Adding Resistance to Bacterial Spot and Speck to Lines with Fungal Resistances in Fresh Market Tomato Lines

Martha Mutschler, Taylor Anderson, Darlene DeJong, Michael Glos, Julie B Bojanowski
Plant Breeding and Genetics Section, SIPS, Cornell University

This foliar disease control program, which had focused on foliar/oomycete blights, has expanded to target bacterial speck and bacterial spot. The information in this report covers work accomplished to date, the work planned for 2018, and estimates on when the first lines that possess resistances to targeted fungal diseases and bacterial diseases should be completed.

Bacterial spot of tomato is caused by up to four species of *Xanthomonas*: *X. euvesicatoria*, *X. vesicatoria*, *X. perforans*, and *X. gardneri* with maximum infection occurring under warm moist conditions. Bacterial speck, caused by *Pseudomonas syringae*, can cause up to 25% loss of crop under cool rainy conditions, and has been increasingly problematic in NE tomato growing regions. Typically, control of bacterial speck and bacterial spot involves multiple applications of copper based bactericide (copper hydroxide or copper sulfate) mixed with mancozeb. However, copper insensitivity in *Xanthomonas* is widespread.

Work elsewhere (Ohio and Florida State Programs) developed sources of bacterial disease resistance, and mapped the resistance genes or QTL associated with these resistances. The Cornell program is combining these resistances with the fungal disease resistances that were the focus of our prior work.

For Bacterial Speck resistance: The *Pto* gene, derived from *S. pimpinellifolium* PI 370093, controls *P. syringae* pv tomato, race 0, and is the standard resistance gene used. *Pto* maps to chromosome 5, and has been cloned, providing sequence data for marker development. For resistance to occur, plants must also possess the active *Prf* gene, which is very tightly linked to *Pto*, and seldom separated by recombination.

For Bacterial spot resistance: *Rx-3*, derived from Hawaii 7998, provides field resistance for T1 strains of *X. euvesicatoria*. *Rx-3* is located on chromosome 5. Resistance to T3 strains of *X. perforans* is derived from either *S. pimpinellifolium* PI 128216 or Hawaii 7981; the two loci map to chromosome 11 and may be allelic. Race non-specific resistance seems to be conferred through the action of several quantitative trait loci with the most important being QTL-11 located on chromosome 11. Francis (at OSU) found that the combination of *Rx-3, Rx-4* and QTL-11 also provide partial resistance/tolerance to *X. gardneri*.

The Cornell and OSU programs are cooperating to combine fungal and bacterial resistance in fresh market and processing tomato, respectively. The goal of this project at Cornell is to transfer the available resistances to bacterial speck and bacterial spot into the best existing CU line possessing the resistances to fungal/ oomycete blights, thereby reducing the need for copper sprays (in addition to the reduction in fungal sprays already achieved in fresh market tomato). Conversely, the program at OSU is transferring the fungal/oomycete resistances into processing tomato lines that already possess the bacterial resistance genes.

Overview Transfer Strategy:

A Cornell fresh market line with resistance to *Septoria* leaf spot and late blight, and strong early blight tolerance was crossed to an OSU line possessing *Pto* and *Rx-3* bacterial resistance genes on chromosome 5 (OH7536) and was also crossed to an OSU line with the other bacterial spot resistances called QTL-11 and *Rx-4* on chromosome 11 (OH7663). The transfer of these sets of bacterial disease resistance genes on chromosomes 5 and 11, respectively, is proceeding separately using backcross breeding, accelerated by the combination of positive selection for the introgressions carrying the bacterial resistance genes and background selection to more rapidly recover the recurrent parent genome on the other 11 chromosomes. We plan to combine the bacterial disease resistance genes on chromosomes 5 and 11 into a single line when their transfers to the Cornell fungal resistance recurrent parent line are completed or close to completion.

In each segregating generation, marker assisted selection is used to screen a very large population to first identify those plants that carry the region of the targeted bacterial resistance genes in that population. The subpopulation with the targeted regions are potted to further develop while their DNA samples are analyzed using SNP markers on all other chromosomes for background selection to identify the plants with the fewest/smallest regions of processing tomato DNA. The best 3 to 7 plants are then used to generate seed of the next generation. This two-step approach minimizes genotyping costs and greenhouse space costs while maximizing time efficiency.

Transfer of Bacterial Spot Resistance on Chromosome 11.

The transfer strategy worked extremely well for transfer of the chromosome 11 region carrying *Rx-4* and QTL-11. The transfer of this region is essentially done, and we are proceeding to the testing phase. The three stages of the transfer were as follows

FIRST: BC1F1: After analysis of a large BC1F1 population, 324 plants were identified that possessed the full chromosome 11 region and were heterozygous for processing tomato DNA on 3 - 5 other chromosomes. This degree of progress is what would be expected for two cycles of backcrossing without background selection. The selected BC1F1 plants were backcrossed to the fresh market parent to produce BC2F1 seed.

SECOND: BC2F1: When this strategy was repeated summer of 2017 using 198 BC2F1 plants, 92 plants were identified that were heterozygous for the full chromosome 11 region and were heterozygous for regions on only 1 to 2 additional chromosomes. The selected BC2F1plants were self-pollinated to produce BC2F2 seed lots.

THIRD: BC2F2: Screening two BC2F2 populations fall of 2017 with PCR markers identified 10 BC2F2 plants that are homozygous for the chromosome 11 region containing the bacterial spot gene *Rx-4* and QTL, QTL-11. SNP/marker data to date indicate that these BC2F2 plants are also homozygous for the DNA of the CU fungal resistant parent line for the remaining 11 chromosomes. Additional plants recombinant for the chromosome 11 region were also saved, in case we later discover linkage drag issues associated with the chromosome 11 region. All of these BC2F2 selections are currently producing self-fruit and BC2F3 seed should be harvested/extracted during March 2018. This seed establishes the new lines combining the fungal resistances of the CU parental line with the *Rx-4* and QTL-11 bacterial speck resistances on chromosome 11.

Work Planned for 2018: Additional denser genotyping will be performed on the new lines to confirm that they are homozygous for fresh market parental DNA throughout the genome, or if any smaller regions remain of heterozygosity or processing tomato DNA in the genome that were not detected by the SNP markers previously used.

During the summer of 2018, the new lines will be tested for plant and fruit characteristics in field trials in three locations in NYS, as well as used in a bacterial spot trial at OSU.

Transfer of Bacterial Spot and Speck Resistances on Chromosome 5.

Transfer of the resistances on chromosome 5 is one generation behind the transfer of the chromosome 11 bacterial resistance genes to the Cornell blight resistant parental line. The two stages of transfer were as follows:

FIRST: Screening of the BC1F1 populations for the chromosome 5 transfer was completed in June 2017 resulting in a selection of 4 plants that were heterozygous for the full chromosome 5 region carrying *Prf*/*Pto* and *Rx-3* and were heterozygous for 3 or 4 additional regions on other chromosomes. These BC1F1 plants were crossed to the recurrent parent to generate BC2F1 seed.

SECOND: The BC2F1 populations were sown Dec 2017 and were screened with chromosome 5 markers in early Jan 2018, identifying 94 plants that were heterozygous for the entire chromosome 5 region carrying both *Prf*/*Pto* and *Rx-3,* and in which heterozygosity for at least one other chromosome was eliminated. These 94 BC2F1 selections might still be heterozygous for processing tomato parent DNA in regions on 2 or 3 other chromosomes. The 94 BC2F1 selections were potted into slightly larger pots, to allow further growth and the start of fruit set while SNP data are being generated.

Work Planned for 2018: SNP data from the 94 BC2F1 plants should be in hand sometime in February, and will be used to select the subset of BC2F1 plants heterozygous for the chromosome 5 region carrying both *Pto/Prf* and *Rx-3* and homozygous for fresh market tomato DNA on all or as many of the other chromosomes as possible.

The final BC2F1 plants selected will be self-pollinated to generate the BC2F2 seed (to be harvested during April 2018). In spring/summer 2018, the resulting BC2F2 populations will be screened with PCR markers to select final lines homozygous for the chromosome 5 region carrying *Pto/Prf* and *Rx-3*. We anticipate that seed from homozygous lines possessing the chromosome 5 bacterial resistance genes would be produced during the fall 2018. We will also save seed from BC2F2 plants with recombinations within the chromosome 5 region, in case later trials indicate a need to reduce introgression size/break linkage drag.

Combining all of these bacterial resistance genes on chromosomes 5 and 11.

Additional, more dense, genotyping will also be performed on the chromosome 5 lines, to determine if they are indeed homozygous for the fresh market parental tomato line throughout the genome, or if any smaller regions of processing tomato DNA remain in the genome that were not detected by the number/locations of the SNPS markers used. Crosses will be made between lines carrying the chromosome 11 and chromosome 5 bacterial resistances. The seed of the chromosome 5 lines and F1 hybrids will be used in the 2019 summer trials.

Mapping Campbell 1943 Stem Early Blight Tolerance and Testing an Alternate Source of Foliar Early Blight Tolerance

Martha Mutschler, Taylor Anderson, Darlene DeJong, Michael Glos, Julie B Bojanowski
Plant Breeding and Genetics Section, SIPS, Cornell University

Prior work at Cornell University produced fresh market tomato lines combining strong resistance to late blight and Septoria leaf spot, and strong tolerance to early blight, resulting in the release of the CUTR fungal resistant tomato lines. The form of early blight tolerance in these lines, derived from Campbell 1943, controls early blight lesion development/expansion on stem and the peduncles (thereby protecting the fruit). However, this tolerance does not provide good control of foliar disease/blighting, although it slightly reduces foliar disease and can reduce the frequency of fungicide sprays required for control of leaf blighting if the plants are homozygous for the tolerance. Although this form of early blight tolerance has been used by breeding programs, and the location of the tolerance has not yet been mapped.

As a side product of the project transferring resistances to bacterial speck and bacterial spot to the Cornell lines with multiple fungal resistances, we surveyed early blight response in the parental OH lines contributing bacterial disease resistance genes. As expected, the Ohio line, which is the source of the bacterial speck and bacterial spot resistance genes, was susceptible to early blight on its stems. This allows us to use the plant materials being used for transfer of bacterial resistances to also map the Campbell 1943 early blight tolerance.

A serendipitous discovery from the early blight screens was the observation that one of the Ohio lines used as a source of resistance to bacterial diseases might have what appears to be some level of foliar early blight tolerance, which is a contrast to fresh market plants with the Campbell 1943 EB stem tolerance. Therefore, we are also using the bacterial resistant populations already developed for additional work to examine this possible form of foliar early blight tolerance, and its inheritance. The goal is to determine whether the foliar early blight tolerance is expressed under heavy field pressure, and if it is confirmed as being of sufficient strength, to determine if it could be combined with the stem tolerance to achieve overall control of early blight on stems and foliage.

Since this project is not yet completed, this report provides information on the work completed to date, and also an overview of the work to be done in 2018.

SUMMER 2017 Inoculated Mist Table and Field Screens Entries and Methods

Entries and setup of the 2017 early blight trials. The F2 seed used was produced by self-pollinating the F1 plants of the cross (CU151095-146 x OH7663). The three way cross seed was generated by the cross (CU151011-170 x OH7663) x OH7536.

CU151095-146 and CU151011-170 are Cornell fungal resistant lines that both possess the strong “Campbell 1943” early blight stem tolerance. The lines differ in that CU151095-146 lacks the SLS-1 gene on chromosome 1 that CU151011-170 possesses.

OH7536 and OH7663 are the OSU lines that possesses the chromosome 5 introgression containing *Pto/Prf* and *Rx-3* bacterial spot and speck resistances, and the chromosome

11 introgression that contains *Rx-4* as well as QTL 11, respectively. Both OSU lines are fully susceptible to early blight disease on stems. In prior mist table experiments, OH7663 is the line that appeared to have potential foliar tolerance to early blight.

The F2 segregating population and its controls, and the three way segregating population and its controls, were grown to 5 weeks of age in greenhouses, and transplanted into separate field experiments, each of which used a random complete block design. The field had been prepared with plastic-covered raised beds and with trickle irrigation. "Fertigation" was used early in the growing season, but stopped mid-July to impose a mild nutrition stress on the plants to favor the development of early blight symptoms.

Mist table screen and phenotypic data:

Cuttings were taken from field F2 and control plants prior to field inoculation, and used in a severe mist table early blight stem screen. In the mist table test, the F2 population segregated 3:1 for F2 plants with susceptible stems : F2 plants with tolerant stems, suggesting that the early blight stem tolerance trait is largely due to a single gene. That the a heterozygous class of plants was not distinguishable from a susceptible class plants for degree of stem disease was unexpected, since in prior work plants heterozygous for stem early blight tolerance usually have disease control that is intermediate in strength or even closer to the tolerant parent than the susceptible parent under natural field infection or in early blight inoculated field tests. The conditions used for the mist table screen might have applied heavier disease pressure than in inoculated or naturally-infected field trials, especially since the cuttings were stressed by being cut and placed into water

Field EB screen inoculation and collection of phenotypic data:

The two field experiments were inoculated mid-July using with *ca.* 60mL/plant of a 37,500 conidia/ml inoculum suspension for the F2 experiment and of an 18,500 conidia/mL inoculum suspension in the three way experiment; application was performed in the evening using backpack sprayers. The weather conditions were very conducive to early blight disease; the disease development was outstanding in both experiments. Readings of the stem early blight disease development were collected alternate weeks for 6 weeks in both the F2 and the three way experiments using a 0 to 5 rating scale for no to high disease, and the resulting data were used to calculate area under disease progress estimates for each plant. Readings of foliar early blight disease development were collected every 10 days for 6 weeks in the F2 experiment using a 0 to 100% defoliation rating scale, and the resulting data used to calculate area under disease progress (AUDPC) estimates for each plant. The final foliar disease rating was collected in week 6 for the three way cross population.

Early blight on stems of controls and F2 population in the F2 experiment.

Visually, the stem lesions on the susceptible parent OH7663 expanded rapidly to fully cover or "girdle" stems. Lesions appeared on stems of the tolerant parent, CU151095-146, after inoculation but remained very small over time (1 mm or less). Lesions appeared on stems of the F1 controls, but remaining moderate in size (up to 2 mm). The stem disease data for the controls in the F2 experiment were analyzed to check the quality of the phenotypic data. Considering the controls of the F2 population experiment, the stem readings from the homozygous tolerant parent (151095-146), homozygous susceptible

parent (OH7663), and their heterozygous F1 were all significantly different from each other, with the AUDPC of the F1 being intermediate between those of the two parents (Table 1). These data are consistent with prior years of breeding and testing of F1 hybrids with this early blight stem tolerance.

Table 1. Early Blight Stem Disease in F2 Population field trial

Genotype	AUDPC for Stem disease rating	df	Lower 95% Confidence interval	Upper 95% Confidence Interval
CU151095-146	16.8 a	50	12.67	20.85
CU151095-146 x OH7663 F1	33.0 b	50	28.90	37.07
OH7663	52.7 c	50	48.56	56.73
F2 population	39.0	276	36.9	41.1

Tukey HSD Letter Grouping (alpha = 0.05 level).
Rating for Stem disease observation used a 0 to 5, no disease to stem fully covered by disease.
AUDPC was calculated using data from 4 observation dates

The curve for the mean stem AUDPC for the F2 population was very similar to that of the F1. However the spread of the F2 plants does not present a smooth curve, but rather appeared to have three peaks, at stem AUDPC values similar to the tolerant parent, F1 and susceptible parent controls (Fig 1). Determination of whether a major gene contributes substantially to this trait, and if so its map location, await completion of the GBS data being generated.

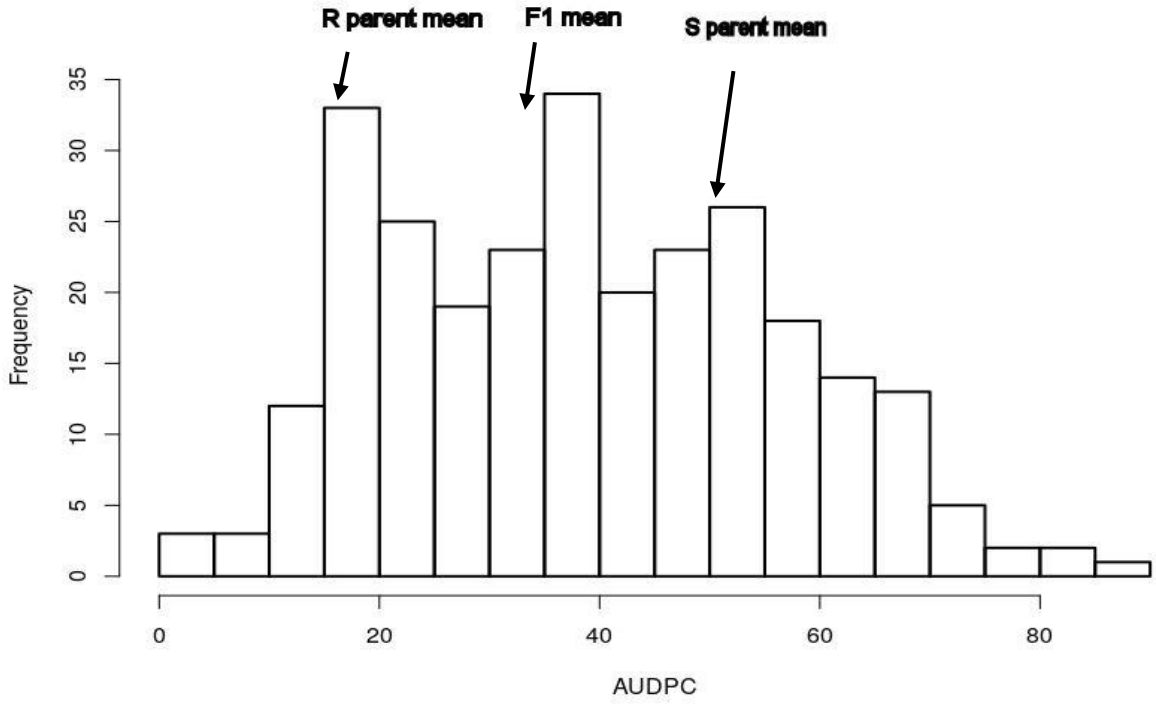

Figure 1. Average area under disease progress curve (AUDPC) for early blight disease on stem of plants in the F2 population in the 2017 inoculated field trial. Means for the stem EB readings for the tolerant parent, the F1, and the susceptible parent controls are shown by legends/arrows at top of this figure.

Early blight on stems of controls and three way population in the three way cross experiment.

The phenotypic data for the controls in the three way experiment were analyzed to check the quality of the phenotypic data (Table 2). The disease observed for the controls in the three way cross experiment were very similar to those observed for the controls in the F2 experiment, with the exception that most of the means were slightly lower than those in the F2 experiment, perhaps due to the lower inoculum concentration used in the three way cross experiment.

The three way experiment included an extra control, CU151095-146, a CU early blight tolerant line which was a parent of the F2 but not of the three way population. The stem readings from the two homozygous tolerant CU lines, the two Ohio homozygous susceptible lines, and the heterozygous F1 in the three way experiment were all significantly different from each other, with the stem F1 being intermediate of the two parents (Table 2). The two Ohio lines were fully susceptible to early blight disease on stems, with lesions rapidly expanding to cover or girdle stems. The CUTR parent 151011-170 showed strong tolerance to EB disease compared to the OH parent also used to generate the evaluated F1; lesions appeared but remained very small (1 mm or less). Plants of the F1 controls are intermediate for EB disease on stems; lesions appeared but remained moderate in size (up to 2 mm).

Table 2. Early Blight Stem Disease in Three Way Cross Population field trial

Genotype	AUDPC for Stem Disease Rating	df	Lower 95% Confidence interval of Mean	Upper 95% Confidence Interval of Mean
CU151095-146	10.6 a	84	7.8	13.4
CU151011-170	13.7 a	84	10.9	16.5
CU151011-170 x OH7663 F1	23.8 b	84	21.0	26.6
OH7663	38.1 c	84	35.3	40.9
OH7536	49.1 d	84	46.3	51.9
Three way cross population	34.2	285	28.5	33.2

Tukey HSD Letter Grouping (alpha = 0.05 level)
CU151095-146 is not a parent in the three way cross, but was included in experiment as an extra control.

Early blight disease on foliage of controls and F2 population in the F2 experiment.

The foliar disease data for the controls in the F2 experiment were analyzed to check the quality of the phenotypic data. This a complex situation since the stem tolerance (Campbell's 1943) does provide very modest foliar control, and the foliar control of OH7663 was first noted in mist table screens on young plants, rather than mature plants under field conditions. The foliar readings on the Cornell parent had foliar ratings that were significantly lower than those of the Ohio parent, and the heterozygous F1 was not significantly different from the Ohio parent (Table 3 on next page). It is not clear if this indicates that the OH7663 line does not have any foliar tolerance under field conditions, or if the tolerance it possesses cannot withstand the heavy inoculation/severe conditions present in the 2017 field trial.

Table 3. Early Blight Foliar Disease in F2 Population field trial

Genotype	AUDPC for Foliar disease rating	df	Lower 95% Confidence interval of mean	Upper 95% Confidence Interval of mean
CU151095-146	402.8 a	50	325.31	480.24
CU151095-146 x OH7663 F1	593.1 b	50	515.59	670.52
OH7663	584.4 b	50	506.98	661.91
F2 population	670.2	276	638.4	701.9

Tukey HSD Letter Grouping (alpha = 0.05 level)
AUDPC includes data from 4 reading, each using 1 to 100 scale. Theoretical min and max for trait would be 1 to 1000

What was particularly striking in for the foliar disease reading in the F2 population was the extreme breadth of foliar disease levels among F2 plants. This breadth of foliar disease expression is also reflected in the curve for the mean stem AUDPC (Fig 2). The spread of the F2 plants for foliar disease does not present a smooth curve, but rather appeared to have two peaks, one, at 400, which is also the mean stem AUDPC for the CU parent line, and another very close to the F2 mean of 670. The F2 population included plants that appeared to combine the stem tolerance trait with much lower degree of foliar disease than the CU parent line. The F2 also included other plants with AUDPC values more than double the means of the parents and the F1 control.

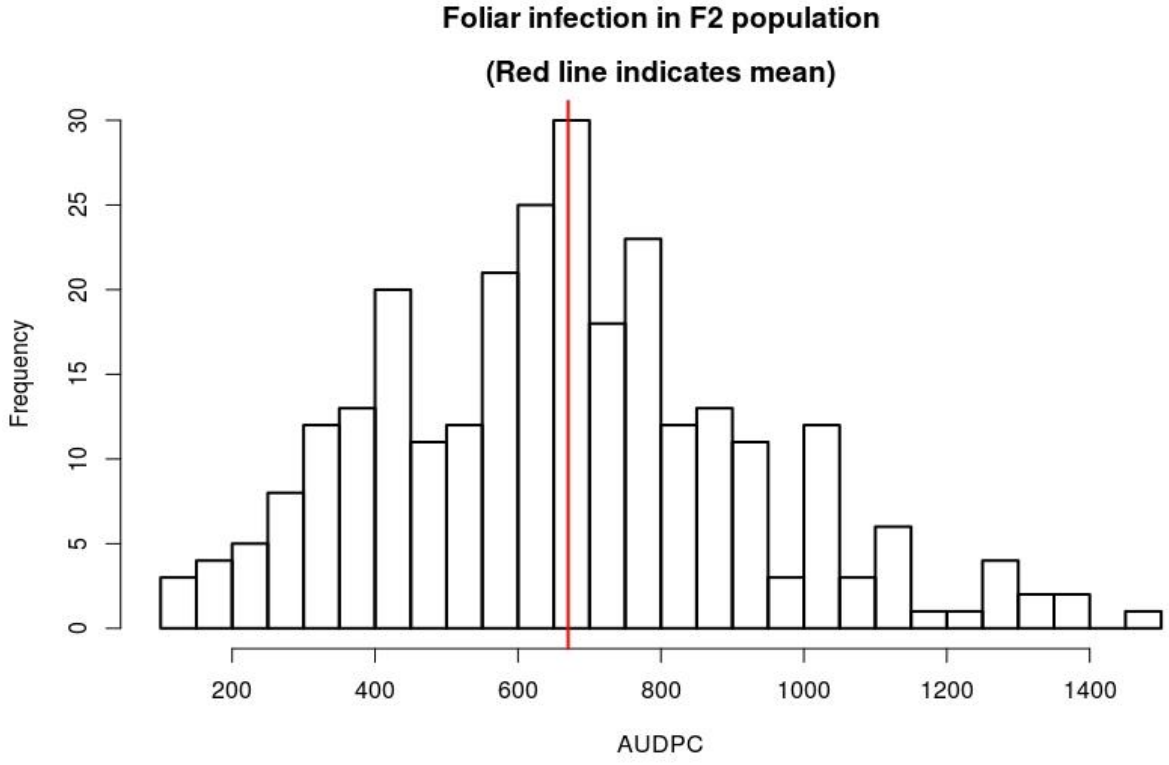

Figure 2. Average area under disease progress curve (AUDPC) for EB stem disease readings on plants of the F2 population in the 2017 inoculated field trial.

Early blight disease observed on foliage in the three way experiment.

The three way cross population seed was produced from cross of (CU151011-170 x OH7663) x OH7536. The parent OH7663 was the line with possible foliar resistance. Therefore, if the F1 control (CU151011-170 x OH7663) did not show signs of foliar resistance, then the putative foliar tolerance trait is not dominant, and would not be detectable in the three way cross population (since the third parent, OH7536, did not appear to have foliar tolerance). Since early observations of foliar early blight of the CU151011-170 x OH7536 F1 control did not indicate any degree of foliar tolerance, and due to labor constraints, foliar disease ratings were only collected for the controls of the three way cross experiment, and they were only collected once, at the end of the scoring period for the F2 experiment. Therefore, the values in Table 4 are the means of the one reading of foliar disease for all of the plants of each entry, rather than mean AUDPC from multiple readings over time.

Table 4. ANOVA of the Three Way Cross Population Controls for Early Blight Foliar Disease Rating; (means are lower since they are from only one reading, not AUDPC)

Genotype	Mean Foliar disease rating, week 6	df	Lower 95% Confidence interval	Upper 95% Confidence Interval
CU151095-146	38.1 a	84	27.08	49.03
OH7663	60.6 b	84	49.58	71.53
CU151011-170	74.4 bc	84	63.47	85.42
CU151011-170 x OH7536 F1	82.2 c	84	71.25	93.20
OH7536	88.6 c	84	77.64	99.58

Tukey HSD Letter Grouping (alpha = 0.05 level)
Disease rating is on a 0 to 100 scale, no disease to dead plants

WORK FOR 2018:

Leaf samples were collected from segregating populations before inoculation, and held at -80C. Plants with extreme early blight disease values were selected for genotyping by GBS. One set of F2 DNAs is from plants within the upper and lower 10% extremes for early blight disease on stems. The other set of F2 DNAs is from the plants within the upper and lower 10% extremes for foliar early blight disease. The libraries for GBS are being sequenced, and should be in hand Feb 2018 for data analysis.

The genotypic data from the F2 plants selected for stem disease extremes will be analyzed with the stem disease data to map the stem tolerance trait. Assuming the putative location of the gene controlling this trait is identified, markers flanking this region will be created, and the DNA from the three way cross population will be genotyped using the markers to confirm the map location. Alternatively we could grow F3 progenies of selected F2 plants to use in early blight mist table screens to confirm location of the gene, or even more finely map its location within the chromosome.

The genotypic data from the F2 plants selected for extremes in foliar early blight disease will also be analyzed with foliar early blight data to examine the inheritance of the putative early blight foliar tolerance, and evaluate its potential value.