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Development of Selected Tobacco Cyst Nematode Isolates on Resistant and Susceptible Cultivars of Flue-cured Tobacco[1]

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Abstract: Tobacco cyst nematode (*Globodera tabacum solanacearum*) isolates were collected from 11 locations in Virginia, 3 in North Carolina, and 1 in Maryland. Isolates from each location were maintained and increased on flue-cured tobacco, *Nicotiana tabacum* cv K326. Plants of flue-cured tobacco cultivars K326 (susceptible) and NC567 (resistant) were each inoculated with 6,000 *G. t. solanacearum* eggs/plant. Tests were conducted over one (6 weeks) or two (14 weeks) generations of the nematode. Shoot and root weights and the number of nematodes present within a 1-g subsample of feeder roots were recorded at completion of the tests. Nematode counts were categorized by nematode life stage (vermiform, swollen, pyriform, and adult). Although significant differences in nematode development were detected among isolates, differences were not consistent across experiments. Results indicate similar virulence among *G. t. solanacearum* isolates on resistant and susceptible flue-cured tobacco cultivars. Therefore, plant breeders may effectively use a single *G. t. solanacearum* isolate when screening tobacco germplasm for resistance.

Key words: cyst nematode, flue-cured tobacco, *Globodera tabacum solanacearum,* nematode, *Nicotiana tabacum,* reproduction, resistance, tobacco cyst nematode.

The tobacco cyst nematode, *Globodera tabacum solanacearum* (Miller and Gray, 1972) Behrens, 1975, is one of the most serious pathogens of Virginia flue-cured tobacco (*Nicotiana tabacum* L.). Currently, *G. t. solanacearum* infests an estimated one-third of all flue-cured tobacco acreage in Virginia, costing farmers an estimated $3 million annually in crop losses and pesticide expenditures (C. S. Johnson, unpubl.). Infested fields average an estimated 15% yield loss annually, and complete crop losses have been reported at times (Komm et al., 1983). Current control measures for *G. t. solanacearum* include crop rotation, sanitation, and application of nematicides (Reed et al., 1997). There are no agronomically desirable *G. t. solanacearum*-resistant cultivars, although some cultivars can reduce the nematode's population densities (Reed et al., 1997). Currently available resistant cultivars perform poorly in terms of yield and quality when compared with susceptible cultivars planted in nematicide-treated soil (Johnson, 1990; Johnson et al., 1989). However, recently developed resistant breeding lines exhibit better yields and quality.

Resistance-breaking biotypes have been reported for potato cyst nematodes (*Globodera pallida* (Stone, 1973) Behrens, 1975 and *G. rostochiensis* (Wollen.Weber, 1923) Behrens, 1975) and soybean cyst nematodes (*Heterodera glycines* Ichinohe, 1952) (Arntzen et al., 1994; Caviness, 1992). The potential stability of resistance to *G. t. solanacearum* was first questioned in the original description of the pathogen, when differences in host range were reported among several isolates of the nematode (Miller and Gray, 1972). Elliot et al. (1986) concluded that *G. t. solanacearum* resistance was stable over a period of several years on the tobacco cultivars PD4 and VA81; however, their study was conducted at a single location and thus involved only one isolate of the pathogen. The potential existence of *G. t. solanacearum* biotypes able to reproduce on resistant cultivars should be investigated to assure plant breeders that a single isolate can be relied upon

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for resistance-screening purposes. In this research, we compared the reproductive ability of 15 *G. t. solanacearum* isolates on susceptible (K326) and resistant (NC567) cultivars of flue-cured tobacco.

Materials and Methods

Site selection and sampling: Fifteen locations infested with *G. t. solanacearum* were selected based on length of infestation, level of infestation, and geographic location. Samples were collected with a 2.0-cm-diam. soil probe from 15 locations between 13 March 1996 and 6 April 1996 (Table 1). Soil cores were collected in a criss-cross pattern (500 to 1,000 cores, 20-cm-deep) over the entire area of the field that had previously been in tobacco production (Barker et al., 1984). Cysts were extracted from soil samples with a modified Fenwick can, from which cysts were caught on a 250-μm-pore sieve (Caswell et al., 1985). Due to variation in sample dates and field histories, each isolate was cultured on the susceptible cultivar K326 for two generations. Cysts were extracted, dried overnight, and stored in capped test tubes at room temperature until needed.

Seedling preparation: Tobacco seedlings were produced in the greenhouse in tin pans containing vermiculite. Five-week-old tobacco seedlings were transplanted to 11-cm-diam. clay pots containing 300 cm^3 steam-pasteurized mixture of one part sandy loam:two parts fine quartz sand (84% sand, 10% silt, 6% clay; pH 5.7). Seedlings were allowed to grow for 1 week before soil infestation with nematodes. Plants were watered with an automatic watering system. In the first two experiments, plants were fertilized with a solution (17% N, 5% P, 24% K, 2% water-soluble Mg, 2.64% S, 0.085% Mo, and 0.055% Zn) calibrated to deliver 125 mg/kg N. Subsequent trials were fertilized with a microinjector to deliver the same rate of N.

Soil infestation: Nematode isolates were randomly divided into two groups to keep experiments at a manageable size (Table 1). The Coffee isolate was included in both groups as a standard. Cysts of each isolate were crushed, and eggs were standardized to 6,000/12 ml tap water for each 11-cm-diam. clay pot. The egg suspension was pipeted into a 5-cm-deep trench around the root zone of a single tobacco seedling. Trenches were covered with 100 cm^3 soil after infestation. Uninfested controls were randomized within all experiments and received 12 ml tap water only. All experiments were conducted on benchtops in the greenhouse, except for the first 14-week trial, which was conducted in a walk-in growth chamber. In all experiments, air temperature ranged from 23 °C to 30 °C.

Experimental design: Initial experiments were arranged in randomized complete blocks, in a complete factorial treatment design with cultivar (NC567 or K326) and nematode isolate as the two factors. Each cultivar × isolate combination was replicated eight times. Uninfested control pots were also included in each experiment for top and root weight comparisons with infected

Table 1. Location and grouping for the 15 sites selected for *Globodera tabacum solanacearum* sampling. Isolates were assigned to groups randomly.

Group I Isolates		Group II Isolates	
Farm	Location	Farm	Location
Baskerville	Dinwiddie Co., VA	Bertie	Bertie Co., NC
Bowman	Nottoway Co., VA	Bing	Mecklenburg Co., VA
Coffee[a]	Lunenburg Co., VA	Clary	Mecklenburg Co., VA
Moore	Lunenburg Co., VA	Coffee[a]	Lunenburg Co., VA
Newburg	Charles Co., MD	Hastings	Amelia Co., VA
Proffitt	Powhatan Co., VA	Lewis	Brunswick Co., VA
SPAREC[b]	Nottoway Co., VA	Rideout	Dinwiddie Co., VA
Warren	Warren Co., NC	Vance	Vance Co., NC

[a] The Coffee isolate was included in both groups as a standard for comparison between groups.
[b] Southern Piedmont Agricultural Research and Extension Center

plants. Each experiment included isolates from either Group I or II and continued for 6 weeks after soil infestation. Experiments were repeated once, so that reproduction of each isolate was evaluated in two separate experiments.

Reproduction of all 15 isolates on the resistant cultivar NC567 was investigated in two additional 6-week experiments and two 14-week experiments. These experiments were conducted in randomized complete blocks with eight replications. Uninfested controls also were included in these tests. In all but the first 6-week experiment, eight plants of the susceptible cultivar K326 were included to compare reproduction on the susceptible and resistant cultivars.

Data collection: Plants were removed from soil 6 or 14 weeks after infestation. Each plant was separated into root and shoot portions, blotted dry, and weighed. One gram of feeder roots was randomly excised from the shoot/root transition area of each root system and washed over a 38-µm-pore sieve. The feeder root subsample and root wash materials were combined and stained with acid fuchsin (Byrd et al., 1983). Nematodes were counted in four categories: vermiform, swollen (distinct sausage shape), pyriform (flask shape), and adult (saccate shape, bearing eggs) according to classification guidelines similar to those of Wang (1996).

Statistical analysis: Due to correlation between variance and treatment means, data were transformed to $\log_{10}(x + 1)$ values for statistical analysis. Analyses of variance and Waller-Duncan means separation procedures (k-ratio = 100) were performed with SAS Software (SAS Institute, Cary, NC). For all statistical analyses, $P \leq 0.05$ was deemed as significant. Where isolate × cultivar interactions were not significant, a combined analysis was performed across cultivars. Statistical analyses were combined across experiments when respective mean square error terms were not significantly different according to *F*-tests (Gomez and Gomez, 1984) and experimental interactions were not significant. Additional *t*-tests were conducted to investigate whether the two groups of isolates developed differently on the two cultivars.

Results

Numbers of vermiform, pyriform, adult, and total nematodes were higher on susceptible K326 than on resistant NC567 for both groups of nematode isolates in both trials (Table 2). Numbers of swollen nematodes were significantly higher on K326 than on NC567 in three of the four experiments.

Reproduction on K326: Relative differences among Group I isolates in the number of nematodes in roots of K326 were inconsistent across trials (Table 3). In trial 1, more vermiform nematodes were counted after infestation with eggs from the Bowman, Cof-

Table 2. *Globodera tabacum solanacearum* development on the resistant cultivar NC567 and the susceptible cultivar K326.

	Nematodes per gram of feeder root									
	Vermiform		Swollen		Pyriform		Adult		Total	
Cultivar	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
					Group I[a]					
K326	21.41 a	14.98 a	10.78 a	3.38 a	3.27 a	4.56 a	0.44 a	10.44 a	35.89 a	33.36 a
NC567	9.61 b	11.27 b	1.39 b	2.62 a	0.14 b	1.23 b	0.00 b	0.52 b	11.14 b	15.63 b
					Group II[a]					
K326	16.81 a	17.40 a	5.84 a	4.23 a	4.00 a	2.82 a	0.52 a	4.61 a	27.17 a	29.06 a
NC567	12.95 b	12.91 b	3.31 b	2.75 b	1.89 b	0.94 b	0.02 b	0.25 b	18.24 b	16.84 b

Values are untransformed means from 64 replications of each cultivar. Data were transformed to $\log_{10}(x + 1)$ values. Means in a column within a group with common letters are not significantly different according to a *t*-test. Isolates were assigned to groups randomly.

[a] Isolates were tested in either of two groups in order to keep each experiment at a manageable size. All isolates except that from the Coffee location were assigned to a group randomly. The Coffee isolate was included in both groups to facilitate evaluation of nematode reproduction between the two groups.

TABLE 3. Development of *Globodera tabacum solanacearum* Group I isolates[a] in flue-cured tobacco cultivar K326.

	Nematodes per gram of feeder root (see Table 2)							
	Vermiform		Swollen		Pyriform		Adult	
Isolate	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Baskerville	15.75 ab	16.75 a	6.25 ab	2.63 b	1.63 a	5.38 a	0.75 a	8.25 ab
Bowman	22.50 a	15.63 a	7.75 ab	2.50 b	2.63 a	7.00 a	0.50 a	8.63 ab
Coffee	31.13 a	12.75 a	13.75 ab	2.25 b	3.13 a	5.63 a	0.63 a	10.25 ab
Moore	19.63 a	16.00 a	10.38 ab	1.13 b	2.88 a	4.00 ab	0.50 a	10.13 ab
Newburg	40.00 a	16.75 a	19.00 ab	2.50 b	6.75 a	5.00 a	0.75 a	12.88 ab
Proffitt	22.00 a	14.25 a	16.88 a	0.88 b	4.25 a	1.38 c	0.00 a	7.13 ab
SPAREC	12.38 bc	16.63 a	7.38 b	8.63 a	3.13 a	5.88 a	0.00 a	15.22 a
Warren	7.88 c	11.13 a	4.88 ab	6.50 a	1.75 a	2.25 bc	0.38 a	4.26 b

Values are untransformed means from eight replications. Mean separations were determined with the Waller-Duncan *k*-ratio *t*-test (*k*-ratio = 100) on data transformed to $\log_{10}(x + 1)$ values. Means within a column followed by a common letter are not significantly different.

[a] Group I was a randomly assigned set of 8 of the 15 isolates, grouped in order to keep each experiment at a manageable size. All isolates except that from the Coffee location were assigned to a group randomly. The Coffee isolate was included in both groups to facilitate evaluation of nematode reproduction between the two groups.

fee, Moore, Newburg, and Proffitt isolates than for the SPAREC and Warren isolates; however, similar differences were not observed in trial 2. Infestation with the Proffitt isolate in trial 1 resulted in more swollen nematodes in roots of K326 than when the SPAREC isolate was used, but the opposite result was observed in trial 2. Although numbers of pyriform nematodes were similar on K326 for the different Group I isolates in trial 1, more pyriform nematodes were observed on K326 in the second trial from the Baskerville, Bowman, Coffee, Newburg, and SPAREC isolates than for the Warren and Proffitt isolates. No relative differences in adult nematodes were observed on K326 among Group I isolates in trial 1; however, more adult nematodes were observed on K326 for the SPAREC isolate than for the Warren isolate in trial 2.

Very few differences were observed among Group II isolates in the number of nematodes on K326 (Table 4). Similar numbers of vermiform, pyriform, and adult nematodes were observed among Group II isolates in roots of susceptible cultivar K326. More swollen nematodes from the Coffee isolate were found on K326 than for the

TABLE 4. Development of *Globodera tabacum solanacearum* Group II[a] isolates in flue-cured tobacco cultivar K326.

	Nematodes per gram of feeder root							
	Vermiform		Swollen		Pyriform		Adult	
Isolate	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Bertie	15.12 a	22.13 a	6.17 ab	3.38 ab	2.95 a	2.75 a	0.00 a	3.38 a
Bing	12.53 a	22.38 a	1.87 b	4.00 ab	1.89 a	3.88 a	0.14 a	4.25 a
Clary	20.02 a	17.13 a	4.54 ab	5.88 a	2.80 a	2.50 a	0.39 a	3.63 a
Coffee	15.84 a	16.88 a	7.68 a	4.25 ab	2.74 a	2.75 a	0.29 a	5.38 a
Hastings	16.73 a	20.83 a	5.22 ab	3.83 ab	3.04 a	3.67 a	0.89 a	6.17 a
Lewis	22.74 a	13.38 a	7.31 ab	3.88 ab	8.21 a	3.63 a	0.98 a	4.13 a
Rideout	17.26 a	13.75 a	7.15 ab	6.13 a	5.66 a	1.88 a	0.43 a	5.38 a
Vance	14.87 a	13.63 a	7.02 ab	2.38 b	5.15 a	1.75 a	1.04 a	5.00 a

Values are untransformed means from eight replications. Mean separations were determined with the Waller-Duncan *k*-ratio *t*-test (*k*-ratio = 100) on data transformed to $\log_{10}(x + 1)$ values. Means within a column followed by a common letter are not significantly different.

[a] Group II was a randomly assigned set of 8 of the 15 isolates, grouped in order to keep each experiment at a manageable size. All isolates except that from the Coffee location were assigned to a group randomly. The Coffee isolate was included in both groups to facilitate evaluation of nematode reproduction between the two groups.

Bing isolate in trial 1, but not in trial 2. More swollen nematodes from the Clary and Rideout isolates were found in the roots of K326 than for the Vance isolate in the second trial, but not in the first.

Reproduction on NC567: Differences observed among Group I *G. t. solanacearum* isolates on the resistant cultivar NC567 were also inconsistent between trials. In the first trial, more vermiform nematodes were observed in roots infected with the Moore isolate than those with the SPAREC isolate (Table 5). However, in the second trial, the Proffitt isolate produced more vermiform nematodes than did the SPAREC, Warren, and Newburg isolates. The Warren isolate produced more swollen nematodes than did the SPAREC and Proffitt isolates in the first trial, but no differences in numbers of swollen nematodes were observed in the second trial. No differences in the numbers of pyriform nematodes were noted among isolates in either trial. In the first trial, no differences were detected in the numbers of adults, but more adults were found in the second trial on the roots of plants infested with the SPAREC isolate than the Newburg and Bowman isolates. Additionally, the Proffitt isolate produced more adults in the roots of NC567 than did the Bowman isolate.

As was the case for K326, fewer differences were noted on NC567 among Group II isolates than among the Group I isolates. In fact, no significant differences were found among Group II isolates at any of the *G. t. solanacearum* life stages on the resistant cultivar NC567 (Table 6).

All isolates on NC567 for one generation: Nematode numbers were similar in roots of NC567 among all isolates in both trials that included all 15 isolates (Table 7). The SPAREC isolate produced more pyriform and adult nematodes on the susceptible cultivar K326 than were produced by any of the isolates on NC567.

All isolates on NC567 for two generations: Combined statistical analyses were performed across the two trials to compare numbers of swollen, pyriform, and adult *G. t. solanacearum.* No significant differences were observed among isolates in numbers of nematodes on NC567 at any individual life stage in the 14-week trials (Table 8). Roots of K326 infected with the SPAREC isolate contained significantly more swollen, pyriform, and adult nematodes than those of NC567 infected with *G. t. solanacearum* from any of the 15 sites.

Discussion

Parasitism by *G. t. solanacearum* was consistently and significantly lower on the resistant

TABLE 5. Development of *Globodera tabacum solanacearum* Group I[a] isolates in flue-cured tobacco cultivar NC567.

	Nematodes per gram of feeder root							
	Vermiform		Swollen		Pyriform		Adult	
Isolate	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Baskerville	8.75 ab	13.50 ab	2.38 ab	2.63 a	0.50 a	1.25 a	0.00 a	0.38 abc
Bowman	7.25 ab	11.57 ab	0.75 ab	2.29 a	0.25 a	1.14 a	0.00 a	0.00 c
Coffee	7.50 ab	11.63 ab	0.88 ab	1.50 a	0.00 a	1.25 a	0.00 a	0.25 abc
Moore	16.13 a	14.00 ab	1.38 ab	1.57 a	0.00 a	0.57 a	0.00 a	0.57 abc
Newburg	4.63 ab	10.29 b	1.25 ab	1.57 a	0.00 a	0.86 a	0.00 a	0.14 bc
Proffitt	17.63 ab	16.75 a	0.38 b	3.75 a	0.00 a	1.50 a	0.00 a	1.25 ab
SPAREC	4.38 b	4.50 c	0.50 b	3.88 a	0.13 a	0.63 a	0.00 a	1.13 a
Warren	10.63 ab	7.71 bc	3.63 a	3.57 a	0.25 a	0.86 a	0.00 a	0.29 abc

Values are untransformed means from eight replications. Mean separations were determined with the Waller-Duncan *k*-ratio *t*-test (*k*-ratio = 100) on data transformed to $\log_{10}(x + 1)$ values. Means within a column followed by a common letter are not significantly different.

[a] Group I was a randomly assigned set of 8 of the 15 isolates, grouped in order to keep each experiment at a manageable size. All isolates except that from the Coffee location were assigned to a group randomly. The Coffee isolate was included in both groups to facilitate evaluation of nematode reproduction between the two groups.

TABLE 6. Development of *Globodera tabacum solanacearum* Group II[a] isolates in flue-cured tobacco cultivar NC567.

	Nematodes per gram of feeder root							
	Vermiform		Swollen		Pyriform		Adult	
Isolate	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Bertie	14.04 a	12.13 a	3.38 a	2.75 a	4.06 a	1.50 a	0.17 a	0.13 a
Bing	12.46 a	13.63 a	3.44 a	2.88 a	2.69 a	1.00 a	0.00 a	0.13 a
Clary	13.17 a	20.00 a	2.20 a	4.38 a	1.00 a	0.50 a	0.00 a	0.38 a
Coffee	10.20 a	11.00 a	2.35 a	3.00 a	0.62 a	1.13 a	0.00 a	0.13 a
Hastings	16.97 a	9.25 a	3.91 a	2.00 a	1.63 a	1.13 a	0.00 a	0.25 a
Lewis	12.45 a	10.88 a	2.95 a	1.88 a	0.95 a	0.50 a	0.00 a	0.13 a
Rideout	9.35 a	16.63 a	5.94 a	3.00 a	2.05 a	1.00 a	0.00 a	0.63 a
Vance	13.24 a	9.75 a	2.86 a	2.13 a	1.70 a	0.75 a	0.00 a	0.25 a

Values are untransformed means from eight replications. Mean separations were determined with the Waller-Duncan *k*-ratio *t*-test (*k*-ratio = 100) on data transformed to $\log_{10}(x + 1)$ values. Means within a column followed by a common letter are not significantly different.

[a] Group II was a randomly assigned set of 8 of the 15 isolates, grouped in order to keep each experiment at a manageable size. All isolates except that from the Coffee location were assigned to a group randomly. The Coffee isolate was included in both groups to facilitate evaluation of nematode reproduction between the two groups.

cultivar NC567 than on the susceptible cultivar K326. Although significant differences were detected in certain trials in the number of vermiform and swollen nematodes found on NC567 and K326, differences in nematode development on the two cultivars seemed to be more consistent and pronounced in the latter nematode life stages. This agrees with previous research showing that resistance to *G. t. solanacearum* inhibits nematode development rather than nematode penetration (Baalawy and Fox, 1971; LaMondia, 1988).

Tobacco cyst nematode development was similar across isolates on the susceptible cul-

TABLE 7. Numbers of pyriform and adult *Globodera tabacum solanacearum* 6 weeks after inoculation of flue-cured tobacco cultivar NC567.

	Nematodes per gram of feeder root			
	Pyriform		Adult	
Isolate	Trial 1	Trial 2	Trial 1	Trial 2
Baskerville	1.00 a	1.75 b	0.00 a	1.13 b
Bertie	1.33 a	2.88 b	0.00 a	1.75 b
Bing	0.88 a	nt[a]	0.13 a	nt
Bowman	1.00 a	2.50 b	0.00 a	1.38 b
Clary	1.63 a	1.88 b	0.25 a	1.25 b
Coffee	1.38 a	1.88 b	0.00 a	1.50 b
Hastings	1.63 a	2.63 b	0.13 a	2.00 b
Lewis	2.25 a	1.50 b	0.25 a	1.88 b
Moore	1.57 a	2.25 b	0.14 a	1.38 b
Newburg	1.75 a	1.63 b	0.13 a	1.63 b
Proffitt	1.75 a	1.75 b	0.25 a	1.50 b
Rideout	2.00 a	2.38 b	0.25 a	2.00 b
SPAREC-NC567	1.63 a	1.50 b	0.25 a	1.38 b
Vance	1.75 a	2.38 b	0.38 a	1.50 b
Warren	2.13 a	1.88 b	0.13 a	1.75 b
SPAREC-K326	nt	10.38 a	nt	16.63 a

Data are means for eight replications. Means within columns followed by the same letter are not statistically different as determined by the Waller-Duncan *k*-ratio *t*-test (*k*-ratio = 100) on data transformed to $\log_{10}(x + 1)$ values.

[a] Not tested.

TABLE 8. Number of swollen, pyriform, and adult *Globodera tabacum solanacearum* 14 weeks after infestation of the flue-cured tobacco cultivar NC567.

	Nematodes per gram of feeder root		
Isolate	Swollen	Pyriform	Adult
Baskerville	2.96 b	1.50 b	1.48 b
Bertie	2.15 b	1.24 b	1.24 b
Bowman	2.38 b	1.13 b	1.13 b
Clary	2.53 b	1.09 b	1.00 b
Coffee	2.52 b	1.19 b	0.88 b
Hastings	2.31 b	1.13 b	1.13 b
Lewis	2.00 b	1.43 b	1.40 b
Moore	2.79 b	1.52 b	1.27 b
Newburg	2.97 b	1.83 b	1.42 b
Proffitt	2.60 b	1.57 b	1.60 b
Rideout	2.52 b	1.50 b	1.00 b
SPAREC-NC	2.27 b	1.27 b	1.17 b
Vance	2.44 b	1.31 b	1.47 b
Warren	2.84 b	1.13 b	1.38 b
SPAREC-K	13.94 a	23.38 a	38.56 a

Data are untransformed means of 16 replications (two trials with eight replications in each). Combined analysis was performed across the two trials, and mean separations were conducted with the Waller-Duncan *k*-ratio *t*-test (*k*-ratio = 100) on data transformed to $\log_{10}(x + 1)$ values. Means followed by the same letter within columns are not significantly different.

tivar K326. Isolates differed in numbers of swollen nematodes in roots of K326 in all four trials when the isolates were broken into groups, but the differences rarely coincided from trial to trial. Differences for the other *G. t. solanacearum* life stages were detected only in one trial out of four. Inconsistencies among experiments could be attributed to several different factors. Slight differences in temperature (5–10 °C) can affect *G. t. solanacearum* reproduction (Adams et al., 1982). Additionally, light quality and day length were found to influence reproduction of potato cyst nematodes (Franco and Evans, 1979). Because our experiments were conducted at different times of the year, these factors could have differed among experiments and influenced our results.

Reproduction on the resistant cultivar NC567 was generally consistent over experiments. This suggests that few, if any, differences in aggressiveness exist among the isolates examined in this study. The lack of significant isolate × cultivar interactions suggests that the expression of resistance in NC567 was similar for all isolates involved in this study. The cultivar NC567 should reduce nematode reproduction of most, if not all, *G. t. solanacearum* isolates. Fifteen isolates from different geographic locations with varying number of years known to be infested should constitute a good representation of all *G. t. solanacearum* isolates. Our results suggest that plant breeders should be able to use a single *G. t. solanacearum* isolate to reliably screen tobacco germplasm, thus saving time and money in developing improved resistant tobacco cultivars.

The experiments in this study spanned only one or two generations of the nematode. Turner (1990) found that *Globodera pallida* isolates behaved differently after 11 generations of reproduction on resistant potato hybrids of *Solanum vernei*. Additionally, the efficacy of resistance to *Globodera pallida* started to decline gradually after four to five generations. Elliott et al. (1986) and Johnson (1990) found that resistance to *G. t. solanacearum* was effective over a 3-year period (approximately 10 to 12 *G. t. solanacearum* generations), but these studies involved only one isolate of the pathogen. A long-term study using several isolates of the pathogen would more fully document the long-term effectiveness of *G. t. solanacearum* resistance. Until the results of such work are available, plant breeders should replenish their screening isolates periodically with fresh field isolates.

In summary, no differences were observed among the *G. t. solanacearum* isolates used in this study in development and reproduction on a resistant and a susceptible flue-cured tobacco cultivar over one or two nematode generations. Therefore, plant breeders may effectively use a single tobacco cyst nematode isolate when screening tobacco germplasm for *G. t. solanacearum* resistance. However, this study does not preclude the possibility that other biotypes might exist that are capable of greater reproduction on currently available *G. t. solanacearum*-resistant genotypes. It may also be possible that resistance-breaking biotypes could develop over numerous nematode generations due to selection pressure from the use of resistant cultivars.

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