Effect of a Chromosome Segment Marked by the Ph_p Gene for Resistance to *Phytophthora nicotianae* on Reproduction of Tobacco Cyst Nematodes

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ABSTRACT

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Host resistance is an important strategy for managing *Globodera tabacum* subsp. *solanacearum* and *G. tabacum* subsp. *tabacum*, important nematode pests of flue-cured tobacco (*Nicotiana tabacum*) in Virginia, and cigar wrapper tobacco (*N. tabacum*) in Connecticut and Massachusetts, respectively. Field research from 1992 to 2005 evaluated reproduction of *G. tabacum* subsp. *solanacearum* on genotypes with and without a chromosome segment from *N. plumbaginifolia* containing a gene (Ph_p) that conferred resistance to race 0 of *Phytophthora nicotianae* (causal agent of tobacco black shank). Ratios of *G. tabacum* subsp. *solanacearum* eggs/500 cm^3 soil at the end versus the beginning of experiments (P_f/P_i) were significantly lower in cultivars and breeding lines possessing the Ph_p-containing chromosome segment from *N. plumbaginifolia* compared with genotypes without the segment. Numbers of vermiform *G. tabacum* subsp. *solanacearum* juveniles in roots were similar among genotypes but numbers of swollen and pyriform nematodes were significantly lower for the known *G. tabacum* subsp. *solanacearum*-resistant cv. NC 567 and in genotypes possessing the Ph_p gene compared with genotypes and cultivars without the gene. In a 2003 greenhouse test, the percentage of plants with visible *G. tabacum* subsp. *tabacum* cysts was also significantly lower for parental and progeny genotypes homozygous and heterozygous, respectively, for Ph_p compared with similar lines without the gene. These results indicate a close linkage or association between a likely single, dominant gene (Ph_p) for resistance to *P. nicotianae* and suppressed reproduction by *G. tabacum* subsp. *solanacearum* and *G. tabacum* subsp. *tabacum*. Further research to accurately elucidate the relationships among these genes could lead to significant improvements in tobacco disease control.

The tobacco cyst nematode species complex is comprised of three subspecies: *Globodera tabacum* subsp. *tabacum* ((Lownsbery & Lownsbery 1954) Behrens 1975), *G. tabacum* subsp. *virginiae* ((Miller & Gray 1968) Behrens 1975), and *G. tabacum* subsp. *solanacearum* ((Miller & Gray 1972) Behrens 1975) (16). Although *G. tabacum* subsp. *virginiae* has been reported primarily from horsenettle (*Solanum carolinense* L.), *G. tabacum* subsp. *tabacum* and *G. tabacum* subsp. *solanacearum* are important pathogens of shade and broadleaf tobacco (*Nicotiana tabacum* L.) in Connecticut, and flue-cured tobacco in Virginia, respectively. Members of the tobacco cyst nematode complex have been reported from over a dozen tobacco-producing countries in North and South America, Europe, North Africa, and Asia; however, few of these reports indicate the subspecies present (16). Yield losses caused by *G. tabacum* subsp. *tabacum* can range from 5 to 45% on shade tobacco (24). Losses in flue-cured tobacco yield due to *G. tabacum* subsp. *solanacearum* may average 15%, but complete crop failures have also occurred (15). Tobacco producers with tobacco cyst nematode-infested fields routinely apply nematicides because the relatively high survival rate of the nematodes requires crop rotation intervals too long to be economically viable.

Host resistance is an attractive strategy for managing *G. tabacum* subsp. *solanacearum* and *G. tabacum* subsp. *tabacum* given the relatively high survival rate of the nematodes and the increasing costs and restrictions on effective nematicides. Resistance to *G. tabacum* subsp. *solanacearum* has been identified in at least nine wild *Nicotiana* spp., including *Nicotiana glutinosa*, *N. longiflora*, and *N. plumbaginifolia*, and in numerous tobacco accessions and cultivars (1,16). Although several shade tobacco cultivars have been developed with resistance to *G. tabacum* subsp. *tabacum* derived originally from *N. longiflora* (23), all currently available flue-cured tobacco cultivars subsequently identified as *G. tabacum* subsp. *solanacearum*-resistant were originally developed for resistance to other tobacco pathogens, such as *Tobacco mosaic virus* (from *N. glutinosa*) and *Pseudomonas syringae* pv. *syringae* (from *N. longiflora*) (13,14).

High levels of resistance to race 0 of *Phytophthora nicotianae* (causal agent of tobacco black shank) have been incorporated into flue-cured tobacco cultivars since the release of flue-cured tobacco cv. Coker 371-Gold (C371G) in 1986. The source of this resistance has been identified as a gene (Ph_p) located on a chromosomal segment originally transferred interspecifically from *N. plumbaginifolia* (6,17). Crowder et al. (9) concluded that C371G also possessed an apparent single dominant gene for resistance to *G. tabacum* subsp. *solanacearum*. The objective of the work presented here was to investigate the possibility of an association between the chromosomal segment containing the single, dominant gene (Ph_p) for resistance to *P. nicotianae* and suppressed reproduction of *G. tabacum* subsp. *solanacearum* and *G. tabacum* subsp. *tabacum*.

MATERIALS AND METHODS

General approaches. A field experiment to evaluate resistance to *G. tabacum* subsp. *solanacearum* was conducted each year in 1992–94, 1998–99, and 2001–05 in *G. tabacum* subsp. *solanacearum*-infested fields at the Southern Piedmont Agricultural Research and Extension Center (SPAREC) near Blackstone, VA. Unless otherwise indicated, all SPAREC experiments were arranged in a randomized complete block design with four replications, and field plots consisted of single 16.1-m-long rows spaced 1.2 m apart and containing approximately 24 plants. A split-plot design was also used in some experiments in order to compare yield and quality traits when cultivars were challenged by reduced versus large nematode populations. Additional trials were conducted in 2001 in four commercial flue-cured tobacco fields in south-central Virginia. All plots were mechanically transplanted and fertilized, and other cultural and pest control practices in all field experiments conformed to recommendations of Virginia Cooperative Extension (26). Additional greenhouse experiments were

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conducted in Connecticut in 2002 to evaluate whether the relationship between resistance to *G. tabacum* subsp. *solanacearum* and *P. nicotianae* also included resistance to *G. tabacum* subsp. *tabacum*.

Nematode data collection. Initial nematode population densities were monitored in each plot by collecting soil samples prior to transplanting, and are reported in each table of this article. Final soil samples were collected at the end of the growing season, except in 2002 and 2005, when follow-up samples were collected on 22 August or 28 July, respectively. At least 24 2-cm-diameter by 16-cm-long soil cores were bulked from each plot to form the plot sample at each sample date. *G. tabacum* subsp. *solanacearum* soil population densities were estimated by extracting cysts from 250 cm^3 of air-dried soil using a modified Fenwick can (7), but are reported in this article on a 500-cm^3 basis to be consistent with Virginia Cooperative Extension nematode assay results. Eggs were released from extracted cysts using a blender for 1 min, stained with 0.005% acid fuchsin (4), and counted at ×20 magnification. Root parasitism by *G. tabacum* subsp. *solanacearum* was monitored in 1994, 1998, and 2002 SPAREC experiments by randomly selecting a single representative plant from each field plot row, excavating the plant, gently washing soil from around plant roots, and randomly selecting a 1-g (fresh weight) subsample from the small, fibrous "feeder roots" of each sampled plant. Feeder root subsamples were stained with 0.007% acid fuchsin to assist nematode identification (5). Nematodes in roots were counted and assigned to one of four classes based on body shape under ×40 magnification as follows: (a) vermiform, (b) swollen, (c) flask-shaped or pyriform, and (d) saccate females bearing eggs (adult). Nematode count data were $\log_{10}$ transformed prior to analysis of variance because the data frequently failed Bartlett's test for homogeneity of error variance (2,25,28). Differences among three or more means were evaluated using the Waller-Duncan *k*-ratio *t* test, *k*-ratio = 100 (SAS: SAS Institute, Cary, NC; Agriculture Research Manager, Gylling Data Management, Inc., Brookings, SD).

Field cultivar screening trials for *G. tabacum* subsp. *solanacearum* resistance. Initial small-plot field experiments were conducted in 1992 and 1993 at SPAREC to screen released flue-cured tobacco cultivars for resistance and tolerance to *G. tabacum* subsp. *solanacearum*. Experiments were laid out in a split-plot experimental design with four replications. Main-plot treatments involved broadcast, preplant-incorporated application of fosthiazate (Nemathorin; Syngenta International AG, Basel, Switzerland) at 7.5 or 6.7 liters/ha in 1992 and 1993, respectively, or a nontreated control. Subplot treatments consisted of single 16.1-m-long rows, spaced 1.2 m apart, and containing approximately 24 plants for each of nine *G. tabacum* subsp. *solanacearum*-susceptible flue-cured tobacco cultivars without the Ph_p *gene* (NC 27NF, NC 37NF, K 394, Reams 134, Reams 158, Coker 319, McNair 944, VA 116, and K 326) and C371G, now known to be homozygous for the Ph_p gene (Ph_pPh_p) (17). Nematode population densities were estimated based on soil samples collected before transplanting (4 and 10 May in 1992 and 1993, respectively) and after final harvest (30 September and 18 October in 1992 and 1993, respectively). Plant samples were excavated from each plot row 44 days after transplanting in a 1994 SPAREC field test to evaluate relative *G. tabacum* subsp. *solanacearum* parasitism of susceptible flue-cured tobacco cv. K 326, known *G. tabacum* subsp. *solanacearum*-resistant cv. NC 567, and C371G (apparently *G. tabacum* subsp. *solanacearum*-resistant, based on 1992 and 1993 results).

Field screening trials were conducted at SPAREC in 1998 and 1999 to compare *G. tabacum* subsp. *solanacearum* parasitism of several newly released hybrid cultivars heterozygous for Ph_p (Ph_pph_p) to that of inbred lines either homozygous for Ph_p or without the gene (ph_pph_p). The specific parents of these hybrids is proprietary information, and was not known. The 1998 experiment was arranged using a randomized complete block design with four replications, while a split-plot experimental design was used in 1999, where main plots consisted of no nematicide or preplant row-fumigation with 1,3-dichloropropene at 84.2 liters/ha (1,3-D or Telone II; Dow AgroSciences LLC, Indianapolis, IN). Cultivar treatments in both years consisted of single 16.1-m-long rows, spaced 1.2 m apart, containing approximately 24 plants of flue-cured tobacco cvs. NC 71 or NC 72 (heterozygous for Ph_p), C371G or SP 168 (homozygous for Ph_p), or K 326, OX 207, SP NF3, NC 567, or SP 172 (without the gene). Plant samples were excavated from each plot in the 1998 test on 7 June, 17 days after transplanting.

Release of additional hybrid cultivars possessing the Ph_p gene prompted further field screening trials at four on-farm locations in 2001 and at SPAREC in 2001 and 2003. All 2001 and 2003 studies were arranged in split-plot experimental designs using nematicide treatment (none versus preplant row-fumigation with 1,3-D at 84.2 liters/ha at SPAREC, Jennings, and Parrish locations; and none versus Temik 15G at 22.4 kg/ha [aldicarb; Bayer CropScience LP, Research Triangle Park, NC] at the Warren and Wright locations) as main plots. Subplot treatments in all tests included Ph_pph_p hybrid cultivars (NC 71, NC 297, RG H51, and SP H20), resistant Ph_pPh_p inbred cv. SP 168, and susceptible ph_pph_p cv. K 326. In 2001, C 319 (ph_pph_p) was included only at the Wright farm, and GL 973 (Ph_pph_p) was not included at the Warren and Wright farms. Flue-cured tobacco cv. NC 810 (Ph_pph_p) was included in the 2001 and 2003 SPAREC tests, but SP 179 (Ph_pph_p), SP 190 (ph_pph_p), and SP 210 (ph_pph_p) were included only in the 2001 study, while NC291 (Ph_pph_p) and GL 737 (Ph_pph_p) were only present in the 2003 experiment. Subplots at each farm site consisted of four rows spaced 1.2 m apart. Plot lengths were 86.9 m at the Jennings site and 57.9 to 83.8 m, 53.3 m, and 82.3 m at the Parrish, Warren, and Wright farms, respectively.

Field testing of doubled haploid progeny lines against *G. tabacum* subsp. *solanacearum*. A 2002 experiment conducted at SPAREC sought to more closely examine the influence of the chromosome segment containing the Ph_p gene on *G. tabacum* subsp. *solanacearum* reproduction by evaluating *G. tabacum* subsp. *solanacearum* parasitism and reproduction on parents and doubled haploid (DH) progeny lines from four different crosses between a flue-cured tobacco parent that contained the Ph_p gene and one that did not. The 2002 SPAREC field study included the resistant parent and a set of nine randomly selected DH progeny lines previously determined to be homozygous for the Ph_p gene (17,18) for each of the four crosses, along with the susceptible parent and nine randomly selected DH progeny lines previously determined to not possess the Ph_p gene (18). The F_1 hybrids were pollinated with *N. africana* Merxmüller to produce haploid plants (3), which were subsequently chromosome doubled to produce the DH progeny lines tested. Cross I resulted from hybridization of NCTG 61 (Ph_pPh_p) with flue-cured tobacco cv. K 326 (ph_pph_p), cross II from hybridizing DH92-3397-13 (Ph_pPh_p) with flue-cured tobacco cv. K 346 (ph_pph_p), cross III from hybridizing DH92-3397-2 (Ph_pPh_p) with K 326, and cross IV from hybridizing DH92-2770-40 (Ph_pPh_p) with K 326. Each line was planted in a randomly assigned location in a *G. tabacum* subsp. *solanacearum*-infested field. Due to the large number of root counts to be performed, data were replicated in time. A single plant was excavated from each plot on 22 July, 25 July, 7 August, and 22 August; 61, 64, 77, and 92 days after transplanting, respectively.

Additional field screening trials were also conducted at SPAREC from 2003 through 2005 to evaluate *G. tabacum* subsp. *solanacearum* reproduction on hybrids and DH entries possessing combinations of genetic factors conditioning resistance to races 1 and 3 of *Meloidogyne incognita* (the *Rk1* gene); and genetic factors (designated *Rk*2) isolated from a South African tobacco line (NOD 30) that may provide resistance to races 2 and 4 of *M. incognita*, *M. javanica*, race 2 of *M.*

arenaria, and the Ph_p gene. These experiments were arranged using a randomized complete block design with four replications.

Greenhouse testing of DH progeny lines against *G. tabacum* subsp. *tabacum*. The DH progeny lines from the same four hybridization crosses were also tested for resistance to *G. tabacum* subsp. *tabacum* in a greenhouse experiment conducted at the Valley Laboratory in Windsor, CT in 2002. Sixteen plants of each line were seeded in Sunshine 3 plant growth media (SunGro Horticulture, Bellevue WA) in 128-cell trays on 19 March and 8 April. Known susceptible plants (Connecticut broadleaf cv. C9) were also grown as positive controls. Second-stage juveniles and juveniles in eggs were obtained from previously collected *G. tabacum* subsp. *tabacum* cysts produced on roots of tobacco and kept air dried in the laboratory. Cysts were hydrated in tap water in a glass test tube for 24 h to soften cyst walls, then crushed with a ceramic tissue macerator in glass test tubes to release eggs and juveniles. Suspended eggs and juveniles were passed through a 180-µm sieve to remove cyst walls and collected on a 38-µm sieve. Volume was adjusted to 2,000 second-stage juveniles and juveniles in eggs per milliliter, and 1 ml of the inoculum suspension was added to each of two 0.75-cm-diameter holes in the potting media per plant, on opposite sides of the plant. Therefore, each greenhouse plant was inoculated with 4,000 hatched and unhatched second-stage *G. tabacum* subsp. *tabacum* juveniles 8 weeks after seeding. Six weeks after inoculation, when white females were visible on the roots of positive control plants, plants were rated and those with no females visible on the outside of the root ball were scored as resistant, while those with one or more white females were considered susceptible. Percentage data were subjected to the arcsine transformation prior to analysis of variance (ANOVA) to homogenize error variance (28) (NCSS; Number Cruncher Statistical Systems, Kaysville, UT). Differences in nematode reproduction between the two parental lines of each cross, as well as between DH progeny lines with or without the Ph_p gene within each cross, were indicated by the results from the ANOVA.

RESULTS

Field cultivar screening trials. Application of a nematicide did not influence relative differences in *G. tabacum* subsp. *solanacearum* reproductive ratios among cultivars in 1992 or 1993 (i.e., interaction effects between nematicide application and cultivars were not statistically significant). Consequently, mean *G. tabacum* subsp. *solanacearum* reproductive ratios could be directly compared among cultivars in the split-plot analysis, using results from both nematicide-treated and nontreated soil (28). Reproductive ratios for *G. tabacum* subsp. *solanacearum* on flue-cured tobacco cv. Coker 371-Gold (C371G) were significantly lower (Waller-Duncan *k*-ratio = 100) than those on all other cultivars except Rms 158 in 1992 and compared with the standard susceptible cv. K 326 in 1993 (Table 1). In the 1994 field experiment, numbers of vermiform *G. tabacum* subsp. *solanacearum* juveniles in roots were not significantly different among C371G, K326, and *G. tabacum* subsp. *solanacearum*-resistant cv. NC567, but fewer swollen, pyriform, and adult *G. tabacum* subsp. *solanacearum* nematodes

Table 2. Parasitism of nine flue-cured tobacco cultivars by *Globodera tabacum* subsp. *solanacearum* at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA on 29 June 1994 and 7 June 1998[x]

		Nematodes/g fresh weight of feeder roots[y]			
Cultivar	Ph_p genotype[z]	Vermiform	Swollen	Pyriform	Adult
1994					
K 326	ph_pph_p	1,498 a	321 a	178 a	34 a
NC 567	ph_pph_p	751 a	30 b	2 c	1 b
C 371G	Ph_pPh_p	795 a	12 b	7 b	2 b
1998					
K 326	ph_pph_p	6.25 ab	3.25 ab	10.50 ab	28.75 a
OX 207	ph_pph_p	6.50 a	5.25 a	9.25 a	42.25 a
SP NF3	ph_pph_p	1.50 ab	1.50 ab	2.25 bc	30.50 a
NC 567	ph_pph_p	1.25 ab	1.25 ab	0.25 c	24.75 a
SP 172	ph_pph_p	3.25 ab	1.75 ab	3.00 ab	15.25 a
NC 71	Ph_pph_p	4.75 ab	0.75 b	2.00 bc	15.00 a
NC 72	Ph_pph_p	0.25 b	0.25 b	0.00 c	28.25 a
SP 168	Ph_pPh_p	1.75 ab	0.50 b	0.25 c	22.00 a
C 371G	Ph_pPh_p	2.50 ab	0.50 b	1.00 bc	11.00 a

[x] Data presented are nontransformed means from four replications. Data were transformed ($\log_{10}[x + 1]$) prior to statistical analysis. Means within a column followed by the same letters are not significantly different according to the Waller-Duncan *k*-ratio *t* test (*k*-ratio = 100).

[y] Mean initial *G. tabacum* subsp. *solanacearum* population densities (eggs/500 cm^3 of soil) ranged from 25,939 to 31,738 on 16 May 1994 and 13,088 to 27,132 on 21 May 1998.

[z] Genotypes: ph_pph_p = without the Ph_p gene, susceptible to race 0 of *Phytophthora nicotianae*; Ph_pph_p = heterozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae*; and Ph_pPh_p = homozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae.*

Table 1. Initial population densities and reproduction of *Globodera tabacum* subsp. *solanacearum* on various flue-cured tobacco cultivars in 1992 and 1993 at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA[v]

		G. tabacum subsp. *solanacearum* eggs/500 cm^3 soil (P_i)[w]		Reproductive ratio (P_f/P_i)[x]	
Cultivar	Ph_p genotype[y]	1992 (4 May)	1993 (10 May)	1992 (30 September)	1993 (18 October)
C 319	ph_pph_p	9,302 a	36,195 a	6.76 a	0.842 ab
K 326	ph_pph_p	10,773 a	35,978 a	4.23 a	1.598 a
K 394	ph_pph_p	10,596 a	40,060 a	3.67 a	0.653 ab
McN 944	ph_pph_p	10,340 a	34,918 a	4.36 a	0.545 ab
NC 27NF	ph_pph_p	13,492 a	39,530 a	4.51 a	0.537 ab
NC 37NF	ph_pph_p	9,929 a	35,249 a	4.64 a	0.586 ab
Rms 134	ph_pph_p	11,976 a	40,994 a	7.61 a	0.759 ab
Rms 158	ph_pph_p	13,583 a	34,268 a	3.16 ab	1.332 ab
VA 116	ph_pph_p	9,810 a	38,156 a	6.92 a	0.630 ab
C 371G[z]	Ph_pPh_p	8,704 a	38,492 a	1.33 b	0.128 b

[v] Nematode count data presented are detransformed means from four replications. Data were transformed ($\log_{10}[x + 1]$) prior to statistical analysis. Means within a column followed by the same letters are not significantly different according to the Waller-Duncan *k*-ratio *t* test (*k*-ratio = 100).

[w] P_i = initial nematode population.

[x] P_f/P_i = ratio of final to initial nematode populations.

[y] Genotypes: ph_pph_p = without the Ph_p gene, susceptible to race 0 of *Phytophthora nicotianae*; and Ph_pPh_p = homozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae.*

[z] Coker 371-Gold (C 371G) was the first flue-cured tobacco cultivar released possessing resistance to race 0 of *P. nicotianae* transferred from *Nicotiana plumbaginifolia*.

were noted for C371G and NC567 compared with K 326 (Table 2). Although initial *G. tabacum* subsp. *solanacearum* population densities were much lower in 1998 versus 1994, fewer vermiform *G. tabacum* subsp. *solanacearum* nematodes were counted in roots of NC 72 (with the Ph_p gene) than for OX 207 (without the Ph_p gene) (Table 2). Reductions in swollen *G. tabacum* subsp. *solanacearum* juveniles were significant for C371G, NC 71, NC 72, and SP 168 compared with OX207, while numbers of pyriform *G. tabacum* subsp. *solanacearum* nematodes were lower for NC 567 and for all cultivars possessing the Ph_p gene, whether homozygous or heterozygous, compared with OX 207 (Table 2). Apparent differences among entries in mean adult *G. tabacum* subsp. *solanacearum* in roots were not significant in 1998 (Table 2) but reproductive ratios based upon nematode eggs extracted from soil were significantly lower for NC 567 and all cultivars either homozygous or heterozygous for the Ph_p gene (C371G, NC 71, NC 72, and SP 168) compared with SP NF3 (Table 3). As in 1992 and 1993, application of a nematicide in 1999 did not influence relative differences in *G. tabacum* subsp. *solanacearum* reproductive ratios among cultivars. Pooled reproductive ratios from both nematicide-treated and nontreated soil were significantly lower in 1999 for NC 567, C 371G, NC 71, NC 72, and SP 168 compared with K 326, OX 207, and SP NF3 (Table 3). Nematicide and cultivar effects also did not interact significantly in similar SPAREC experiments performed in 2001 and 2003, which featured lower pooled reproductive ratios for all Ph_p gene cultivars tested (GL 737, NC 71, NC 291, NC 297, NC 810, RG H51, SP 168, and SP H20) compared with K 326 (Table 3). Reproductive ratios were also lower for SP 168 (homozygous for the Ph_p gene) and for GL 973, NC 71, NC 297, RG H51, and SP H20 (all heterozygous for the Ph_p gene) than for *G. tabacum* subsp. *solanacearum*-susceptible K 326 (or C 319 at the Wright farm) in four on-farm experiments conducted in 2001 (Table 4).

Table 3. Reproductive ratios of *Globodera tabacum* subsp. *solanacearum* on various flue-cured tobacco cultivars in 1998, 1999, 2001, and 2003 at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA[x]

		Reproductive ratio (P_f/P_i)[y]			
Cultivar	**Ph_p genotype[z]**	**1998**	**1999**	**2001**	**2003**
VPI 1115	ph_ph_p	nt	4.23 a	nt	nt
K 326	ph_ph_p	1.93 ab	3.06 ab	2.89 a	7.31 a
OX 207	ph_ph_p	1.43 ab	3.13 ab	nt	nt
SP NF3	ph_ph_p	2.72 a	3.05 ab	nt	nt
NC 567	ph_ph_p	0.16 b	0.55 d	nt	nt
SP 172	ph_ph_p	1.57 ab	2.48 bc	nt	nt
SP 190	ph_ph_p	nt	nt	1.42 b	nt
SP 210	ph_ph_p	nt	nt	1.13 b	nt
NC 71	Ph_ph_p	0.11 b	0.58 d	0.42 c	0.36 b
NC 72	Ph_ph_p	0.14 b	1.09 cd	nt	nt
GL 737	Ph_ph_p	nt	nt	nt	0.33 b
GL 973	Ph_ph_p	nt	nt	0.24 c	nt
NC 291	Ph_ph_p	nt	nt	nt	0.12 b
NC 297	Ph_ph_p	nt	nt	0.39 c	0.31 b
RG H51	Ph_ph_p	nt	nt	0.29 c	0.22 b
SP 179	Ph_ph_p	nt	nt	0.34 c	nt
SP H20	Ph_ph_p	nt	nt	0.37 c	0.56 b
NC 810	Ph_pPh_p	nt	nt	0.61 c	0.70 b
SP 168	Ph_pPh_p	0.19 b	1.06 cd	0.49 c	0.89 b
C 371G	Ph_pPh_p	0.14 b	0.27 d	nt	nt

[x] Data presented are detransformed means from four replications. Data from 1998 and 1999 were transformed ($\log_{10}[x + 1]$) prior to statistical analysis. Means within a column followed by the same letters are not significantly different according to the Waller-Duncan *k*-ratio *t* test (*k*-ratio = 100).

[y] P_f/P_i = ratio of final to initial nematode populations, determined on 15, 13, 12, and 6 October in 1998, 1999, 2002, and 2003, respectively. Initial *G. tabacum* subsp. *solanacearum* population densities ranged from 13,088 to 27,132 eggs/500 cm^3 in 1998, 9,245 to 12,880 in 1999, 14,108 to 18,848 in 2001, and 2,034 to 3,979 in 2003; nt = not tested.

[z] Genotypes: ph_ph_p = without the Ph_p gene, susceptible to race 0 of *Phytophthora nicotianae*; Ph_ph_p = heterozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae*; and Ph_pPh_p = homozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae.*

Field testing of DH progeny lines against *G. tabacum* subsp. *solanacearum*. As in the 1994 and 1998 evaluations of released cultivars, numbers of vermiform *G. tabacum* subsp. *solanacearum* were similar in 2002 among lines with or without the Ph_p gene (Table 5). However, fewer swollen and pyriform *G. tabacum* subsp. *solanacearum* nematodes were present in roots of parents (homozygous) and progeny (heterozygous) lines possessing the Ph_p gene compared with those that did not. Although differences in the number of *G. tabacum* subsp. *solanacearum* adults within each cross were not statisti-

Table 4. Reproduction of *Globodera tabacum* subsp. *solanacearum* on various flue-cured tobacco inbred and hybrid cultivars at the Southern Piedmont Agricultural Research and Extension Center (SPAREC) and on commercial farms in Southside, VA in 2001[x]

		Reproductive ratio (P_f/P_i)[y]				
Cultivar	**Ph_p genotype[z]**	**SPAREC (11 April)**	**Parrish (29 Sept.)**	**Warren (24 Sept.)**	**Jennings (24 Sept.)**	**Wright (24 Sept.)**
C 319	ph_ph_p	nt	nt	nt	nt	5.95 a
K 326	ph_ph_p	2.89 a	37.46 a	15.05 a	10.82 a	nt
SP 190	ph_ph_p	1.42 b	nt	nt	nt	nt
SP 210	ph_ph_p	1.13 b	nt	nt	nt	nt
GL 973	Ph_ph_p	0.24 c	1.05 c	nt	1.19 bc	nt
NC 71	Ph_ph_p	0.42 c	1.53 b	1.60 b	0.51 d	0.45 c
NC 297	Ph_ph_p	0.39 c	0.85 c	1.01 d	1.24 bc	0.81 b
RG H51	Ph_ph_p	0.29 c	1.41 b	1.12 cd	1.00 c	0.39 cd
SP H20	Ph_ph_p	0.37 c	1.02 c	1.02 d	1.32 b	0.27 d
SP 168	Ph_pPh_p	0.49 c	nt	1.48 bc	nt	0.46 c
SP 179	Ph_pPh_p	0.34 c	nt	nt	nt	nt
NC 810	Ph_pPh_p	0.61 c	nt	nt	nt	nt

[x] Data presented are detransformed means from four replications. Data were transformed ($\log_{10}[x + 1]$) prior to statistical analysis. Means within a column followed by the same letters are not significantly different according to the Waller-Duncan *k*-ratio *t* test (*k*-ratio = 100).

[y] P_f/P_i = ratio of final to initial nematode populations. Initial *G. tabacum* subsp. *solanacearum* population densities ranged from 14,108 to 18,848 eggs/500 cm^3 at SPAREC, 598 to 892 at Parrish, 2,046 to 5,894 at Warren, 2,041 to 4,495 at Jennings, and 3,287 to 5,050 at Wright locations; Sept. = September; nt = not tested.

[z] Genotypes: ph_ph_p = without the Ph_p gene, susceptible to race 0 of *Phytophthora nicotianae*; Ph_ph_p = heterozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae*; and Ph_pPh_p = homozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae.*

cally significant, these differences were significant in the pooled data including all crosses (Table 5). In field tests conducted in 2003–05, reproductive ratios for similar parental and DH progeny lines homozygous or heterozygous for the Ph_p gene were also significantly lower than those for K 326 (Table 6). Reproductive ratios for entries containing *Rk*2 (a gene conferring resistance to *M. javanica*, *M. arenaria*, and races 2 and 4 of *M. incognita*) were intermediate between those for susceptible K 326 and lines possessing Ph_p.

Greenhouse testing of DH progeny lines against *G. tabacum* subsp. *tabacum*. In a greenhouse screening test for resistance to *G. tabacum* subsp. *tabacum*, the percentage of resistant plants (with one or more white *G. tabacum* subsp. *tabacum* females visible on roots) was higher for black shank-resistant plants than plants susceptible to the black shank disease (Table 7). For parental lines, 81.6% of plants resistant to black shank were also resistant to *G. tabacum* subsp. *tabacum*, and only 28.8% of the black shank-susceptible parents did not have mature females visible on the roots 6 weeks after inoculation. For DH progeny, black shank-resistant plants were more resistant to *G. tabacum* subsp. *tabacum* than black shank-susceptible plants ($P = 0.0003$). There were no significant differences among crosses or interactions between crosses and resistance to tobacco black shank.

Table 5. Tobacco cyst nematodes (*Globodera tabacum* subsp. *solanacearum*) on 22 July 2002 associated with presence or absence of the Ph_p gene for resistance to *Phytophthora nicotianae* (tobacco black shank) in selected doubled-haploid flue-cured tobacco lines developed from F_1 hybrids heterozygous for the chromosomal segment containing Ph_p[x]

		Nematodes/g of feeder root[y]			
Cross	**Ph_p genotype[z]**	**Vermiform**	**Swollen**	**Pyriform**	**Adult**
All four crosses	ph_pph_p	103.1 a	80.3 a	17.0 a	12.7 a
All four crosses	Ph_pPh_p	89.9 a	10.7 b	1.9 b	9.2 b
K326 × NCTG-61	ph_pph_p	89.9 a	75.4 a	14.2 a	13.3 a
K326 × NCTG-61	Ph_pPh_p	87.6 a	10.5 b	2.1 b	8.2 a
K346 × DH92-3397-13	ph_pph_p	133.9 a	72.3 a	14.5 a	11.4 a
K346 × DH92-3397-13	Ph_pPh_p	89.6 a	6.6 b	1.6 b	7.9 a
K326 × DH92-3397-2	ph_pph_p	98.1 a	86.1 a	21.3 a	16.2 a
K326 × DH92-3397-2	Ph_pPh_p	92.5 a	13.7 a	1.5 a	13.3 a
K326 × DH92-2770-40	ph_pph_p	90.5 a	87.5 a	18.1 a	10.1 a
K326 × DH92-2770-40	Ph_pPh_p	89.7 a	12.1 b	2.5 b	7.4 a

[x] Data presented for all crosses are means from 160 observations; means for each cross are averages of 40 observations. Data were transformed ($\log_{10}[x + 1]$) prior to statistical analysis. Means followed by the same letter, within a column and cross, are not significantly different according to a Fisher's protected least significant difference ($P \leq 0.05$).

[y] Nematodes/g of feeder root are means from one-plant samples collected from each plot on 22 July, 25 July, 7 August, and 22 August, 61, 64, 77, and 92 days after transplanting, respectively.

[z] Genotypes: ph_pph_p = without the Ph_p gene, susceptible to race 0 of *Phytophthora nicotianae*; and Ph_pPh_p = homozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae.*

DISCUSSION

Based upon a generation means analysis of greenhouse data, Crowder et al. (9) demonstrated that C371G possesses what appears to be a single dominant gene conferring resistance to *G. tabacum* subsp. *solanacearum*. Carlson et al. (6) showed that C371G also possesses a "single" dominant gene that provides complete resistance to race 0 of *P. nicotianae*. Johnson et al. (17,18) published a set of randomly amplified polymorphic DNA markers that are completely linked to the Ph_p gene, and demonstrated that Ph_p and the markers in coupling phase linkage to Ph_p are present in a chromosome segment originally derived from *N. plumbaginifolia*. Johnson et al. failed to detect recombination among coupling phase markers associated with Ph_p, or among repulsion phase markers in ph_p genotypes, in more than 250 DH lines derived from Ph_pph_p F_1 hybrids, indicating that recombination is not occurring between genes within the chromosomal segment that contains Ph_p or, if it does, such recombination is extremely rare. The results presented here demonstrate that a gene (or genes) conferring resistance to *G. tabacum* subsp. *solanacearum* and *G. tabacum* subsp. *tabacum* is associated or linked with the chromosomal segment from *N. plumbaginifolia* containing Ph_p. Thus, we have used the presence or absence of Ph_p to discern the presence or absence of the entire chromosomal segment. The lack of recombination between this segment and a potential "homologous" *N. tabacum* chromosome segment indicates that every gene in the *N. plumbaginifolia* chromosome segment behaves as a single unit. Consequently, our results may not prove that Ph_p is a single gene or that the resistance to *G. tabacum*

Table 6. Initial population densities and reproduction of *Globodera tabacum* subsp. *solanacearum* on flue-cured tobacco breeding lines and cultivars with and without the *Rk*1, *Rk*2, and Ph_p genes at the Southern Piedmont Agricultural Research and Extension Center, Blackstone, VA in 2003–05[u]

	Genotype			(P_m/P_i)[v]			(P_f/P_i)[w]	
Entry	***Rk1*[x]**	***Rk2*[y]**	**Ph_p[z]**	**18 July 2003**	**16 July 2004**	**28 July 2005**	**6 October 2003**	**8 October 2004**
K 326	*Rk*1*Rk*1	*rk*2*rk*2	ph_pph_p	1.15 a	1.26 a	2.39 a	72.78 a	20.99 a
DH NC01-619-7	*Rk*1*Rk*1	*Rk*2*rk*2	ph_pph_p	…	0.62 a	0.91 bc	…	11.97 ab
DH NC99-119-4	*Rk*1*Rk*1	*Rk*2*Rk*2	ph_pph_p	0.97 ab	0.44 a	1.71 ab	29.96 b	9.28 bc
DH NC99-119-5	*Rk*1*Rk*1	*Rk*2*Rk*2	ph_pph_p	0.16 b	0.45 a	2.72 a	13.53 b	5.70 c
NC61 × DH NC99-119-4	*Rk*1*Rk*1	*Rk*2*rk*2	Ph_pph_p	0.20 b	0.46 a	0.40 c	0.53 b	0.56 de
NC99-119-4 × DH95-1816-5	*Rk*1*Rk*1	*Rk*2*rk*2	Ph_pph_p	0.24 b	0.45 a	1.49 ab	1.81 b	0.31 de
NC61 × NC01-619-7	*Rk*1*Rk*1	*Rk*2*rk*2	Ph_pph_p	…	0.82 a	0.56 bc	…	0.57 de
NC 71	*Rk*1*rk*1	*rk*2*rk*2	Ph_pph_p	…	0.30 a	0.27 c	…	0.32 de
Coker 371-G	*rk*1*rk*1	*rk*2*rk*2	Ph_pPh_p	…	0.16 a	0.38 c	…	0.21 e
DH NC01-1459-041	*Rk*1*Rk*1	*Rk*2*Rk*2	Ph_pPh_p	…	0.50 a	0.82 bc	…	1.34 d

[u] Data presented are detransformed means from four replications. Data were transformed ($\log_{10}[x + 1]$) prior to statistical analysis. Means within a column followed by the same letters are not significantly different according to the Waller-Duncan *k*-ratio *t* test (*k*-ratio = 100).

[v] P_m/P_i = ratio of midseason to initial nematode populations. Mean initial *G. tabacum* subsp. *solanacearum* population densities ranged from 228 to 464 eggs/500 cm^3 of soil in 2003, 7,077 to 19,501 in 2004, and 4,566 to 8,814 in 2005. Mean midseason *G. tabacum* subsp. *solanacearum* population densities ranged from 39 to 324 eggs/500 cm^3 of soil in 2003, 180 to 9,300 in 2004, and 2,262 to 27,516 in 2005.

[w] P_f/P_i = ratio of final to initial nematode populations; not available in 2005 because the experiment was terminated in late July that year.

[x] Genotypes: *Rk1Rk1* = homozygous for the *Rk1* gene for resistance to races 1 and 3 of *Meloidogyne incognita*; *rk1rk1* = without the *Rk1* gene for resistance to races 1 and 3 of *Meloidogyne incognita*.

[y] Genotypes: *Rk2Rk2* = homozygous for the genetic factor *Rk2* for resistance to *M. javanica, M. arenaria*, and races 2 and 4 of *M. incognita*; *rk2rk2* = without the *Rk2*gene.

[z] Genotypes: ph_pph_p = without the Ph_p gene, susceptible to race 0 of *Phytophthora nicotianae*; Ph_pph_p = heterozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae*; and Ph_pPh_p = homozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae.*

subsp. *solanacearum* and *G. tabacum* subsp. *tabacum* arises from a single gene but, rather, demonstrate that resistance traits for these two subspecies of *G. tabacum* are linked to that for *P. nicotianae* on the chromosomal segment from *N. plumbaginifolia*, so that resistance to these three pathogens behaves as though conferred by a single gene.

Links between resistance to *Phytophthora* and *Globodera* spp. have been noted in other solanaceous species. The *R1* gene for resistance to *P. infestans* was found to be clustered with the *Gpa* and *Grp1* genes for resistance to *G. pallida* and *G. rostochiensis* on chromosome 5 of potato, and two R loci for *P. infestans* in tomato were located in roughly corresponding locations to resistance alleles in potato against *Globodera* spp. (11,31). Our results build on multiple previous observations of linkages between genes for resistance to tobacco diseases. Baalawy and Fox found resistance to *G. tabacum* subsp. *solanacearum* in *N. glutinosa*, *N. longiflora*, *N. paniculata*, and *N. plumbaginifolia* (1,23). Testing an association originally reported by Komm and Terrill (22), Hayes found a highly significant correlation ($r = 0.73$) between resistance to *G. tabacum* subsp. *solanacearum* and to wildfire, caused by *Pseudomonas syringae* pv. *syringae* (13). LaMondia concluded that the likely source of resistance to *G. tabacum* subsp. *tabacum* transferred through burley tobacco lines was *N. longiflora*, which was originally introduced into burley for resistance to tobacco wildfire (8,14,23). *N. longiflora* was also the source of a major gene for resistance to *Phytophthora nicotianae* (Ph_l) transferred into burley tobacco (30). This gene (Ph_l) is located approximately 3 centimorgans from the Ph_p gene from *N. plumbaginifolia,* and both genes provide near complete resistance to race 0 of *P. nicotianae* but none against race 1 of the pathogen (17). Further work is necessary to elucidate whether or not these multiple correlations between resistance genes to wildfire, black shank, and tobacco cyst nematodes indicate a resistance gene cluster, pleiotropic effects, or some combination.

Pathogen avoidance is among the mechanisms of resistance to tobacco black shank (19–21). Black-shank-resistant cultivars were initially evaluated for *G. tabacum* subsp. *solanacearum* reproduction to test the hypothesis that smaller root systems might avoid *G. tabacum* subsp. *solanacearum* juveniles as well as *Phytophthora* zoospores. However, vermiform *G. tabacum* subsp. *solanacearum* juveniles penetrated roots of the resistant and susceptible cultivars tested in similar numbers, indicating that the observed suppression of *G. tabacum* subsp. *solanacearum* reproduction resulted from inhibition of feeding site establishment. This mechanism is similar to that observed in flue-cured tobacco cv. NC 567, a *Tobacco mosaic virus*-resistant cultivar that may have gained *G. tabacum* subsp. *solanacearum* resistance from either *N. glutinosa* or *N. longiflora* (14,32).

The consistency of suppressed *G. tabacum* subsp. *solanacearum* reproduction by cultivars and entries possessing the chromosome segment from *N. plumbaginifolia* containing Ph_p highlights the longer-term impact of host resistance on nematode population dynamics compared with crop protection agents (CPAs) such as nematicides. A nematicide treatment was included in some of the experiments reported here in order to compare yield and quality traits among cultivars and breeding lines with or without the Ph_p gene in soil with low versus high initial *G. tabacum* subsp. *solanacearum* population densities. Yield and quality effects are beyond the scope of this article; however, potential interactions in *G. tabacum* subsp. *solanacearum* reproduction between main plots (nematicide treatment versus none) and subplots (tobacco cultivars or breeding lines) were never statistically significant in any of the experiments reported here. Even the most effective CPA degrades over time, usually days for soil fumigants and weeks for nonfumigant nematicides, leaving an escaped population of plant-parasitic nematodes. Nematicide efficacy is also frequently influenced by environmental conditions at application, including soil temperature and moisture (12). Once CPA residues in soil drop below toxic levels, the susceptible root systems that have been protected are fully available to the remaining nematode population. This scenario gives the crop a "head start" that enables it to produce an acceptable yield but, for plant-parasitic nematode species with generation times short enough to allow multiple generations in a growing season, this scenario also frequently results in "density-dependent reproduction," evidenced by higher final nematode population densities in treated soil compared with untreated controls or poorly performing treatments (27). On the other hand, the resistance or susceptibility of host roots remains constant over the growing season. Consequently, nematode population densities at the end of the growing season will be low for resistant genotypes, whether or not soil was treated with a nematicide, while final populations may be high for susceptible lines, even when planted in nematicide-treated soil. The results of the experiments reported here are consistent with this scenario and illustrate one of the benefits of host resistance in managing plant-parasitic nematodes.

Prior to the release of hybrid cultivars containing the Ph_p gene, the inferior agronomic quality of the *G. tabacum* subsp. *solanacearum*-resistant flue-cured tobacco cultivars and their inability to withstand nematode infestations at high initial population densities prevented their widespread adoption (9,15,33). However, hybrid cultivars of flue-cured tobacco possessing the Ph_p gene have been very popular among farmers over the past 10 years due to their resistance to race 0 of the black shank pathogen and their desirable agronomic traits. Incidence of race 1 of *P. nicotianae* has increased correspondingly, resulting in recommendations to rotate cultivars with and without the Ph_p gene in order to shift *P. nicotianae* populations from predominantly race 1 back to mostly race 0 (10,29). However, such a strategy could also result in rebounding populations of *G. tabacum* subsp. *solanacearum* in fields where both pathogens are present. Widespread planting of cultivars depending too heavily upon Ph_p gene resistance to tobacco black shank is already leading to

Table 7. Resistance to *Globodera tabacum* subsp. *tabacum* among parental and progeny tobacco entries with and without the Ph_p gene for resistance to tobacco black shank[w]

		Resistant or susceptible plants (%) ($n = 16$)	
Cross	**Ph_p genotype[x]**	**Susceptible ≥1 female**	**Resistant 0 females**
Parental line[y]	ph_pph_p	71.18	28.82
Parental line	Ph_pPh_p	18.43	81.57
K326 × NCTG-61[z]	ph_pph_p	64.45	23.93
K326 × NCTG-61	Ph_pPh_p	48.14	41.26
K346 × DH92-3397-13	ph_pph_p	78.04	18.08
K346 × DH92-3397-13	Ph_pPh_p	32.70	49.33
K326 × DH92-3397-2	ph_pph_p	82.53	16.75
K326 × DH92-3397-2	Ph_pPh_p	42.53	45.69
K326 × DH92-2770-40	ph_pph_p	67.72	27.65
K326 × DH92-2770-40	Ph_pPh_p	25.90	62.64

[w] Data presented are means from 16 replications (2002 greenhouse data). Data were analyzed by analysis of variance after arcsine transformation.

[x] Genotypes: ph_pph_p = without the Ph_p gene, susceptible to race 0 of *Phytophthora nicotianae*; and Ph_pPh_p = homozygous for the Ph_p gene, resistant to race 0 of *P. nicotianae.*

[y] Differences in percentage of plants resistant or susceptible to *G. tabacum* subsp. *tabacum* among parental lines with (NCTG-61, DH92-3397-13, DH92-3397-2, and DH92-2770-40) or without (K326 and K346) the Ph_p gene were statistically significant at $P = 0.02$.

[z] Differences among doubled-haploid hybrid progeny lines with or without the Ph_p gene were significant at $P = 0.0003$; differences among crosses were not statistically significant ($P = 0.23$), nor were interaction effects statistically significant ($P = 0.79$).

renewed dependence on soil fungicides to maintain adequate control of this disease. Further research is needed to clarify the link between resistance to *G. tabacum* subsp. *solanacearum* and *G. tabacum* subsp. *tabacum* and that to race 0 of the black shank pathogen in order to facilitate development of cultivars possessing an optimal combination of resistance to all three pathogens.

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