Chapter 2: Development of Selected Tobacco Cyst Nematode Populations on Resistant and Susceptible Cultivars of Flue-cured Tobacco.

Abstract: Tobacco cyst nematode (*Globodera tabacum solanacearum*) populations were sampled from eleven Virginia locations, three North Carolina sites, and one Maryland farm. Populations from each location were maintained and increased on the flue-cured tobacco cultivar K326 through the duration of the experiments. Plants of flue-cured tobacco cultivar K326 (susceptible) and NC567 (resistant) were each inoculated with 6,000 TCN eggs per plant. Tests were conducted over one generation (6 wk) and two generations (14 wk). Shoot and root weights and the number of nematodes present within a one gram subsample of feeder roots were recorded at completion of the tests. Nematode counts were subdivided by nematode life stage (vermiform, swollen, pyriform, and adult). Although significant differences in nematode development were detected among populations, differences were not consistent across experiments. Results indicate similar pathogenicity among TCN populations on resistant and susceptible flue-cured tobacco cultivars.

Key Words: Tobacco cyst nematodes, resistance, flue-cured tobacco.

The tobacco cyst nematode (TCN), *Globodera tabacum solanacearum* (Miller and Gray, 1972) Behrens, 1975, is one of the most serious pathogens Virginia flue-cured tobacco (*Nicotiana tabacum* L.) farmers must deal with. Currently, TCN infests an estimated one-third of all flue-cured tobacco acreage in Virginia, costing farmers an estimated $3,000,000 annually in crop losses and pesticide expenditures (C.S. Johnson, 1998, personal communication). Infested fields average an estimated 15% yield loss annually, and some complete crop losses have been reported (Komm *et al.*, 1983). Current control measures for TCN include crop rotation, sanitation, and application of nematicides (Reed *et al.*, 1997). There are no agronomically desirable TCN-resistant cultivars available for farmers, although flue-cured tobacco cultivars such as Coker 371-Gold, Speight G-80, and NC567 can reduce TCN population densities (Reed *et al.*, 1997). However, heavy yield losses may occur when these cultivars are planted in highly infested fields, even though TCN levels are reduced. Tobacco breeding programs may be close to the introduction of an agronomically desirable TCN-resistant cultivar.

Sources of TCN resistance have been reported to include *Nicotiana longiflora* L., *N. glutinosa* L., and *N. plumbiginiflora* L. (Baalawy and Fox, 1971). Additional sources include older dark-fired (DVA 606) and burley (BVA 523) tobacco breeding lines, as well as in more recent flue-cured cultivars (PD4 and VA81) (Elliott *et al.*, 1986; Miller *et al.*, 1972; Spasoff *et al.*, 1971). Early studies involving these sources indicated that TCN resistance may be of a multigenic nature (Elliot *et al.*, 1986; Miller *et al.*, 1972). TCN resistance has also been linked to resistance to wildfire, caused by the pathogen *Pseudomonas syringae* pv. *tabaci* (Spasoff *et al.*, 1971). However, recent studies have

shown that the association between resistance to the two pathogens may be weaker than originally thought (Gwynn *et al.*, 1986; Hayes *et al.*, 1997). Currently available flue-cured tobacco cultivars with TCN-resistance perform poorly in terms of yield and quality when compared to susceptible cultivars planted in nematicide-treated soil (Johnson, 1990; Johnson *et al.*, 1989). However, more recently developed TCN resistant breeding lines exhibit better yield and quality.

Resistance breaking biotypes have been reported for the potato cyst nematodes (*Globodera pallida* (Stone, 1973) Behrens, 1975 and *G. rostochiensis* (Woll., 1923) Behrens, 1975) and the soybean cyst nematode (*Heterodera glycines* Ichinohe, 1952) (Arntzen *et al.*, 1994; Caviness, 1992). The potential stability of resistance to TCN was questioned in the original description of the pathogen, when differences in host range were reported among several populations of the nematode (Miller and Gray, 1972). Elliot *et al.* (1986) concluded that TCN resistance was stable over a period of several years on the tobacco cultivars PD4 and VA 81. However, this study was conducted at a single location, and thus involved only one isolate of the pathogen. The potential existence of TCN biotypes able to reproduce on resistant cultivars should be investigated to assure plant breeders that a single TCN isolate can be relied upon for resistance screening purposes. This research compared the reproductive ability of 15 geographically distinct TCN populations on a susceptible (K326) and resistant (NC567) cultivar of flue-cured tobacco.

Materials and Methods

Site Selection and Sampling-Fifteen locations infested with *Globodera tabacum solanacearum* were selected based on age of infestation, level of infestation, and geographic location. Soil was sampled from the fifteen locations between 13 March 1996 and 6 April 1996. Table 2.1 presents the location and identification for each site. All fields had been planted in tobacco the previous year. Nematicides used in sampled fields included fenamiphos, 1,3-dichloropropene, methyl bromide, ethroprop, or chloropicrin. Soil was collected randomly within each location using a standard soil probe taking cores 2 cm in diameter to a maximum depth of 20 cm. Sampling was conducted over the entire area of the field previously in tobacco production. A criss-cross sampling procedure was used to obtain an adequate representation of the nematode population in the entire field (Barker *et al.*, 1984). Twenty 1,000 cm^3 soil samples were collected at each location and stored in polyethylene-lined paper bags at room temperature. Cysts were extracted from soil samples using a modified Fenwick Can (Caswell *et al.*, 1985). Cysts were washed off the sieve and poured onto filter paper, where they were allowed to dry overnight. Finally, cysts were stored in capped test tubes at room temperature until further use. Population densities in the samples from each site are presented in Table 2.1.

Soil Analysis-Soil texture and pH were analyzed for each of the 15 sites. Day's (1965) procedure was used to classify each soil based on clay, sand, and silt content. Results from these tests are presented in Table 2.1.

Seedling Preparation-Five week-old tobacco seedlings were transplanted to 11 cm clay pots containing 300 cm^3 of a steam sterilized one part steam sandy loam: two

parts fine quartz sand mix (84% sand, 10% silt, 6% clay; pH 5.7). Soil mix was chosen in order to allow adequate water drainage. Seedlings were allowed to grow for one week prior to inoculation. Plants were watered using an automatic watering system. The amount of water delivered was determined during the one week growth period prior to inoculation and an amount was selected that maintained soil moisture, but did not produce standing water in the pots. In the first two experiments, plants were fertilized manually with a 17-5-24 (17% nitrogen, 5% phosphorus, 24% soluble potash, 2% water soluble magnesium, 2.64% sulfur, 0.085% molybdenum, and 0.055% zinc) solution calibrated to deliver 125 mg/kg nitrogen. Subsequent trials were fertilized using a microinjector to deliver the same 125 mg/kg nitrogen rate.

Physiological State Homogeneity and Population Proliferation-Due to variation in sample dates and field histories, cysts from each population were inoculated onto the susceptible cultivar K326 to increase each population and to standardize the physiological state of the populations. One hundred cysts from each population were placed in a trench cut around the root zone of a single six week old tobacco seedling growing in 300 cm^3 of the sterilized 1:2 soil:sand mix. Trenches were covered with an additional 100 cm^3 of soil after inoculation. Four pots were maintained for each population. Inoculated plants were maintained in root zone chambers at 27°C during the day and 25°C at night. Twelve weeks after inoculation, cysts were extracted using a modified Fenwick can, dried overnight, and stored in capped test tubes at room temperature until use.

Inoculation of Main Tests-Populations were randomly divided into two groups to keep experiments at a manageable size (Table 2.1). The Coffee population was included

in both groups as a standard to compare overall virulence across experiments. Groups I and II each contained seven populations, as well as the Coffee population and a non-inoculated control. Stored cysts were crushed using a blender for one minute prior to inoculation. Inoculum of each population was standardized to 6,000 TCN eggs in 12 mL of tap water for each 11 cm clay pot. Inoculations were performed by pipetting the egg suspension into a trench around the root zone of a single tobacco seedling. Trenches were covered with an additional 100 cm^3 of the 1:2 soil:sand mix after inoculation. Experiments were conducted on benchtops in the greenhouse or in a walk-in growth chamber. In all experiments, temperature was stable and ranged from 23°C to 30°C

Experimental Design-Initial experiments were arranged in randomized complete blocks, using a factorial treatment design with cultivar (NC567 or K326) and nematode population as the two factors. Each cultivar-population combination was replicated eight times. Non-inoculated control pots were also included in each experiment for top and root weight comparisons with inoculated plants. Each experiment included populations from either Group I or II and continued for six weeks after inoculation. Experiments were repeated once, so that reproduction of each population was evaluated in two separate experiments. Reproduction of all 15 populations on the resistant cultivar NC567 was investigated in two additional six week experiments. These experiments were conducted using a randomized complete block design with eight replications. Non-inoculated controls were also included in these tests. In the second test, eight plants of the susceptible cultivar K326 were also included to compare reproduction on the susceptible and resistant cultivars.

Two additional tests were conducted to evaluate reproduction of fourteen TCN populations on the resistant cultivar NC567 for approximately two generations (14 wk). These experiments were inoculated and maintained as previously described. Plants of the susceptible cultivar K326 were again included in the study to compare reproduction on the susceptible cultivar and the resistant cultivar.

Data Collection-Plants were carefully removed from the soil six or fourteen weeks after inoculation. Each plant was separated into root and shoot portions and blotted dry. One gram of feeder roots was randomly excised from the shoot/root transition area of each root system and washed over a 400-mesh sieve. The feeder root subsample and root wash materials were then combined and stained with acid fuchsin (Byrd *et al.*, 1983). Nematodes were divided into four categories: vermiform, swollen (distinct sausage shape), pyriform (flask shape), and adult (saccate shape bearing eggs) using classification guidelines similar to those of Wang (1996).

Statistical Analysis-Due to increasing variance with an increase in treatment means, data were subjected to log transformation [$\log_{10}(x + 1)$] prior to statistical analysis. Analyses of variance were performed using SAS and the Waller-Duncan means separation procedure (k-ratio = 100) (SAS Institute, 1989). Where population-cultivar interactions were not significant ($P > 0.05$), combined analysis was performed across cultivars. Statistical analyses were combined across experiments when respective mean square error terms were not significantly different ($P > 0.05$) according to an F-test (Gomez and Gomez, 1984) and experimental interactions were deemed non-significant (P

> 0.05). Additional T-tests were conducted to investigate whether the populations as a group developed differently on the two cultivars.

Results

Total numbers of vermiform, pyriform, adult, and total nematodes were higher ($P \leq 0.05$) on the susceptible K326 than on NC567 for both groups of nematode populations in both trials (Table 2.2). 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 populations in the number of nematodes in roots of K326 were inconsistent across trials (Table 2.3). In trial 1, more vermiform nematodes were counted after inoculation with eggs from the Bowman, Coffee, Moore, Newburg, and Proffitt locations than for the SPAREC and Warren populations. However, similar differences were not observed in trial 2. Inoculation with the Proffitt population in trial 1 resulted in more swollen nematodes in roots of K326 than when the SPAREC population 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 populations in trial 1, more pyriform nematodes were observed on K326 in the second trial from the Baskerville, Bowman, Coffee, Newburg, and SPAREC populations than for nematodes from the Warren and Proffitt sites. No relative differences in adult nematodes were observed on K326 among Group I populations in trial 1. However, more adult nematodes were observed on K326 for the SPAREC population than for the Warren site in trial 2.

Very few differences were observed among Group II populations in the number of nematodes on K326 (Table 2.3). Similar numbers of vermiform, pyriform, adult, and total nematodes were observed among Group II populations in roots of susceptible K326. More swollen nematodes from the Coffee location were found on K326 than for the Bing location in trial 1, but not in trial 2. More swollen nematodes from the Clary and Rideout populations were found in the roots of K326 compared to the Vance population in the second trial, but not in the first. Consequently, when differences were observed among Group II populations in reproduction on K326, these differences were inconsistent across experiments.

Reproduction on NC567-Differences observed among Group I TCN populations on the resistant cultivar NC567 were also inconsistent across trials. In the first trial, more vermiforms were observed in roots inoculated with the Moore population than those inoculated with the SPAREC population (Table 2.4). However, in the second trial, the Proffitt population produced more vermiform nematodes than did the SPAREC, Warren, and Newburg populations. No differences in number of swollen nematodes were observed in the second trial, but the Warren population produced more swollen nematodes than did the SPAREC and Proffitt populations in the first trial. No differences in the number of pyriform nematodes were noted among populations in either trial. In the first trial, no differences were detected in the number of adults, but more adults were found in the second trial on the roots of plants inoculated with the SPAREC population than the Newburg and Bowman populations. Additionally, the Proffitt population produced more adults in the roots of NC567 than did the Bowman population.

As was the case for K326, fewer differences were noted on NC567 for Group II populations than among the Group I populations. In fact, no significant differences were found among Group II populations at any of the TCN life stages when inoculated on the TCN-resistant cultivar NC567 (Table 2.4).

All Populations on NC567 for one generation-Nematode numbers were similar in roots of NC567 for all populations in both trials that included all 15 populations (Table 2.5). The SPAREC population produced more pyriform and adult nematodes on the susceptible cultivar K326 than were produced by any of the populations on NC567. Total nematode numbers were higher for the SPAREC population on K326 than for any of the populations on NC567, except for TCN from the Bertie and Hastings locations.

All populations on NC567 for two generations-Statistical analyses of numbers of vermiform and total nematodes in roots of NC567 over 14 wk (~two generations) could not be combined due to significant experiment by treatment interactions. Combined statistical analyses were performed across the two trials comparing numbers of swollen, pyriform, and adult TCN. No significant differences were observed among populations in number of nematodes on NC567 at any individual life stage in the 14 wk trials (Table 2.6). Fewer total TCN were observed on NC567 for the Coffee and Hastings populations compared to the Proffitt and Vance populations in the first 14 wk experiment, but differences in total nematode numbers were absent among populations on NC567 in the second 14 wk trial. Roots of K326 inoculated with the SPAREC population contained more swollen, pyriform, adult, and total nematodes than those of NC567 inoculated with TCN from any of the 15 sites.

Fresh Weights-Combined analysis was performed on fresh weight results across cultivars and experiments because no significant interactions were present and mean square error terms were similar. Neither Group I or Group II populations reduced shoot weight of either cultivar (Table 2.7). Group I populations did not reduce shoot weight of either cultivar. Only the Bing and Hastings populations reduced root weight among the Group II populations.

No differences were observed in the root and shoot weight of NC567 among populations in the first single generation test (Table 2.8). Shoot weight was higher in the second single generation trial for plants inoculated with the Baskerville or Moore populations than for plants inoculated with TCN from the Clary farm. Root weight was also higher in the second single generation trial when inoculum originated from the Moore versus the Newburg population. Inoculation with TCN had no effect on fresh weight of shoots or roots of NC567 in the multiple generation experiments.

Discussion

Parasitism by *Globodera tabacum solanacearum* was consistently and significantly lower on the TCN-resistant cultivar NC567 than on the TCN-susceptible cultivar K326. Difference 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 which showed that resistance to TCN inhibits nematode development rather than nematode penetration (Baalawy and Fox, 1971; LaMondia, 1988). However, significant differences were detected in certain trials in the number of vermiform and swollen nematodes found on NC567 and K326. The lack of significant population-

cultivar interactions suggests that the expression of resistance in NC567 was similar for all populations involved in this study. The cultivar NC567 should reduce nematode reproduction of most, if not all, TCN populations.

Tobacco cyst nematode development was similar among the populations tested on the TCN-susceptible cultivar K326. Numbers of swollen nematodes in roots of K326 differed among populations in all four trials where the populations were broken into groups, but differences for the other TCN life stages were only detected in one trial out of four. The differences detected among populations also rarely coincided from trial to trial. Variation between experiments could be attributed to several different factors. Slight differences in temperature have been known to affect TCN reproduction (Adams *et al.*, 1982). Additionally light quality and daylength were found to influence reproduction of potato cyst nematodes (Franco and Evans, 1979). Since these experiments were conducted at different times of the year, these factors could have differed slightly between experiments.

Reproduction on the TCN-resistant cultivar NC567 was generally consistent over experiments. This result suggests that few, if any, differences in aggressiveness among the populations examined in this study. Fifteen populations from a large range of geographic area and length of infestation should be a good representation of all TCN populations. This conclusion should enable breeders to reliably screen tobacco germplasm against a single TCN population, thus saving time and money in developing improved TCN-resistant tobacco cultivars.

The experiments in this study only spanned one or two generations of the nematode. Turner (1990) found that potato cyst nematode (*Globodera pallida*) populations behaved differently after 11 generations of reproduction on resistant potato hybrids of *Solanum vernei* (Bitt and Wittm.). Additionally, it was found that the efficacy of resistance to the potato cyst nematode started to decline gradually after four to five generations. Elliott *et al.* (1986) and Johnson (1990) found that resistance to TCN was effective over a three year period (approximately 10 to 12 TCN 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 TCN resistance.

In summary, the TCN populations used in this study developed and reproduced similarly upon a resistant (NC567) and a susceptible (K326) flue-cured tobacco cultivar over one or two nematode generations. Therefore, plant breeders may effectively use only one tobacco cyst nematode population when screening future tobacco germplasms for TCN resistance. However, this study does not preclude the possibility that resistant breaking biotypes could develop over numerous nematode generations due to selection from a resistant cultivar. Additional research should focus on the long term stability of tobacco cyst nematode resistance prior to the widespread release of a commercially available TCN-resistant cultivar.

Table 2.1. Group assignments, soil pH, texture, and nematode population densities from fifteen tobacco cyst nematode infested locations.

Group	Site Location	Identification	Soil Characteristics					TCN Eggs per 500 cc soil
			Soil pH	% Clay	% Sand	% Silt	Classification	
I	Charles Co., MD	Newburg	6.13	7.1	50.7	42.2	Sandy Loam	29,777
I	Dinwiddie Co., VA	Baskerville	4.83	10.2	44.0	45.8	Loam	19,426
I	Lunenburg Co., VA	Moore	5.35	7.1	69.7	23.2	Sandy Loam	13,908
I	Nottoway Co., VA	SPAREC[a]	5.37	9.9	74.5	15.6	Sandy Loam	11,902
I	Nottoway Co., VA	Bowman	5.09	9.4	74.4	16.2	Sandy Loam	6,977
I	Powhatan Co., VA	Proffitt	5.99	10.0	70.9	19.1	Sandy Loam	15,550
I	Warren Co., NC	Warren	5.83	15.5	67.8	16.7	Sandy Loam	13,133
I&II	Lunenburg Co., VA	Coffee	4.92	7.4	79.1	13.5	Loamy Sand	19,015
II	Amelia Co., VA	Hastings	5.66	12.5	72.8	14.7	Sandy Loam	15,823
II	Bertie Co., NC	Bertie	5.06	7.8	79.1	13.1	Loamy Sand	9,713
II	Brunswick Co., VA	Lewis	5.52	7.4	75.8	16.7	Loamy Sand	6,110
II	Dinwiddie Co., VA	Rideout	5.63	18.6	69.9	11.5	Sandy Loam	5,335
II	Mecklenburg Co., VA	Bing	5.31	15.5	69.5	15.0	Sandy Loam	14,364
II	Mecklenburg Co., VA	Clary	5.72	10.7	71.1	18.2	Sandy Loam	6,840
II	Vance Co., NC	Vance	5.77	9.7	79.4	10.9	Loamy Sand	5,335

[a]Southern Piedmont Agricultural Research and Extension Center

Table 2.2. Development of a tobacco cyst nematode (*Globodera tabacum solanacearum*-TCN) on the TCN-resistant cultivar NC567 versus the TCN-susceptible cultivar K326.

	Nematodes per gram of feeder root for Group I Populations[a]									
	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
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
p-value	0.0001*	0.0013*	0.0001*	0.6284	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*	0.0001*

	Nematodes per gram of feeder root for Group II Populations[a]									
	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
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
p-value	0.0113*	0.0033*	0.0032*	0.0010*	0.0002*	0.0001*	0.0001*	0.0001*	0.0005*	0.0001*

[a]All studies trials were conducted on a benchtop in the greenhouse. For Group I populations, Trial 1 was conducted Nov.-Dec., 1996 and Trial 2, July-Aug., 1997. For Group II populations, Trial 1 was conducted Jan.-Feb., 1997, and Trial 2, Sept.-Oct., 1997.

Values indicate p-values and non-transformed means from 64 observations. T-testing was performed at $P \leq 0.05$ significance on log transformed data [$\log_{10}(x+1)$].

Table 2.3. Comparison of *Globodera tabaucm solanacearum* population development on the tobacco cultivar K326.

	Nematodes per gram of feeder root: Group I Populations[a]									
	Vermiform		Swollen		Pyriform		Adult		Total	
Population	Trial 1	Trial 2	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	24.38 ab	33.00 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	33.38 a	33.75 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	33.38 a	31.25 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	66.50 a	37.13 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	43.13 a	23.63 b
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	22.88 b	50.50 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	14.88 b	26.75 b
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	48.63 a	30.88 ab
	Nematodes per gram of feeder root: Group II Populations[a]									
	Vermiform		Swollen		Pyriform		Adult		Total	
Population	Trial 1	Trial 2	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	24.24 a	31.63 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	16.44 a	34.50 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	27.74 a	29.13 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	25.88 a	34.50 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	39.23 a	25.00 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	30.49 a	27.13 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	28.08 a	22.75 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	26.54 a	29.25 a

[a]All studies conducted on greenhouse benchtops. For Group I populations, Trial 1 was conducted Nov.-Dec., 1996 and Trial 2, July-Aug., 1997. For Group II populations, Trial 1 was conducted Jan.-Feb., 1997 and Trial 2, Sept.-Oct., 1997. Data were transformed [$\log_{10}(x+1)$] prior to statistical analysis. Means followed by the same letter(s) are not significantly different according to the Waller-Duncan test (k-ratio=100). Data represent non-transformed means of eight replications.

Table 2.4. Comparison of *Globodera tabaucm solanacearum* population development on the tobacco cultivar NC567.

	Nematodes per gram of feeder root: Group I Populations[a]									
	Vermiform		Swollen		Pyriform		Adult		Total	
Population	Trial 1	Trial 2	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	11.63 ab	17.75 ab
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	8.25 ab	15.00 ab
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	17.50 a	18.57 ab
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	5.88 b	12.86 ab
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	18.00 ab	23.25 a
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	5.00 b	10.13 b
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	14.50 ab	12.43 ab
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	8.38 ab	14.63 ab
	Nematodes per gram of feeder root: Group II Populations[a]									
	Vermiform		Swollen		Pyriform		Adult		Total	
Population	Trial 1	Trial 2	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	21.65 a	16.50 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	18.59 a	17.63 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	16.37 a	25.25 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	22.51 a	12.63 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	16.34 a	13.38 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	17.34 a	21.25 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	17.80 a	12.88 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	13.16 a	15.25 a

[a]All studies conducted on greenhouse benchtops. For Group I populations, Trial 1 was conducted Nov.-Dec., 1996 and Trial 2, July-Aug., 1997. For Group II populations, Trial 1 was conducted Jan.-Feb., 1997 and Trial 2, Sept.-Oct., 1997. Data were transformed [$\log_{10}(x+1)$] prior to statistical analysis. Means followed by the same letter(s) are not significantly different according to the Waller-Duncan test (k-ratio=100). Data represent non-transformed means of eight replications.

Table 2.5. Development of 15 populations of *Globodera tabacum solanacearum* on the flue-cured tobacco cultivar NC567 over a six week period.

Trial 1[a]	Nematode per gram of feeder root				
Population	Vermiform	Swollen	Pyriform	Adult	Total
Baskerville	20.00 a	3.83 a	1.00 a	0.00 a	24.83 a
Bertie	18.83 a	4.33 a	1.33 a	0.00 a	24.50 a
Bing	16.63 a	3.13 a	0.88 a	0.13 a	20.75 a
Bowman	15.11 a	3.75 a	1.00 a	0.00 a	19.75 a
Clary	15.05 a	3.88 a	1.63 a	0.25 a	23.50 a
Coffee	15.88 a	3.38 a	1.38 a	0.00 a	20.63 a
Hastings	17.50 a	2.75 a	1.63 a	0.13 a	22.00 a
Lewis	19.75 a	3.88 a	2.25 a	0.25 a	26.13 a
Moore	18.57 a	3.71 a	1.57 a	0.14 a	24.00 a
Newburg	18.50 a	4.00 a	1.75 a	0.13 a	24.38 a
Proffitt	16.38 a	4.13 a	1.75 a	0.25 a	22.50 a
Rideout	17.88 a	3.50 a	2.00 a	0.25 a	23.63 a
SPAREC	15.38 a	2.25 a	1.63 a	0.25 a	19.50 a
Vance	16.38 a	3.88 a	1.75 a	0.38 a	22.38 a
Warren	16.25 a	3.25 a	2.13 a	0.13 a	21.75 a
Trial 2[a]					
Baskerville	26.75 a	3.38 a	1.75 b	1.13 b	33.00 b
Bertie	36.25 a	5.75 a	2.88 b	1.75 b	46.63 ab
Bowman	28.50 a	4.00 a	2.50 b	1.38 b	36.38 b
Clary	35.13 a	3.50 a	1.88 b	1.25 b	41.75 b
Coffee	22.88 a	3.38 a	1.88 b	1.50 b	29.63 b
Hastings	34.38 a	5.25 a	2.63 b	2.00 b	44.25 ab
Lewis	27.25 a	4.00 a	1.50 b	1.88 b	34.63 b
Moore	23.63 a	3.75 a	2.25 b	1.38 b	31.00 b
Newburg	26.00 a	2.88 a	1.63 b	1.63 b	32.13 b
Proffitt	25.13 a	3.63 a	1.75 b	1.50 b	32.00 b
Rideout	29.63 a	3.63 a	2.38 b	2.00 b	37.63 b
SPAREC-NC	27.00 a	4.00 a	1.50 b	1.38 b	33.88 b
Vance	29.75 a	4.25 a	2.38 b	1.50 b	37.88 b
Warren	25.00 a	3.00 a	1.88 b	1.75 b	31.63 b
SPAREC-K	27.75 a	5.38 a	10.38 a	16.63 a	60.13 a

[a]Trial 1 was conducted Oct.-Nov. 1997, Trial 2 conducted Jan.-Feb. 1998 on greenhouse benchtops. Data were transformed [$\log_{10}(x+1)$] prior to statistical analysis. Means followed by the same letter(s) are not significantly different according to Waller-Duncan test (k-ratio=100). Data represent non-transformed means of eight replications.

Table 2.6. Numbers of tobacco cyst nematodes (*Globodera tabacum solanacearum*) in roots of the cultivar NC567 fourteen weeks after inoculation.

	Nematodes per gram of feeder root						
	Vermiform		Swollen	Pyriform	Adult	Total	
Population	Trial 1[a]	Trial 2[a]	Trial 1&2	Trial 1&2	Trial 1&2	Trial 1	Trial 2
Baskerville	26.22 a	31.63 b	2.96 b	1.50 b	1.48 b	31.23 bc	38.50 b
Bertie	23.97 a	26.14 b	2.15 b	1.24 b	1.24 b	27.95 bc	31.43 b
Bowman	24.50 a	27.50 b	2.38 b	1.13 b	1.13 b	28.25 bc	33.00 b
Clary	24.05 a	28.57 b	2.53 b	1.09 b	1.00 b	27.13 bc	34.71 b
Coffee	21.79 a	31.00 b	2.52 b	1.19 b	0.88 b	24.57 c	37.38 b
Hastings	21.50 a	28.25 b	2.31 b	1.13 b	1.13 b	24.13 c	33.88 b
Lewis	25.76 a	28.50 b	2.00 b	1.43 b	1.40 b	30.29 bc	33.63 b
Moore	26.14 a	29.38 b	2.79 b	1.52 b	1.27 b	30.96 bc	35.63 b
Newburg	27.01 a	31.50 b	2.97 b	1.83 b	1.42 b	33.06 bc	37.88 b
Proffitt	30.85 a	25.75 b	2.60 b	1.57 b	1.60 b	35.37 b	32.75 b
Rideout	23.03 a	26.63 b	2.52 b	1.50 b	1.00 b	26.82 bc	32.88 b
SPAREC	25.17 a	26.75 b	2.27 b	1.27 b	1.17 b	28.40 bc	32.75 b
Vance	30.69 a	25.13 b	2.44 b	1.31 b	1.47 b	35.62 b	30.63 b
Warren	27.69 a	28.75 b	2.84 b	1.13 b	1.38 b	32.01 bc	35.13 b
SPAREC-K	30.63 a	64.13 a	13.94 a	23.38 a	38.56 a	74.25 a	172.25 a

[a]Trial 1 conducted Jan.-May 1998 in a walk-in growth chamber, Trial 2 conducted April-June, 1998 on greenhouse benchtops.

Data were transformed [$\log_{10}(x+1)$] prior to statistical analysis. Means followed by the same letter(s) are not significantly different according to the Waller-Duncan test (k-ratio = 100).

Data represent non-transformed means of eight replications when trials were analyzed separately and 16 replications where combined analysis across trials was performed.

Table 2.7. Effects of Group I and Group II populations on root and shoot weight of tobacco.

Group I	Fresh Weight (g)		T-test for Ho: K326=NC67	
Population	Shoot	Root	Shoot	Root
Baskerville	15.50 a	6.30 a	0.4664	0.2307
Bowman	15.26 a	6.82 a	0.6394	0.7358
Coffee	15.91 a	6.70 a	0.8455	0.9965
Moore	14.70 a	6.08 a	0.4545	0.7442
Newburg	16.41 a	6.68 a	0.8791	0.7190
Proffitt	15.73 a	6.92 a	0.8234	0.7032
SPAREC	15.11 a	6.00 a	0.2801	0.1695
Warren	17.46 a	7.15 a	0.6702	0.5929
UTC	16.20 a	6.91 a	0.1772	0.0384*
Group II				
Bertie	31.91 a	12.11 ab	0.5655	0.3789
Bing	32.37 a	11.67 b	0.7772	0.4976
Clary	35.19 a	12.68 ab	0.8423	0.8440
Coffee	31.09 a	10.36 ab	0.5872	0.3075
Hastings	32.26 a	12.03 b	0.9497	0.8803
Lewis	37.57 a	12.00 ab	0.9092	0.6771
Rideout	37.75 a	12.23 ab	0.5623	0.9376
Vance	35.09 a	12.42 ab	0.7098	0.6036
UTC	33.30 a	14.77 a	0.9351	0.9416

Data were transformed [$\log_{10}(x+1)$] prior to statistical analysis. Means followed by the same letter(s) are not significantly different according to the Waller-Duncan test (k-ratio=100). Data represent non-transformed means of 16 replications.

Table 2.8. Fresh shoot and root weights for trials involving all tobacco cyst nematode populations inoculated on the tobacco cultivar NC567.

	Fresh Wt. (g)					
	Single Generation Tests				Multiple Generation Tests	
	Trial 1		Trial 2		Trials 1 and 2	
Population	Shoot	Root	Shoot	Root	Shoot	Root
Baskerville	53.68 a	14.60 a	54.68 a	5.15 ab	116.70 a	18.20 a
Bertie	54.86 a	19.65 a	47.04 ab	4.55 ab	120.57 a	15.72 a
Bing	49.31 a	15.38 a	nt[a]	nt	nt	nt
Bowman	56.30 a	15.11 a	49.41 ab	4.90 ab	75.28 a	9.93 a
Clary	57.10 a	15.05 a	41.76 b	5.06 ab	84.79 a	16.38 a
Coffee	56.49 a	14.81 a	51.98 ab	6.31 ab	135.60 a	15.44 a
Hastings	56.04 a	14.07 a	50.94 ab	5.14 ab	126.71 a	18.35 a
Lewis	67.52 a	19.82 a	51.51 ab	5.07 ab	96.17 a	13.34 a
Moore	56.65 a	16.73 a	57.29 a	6.69 a	104.76 a	16.40 a
Newburg	49.45 a	15.55 a	43.88 ab	4.21 b	118.66 a	17.02 a
Proffitt	57.34 a	15.26 a	50.48 ab	5.31 ab	103.47 a	12.06 a
Rideout	56.01 a	12.32 a	51.49 ab	5.96 ab	99.89 a	12.30 a
Vance	57.14 a	15.80 a	46.85 ab	5.14 ab	118.47 a	14.48 a
Warren	58.15 a	11.76 a	47.84 ab	5.92 ab	112.66 a	16.90 a
SPAREC-NC	52.06 a	14.78 a	53.70 ab	5.70 ab	91.22 a	14.07 a
SPAREC-K	nt	nt	50.56 ab	6.02 ab	118.82 a	15.94 a
UTC	55.16 a	13.78 a	52.12 ab	6.72 a	109.65 a	16.06 a

[a]nt-not tested

Data were transformed [$\log_{10}(x+1)$] prior to statistical analysis. Means within columns followed by the same letter(s) are not significantly different according to the Waller-Duncan test (k-ratio=100). Data represent non-transformed means of 8 replications when trials were analyzed separately and 16 replications when combined.

Literature Cited

Adams, H.S., W.W. Osborne, and A.J. Webber, Jr. 1982. Effect of temperature on development and reproduction of *Globodera solanacearum*. Nematropica 12:305-311.

Arntzen, F.K., J.H.M. Visser, and J. Hoogendoorn. 1994. Differences in virulence between some potato cyst nematode populations to potato genotypes with monogenic resistance to *Globodera pallida* from *Solanum multidissectum* or *S. tuberosum* ssp. *Andigena* CPC1673. Fundamental and Applied Nematology 17:190-192.

Baalawy, H.A. and J.A. Fox. 1971. Resistance to Osborne's cyst nematode in selected *Nicotiana* species. Journal of Nematology 3:395-398.

Barker, K.R., D.P. Schmitt, and J.P. Noe. 1984. Role of sampling for crop-loss assessment and nematode management. Agriculture, Ecosystems, and Environment 12: 355-369.

Byrd, D.W., T. Kirkpatrick, and K.R. Barker. 1983. An improved technique for clearing and staining plant tissues for detection of nematodes. Journal of Nematology 15:142-143.

Caswell, E.P., I.J. Thomason, and H.E. McKinney. 1985. Extraction of cysts and eggs of *Heterodera schachtii* from soil with an assessment of extraction efficiency. Journal of Nematology 17:337-340.

Caviness, C.E. 1992. Breeding for resistance to soybean cyst nematode. Pp. 143-156 in Biology and Management of Soybean Cyst Nematode, R.D. Riggs and J.A. Wrather, eds., APS Press, St. Paul, MN. 186pp.

Day, P.R. 1965. Particle fractionation and particle-size analysis. Pp. 545-567 in Methods of Soil Analysis. C.A. Black, ed. American Society of Agronomy, Madison WI. 770 pp.

Elliott, A.P., P.M. Phipps, and T.R. Terrill. 1986. Effects of continuous cropping of resistant and susceptible cultivars on reproduction potentials of *Heterodera glycines* and *Globodera tabacum solanacearum*. Journal of Nematology 18:375-379.

Franco, J. and K. Evans. 1979. Effects of daylight on the multiplication of potato cyst nematodes (*Globodera* spp.) populations. Nematologica 25:184-190.

Gomez, K.A. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research 2nd edition. John Wiley and Sons, Inc., New York. 680 pp.

Gwynn, G.R., K.R. Barker, J.J. Reilly, D.A. Komm, L.G. Burk, and S.M. Reed. 1986. Genetic resistance to tobacco mosaic virus, cyst nematodes, root-knot nematodes, and

wildfire from *Nicotiana repanda* incorporated into *N. tabacum*. Plant Disease 70:958-962.

Hayes, A.J., C.A. Wilkinson, and C.S. Johnson. 1997. Evaluation of tobacco accessions for resistance to tobacco cyst nematode and wildfire. Crop Science 37:586-591.

Johnson, C.S. 1990. Control of *Globodera tabacum solanacearum* by rotating susceptible and resistant flue-cured tobacco cultivars. Supplement to Journal of Nematology 22(4S):700-706.

Johnson, C.S., D.A. Komm, and J.L. Jones. 1989. Control of *Globodera tabacum solanacearum* by alternating host resistance and nematicide. Journal of Nematology 21:16-23.

Komm, D.A., J.J. Reilly, and A.P. Elliott. 1983. Epidemiology of a tobacco cyst nematode (*Globodera solanacearum*) in Virginia. Plant Disease 67:1249-1251.

LaMondia, J.A. 1988. Tobacco resistance to *Globodera tabacum.* Annals of Applied Nematology 2:77-80.

Miller, L.I., J.A. Fox, and L. Spasoff. 1972. Genetic relationship of resistance to *Heterodera solanacearum* in dark-fired and burley tobacco. Phytopathology 62:778. (Abstr.).

Miller, L.I., and B.J. Gray. 1972. *Heterodera solanacearum* n. sp., a parasite of solanaceous plants. Nematologica 18:404-413.

Reed, T.D., J.L. Jones, C.S. Johnson, P.J. Semtner, B.B. Ross, and C.A. Wilkinson. 1997. 1998 Flue-Cured Production Guide. Virginia Cooperative Extension. Publication 436-048. 116Pp.

SAS Institute, Inc. 1989. SAS/STAT user's guide, version 6, 4th ed., vol. 1. Cary, NC: SAS Institute, Inc.

Spasoff, L., J.A. Fox, and L.I. Miller. 1971. Multigenic inheritance of resistance to Osborne's Cyst Nematode. Journal of Nematology 3:329-330. (Abstr.).

Turner, S.J. 1990. The identification and fitness of virulent potato cyst nematode populations (*Globodera pallida*) selected on resistant *Solanum vernei* hybrids for up to eleven generations. Annals of Applied Biology 117:385-397.

Wang, J.. 1996. Characterizing resistance in flue-cured tobacco to *Globodera tabacum solanacearum*. Ph.D. dissertation. Virginia Polytechnic Institute and State University. Pp. 126.