce a population of hybrid trees with the chestnut blight resistance of Asian chestnut species but with all other phenotypic traits of the American chestnut. Researchers have pursued this goal in earnest since the early 1980s (Burnham 1988); however, the need to produce American-type hybrids with PRR resistance has only become apparent within the last decade. A population of American-type trees with resistance to both chestnut blight and PRR would facilitate successful restoration in the southern portion of the American chestnut's distribution, where PRR-induced planting failures have been most common. To introgress PRR resistance into current restoration breeding populations, two research programs are in progress: (1) the identification of sources of PRR resistance from Asian chestnut species, and (2) the use of genomic approaches to identify the loci involved in PRR resistance, which can inform breeding strategies. Multiple PRR resistance screening facilities, like those at Chestnut Return Farm and UTC, may be required to identify sources of PRR resistance in each state chapter's existing backcross lines. Chapter-specific screening facilities would also be environmentally responsible, if efforts are made to prevent the transport of *P. cinnamomi* to new areas. If *P. cinnamomi* isolates used in the screening experiments are obtained from pre-existing TACF orchards, selected plants from the

screening facilities are only transplanted into *P. cinnamomi*-positive orchards, and only pollen and harvested seed are transferred to new orchards, then breeders and landowners can be more confident that *P. cinnamomi* will not be moved to new lands as a part of the American chestnut restoration process.

As a first step toward producing populations of PRR resistant hybrids for the Tennessee Chapter's breeding program, we have screened fifteen backcross families for PRR resistance. This study represents the first attempt to identify sources of PRR resistance from the following *C. mollissima* cultivars and selections: 'Amy', 'Byron', 'Gideon', 'Lindstrom 99', 'Neel 3-262', and 'Payne'. Several aspects of our experimental design were borrowed from the methods already developed at Chestnut Return Farm, in South Carolina (Jeffers et al. 2009; James 2011a,b). Specifically, these included the replication of experimental families in large planting containers, the use of a phenotyping system that accounted for survival and root lesions, and transplanting the least symptomatic plants into a *P. cinnamomi*-positive orchard after the first year of assessment in the nursery. It should be noted that much progress has been made in this field of research by Dr. Rita Costa and colleagues in Portugal (reviewed by Santos et al. 2016). Importantly, Santos et al. (2015) recently found that an excised shoot inoculation test may be an appropriate way to identify PRR-resistant progeny, especially when direct root inoculations are too expensive or laborious. Because of the low cost and resource requirements of this method, excised shoot inoculations may be an most appropriate method for the various TACF chapters to identify PRR resistant plants.

Results of the chi-square and Fisher's exact tests of independence were used to answer two questions: (1) are the backcross families more resistant than *C. dentata*?, and (2) are the backcross families as resistant as *C. mollissima* and *C. henryi* controls? In general, the backcross families displayed levels of PRR resistance intermediate to the *C. dentata* and Asian chestnut control groups. A majority, seven of 11, backcross families were significantly more resistant than *C. dentata* as evidenced by their significantly higher proportion of asymptomatic individuals than the American chestnut control group (Table 4). Only two backcross families were as resistant as *C. mollissima* and *C. henryi* (Table 4). The two families that were not significantly different from the Asian chestnut control groups were UTC1 (TTU A4 × ALA Frames 4) and UTC12 (TTU A4 × ALA Frames 5). Interestingly, both of these families are derived from the same F_1 mother tree, TTU A4. One possible explanation for the high rate of asymptomatic individuals in UTC1 and UTC12 is that some individuals may have "escaped" inoculation; even three of the 13 *C. dentata* were asymptomatic. A second possibility is that some of the progeny in the UTC1 and UTC12 families are outcrosses to a neighboring Chinese chestnut tree or to neighboring male-fertile F_1 trees. Outcrosses to these two unintended pollen donors would result in some progeny that are homozygous for PRR resistance alleles. However, since we did not observe any outcrosses in our "no pollen control" flowers, and since the open pollinated family UTC4 (TTU A4 × OP) had a significantly higher proportion of symptomatic individuals than the Asian control groups, it appears very unlikely that outcrosses affected these results. The third potential explanation for this result is that the *C. mollissima* 'Gideon' parent of TTU A4 is

strongly resistant to PRR. To evaluate the putative high strength of resistance in TTU A4, we are screening additional families from this mother tree in our second year of trials.

SQ values of all statistically-analyzed backcross families (i.e., those with more than six individuals planted) were higher than the SQ value of the *C. dentata* control group (48%), but lower than SQ values of the *C. mollissima* (100%) and *C. henryi* (100%) control groups. These statistics characterize the families as a whole, but it should be noted that many of the individual plants scored as "asymptomatic" and "lesions on lateral roots only" survived planting in a *P. cinnamomi* infested orchard. In a comparison of the nursery-screened backcross families to backcross families that had not been screened for resistance, a higher proportion of the pre-screened families survived the first half-season of growth in the *P. cinnamomi*-positive orchard (72% survival in pre-screened backcross individuals vs. 14% survival in backcross individuals that had not been pre-screened). These results suggest that levels of PRR resistance at the first-backcross generation are sufficient to introgress this trait into TACF's breeding program. Nevertheless, some aspects of the present study may have complicated identification of the most resistant hybrid progeny during nursery resistance screening trials.

In a comparison of the present study's SQ values to the results of Jeffers et al. (2009), all of the crosses analyzed here are more resistant than any cross reported in the earlier study. Only two crosses, UTC3: TTU A4 × ALA Frames 4 and UTC9: TNMac1 × TNSM1, had SQ values (56.5% and 58.3%, respectively) similar to that of the most resistant cross reported by Jeffers et al. (2009), Hyko × JB575 (56.3%). There are a few possible explanations for this result. First, all of the families studied by Jeffers et al. (2009) were the descendants of a greater number of recurrent crosses to *C. dentata* than any of the families reported here. Specifically, Jeffers et al. (2009) screened BC_2, BC_3, and BC_4 progeny, while we screened nine BC_1 families, three better backcross families, and one BC_4 family. Because PRR resistance in chestnut is thought to be a polygenic trait (Santos et al. 2015), it would be expected that Asian-American BC_1 hybrids would retain more alleles for resistance than later generation backcrosses to *C. dentata*, if plants were not selected for PRR resistance at every generation. Second, the strength of selection in the study of Jeffers et al. (2009) may be higher. In other words, the environment in the tubs at Chestnut Return Farm may have been more favorable for *P. cinnamomi* or our inoculum may not have been entirely successful. The latter scenario would have resulted in an uneven distribution of *P. cinnamomi* in the tubs. The existence of three asymptomatic *C. dentata* does suggest that *P. cinnamomi* was not uniformly successful within the tubs.

The listed explanations are not mutually exclusive, however, and future problems like those mentioned can be prevented through a few modifications to the experimental design. For example, two inoculations of *P. cinnamomi* during the growing season should decrease the probability of some plants escaping inoculation. An additional benefit of a second inoculation would be increased mortality in the nursery screening trials, which would allow fewer plants rated as "1" or "2" to eventually succumb to PRR after orchard planting. To address the possibility of non-uniform conditions within the tubs (e.g., higher temperatures in certain parts of the tubs, because of greater exposure to direct sunlight), we have conducted our second year of

nursery screenings inside the greenhouse, with each seedling planted in an individual container. This design should obviate the problem of non-uniform environment within replicates, and it may also allow greater control over moisture levels within the containers.

Future directions for our PRR resistance screening program include screening in individual containers (as mentioned), repeating and re-screening some of the crosses that displayed lowest symptom severity, generating *C. henryi* × *C. dentata* F_1 hybrids, and screening more progenies derived from untested sources of disease resistance. In 2017, we also plan to genotype the most resistant progeny at the major effect locus on linkage group E that was previously identified by Zhebentyayeva et al. (2014). An important question in this area of research is concerned with whether the same loci encode PRR resistance in different *C. mollissima* cultivars. The results obtained in the next phase of this project are expected to help answer this question. Besides the future directions mentioned above, we expect the backcross progeny screened here to become a valuable resource for future chestnut breeding efforts in the Southeast.

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Appendix I: Tables

Table 1. Backcross families evaluated for PRR resistance. All families were derived from *C. dentata* from Tennessee and Alabama, Tennessee Chapter F_1s, and Chinese chestnut cultivars accessioned at UTC's cultivar trial (Smith Farm, Hamilton Co., TN).

Family	Pedigree	Cross type	Source of resistance	Has this source been tested previously?
UTC1	TTU A4 × ALA Frames 1	BC_1	*C. mollissima* 'Gideon'	No
UTC2	McInturff FF-1 × OP	"Better backcross" (BB) = F_1 × selected 'Clapper' BC_4	*C. mollissima* 'Nanking'	Yes (Olukolu *et al.* 2012, Zhebentyayeva *et al.* 2014)
UTC3	TTU A4 × ALA Frames 4	BC_1	*C. mollissima* 'Gideon'	No
UTC4	TTU A4 × OP	BB	*C. mollissima* 'Gideon'	No
UTC5	TNSum1 × Neel 6-193	BC_1	*C. mollissima* 'Payne'	No
UTC6	TNMac1 × Neel 4-195	BC_1	*C. mollissima* 'Amy'	No
UTC7	TNMac1 × Neel 6-268	BC_1	*C. mollissima* 'Byron'	No
UTC8	TNMac1 × Neel 2-127	BC_1	*C. mollissima* 'Lindstrom '99'	No
UTC9	TNMac1 × TNSM1	BC_4	'Clapper' BC_1	Yes (Jeffers *et al.* 2009)
UTC10	McInturff DD-1 × OP	BB	*C. mollissima* 'Nanking'	Yes (Olukolu et al. 2012, Zhebentyayeva et al. 2014)
UTC11	McInturff II-1 × OP	BB	*C. mollissima* 'Nanking'	Yes (Olukolu *et al.* 2012, Zhebentyayeva *et al.* 2014)
UTC12	TTU A4 × ALA Frames 5	BC_1	*C. mollissima* 'Gideon'	No
UTC13	Neel 5-238 × ALA Frames 1	BC_1	*C. mollissima* 'Byron'	No
UTC14	Neel 3-262 × TNCarroll1	BC_1	Unnamed *C. mollissima* seedling	No
UTC15	Neel 6-268 × AL T3	BC_1	*C. mollissima* 'Byron'	No
UTC16	*C. mollissima*	Resistant control	N/A	N/A
UTC17	*C. dentata*	Susceptible control	N/A	N/A
UTC18	*C. henryi*	Resistant control	N/A	N/A (but see Crandall et al. 1945)

Table 2. Proportions of symptomatic and asymptomatic individuals in the backcross families screened for PRR resistance.

Family	Pedigree	% symptomatic	% asymptomatic	*N*
UTC1	TTU A-4 × ALA Frames 1	14.8	85.2	27
UTC2	McInturff FF-1 × OP	51.9	48.1	77
UTC3	TTU A-4 × ALA Frames 4	56.5	43.5	23
UTC4	TTU A-4 × OP	47.4	52.6	116
UTC5	TNSUM1 × Neel 6-193	40	60	15
UTC6	TN MAC1 × Neel 4-195	40	60	40
UTC7	TN MAC1 × Neel 6-268	50	50	2
UTC8	TN MAC1 × Neel 2-127	44.4	55.6	27
UTC9	TN MAC1 × TNSM1	50	50	6
UTC10	McInturff DD-1 × OP	0	100	1
UTC11	Mcinturff II-1 × OP	100	0	1
UTC12	TTU A-4 × ALA Frames 5	16.7	83.3	12
UTC13	Neel 5-238 × ALA Frames 1	34.5	65.5	29
UTC14	Neel 3-262 × TN Carroll Co. 1	28.6	71.4	49
UTC15	Neel 6-268 × AL T3	0	100	4
UTC16	*C. mollissima* control	0	100	11
UTC17	*C. dentata* control	76.9	23.1	13
UTC18	*C. henryi* control	0	100	17
All BC plants		41.8	58.2	424
Total plants		40.2	59.8	465

Table 3. Survival quotients of backcross families screened for PRR resistance. Families too small to be replicated in all five tubs (i.e., families with fewer than five individuals) were not used for calculation of SQ values.

Family	Pedigree	Cross type	*N*	SQ
UTC1	TTU A-4 × ALA Frames 1	BC_1	27	87.0
UTC2	McInturff FF-1 × OP	BB	77	61.4
UTC3	TTU A-4 × ALA Frames 4	BC_1	23	56.5
UTC4	TTU A-4 × OP	BB	116	67.5
UTC5	TNSUM1 × Neel 6-193	BC_1	15	71.7
UTC6	TN MAC1 × Neel 4-195	BC_1	40	70.6
UTC8	TN MAC1 × Neel 2-127	BC_1	27	66.7
UTC9	TN MAC1 × TNSM1	BC_4	6	58.3
UTC12	TTU A-4 × ALA Frames 5	BC_1	12	89.6
UTC13	Neel 5-238 × ALA Frames 1	BC_1	29	72.4
UTC14	Neel 3-262 × TN Carroll Co. 1	BC_1	49	76.0
UTC16	*C. mollissima* control	Resistant control	11	100
UTC17	*C. dentata* control	Susceptible control	13	48.1
UTC18	*C. henryi* control	Resistant control	17	100

Table 4. Results of chi-square tests of independence and Fisher's exact test of independence between backcross families and control groups. Tests were performed using the proportions of families in the asymptomatic (i.e., plants rated as "0") and symptomatic (i.e., plants rated as "1", "2", or "3") classes. The chi-square test of independence was used to compare each backcross family to each of the three control groups. For comparisons where the expected number of plants in one of the symptomatic or asymptomatic categories was less than five, Fisher's exact test was also used. Asterisks (*) indicate differences between groups that are significant at the $P < 0.05$ level. Daggers (†) indicate significant differences (at the $P < 0.05$ level) from Fisher's exact test. Results of the Fisher's exact test agreed with those of the chi-square test in every comparison.

Family	***C. dentata* control**	***C. mollissima* control**	***C. henryi* control**
UTC1 TTU A-4 x ALA Frames 1	$\chi^2 = 14.879$; p= 0.0001*†	$\chi^2 = 1.821$; p = 0.177	$\chi^2 = 2.770$; p = 0.096
UTC2 McInturff FF-1 x OP	$\chi^2 = 2.81$; p = 0.094	$\chi^2 = 10.476$; p = 0.001*	$\chi^2 = 15.373$; p = 0.00009*
UTC3 TTU A-4 x ALA Frames 4	$\chi^2 = 1.498$; p = 0.221	$\chi^2 = 10.066$; p = 0.002*	$\chi^2 = 14.235$; p = 0.0002*
UTC4 TTU A-4 x OP	$\chi^2 = 4.072$; p = 0.044*	$\chi^2 = 9.2$; p = 0.002*	$\chi^2 = 13.744$; p = 0.0002*
UTC5 TTU SUM1 x Neel 6-193	$\chi^2 = 3.877$; p = 0.049*	$\chi^2 = 5.720$; p = 0.017*†	$\chi^2 = 8.369$; p = 0.004*†
UTC6 TN MAC1 x Neel 4-195	$\chi^2 = 5.352$; p 0.021*	$\chi^2 = 6.411$; p = 0.011*†	$\chi^2 = 9.454$; p = 0.002*†
UTC8 TN MAC1 x Neel 2-127	$\chi^2 = 3.74$; p = 0.053	$\chi^2 = 7.145$; p = 0.008*†	$\chi^2 = 10.389$; p = 0.001*†
UTC9 TN MAC1 x TNSM1	$\chi^2 = 1.377$; p = 0.241	$\chi^2 = 6.679$; p =0.010*†	$\chi^2 = 9.775$; p = 0.002*†
UTC12 TTU A-4 x ALA Frames 5	$\chi^2 = 9.077$; p = 0.003*	$\chi^2 = 2.008$; p = 0.156	$\chi^2 = 3.043$; p = 0.081
UTC13 Neel 5-238 x ALA Frames 1	$\chi^2 = 6.482$; p = 0.011*	$\chi^2 = 5.507$; p = 0.03*†	$\chi^2 = 7.49$; p = 0.006*†
UTC14 Neel 3-262 x TN Carroll County #1	$\chi^2 = 10.124$; p = 0.001*	$\chi^2 = 4.099$; p = 0.043*†	$\chi^2 = 6.165$; p = 0.013*†

Appendix II: Figures

Figure 1. Families were planted in a randomized complete block design, with five replications, in 229 L plastic nursery containers.

Figure 2. Examples of plants in the four symptom severity classes used in this study. Plants with no root lesions were scored as "0" (see Fig. 2a). Plants with lesions on the lateral roots, but not the tap root were scored as "1" (see Fig. 2b). Plants with lesions on the tap root were scored as "2" (see Fig. 2c). Dead plants were scored as "3" (see Fig. 2d). Thorough descriptions of the symptom severity classes are provided by Jeffers et al. (2009).

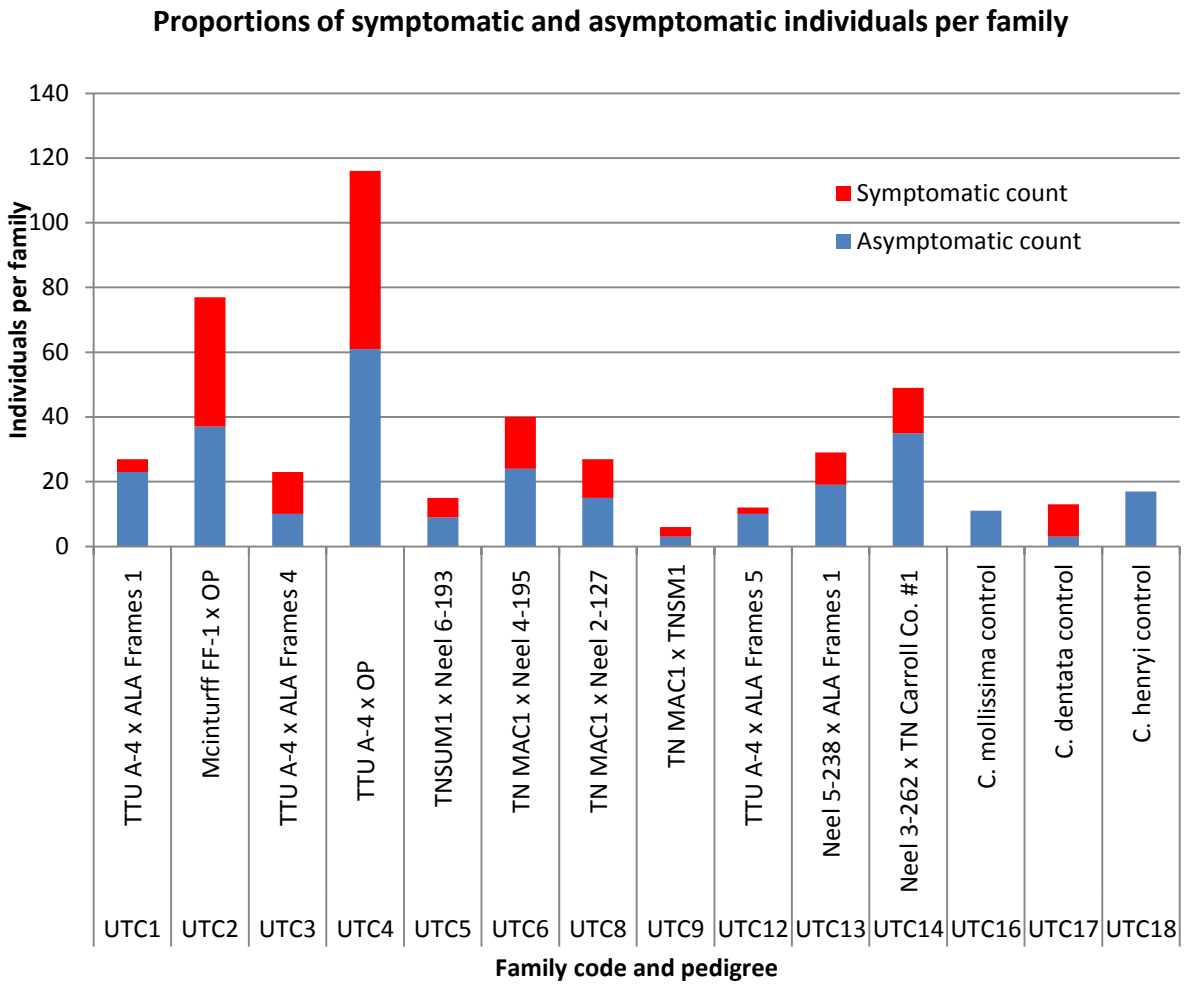

Figure 3. Proportions of symptomatic and asymptomatic individuals per family. The asymptomatic class includes plants scored as "0" and the symptomatic class includes plants scored as "1", "2", or "3". Families UTC7, UTC10, UTC11, and UTC15 are not reported here because too few progeny were available for statistical analysis.