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Vertical Distribution of *Rotylenchulus reniformis* in Cotton Fields

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Abstract: The possible impact of *Rotylenchulus reniformis* below plow depth was evaluated by measuring the vertical distribution of *R. reniformis* and soil texture in 20 symptomatic fields on 17 farms across six states. The mean nematode population density per field, 0 to 122 cm deep, ranged from 0.4 to 63 nematodes/g soil, and in 15 fields more than half of the *R. reniformis* present were below 30.5 cm, which is the greatest depth usually plowed by farmers or sampled by consultants. In 11 fields measured, root density was greatest in the top 15 cm of soil; however, roots consistently penetrated 92 to 122 cm deep by midseason, and in five fields in Texas and Louisiana the ratio of nematodes to root-length density within soil increased with depth. Repeated sampling during the year in Texas indicated that up to 20% of the nematodes in soil below 60 cm in the fall survived the winter. Differences between Baermann funnel and sugar flotation extraction methods were not important when compared with field-to-field differences in nematode populations and field-specific vertical distribution patterns. The results support the interpretation that *R. reniformis* below plow depth can significantly impact diagnosis and treatment of cotton fields infested with *R. reniformis*.

Key words: cotton, *Gossypium hirsutum,* management, nematode, reniform, *Rotylenchulus reniformis,* vertical distribution.

Upland cotton (*Gossypium hirsutum*) is a deeply rooted plant and the Mexican and Caribbean racestocks from which modern cultivars were developed are adapted to semiarid environments. Historically, most studies on the nematodes of cotton in the United States focused on the root-knot nematode *Meloidogyne incognita,* which markedly deforms and stunts cotton roots, restricting most nematode feeding sites to the top 30 cm (Robinson, 1999). The reniform nematode *Rotylenchulus reniformis,* which is increasingly recognized as a yield-limiting factor in cotton in the United States east of New Mexico (Blasingame and Patel, 2004; Overstreet and McGawley, 1997), has a weaker influence than *M. incognita* on cotton root development, and yield losses are attributed primarily to fruit set delay and nutrient uptake impairment. Thus *R. reniformis* would be expected to feed and reproduce more deeply than *M. incognita* in the soil profile because *R. reniformis* allows roots to grow deeper.

Most studies of *R. reniformis* on cotton monitor nematodes only within the top 30 cm of soil, but deeper occurrences are reported. Heald and Thames (1980) found *R. reniformis* 1.75 m deep in a Texas Lower Rio Grande Valley (LRGV) field, which subsequently was maintained in cotton monoculture and re-sampled in 1998 (Robinson and Cook, 2001). In 1998 the highest population density (20 nematodes/gram of soil = 15 nematodes/cm^3 at 1.3 soil bulk density) of *R. reniformis* was 100 cm below the surface, in striking contrast with *Pratylenchulus agilis,* which was undetected deeper than 75 cm and at greatest density in the top 15 cm. Recently, *R. reniformis* was found below plow depth (30 cm) in Tennessee (Newman and Stebbins, 2002) and Mississippi (Lee et al., 2003). The vertical distributions of other nematodes found deep in the soil profile are reviewed by Westphal and Smart (2003). Fumigation experiments in two Texas fields indicated that half of the total cotton yield suppression caused by *R. reniformis* in a field may result from nematodes below 30 cm (Robinson et al., 2005; Westphal et al., 2004).

The objective of this research was to test the hypothesis that deep occurrence of *R. reniformis* in cotton is sufficiently common and pronounced to expect an important impact on diagnosis and treatment of infested fields. The hypothesis was tested by collecting and analyzing soil cores from selected cotton fields across the southern United States and examining nematode population densities in relation to depth, geographic region, soil texture and moisture, root growth, and nematode extraction technique.

Materials and Methods

Sampling site selection: Seventeen representative cotton farms with damaging levels of *R. reniformis* were identified by agricultural researchers and extension specialists in Alabama, Arkansas, Georgia, Louisiana, Missis-

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sippi, and Texas. Altogether, 20 fields on these farms were selected for sampling based on previous nematode samples and cropping history (Fig. 1). Two sides of the "Quarter" field at Sumner, Mississippi, were considered separate fields because cotton was inexplicably stunted on one side and not stunted on the other. Soil cores were collected from five fields with a 14-cm-diam. posthole digger, at 13 fields with a 3.3-cm-diam. 122-cm-long Environmentalists Subsoil Probe Plus (Clements Associates Inc., Newton, IA), and at two fields with a tractor-mounted 4.5-cm-diam., 122-cm-long hydraulic core sampler (Giddings Machine Co., Windsor, CO). All samples from 17 fields were collected by or under the direct supervision of the same person (A. F. Robinson). An average of 5.7 soil cores were collected from each field not counting the North farm in south Texas, where 126 cores were collected spanning 19 collection dates across 5 years. At only one field was a single core collected. At six fields, soil cores were collected on two or more dates. Cores were collected from the planting bed when plants were present.

Soil core analysis: All soil samples except those from Holly Grove, Arkansas, and Monte Alto, Texas, were processed in the same laboratory by the same technicians. Cores were separated into 15.2-cm vertical increments, each of which was thoroughly mixed and divided into 100-g or 40-g subsamples for analysis. Nematodes were extracted by the Baermann funnel technique (Robinson and Heald, 1991) or by centrifugal flotation (Jenkins, 1964; Liu et al., 2002). Soil texture determinations were made by the Bouyoucos method (Piper, 1944). Soil moisture determinations were based on weight loss overnight at 105 °C divided by the soil dry weight (Richards et al., 1954). Roots were extracted by suspending a 40-g subsample in 8 liters water and decanting into nested sieves with openings of 425, 180, and 150 µm. Root fragments were transferred with forceps from the 150-µm sieve to 2% formaldehyde solution and stored at room temperature. Total root length per sample was measured with a Win/Mac Rhizo root scanner (Regents Instruments, Ltd., Quebec, Canada).

Statistical analyses: Nematode counts and root measurements were subjected to analysis of variance, and treatment means for soil layers were separated by the protected LSD when *F* values were significant.

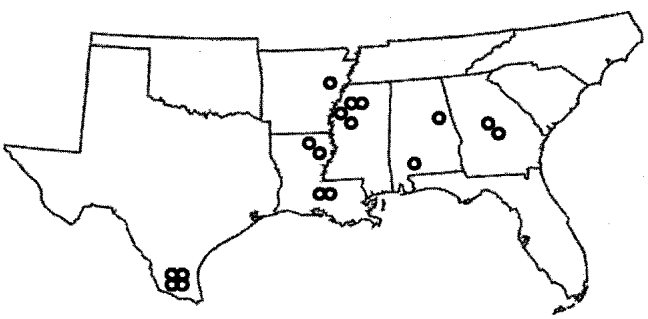

FIG. 1. Location of the 20 fields where 122-cm-deep soil cores were collected and analyzed for *Rotylenchulus reniformis,* soil texture, soil moisture, and cotton roots.

RESULTS AND DISCUSSION

Most fields had deep alluvial soils that were texturally uniform from the surface to 122 cm with the A horizon extending 90 cm or deeper (Figs. 2,3). Notable exceptions included the Lawrence test field and the Mitchner farm Quarter field at Sumner, Mississippi, where an abrupt increase in clay occurred at 76 to 92 cm, and in fields at the Delta Research Center, where sand content increased abruptly at 76 to 92 cm. The clay content varied over a relatively narrow range (20% to 40%) and most site-to-site variance was due to sand and silt content. Twelve fields had 40% to 60% silt and the other eight had 30% to 65% sand with less than 25% silt. In 11 of 14 cases where soil moisture was measured, the bottom half of the 122-cm core was wetter than the top half (Fig. 3).

The greatest population density of *R. reniformis* observed for any core within a 15-cm layer was 321 nematodes/g in the top 15 cm at Rayville, Louisiana. The mean nematode population density per field, 0 to 122 cm deep, ranged from 0.04 in the "normal" side of the Quarter field at the Mitchner farm at Summer, Mississippi, to 63 nematodes/g soil at the farm sampled at Rayville, Louisiana. The three next-highest mean population densities (33, 24, and 20 nematodes/g soil) were at Crowville, Louisiana; the Burden Plantation on the Louisiana State University campus at Baton Rouge; and the North farm in south Texas.

Starting at the soil surface, the average depth (across all field samples for both nematodes and roots) at which 50% of the nematodes were accumulated was 38.9 cm (range: 14 to 74 cm) and the corresponding depth for 50% of the roots was 24.7 cm (range: 11.3 to 49.0 cm). Thus, the ratio of the overall nematode median depth to the root median depth was 1.6. The field-specific nematode:root median depth ratios for the 11 fields where roots were collected were: 0.7, 0.9, 1.0, 1.1, 1.5, 1.5, 1.6, 1.9, 2.3, 2.8, and 5.1. Consequently, in 8 of 11 fields, the median depth for nematodes was 10% to 410% greater than the median depth for roots.

Most of the roots collected at all depths were less than 1 mm in diameter. The greatest root density was always in the top 15 cm of soil, although roots consistently penetrated 92 to 122 cm deep by mid season (Figs. 2,3,4). At the five fields in Alabama, Louisiana, and Texas where root extractions were conducted, the ratio of nematodes to roots in soil increased with depth, suggesting more feeding sites per centimeter of root on average in deep than in shallow soil layers. This pattern was not seen at any of the other six fields in Mississippi, all of which had 2 to 4 times the silt content of the Texas fields. It was noted, however, that the Burden field at Baton Rouge where the pattern did occur, unlike the Texas fields where it occurred, had a high silt

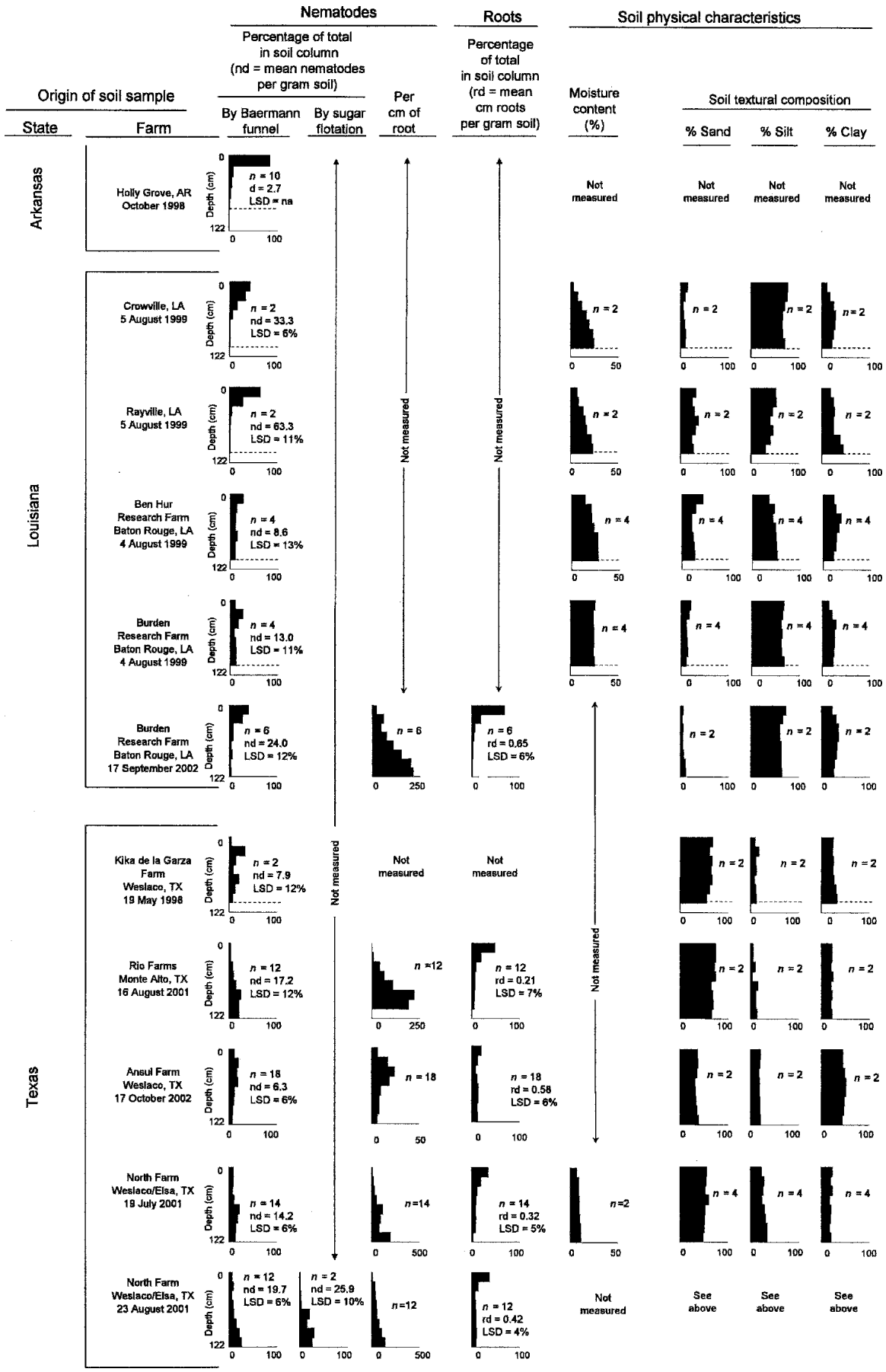

FIG. 2. Data from 122-cm-deep cores collected in cotton fields in Arkansas, Louisiana, and Texas, and analyzed for *Rotylenchulus reniformis,* cotton roots, soil moisture, and soil texture. Dashed line indicates deepest level sampled; *n* = number of cores averaged; nd = number of nematodes per gram soil; rd = cm roots per gram soil. LSD for Holly Grove, Arkansas, not available.

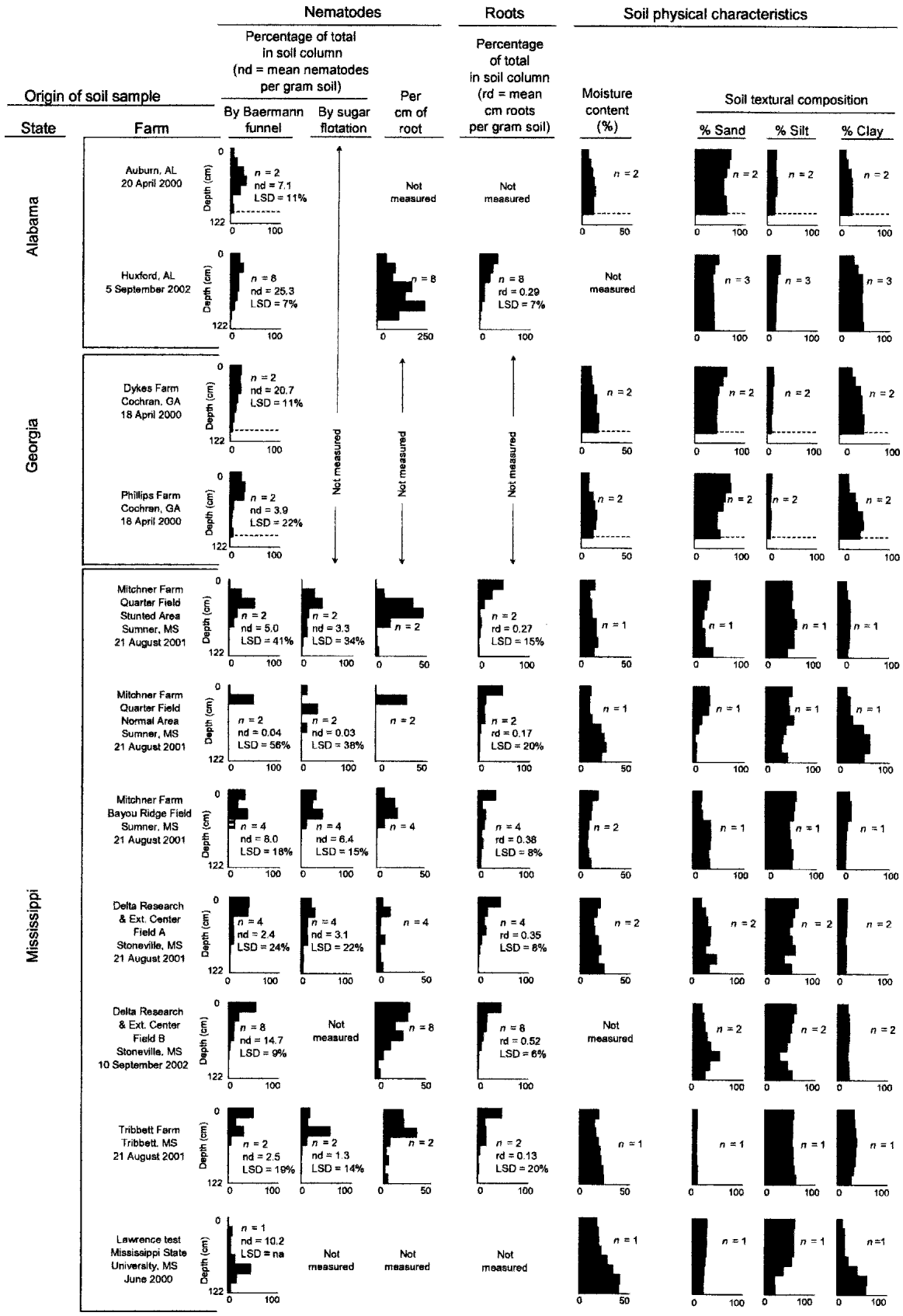

FIG. 3. Data from 122-cm-deep cores collected in cotton fields in Alabama, Georgia, and Mississippi, and analyzed for *Rotylenchulus reniformis*, cotton roots, soil moisture, and soil texture. Dashed line indicates deepest level sampled; *n* = number of cores averaged; nd = number of nematodes per gram soil; rd = cm roots per gram soil.

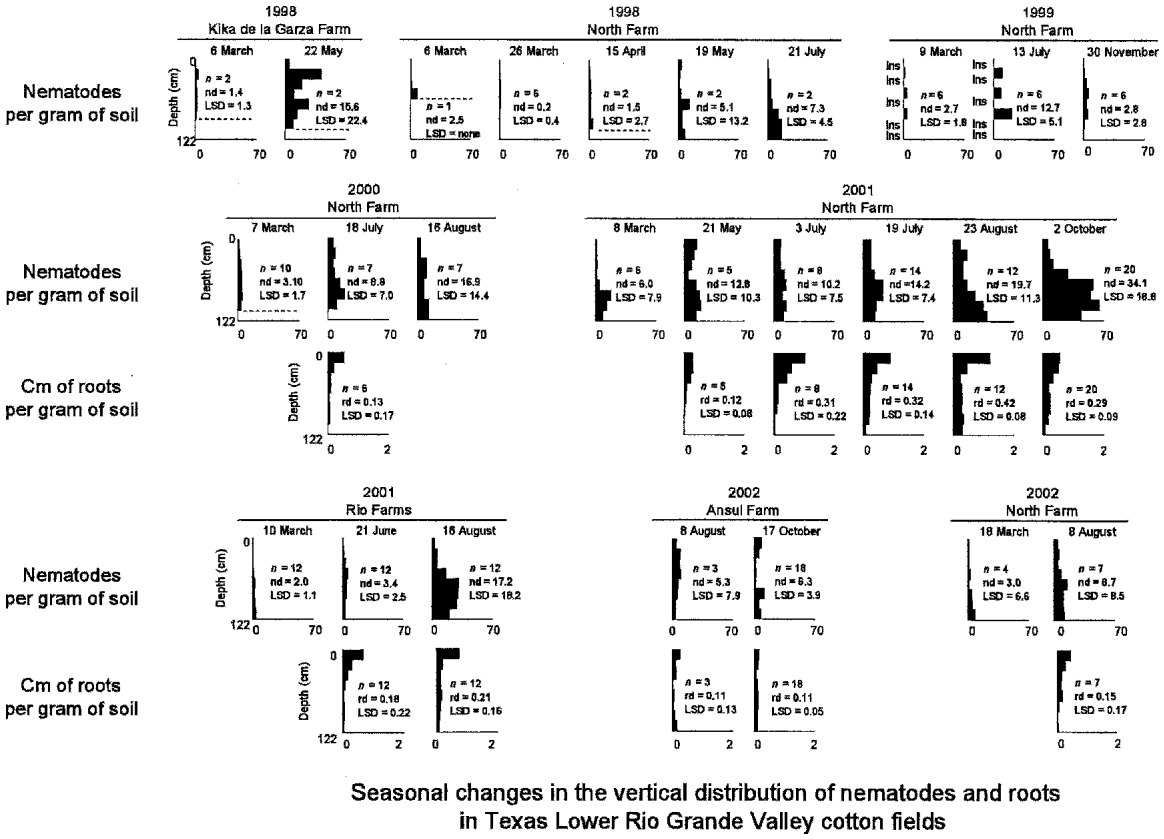

FIG. 4. Nematode population and root density data from repeated sampling of four Texas cotton fields illustrating seasonal changes in *Rotylenchulus reniformis* and cotton roots and persistent deep occurrence of nematodes in soil. Dashed line indicates deepest level sampled; *n* = number of cores averaged; nd = number of nematodes per gram soil; rd = cm roots per gram soil.

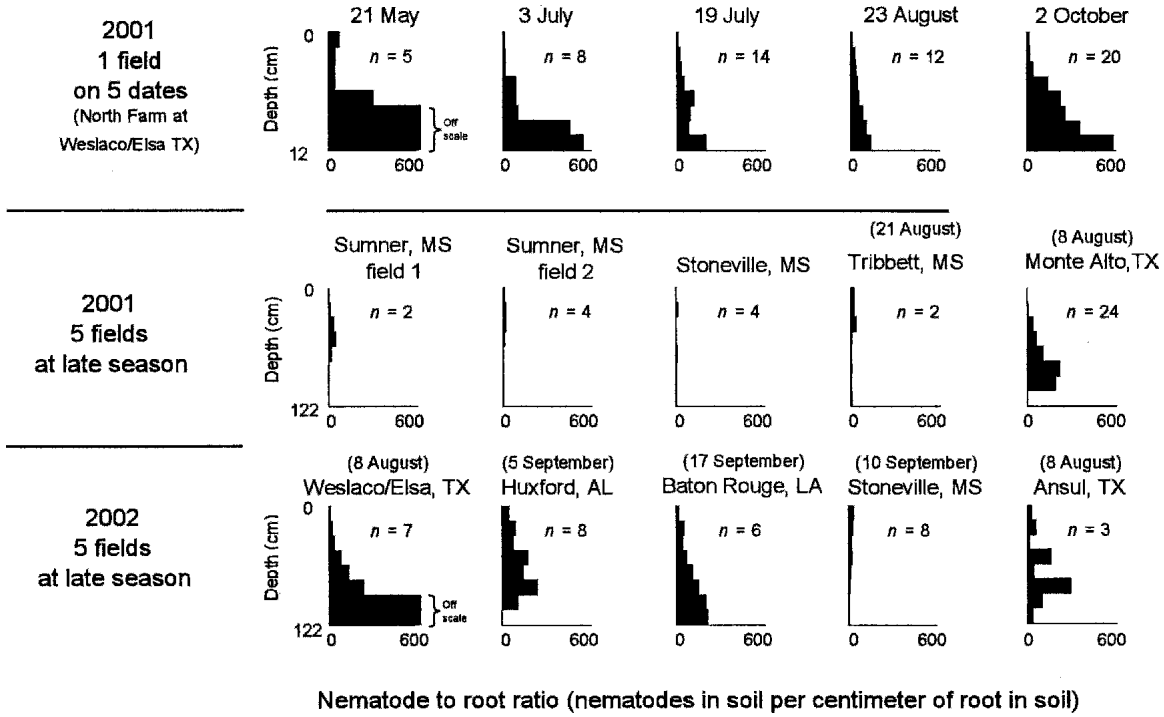

FIG. 5. Ratios of *Rotylenchulus reniformis* vermiform nematodes to cotton roots down through the soil profile at five points during the course of the growing season at one field in Texas in 2001, and compared at late season across 10 fields in 2001 and 2002. 1ns = layer not sampled.

content (70%) that was exceeded only at Crowville, Louisiana.

Temporal changes in the ratio of nematodes to roots in the soil profile across five dates in 2001 at the North farm field in Texas (Fig. 4,5) could be explained by changes in the density of roots and nematodes through time. High ratios in May, for example, may have resulted from presence of nematodes that had overwintered before roots penetrated deeply. *Rotylenchulus reniformis* can survive in soil for more than a year without a host (Heald and Inserra, 1988; Robinson et al., 1997). Low ratios in July, on the other hand, could be explained by increased root density before nematodes had time to reproduce. Other plausible explanations include downward movement by nematodes, high suitability of deep roots for feeding court establishment, and suppression of nematodes in the upper profile by heat or biological antagonists.

Sampling method effects were apparent but not important. In the six fields where Baermann funnel and centrifugal flotation extraction techniques were compared, 89% as many nematodes were extracted on average by centrifugal flotation as by Baermann funnel (Figs. 2,3). The ratio of nematodes obtained by Baermann funnel to those obtained by sugar flotation ranged from 50% for the Tribbett farm samples to 132% for the Delta Research and Extension Center samples. The Tribbett farm had an exceptionally low sand content (ca. 10%) and a high clay content (40%) throughout the soil profile. Finely textured soils with high clay content are typically difficult to process by centrifugal flotation. Soil from the Delta Research and Extension Center area was reported previously to be more effectively extracted for *R. reniformis* by centrifugal flotation than by Baermann funnel (Liu et al., 2002). The important result, however, is that mean nematode population densities and vertical nematode population patterns at each site were influenced little by extraction techniques when compared with field-to-field differences. Thus, extraction methods were unimportant compared to the sampling protocol implemented to evaluate the nematode situation in a given field.

In 15 of the 20 fields sampled, more than half of the nematodes present were below 30.5 cm, and thus below usual plow and soil sampling depths in cotton. The recommended treatment thresholds for *R. reniformis* vary across states and sample collection date, but fall between 1.54 and 15.4 nematodes/g soil (equivalent to 1,000 and 10,000 nematodes/500 cm^3 of soil, respectively, at 1.3 soil bulk density), based on samples collected in the top 30 cm of soil (Koenning, 2002; Komar et al., 2003; Overstreet, 2001; Sciumbato et al., 2004). In this study, in 12 of 20 fields sampled the greatest density below 30 cm was greater than the 15.4-nematode threshold, and in seven other fields it was above the 1.54-nematode threshold.

In cotton, deep roots play an important role in sustaining plant development (Hons and McMichael, 1986; McMichael and Quinsenberry, 1993; Oosterhuis and Bourland, 2001). Because only a small portion of the root system of a plant may be responsible for a large part of the total water uptake, deep roots can be critical to crop water status even when they comprise a small portion of the total root volume (Stone et al., 1976). In two Texas fields with different soils (Robinson et al., 2005; Westphal et al., 2004), comparisons between yield responses to fumigation at various depths indicated 33% yield losses were attributable to *R. reniformis* below 30 cm when at concentrations similar to those in fields examined in this study. Thus, our results support the interpretation that *R. reniformis* below plow depth may significantly impact diagnosis and treatment of cotton fields infested with *R. reniformis* across major areas of the United States cotton belt. For example, further study may show that yield response to nematicide treatment in a given field can be predicted by the proportion of the nematode population that will escape nematicide treatment due to deep nematode distribution. Alternatively, we may find that resistant rotational crops, and resistant cotton cultivars when they become available, can be used to suppress populations within deeper soil layers, which are not feasible to treat with nematicides.

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