Multiple Disease Resistance Loci Affect Soilborne Disease Resistance in Tobacco (*Nicotiana tabacum*)

Katherine Drake-Stowe, Nicolas Bakaher, Simon Goepfert, Berangere Philippon, Regis Mark, Paul Peterson, and Ramsey S. Lewis†

First and seventh authors: Crop and Soil Science Department, North Carolina State University, Raleigh; second, third, fourth, and fifth authors: Philip Morris International R&D, Philip Morris Products S.A., Neuchatel, Switzerland (part of Philip Morris International group of companies); and sixth author: Pee Dee Research and Education Center, Clemson University, 2200 Pocket Rd., Florence, SC.
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ABSTRACT

Phytophthora nicotianae and *Ralstonia solanacearum* are two of the most important pathogens affecting tobacco worldwide. Greater insight regarding genetic systems controlling resistance to these two soilborne pathogens, as well as identification of DNA markers associated with genomic regions controlling this resistance, could aid in variety development. An evaluation of 50 historical tobacco lines revealed a high positive correlation between resistances to the two pathogens, preliminarily suggesting that some genomic regions may confer resistance to both pathogens. A quantitative trait loci (QTL) mapping experiment designed to investigate the genetic control of soilborne disease resistance of highly resistant ‘K346’ tobacco identified four QTL significantly associated with resistance to *P. nicotianae* (explaining

60.0% of the observed phenotypic variation) and three QTL to be associated with *R. solanacearum* resistance (explaining 50.3% of the observed variation). The two QTL with the largest effect on *Phytophthora* resistance were also found to be the QTL with the greatest effects on resistance to *Ralstonia*. This finding partially explains previously observed associations between resistances to these two pathogens among U.S. current cultivars and within breeding populations. Further study is needed to determine whether these relationships are due to the same genes (i.e., pleiotropy) or favorable coupling-phase linkages that have been established over time.

Additional keywords: bacterial wilt, black shank, *Nicotiana*.

Black shank and bacterial wilt, caused by the soilborne pathogens *Phytophthora nicotianae* and *Ralstonia solanacearum*, respectively, are the most important diseases affecting tobacco production in many parts of the world. Strategies used to reduce economic loss from both diseases include crop rotation, chemical inputs, and the use of genetic resistance. Genetic resistance is usually the most cost-effective component of integrated disease management systems if it can be incorporated into cultivars without adversely affecting yield or cured leaf quality.

A number of sources of genetic resistance to black shank have been investigated but no known source provides complete resistance to all races of the black shank pathogen. Monogenic resistance conferred by the *Php* and *Phl* genes has been introgressed from *Nicotiana plumbaginifolia* (Apple 1962; Chaplin 1962) and *N. longiflora* (Valleau et al. 1960), respectively. These genes provide immunity only to race 0 of *P. nicotianae*. Very high levels of resistance to both race 0 and race 1 of the black shank pathogen have also been associated with an introgressed *N. rustica* region designated as *Wz* (Drake and Lewis 2013; Drake et al. 2015). Widespread adoption of varieties containing either *Php* or *Phl* has been shown to lead to pathogen race shifts and the ultimate predominance of alternative races (Sullivan et al. 2005). It is possible that *P. nicotianae* populations may also rapidly adapt to *Wz* (K. L. McCorkle, K. E. Drake-Stowe, R. S. Lewis, and H. D. Shew, unpublished data). Because of low durability of monogenic resistance mechanisms, tobacco breeders should focus on combining monogenic resistance with medium to high levels of polygenic resistance, which tends to be more durable over time.

Based on historical pedigree information, most polygenic black shank resistance in flue-cured and burley tobacco is believed to have

been derived from ‘Florida 301’ cigar tobacco (Tisdale 1931). However, no comparison has been made between black shank resistance quantitative trait loci (QTL) discovered to control resistance in Florida 301 and those found to influence resistance in modern U.S. flue-cured tobacco. It is known, however, that this type of resistance is of the classic polygenic type, where resistance is partial, controlled by multiple genes, and nonrace specific (Chaplin 1966; Smith and Clayton 1948; Xiao et al. 2013).

Considerably less is known about genetic resistance of *N. tabacum* to bacterial wilt. This is likely due to greater sensitivity of *R. solanacearum* to environmental conditions and also large differences in pathogenic potential of various strains (Katawczik and Mila 2012). Strains of *R. solanacearum* isolated from tobacco and tomato across the southeastern United States have been classified as race 1, biovar 1, and belong to phylotype II (Hong et al. 2012; Katawczik et al. 2016). Resistance from *N. tabacum* germplasm accession TI 448A has been described as polygenic in nature and is reported to be the primary source of bacterial wilt resistance incorporated into U.S. flue-cured tobacco cultivars (Smith and Clayton 1948; Valleau 1952). The *Rps* and *Rxa* genes have also been described and reportedly utilized by Japanese tobacco breeders (Matsuda 1977; Matsuda and Ohashi 1973), although the system used to identify and declare the presence of these genes is not clear.

There is anecdotal evidence of a correlation between genetic resistance to black shank and bacterial wilt in tobacco, because many flue-cured commercial cultivars that exhibit high levels of resistance to black shank also display medium to high levels of resistance to bacterial wilt, and vice versa (North Carolina Cooperative Extension 2015). Taken alone, these observations do not indicate that the same genes or genomic regions controlling resistance to one disease also affect resistance to the second disease. Such a genetic relationship is possible, however, and the subject of plant genomic regions controlling multiple disease resistance (MDR) has recently become of increased interest (Wiesner-Hanks and Nelson 2016).

†Corresponding author: R. S. Lewis; E-mail: ramsey_lewis@ncsu.edu

*The e-Xtra logo stands for “electronic extra” and indicates that one supplementary figure is published online.

Mapping of QTL can be used to identify *N. tabacum* genomic regions that influence resistance to both *P. nicotianae* and *R. solanacearum*. Previous mapping studies have identified QTL controlling black shank resistance in highly resistant ‘Beinhart 1000’ (Vontimitta and Lewis 2012a,b) and Florida 301 (Xiao et al. 2013) cigar tobacco. The objective of the current research was to further investigate the genetic basis of soilborne disease resistance in *N. tabacum*. First, a set of 50 tobacco lines of historical breeding importance was evaluated in replicated testing over multiple environments for resistance to both black shank and bacterial wilt to gain preliminary insight into the correlation between these two traits. Second, we studied the correlation between resistance to these two diseases using a recombinant inbred line (RIL) population derived from a cross between disease-susceptible tobacco accession ‘TI 1068’ and ‘K346,’ a representative of U.S. flue-cured tobacco cultivars that exhibits very high levels of resistance to both black shank and bacterial wilt. Comparison of QTL identified for resistance to each disease was used to identify genomic regions carrying alleles that influence multiple disease resistance. Finally, we determined the extent to which QTL significantly associated with black shank resistance in modern K346 tobacco overlap with those previously identified to control resistance in Florida 301, the reported source of polygenic black shank resistance in modern U.S. tobacco. Comparisons may be of value in connection with future work to exploit cigar tobacco germplasm as a resource for black shank resistance in flue-cured and burley tobacco.

MATERIALS AND METHODS

Genetic materials and disease evaluations. A set of 50 tobacco genotypes was selected to study the phenotypic ranges and correlations for resistance to black shank and bacterial wilt (Table 1). The set of lines included Florida 301 and ‘TI 448A’, the reported sources of soilborne disease resistance in flue-cured tobacco. Eight old-line cultivars were selected to represent flue-cured tobacco germplasm prior to the initiation of science-based plant breeding to improve soilborne disease resistance. Also included were 39 flue-cured tobacco varieties of historical importance that represented decades of tobacco improvement from the 1940s to the 1990s. No hybrids were included and no varieties carrying the *Php* gene were included, because the primary interest was to study polygenic resistance. Finally, Beinhart 1000 was included in the set of 50 lines as a genotype that exhibits exceptionally high levels of polygenic resistance to black shank. These 50 lines were evaluated for black shank resistance at two North Carolina locations naturally infested with the black shank pathogen (Lower Coastal Plain Research Station, Kinston, NC and Upper Coastal Plain Research Station, Rocky Mount, NC) in 2010 and 2011 (for a total of four environments). The set of 50 lines was also evaluated for bacterial wilt resistance in a disease nursery at the Upper Coastal Research Station during 2014 and 2015; a bacterial wilt field nursery at the Pee Dee Research and Education Center at Florence, SC during 2015; and in a naturally infested private farm field near Selma, NC during 2015 (for a total of four environments). The experimental design for each environment was a randomized complete block design with three replications. Experimental units consisted of single 22-plant plots, each 14 m in length. Starting at approximately 40 days after transplanting, the number of plants killed by black shank or bacterial wilt was recorded. This continued throughout the growing season at intervals of approximately 14 days. At the end of the growing season, area under the disease progress curve (AUDPC) was calculated for each plot using the trapezoidal method of Madden et al. (2007).

A population consisting of 180 F_{4:5} RILs was generated from a cross between highly bacterial wilt- and black-shank-resistant flue-cured tobacco cultivar K346 and disease-susceptible germplasm accession ‘TI 1068’. The RIL population and parental lines were evaluated for black shank incidence at the Lower Coastal Plain Research Station and Upper Coastal Plain Research Station during

2014 and 2015 (for a total of four environments). The population was also evaluated for bacterial wilt incidence in a disease nursery at the Upper Coastal Plain Research Station in 2014 and 2015 and in one naturally infested field on a private farm near Selma, NC, in 2015 (for a total of three environments). The experimental design for each environment was a 10-by-18 α -lattice with three replications. The two parents were also randomized within each block for a total of 20 entries per block. Experimental units consisted of 12-plant plots of 7 m in length. Starting at approximately 40 days after transplanting, the number of plants killed by black shank or bacterial wilt was recorded. This continued throughout the growing season at intervals of approximately 14 days. AUDPC was calculated for each plot at the end of the growing season according to Madden et al. (2007).

Statistical analysis. An analysis of variance for AUDPC was performed on the 50 historical lines using PROC MIXED of SAS 9.4 (SAS Institute Inc. 2013) for each disease separately. Genotype was treated as a fixed effect, whereas environment and replication were treated as random effects. Correlation coefficients between entry means for black shank and bacterial wilt AUDPC were calculated using PROC CORR of SAS 9.4.

A square-root transformation was first performed on the AUDPC data from the RIL population to reduce heterogeneity of error variances. ASReml version 3 (Gilmour et al. 2009) was then used to obtain a best linear unbiased prediction (BLUP) for each RIL across all location–year combinations for each disease. A mixed linear model was fit with random effects for RIL, environment, RIL–environment, replication within environment, and block within replication or environment. Variance components estimated from this model were used to calculate heritabilities on an entry mean basis per a univariate mixed-model analysis, as described by Holland et al. (2003). Subsequent QTL analyses were carried out using the BLUPs.

DNA marker genotyping. Leaf tissue samples were collected for the parental lines K346 and TI 1068 and from 10 plants from each F_{4:5} family and combined into single tubes. Genomic DNA was isolated using a modified cetyltrimethylammonium bromide procedure (Afandor et al. 1993; Johnson et al. 1995).

The two parental lines were initially genotyped using a total of 825 previously described primer pairs selected to amplify well-spaced microsatellite markers across each *N. tabacum* linkage group (LG) (Bindler et al. 2011). Polymerase chain reactions (PCR) were performed in 10- μ l volumes containing approximately 1 to 15 ng of template DNA, 6 μ l of True Allele PCR Premix (Life Technologies, Carlsbad, CA), and 10 μ M each primer. Reaction conditions consisted of 45 PCR cycles at an annealing temperature of 55°C, according to Bindler et al. (2011). Forward primers were labeled with FAM, HEX, or AT550 for fragment analysis on an Applied Biosystems 3130xL Genetic Analyzer (Life Technologies). Samples containing 1 μ l of PCR product for each marker, 1 μ l of Gene Scan 400 HD ROX dye standard (Life Technologies), and 9 μ l of Hi-Di formamide (Life Technologies) were denatured for 5 min at 95°C. Fragment analysis was performed as a multiloading assay simultaneously analyzing up to three markers labeled with different dyes. Alleles were scored using the GeneMapper 5.0 software package (Life Technologies). Markers identified to be polymorphic between the parental lines were used to genotype the entire RIL population.

Linkage map construction and QTL identification. A linkage map was created with R/qtl (Broman et al. 2003). Heterozygous markers were treated as missing data. Markers with genotypes on less than 130 individuals were excluded from the analysis, as well as lines with genotypes for less than 50 markers. Markers were organized into roughly 24 LG based on a minimum logarithm of odds (LOD) score of 4.5. Marker orders and genetic distances were calculated with the Kosambi mapping function (Kosambi 1943) using ripple and a genotyping error probability of 0.0001. A final ripple was performed to ensure that the most likely marker order had been selected.

Following linkage map construction, QTL mapping was performed using phenotypic BLUPs for both black shank and bacterial wilt

resistance using Haley-Knott regression (Haley and Knott 1992) in R/qtl (Broman et al. 2003). QTL were selected using a forward/backward stepwise model selection approach with the function stepwiseqtl (Broman and Wu 2017). All pairwise interactions were considered but no higher-order interactions were allowed. QTL were identified using a penalized LOD score criterion with three penalties (main effects penalty, heavy penalty on interaction, and light penalty on interaction) (Manichaikul et al. 2009). Values for penalties were

calculated from 1,000 permutation results with scantwo. After the model was finalized, the effect of each QTL was estimated using fitqtl.

RESULTS

Analyses of historical lines. The set of materials outlined in Table 1 was evaluated for black shank resistance in a total of four environments and for bacterial wilt resistance in a total of four

TABLE 1. List of 50 lines of historical importance to flue-cured tobacco breeding that were evaluated for black shank and bacterial wilt resistance^a

Entry number	Cultivar	Year	Number or source	Pedigree
1	Florida 301	...	PI 552629	Big Cuba/Little Cuba
2	Beinhart 1000	...	...	Selection from Quin Diaz
3	TI 448A	...	TC 594	Columbian accession collected during 1936 expedition
4	Warne	FV	PI 552335	Farmer variety
5	Virginia Bright Leaf	FV	PI 552385	Farmer variety
6	Gold Dollar	FV	PI 552310	Farmer variety
7	Hicks Broadleaf	FV	PI 552397	Farmer variety
8	Jamaica	FV	PI 552315	Farmer variety
9	White Stem Orinoco	FV	PI 552337	Farmer variety
10	Yellow Special	FV	PI 552340	Harrison Special/Lizzard Tail
11	400	FV	PI 552341	A farmer selection likely from Harrison Special/Silk Leaf
12	Vesta 30	1940s	PI 552769	Parentage is a BS resistant strain, Warne, and Yellow Special
13	Vesta 64	1940s	PI 552772	[FLA 301/White Stem/White Stem/3/White Stem] × Yellow Special
14	Oxford 1	1942	PI 552320	FLA 301/Virg. Bright Leaf/Virg. Bright Leaf/3/Virg. Bright Leaf
15	Oxford 2	1942	PI 552321	FLA 301/Virg. Bright Leaf/Virg. Bright Leaf/3/Virg. Bright Leaf
16	Oxford 3	1942	PI 552322	FLA 301/White Stem/White Stem/3/White Stem
17	Yellow Special A	1943	PI 552378	Yellow Special/Yellow Mammoth
18	Virginia Gold	1947	PI 552334	Yellow Special/Cash
19	Oxford 1-181	1948	PI 552401	FLA 301/Virg. Bright Leaf/Virg. Bright Leaf/3/Virg. Bright Leaf
20	Golden Wilt	1949	PI 552393	Virginia Bright Leaf/TI 448A
21	White Gold	1949	PI 552400	Huggins Golden Yellow/402
22	Dixie Bright 101	1949	PI 552383	[TI 448A/400/Oxford 1] × [FLA 301/400/400]
23	Dixie Bright 102	1949	PI 552384	[TI 448A/400/Oxford 1] × [FLA 301/400/400]
24	Golden Cure	1950	PI 552390	Ducane/TI 706/Ducane/3/Ducane
25	Vesta 5	1957	PI 552396	Vesta 11/Oxford 26
26	Coker 139	1954	PI 552389	Coker Golden Cure/Oxford 1-181/Coker Golden Wilt/D. Bright 101
27	Dixie Bright 244	1955	PI 551300	Dixie Bright 101/Dixie Bright 102/Bottom Special
28	Coker 187	1956	PI 552391	Golden Wilt/Oxford 1-181
29	Coker 187-Hicks	1957	PI 552392	Coker 187/Hicks
30	SC 58	1959	PI 552344	Yellow Special X [FLA 301/Warne] × [400/TI 448A]
31	McNair 10	1960	PI 552422	McNair 12/Ox 1-181
32	Coker 319	1962	PI 552426	Coker 139/Hicks
33	McNair 20	1962	PI 552429	White Gold/224G//4*Hicks
34	McNair 30	1962	PI 552430	White Gold/224G//4*Hicks
35	Coker 298	1964	PI 552445	Coker 139/Coker 156
36	NC 2326	1964	PI 552453	Hicks/9102/Hicks/3/Hicks/4/Hicks
37	Virginia 115	1964	PI 552458	Hicks/Coker 139
38	Coker 258	1966	PI 552461	Coker 319/NC 95
39	Speight G-28	1969	PI 551318	Coker 139/Oxford 1-181/NC 95
40	McNair 135	1970	PI 551304	McNair 30/Speight G-10
41	McNair 944	1973	PI 552494	Speight G-10/McNair 30
42	NC 82	1979	PI 551311	6129/Coker 319
43	K326	1982	GLS	McNair 225/McNair 30/NC95
44	K 394	1984	PI 552525	Speight G-28/McNair 944
45	K149	1990	PI 549109	[Spt G28/Coker 354]X[CB-139XF-105] × [Spt G28/Coker 354]XMcNair 399
46	K346	1990	GLS	McNair 926/80241
47	NC 55	1996	GLS	K 326/DH 1220//K326/Coker 371-Gold
48	Oxford 207	1996	GLS	Coker 319/K 399
49	NC 606	1999	NCSU	NC 729/NC 82
50	NC 810	2001	NCSU	Oxford 2101/NC 729

^a Abbreviations: FV = farmer variety, GLS = Gold Leaf Seed Company, and NCSU = North Carolina State University.

environments. Analyses of variance for black shank and bacterial wilt resistance across all environments revealed highly significant differences ($P < 0.0001$) among the 50 entries. Black shank and bacterial wilt resistance for the 50 historical lines, as measured by AUDPC, were highly correlated measured characters; $r_{\text{Pearson}} = 0.85$ ($P < 0.0001$) (Fig. 1). The “farmer” varieties, as a group, were the most susceptible to both soilborne pathogens. Modern flue-cured tobacco cultivars K346 and ‘NC606’ displayed the best combinations of resistances to both black shank and bacterial wilt.

Analyses of RIL population. Phenotypic evaluation. The set of 180 RIL and their parental lines (K346 and TI 1068) were evaluated for black shank resistance in a total of four environments and for bacterial wilt resistance in a total of three environments. Mixed-model analyses revealed extremely significant differences among the RIL for resistance to both diseases ($P < 0.0001$). Entry mean heritabilities for black shank and bacterial wilt AUDPC were calculated to be $H = 0.95$ (standard error [SE] = 0.01) and $H = 0.65$ (SE = 0.05), respectively. A high genetic correlation between resistance to the diseases was observed ($r_g = 0.89$; $P < 0.0001$) (Fig. 2).

Genetic linkage map. Of the 825 microsatellite primer pairs tested, 258 (31.2%) were found to produce distinguishable, polymorphic alleles between K346 and TI 1068. In total, 252 markers were used for linkage map construction. The 252 markers were assigned to 24 LGs spanning a total length of 2,020 centimorgans (cM) (Supplementary Fig. S1). The number of markers on each LG ranged from 9 to 13, with an average of 10.5 markers per LG and an average spacing of 8.8 cM per marker. Marker order in this population was largely congruent with the high-density map published by Bindler et al. (2011). Exceptions included the reordering of some markers within an LG, smaller pairwise differences, and a reduced overall map length.

QTL identification in RIL population. Using Haley-Knott regression, the stepwise model selection approach selected a final model for black shank AUDPC that included four QTL located on LG 6, 7, 14, and 23, with no significant QTL–QTL interaction effects (Table 2; Fig. 3). The favorable allele at the QTL on LG 6, 7, and 23 was contributed by K346, whereas the favorable allele at the QTL on LG 14 was contributed by TI 1068. The final model for bacterial wilt AUDPC included three QTL located on LG 6, 7, and 19, also with no significant QTL–QTL interaction effects (Table 2;

Fig. 3). The favorable allele was contributed by K346 for all three QTL. QTL for both black shank and bacterial wilt AUDPC colocalized on the same regions on LG 6 and 7. The QTL on LG 7 explained 38.0 and 32.1% of the phenotypic variation for black shank and bacterial wilt AUDPC, respectively. The QTL on LG 6 explained 6.1 and 8.4% of the phenotypic variation for black shank and bacterial wilt AUDPC, respectively. Additional QTL affecting resistance to both diseases explained less than 5% of the total phenotypic variation. The final QTL model for black shank AUDPC explained 60.0% of the observed phenotypic variation, whereas the final QTL model for bacterial wilt AUDPC explained 50.3% of the observed phenotypic variation.

DISCUSSION

Previous anecdotal evidence suggested a possible genetic relationship between tobacco resistance to black shank and bacterial wilt, caused by the soilborne pathogens *P. nicotianae* and *R. solanacearum*, respectively. We were interested in investigating the genetic control of resistance to both of these diseases using QTL mapping and determining whether there was significant overlap in the genomic regions influencing resistance to each disease. To gain initial insight regarding this question, we evaluated a set of 50 tobacco lines of historical breeding importance. As compared with old-line farmer varieties, tested modern cultivars were found to be much more resistant to both pathogens, thus demonstrating the impact of directed hybridization and selection for disease resistance over decades of science-based tobacco breeding. Cultivars exhibiting high levels of resistance to black shank generally also displayed medium to high levels of resistance to bacterial wilt, and a correlation ($r_{\text{Pearson}} = 0.85$) was observed between resistance to both diseases. K346 and NC606 were found to be excellent examples of cultivars with high levels of resistance to both diseases. These findings provided initial support for the hypothesis that at least some tobacco genomic regions controlling resistance to black shank also contribute to bacterial wilt resistance. However, genome-wide genotypes were not available for the set of 50 lines, and some of the observed high correlation could possibly be due to population structure or kinship.

If pleiotropic effects were contributing factors to the strong genetic correlations described above, one would expect the two reported sources of resistance in K346 (Florida 301 for black shank resistance and TI 448A for bacterial wilt resistance) to exhibit some level of resistance to both diseases. As expected, Florida 301 exhibited very high field resistance to black shank but also showed a moderate level of resistance to bacterial wilt. In our experiments,

Fig. 1. Area under disease progress curve (AUDPC) means for black shank and bacterial wilt for 50 cultivars or breeding lines of historical breeding importance. Numbers correspond to entry numbers defined in Table 1. White circles correspond to farmer varieties that existed prior to the initiation of science-based tobacco breeding. Shaded circles represent varieties developed via directed hybridization.

Fig. 2. Area under disease progress curve (AUDPC) means for black shank and bacterial wilt for 180 recombinant inbred lines derived from a cross between K346 and TI 1068.

however, TI 448A exhibited only low to moderate levels of resistance to bacterial wilt, along with very low levels of resistance to black shank. Reasons for these observations may include (i) the *Ralstonia* populations are different today than they were in the 1940s when TI 448A was classified as resistant, (ii) TI 448A is not the source of bacterial wilt resistance in K346, (iii) the current seed source for TI 448A in the *Nicotiana* germplasm collection is not correct, or (iv) the high level of bacterial wilt resistance in varieties such as K346 is due to one or a few TI 448A-derived QTL coupled with a series of additional QTL derived from alternative source material.

To more thoroughly investigate the high level of genetic resistance to both black shank and bacterial wilt in flue-cured tobacco cultivar K346, a mapping population derived from hybridization between K346 and susceptible germplasm accession TI 1068 was studied. The mapping study identified four QTL on LG 6, 7, 14, and 23 associated with black shank resistance and three QTL on LG 6, 7, and 19 associated with resistance to bacterial wilt. The QTL on LG 6 and 7 affecting resistance to both diseases colocalized to the same genomic regions. The QTL identified on LG 7 had the greatest effect and controlled the largest percentage of phenotypic variation for both black shank (38.0%) and bacterial wilt (32.1%) resistance. This QTL was also found to have the largest effect on and to control the largest percentage of variation for black shank resistance in a Florida 301 × ‘Hicks’ RIL population (16.9%) (Xiao et al. 2013) (Table 2) and a Beinhart 1000 × Hicks doubled-haploid population (25.4%) (Vontimitta and Lewis 2012b). The number of shared markers between the three studies was limited, making comparison of the location of the associated region somewhat challenging. In both the K346- and Beinhart 1000-derived populations, however, the interval for the QTL was positioned over marker locus PT61472. In the Beinhart 1000- and Florida 301-derived populations, the interval was positioned over marker locus PT30174, suggesting a role for the same region in each population. The identification of this QTL in a third population provides further validation for the position and importance of allelic variation in this region in black shank resistance in *N. tabacum*. Given the confirmed importance of the QTL on LG 7 for black shank resistance in combination with newfound importance it has on bacterial wilt resistance, fine mapping this interval for resistance to both soilborne diseases would be valuable for moving in the direction of the identification of the genes underlying this QTL. Indeed, this work is already underway (Ma 2017) and should aid in the transfer of soilborne disease resistance to other cultivars and provide insight regarding the question of linkage versus pleiotropy as reasons contributing to the correlation between the two traits.

The QTL on LG 6 had an intermediate effect for both diseases, explaining 6.1 and 8.1% of the phenotypic variation in black shank and bacterial wilt, respectively. A QTL on LG 6 was also found to

have an intermediate effect on bacterial wilt resistance (5.4%) in a ‘Yanyan 97’ (which has TI 448A in its pedigree) × ‘Honghua Dajinyuan’ F_{2:3} mapping population (Lan et al. 2014), and to have an intermediate effect on black shank resistance (7.1%) in a Florida 301 × Hicks RIL mapping population (Xiao et al. 2013). The fact that few markers were shared between these studies again makes comparison difficult but the confidence interval for this QTL was positioned over marker TM10089 in both the Yanyan 97 and Florida 301 populations, strengthening the evidence for the importance of this region for both black shank and bacterial wilt resistance.

Surprisingly, a QTL on LG 24 was identified in both the Florida 301 and Beinhart 1000 black shank resistance mapping studies as well as in the Yanyan 97 bacterial wilt resistance mapping study but not in the current K346 mapping study. Limited overlapping markers make it difficult to determine whether the same region on LG 24 was identified in all three studies but this seems likely. K346 is representative of modern flue-cured tobacco cultivars possessing high levels of polygenic resistance to multiple soilborne pathogens. Because this QTL was not identified in the K346 mapping population (Table 2), it suggests that this region may not have been incorporated in modern U.S. cultivars. High-density genotyping of the 50 historical lines in the genomic regions of interest would help confirm this speculation. If modern varieties do not carry an incorporated Florida 301 region at this genomic location, it could be utilized as a novel source of resistance to both black shank and bacterial wilt. Markers associated with this region could be useful in transferring this resistance to desired genetic backgrounds. Given the low to moderate effect associated with this QTL in the other populations, it would be most useful in backgrounds that already possess the QTL on LG 6 and 7.

The finding that the two largest QTL affecting resistance to black shank in the K346 × TI 1068 population overlapped almost perfectly with the two QTL with greatest effects against bacterial wilt in the same population is interesting. Further study is necessary to determine whether this relationship is due to the same genes affecting resistance to both diseases (i.e., pleiotropy) or due to the result of favorable coupling phase linkages that were produced via selective breeding. The concept of MDR has recently been reviewed by Wiesner-Hanks and Nelson (2016). Lines exhibiting MDR could contain many genes acting independently to confer resistance, or single genes conferring resistance to multiple pathogens. Single genomic regions and single genes conferring resistance to multiple plant pests have recently been identified. For example, the qualitative *Mi-1* gene of tomato confers resistance to both the root-knot nematode and potato aphid (Rossi et al. 1998), and the *RPS1* and *RPS4* *Arabidopsis* genes work together to confer resistance to both fungal and bacterial pathogens (*Colletotrichum higginsianum*, *Pseudomonas syringae*, and *R. solanacearum*) (Narusaka et al. 2009). Quantitative resistance loci associated with MDR have also been reported in crops

TABLE 2. Summary of quantitative trait loci results for evaluation of tobacco mapping populations for soilborne disease resistance

Linkage group ^a	K346 × TI1068 recombinant inbred line mapping population												Florida 301 × Hicks mapping population ^c					
	Black shank resistance						Bacterial wilt resistance						Black shank resistance					
	Left marker	Right marker	cM	Effect (sqAUDPC) ^b	LOD	R ²	Left marker	Right marker	cM	Effect (sqAUDPC)	LOD	R ²	Left marker	Right marker	cM	Effect (AUDPC)	LOD	R ²
7	PT61472	PT52970	6.6	−0.83 ^d	25.4	38.0	PT61472	PT52970	6.6	−0.45	19.1	32.1	PT30174	PT30165	62.0	−45.09	8.79	16.9
24													TME0466	PT60308	9.5	−32.09	3.52	9.2
6	PT61581	PT50885	71.0	−0.42	5.4	6.1	PT61581	PT50885	69.0	−0.28	6.0	8.4	TM10470	TM10922	67.4	−28.24	4.19	7.1
24													PT51264	PT61153	37.3	−26.29	2.60	6.1
19							PT60099	PT53353	87.7	−0.18	4.0	5.5						
14	PT53339	PT53687	60.8	0.29	4.4	4.4							TM10552	TM10983	4.9	−28.05	3.17	6.0
4													TM10821	TM10914	83.8	−24.96	2.79	4.2
23	PT54270b	PT50062	0.0	−0.28	4.0	4.0												
22													TM10368	TM11161	33.6	−22.69	2.49	3.7
20													TME0324a	TM10386	15.1	−25.30	3.21	4.3

^a Linkage groups correspond to those published by Bindler et al. (2011).

^b sqAUDPC indicates the effect for square root-transformed area under the disease progress curve data.

^c Data from Xiao et al. (2013).

^d Negative effect indicates that the favorable allele was derived from K346 or Florida 301.

such as rice, where overlapping QTL affecting resistance to rice blast, sheath blight, and bacterial blight were detected on the same chromosome segment (Wisser et al. 2005). In addition, multiple genomic regions were found to be associated with gray leaf spot, northern leaf blight, and southern leaf blight in maize (Wisser et al. 2011; Zwonitzer et al. 2010). The marker-trait associations identified here, coupled with the recently published increased-quality *N. tabacum* genome sequence (Edwards et al. 2017), should be useful for further studies to determine whether the overlap of bacterial wilt and black shank resistance QTL on *N. tabacum* LG 6 and 7 is due to genes with pleiotropic effects or due to favorable coupling phase genetic linkages that were generated via selective breeding.

The data reported here describe a polygenic system contributing to the high levels of field resistance to bacterial wilt in K346 flue-cured tobacco. Bacterial wilt resistance in the Solanaceae family has been most thoroughly investigated in tomato, where resistance appears to be controlled in a manner similar to that in tobacco. In tomato, bacterial wilt resistance is largely quantitative in nature, with many QTL mapped and no major resistance genes identified (Thoquet et al. 1996a,b; Wang et al. 2000, 2013; Young and Danesh 1994). Both strain-specific and broad-spectrum QTL have been

identified. A major broad-spectrum QTL on tomato chromosome 6 was identified that is effective against both phyto-type I and II strains, and the local genomic region is a hot spot for disease resistance genes (Carmeille et al. 2006; Wang et al. 2013). Resistance genes in this region include *Mi* for root-knot nematode and potato aphid resistance, *Cf2* and *Cf5* for *Cladosporium fulvum* (tomato leaf mold) resistance, *Ol-1* for *Oidium lycopersicon* (powdery mildew) resistance, *Ty-1* for *Tomato yellow leaf curl virus* resistance, and *Am* for *Alfalfa mosaic virus* resistance (Kim et al. 2016).

Development of tobacco cultivars with high levels of resistance to root diseases and soilborne pathogens while maintaining yield and quality is a primary objective of tobacco breeding programs. Results from the current study provide a greater understanding of the genetic control of polygenic black shank and bacterial wilt resistance in K346 tobacco, as well as a greater understanding of resistance to major soilborne pathogens available in tobacco germplasm as a whole. The identification of regions associated with resistance, and molecular markers associated with those regions, will hopefully aid in the development of new tobacco varieties that have high levels of soilborne disease resistance coupled with acceptable yield and quality. This information should also contribute to future efforts to identify genes

Fig. 3. Logarithm of odds score peaks for linkage groups (LG) found to be significantly associated with resistance to bacterial wilt and black shank. Solid and dashed lines relate to data for black shank and bacterial wilt, respectively.

contributing to high levels of partial resistance to soilborne pathogens in *N. tabacum*.

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