Chapter 3: The Effect of Soil Edaphic and Biological Factors on Reproduction of the Tobacco Cyst Nematode.

Abstract: The tobacco cyst nematode (TCN), *Globodera tabacum solanacearum*, continues to spread and poses a serious threat to flue-cured tobacco production in Virginia and surrounding states. Soils were sampled from six non-infested and one infested flue-cured tobacco producing locations. Soil analysis was conducted on these soils to determine 23 edaphic characteristics to determine if any of them correlated with TCN reproduction. Additionally, comparisons were made between heat sterilized and non-sterilized soils to determine if a biological organism was present that suppressed TCN reproduction in currently non-infested areas. Differences in TCN reproduction among the soils were noted for all tests. However, results from these experiments were very inconsistent across two trials. The only consistent result was that soil acidity showed a weak negative correlation in both trials. Further testing is needed to determine which, if any, edaphic factors influence TCN reproduction. *Globodera tabacum solanacearum* reproduced more efficiently in non-sterilized soils as compared to sterile soils. This phenomenon may be contributed to predisposition by other soilborne pathogens. The occurrence of a TCN suppressing organism was not observed.

Key terms-Tobacco cyst nematode, soil edaphic factors, biological control, soil analysis.

The tobacco cyst nematode (TCN), *Globodera tabacum solanacearum* (Miller and Gray, 1972) Behrens, 1975, costs Virginia flue-cured tobacco (*Nicotiana tabacum* L.) 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, although complete crop losses have been reported (Komm *et al.*, 1983).

Globodera tabacum solanacearum was first found parasitizing flue-cured tobacco in Amelia County, Virginia in 1961 (Osborne, 1961). By 1972, the nematode was known to infest 18 farms in Amelia, Dinwiddie, and Nottoway counties (Miller and Gray, 1972). Komm *et al.* (1983) reported that TCN infested 148 flue-cured tobacco farms in ten Virginia counties. Currently, TCN infests an estimated one-third of Virginia's flue-cured tobacco acreage in eleven counties. The first report of TCN outside of Virginia occurred in 1990, from Warren County, North Carolina (Melton and Phillips, 1991). Since that time, TCN has been reported to infest farms in seven North Carolina counties (T.A. Melton, 1998, personal communication). Additionally, TCN 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. Tobacco cyst nematodes currently do not infest counties in the western portion of the Virginia flue-cured tobacco producing region. An edaphic or biological factor present within soils of the non-infested region might explain this phenomenon. Nematode-suppressive soils have also been reported for other cyst nematodes, such as the soybean cyst nematode

(*Heterodera glycines* Ichinohe, 1952) (Chen *et al*., 1994; Kim *et al.*, 1998), the potato cyst nematodes (*Globodera rostochiensis* (Woll., 1923) Stone, 1973 and *G. pallida* (Stone, 1973) Behrens, 1975) (Clovis and Nolan, 1983; Goswami and Rumpenhorst, 1978), and the sugar beet cyst nematode (*Heterodera schachtii* Schmidt, 1871) (Burnsall and Tribe, 1974; Tribe, 1979). Todd and Pearson (1988) found more cysts of the soybean cyst nematode (SCN) in sandy loam soils than in a heavier silty loam soil. Heatherly and Young (1991) indicated that SCN did not survive in clay soils as well as in silty loam 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) also correlated SCN levels positively with soil pH, as well as Mg levels. He also found a negative correlation between levels of Cu in soil and nematode density. 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 TCN parasitism among soils from TCN-infested and non-infested locations. Possible effects of soil sterilization and edaphic factors were also investigated.

Materials & Methods

Site Selection and Sampling-Six locations not infested with the tobacco cyst nematode (TCN-*Globodera tabacum solanacearum*) were chosen based on geographic location and differences in soil type. A seventh known TCN-infested location was chosen to serve as a control in the experiments. Soil was sampled from all locations during a three week period in February, 1998. The sample location for each soil is

presented in Table 3.1. Soil was collected randomly within a chosen field at each location using a shovel at a maximum depth of 20 cm. Total soil collected was approximately 60 L 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). Selected fields had all been planted with tobacco the preceding year (1997). Sampled soil was stored in 68 L plastic containers at room temperature. Soil samples were stirred and moistened with tap water every other day to ensure integrity of the soil microflora.

Soil Testing-A random sample was taken from each soil and analyzed for percent organic matter, phosphorus, potassium, magnesium, calcium, sodium, soil pH, cation exchange capacity (a measurement of the ability of the soil to hold exchangeable cations, or the leaching ability of the soil), percent base saturation for K, Mg, Ca, Na, and H (a measure of the percentage of a specific cation of the total cations in the soil), soil acidity (H), zinc, manganese, and copper. Additionally, percent clay, sand, and silt was determined for the soil from each location (Day, 1965). Chemical and physical soil analysis was conducted by A&L Laboratories in Richmond, Virginia. Two 250 cm^3 sub-samples of soil from each location were also 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 clay pots containing 300 cm^3 of soil from each location. Seedlings were allowed to grow for one week prior to inoculation to allow seedlings to adjust to the new environment. Plants were watered and fertilized using a 17-5-24 fertilizer solution (17% nitrogen, 5% phosphorus, 24% soluble potash, 2% water soluble

magnesium, 2.64% sulfur, 0.085% molybdenum, and 0.055% chelated zinc) delivered using an automated water system that included a microinjector calibrated at a rate of 125 mg/kg nitrogen. Pots were watered so that standing water was not present and the soil remained moist throughout the experiment.

Inoculum Source and Maintenance-Inoculum for this study was obtained by extracting cysts of *G. t. solanacearum* from infested soil collected at the Virginia Tech Southern Piedmont Agricultural Research and Extension Center (SPAREC) in Blackstone, Virginia. The susceptible flue-cured tobacco cultivar K326 was inoculated with extracted cysts for proliferation and homogeneity. After a twelve week incubation period, cysts were extracted from soil and stored in capped test tubes at room temperature.

Experimental Treatments and Design-This study involved the following treatments: 1) non-sterilized, non-inoculated soil, 2) sterilized soil, inoculated with TCN, and 3) non-sterilized soil, inoculated with TCN. Prior to transplanting, all soils were ground in a soil grinder to break up clods. Approximately one-third of the soil from each location was steam-sterilized at 155°C for 3 hr 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 once, for a total of two experiments. The first test was performed on greenhouse benches and the second was conducted in root zone chambers within a greenhouse. Soil temperatures for the first test ranged between 25 to 30°C. Temperatures in the root zone chamber were 27°C during the day and 25°C at night.

Inoculation of Trials-Inoculum was prepared by crushing cysts in tap water and standardizing egg suspensions to deliver 6,000 eggs in 10 ml per pot. Pots containing one tobacco seedling were inoculated by pipetting the egg suspension into a 2 cm trench cut around the root zone. Trenches were covered with an additional 100 cm^3 of the appropriate soil after inoculation. Pots were placed on the benchtop or root zone chambers and maintained as previously described.

Data Collection-Tests were harvested 6 wk after inoculation. The fresh weight of the root and shoot of each plant was determined after the root system of each plant was removed from soil. 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 in order to capture cysts from the surface of the root system. Bags containing soil were allowed to air dry for 2 weeks. After soil had dried for two weeks, cysts were extracted using a modified Fenwick can (Caswell *et al.*, 1985). Counted cysts were crushed in a blender for 1 min and the eggs were suspended in tap water and stained with acid fuchsin (Daykin and Hussey, 1985). The number of TCN eggs in two 10 ml aliquots were counted for each sample and averaged to express the total number of eggs per plant. Total eggs per plant were then divided by the total number of cysts for each sample in order to estimate an average egg content for the cysts from each sample.

Statistical Analysis-All data was subjected to log transformation [log(x+1)] prior to analysis of variance (SAS Institutue, 1989). The Waller-Duncan test (k-ratio=100) was conducted to evaluate soil differences. Tukey's mean separations ($P < 0.05$) were performed to test for the effects of soil sterilization. A combined statistical analysis of

the results from the two trials was performed when mean square error terms were not significantly different according to an F-test (Gomez and Gomez, 1984) and when no significant treatment-experiment interactions were present. In order to isolate soil edaphic characteristics, nematode data from sterilized soil was correlated with edaphic factors using Pearson's correlation coefficients for both experiments (SAS Institute, 1989).

Results

Soil analysis-Based upon soil texture, four of the soils used in this study were sandy loams, two were sandy clay loams, and one was a true sand (Table 3.1). Edaphic characteristics for each soil are presented in Table 3.2. Plant-parasitic nematodes detected in each of the soils are listed in Table 3.3. Cyst nematodes (*Globodera* spp.) were detected in soil from the SPAREC and Pittard locations.

Soil effect on nematode reproduction-In the first experiment, more cysts were recovered from the Giles and SPAREC soils than from any of the others except for the Vaughn soil (Table 3.4). More cysts were recovered from the Vaughn soil than from the Pittard, Jones, and Bullard locations. In the second trial, more cysts were observed for the Giles and Bullard locations than from any of the other locations. Although more cysts were recovered from the Bullard soil than from all the other soils in trial 2, fewer cysts were observed in the Bullard soil compared to all of the other locations in trial 1.

Egg production was higher in soil from the Giles location than in soils from the Watts, Bullard, Jones, and Pittard sites in trial 1 and compared to all of the locations except the Bullard site in the second trial (Table 3.4). Egg production was lower in soil

from the Jones and Pittard sites compared to the Giles, SPAREC, and Vaughn locations in trial 1 and relative to the Bullard site in trial 2.

Differences in nematode fecundity among soils were inconsistent across experiments. In the first trial, the Giles and SPAREC soils were associated with significantly higher eggs per cyst ratios than the Bullard and Pittard soils (Table 3.4). Nematode fecundity was also higher in the Vaughn soil compared to that from the Pittard location. Additionally, the Bullard location had a higher egg/cyst ratio than did the Watts and SPAREC soils.

. *Soil treatment effect on nematode reproduction*-Soil sterilization had an inconsistent influence on number of cysts recovered (Table 3.5). Cyst production was similar in sterilized and non-sterilized soil in the first trial. More cysts and eggs ($P \leq 0.05$) were associated with the non-sterilized soil in trial 2 than within the sterilized soil. The number of eggs recovered from non-sterilized soil was significantly ($P \leq 0.0087$) higher than that in sterilized soil in both experiments. However, no significant differences were observed in the egg/cyst ratio between the two soil treatments in either test.

Correlation coefficients-Organic matter content correlated ($P \leq 0.05$) negatively with numbers of cysts and eggs in the second experiment, but not in the first (Table 3.6). Magnesium and percent base saturation of magnesium exhibited a strong positive correlation with cysts and eggs for both factors in the first experiment, but not in the second trial. A negative correlation was found between percent base saturation of sodium and numbers of cysts and eggs in the first experiment, but not in the second. No

correlation was found with sodium in the first experiment, but a weak negative association was found between sodium and number of cysts and eggs in the second trial. Factors involving soil acidity also produced contradictory results. There was a positive association between soil pH and numbers of cysts and eggs in the first experiment, but not the second. Buffer index values produced a weak positive association with numbers of eggs and cysts in the second trial, but not the first. Percent base saturation of hydrogen correlated negatively with numbers of eggs and cysts in the first experiment, but not in the second. A weak association was observed between soil acidity (H) and the number of cysts and eggs in both experiments. A positive correlation was found between zinc and number of cysts and eggs in the second experiment, but not in the first trial. Soil texture seemed to have little influence on nematode reproduction. The only association between soil factors and egg/cyst ratio was a negative correlation with manganese in the second experiment, but not in the first.

Top Weights-A combined analysis across trials could not be conducted on shoot weight data due to a significant soil-experiment interaction. The interaction stemmed from higher shoot weights associated with the Bullard soil versus soil from the Giles location in the first trial, but not in the second. However, other treatment-soil interactions were not significant for either experiment, so shoot weight data was analyzed across soil treatments (Table 3.4). In both experiments, the Bullard soil (sand) was associated with the highest shoot weight, whereas the Jones soil (sandy clay loam) produced the lowest. Shoot weight was significantly lower for the Jones soil than for the rest of the soils in

both experiments. The Giles and Pittard soils produced the second highest and sixth highest shoot weight, respectively, in both experiments.

Discussion

Recovery of TCN cysts and eggs was lowest from the Bullard soil in the first trial, but highest in the second experiment. The other soils examined in this study behaved similarly between experiments. However, nematode increase differed between the two sandy clay loams (Giles and Jones) used in this study. Significantly more nematodes were found in the Giles soil than for the Jones soil in both experiments. Correlations relating nematode reproduction with soil texture factors were also not statistically significant. This variation suggests that environmental conditions may influence TCN reproduction more than soil factors. Differences in daylength, light quality, and temperature between the two trials could have produced the inconsistencies between the two experiments. Egg per cyst ratio data was also inconsistent across trials. However, soils that allowed higher TCN reproduction tended to have higher eggs per cyst ratios as well. The fitness of reproducing cysts may also be reduced in soils where reproduction is inhibited. Overall plant health also influences cyst fecundity. A study of potato cyst nematodes (*Globodera rostochiensis*) showed reduced cyst production and fecundity upon plants with reduced root systems compared to plants with larger root systems (Rawsthorne and Brodie, 1986). It is possible that some of the differences observed in this study could have arisen from differences in plant root weights within the various

soils. Fertility and moisture content of the different soils could have been responsible for the differences seen in overall plant health.

Nematode reproduction was similar or lower in sterile versus non-sterilized soil. These results indicate that there was not a TCN-suppressive parasite present in the soils tested. The increased nematode numbers seen in the non-sterilized soil suggests that a biological factor in the soil may enhance TCN penetration and development. It is also possible that reduced TCN reproduction in sterilized soil resulted from alterations caused by the sterilization process, which hindered either nematode or plant development.

Correlations between nematode data and soil edaphic characteristics for the two experiments were inconsistent across trials. An additional test is currently being conducted to verify our results. Where significant associations were found, they were weak for the most part. Some notable correlations involved soil acidity, soil pH, and buffer index. Since buffer index and soil pH are inverses of soil acidity (H), it may be possible that TCN can survive better at lower levels of acidity in the soil (higher pHs). This trend would be similar to that reported for the soybean cyst nematode (Anand *et al.*, 1995). Further research correlating soil edaphic factors with TCN reproduction needs to focus on a few specific soil characteristics, especially soil acidity. Interactions that occur between a multitude of factors may obscure the effect of individual factors. Additionally, the soil chemistry could have changed over the course of the study. Fertilizer applied to the pots could have effected edaphic factors of the soil and thus affected nematode reproduction. Soil chemistry samples taken at the end of the study could have been beneficial in order to indicate the nature of these changes.

Despite the differences in TCN reproduction among the soils used in this study, it seems that TCN is able to reproduce in most, if not all, flue-cured tobacco soils. Although the soils used in this study do not represent the full range of soils in flue-cured tobacco production, they do represent the majority of soils used. Our results, therefore indicate that TCN has the capability to spread throughout flue-cured producing areas of the eastern United States. However, TCN spread may be inhibited by other factors, such as competition with root-knot (*Meloidogyne* spp.) or lesion (*Pratylenchus* spp.) nematodes. Research with *Globodera tabacum tabacum* (Miller and Gray, 1968) Behrens, 1975 and *Pratylenchus penetrans* (Cobb, 1917) Chitwoodi and Oteifa, 1952 showed mutual suppression between the two nematodes. However, tobacco cyst nematodes reduced lesion nematode populations to a greater extent than vice versa, signifying that lesion nematodes may not inhibit TCN spread (Miller and Wihrheim, 1968; Miller, 1970). Research examining competition between TCN and root-knot and other nematodes common in flue-cured tobacco soils would clarify the importance of these interactions.

Table 3.1. Location, particle size distribution, and classification of seven flue-cured tobacco soils in 1998.

Farm	Location	% Sand	% Silt	% Clay	Soil Classification
Bullard	Cumberland Co., NC	92.4	5.2	2.4	Autryville Sand
Giles	Pittsylvania Co., VA	60.4	19.2	20.4	Cecil Sandy Clay Loam
Jones	Appomattox Co., VA	46.4	25.2	28.4	Cecil Sandy Clay Loam
Pittard	Mecklenburg Co., VA	76.4	9.2	14.4	Appling Sandy Loam
SPAREC*	Nottoway Co., VA	72.4	15.2	12.4	Appling Sandy Loam
Vaughn	Charlotte Co., VA	66.4	25.2	8.4	Appling Sandy Loam
Watts	Halifax Co., VA	74.4	15.2	10.4	Appling Sandy Loam

*Southern Piedmont Agricultural Research and Extension Center

Table 3.2. Edaphic characteristics of seven tobacco soils sampled in 1998.

	Phosphorus (mg/kg)		K	Mg	Ca	Na	Zn	Mn	Cu	
Farm	Bray P1	Bray P2	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	mg/kg	CEC
Bullard	104	116	41	38	220	12	2.2	6	0.9	2.4
Giles	66	95	132	135	410	14	1.6	11	1.1	4.1
Jones	51	57	225	94	420	25	1.5	11	1.4	5.4
Pittard	38	53	71	52	230	15	1.0	9	0.6	3.1
SPAREC	16	22	73	75	250	15	0.7	4	0.5	3.2
Vaughn	143	159	99	66	480	14	1.8	31	1.0	3.9
Watts	86	96	134	72	280	14	2.6	25	1.1	4.5

	Organic Matter		pH			% Base Saturation				
Farm	%	kg/ha	Soil	Buffer In.	Acidity H	K	Mg	Ca	Na	H
Bullard	1.1	77	5.2	6.9	0.8	4.4	13.3	46.0	2.2	34.1
Giles	1.2	76	6.2	6.9	0.5	8.3	27.7	50.4	1.5	12.1
Jones	2.2	96	5.2	6.8	1.8	10.7	14.6	39.1	2.0	33.5
Pittard	1.1	76	5.0	6.8	1.3	5.9	14.0	37.1	2.1	40.9
SPAREC	1.8	91	5.2	6.8	1.1	5.8	19.4	38.7	2.0	34.1
Vaughn	1.7	87	6.0	6.9	0.6	6.6	14.3	62.2	1.6	15.4
Watts	1.2	76	4.8	6.7	2.1	7.6	13.3	31.1	1.4	46.6

Table 3.3. Plant-parasitic nematode species in soil previously planted (1997) in flue-cured tobacco at six Virginia sites and one North Carolina location in 1998.

	Nematodes per 500 cc of soil					
Farm	*Meloidogyne* (Root-Knot)	*Globodera* (Cyst)	*Pratylenchus* (Lesion)	*Criconomella* (Ring)	*Tylenchorhynchus* (Stunt)	*Helicotylenchus* (Spiral)
Bullard	35	0	0	250	0	20
Giles	80	0	0	0	20	0
Jones	20	0	0	0	25	0
Pittard	0	30	0	15	5	95
SPAREC	0	475	25	0	0	5
Vaughn	25	0	0	5	0	0
Watts	60	0	0	0	0	40

Table 3.4. Reproduction over a six week period of a tobacco cyst nematode (*Globodera tabacum solanacearum*) in soils from seven different locations in 1998 greenhouse trials, conducted at the Southern Piedmont Agricultural Research and Extension Center, in Nottoway County, Virginia.

Soil		Shoot wt. (g)		Cysts per pot		Eggs per pot		Egg/Cyst Ratio[d]	
Location	Type[a]	Trial 1[b]	Trial 2[c]	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Bullard	sand	91.07 a	70.62 a	6.17 c	55.58 a	1,071.9 c	9,443.8 a	151.15 bc	175.26 a
Giles	scl	74.07 b	70.05 a	70.75 a	51.91 a	13,114.6 a	8,453.4 a	188.91 a	161.19 ab
Jones	scl	33.11 d	43.55 c	15.25 c	10.08 b	2,638.5 c	1,702.1 b	153.15 abc	156.77 ab
Pittard	sl	49.24 c	50.38 b	16.70 c	15.75 b	2,643.8 c	2,778.1 b	138.97 c	164.16 ab
SPAREC	sl	57.86 c	55.08 b	69.50 a	26.83 b	12,837.5 ab	4,302.1 b	192.45 a	147.86 b
Vaughn	sl	55.33 c	55.41 b	37.58 ab	11.11 b	7,040.6 ab	1,851.4 b	173.01 ab	155.63 ab
Watts	sl	73.12 b	51.07 b	17.50 bc	13.55 b	3,459.4 bc	2,168.2 b	164.27 abc	145.32 b
Range		57.96	27.07	64.58	45.50	12,042.7	7,741.7	53.48	29.94
Mean		61.97	56.59	33.35	26.40	6,115.2	4,385.6	165.99	158.03
%Variance		93.53	47.84	193.64	172.35	196.9	176.5	32.22	18.95

Data presented in table are non-transformed means from six replications. Means separations were performed using the Waller-Duncan test (k-ratio = 100) on log-transformed data [$\log_{10}(x+1)$]. Data points followed by the same letter are not statistically different.

[a]For soil type: scl=sandy clay loam and sl=sandy loam.

[b]Trial 1 was conducted on a benchtop in the greenhouse from March-May, 1998.

[c]Trial 2 was conducted in root zone chambers in the greenhouse from April-June, 1998.

[d]Egg/cyst ratio was determined for each sample and these numbers were averaged for each soil.

Table 3.5. Effects of soil sterilization prior to inoculation as determined in 1998 greenhouse trials conducted at the Southern Piedmont Agricultural Research and Extension Center, in Nottoway County, Virginia on the reproduction of a tobacco cyst nematode (*Globodera tabacum solanacearum*).

	Shoot Wt. (g)	Cysts/pot		Eggs/pot	Eggs/Cyst
Soil Treatment	Both Exps.[a]	Trial 1	Trial 2	Both Exps.	Both Exps.
Sterile, Inoculated	60.28 a	34.8 a	24.2 b	5,306.4 b	159.29 a
Non-Sterile, Inoculated	58.34 a	32.7 a	29.4 a	5,438.8 a	169.51 a
Non-Sterile, Non-Inoculated	59.58 a	N/A[b]	N/A	N/A	N/A

[a]Trial 1 was conducted on a benchtop in the greenhouse from March-May, 1998 and Trial 2 was conducted in root zone chambers in the greenhouse from April-June, 1998.

[b]Indicates data was not included in this analysis

Means followed by different letters are statistically different as determined by Tukey's test ($P \leq 0.05$) on log transformed data [$\log_{10}(x+1)$]. Data presented are non-transformed means of 84 observations from a combined analysis of two experiments (42 observations where trials were analyzed separately).

Table 3.6. Pearson correlation coefficients between numbers of cysts, eggs, and eggs per cyst and edaphic soil characteristics of soils from six locations in Virginia
and one North Carolina site.

	Pearson Correlation Coefficients					
	Cysts		Eggs		Egg/Cyst	
Soil Factors	Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Org. M (%)	ns[a]	-0.409	ns	-0.412	ns	ns
Org. M (lb)	ns	-0.409	ns	-0.409	ns	ns
Magnesium	0.513	ns	0.503	ns	ns	ns
Sodium	ns	-0.356	ns	-0.362	ns	ns
Soil pH	0.475	ns	0.468	ns	ns	ns
Buffer Index	ns	0.336	ns	0.338	ns	ns
Acidity (H)	-0.336	-0.319	-0.346	-0.319	ns	ns
Zinc	ns	0.326	ns	0.330	ns	ns
Manganese	ns	ns	ns	ns	ns	-0.789
%BaseSat Mg	0.592	ns	0.600	ns	ns	ns
%BaseSat Na	-0.342	ns	-0.334	ns	ns	ns
%BaseSat H	-0.453	ns	-0.447	ns	ns	ns
% Silt	ns	ns	ns	-0.310	ns	ns

[a]Indicates that the association was not significant at the $P \leq 0.05$ level.

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