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Reproduction of *Globodera tabacum solanacearum* in Seven Flue-Cured Tobacco-Producing Soils[1]

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Abstract: The tobacco cyst nematode (*Globodera tabacum solanacearum*) continues to pose a serious threat to flue-cured tobacco production in Virginia and nearby states. Soils were sampled from five uninfested and two infested flue-cured tobacco-producing locations. Twenty-three edaphic factors were characterized to determine if any were correlated with *G. t. solanacearum* reproduction. Comparisons were also made between pasteurized and natural soils to determine if biological suppression of *G. t. solanacearum* reproduction might be occurring in currently uninfested areas. Differences in *G. t. solanacearum* reproduction were noted among the soils, but results were inconsistent across the three trials conducted in this study. Only soil pH correlated significantly with nematode reproduction, and then only in one of three trials. *Globodera tabacum solanacearum* reproduced with similar efficiency in natural and pasteurized soils.

Key words: cyst nematode, flue-cured tobacco, *Globodera tabacum solanacearum,* nematode, *Nicotiana tabacum,* soil edaphic factors, suppressive soil, tobacco cyst nematode.

The tobacco cyst nematode *Globodera tabacum solanacearum* (Miller and Gray) Behrens costs Virginia flue-cured tobacco (*Nicotiana tabacum* L.) farmers an estimated $1.5 million annually in crop loss and pesticide expenditures (C.S. Johnson, unpubl.). Infested fields average an estimated 15% yield loss annually, although total crop losses in some fields have been reported (Komm et al., 1983).

Globodera tabacum solanacearum was first reported on flue-cured tobacco in Amelia County, Virginia, in 1961 (Osborne, 1961). By 1972 the nematode had been found on 18 farms in Amelia, Dinwiddie, and Nottoway counties (Miller and Gray, 1972) and by 1983 Komm et al. (1983) reported that *G. t. solanacearum* infested 148 flue-cured tobacco farms in 10 Virginia counties. Currently, *G. t. solanacearum* infests an estimated one-third of Virginia's flue-cured tobacco acreage in 13 counties (C.S. Johnson, unpubl.) The first report of *G. t. solanacearum* outside Virginia occurred in 1990, from Warren County, North Carolina (Melton et al., 1991), and it has been found on farms in seven North Carolina counties (T.A. Melton, pers. comm.). Additionally, *G. t. solanacearum* was found on a tobacco farm in Charles County, Maryland, in 1995 (Johnson, 1998).

Globodera tabacum solanacearum has spread mainly to the south, despite movement of equipment and plant materials in a westerly direction. An edaphic or biological factor present within soils of the uninfested region might explain this phenomenon. Nematode-suppressive soils have also been reported for other cyst nematodes, such as the soybean cyst nematode (*Heterodera glycines*) (Chen et al., 1994; Kim et al., 1998), the potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) (Clovis and Nolan, 1983; Goswami and Rumpenhorst, 1978), and the sugar beet cyst nematode (*Heterodera schachtii*) (Burnsall and Tribe, 1974; Tribe, 1979).

Edaphic factors can influence nematode reproduction. Todd and Pearson (1988) found more cysts of *H. glycines* in sandy loam than in silty loam soil. The frequency and density of *H. glycines* populations were inversely correlated with clay content in no-till fields (Workneh et al., 1999). Heatherly and Young (1991) indicated that *H. glycines* did not survive in clay soils as well as in silty loam

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soils. Anand et al. (1995) found higher numbers of soybean cyst nematode females at pH 6.5 and 7.5 than at pH 5.5. Francl (1993) correlated *H. glycines* levels positively with soil pH and magnesium levels. He also found a negative correlation between levels of copper in soil and nematode numbers. Research involving other nematodes has shown correlations between organic matter content (Norton and Hoffmann, 1974), cation exchange capacity (Norton et al., 1971), and sodium levels in the soil (Noe and Barker, 1985).

The purpose of this study was to compare *G. t. solanacearum* parasitism in soils from *G. t. solanacearum*-infested and uninfested locations and to evaluate the effects of soil pasteurization and edaphic factors on nematode reproduction.

Materials and Methods

Site selection and sampling: Six locations that were considered uninfested with the tobacco cyst nematode (*G. t. solanacearum*) were chosen based on geographic location and differences in soil type. Low numbers of the nematode were detected at one of these sites. A seventh location known to be infested with *G. t. solanacearum* was chosen to serve as a control in the experiments. Soil was sampled from Cumberland County, North Carolina, and Charlotte, Nottoway, Halifax, Mecklenburg, Pittsylvania, and Appomattox counties in Virginia during a 3-week period in February 1998. Soil was collected with a shovel randomly within a chosen field at each location to a maximum depth of 20 cm. Approximately 60 liters of soil was collected from each location. Fields were sampled thoroughly in a criss-cross pattern to obtain an accurate representation of the entire field (Barker et al., 1984), and all fields had been planted with tobacco in 1997. Soil was stored in 68-liter plastic containers at room temperature. Soil samples were stirred and moistened with tap water every other day to help maintain the integrity of the soil microflora.

Soil testing: Percent sand, silt, and clay were determined for the soil from each location (Day, 1965). A random sample also was taken from each soil and analyzed for percent organic matter; phosphorus; potassium; magnesium; calcium; sodium; soil pH; cation exchange capacity; percent base saturation for K, Mg, Ca, Na, and H; soil acidity (H^+); zinc; manganese; and copper. Chemical and physical soil analyses were conducted by A&L Laboratories (Richmond, VA). Two 250-cm^3 subsamples of soil from each location also were assayed for nematodes by elutriation and sugar flotation (Jenkins, 1964).

Seedling preparation: Five-week-old seedlings of the flue-cured tobacco cultivar K326 were transplanted to 11-cm-diam. clay pots containing 300 cm^3 of soil from each location. Seedlings were allowed to grow for 1 week prior to inoculation. Plants were watered and fertilized with a 17-5-24 fertilizer solution (17% N, 5% P, 24% K, 2% Mg, 2.64% S, 0.085% Mo, and 0.055% chelated Zn) delivered through an automated water system that included a microinjector calibrated at a rate of 125 mg/kg nitrogen.

Inoculum source and maintenance: Cyst inoculum for this study was collected from the Virginia Tech Southern Piedmont Agricultural Research and Extension Center (Nottoway County) in Blackstone, Virginia, and increased on the susceptible flue-cured tobacco cultivar K326. After a 12-week incubation period, cysts were extracted from soil with a modified Fenwick can and stored in capped test tubes at room temperature (Caswell et al., 1985).

Inoculation of trials: Inoculum was prepared by crushing cysts in tap water and standardizing egg suspensions to deliver 6,000 eggs in 10 ml of tap water per pot. Inoculum for unpasteurized soils from the Nottoway and Mecklenburg sites was adjusted to supplement the nematode population already present so that a total of 6,000 eggs/pot was not exceeded. Pots containing one tobacco seedling each were infested by pipeting the egg suspension into a 2-cm-deep trench cut around the root zone. Trenches were covered with an additional

100 cm^3 of the appropriate soil after infestation.

Experimental treatments and design: This study was composed of three treatments: (i) unpasteurized, uninfested soil; (ii) pasteurized soil infested with *G. t. solanacearum;* and (iii) unpasteurized soil infested with *G. t. solanacearum.* Approximately one-third of the soil from each location was steam-pasteurized at 155 °C for 3 hours and allowed to cool overnight prior to transplanting the next day. Experiments were arranged in a randomized complete-block design with six replications of each soil-treatment combination. The test was repeated twice, for a total of three experiments. The first and third tests were performed on greenhouse benches, where soil temperatures ranged between 25 and 30 °C. The second test was conducted in soil temperature tanks that maintained soil temperatures at 27 °C during the day and 25 °C at night.

Data collection: Tests were harvested 6 weeks after infestation, when plants were removed from pots. Soil from roots and remaining soil from pots were placed in a polyethylene-lined paper bag. The root system of each plant was rinsed gently over the bag to capture cysts from the surface of the root system. After soil had air-dried for 2 weeks, cysts were extracted with a modified Fenwick can (Caswell et al., 1985), crushed in a blender for 1 minute, and the eggs were suspended in tap water and stained with acid fuchsin (Daykin and Hussey, 1985). The number of *G. t. solanacearum* eggs in two 10-ml aliquots was counted for each sample and averaged to express the total number of eggs per plant. Total eggs per plant was then divided by the total number of cysts for each sample to estimate an average egg content for the cysts. For the final trial, only egg counts were recorded.

Statistical analysis: All data was subjected to log transformation ($\log_{10}[x + 1]$) prior to analysis of variance (SAS Institute, Inc. Cary, NC). Waller-Duncan mean separation procedures (k-ratio = 100) were conducted to evaluate soil differences. Tukey's test ($P \leq 0.05$) was used to separate means for the effects of soil pasteurization. A combined statistical analysis of the results was performed when mean square error terms were not significantly different according to *F*-tests (Gomez and Gomez, 1984) and when no significant ($P > 0.05$) treatment by experiment interactions was present. To isolate soil edaphic characteristics, average nematode data from each experiment for the pasteurized soils were correlated with edaphic factors using Pearson's correlation coefficients (SAS Institute Inc., Cary, NC).

Results

Soil texture, pH, and organic matter for the soils used in this study are presented in Table 1. Results from nematode assays conducted prior to our experiments are presented in Table 2.

Soil texture effects on nematode reproduction: More cysts were extracted in the first experiment from the Pittsylvania County sandy clay loam and from the Nottoway County sandy loam than from any of the other soils except for the sandy loam from Charlotte

Table 1. Location, particle size distribution, soil pH, and soil classification of seven flue-cured tobacco soils in 1998.

Location (county, state)	Soil classification	Percent sand	Percent silt	Percent clay	Soil pH	Percent organic matter
Cumberland, NC	Autryville sand	92.4	5.2	2.4	5.2	1.1
Charlotte, VA	Appling sandy loam	66.4	25.2	8.4	6.0	1.7
Nottoway, VA	Appling sandy loam	72.4	15.2	12.4	5.2	1.8
Halifax, VA	Appling sandy loam	74.4	15.2	10.4	4.8	1.2
Mecklenburg, VA	Appling sandy loam	76.4	9.2	14.4	5.0	1.1
Pittsylvania, VA	Cecil sandy clay loam	60.4	19.2	20.4	6.2	1.2
Appomattox, VA	Cecil sandy clay loam	46.4	25.2	28.4	5.2	2.2

TABLE 2. Plant-parasitic nematode taxa detected in soil from seven flue-cured tobacco fields in 1998. All fields had been previously planted with flue-cured tobacco in 1997.

Location (county, state)	Soil[a]	Nematodes per 500 cm^3 of soil					
		Meloidogyne spp.	*Globodera* spp.	*Pratylenchus* spp.	*Criconemella* spp.	*Tylenchorhynchus* spp.	*Helicotylenchus* spp.
Cumberland, NC	Sand	35	0	0	250	0	20
Charlotte, VA	SL	25	0	0	5	0	0
Nottoway, VA	SL	0	475	25	0	0	5
Halifax, VA	SL	60	0	0	0	0	40
Mecklenburg, VA	SL	0	30	0	15	5	95
Pittsylvania, VA	SCL	80	0	0	0	20	0
Appomattox, VA	SCL	20	0	0	0	25	0

[a] SL = Sandy loam; SCL = Sandy clay loam.

County (Table 3). More cysts were collected from the Charlotte County sandy loam soil than from the Mecklenburg County sandy loam, the Appomattox County sandy clay loam, or the sand soil from Cumberland County, North Carolina. In the second trial, more cysts were extracted for the Pittsylvania County sandy clay loam and the sand from Cumberland County, North Carolina, than from any of the other soils. Although more cysts were collected from the sand than from any of the other soils in trial 2, fewer cysts were associated with the sand compared to all of the other soils in trial 1.

In trial 1, egg production was higher in the Pittsylvania County sandy clay loam soil than in the sandy clay loam from Appomattox County, the sandy loams from Halifax and Mecklenburg Counties, or the sand from Cumberland County, North Carolina (Table 3). In the second trial, egg numbers for the sandy clay loam from Pittsylvania County were higher than those from any of the other soils except the sand. Egg production was lower in the sandy clay loam and sandy loam soils from the Appomattox and Mecklenburg County sites than the Pittsylvania County sandy clay loam, the sandy loams from Nottoway and Charlotte counties in trial 1, and the sand in trial 2. In the third trial, more eggs were found in the sandy loam soil from Charlotte County than in the other soils, except for the Cumberland County sand and the sandy clay loam from Pittsylvania County. The sandy clay loam from Appomattox County and the sandy loam soil from Halifax County supported less egg production than any of the other soils.

Differences in nematode fecundity among

TABLE 3. Reproduction of the tobacco cyst nematode (*Globodera tabacum solanacearum*) in soils from seven different locations. The greenhouse experiments were conducted at the Southern Piedmont Agricultural Research and Extension Center in Blackstone, Virginia.

Location (county, state)	Soil[a]	Cysts per pot		Eggs per pot			Egg/Cyst ratio	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2
Cumberland, NC	Sand	6 c	56 a	1,072 c	9,444 a	313 abc	151 bc	175 a
Charlotte, VA	SL	38 ab	11 b	7,041 ab	1,851 b	681 a	173 ab	156 ab
Nottoway, VA	SL	70 a	27 b	12,838 ab	4,302 b	231 bc	192 a	148 b
Halifax, VA	SL	18 bc	14 b	3,459 bc	2,168 b	74 d	164 abc	145 b
Mecklenburg, VA	SL	17 c	16 b	2,644 c	2,778 b	229 c	139 c	164 ab
Pittsylvania, VA	SCL	71 a	52 a	13,115 a	8,453 a	572 ab	189 a	161 ab
Appomattox, VA	SCL	15 c	10 b	2,639 c	1,702 b	110 d	153 abc	157 ab

Data presented are means from six pots. Analysis was performed on $\log_{10}$ (X + 1)-transformed data; untransformed means are presented. Means followed by a common letter are not significantly different according to the Waller-Duncan k-ratio *t*-test ($P < 0.05$).

[a] SL = Sandy loam; SCL = Sandy clay loam.

soils were inconsistent across experiments (Table 3). In the first trial, the sandy clay loam from Pittsylvania County and the sandy loam from Nottoway County were associated with significantly higher egg/cyst ratios than the sand from Cumberland County and the sandy loam from Mecklenburg County. Nematode fecundity was also higher in the sandy loam from Charlotte County than that from the Mecklenburg County location. In the second trial, the sand from Cumberland County had a higher egg/cyst ratio than the sandy loams from the Halifax and Nottoway County sites.

Soil treatment effect on nematode reproduction: Soil pasteurization did not influence cyst production in the first trial, but more cysts ($P \leq 0.05$) were found in the unpasteurized soil compared to pasteurized soil in the second experiment (Table 4). No significant differences were observed in the number of eggs per pot or the egg/cyst ratio between the two soil treatments in combined analyses across three trials. Differences in nematode reproduction due to soil pasteurization, or lack thereof, were consistent across all seven soils in the study.

Correlation coefficients: A significant positive correlation was found in the first trial between soil pH and numbers of cysts and eggs per pot (Table 5). A similar trend was observed for magnesium and percent base magnesium. However, neither trend was observed in the second or third trial, and all other correlations between soil edaphic factors and nematode data were not significant.

Discussion

This study suggests that *G. t. solanacearum* will reproduce similarly in most flue-cured tobacco-producing soils. Consistent differences in nematode reproduction among soils of different textures were not observed. Investigations of the influence of soil texture on soybean cyst nematode reproduction have shown significant effects only in soils with high clay content (silty clay loams or clays) (Workneh, et al., 1999). The clay contents of the soils used in this study were less than 30% and may have been too low to significantly influence reproduction of *G. t. solanacearum*. Although other soil characteristics rarely correlated with nematode reproduction, the positive correlations between soil pH and magnesium with numbers of eggs and cysts (in trial 1) were similar to trends reported for the soybean cyst nematode (Anand et al., 1995; Francl, 1993). The consistent lack of a response to soil pasteurization across soils from *G. t. solanacearum*-infested and uninfested areas suggests that soil biota may not influence the spread of *G. t. solanacearum*. The soils used in this study represent a majority of the soils used to produce flue-cured tobacco in the mid-Atlantic and southeastern United States. Taken together, our results indicate that *G. t. solanacearum* should continue to spread to flue-cured tobacco-producing areas beyond its current distribution.

Competition with root-knot (*Meloidogyne* spp.) or lesion (*Pratylenchus* spp.) nematodes could also limit the geographic distri-

Table 4. Effects of soil pasteurization on reproduction of *Globodera tabacum solanacearum* (*Gts*) in greenhouse trials conducted at the Southern Piedmont Agricultural Research and Extension Center in Blackstone, Virginia.

Soil treatment	Cysts per pot		Eggs per pot[a]	Eggs per cyst[b]
	Trial 1	Trial 2		
Pasteurized, infested with *Gts*	35 a	24 b	3,571 a	159 a
Unpasteurized, infested with *Gts*	33 a	29 a	3,700 a	170 a
Number of observations[c]	42	42	126	84

Analysis was performed on $\log_{10}$ (x + 1)-transformed data; untransformed means are presented. Means followed by a common letter are not significantly different according to Tukey's test ($P < 0.05$).

[a] Data for trials 1, 2, and 3 were combined.

[b] Data for trials 1 and 2 were combined.

[c] Number of observations per treatment for each analysis.

TABLE 5. Pearson correlation coefficients ($P < 0.05$) between numbers of cysts or eggs of *Globodera tabacum solanacearum* and soil characteristics from six locations in Virginia and one location in North Carolina.

	Pearson correlation coefficient				
	Cysts per pot		Eggs per pot		
Soil factor	Trial 1	Trial 2	Trial 1	Trial 2	Trial 3
Magnesium	0.83	ns	0.79	ns	ns
Base saturation percent-Magnesium	0.95	ns	0.94	ns	ns
Soil pH	0.77	ns	0.74	ns	ns

bution of *G. t. solanacearum*. *Globodera tabacum tabacum* (Miller and Gray) Behrens and *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa mutually suppressed each other. However, tobacco cyst nematodes suppressed lesion nematode populations to a greater extent than vice versa, indicating that lesion nematodes may not inhibit *G. t. solanacearum* spread (Miller, 1970; Miller and Wihrheim, 1968). Research examining competition between *G. t. solanacearum* and root-knot and other nematodes common in flue-cured tobacco soils would clarify the importance of these interactions.

Differences in day length, light quality, and temperature between the trials could have been responsible for the inconsistencies among the three experiments. Some of the differences observed in this study could have arisen from differences in plant root growth in the various soils. *Globodera rostochiensis* had reduced cyst production and fecundity on plants with reduced root systems compared to plants with larger root systems (Rawsthorne and Brodie, 1986). Differences in fertility and moisture-holding capacity among the soils also could have been responsible for variability in nematode reproduction caused by variation in overall plant health.

In summary, our results suggest that neither edaphic nor biological characteristics are major factors influencing the spread of tobacco cyst nematodes within Virginia and perhaps North Carolina, as well. Other factors, however, may be influencing spread of these nematodes. Environmental characteristics such as soil temperature, antagonistic or competing nematode genera, and crop production practices may play a major role. Our results emphasize the need for consistent application of sound nematode management practices, including sanitation, to limit the spread of these nematodes to other tobacco-producing regions of the United States.

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