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Impact of *Rotylenchulus reniformis* on Cotton Yield as Affected by Soil Texture and Irrigation

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Abstract: The effects of soil type, irrigation, and population density of *Rotylenchulus reniformis* on cotton were evaluated in a two-year microplot experiment. Six soil types, Fuquay sand, Norfolk sandy loam, Portsmouth loamy sand, Muck, Cecil sandy loam, and Cecil sandy clay, were arranged in randomized complete blocks with five replications. Each block had numerous plots previously inoculated with *R. reniformis* and two or more noninoculated microplots per soil type, one half of which were irrigated in each replicate for a total of 240 plots. Greatest cotton lint yields were achieved in the Muck, Norfolk sandy loam, and Portsmouth loamy sand soils. Cotton yield in the Portsmouth loamy sand did not differ from the Muck soil which averaged the greatest lint yield per plot of all soil types. Cotton yield was negatively related to *R. reniformis* PI (initial population density) in all soil types except for the Cecil sandy clay which had the highest clay content. Supplemental irrigation increased yields in the higher yielding Muck, Norfolk sandy loam, and Portsmouth loamy sand soils compared to the lower yielding Cecil sandy clay, Cecil sandy loam, and Fuquay sand soils. The Portsmouth sandy loam was among the highest yielding soils, and also supported the greatest *R. reniformis* population density. Cotton lint yield was affected more by *R. reniformis* Pi with irrigation in the Portsmouth loamy sand soil with a greater influence of Pi on lint yield in irrigated plots than other soils. A significant first degree PI × irrigation interaction for this soil type confirms this observation.

Key words: cotton, *Gossypium hirsutum*, irrigation, microplot, nematode, reniform nematode, *Rotylenchulus reniformis*, soil texture, soil moisture, volumetric water content, yield loss.

The reniform nematode, *Rotylenchulus reniformis*, is an important pathogen of cotton, *Gossypium hirsutum*, in the southeastern United States (Koenning et al., 2004; Robinson, 2007). A rapid reproductive rate, ability to colonize deep in the soil profile, and the ability to enter into an anhydrobiotic state improve over winter survival and make control of this nematode particularly challenging (Heald and Orr, 1984; Koenning et al., 2004; Robinson et al., 2005). As with many nematode infestations in cotton production systems, crop rotation and nematicide use are the major management tactics for *R. reniformis*. Though rotation is effective in managing this nematode, crop rotation is not a viable solution in many areas where cotton is grown in the US (Davis et al., 2003). Cultivars resistant and or tolerant to *R. reniformis* have promise to alleviate yield loss, but these are not presently available and the efficacy of tolerant cultivars has been questioned (Koenning et al. 2000; Starr et al. 2007). Nematicide application is an expensive alternative to rotation, and environmental concerns make their usage problematic.

The reniform nematode has been associated with soil types having higher silt and (or) clay contents compared with most plant-parasitic nematodes (Robinson et al., 1987; Heald and Robinson, 1990; Koenning et al., 1996; Koenning et al., 2004; Moore et al., 2008). Soil type and texture are important factors that can affect not only nematode population densities but also the ability of the host plant to thrive and the subsequent crop-yield suppression caused by plant-parasitic nematodes. Additionally, potential changes in climate may result in a greater frequency in drought that may enhance damage caused by plant-parasitic nematodes.

Recent advances in nematode management have included the use of precision agricultural systems to improve application of fertilizers and pesticides (Monfort et al., 2007; Starr et al., 2007). Precision application of nematicides using GIS/GPS systems has the potential to lower the costs of nematicidal treatments by limiting their placement to portions of fields where they are most beneficial (Monfort et al., 2007). Still, more basic knowledge about host-parasite relationships is required before precision application technology can be effectively utilized. Of particular importance are interactions of edaphic factors, plant-parasitic nematodes, and the production environment.

The purpose of this study was to evaluate the effects of soil texture and soil moisture on cotton - *R. reniformis* interactions. Specific objectives of this research were to 1) determine the influence of irrigation and soil type on cotton lint yield in the presence of *R. reniformis*, and 2) evaluate the effects of interactions between soil moisture and *R. reniformis* population density on yield.

Materials and Methods

The soil type/irrigation experiment was conducted in 2008 and 2009 in fiberglass microplots located at the Central Crops Research Station located near Clayton, NC. Selected plots were previously infested with *R. reniformis* at various levels including noninfested controls (Koenning et al., 1996). Each microplots contained one of six soil types. Norfolk sandy loam, Portsmouth loamy sand, Muck, Cecil sandy loam, and Cecil sandy clay soils were collected from the plow layer (Ap horizon material) of other sites and transported to this location. The sixth soil was the indigenous Fuquay sand (Rich and Barker, 1984; Windham and Barker, 1986;

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Schmitt et al., 1987; Windham and Barker, 1988; Barker et al., 1988; Barker and Weeks, 1991; Koenning and Barker, 1995; Koenning et al., 1996). The soils were arranged in five randomized complete blocks in microplots 0.76-m-diam. approximately 0.61-m deep (Barker et al., 1979). The taxonomic classification and the sand, silt, clay, and organic matter percentages (Bouyoucos, 1962) of these soils as taken from their native sites are listed in Table 1, as well as the initial population level (Pi) of *R. reniformis* ± standard error with noninfested controls excluded.

Plots within a given soil type were randomly assigned either to a high or low moisture regime in 2008 following initial population sampling (Table 1). Combinations of population density and soil type were equally represented by moisture level and plots retained their moisture regime for both years of the study. Plots assigned to the high moisture regime were irrigated three days a week in three one hour increments using trickle irrigation. Irrigation levels differed based on the drainage properties of each soil to achieve an approximation of field capacity for each irrigated plot. The Muck soil received an irrigation rate of 16 l/hr, Fuquay sand and Norfolk sandy loam soils a rate of 8 l/hr and the remaining soil types a rate of 4 l/hr. All plots were covered with opaque white polyethelene tops manufactured in our lab to fit the plots in order to reduce the effects of rainfall events. Low moisture treatments only received irrigation (approximately 12 l/plot regardless of soil type) after significant dry periods which threatened the life of the plants.

Moisture•point (Model MP-917, E.S.I. Environmental Sensors Inc., Sidney BC Canada) probes were installed to a depth of 0.4 m in 24 of the plots to monitor soil moisture. Plots for probe installation were selected using a random numbers table. Each population density and moisture regime combination was represented per soil type and probes remained in selected plots throughout the study. Measurements of soil moisture were taken prior to the application of irrigation following two weeks of the plots being covered in order to establish a baseline. During the growing season soil moisture measurements were taken at midseason and at the time of final harvest.

Microplots were planted in mid-June in 2008 and mid-May in 2009 with cotton cv. ST5327B2RF (Stoneville Pedigreed Seed, Stoneville MS). Cotton seed was commercially treated with imidacloprid (Gaucho®, Bayer Crop Science, Research Triangle Park, NC) for prevention of early season insect damage. Cotton was grown in accordance with practices recommended by North Carolina Cooperative Extension (Edmisten, et al., 2005). Plots were fertilized and limed based on a soil-test of each microplot (North Carolina Department of Agriculture & Consumer Services, Raleigh, NC). Seed cotton yield for 2008 was determined by conducting four hand harvests commencing with boll opening over a 9-week-period, in order to observe any delays in lint production and each harvest was weighed individually. Due to differences in weather conditions only three harvests were conducted over a 6-week-period in 2009. Lint from all four harvests in 2008 were combined by plot and ginned to obtain a total lint yield for the year. In 2009, lint yields from the first two harvests were ginned with a commercial grade cotton gin and a total lint yield was calculated based on the ratio of seed cotton yield to lint.

Samples for nematode population assessment were collected prior to planting (Pi), mid-season (Pm), and following the final harvest (Pf) during both years. Soil samples were comprised of six to eight cores (2.5-cm-diam.) taken to a depth of 15 cm. A 250 cm^3 sub-sample was processed by elutriation and centrifugation to extract vermiform nematodes from soil. (Byrd et al., 1976; Jenkins, 1964).

Nematode data were transformed $\log_{10}$ (x + 1) to standardize the variance and PROC GLM of SAS, version 9 (SAS Institute, Cary, NC) was used for analysis of all data. Initial population density was used as a covariate in analysis for mid-season and harvest nematode densities as well as cotton lint yields. Least squared means and standard errors are presented in figures. The Waller Duncan k-ratio *t* test and Tukey's Studentized Range test (HSD) were used for mean separation comparisons between soil types. Scatter plots and regressions of yield and Pi were produced using SigmaPlot 8.0 (Systat Software Inc., Chicago, IL).

Results

When data was combined from both years, soil type affected volumetric water content (θ) independent of

Table 1. Soil common name, taxonomic classifications, percentages of sand, silt, clay, and organic matter content, and mean and standard error (S.E.) of the initial *Rotylenchulus reniformis* population density (Pi) per 250 cm^3 of soils used in this study.

Soil	Taxonomic Classification	% Sand	% Silt	% Clay	% Organic Matter	Pi	S.E.
Fuquay sand	Loamy, siliceous, thermic, arenic, plinthic Kandiudults	91	6	3	0.6	5,235	2,491
Norfolk sandy loam	Fine-loamy, siliceous, thermic Kandiudults	84	12	4	1.4	7,421	2,127
Portsmouth loamy sand	Fine-loamy, over sandy or sandy- skeletal, mixed thermic, Typic Umbraquelts	72	18	10	3.8	15,698	3,283
Muck	Medisaprists	58	33	9	>30	4,967	1,947
Cecil sandy loam	Clayey, kaolinitic, thermic, Typic Kanhapludults	53	18	29	2.2	6,196	2,213
Cecil sandy clay	Clayey, kaolinitic, thermic, Typic Kanhapludults	48	13	39	0.9	5,048	2,176

irrigation at the 15-cm depth ($P = 0.0018$) based on the mean of all sample dates. The Portsmouth sandy loam and Muck soils had the greatest differences between irrigated and non-irrigated average θ. All soils had greater θ at the 15-cm depth when irrigation was applied [Waller Duncan k-ratio *t* test (k-ratio = 100)] (Fig. 1). When irrigated and non-irrigated plots were compared in the analysis, the Portsmouth loamy sand and the Cecil soils had similar soil θ at the 15-cm depth. However, the Cecil sandy loam differed from the rest of the soil types (k-ratio = 100). The Portsmouth loamy sand and Cecil sandy clay soils were similar to the Muck but not to the Norfolk sandy loam or the Fuquay sand soils (k-ratio = 100). The Portsmouth loamy sand and Cecil sandy loam soils had equivalent average θ to but varied little from the Muck soil (k-ratio = 100).

Comparisons of θ between irrigated plots at the 15-cm depth, the Cecil sandy loam and Fuquay sand soils differed from each other according to the Waller Duncan k-ratio *t* test (k-ratio = 100) (Fig. 1). The Cecil sandy loam had the greatest θ and the Fuquay the lowest θ of all soil types. The test also indicated that the Cecil sandy clay and Cecil sandy loam soils differed from the Muck soil in non-irrigated plots, but no other differences were statistically evident (k-ratio = 100) (Fig. 1).

Soil type was associated with *R. reniformis* population variation at every sampling date in both years and non-irrigated plots typically had greater population densities at both mid-season and harvest ($P = 0.0907$ and $P = 0.0647$, respectively) (Table 2; Figs. 2, 3). Mid-season and Pf *R. reniformis* nematode population densities in the Portsmouth sandy loam soil were greater than all other soil types according to the Waller Duncan k-ratio *t* test (k-ratio = 100) (Figs. 2, 3). Both irrigation and soil texture had an effect on Pm, however the soil type × irrigation interaction was not significant ($P \leq 0.10$).

TABLE 2. Analysis of variance for effects of soil and irrigation on *Rotylenchulus reniformis* population densities at planting (Pi), midseason (Pm), and cotton harvest (Pf) for 2008 and 2009.

Source	DF[a]	$P > F$[a]		
		Pi	Pm	Pf
Year	1	0.0133	0.4574	0.5717
Irrigation (Ir)	1	0.7074	0.0907	0.0647
Year × Ir	1	0.9851	0.1297	0.6481
Soil type (Soil)	5	0.0020	0.0070	0.0001
Ir × Soil	5	0.846	0.8610	0.5229
Year × Soil	5	0.371	0.2236	0.0286
Year × Ir × Soil	5	0.9461	0.1398	0.3443
Pi (covariate)	1	-	0.0049	0.1253

[a] DF is degrees of freedom, and $P > F$ indicates the probability of a larger *F* value from ANOVA and General Linear Models of SAS with $\log_{10}$ (Pi +1) used as a covariate.

The Fuquay sand, Muck, and Cecil sandy loam soils generally had lower population densities at most sampling dates than other soils according to the Waller Duncan k-ratio *t* test (k-ratio = 100).

Increasing *R. reniformis* Pi consistently suppressed cotton lint yield in all soil types except the Cecil sandy loam soil during both years ($P = 0.0001$) (Table 3, Fig. 4). Similarly irrigation increased cotton lint yield and soil types differed in main effects means ($P = 0.0001$ and $P = 0.0017$, respectively). The Portsmouth sandy loam and Muck soils had the greatest average lint yield when the data was combined for both years (k-ratio = 100). The Fuquay sand soil had the lowest average lint yield per plot over the two year period, but did not differ from the Cecil sandy loam, Cecil sandy clay, and Norfolk sandy loam soils (k-ratio = 100). Lint yield was unaffected by year ($P = 0.45$) and first or second order interactions of year with other factors were not significant (P ≤ 0.10). The first order and second order

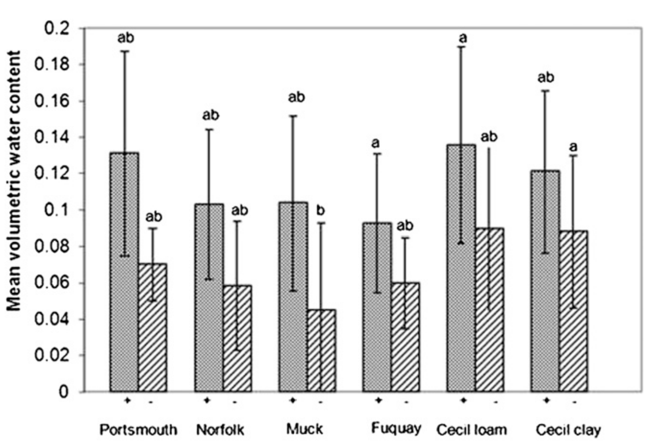

FIG. 1. Mean volumetric soil moisture (θ in m^3/m^{-3}) at 15 cm in irrigated (+) and nonirrigated (-) plots. Data was combined across dates for each soil type and is the mean and standard error (N = 20). Across soils, irrigated plots had greater soil moisture content ($P = 0.01$) than nonirrigated plots. Means between soil types within an irrigation level followed by the same letter are not different according to Tukey's Studentized Range (HSD) test ($P = 0.05$).

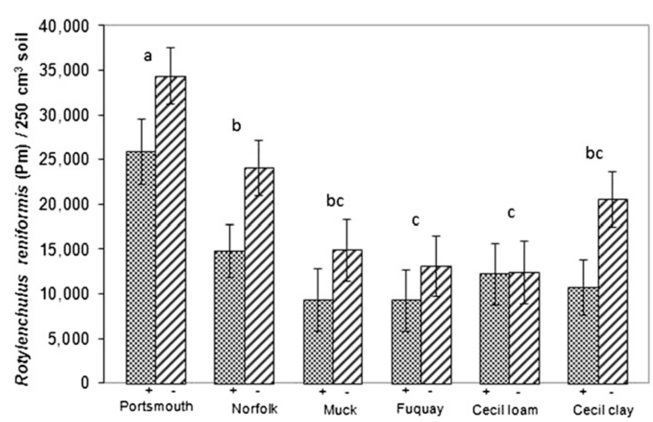

FIG. 2. Least squared means and standard errors for influence of soil type and irrigation (+ denotes irrigated plots, - denotes non-irrigated plots) on mid-season numbers (Pm) of *Rotylenchulus reniformis* per 250 cm^3 soil in microplots from 2008 and 2009 near Clayton, NC. Irrigated plots had significantly lower population densities of *R. reniformis* at midseason ($P = 0.0907$). Nematode numbers differed by soil type ($P < 0.0001$). Main effect means by soil type with the same letter do not differ according to the Duncan k-ratio *t* test (k-ratio = 100).

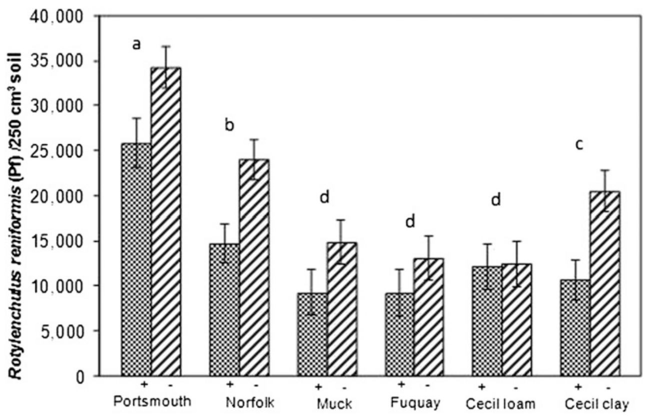

FIG. 3. Least squared means and standard errors for influence of soil type and irrigation (+ denotes irrigated plots, - denotes non-irrigated plots)on harvest population densities (Pf) of *Rotylenchulus reniformis* per 250 cm^3 soil in microplots from 2008 and 2009 near Clayton, NC. Irrigated plots had significantly lower population densities of *R. reniformis* at midseason ($P = 0.0647$). Nematode numbers differed by soil type ($P < 0.0001$). Main effect means by soil type with the same letter do not differ according to the Duncan k-ratio *t* test (k-ratio = 100).

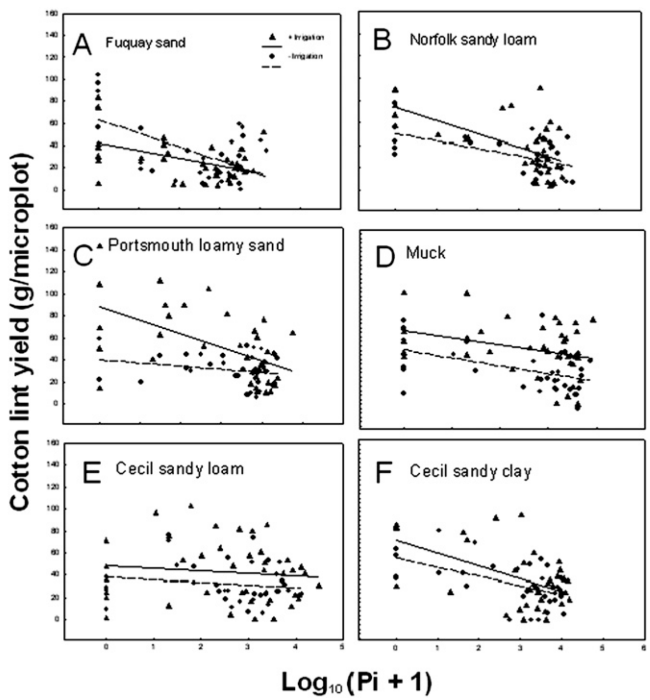

FIG. 4. Effects of $\log_{10}$ density per 250 cm^3 soil (Pi) of *Rotylenchulus reniformis* and of irrigation on lint yield of cotton cultivar ST5327B2RF combined for 2008 and 2009. A) Fuquay sand regression equations: irrigated $\hat{y} = 42 - 6.7x$ ($R^2 = 0.23$, $P = 0.0120$), nonirrigated $\hat{y} = 63 - 12.2x$ ($R^2 = 0.35$, $P = 0.0030$). B) Norfolk sandy loam regression equations: irrigated $\hat{y} = 73 - 11.7x$ ($R^2 = 0.42$, $P = 0.0016$), nonirrigated $\hat{y} = 50.5 - 6.6x$ ($R^2 = 0.30$, $P = 0.0046$). C) Portsmouth loamy sand regression equations: irrigated $\hat{y} = 87.8 - 12.1x$ ($R^2 = 0.31$, $P = 0.0035$), nonirrigated $\hat{y} = 40.5 - 2.9x$ ($R^2 = 0.06$, $P = 0.1634$). D) Muck regression equations: irrigated $\hat{y} = 69.4 - 6.2x$ ($R^2 = 0.11$, $P = 0.0726$), nonirrigated $\hat{y} = 52.1 - 7.0x$ ($R^2 = 0.26$, $P = 0.0207$). E) Cecil sandy loam regression equations: irrigated $\hat{y} = 48.4 - 2.4x$ ($R^2 = 0.01$, $P = 0.4532$), nonirrigated $\hat{y} = 38.1 - 2.3x$ ($R^2 = 0.02$, $P = 0.3512$). F) Cecil clay irrigated $\hat{y} = 70.0 - 11.1x$ ($R^2 = 0.28$, $P = 0.0420$), nonirrigated $\hat{y} = 54.4 - 8.0$ ($R^2 = 0.03$, $P = 0.3480$).

interactions, however, were significant for irrigation × soil, irrigation × Pi, and irrigation × soil × Pi ($P = 0.0092$, $P = 0.0437$, and $P = 0.0101$, respectively). These interactions of irrigation with Pi and soil demonstrate that nematode damage was increased by irrigation (Fig. 4), and the interaction tended to be more prominent in some of the soils with the highest yield potential (Portsmouth loamy sand and Norfolk sandy loam soils [$P = 0.0081$ and $P = 0.0737$, respectively]) as opposed to the lower yielding clay soils. The yield in the Muck soil was an obvious exception, but soils with this high level of organic material may be a special case.

DISCUSSION

The differences established in soil volumetric moisture content were sufficient for the goals of this research project. Higher rates of irrigation were used in the Fuquay sand, Norfolk sandy loam and Muck soils based on previous experience with the drainage characteristics of these soils (Koenning and Barker, 1995). Differences in θ associated with irrigation appeared to be more pronounced at a soil depth of 15 cm than at 30 cm as expected. This may have been due to a clay sub-soil beneath the plots, which would result in greater water retention at this depth.

The impact of soil type on *R. reniformis* was similar to results obtained in previous studies (Koenning et al., 1996). The Portsmouth sandy loam was one of the greatest yielding soils and consistently had the largest nematode population densities. The influence of irrigation on *R. reniformis* population densities with greater numbers of this nematode at lower moisture levels was not expected. This result, however, is not without precedent. Other research has noted a negative impact of moisture level on *R. reniformis* Pf (Koenning et al., 2000).

Soils with a high percentage of clay, the Cecil sandy clay and Cecil sandy loam, had better water retention than very sandy soils such as the Fuquay sand, and these two soils showed no effect of irrigation on lint yield. In

TABLE 3. Partial analysis of variance combined over years 2008 and 2009 for cotton lint yield[a].

Source	DF[b]	$P > F$[b] Lint Yield
Irrigation	1	0.0001
Pi	1	0.0001
Soil	5	0.0174
Year	1	0.4560
Replication	4	0.7589
Irrigation × Soil	5	0.0092
Irrigation × Pi	1	0.0437
Irrigation × Soil × Pi	10	0.0101

[a] Yield was calculated as grams per microplot.
[b] DF is degrees of freedom, and $P > F$ indicates the probability of a larger F value from ANOVA and General Linear Models of SAS with $\log_{10}$ (Pi +1) used as a covariate.

the Cecil sandy loam and Cecil sandy clay soils, it is likely that the presence of plant available water even in nonirrigated plots may have resulted in a similar growth pattern to those receiving irrigation, resulting in no statistical differences in lint yield. Irrigation water drained rapidly through the Fuquay sand soil before it could be taken up by the root system which may explain the lack of obvious improvement in lint yield due to irrigation.

The Portsmouth sandy loam and Norfolk loamy sand were the soils in which the nematode population level × irrigation interaction was significant on lint yield. Although there appears to be in interaction with irrigation and Pi for Fuquay sand soil, lint yield was not improved by irrigation, hence no significant interaction. In all other soils Pi and irrigation appeared to behave independently if there were any effects at all. The observed interactions in the Norfolk sandy loam and particularly in the Portsmouth loamy sand could have been due to the extremely high population levels that were reached in these soils and may explain why this was not also apparent in other soil types.

In conclusion, the most significant finding of this research was the interaction of irrigation with Pi in certain soil types. Greater damage caused by *R. reniformis* with irrigation tended to be more pronounced in the most productive soils, which may indicate that nematode damage increases with increasing productivity. In environments with high yield potential control of *R. reniformis* becomes more profitable. Koenning et al. (2007) found it was more cost effective to treat for *R. reniformis* in situations of high productivity versus in areas with low average production yield. Soils such as the Cecil sandy loam, which showed no effect of Pi on lint yield in this study, typically yield very poorly and application of nematicidal treatments would likely be uneconomical. As mentioned previously, this also has major implications in precision application of nematicides in fields with mixed soil types and within field variation in productivity and should be considered. Based on the current research, *R. reniformis* needs to be managed more intensively in the more productive areas of the field, whereas areas with limited productivity are unlikely to benefit from application of nematicides.

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