Natural Migration of *Rotylenchulus reniformis* In a No-Till Cotton System

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Abstract: *Rotylenchulus reniformis* is the most damaging nematode pathogen of cotton in Alabama. It is easily introduced into cotton fields via contaminated equipment and, when present, is difficult and costly to control. A trial to monitor the natural migration of *R. reniformis* from an initial point of origin was established in 2007 and studied over two growing seasons in both irrigated and non-irrigated no-till cotton production systems. Vermiform females, juveniles and males reached a horizontal distance of 200 cm from the initial inoculation point, and a depth of 91 cm in the first season in both systems. Irrigation had no effect on the migration of vermiform females and juveniles, but males migrated faster in the irrigated trial than in the non-irrigated trial. Population density increased steadily in the irrigated trial during both years, exceeding the economic threshold of 1,000 per 150 cm², but was highly correlated with rainfall in the non-irrigated trial. The average speed of migration ranged from 0- to 3.3-cm per day over 150 days. *R. reniformis* was able to establish in both the irrigated and non-irrigated trials in one season and to increase population density significantly.

Key words: Behavior, cotton, *Gossypium hirsutum*, host-parasite relationship, movement, no-till, population dynamics, root growth, *Rotylenchulus reniformis*.

The reniform nematode, *Rotylenchulus reniformis* (Linford and Oliveira), is currently the most damaging nematode pathogen of cotton in Alabama. It is well established in 24 of the 59 cotton producing counties throughout the state (Gazaway and McLean, 2003) and has caused an estimated 7% annual yield loss totaling nearly $126 million over the past decade (Blasingame et al., 2009). The aboveground symptoms of damage to the plant by *R. reniformis* generally are patches of irregular plant growth that may include stunting, wilting, and intervinal chlorosis (Lawrence and McLean, 2001). Over time, these symptoms can become uniform throughout an entire field, resulting in yield decline. However, the time required for, and factors related to infestation, colonization, and temporal population increases are unknown.

Multiple studies have been published on *R. reniformis* distribution within a cotton field, particularly depth distribution. Heal and Thames (1980) reported *R. reniformis* at depths of up to 1.75 m from the surface and suggested that depth distribution through the soil horizon is correlated with cotton root growth. Robinson et al. (2005a) conducted a multi-state survey of *R. reniformis* depth distribution and reported population concentrations at varying depths, regardless of the depth reached by the cotton roots. This suggested that factors other than root growth alone are involved in soil horizon infestation by the nematode. Similarly, Lee et al. (2003) observing *R. reniformis* populations at 15-cm intervals to a depth of 1.2 m found populations at each depth to fluctuate throughout the season. The impact of deep populations of *R. reniformis* on cotton yield was demonstrated by Newman and Stebbins (2002), using late season side-dress applications of aldicarb, and by Robinson et al. (2005b), using 39 and 77 L/ha of 1,3-dichloropropene at both 43 and 81 cm. Both methods significantly reduced *R. reniformis* in the lower horizons and increased cotton yield.

Soil texture has been shown to affect both the distribution of *R. reniformis* throughout a field and population development. Starr et al. (1993) surveyed cotton fields in Texas and reported that only 12% of samples containing *R. reniformis* had a sand content of greater than 40%. In a similar survey in the Lower Rio Grande Valley of Texas, Robinson et al. (1987) observed that *R. reniformis* was found more often in fields with higher clay and silt contents than in fields with higher sand contents. Although *R. reniformis* has been shown to prefer finer textured soils, it does exist above economic thresholds in a wide variety of soil types (Gazaway and McLean, 2003).

*R. reniformis* has been found to exist in a wide variety of soil types at depths of up to 1.75-m, however factors influencing *R. reniformis* dispersion and population development once introduced into a cotton field are unknown. Considerable dispersion was reported under a minimum tillage production system in an Arkansas cotton field by Monfort et al. (2008), but no information is available on the potential of this nematode to spread in a no-till system. The rate of root growth or the movement of water through the field during a rain event or irrigation are two factors possibly affecting the migration of *R. reniformis*. As such, the objectives of these trials were to 1) monitor the vertical, horizontal and temporal migration of *Rotylenchulus reniformis* through a cotton field from an initial point of infestation, 2) determine if irrigated or non-irrigated production systems differentially affect the nematode migration, and 3) measure the population increase associated with field colonization.

**MATERIALS AND METHODS**

Two test fields (irrigated and non-irrigated) were established at the Tennessee Valley Research and Extension

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Center of Auburn University near Belle Mina, Alabama to document the natural migration of *R. reniformis* through the soil profile during the 2007 and 2008 growing seasons. The soil in both fields was classified as a Decatur silt loam (fine, kaolinitic, thermic, Rhodic Paleudults: 23% sand, 49% silt, 28% clay; 1% organic matter; pH 6.2) that had been continuously cropped in cotton under a no-till cultivation system for at least ten years. Both trial areas were sampled extensively in 2006 prior to the initiation of our trial to confirm the absence of *R. reniformis* (n = 30 samples across 2.8 ha). No other plant parasitic nematodes of economic importance to cotton in Alabama that could potentially compete with *R. reniformis* (e.g., *Meloidogyne incognita*) were observed within our test fields. Each test field contained five replicate plots consisting of seven rows of Delta and PineLand (DP 444 BGRR) cotton, planted using a John Deere 1700, four row vacuum planter. Rows were 7.8-m long on 1-m centers and the plots were separated by 4.6-m alleys. In each plot, rows one and five were inoculated with nematodes at 0 DAP (days after planting) in 2007 using an in-furrow spray system equipped with 8002 nozzles (R&D Sprayers, Opelousas, LA) placed horizontally over the row at a pressure of 2.1 kg/cm². *R. reniformis* was applied directly into the planting furrow in a 2.5-cm band to a depth of 5 cm at the rate of 8,300 vermiform life stages per meter of row in 46.8 liters/ha. Irrigation was applied in the irrigated field as needed throughout both seasons using an overhead center-pivot system.

Nematodes for this work were obtained from stock cultures grown on ST 5599 BG/RR cotton at the Auburn University Plant Science Research Center. The nematodes were increased in 10-cm diameter polystyrene pots containing 500 cm³ of a loamy sand soil (72.5% sand, 25% silt, 2.5% clay; 1% organic matter; pH 6.4). The soil was sterilized by autoclaving at 121 °C and 103.4 kPa for two hours on two successive days. Nematode inoculum consisted of *R. reniformis* eggs and vermiform life stages extracted from the soil and root systems of cotton plants using combined gravity screening and sucrose centrifugal flotation (Jenkins, 1964). Eggs were extracted by agitating the root system for 4 min in a 0.6% sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973) and collected on a 25-µm screen.

At 30 d increments throughout the 150 d growing season, horizontal nematode migration was assessed by taking ten, 15-cm deep soil samples from each 7.8-m row using a hand-held soil probe (Fig. 1). The samples were collected beginning in the rows farthest from the inoculated rows and working toward the inoculated rows. Soil probes were washed between samples to prevent the spread of the nematodes. Samples were taken directly from the seven row centers and 50-cm away from each row (row middles). The ten samples were combined and mixed, and a 150-cm³ sub-sample was extracted by combined gravity screening and sucrose centrifugal flotation and nematodes were enumerated using a Nikon TS100 inverted microscope at x40 magnification.

The vertical distribution of *R. reniformis* was determined by taking three core samples, 91-cm deep and 4.5-cm diameter, from each of the inoculated rows (rows one and five) and in rows three and seven (non-inoculated) of each plot using a #5-UV4 Model GSRSUV4G soil sampler (Giddings Machine Company, Windsor, CO) (Fig. 1). The three core samples from each plot were cut into sub-sections at 15-cm intervals, pooled, mixed thoroughly, and extracted as previously described.

The data for horizontal movement exhibited a non-normal distribution and was analyzed using SAS (SAS Institute, Inc, Cary, NC) using generalized linear models with a lognormal distribution. A fitted regression model was also utilized with the formula

\[
\text{nematode population} = \text{sampling date} + \text{distance} \times \text{distance (sampling date)}
\]

Residuals were modeled with a compound symmetry structure to account for correlation among sampling dates. The data for vertical movement was analyzed using generalized linear models with normal distribution. Least squares means of fixed effects were used to determine significance (*P* ≤ 0.10) by depth. Total population data were analyzed utilizing either Fisher’s Least Significant Difference (LSD) test, or Pearson product-moment correlation coefficients.

**Results**

*Horizontal migration and population growth:* The rate of horizontal migration of *R. reniformis* vermiform females and juveniles was identical within the irrigated and non-irrigated trials in 2007 (Table 1). Initially, vermiform females and juveniles were concentrated within the inoculated row and up to 50 cm on either side of the inoculated row. At 90 days after planting (DAP), the nematodes reached a distance of 150 cm from the

![Image](https://example.com/fig1.png)

**Fig. 1.** Sampling scheme for one replicate field plot. Samples for horizontal migration were taken from each of the cotton rows (solid lines) and 50 cm from each row (dashed line). A • represents where deep core samples were taken for vertical migration.
inoculated row and remained concentrated there through 120 DAP. The vermiform females and juveniles were detected at the maximum sampling distance of 200 cm from the inoculated row at 150 DAP. The rate of spread of vermiform females and juveniles averaged 0.5 to 3.3 cm per day during 30-day intervals over 150 days.

*R. reniformis* vermiform females and juveniles in both the irrigated and non-irrigated trials in 2008 developed first within the cotton rows before spreading to the row middles (Table 1). Within the irrigated trial, nematodes were observed in the inoculated row and the adjacent rows (100 cm) at 30 DAP, but not between these two rows at the 50-cm distance. At 60 DAP, nematodes were found in the inoculated row and at 50-, 100-, and 200-cm, but not at 150 cm. Populations were detected at every distance at 90 DAP and throughout the entire trial at 150 DAP. Within the non-irrigated trial, nematodes were detected in all cotton rows (0-, 100- and 200-cm) at 30 DAP, but not in the row middles (50- and 150-cm). At 60 DAP nematodes were found only in the inoculated row and adjacent rows (100 cm). Nematodes were detected in the inoculated row, 50-, and 100-cm at 90 DAP and at every sampling point with the exception of 150 cm at 120 DAP. Populations were detected across the entire trial at 150 DAP. *R. reniformis* males were not enumerated separately at 30 DAP in 2007. However at 60 DAP, it became apparent that males had migrated farther than the vermiform females and juveniles in both trials. Within the irrigated trial, males were detected at 60 DAP 150 cm from the inoculated row (Table 2); a distance 100-cm greater than the vermiform females and juveniles. At 90 and 120 DAP, males had migrated across the entire trial (200-cm). However, population levels dropped to undetectable levels at 150 DAP. Within the non-irrigated trial, males were detected 100 cm from the inoculated row at 60 DAP (Table 2), which was 50 cm

**Table 1.** Populations of *R. reniformis* vermiform females and juveniles for both the irrigated and non-irrigated trial over two seasons (2007 and 2008).

<table>
<thead>
<tr>
<th>Distance (cm)</th>
<th>2007 Irrigated</th>
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<tr>
<td></td>
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<td>200</td>
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*aHorizontal Distance (cm) from the inoculated row.  
*bDays after planting.  
*cLSD(0.10) comparisons of mean populations by distance at each sampling date. Means followed by the same letter are not significantly different.

**Table 2.** Populations of *R. reniformis* males for both the irrigated and non-irrigated trial over two seasons (2007 and 2008).

<table>
<thead>
<tr>
<th>Distance (cm)</th>
<th>2007 Irrigated</th>
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<tr>
<td></td>
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<td>200</td>
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*aHorizontal Distance (cm) from the inoculated row.  
*bDays after planting.  
*cNA = data not collected.  
*dLSD(0.10) comparisons of mean populations by distance at each sampling date. Means followed by the same letter are not significantly different.
farther than vermiform females and juveniles in the non-irrigated trial, but 50-cm less than males in the irrigated trial. Males in the non-irrigated trial were detected across the entire trial by 90 DAP, equaling the distance reached by males in the irrigated trial. The rate of migration for *R. reniformis* males averaged 0- to 3.3-cm per day during 30-day intervals over 150 days, equaling that for the vermiform females and juveniles.

In 2008, males were detected within the irrigated trial at every sampling distance from 30 – 120 DAP (Table 2), and in the inoculated row, 50-, and 100-cm at 150 DAP. Within the non-irrigated trial males were detected only within the cotton rows at 60 DAP (inoculated row, 100-, and 200-cm) (Table 2). At 90 and 120 DAP, males again covered the entire trial, and were detected at every distance with the exception of 100 cm at 150 DAP.

Overall population density of *R. reniformis* in the irrigated trial from seed germination through cotton boll maturity, or 0 to 120 DAP, increased in a linear fashion in 2007 under irrigation. Population growth of *R. reniformis* in 2007 was best described as a linear relationship over time with $y = 69.35 + 6.4127x$, where $x$ represents the days after planting, with an $r^2$ value of 0.97. However, population growth in 2008 were best described by a second order polynomial relationship between nematode populations over time with $y = 167.22 - 2.4523x + 0.1061x^2$, where $x$ represents the days after planting ($r^2 = 0.98$). *R. reniformis* populations within the inoculated row increased to economic threshold levels (> 1,000/150 cm$^3$) within 120 DAP in the second year after infestation (Table 1). This is a reproductive factor value (RF) of 8.35 in the second growing season.

Overall population density within the non-irrigated trial was closely correlated to rainfall (Fig. 2). The Pearson correlation coefficient for the comparison between rainfall and *R. reniformis* density was 0.95 ($P = 0.024$; Fig. 2). Similarly, the comparison of the 2008 population densities of *R. reniformis* and 2008 rainfall amounts between sampling dates produced a Pearson correlation coefficient of 0.92 ($P = 0.034$; Fig. 2).

Vertical migration and population growth: In the irrigated trial, *R. reniformis* moved downward through the soil profile from the initial inoculation depth of 5 cm to 90 cm within 150 days in 2007 (Fig 3A, B). Samples taken in the inoculated rows at harvest in 2007 revealed the highest nematode concentration in the top 15 cm of the profile (Fig. 3A). Samples taken in the non-inoculated rows (200 cm) yielded greater ($P \leq 0.10$) population densities within the 15- to 30-cm portion of the profile compared to all other depths (Fig. 3B). No other depths differed significantly ($P \leq 0.10$) but declined progressively with depth.

*R. reniformis* was observed at all sampling depths in the inoculated row of the irrigated trial at planting in 2008 with the exception of the 76- to 91-cm depth (Fig. 3A). Population densities were significantly higher ($P \leq 0.10$) in the top 15 cm of the inoculated row. The population densities at all other depths were not significantly different. Samples 200 cm from the inoculated row contained no detectable nematode populations at planting in 2008 (Fig. 3B). Population densities of *R. reniformis* within the inoculated rows at harvest in 2008 were significantly higher ($P \leq 0.10$) in the top 15 cm than at all other depths (Fig. 3A). Population densities 200-cm away were significantly higher ($P \leq 0.10$) in the top 15 cm and 76- to 91-cm depths than all other depths (Fig. 3B).

*R. reniformis* was detected in the non-irrigated trial to the maximum sampling depth of 91 cm in both the inoculated row and 200 cm from the inoculated row in 2007 (Fig. 3C, 3D). Population densities in the inoculated row were concentrated towards the upper half of the profile, and were significantly higher ($P \leq 0.10$) in the upper 30 cm of the profile (Fig. 3C). Samples taken 200 cm from the inoculated row contained significantly higher ($P \leq 0.10$) populations within the 0- to 15-cm and 30- to 46-cm depths (Fig. 3D). Population densities within the inoculated row at plant in 2008 were significantly higher ($P \leq 0.10$) in the top 15 cm of the profile (Fig. 3C), and the only nematodes observed 200 cm from the inoculated row at plant in 2008 were detected at the 30- to 50-cm.
45-cm depth (Fig. 3D). Final population densities within the inoculated row in 2008 were significantly higher \((P < 0.10)\) in the 0- to 45-cm depths (Fig. 3C) with no nematodes observed at the 75- to 91-cm depth. Population densities at 200 cm from the inoculated row were significantly higher \((P < 0.10)\) in the top 30 cm (Fig. 3D) with no nematodes detected at the 60- to 75-cm depth.

**DISCUSSION**

The horizontal migration of *R. reniformis* in our study was slower for the females and juveniles compared to the males. However, both were able to migrate at least 200 cm from the introduction site. We hypothesize that *R. reniformis* populations remained concentrated in the root zone of the inoculated row until lateral roots from the originally inoculated row and the next row either came into contact, or were close enough to warrant movement from one to the other. Once established within the first row away from the originally inoculated row, populations increased and continued to move along roots to the next row.

Although cotton root distribution was not assessed, migration patterns of the females and juveniles closely followed cotton root growth patterns described in numerous studies (Pearson and Lund, 1968; Taylor and Ratliff, 1969; Taylor and Klepper, 1974; Taylor and Klepper, 1978). Males, however, progressed more rapidly than did the females. As males do not colonize the root systems, we speculate that the search for females along the cotton roots and possibly soil water movement may play a role in their migration. Differences between the irrigated and non-irrigated trial in distance moved by males at 60 DAP is apparently due in large part to moisture availability, which could have direct effects on the nematodes and possibly influence lateral root proliferation. Both factors are likely to contribute, as soil water potential has been suggested to effect nematode locomotion (Hunt et al., 2001), and cotton root distribution is often affected by soil water content, especially when moisture is deficient (Taylor, 1983).

The difference between the irrigated and non-irrigated trials in overall *reniform* population growth in the top 15 cm of soil also is most reasonably attributed to cotton plant responses to adequate moisture. The expansion of cotton roots, and subsequently available feeding sites and reproductive potential, is dependent on environmental conditions until the plants begin producing fruit (McMichael, 1980). The increase in *R. reniformis* populations during the first 120 days of the 2007 and 2008 irrigated trials corresponds to root expansion. Irrigation and rainfall combined for an average of 23.4 mm and 14.7 mm weekly during both 2007 and 2008, respectively. Conversely, *R. reniformis*...
populations within the non-irrigated cotton were smaller than those within the irrigated trial, likely due to the lack of moisture available for cotton plant development. Rainfall in the non-irrigated trial averaged only 7.3 mm and 6.8 mm of rainfall weekly in 2007 and 2008 (i.e., 69% and 54% less than the irrigated trial). Root density, and corresponding feeding site availability, has been shown to decrease as soil moisture decreases (Taylor and Klepper, 1974). Additionally, lower soil moistures have been shown to decrease root elongation rates (Taylor, 1983), and promote root proliferation deeper in the soil profile while altering it in the upper parts of the profile (Taylor and Klepper, 1974; Browning et al., 1975) both of which could reduce reniform populations within the top 15 cm.

The detection of R. reniformis to the maximum sampling depth of 91 cm corresponds to multiple reports of populations being found at depths of greater than 100 cm (Heald and Thames, 1980; Newman and Stebbins, 2002; Lee et al., 2003; Robinson et al., 2005a). Populations were generally higher in the upper portion of the profile where the relative majority of lateral roots are found (Taylor and Klepper, 1978). However, the detection of the nematode to the depth of 91 cm in one season indicates migration vertically within the profile. This deep soil profile population occurred more quickly than anticipated in the newly colonized field. R. reniformis populations below the plow layer have been shown to be a source for the increase in surface populations in the first 30 days after cotton planting following non-host rotations in R. reniformis infested fields (Lee et al., 2003). However, our study is the first to show that the reniform nematode can establish in these deep soil profiles within one season. This is further evidence that once the soil is infested with R. reniformis the nematode will likely continue to survive and increase in that field.

R. reniformis movement through a cotton field occurs much more rapidly than anticipated with or without irrigation. Once a row is colonized, populations can migrate with lateral root expansion from row to row as long as growing conditions are favorable. Populations will develop quickly with adequate moisture and a susceptible host. If adequate moisture is not present at points during a season, R. reniformis can survive until it becomes available and again progress rapidly. If great care is not taken to prevent the spread of R. reniformis into a non-infested field, the colonization of the soil profile can occur quickly, and be irreversible and unstoppable.

**Literature Cited**


