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Spread of *Rotylenchulus reniformis* in an Arkansas Cotton Field Over a Four-Year Period

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Abstract: *Rotylenchulus reniformis* was first detected in a single grid (100 m^2) in May 2001 in a cotton field in Ashley County, AR, that was being utilized to evaluate the utility of grid-sampling for detection of *Meloidogyne incognita*. A total of 512 grids were sampled in the 6-ha field in the spring and fall for four years (2001 - 2004), nematode populations were determined for each grid, and nematode population density maps were constructed utilizing Global Positioning Systems and Geographic Information Systems. In May 2001, *R. reniformis* population density in the single grid where it was detected was 6,364 juveniles and adult reniform nematodes/500 cm^3 soil. By the end of the first year (October 2001), the nematode was found in 17 of the 512 plots with population densities ranging from 682 to 10,909 nematodes/500 cm^3 soil. Over the course of the 4-yr period, reniform nematode incidence increased to 107 of 512 plots, with population density ranging from 227 to 32,727 nematodes/500 cm^3 soil. Reniform nematode spread could be explained by the direction of tillage and water flow in the low end of the field. Highest population densities were observed in the areas of the field with soil types ranging from 54% to 60% silt fraction. In addition to *R. reniformis*, *Meloidogyne incognita* was commonly detected in many of the grids, and *Tylenchorhynchus* spp., *Helicotylenchus* spp., *Paratrichodorus minor* and *Hoplolaimus magnistylus* were detected occasionally.

Key words: Reniform nematode incidence, spatial correlation, soil texture, geographically weighted regression, management, detection, ecology.

The reniform nematode, *Rotylenchulus reniformis*, was first observed in Arkansas in 1979 in a Crawford County soybean field (R.T. Robbins, University of Arkansas, pers. com.) and in a few Jefferson and Monroe County cotton fields in 1986 to 1988 (Robbins et al., 1989). By 2003, reniform nematodes had been documented in 262 cotton fields from 13 different counties in Arkansas (Bateman et al., 2003).

The rather rapid spread of this nematode pest through Arkansas has prompted considerable concern among cotton producers. Movement of the nematode by farming equipment has been suggested as a major means of spread (Koenning et al., 2004). Although sanitation measures have been suggested for limiting the spread of the nematodes from field to field, very few producers actively practice preventive sanitation on their farms. In addition to spread to new geographical regions, spread within fields is of considerable concern once the nematode has been introduced. Reniform nematode reproduces readily and achieves high population densities in fine-textured soils (Koenning et al., 1996) and can be uniformly distributed within fields (Koenning et al., 2004). There is currently little information on the dynamics of reniform nematode movement within individual fields.

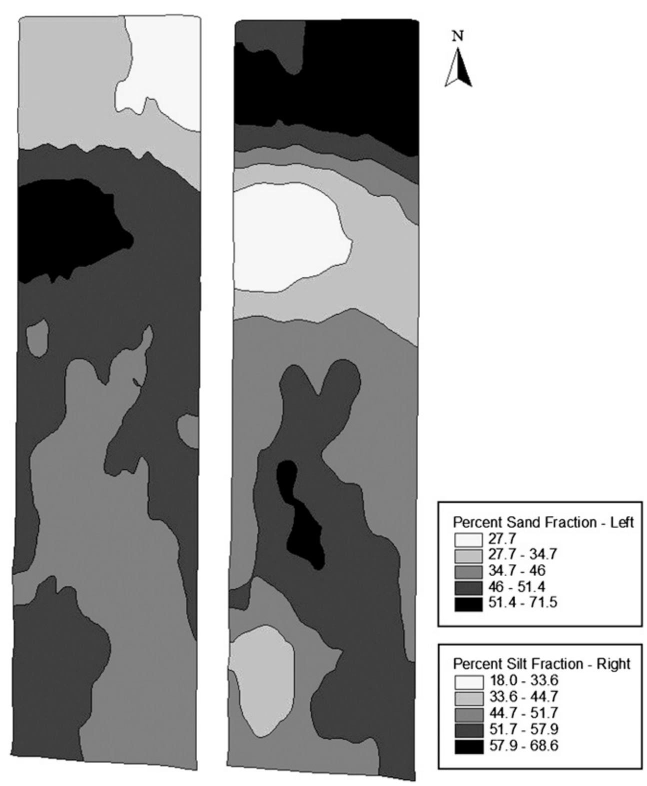

Fig. 1. Kriging maps of the spatial distribution of percent sand (left) and percent silt (right) soil fractions of a 6.1 ha cotton field in Southeastern Arkansas. Spatial distribution maps were constructed utilizing the Latitude/Longitude coordinates of each plot in the GIS software SSToolBox (SST Development Group, Inc., Stillwater, OK).

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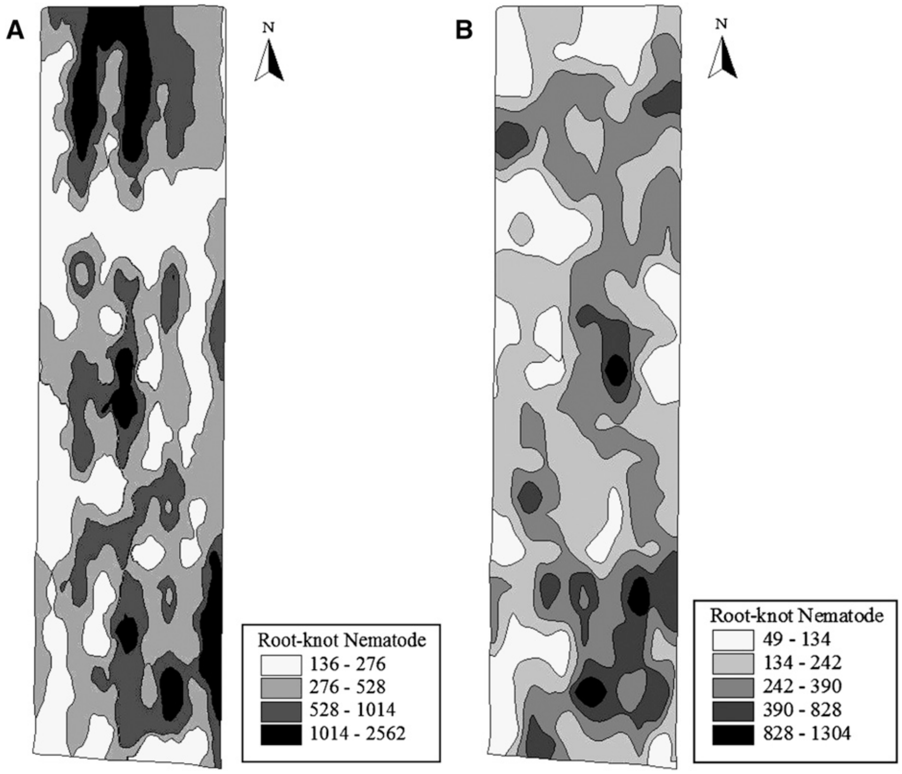

FIG. 2. Kriging maps of the spatial distribution of root-knot (*Meloidogyne incognita*) nematode in a 6.1 ha cotton field in southeastern Arkansas in Spring 2001 (Fig. 2A) and Spring 2004 (Fig. 2B). Spatial distribution maps were constructed utilizing the Latitude/Longitude coordinates of each plot in the GIS software SSToolBox (SST Development Group, Inc., Stillwater, OK). Root-knot nematode population densities represent number of juveniles per 500 cm^3 soil.

The relatively wide availability of Global Positioning Systems (GPS) and Geographic Information Systems (GIS) provides a means of documenting nematode movement over time with considerable precision. In 2001, a study to investigate the influence of soil texture on the damage potential of *Meloidogyne incognita* was established in a production field in Ashley County, AR. A component of this study was monitoring nematode population densities from 512 individual grids (100 m^2) over a 4-yr period. Initial samples in the spring of 2001 indicated the presence of *R. reniformis* as well as the Southern root-knot nematode, *Meloidogyne incognita,* in a single grid, so a second objective of the study was to document the spread of *R. reniformis* in the field over the 4-yr study.

Materials and Methods

The study site was a commercial cotton field (6.1 ha) in Ashley County, AR. This field had been planted in cotton each year for at least 10 yr prior to initiation of the study and had been identified by the grower as a problem field due to *Meloidogyne incognita.* Individual sampling grids (512 total) that were 3.6 m (four rows) wide × 30.5 m in length were established in March 2001. The geographic location of each plot was identified using a GPS receiver (Trimble, Sunnyvale, CA) and Site-Mate, a GPS mapping software (Farmworks, Hamilton, IN). The individual plot size was established to accommodate collecting yield using an Ag Leader PF3000 yield monitor (Ag Leader Technology, Ames, IA) that recorded yield once per second mounted on a 4-row John Deere 9970 cotton picker. The 30.5 m length ensured that each plot had at least seven individual yield recordings. A component of the original study was the application of 1,3 dichloropropene (Telone II) at rates of 14.1 to 42.2 liters/ha in strips in the field to ensure that there were differences in *M. incognita* population densities across the field (Monfort et al., 2007). Cotton was grown in the field each year of the study under a reduced-tillage system. Stoneville 4892 BR, a glyphosate-tolerant cotton cultivar, was planted each year. Crop fertilization, irrigation (center-pivot) and insect/weed management were performed by the grower according to his normal farming practices.

Soil samples were taken from each grid in April (planting) and October (harvest) each year from 2001 through 2003, and a final sample was collected in April 2004. Each sample consisted of a composite of 16 soil cores collected from the root zone (bed) to a depth of 30 cm in the center two rows of each grid. Nematodes were extracted from the composite samples using the semi-automatic elutriator (Byrd et al., 1976) followed by centrifugal flotation (Jenkins, 1964), and nematodes were identified and quantified with a stereoscope at

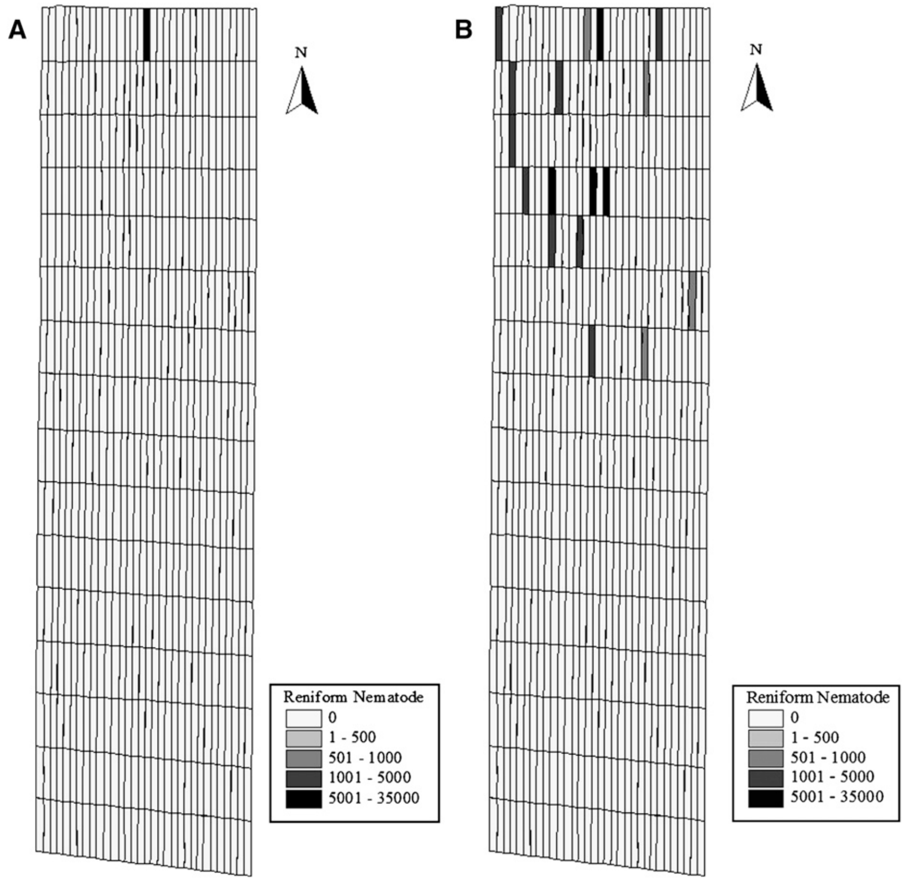

FIG. 3. Spatial distribution maps of reniform (*Rotylenchulus reniformis*) nematode in a 6.1 ha cotton field in southeastern Arkansas in Spring 2001 (Fig. 3A) and Fall 2001 (Fig. 3B). Spatial distribution maps were constructed utilizing the Latitude/Longitude coordinates of each plot in the GIS software SSToolBox (SST Development Group, Inc., Stillwater, OK). Reniform nematode population densities represent number of adults and juveniles per 500 cm^3 soil.

×40 to ×60 magnification. Soil particle size distribution also was assessed for each plot utilizing the hydrometer particle-size analysis (Arshad et al., 1996) in order to correlate soil particle size distribution with nematode population density and spread.

Analysis of spatial correlation between nematode distribution and soil particle size distribution was conducted using geographically weighted regression (GWR). The nematode population densities at each sampling time were merged with their corresponding GPS location in the field, and spatial distribution maps were created using the GIS software SSToolBox (SST Development Group, Inc., Stillwater, OK). Spatial distribution maps of the reniform nematode were developed for each sampling period from 2001 to 2004.

RESULTS

The soil texture in individual grid plots ranged from 21% to 81% sand and 18% to 68% silt (Fig. 1). *Meloidogyne incognita* remained widely distributed throughout the field during the 4-yr period (Fig. 2A,B). In addition to *M. incognita*, several nematode species that are not considered to be of economic significance in cotton (Robbins, et al., 1998; Koenning et al., 2004), including spiral (*Helicotylenchus* spp.), stunt (*Tylenchorhynchus* spp.), *Paratrichodorus minor* and *Hoplolaimus magnistylus* were found occasionally in the site (data not shown).

Throughout this study, the reniform nematode was found in grid plots representing a range of soil textures and *M. incognita* population densities. No significant correlation ($P \leq 0.05$) was found between the population density of the reniform nematode and existing *M. incognita* population density at any sampling period (data not shown). In the spring of 2001, *R. reniformis* was detected in only one of the 512 grid plots in the northern end of the field at a population density of 6,364 juveniles and adults/500 cm^3 soil (Fig. 3A). The soil particle size distribution in this plot was 29% sand, 11% clay, and 60% silt, a silt loam texture. By the end of the 2001 season, reniform nematodes were detected in 17 of the grid plots, with population densities ranging from 682 to 10,909 nematodes/500 cm^3 of soil (Fig. 3B). The nematodes appeared to have spread during the first growing season both in the same direction as the rows (north to south) and across rows, although incidence was confined to the northern one-third of the field.

TABLE 1. Spatial correlation between the spread of reniform nematode and the soil particle size distribution (% silt) within a 6.1 ha cotton field in southeastern Arkansas.[a]

	2001	2002	2003
Number of observations	512	512	512
Sigma	740	1,947	2,337
Akaike Information Criterion	8,260	9,268	9,523
Coefficient of Determination	0.13	0.41	0.63
Adjusted r-square	0.07	0.34	0.54
P-value[b]	0.99	< 0.0001	<0.0001

[a]Results based on the geographically weighted regression analysis for determining spatial relationships between parameters: percentage silt and reniform populations per 500 cm^3 soil.

[b]Statistical significance of parameters based on the Monte Carlo significance test procedure (Hope, 1968).

In 2002, reniform nematode infestations were detected in a majority of the plots where they were found the year before (Fig. 4A) and in two grid plots toward the southern end of the field where the nematode had not been detected the previous fall. By the end of the growing season in 2002, reniform nematodes had spread to a total of 84 of the grid plots in the field, with population densities ranging from 227 to 31,364 nematodes/500 cm^3 soil (Fig. 4B). The highest population densities were in grid plots that were either adjacent to or near the site of the original detection. Nematode incidence and population densities continued to show the same trend in 2003, and, by the time cotton was harvested, nematode incidence was almost field–wide, with 107 of the grid plots infested (Fig. 5A,B). The final sampling of the experiment was made in the spring of 2004 shortly before planting (Fig. 6). Although nematodes were not detected in all grid plots that had recorded nematode population densities the previous fall, densities were considerably higher in plots near and adjacent to the grid plot where the nematode was originally detected in 2001. In general, throughout the study, reniform nematode population densities declined each year during the winter months, but incidence of the nematode increased each year in both spring and fall compared to the previous year. Based on the GWR analysis, the spread of reniform nematodes was significantly correlated with soil particle size distribution (percentage silt) (Table 1).

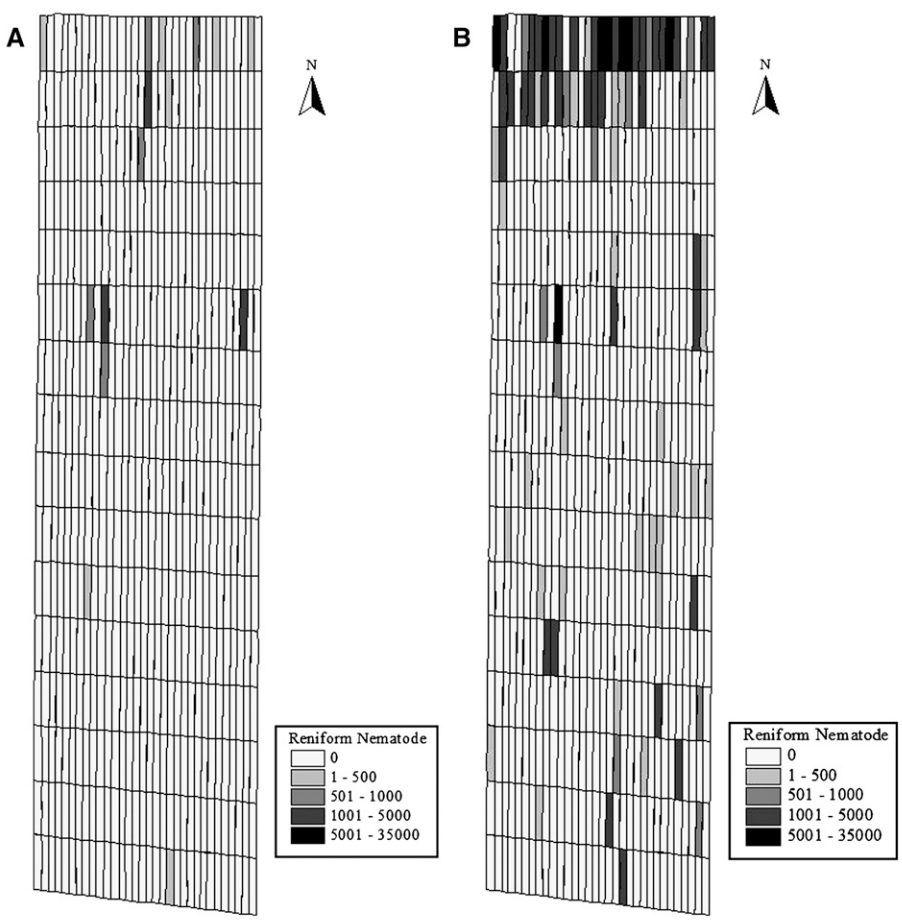

FIG. 4. Spatial distribution maps of reniform (*Rotylenchulus reniformis*) nematode in a 6.1 ha cotton field in southeastern Arkansas in Spring 2002 (Fig. 4A) and Fall 2002 (Fig. 4B). Spatial distribution maps were constructed utilizing the Latitude/Longitude coordinates of each plot in the GIS software SSToolBox (SST Development Group, Inc., Stillwater, OK). Reniform nematode population densities represent number of adults and juveniles per 500 cm^3 soil.

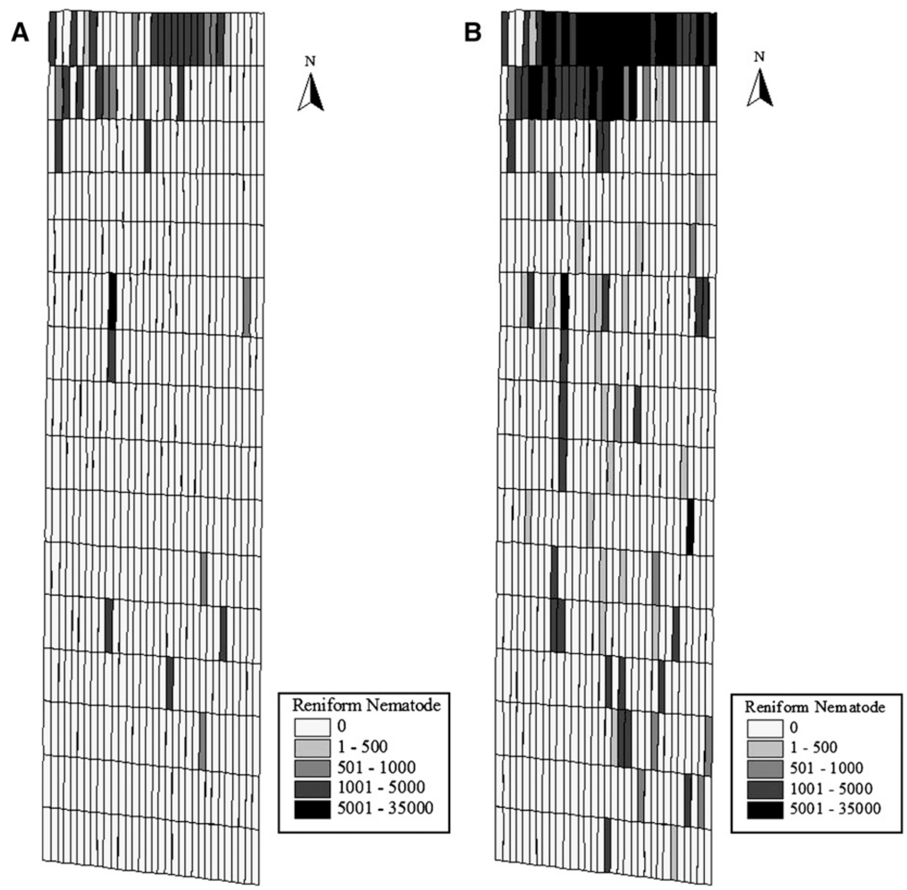

FIG. 5. Spatial distribution maps of reniform (*Rotylenchulus reniformis*) nematode in a 6.1 ha cotton field in southeastern Arkansas in Spring 2003 (Fig. 5A) and Fall 2003 (Fig.5B). Spatial distribution maps were constructed utilizing the Latitude/Longitude coordinates of each plot in the GIS software SSToolBox (SST Development Group, Inc., Stillwater, OK). Reniform nematode population densities represent number of adults and juveniles per 500 cm^3 soil.

DISCUSSION

The reniform nematode spread from one point in the northern part of a 6.1-ha field to become essentially field-wide in a period of four years. Incidence was relatively localized during the first year, with nematodes confined to the northern part of the field in the vicinity of the original detection. Soil in the northern one-third of this field tended to have a higher silt content, which may have been more conducive to establishment of the nematode (Koenning, et al., 1996). This was supported by the GWR analysis indicating that there was a significant spatial correlation between the spread of reniform nematode and percentage silt (Table 1).

During years two, three and four, the number of plots with detectible reniform nematode populations increased throughout the field, and population densities increased dramatically. Several possible mechanisms may have been involved in the increased incidence. Heavy rains fell in April 2002 immediately prior to planting, causing water to pool in the north end of the field. To improve drainage so planting could be accomplished, the grower cut surface drain furrows across (east to west) the north end of the field. It is likely that nematodes were moved across the field to new locations by equipment used to cut drain furrows and surface water drainage. Spread of the nematode parallel to the row direction (north to south) also was apparent, although much less uniform. While minimum-tillage was practiced in all years of the study, some movement of equipment through the field (herbicide, nematicide and fertilizer applications) occurred each year. It is possible that infested soil was carried to new areas along the row direction by equipment.

In addition to equipment and moving water, other possible means of transport of nematodes must be considered. A center-pivot irrigation system was used each year in the field. It is possible that infested soil was moved across the field to new sites on the wheels as they traveled through the field during irrigation events. Movement by this mechanism could explain the occurrence of individual grid plots toward the center of the field that appeared somewhat isolated from the main areas of infestation. Although some measures were taken to avoid spread of the nematode during sampling, it is also possible that the nematode was introduced to new areas due to the intensive soil-sampling regime that was practiced during the study.

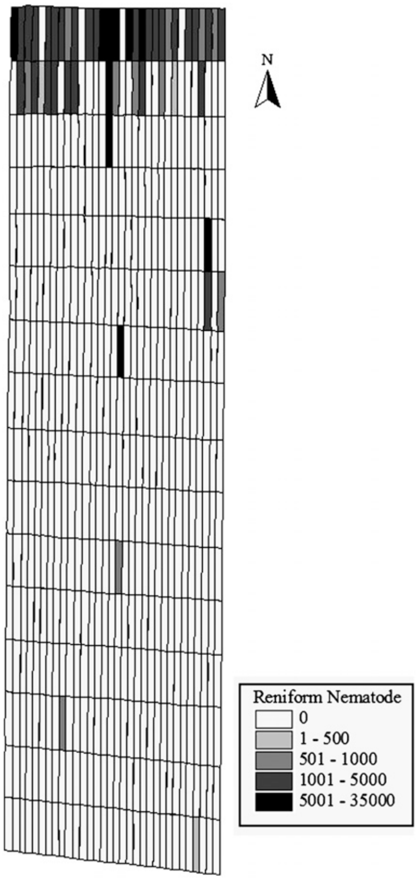

FIG. 6. Spatial distribution maps of reniform (*Rotylenchulus reniformis*) nematode in a 6.1 ha cotton field in southeastern Arkansas in Spring 2004. Spatial distribution maps were constructed utilizing the Latitude/Longitude coordinates of each plot in the GIS software SSToolBox (SST Development Group, Inc., Stillwater, OK). Reniform nematode population densities represent number of adults and juveniles per 500 cm^3 soil.

Personnel were advised to be careful to clean sampling equipment before moving to each new plot, but no attempt was made to surface sterilize the sampling tubes between plots.

It is possible that the reniform nematode was already present below detectible levels in some of the grid plots prior to the spring of 2001. This nematode has been reported to be able to survive for long periods of time in an anhydrobiotic state (Birchfield and Martin, 1967), and it may be found at considerable depths in the soil profile. However, given the fact that the field had a long history of continuous cotton production, it is likely that, if the nematode had been present prior to 2001, detectible levels would have been achieved before the study began. In our study, reniform nematode population densities increased dramatically in most grid plots following their original detection.

The reniform nematode seemed to be most successful in establishing in the higher silt content soils (51% to 68% silt) in the northern end of the field as supported by the GWR spatial analysis, indicating that there was a spatial relationship between the soil particle size distribution and the spread of reniform nematode. Reniform nematodes reproduce well and readily achieve high population densities in fine-textured soil (Koenning et al., 1996). The presence of the root-knot nematode did not appear to influence the success of the reniform nematode in becoming established in new areas, and, with very few exceptions, once reniform nematode was detected in a plot, both it and *M. incognita* were detected concomitantly in subsequent samplings.

The results suggest that the reniform nematode is capable of spreading successfully and reproducing aggressively across a broad range of soil textures in a relatively short period of time. The potential for within-field spread appears to be high, and the data imply that longer distance spread could be of major concern. Therefore, it is important that growers be aware of the presence of this species in individual fields so measures can be taken to minimize its introduction into uninfested fields or areas.

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