Dimensional Movement of *Rotylenchulus reniformis* Through a Silt Loam: Observations of Movement and Population Growth from an Initial Point of Inoculation

by

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Abstract

Rotylenchulus reniformis was evaluated for total distance moved, rate of movement and population growth upon initial introduction into a cotton (*Gossypium hirsutum)* field under irrigated and non-irrigated conditions. The soil type in both fields was a Decatur silt loam (fine, kaolinitic, thermic, Rhodic Paleudults: S-S-C=23-49-28, 1% organic matter, CEC of 9 – 10, average pH 6.2). *Rotylenchulus reniformis* vermiform females and juveniles in both the irrigated and non-irrigated trials migrated to the row adjacent to the inoculated row (100cm) at 90 days after planting (DAP) and the second row from the inoculated row (200cm) at 150 DAP. Males within the irrigated trial reached a distance of 150cm at 60 DAP and 200cm at 90 DAP. Males within the non-irrigated trial reached only 100cm at 60 DAP and 200cm at 90 DAP. Rate of movement for *R. reniformis* averaged 1.7 cm per day. Overall populations of *R. reniformis* were the highest in the inoculated row, and were higher on average within the cotton rows compared to the row middles. Populations of *R. reniformis* were observed to the maximum sampling depth of 91 cm at 150 DAP and were highest within the top 15 cm of soil.

A trial to evaluate the effect of water infiltration and root growth on the downward migration of *R. reniformis* was conducted in 7.62 cm diameter by 75 cm deep soil cores of the same soil type as the field trial. Water infiltration minimally affected downward movement. The 25.4 mm rainfall treatment enabled the nematodes to reach a 30 cm depth. Rainfall of 76.2 mm was required to reach a 45 cm depth and 127 mm of rainfall was needed to surpass 45 cm. Root growth was closely related to populations of *R. reniformis* throughout the soil profile.

Cotton roots reached the maximum sampling depth of 75 cm at 60 DAP. Vermiform life stages of *R. reniformis* were detected at a depth of 75 cm at 45 DAP, however were not observed to colonize the root system at the 75 cm depth until 90 DAP. *Rotylenchulus reniformis* will migrate through the soil profile both vertically and horizontally in the search of their food source, the cotton roots, or *R. reniformis* males migrate in search of females to mate with. Once introduced into a cotton system *R. reniformis* will quickly colonize and irreversibly become established within the entire soil profile.

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Introduction and Literature Review

The reniform nematode (*Rotylenchulus reniformis* Linford and Oliveira) is the most economically damaging pest of cotton (*Gossypium hirsutum* L.) in Alabama. Since the first report of its presence in 1958 (Minton & Hopper, 1959), the reniform nematode has become the most widespread nematode pathogen in the state (Gazaway & McLean, 2003). Current damage estimates report a 7% annual yield loss equaling nearly $126 million over the past decade (Blasingame *et al*., 2009).

Life Cycle:

The life cycle of *Rotylenchulus reniformis* begins with a one-celled egg being oviposited by a mature female. The first stage juvenile (J1) appears in the egg and undergoes the first molt in the egg (Nakasono, 1973). After the first molt, the second stage juvenile (J2) emerges from the egg and undergoes two more molts to produce the third and fourth stage juvenile (J3 and J4). At this point, the cuticles from the previous two molts are retained and are visible as a result of *R. reniformis* becoming smaller and shorter instead of larger and longer (Bird, 1983). The final molt gives rise to approximately equal numbers of vermiform parasitic females and non-parasitic males; however, *R. reniformis* has been observed to reproduce parthenogenetically (Dasgupta and Raski, 1968; Nakasono, 1983) in which the majority of a population of vermiform adults is females. Once the infective females are in the vicinity of a host, they will penetrate the root cortex intracellularly (Rebois, 1980) and establish a permanent feeding site in the stele

(Cohn, 1973). The stelar penetration of the root results in the formation of syncytia, primarily from altered pericycle cells (Jones and Dropkin, 1975; Razak and Evans, 1976; Taha and Kassab, 1979). The anterior portion of the body will remain embedded, while the posterior portion protrudes from the root forming into a kidney-shape with the maturation of the reproductive system (Robinson *et al*., 1997). The female will then secrete a gelatinous matrix that flows from the vulva and is produced by vaginal glands (Sivakamur and Seshadri, 1971). Males are observed within these gelatinous matrices five days to two weeks after root penetration, (Robinson, 2007) facilitating sexual reproduction. These gelatinous egg matrices normally contain up to 60 eggs each, however up to 200 eggs have been observed in a matrix (Sivakamur and Seshadri, 1971). The life cycle on cotton from egg to egg was observed to be 17-23 days at room temperature by Birchfield in 1962; however, this varies depending on temperature (Bird, 1984; Heald and Inserra, 1988). In the absence of a host, *R. reniformis* can lie in a state of dormancy for long periods of time, even under extreme moisture stress (Birchfield and Martin, 1967; Guar and Haque, 1986) or prolonged exposure to cold (McLean *et al*., 2003), and still become infective.

Reniform-Cotton Interaction:

Symptoms of nematode damage on cotton are initially observed as areas of irregular, uneven stunting, which becomes homogeneous across the entire field over time. Other symptoms, as described by Robinson (2007), are a slowing of growth at the third or fourth leaf stage, a uniform slightly light or off-green appearance or purple cast of the leaves, and a delaying of flowering and fruit set. In general, the reniform nematode restricts the flow of water and nutrients from the roots which results in an overall unhealthy plant and can suppress yield up to 40% of the yield potential (Robinson, 2007). Intervienal chlorosis of the cotton leaf has been reported in some areas of the southeastern U.S. (Lawrence and McLean, 2001). From

1954-1956, Jones *et al*., (1959) initially observed the effects of the reniform nematode on yield, plant characters, and fiber properties of cotton in both greenhouse and field situations. There was a 12.6% reduction in total green weight of plants, a 21.3% reduction in total boll weight, and a 20.7% reduction in number of bolls attributed to an initial inoculation of 320 vermiform life stages per pint of soil in the greenhouse trial. The field trial produced similar results with significant reduction in total yield, weight per boll, and lint percentage.

Depth Distribution:

Rotylenchulus reniformis is most often observed in the top 30 cm of soil, simply because this is where soil samples are readily collected. However, *R. reniformis* has been documented to depths of up to 1.75 m, and the populations there have significant effects on cotton production and are in some cases higher. One study of the vertical distribution of *R. reniformis* in a cotton field (Heald and Thames, 1980) found populations at depths of 1.75 m. The highest populations occurred in the upper 1 meter and a transition zone between high and low populations occurred at approximately 1.25 m. Also noted was the significant decrease in roots at 1.5 and 1.75 m. A similar study was conducted on the vertical distribution of *R. reniformis* in cotton fields in Arkansas, Louisiana, and Texas (Robinson *et al*., 2000). Here, *R. reniformis* was found at high population densities down to below 100 cm in approximately half the fields sampled. Often in these fields, more than half of the *R. reniformis* found were below 30 cm. In contrast, the authors noted that some fields contained populations of *R. reniformis* only in the top 45 cm with the greatest numbers being in the top 15 cm. While the results observed by Heald and Thames (1980) were suggestive of a possible direct correlation between cotton root presence and

location of *R. reniformis*, the results of Robinson *et al*., (2000) suggests that there may be other factors involved in depth distribution.

A study of the vertical distribution of *R. reniformis* in a continuous cotton field and a cotton-corn rotation in Mississippi (Lee *et al*., 2002) found reniform at depths exceeding one meter in both fields. The authors point out that throughout the season, populations of *R. reniformis* fluctuated at all depths depending when during the growing season the sample was taken. The authors also suggest that the ability of *R. reniformis* to survive at lower depths in the profile could aid in explaining how populations can reach high levels in rotations with a non-host crop, such as corn. Newman and Stebbins (2002) studied the vertical distribution of *R. reniformis* in Tennessee and also found populations of *R. reniformis* to a depth of 1 m. The authors reported that the seasonal increase of *R. reniformis* that is observed in the upper 30 cm of the profile also takes place at the 31-100 cm depths. The authors observed that applications of 5.6 kg/ha aldicarb in-furrow at planting did nothing to reduce populations of *R. reniformis* below 50 cm. However, a 5.6 kg/ha side dress application later in the season did reduce populations in the lower 50 cm and increased yield an additional 40.4 kg lint/ha over the 5.6 kg/ha in-furrow application at planting only. This is evidence that *R. reniformis* below plow depth can have a definite impact on yield. Robinson *et al*., (2005a) examined nematode depth population densities in relation to geographic region, soil texture, soil moisture, and root growth. The results of this survey showed the clay content of fields ranging from 20% - 40% and sand and silt contents varying widely. The moisture level was observed to be higher in the bottom half of the 122 cm deep samples in 79% of the samples taken. In 75% of the fields sampled, the largest populations of *R. reniformis* were observed below the 30 cm depth and the median depth for nematodes was observed to be greater than the median depth for roots in 72% of the fields sampled. This study suggested that *R. reniformis* below plow depth can significantly impact

yield; it also referenced the results of Stone *et al*., (1976), which found that deep roots can be critical to crop water status, especially when moisture levels have been depleted in the upper horizon of the profile.

Fumigation experiments in the lower profiles of cotton fields have been conducted to measure the effects of *R. reniformis* below plow depth affecting cotton yield. Westphal *et al*., (2004) studied the depth distribution of *R. reniformis* under crops of both host and non-host status after fumigation with 107 L/ha of 1, 3-dichloropropene (1, 3-D) (Telone II, Dow Agro Sciences, Indianapolis, IN, USA) injected using shanks at 51 cm spacing. *Rotylenchulus reniformis* populations were the greatest under host crops at all depths sampled (1-120 cm) without fumigation. Also observed was that successive years populations were the highest in all depths under cotton when it followed another host of *R. reniformis*. Fumigation in the 0-60 cm depths was observed to reduce numbers in the 0-60 cm depths. Fumigation in the bottom half of the profile (60-120 cm) reduced numbers of *R. reniformis* to undetectable levels below 30 cm and resulted in the largest increase in yield of all treatments. The results of this study are, once again, evidence that *R. reniformis* below normal plowing and treatment depths has a large impact on yield. Observations from many of the aforementioned studies (Robinson *et al*., 2000; Lee *et al*., 2002; Newman and Stebbins 2002; Westphal *et al*., 2004, Robinson *et al*., 2005a) in addition to others, (Westphal and Smart 2003; Robinson *et al*., 2005b) observed higher populations at depths below 30 cm in many cases. The resurgence of populations of *R. reniformis* in the plow layer after nematicide application or rotation to a non-host crop has been attributed to these deeper living populations (Lee *et al*., 2002).

Soil texture has also been found to influence the distributions of *R. reniformis*. A microplot study of the effect of six different soil types on the reproductive and damage

potentials of *R. reniformis* (Koenning *et al*., 1996) observed reniform to be favored by moderate levels of clay plus silt. The six soil types tested are listed by series name, description, and sand, silt, clay, and organic matter content: Fuquay sand (loamy, siliceous, thermic, arenic, plinthic Kandiudults) 91, 6, 3, 0.6; Portsmouth loamy sand (fine-loamy over sandy or sandy-skeletal, mixed, thermic, Typic Umbraquelts), 72, 18, 10, 3.8; Norfolk sandy loam (fine-loamy, siliceous, thermic, Kandiudults), 84, 12, 4, 1.4; muck (Medisaprists), 58, 33, 9, <30; Cecil sandy loam (clayey, kaolinitic, thermic, Typic Kanhapludults), 53, 18, 29, 2.2; and Cecil sandy clay (clayey, kaolinitic, thermic, Typic Kanhapludults), 48, 13, 39, 0.9. The authors point out that soil type did have a significant effect on final populations (Pf) of *R. reniformis* in both years of the trial with the greatest Pf occurring in the Norfolk sandy loam and Portsmouth loamy sand (silt + clay = 16 and 28% respectively), intermediate Pf levels in the muck soil and Fuquay sand (silt + clay = 42 and 9% respectively), and the lowest Pf in the Cecil sandy loam and Cecil sandy clay (silt + clay = 47 and 52% respectively). However, the effect on yield in the differing soil types did not necessarily correlate with *R. reniformis* Pf values. The authors report that in the first year of the study, cotton yields were suppressed in the muck soil and Fuquay sand, while in the second year, yields were suppressed in all soil types. This is an example, as the authors note, of *R. reniformis* having high reproductive and damage potentials in varying soil types; however, certain silt + clay percentages may provide a competitive advantage over other cotton nematode pathogens.

A study of the association of *R. reniformis* and soil textures in Texas cotton fields was conducted from 1989-92. Starr *et al*., (1993) reported finding *R. reniformis* associated with finely textured soils, with only 12% of all samples containing *R. reniformis* having more than 40% sand content. A similar study of the distributions of *R. reniformis* as related to soil texture in the Lower Rio Grande Valley (LRGV) of Texas also found *R. reniformis* more commonly associated

with finer-texture soils (Robinson *et al*., 1987). The authors reported high incidences of *R. reniformis* in Harlington, Mercedes, and Benito clays (45-78% clay), as well as in silty clays and silty clay loams of the Laredo-Olmito association (32-42% clay). Moderate levels of *R. reniformis* were observed in the sandy clay loams and clay loams of the north-central LRGV, but were rarely observed in the sandy loams of the western LRGV, and never observed in the western, gravelly loams and relatively low-clay soils (12-22% clay) of the Rio Grande-Matamoros association along the western flood terraces of the modern-day Rio Grande. *Rotylenchulus reniformis* has been observed to prefer finer-textured soils; however, throughout many areas of the United States, it can be found in sandy soils as well (Heald and Robinson, 1990). A survey of the distribution of *R. reniformis* in Alabama (Gazaway and McLean, 2003) observed *R. reniformis* above economic threshold levels in the Tennessee River Valley of north Alabama where soils are generally silt loams to silty clay loams (12 – 40% clay, < 20% sand), as well as in the coastal plain soils of central Alabama where soils are generally sandy loams or loamy sands (<20% clay, 52-85% sand). Although the studies illustrate that *R. reniformis* is a very widespread cotton parasite, direct determinations of preference and movement within different soil types has yet to be determined.

Movement

A study of the temporal spread of *R. reniformis* was done in a cotton field in Arkansas from 2001 – 2004 (Monfort and Kirkpatrick, 2005). From the first observation of *R. reniformis* in the field in May 2001, a grid-sampling experiment was conducted sampling 512 grids (grid = 30.5 m *x* 4 m) throughout the field and mapping observed populations using GPS/GIS technology. The authors report that in the first year of the experiment, observations of *R. reniformis* increased from one of 512 grids at the beginning of the season, to 17 of 512 grids at the end of the year.

Over the course of the study, *R. reniformis* spread to 107 of the 512 grids sampled. The authors also noted that the highest population densities of all grids were the grids with soil types ranging from 26-30% sand. A study to examine the effects of temperature on the movement of *R. reniformis* was conducted in acrylic tubes filled with sand and subjected to natural temperature gradient fluctuations (Robinson, 1994). The nematodes were placed in the sand-filled tubes in vials 20-36 hours prior to the experiment to allow for adaptation to the environment, and then subjected to either upward, downward, or horizontal heat with the sand-filled tubes oriented either horizontally or vertically. When heat waves were propagated upward, downward, or horizontally, *R. reniformis* moved away from the thermal surface irrespective of gravity or tube orientation, apparently striving to remain close to a thermal preference. The author suggests as a result of this observed thermal preference, that as the soil was warmed by solar energy, *R. reniformis* would move away from heat or most likely, downward. Likewise, when the soil began to cool, *R. reniformis* would move toward heat, again most likely downward, to remain closer to a thermal preference. A study of the movement of *R. reniformis* from soil in Baermann funnels subjected to temperature gradients (Robinson and Heald, 1989) revealed patterns of response to ambient temperatures when shifted in the direction of the storage temperature of the nematodes. A study of movement of *R. reniformis* affected by temperature was conducted on populations of *R. reniformis* stored at different temperatures and placed in 4 cm diameter tubes of either 0.75% water agar or moist sand of two different particle sizes (150-250 µm and 250-425 µm), and subjected to different temperatures (Robinson and Heald, 1993). The study revealed similar results to the study of movement from soil in Baermann funnels. The authors reported that *R. reniformis* stored at 17°C moved toward the cool ends of cylinders when the mean temperature was between 24 and 34°C, and moved toward heat only when placed below approximately 23°C. Nematodes stored at 30°C moved toward cool only when placed above

30°C, and moved toward heat when placed between 25 and 30°C. These results illustrate an apparent preference of approximately 30°C for nematodes stored at 30°C, while nematodes stored at 17°C had a preference to move toward temperatures of approximately 23°C.

Rotylenchulus reniformis will, over time, become distributed throughout a cotton field once it is introduced. Many factors can have influence on movement and behavior of *R. reniformis*; however the predominant factors driving migration are unknown. The objectives of this study are to 1) Observe the natural migration of *R. reniformis,* both horizontally and vertically, within a cotton field from the initial point of inoculation; 2) Determine the effect of soil moisture on the migration of *R. reniformis*; 3) Observe the population development of *R. reniformis* with the migration; and 4) Determine the effects of water infiltration and root growth on the vertical migration of *R. reniformis*.

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Natural Movement of *Rotylenchulus reniformis* Upon Introduction Into a No-Till Cotton System

Abstract

Rotylenchulus reniformis is the most economically damaging 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 spread of *R. reniformis* from an initial point of origin was established in 2007 and monitored over two years in both irrigated and non-irrigated no-till cotton production systems. Both vermiform females and 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 trials. Moisture had no effect on the movement of vermiform females and juveniles; however, males moved faster in the irrigated trial than in the non-irrigated trial. Males in the irrigated trial at 60 days after planting reached a distance of 150 cm from the initial point of inoculation, while in the non-irrigated trial they were detected only to the 100 cm distance. Populations of *R. reniformis* continued to rise throughout the second year reaching greater than 1,000 per 150 cm^3. Reproduction within the irrigated trial increased linearly both years, whereas within the non-irrigated trial was highly correlated with rainfall amount. Movement of vermiform females and juveniles averaged between 0 and 3.3 cm per day and corresponded with published cotton root growth patterns. Males exhibited faster movement under irrigation, and also achieved rates as high as 3.3 cm per day. Overall population growth showed a dependency on soil moisture throughout both years. *Rotylenchulus reniformis* was able to colonize both the irrigated and non-irrigated trials in one season, and make significant increases in population. While this may not be surprising in a conventional tillage operation, the

outcome of this trial illustrates that *R. reniformis* can colonize a field unaided and have a rapid impact in a no-till system.

The reniform nematode, *Rotylenchulus reniformis*, is currently the most economically damaging nematode pathogen of cotton in Alabama. It is well established in 24 of the 59 cotton producing counties throughout the state, (Gazaway & McLean, 2003) and has caused an estimated 7% annual yield loss equaling nearly $126 million over the past decade (Blasingame *et al*., 2009). The foliar symptoms of damage to the plant by *R. reniformis* are generally patches of irregular plant growth such as stunting, wilting and inter-venal chlorosis. These symptoms can, over time, become uniform throughout an entire field with yields steadily declining. However, the time and factors related to infestation, colonization, and temporal population increases are unknown.

Multiple studies have been published on *Rotylenchulus reniformis* distribution within a cotton field, particularly depth distribution. Heald and Thames (1980) reported *R. reniformis* at depths of up to 1.75 m from the surface and suggest that depth distribution through the soil horizon is correlated with cotton root growth. Robinson (2000) conducted a multi-state survey of *R. reniformis* depth distribution and reported population concentrations of *R. reniformis* at varying depths at different locations. This suggested that factors other than root growth alone are involved in soil horizon infestation by the nematode. Similarly, Lee *et al*., (2002) observed *R. reniformis* populations at depths up to 1 m fluctuating throughout the growing season. The impact of *R. reniformis* distributions deep into the soil profile was observed by Newman and Stebbins (2002). The authors found that late season side-dress applications of aldicarb could lower *R. reniformis* populations in the lower horizons and thereby increase cotton yields.

Soil texture has also been well documented to affect the distribution of *R. reniformis* throughout a field, as well as population development. Starr *et al*., (1993) conducted a survey of cotton fields in Texas and reported that only 12% of samples containing *R. reniformis* had a sand

content of greater than 40%. Similarly, Robinson *et al*., (1987) conducted a survey in the Lower Rio Grande Valley of Texas and 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).

Movement of *R. reniformis* as affected by temperature was observed by Robinson (1994) using sand-filled tubes subjected to temperature change. The author reported that once *R. reniformis* was at an ambient temperature, it would move toward or away from the thermal surface in an effort to remain close to that ambient temperature. The same phenomenon was also observed earlier by Robinson and Heald (1989) utilizing Baermann funnels subjected to temperature gradients.

Rotylenchulus reniformis has been shown to exist in a wide variety of soil types at depths of up to 1.75 m and have preferential movement towards an ambient temperature in vitro. However, once in a cotton field, the sum of these factors and other possible factors on *R. reniformis* dispersion and population development are unknown. Conventional wisdom of the movement of *R. reniformis* has been that the nematodes can only move a few centimeters in their lifetime and tillage practices are the cause of dispersion. As such, the expected outcomes of these trials are that *R. reniformis* will move, at most, one row (100cm) in one season under the influence of irrigation, and much less in the non-irrigated trial. Populations in the irrigated trial are also expected to reach much higher levels compared to non-irrigated populations. Thus the objective of the trials included here are to: 1) monitor the vertical, horizontal and temporal movement of *Rotylenchulus reniformis* through a cotton field from an initial point of inoculation;

2) determine if irrigated and dry land conditions affect the nematode distribution; and 3) measure the population iincrease associated with field colonization.

MATERIALS AND METHODS

Two tests were established in May 2007 at the Tennessee Valley Research and Extension Center near Belle Mina, Alabama to document the natural spread of *R. reniformis* through the soil profile over the growing season. The tests were established under irrigated and dry land cropping conditions. The soil in both fields was classified as a Decatur silt loam (fine, kaolinitic, thermic, Rhodic Paleudults: 23%, 49%, 28%, S-S-C, 1% organic matter, CEC of 9 – 10, average pH 6.2), that had been continuously cropped in cotton under a no-till cultivation system for at least 10 years. The test was planted in eight rows of Delta and PineLand (DPL 444 BGRR) cotton, using a John Deere 1700, 4 row vacuum planter. Plots were 7.8 m long on 1 m centers and the adjacent five blocks were separated by 4.6 m alleys. In each plot, two rows were inoculated at planting using the in-furrow spray system equipped with 8002 nozzles placed horizontally over the row. *Rotylenchulus reniformis* was applied at 8,300 vermiform life stages per meter of row in 46.8 L/ha.

Rotylenchulus reniformis (Linford and Oliveira) used to infest the fields were increased from stock cultures grown on DPL 5599 BG/RR cotton at the Auburn University Plant Science Research Center located on the Auburn University campus. The nematodes were increased in 10 cm diameter polystyrene pots containing 500 cm^3 of a loamy sand soil (72.5% sand, 25% silt, 2.5% clay, OM 1%, 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 (specific gravity = 1.13) centrifugal flotation. Eggs were extracted

by agitating the root system for 4 minutes in a 0.6 % sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973).

At planting and at harvest, vertical populations of *R. reniformis* were determined by taking three core samples, 91 cm deep and 4.5 cm diameter, from each replication in the inoculated rows, one and five, and in rows three and seven of each plot using a #5-UV4 Model GSRPSUV4G (Giddings Machine Company, Windsor, CO). These samples were cut into sub-sections at 15 cm intervals, mixed thoroughly and evaluated for number of nematodes and soil moisture content. Horizontal observations of nematode population movement were determined by taking 15 cm deep soil samples at 30 day increments throughout the season. The samples were taken from directly in the row, and 25 cm and 50 cm away from the row. These samples were evaluated for number of nematodes and soil moisture content. Figure 2.1 illustrates the sampling scheme for one replication. A 150 cm^3 sub-sample was taken from every soil sample for nematode extraction. Each sub-sample was extracted by combined gravity screening and sucrose centrifugal floatation and enumerated using a Nikon TS100 inverted microscope at 40x magnification. Twenty grams of soil from each sample was weighed, dried at 100°C, and measured for soil moisture content.

The data for horizontal movement exhibited a non-normal distribution and was analyzed by SAS (SAS Institute, Inc) using generalized linear models with a lognormal distribution. A fitted regression model was also utilized with the formula count = sampling date + distance*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 ($P \leq 0.10$) by depth. Total population data were

analyzed utilizing either a linear regression over time, or correlating factors using Pearson product-moment correlation coefficients.

RESULTS

Irrigated Trial: The *R. reniformis* vermiform females and juveniles in the top 15 cm of the irrigated trial migrated laterally at an average of 1.67 cm per day in the 2007 season, (Fig. 2.2). At 30 DAP, populations were found to have expanded 50 cm on either side of the originally inoculated row. Nematode population levels formed an inverted parabola with higher numbers present in the row middles than in the center of the inoculated row. The nematodes were found in this same region at 60 DAP; however, populations had increased in the originally inoculated row reversing the crux of the parabola. Between 60 and 90 DAP, vermiform females and juveniles were observed to have migrated 150 cm from the originally inoculated row, or 1.5 cotton rows. This distance corresponds with *R. reniformis* being concentrated in the root zone of the cotton plants one row away from the originally inoculated row, just as they increased in the originally inoculated row at 60 DAP. Movement of the vermiform females and juveniles between 90 and 150 DAP mimicked the migration observed between 0 and 90 DAP. At 90 DAP, vermiform females and juveniles were observed to be concentrated in the originally inoculated row with populations spreading 150 cm laterally from that row and remaining in this zone through 120 DAP. The next wave of expansion occurred at 150 DAP for a total expansion distance of 200 cm, or two cotton rows. Total distance migrated by *R. reniformis* vermiform females and juveniles in the 2007 irrigated trial was 200 cm from the originally inoculated row. The rate of movement ranged from 0 – 3.3 cm per day, with an average movement of 1.7 cm per day.

Population development of vermiform females and juveniles within the inoculated row increased until plant maturity in 2007 (Fig. 2.3). At 30 DAP, populations averaged 77 per 150 cm^3

of soil and increased an average of 41% at each sampling date for populations of 108, 147 and 216 per 150 cm^3 at 60, 90, and 120 DAP, respectively. Vermiform females and juveniles decreased 94% to 13 per 150 cm^3 at harvest (150 DAP). Populations in the row middles, or between the inoculated row and the adjacent row (50 cm), averaged 97 per 150 cm^3 and were similar to those in the inoculated row at 30 DAP. Sixty day populations decreased 91.8% from 30 DAP to 8 per 150 cm^3, followed by an increase to 19 per 150 cm^3 at 90 DAP. Populations at 50 cm peaked at 120 DAP averaging 100 per 150 cm^3 before declining to a population of 18 per 150 cm^3 at 150 DAP. The row adjacent to the inoculated row (100 cm) yielded its' first detectable and highest populations of 2007 at 90 DAP, averaging 120 per 150 cm^3. Populations declined through the remainder of the season, averaging 89 and 25 per 150 cm^3 at 120 and 150 DAP, respectively. Samples taken at 150 cm detected a nematode population of 8 per 150 cm^3 at both 90 and 120 DAP with a subsequent decline to 2 per 150 cm^3 at 150 DAP. The *R. reniformis* vermiform females and juveniles had migrated 200 cm, or two rows, from the inoculated row by 150 DAP; however, the population numbers were low averaging 8 per 150 cm^3. Populations within the inoculated row were significantly higher ($P \leq 0.10$) over all other populations at 60, 90, and 120 DAP; with the exception of the 100 cm samples at 90 DAP. The number of vermiform females and juveniles within cotton rows were higher ($P \leq 0.10$) in comparison to samples taken from the row middles, or between the rows, at 90 DAP. However, these population differences were significant ($P \leq 0.10$) only at 90 DAP.

Rotylenchulus reniformis males in the 2007 irrigated trial were observed to migrate more rapidly than the vermiform females and juveniles (Fig. 2.4). Although present at 30 DAP, the distance moved compared to the vermiform females and juveniles at 60 DAP was drastically different. At 60 DAP, *R. reniformis* males were observed to have migrated 150 cm from the originally inoculated row. This is a 100 cm increase in distance as compared to the 50 cm

migration of the vermiform females and juveniles. In only 90 days, *R. reniformis* males were observed at 200 cm, or two cotton rows, from the originally inoculated row. The vermiform females and juveniles were found at 200 cm, but not for another 60 days. The total distance traveled by the *R. reniformis* males in the 2007 irrigated trial was 200 cm with a rate of migration for males as high as 3.3 cm per day, and a seasonal average of 1.7 cm per day.

Male populations did not proliferate as did the vermiform females and juveniles in 2007 (Fig. 2.5). Within the inoculated row, populations averaged 23 per 150 cm^3 at 60 DAP, doubled to 46 per 150 cm^3 at 90 DAP, and increased again to 70 per 150 cm^3 at 120 DAP. In contrast to the steadily increasing populations within the inoculated row, males at 50 cm were the highest at 60 DAP, averaging 89 per 150 cm^3, and declined to 24 and 27 per 150 cm^3 at 90 and 120 DAP. Within the row adjacent to the inoculated row, males averaged 50 per 150 cm^3 at 60 DAP, declined to 4 per 150 cm^3 at 90 DAP, and rebounded to 31 per 150 cm^3 at 120 DAP. Samples taken at 150 cm from the inoculated row yielded average populations at 60 DAP of 77 per 150 cm^3, decreased to 27 per 150 cm^3 at 90 DAP, and had a final population of 47 per 150 cm^3 at 120 DAP. Males were not detected at 60 DAP in the 200 cm samples; however populations at 90 and 120 DAP averaged 35 and 16 per 150 cm^3, respectively. There were no detectable levels of *R. reniformis* males at 150 DAP. Male populations fluctuated throughout the trial at all distances in 2007 however they were higher ($P \leq 0.10$) in between the cotton rows at 60 DAP.

Irrigated populations of *R. reniformis* vermiform females and juveniles in 2008 developed first within the cotton rows before spreading to the row middles (Fig. 2.6). Initially, at cotton planting, nematodes were only detected within the inoculated row. However populations were observed within 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, populations were

observed within the inoculated row, and at 50, 100, and 200 cm, but not at 150 cm. Populations were detected at every distance at 90 DAP, regressed to a maximum distance of 150 cm at 120 DAP, and again covered the entire trial at 150 DAP.

Populations within the inoculated row increased dramatically during active plant growth in 2008 (Fig. 2.7). At 30 DAP, vermiform females and juveniles averaged 54 per 150 cm^3, increased to 131 per 150 cm^3 at 60 DAP, 409 per 150 cm^3 at 90 DAP, and peaked at 1,027 per 150 cm^3 at 120 DAP. At harvest (150 DAP) 448 per 150 cm^3 vermiform females and juveniles remained in the inoculated row. Populations 50 cm from the inoculated row remained low throughout 2008 beginning at 4 per 150 cm^3 at 60 DAP, increasing to 8 per 150 cm^3 at 90 DAP, and peaking at 16 per 150 cm^3 at both 120 and 150 DAP. The row adjacent to the inoculated row (100 cm) gradually increased during active plant growth. Thirty day populations averaged 39 per 150 cm^3, increased to 43 per 150 cm^3 at 60 DAP, again at 90 DAP to 58 per 150 cm^3 and reached the maximum population of 105 per 150 cm^3 at 120 DAP before a decline to 27 per 150 cm^3 at harvest (150 DAP). As at the 50 cm distance, populations at 150 cm remained low in 2008. Although not detected at 30 DAP, vermiform females and juveniles appeared at 60 DAP two rows from the inoculated row (200 cm) before being detected at the 150 cm distance. Populations 200 cm from the inoculated row at 60 DAP averaged 23 per 150 cm^3, 15 per 150 cm^3 at 90 DAP, were not detected at 120 DAP and ended the season (150 DAP) at 31 per 150 cm^3. The inoculated row yielded populations of vermiform females and juveniles remarkably higher than at all other distances and were significantly elevated ($P \leq 0.10$) at all sampling dates with the exception of the 100 cm distance at 30 DAP. Populations within the inoculated row increased to economic threshold levels within 120 DAP in the second year after infestation. This is a reproductive factor value (Rf) of 8.35 in the second growing season. *Rotylenchulus*

reniformis vermiform females and juveniles developed first within the cotton rows colonized in 2007, and subsequently expanded to the row middles.

Rotylenchulus reniformis males were detected only within the inoculated row at planting; however, they were in all rows sampled at 30 DAP in 2008 and remained through 120 DAP (Fig. 2.8). No males were detected at the 100 or 200 cm distances at 150 DAP. Male populations remained low throughout the entire trial in 2008 (Fig. 2.9). Within the inoculated row, populations averaged 15 and 8 per 150 cm^3 at 30 and 60 DAP, increased to 85 per 150 cm^3 at 90 DAP, declined to 77 per 150 cm^3 at 120 DAP and ended the season at 116 per 150 cm^3. The 50 cm distance averaged 23 and 24 per 150 cm^3 at 30 and 60 DAP, declined to 12 per 150 cm^3 at 90 DAP, increased to 39 per 150 cm^3 at 120 DAP, and declined again to 20 per 150 cm^3 at 150 DAP. Populations within the row adjacent to the inoculated row (100 cm) averaged 43 per 150 cm^3 at 30 DAP and declined gradually to 27, 12, and 15 per 150 cm^3 at 60, 90, and 120 DAP, respectively. No males were detected 150 DAP at the 100 cm distance. A constant population of 12 per 150 cm^3 was detected 150 cm from the inoculated row at 30, 60, and 90 DAP. These populations increased to 31 per 150 cm^3 at 120 DAP before reducing to 8 per 150 cm^3 at 150 DAP. Populations fluctuated at the 200 cm distance with averages of 9, 35, 16 and 23 per 150 cm^3 at 30, 60, 90, and 120 DAP, respectively. No males were detected 150 DAP at the 200 cm distance.

Overall populations of *R. reniformis* from seed germination through cotton boll maturity, or 0 to 120 DAP, increased in a linear fashion in 2007 (Fig. 2.10) under irrigation. The availability of consistent soil moisture enabled uniform growth of both the cotton plant and reproduction rates of the nematodes. *Rotylenchulus reniformis* populations exhibited a linear population increase over time. Populations in 2007 were best described as a linear relationship

over time with populations or y = 69.35 + 6.4127x, where x is the sampling date, and an r-squared value of 0.97. However, populations in 2008 were best described by a second order polynomial relationship between nematode populations over time with y = 336.3 - 264.59x + 95.511x^2, where x is the sampling date. The significant r-squared value was 0.98. In 2007 and 2008, the variation in sampling date could be used to explain 97 and 98% of the variation in population growth.

Rotylenchulus reniformis was able to successfully infest the cotton rows observed during the 2007 – 08 irrigated trial (Fig. 2.12). Populations were injected into the designated row (inoculated row) at a rate of 163 per 150 cm^3 at plant in 2007. No populations were present one (100 cm) or two (200 cm) rows from the inoculated row. Harvest populations of *R. reniformis,* which are the inoculum for the subsequent growing season, averaged 229 per 150 cm^3 within the inoculated row, 18 per 150 cm^3 at 100 cm and 34 per 150 cm^3 at 200 cm. At planting in 2008, populations were detected only within the inoculated row at an average of 123 per 150 cm^3, a decline of 46% from the previous years' final populations. However, populations within the inoculated row at harvest in 2008 averaged 641 per 150 cm^3; an increase of 521% over planting populations and 280% above 2007 final populations. Final populations at 100 and 200 cm in 2008 increased 11% and 3%, respectively, over final populations in 2007 despite having undetectable 2008 initial populations. These numbers illustrate *R. reniformis'* ability to establish within a cotton row and survive from year to year.

Rotylenchulus reniformis moved downward through the soil profile from the initial inoculation depth of 5 cm to 91 cm within 150 days in 2007 (Fig. 2.13). Samples taken in the inoculated rows revealed the highest population concentration in the top 15 cm of the profile. Populations were significantly higher ($P \leq 0.10$) in the top 15 cm compared to all other depths

and averaged 230 individuals per 150 cm^3 of soil. *Rotylenchulus reniformis* was detected in all sampling depths to the maximum depth of 91 cm. Populations averaged 23, 39, 10, 13 and 5 individuals per 150 cm^3 of soil at depths of 15 – 30 cm, 30 – 46 cm, 46 – 61 cm, 61 – 76 cm, and 76 – 91 cm, respectively, with no differences between the numbers at any depth. Samples taken in the non-inoculated rows (200 cm) yielded higher ($P \leq 0.10$) *R. reniformis* populations within the 15 – 30 cm portion of the profile compared to all other depths. The average population at the 15 – 30 cm depth was 98 individuals per 150 cm^3 of soil. No other depths differed significantly ($P \leq 0.10$) but declined progressively with depth and had average populations of 34, 23, 18, 13, and 3 individuals per 150 cm^3 of soil at depths of 0 – 15 cm, 30 – 46 cm, 46 – 61 cm, 61 – 76 cm, and 76 – 91 cm, respectively.

Populations of *R. reniformis* were observed at all sampling depths in the inoculated row at planting with the exception of the 76 – 91 cm depth, (Fig. 2.14).Populations were significantly higher ($P \leq 0.10$) in the top 15 cm of the inoculated row at planting in 2008, averaging 124 individuals per 150 cm^3 of soil. The populations at all other depths were not significantly different, but were detectable averaging 8 individuals per 150 cm^3 of soil at the 15 – 30 cm and 61 – 76 cm depths, and 23 individuals per 150 cm^3 of soil at all depths between 30 and 61 cm. Samples 200 cm from the inoculated row found no detectable nematode populations at planting in 2008. Populations of *R. reniformis* within the inoculated rows at harvest were significantly higher ($P \leq 0.10$) in the top 15 cm than at all other depths, averaging 703 individuals per 150 cm^3 of soil (Fig. 2.15). Populations 200 cm away were similar throughout the profile to a depth of 91 cm. In the second year the populations ranged from 23 to 85 individuals per 150 cm^3 of soil with no ($P \leq 0.10$) differences between nematode populations.

Non-irrigated Trial: Rotylenchulus reniformis vermiform females and juveniles in the 2007 non-irrigated trial behaved much the same as in the irrigated trial with populations remaining concentrated about the cotton root zones and moving from row to row at 60 – 90 day intervals (Fig. 2.16). Populations were observed to have moved to a distance of 50 cm from the inoculated row at 30 DAP, and remained at that distance through 60 DAP. At 90 DAP, vermiform females and juveniles populations had risen to detectable levels to a distance of 150 cm from the inoculated row; a 100 cm increase within the 30 day period between sampling dates. Populations did not advance any farther between 90 and 120 DAP, remaining at a distance of 150 cm from the inoculated row. As observed in the irrigated trial, non-irrigated populations remained concentrated about the root zone of the inoculated row (50 cm) for the first 60 days, and upon infestation of the adjacent row (100 cm) remained concentrated within the root zone of that row (150 cm). At 150 DAP, *R. reniformis* populations were detected two rows away from the inoculated row (200 cm). Vermiform females and juveniles once again moved to the row adjacent to a colonized row within a 60 – 90 day period of infestation of that colonized row. Sixty to ninety days after infestation of the originally inoculated row, *R. reniformis* populations were detected within the row adjacent to the inoculated row. Subsequently, 60 – 90 days after the row adjacent to the inoculated row was colonized, the second row from the inoculated row was colonized. The total distance moved by *R. reniformis* vermiform females and juveniles in the 2007 non-irrigated was 200 cm from the inoculated row. The rate of movement averaged between 0 – 3.3 cm per day, with an average movement of 1.7 cm per day.

Populations of vermiform females and juveniles were the highest within the inoculated row and declined with distance throughout 2007 (Fig. 2.17). At 30 DAP, populations of vermiform females and juveniles averaged 193 per 150 cm^3 within the inoculated row. A decline to 147 per 150 cm^3 was observed at 60 DAP, followed by a 300% increase to the highest

population of 2007 of 440 per 150 cm^3. At 120 DAP populations declined again to an average of 185 per 150 cm^3 and increased at 150 DAP for a final population of 252 per 150 cm^3. Populations at 50 cm in 2007 followed the same pattern of fluctuation as the inoculated row through 120 DAP. At 30 DAP, populations averaged 78 per 150 cm^3, declined at 60 DAP to 15 per 150 cm^3, increased at 90 DAP to a seasonal high of 114 per 150 cm^3, and then declined through the end of the season to 66 and 22 per 150 cm^3 at 120 and 150 DAP, respectively. The first detected populations within the row adjacent to the inoculated row (100 cm) at 90 DAP averaged the highest numbers in 2007 at 120 per 150 cm^3. Populations then declined at 120 DAP to 42 per 150 cm^3 before ending the season with a final population of 93 per 150 cm^3. At 90 DAP, populations averaging 8 per 150 cm^3 were detected at 150 cm from the inoculated row. At 120 DAP, in contrast with all other distances in 2007, populations rose from 90 DAP to an average of 19 per 150 cm^3 and then declined to a final population of 9 per 150 cm^3. An average of 12 per 150 cm^3 was observed at 200 cm from the inoculated row at 150 DAP, and were the only vermiform females and juveniles detected at that distance in 2007. At all sampling dates, populations were higher ($P \leq 0.10$) within the inoculated row over populations at all other distances.

Male populations of *R. reniformis* in the 2007 non-irrigated trial spread more rapidly than did vermiform females and juveniles. At 60 DAP populations were detected at 100 cm from the inoculated row (Fig. 2.18), 50 cm farther than the vermiform females and juveniles. At 90 DAP, males were detected at 200 cm from the inoculated row and remained there throughout the rest of the season. Populations within the inoculated row grew steadily from 31 per 150 cm^3 at 60 DAP to 147 per 150 cm^3 at 120 DAP before declining to a final population of 21 per 150 cm^3 at 150 DAP (Fig. 2.19). At 50 cm from the inoculated row, populations began at 15 per 150 cm^3, peaked at 90 DAP, averaging 93 per 150 cm^3, and declined throughout the

remainder of the season to a final population of 13 per 150 cm^3. Similarly, populations at 100 cm began low, averaging 8 per 150 cm^3, peaked at 90 DAP with 81 per 150 cm^3, and declined to 14 per 150 cm^3 at both 120 and 150 DAP. Average populations at 90 DAP for the 150 and 200 cm distances were 62 and 35 per 150 cm^3, respectively, and declined through end season (150 DAP) to 14 and 16 per 150 cm^3. Populations within the inoculated row were higher at each sampling date compared with all other distances, and significantly so ($P \leq 0.10$) at 120 DAP. No differences ($P \leq 0.10$) in populations were observed comparing populations within the cotton rows to those in between cotton rows. Ninety day populations declined linearly from the inoculated row to 200 cm with 98% of the variation in population attributed to the variation in distance ($r^2 = 0.98$).

Vermiform females and juveniles populations were retarded by drought in 2008. Thirty day samples detected populations within the cotton rows only (the inoculated row, 100 and 200 cm) (Fig. 2.20). However at 60 DAP populations were detected within the inoculated row and the row adjacent (100 cm) but not at the 200 cm distance. Ninety day samples detected populations within the inoculated row, and at 50 and 100 cm from the inoculated row. At 120 DAP, populations reappeared at the 200 cm distance and were detected at all other sampling points with the exception of 150 cm. Samples taken at 150 DAP revealed vermiform females and juveniles at every sampling point throughout the trial.

Populations within the inoculated row at 30 DAP averaged 185 per 150 cm^3, declined 21% to 147 per 150 cm^3 at 60 DAP, and declined even further, 63 %, at 90 DAP to an average of 54 per 150 cm^3 (Fig. 2.21). The highest populations of the season occurred at 120 DAP, averaging 402 per 150 cm^3; a 744% increase over the 90 day populations. Final populations within the inoculated row averaged 162 per 150 cm^3 at 150 DAP. Populations at 50 cm from the

inoculated row were first detected at 90 DAP, at an average of 15 per 150 cm^3. An increase to 20 per 150 cm^3 was observed at 120 DAP, and populations at end season (150 DAP) averaged 19 per 150 cm^3. The row adjacent to the inoculated row (100 cm) contained detectable populations throughout 2008. Thirty day populations averaged 97 per 150 cm^3 and were the highest of the season. Populations were then observed to fluctuate throughout the rest of the season averaging 23 per 150 cm^3 at 60 DAP, 31 per 150 cm^3 at 90 DAP, a seasonal low of 12 per 150 cm^3 at 120 DAP, and a final population of 27 per 150 cm^3 at 150 DAP. At 150 cm from the inoculated row, populations were only detected at 150 DAP at an average of 23 per 150 cm^3. Subsequent to the initial detection of populations averaging 23 per 150 cm^3 at 30DAP, vermiform females and juveniles were not detected at the 200 cm distance again until 120 DAP. At both 120 and 150 DAP, populations averaged 12 per 150 cm^3. Populations were significantly higher ($P \leq 0.10$) within the inoculated row throughout 2008, and were higher ($P \leq 0.10$) within the cotton rows at 30 and 60 DAP compared with the row middles.

Males in 2008 were found to the maximum sampling distance of 200 cm throughout the season (Fig. 2.22). Populations within the inoculated row were low throughout the season, averaging 8 per 150 cm^3 at 30, 60, and 150 DAP, and 39 per 150 cm^3 at 90 and 120 DAP (Fig. 2.23). At 50 cm from the inoculated row, populations averaged 8 per 150 cm^3 at 30 DAP, were absent at 60 DAP, increased to a seasonal high of 39 per 150 cm^3 at 90 DAP, and declined through the end of the season to a final population of 20 per 150 cm^3 at 150 DAP. The 100 cm distance averaged 4 per 150 cm^3 at both 30 and 60 DAP, increased to 82 per 150 cm^3 at 90 DAP, and decreased to 8 per 150 cm^3 at 120 DAP. No populations were detected at 150 DAP. At 150 cm, populations averaged 4 per 150 cm^3 at 30 DAP, were absent at 60 DAP, reached a seasonal high of 35 per 150 cm^3 at 90DAP, and decreased through 150 DAP to a final population of 8 per 150 cm^3. Populations were significantly higher ($P \leq 0.10$) at 200 cm from the inoculated row at

both 30 and 60 DAP compared with all other distances, averaging 12 and 23 per 150 cm^3, respectively. Males increased to an average of 66 per 150 cm^3 at 90 DAP, and declined through 150 DAP to an average of 4 per 150 cm^3. Male populations were significantly ($P \leq 0.10$) higher within the cotton rows compared to the row middles at 60 DAP. However, at 120 and 150 DAP, male populations were similar in the row middles compared to within the cotton rows.

Overall populations within the non-irrigated trial were closely related to rainfall. In both 2007 and 2008, average population changes of *R. reniformis* paralleled the increase or decline of rainfall amounts between sampling dates (Table 2.1; Figs. 2.24, 2.25). Comparisons of 2007 *R. reniformis* populations and 2007 rainfall amounts between sampling dates produced a strong correlation between the two parameters. The Pearson correlation coefficient for the comparison is equal to 0.93 with $p = 0.024$. This affirms that 92% of the variation of *R. reniformis* populations is explained by the variation in rainfall. Similarly, the comparison of the 2008 populations of *R. reniformis* and 2008 rainfall amounts between sampling dates produced a Pearson correlation coefficient of 0.92 with $p = 0.034$. The variation in 2008 rainfall amounts explains 92% of the variation in 2008 *R. reniformis* populations.

As was observed in the irrigated trial, the populations of *R. reniformis* injected into the inoculated row at plant in 2007 were able to survive and increase from year to year (Fig. 2.26). Populations within the inoculated row increased 155% for the 2007 season. Of the 2007 final populations, 18% were detected at planting in 2008 for an average of 46 per 150 cm^3. Final 2008 populations averaged 232 per 150 cm^3, an increase of 504% from initial populations, but 8% less than 2007 final populations. Final 2007 populations within the row adjacent to the inoculated row (100 cm) averaged 84 per 150 cm^3. No populations were detected at 100 cm at plant in 2008, and final 2008 populations averaged 23 per 150 cm^3. Similar, but more pronounced, to the

inoculated row, 2008 final populations were lower (73%) than the final populations of 2007. In contrast to both the inoculated row and the row adjacent, the second row from the inoculated row (200 cm) produced an increase in final populations from 2007 to 2008. 2008 final populations averaged 58 per 150 cm^3, a 322% increase over the final 2007 population of 18 per 150 cm^3 despite no detectable populations at plant in 2008.

Rotylenchulus reniformis populations in the non-irrigated trial were detected to the maximum sampling depth of 91 cm in both the inoculated row and 200 cm from the inoculated row in 2007 (Fig. 2.27). Populations 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 at an average of 102 per 150 cm^3 compared to the 30 – 91 cm depth which averaged 24 per 150 cm^3. Samples taken 200 cm from the inoculated row contained significantly higher ($P \leq 0.10$) populations within the 0 – 15 cm and 30 – 46 cm depths, averaging 48 per 150 cm^3 compared with all other depths which averaged 12 per 150 cm^3. Populations within the inoculated row at plant in 2008 were significantly higher ($P \leq 0.10$) in the top 15 cm of the profile at an average of 46 per 150 cm^3 compared to the 15 – 91 cm depths, which averaged 11 per 150 cm^3 (Fig. 2.28). The only populations observed 200 cm from the inoculated row at plant in 2008 were detected at the 30 – 46 cm depth and averaged 8 per 150 cm^3. Final populations within the inoculated row in 2008 were significantly higher ($P \leq 0.10$) in the 0 – 46 cm depths averaging 203 per 150 cm^3 compared to the 46 – 76 cm depths which averaged 16 per 150 cm^3 (Fig. 2.29). No *R. reniformis* were observed at the 76 – 91 cm depth. Populations at 200 cm from the inoculated row were significantly higher ($P \leq 0.10$) in the top 30 cm at an average of 74 per 150 cm^3 compared with the 30 – 91 cm depths, which averaged 15 per 150 cm^3. No *R. reniformis* were detected at the 61 – 76 cm depth.

DISCUSSION

The horizontal movement of *R. reniformis* in our study corresponded to published root growth patterns. Cotton roots can extend horizontally from the taproot to a length of two meters (Taylor and Klepper, 1978) with the majority of lateral growth occurring in the upper meter of the soil horizon. Growth rates under ideal conditions can reach 6 cm per day (Taylor and Ratliff, 1969) until root growth ceases at fruit formation (Taylor and Klepper, 1974; Pearson and Lund, 1968). *Rotylenchulus reniformis* vermiform females and juveniles movement closely follows this pattern of root development during the 2007 season. 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. Males progressed along the roots more rapidly than did the females. Once the lateral roots of the cotton rows were linked to the adjacent rows, the males were able to progress quickly from row to row in search of females uninhibited by the aspiration to colonize available feeding sites. 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, whether it is a direct effect on the nematodes or on lateral root proliferation. Both conclusions are probable, as soil water potential has been suggested to effect nematode locomotion (Hunt *et al*., 2001) and cotton root distribution is often altered by soil water content, especially when moisture is deficient (Taylor, 1983).

The difference in overall population growth of the top 15 cm of soil between the irrigated and non-irrigated trials is most reasonably attributed to cotton plant responses to soil moisture. Cotton roots, and subsequently available feeding sites and reproductive potential,

expand dependant on environmental conditions until the plants begin producing fruit (McMichael, 1980). The increase in *R. reniformis* populations during the first 120 days of 2007 – 08 irrigated trials corresponds to this root expansion. Irrigation and rainfall combined for an average of 23.3 mm and 14.6 mm weekly during both 2007 and 2008, respectively, and provided ample moisture during these trials for the cotton plant to develop relatively free of moisture stress. Furthermore, photosynthetic activity was not lowered due to water stress, thus the nutrition source remained more consistent within the irrigated trial. Conversely, *R. reniformis* populations within the non-irrigated cotton were less than those within the irrigated trial most probably due to the lack of moisture available for cotton plant development. 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 (Browning, *et al*., 1975; Taylor and Klepper, 1974) both of which would reduce populations within the top 15 cm. Under water stress, cotton plants reduce photosynthetic activity and reallocate exported assimilates from sources (Krieg and Sung, 1979) which can deprive embedded females of nutrition, thus slowing or reducing reproduction.

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 one meter deep (Heald and Thames, 1980; Robinson *et al*., 2000; Lee *et al*., 2002; Newman and Stebbins, 2002; Robinson *et al*., 2005). Populations were generally higher in the upper portion of the profile in both trials where the relative majority of lateral roots are found (Taylor and Klepper, 1978).

Rotylenchulus reniformis movement through a cotton field occurs much more rapidly than anticipated with or without irrigation. Once a row is colonized, populations will follow lateral roots when available from row to row as long as growing conditions are favorable. Populations will develop quickly with adequate moisture. 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.

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Table 2.1. 2007 – 2008 rainfall and irrigation amounts (mm) between sampling dates.

	2007		2008	
DAP	**Rain**	**Irrigation**	**Rain**	**Irrigation**
0 – 30	21.8	48.3	40.6	12.7
31 – 60	10.9	121.9	26.4	30.5
61 – 90	74.9	50.8	34.2	94
91 – 120	21.6	124.5	36.9	30.5
121 – 150	27.2	0	8.0	0
Total	156.4	345.5	146.1	167.6

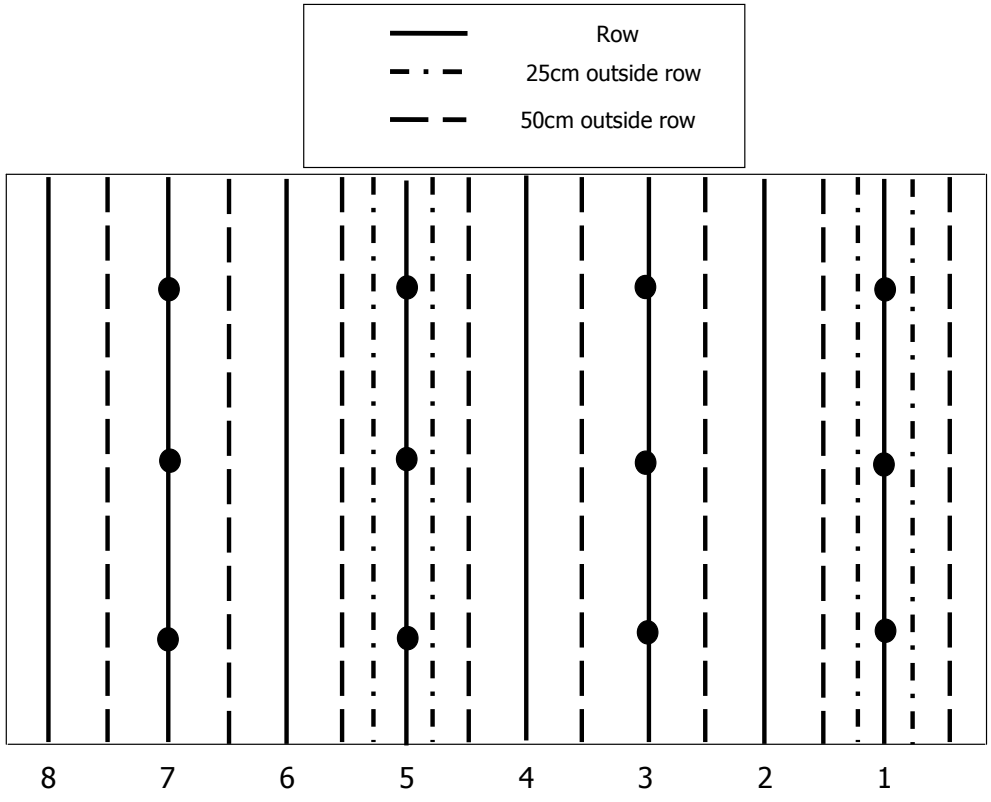


Figure 2.1. Sampling scheme for one replication. A • represents where a core sample was collected.

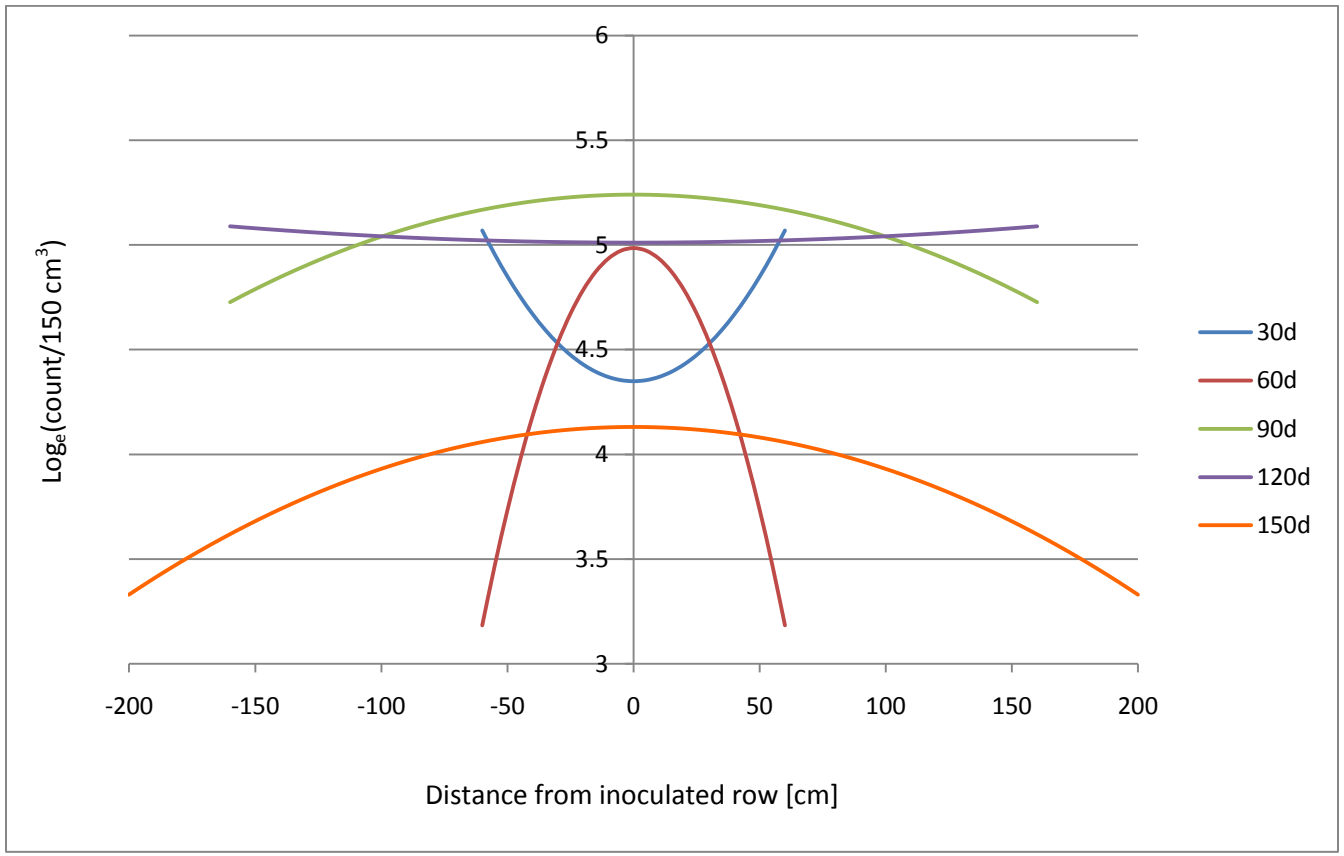


Figure 2.2. 2007 Irrigated Trial: *Rotylenchulus reniformis* vermiform females and juveniles Log$_e$ count per 150cm^3 of soil at the 0 – 15 cm depth for each sampling date, 30d, 60d, 90d, 120d, and 150d days after planting as measured at set distances (cm) from the originally inoculated row (0).

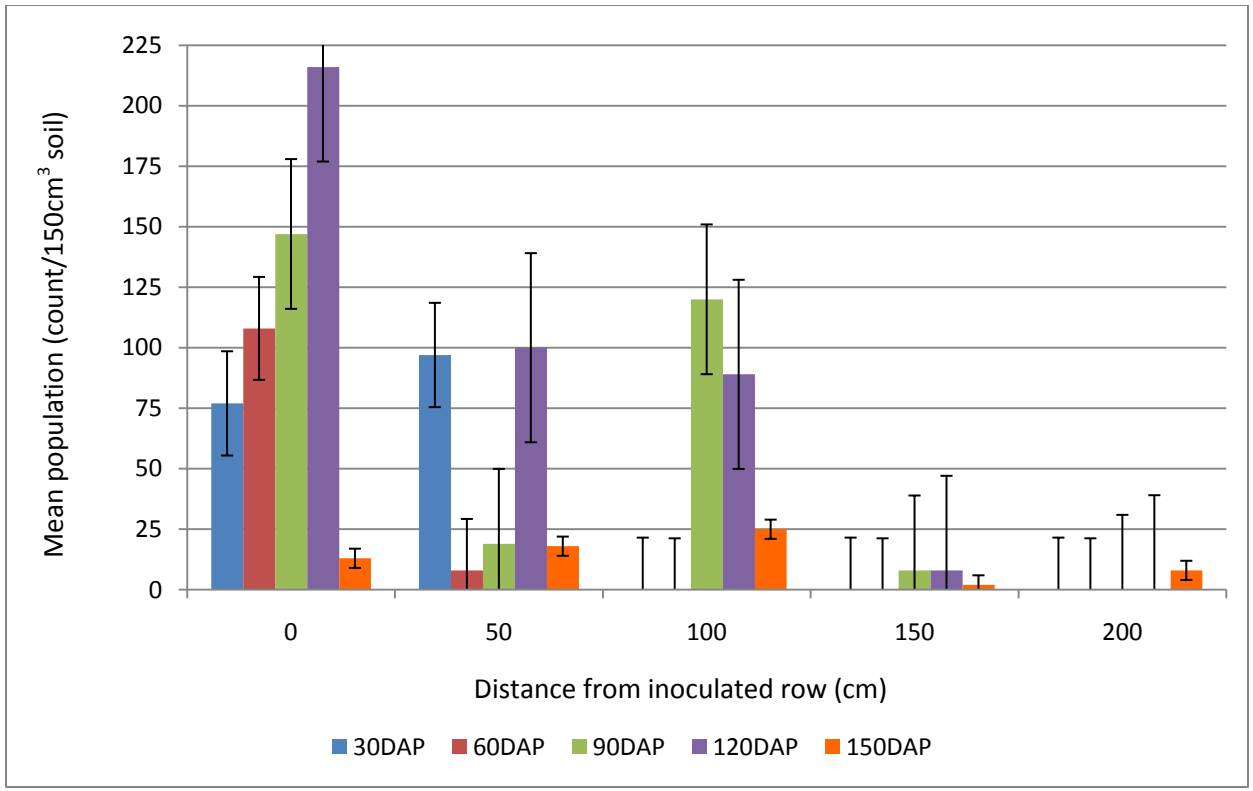


Figure 2.3. 2007 mean populations of *R. reniformis* vermiform females and juveniles per 150cm^3 of soil at the 0 – 15 cm depth at 30, 60, 90, 120 and 150 days after planting by distance (cm) from the inoculated row.

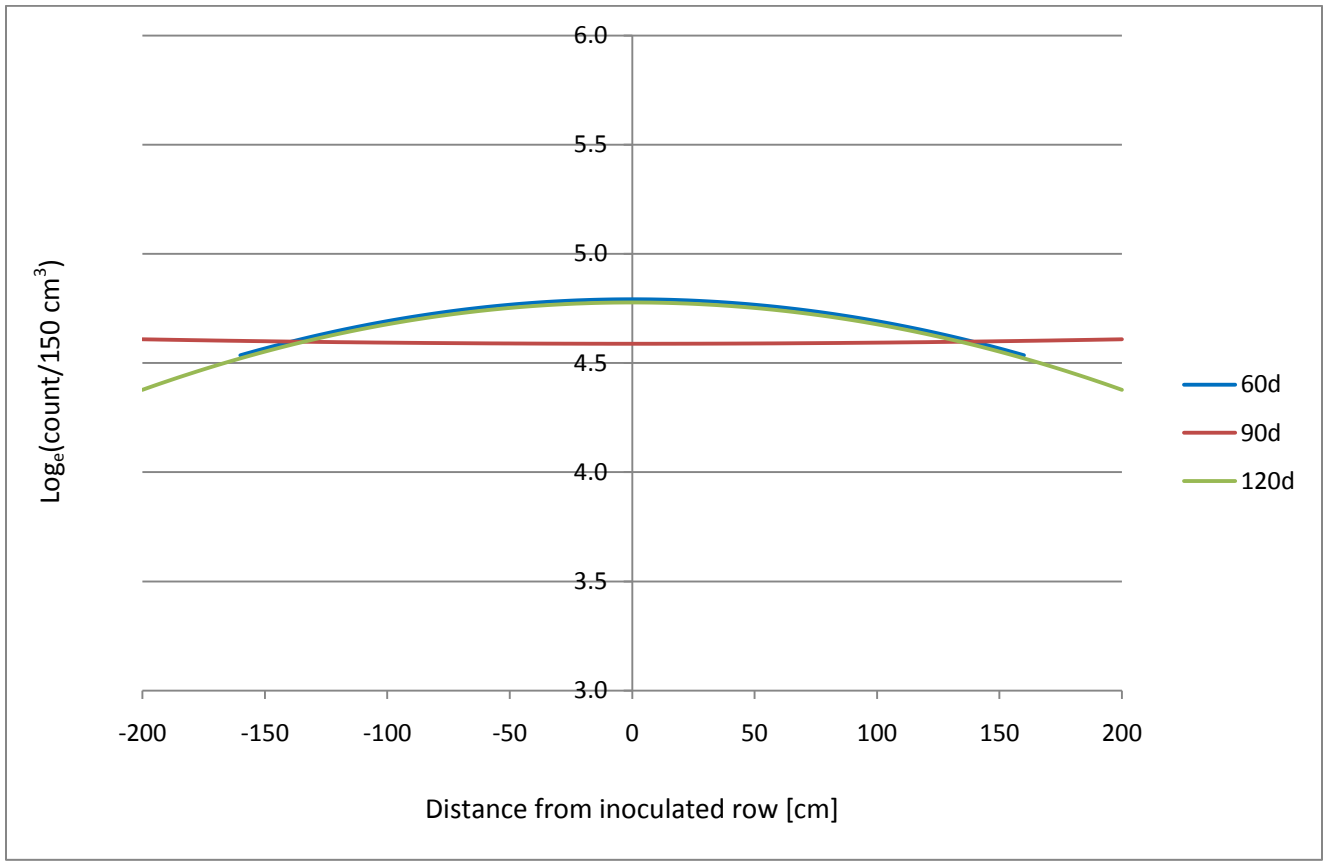


Figure 2.4. 2007 Irrigated Trial: *Rotylenchulus reniformis* males Log$_e$ count per 150cm^3 of soil at the 0 – 15 cm depth for each sampling date, 60d, 90d, and 120d days after planting as measured at set distances (cm) from the originally inoculated row (0).

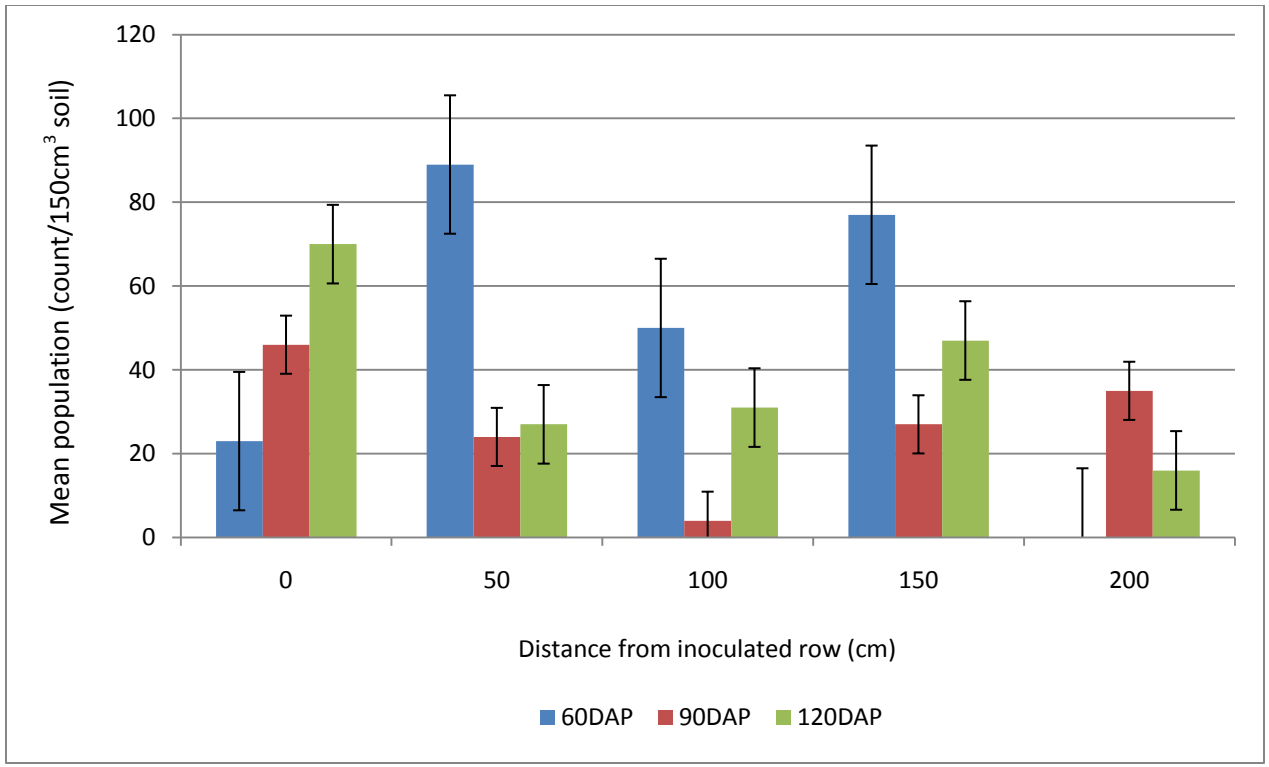


Figure 2.5. 2007 mean populations of *R. reniformis* males per 150cm^3 at the 0 – 15 cm depth of soil at 60, 90, and 120 days after planting by distance (cm) from the inoculated row.

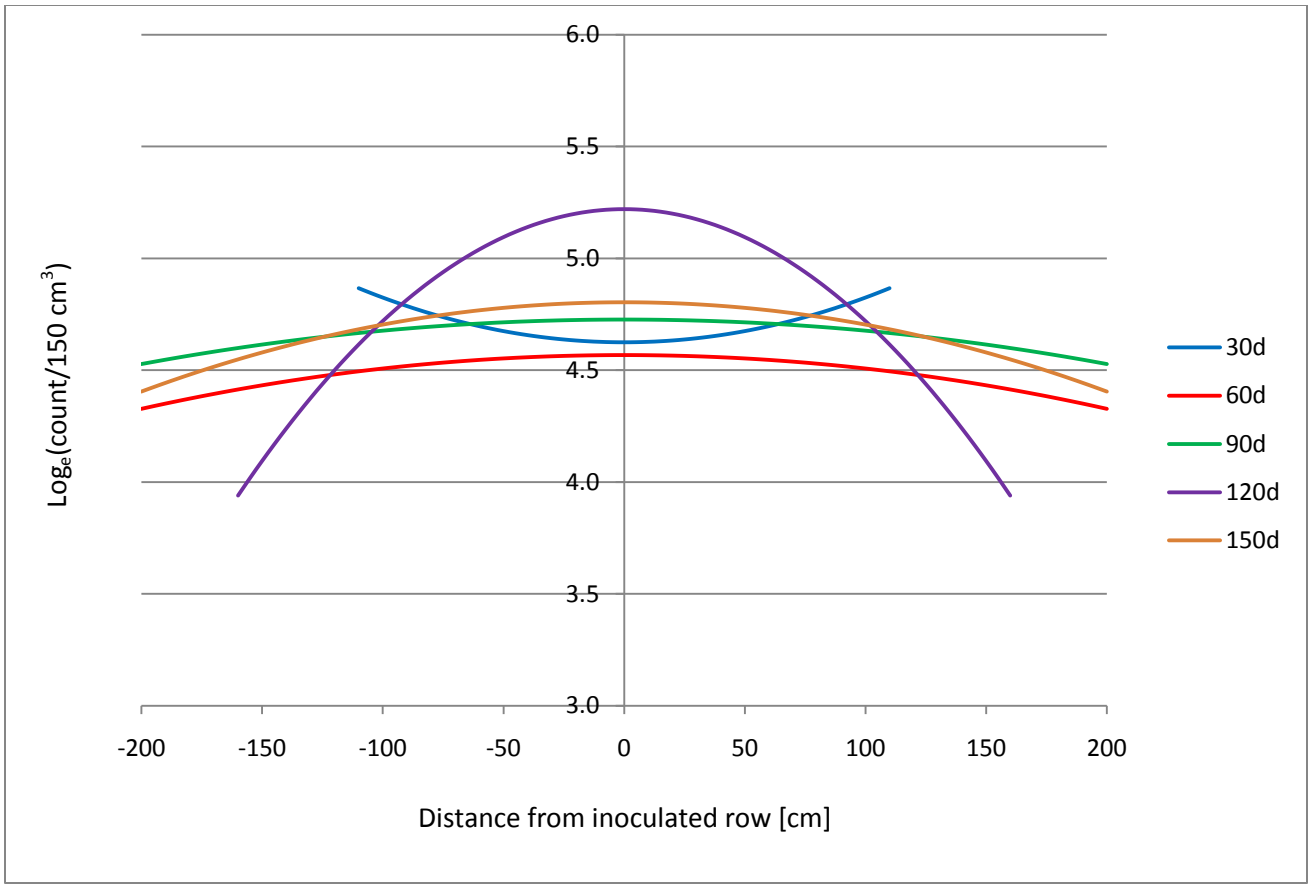


Figure 2.6. 2008 Irrigated Trial: *Rotylenchulus reniformis* vermiform females and juveniles Log$_e$ count per 150cm^3 of soil at the 0 – 15 cm depth for each sampling date, 30d, 60d, 90d, 120d, and 150d days after planting as measured at set distances (cm) from the originally inoculated row (0).

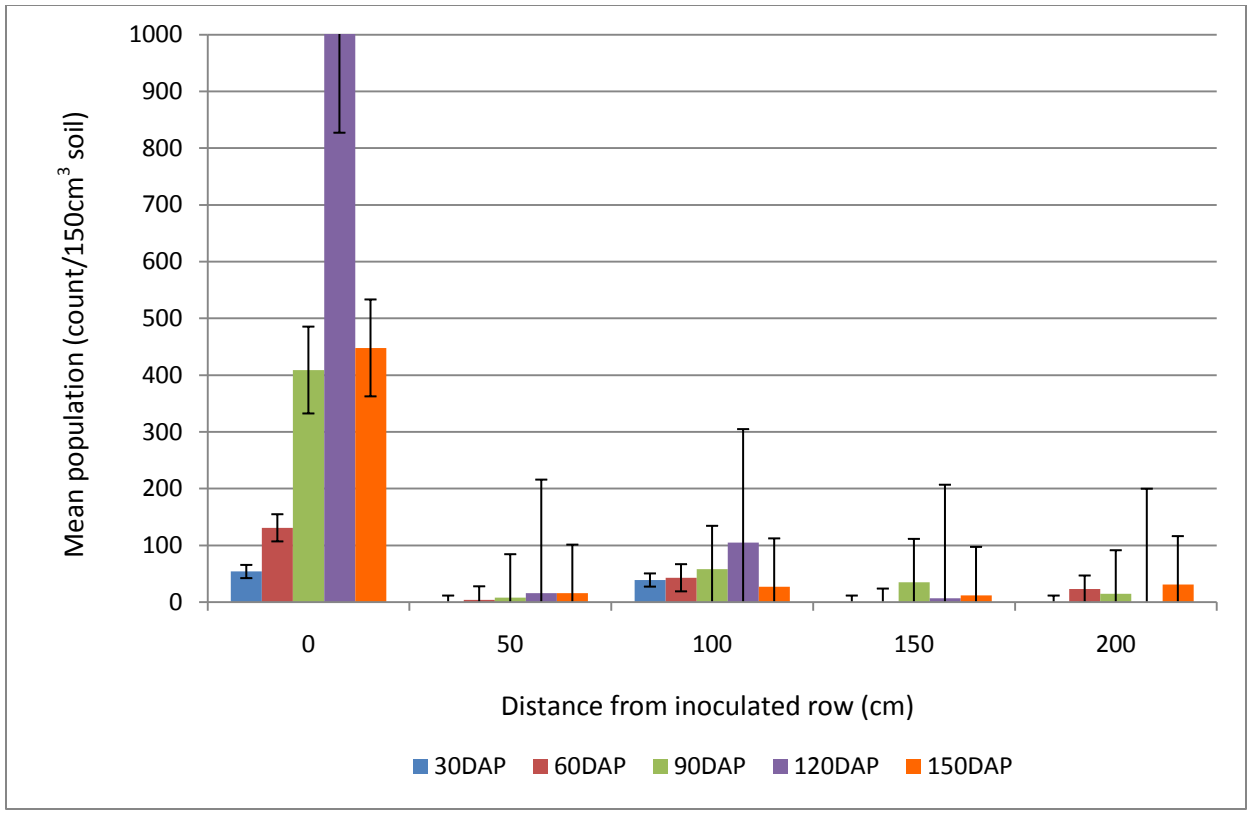


Figure 2.7. 2008 mean populations of *R. reniformis* vermiform females and juveniles per 150cm^3 of soil at the 0 – 15 cm depth at 60, 90, 120, and 150 days after planting by distance (cm) from the inoculated row.

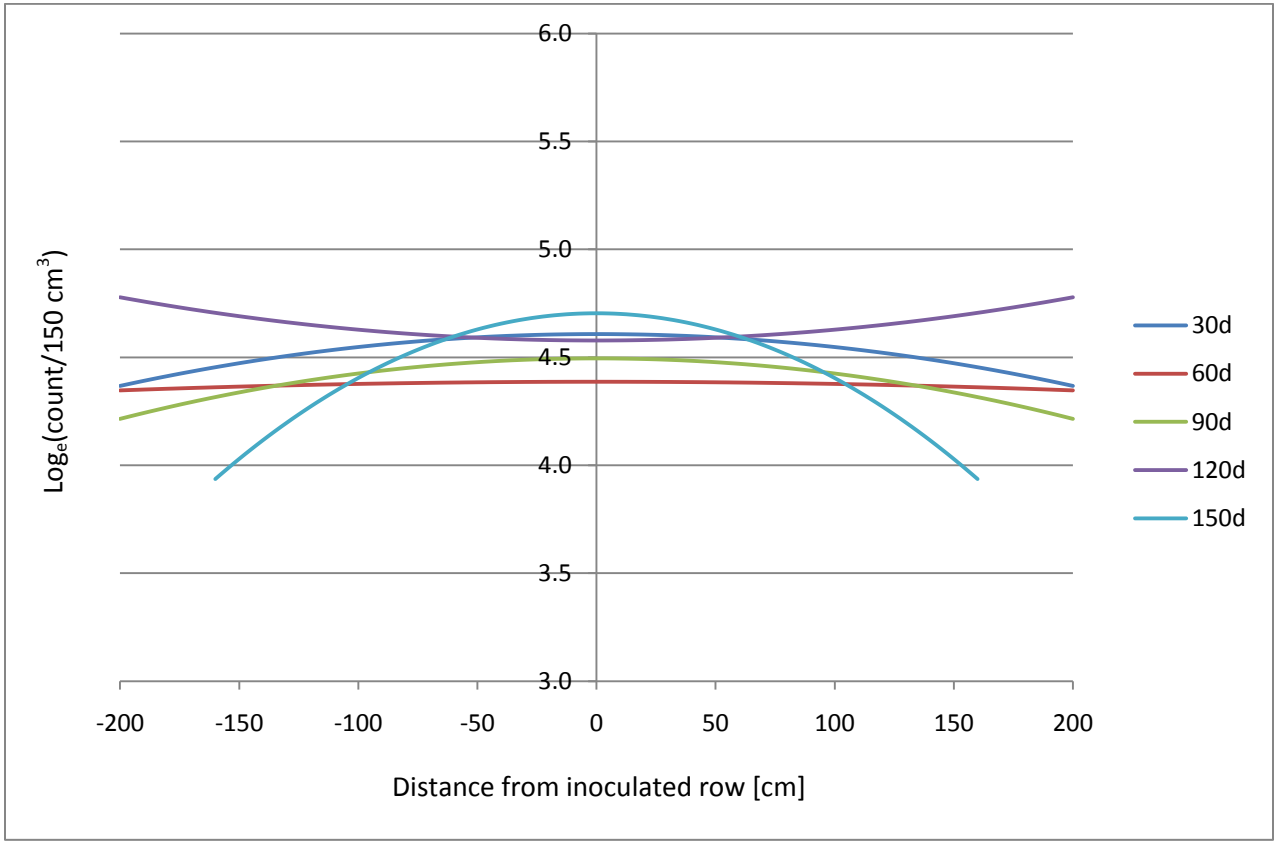


Figure 2.8. 2008 Irrigated Trial: *Rotylenchulus reniformis* males Log_e count per 150cm^3 of soil at the 0 – 15 cm depth for each sampling date, 30d, 60d, 90d, 120d, and 150d days after planting as measured at set distances (cm) from the originally inoculated row (0).

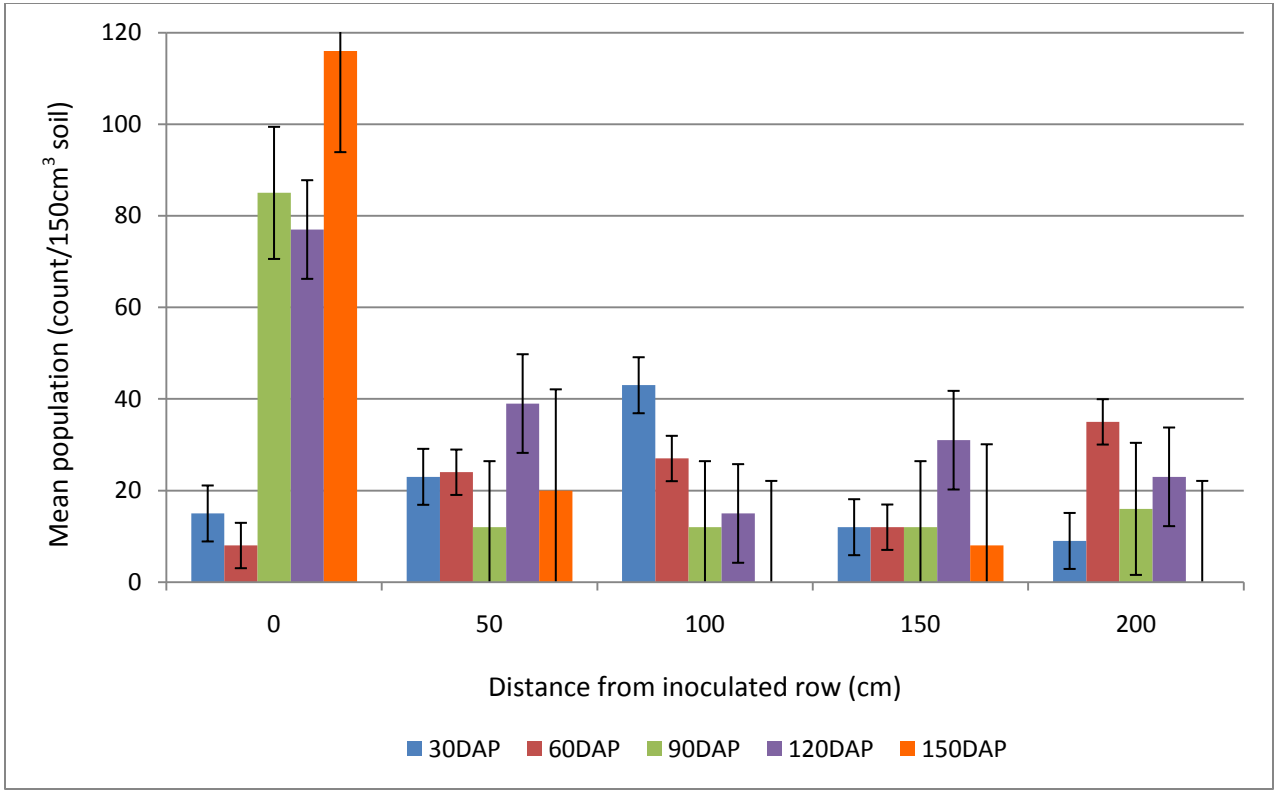


Figure 2.9. 2008 mean populations of *R. reniformis* males per 150cm^3 of soil at the 0 – 15 cm depth at 60, 90, 120 and 150 days after planting by distance (cm) from the inoculated row.

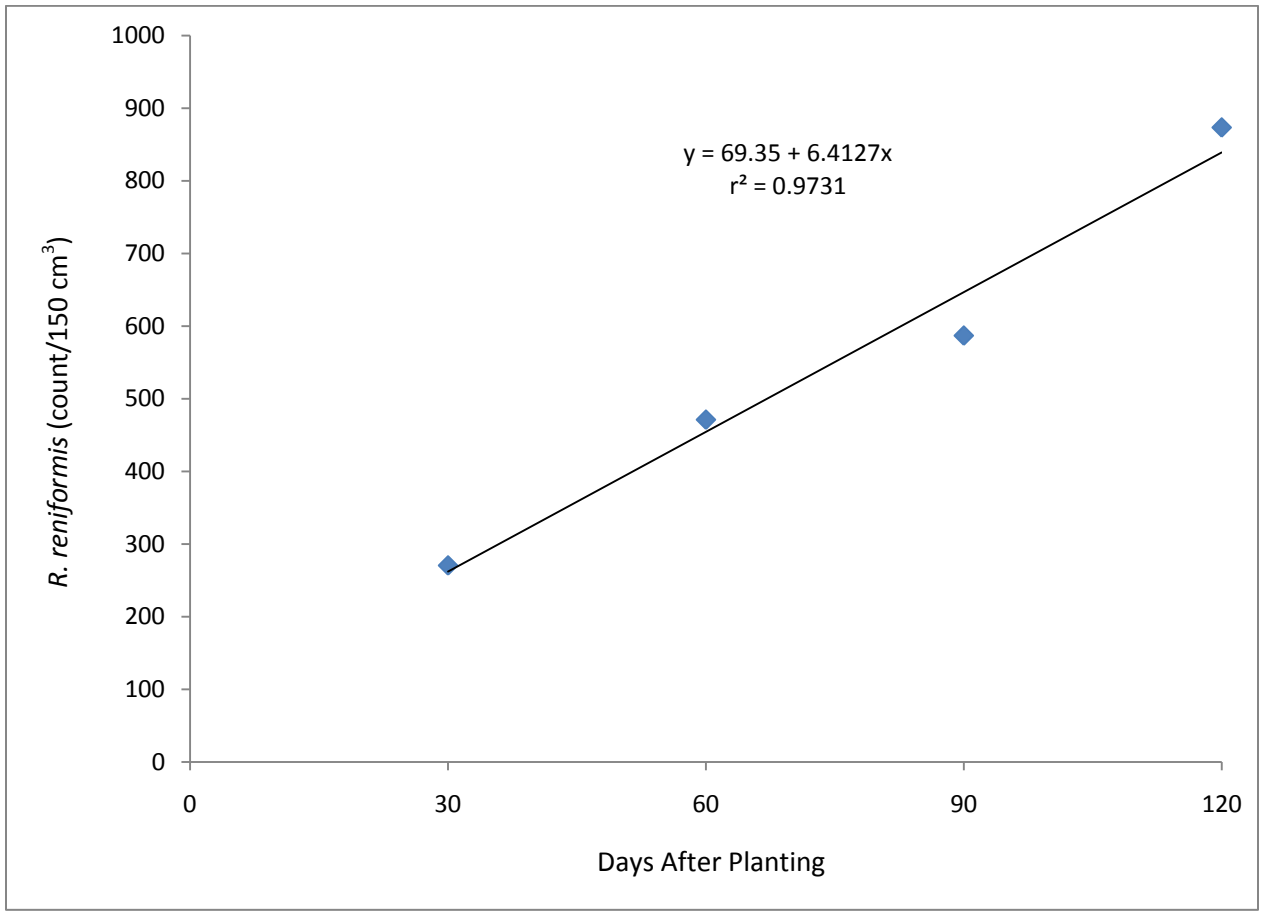


Figure 2.10. Linear growth of *R. reniformis* total populations at the 0 – 15 cm depth, 2007 irrigated trial.

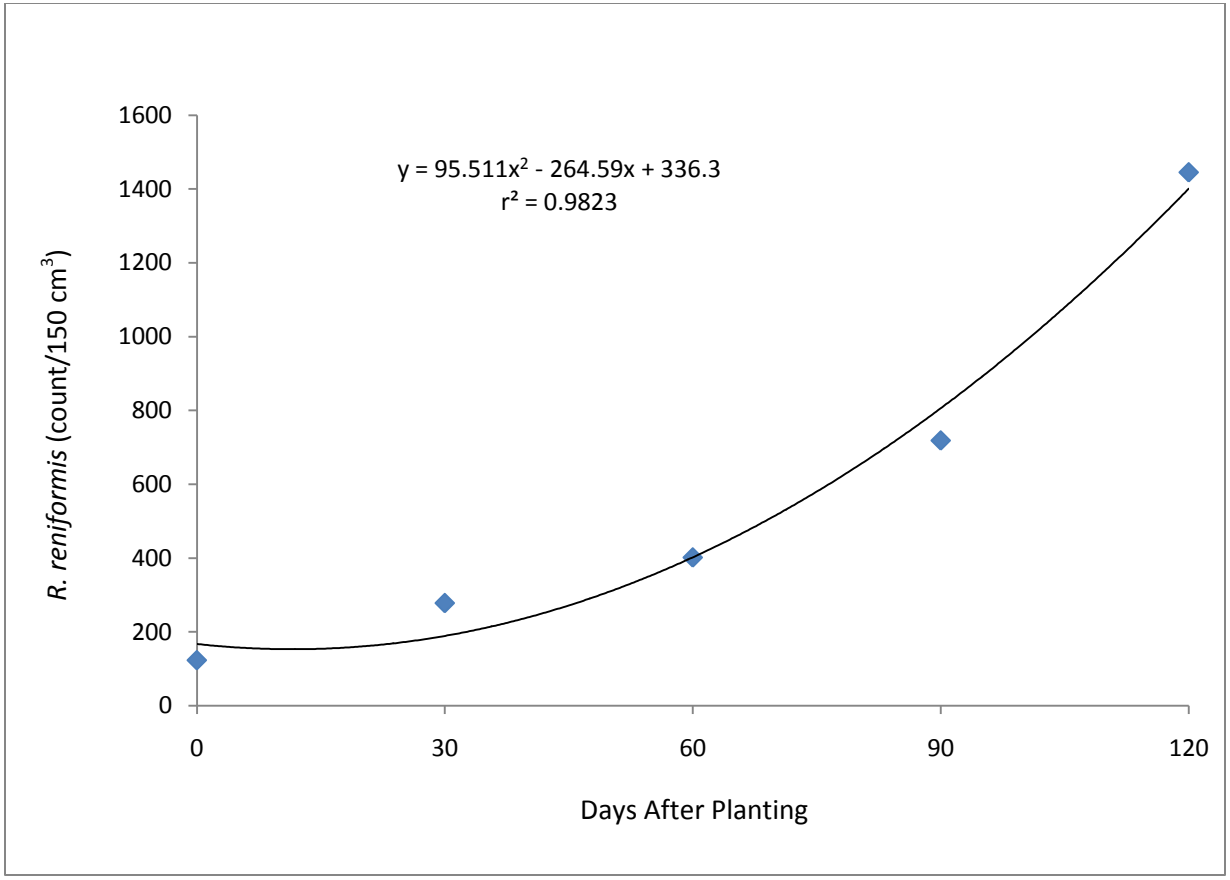


Figure 2.11. Growth of *R. reniformis* total populations at the 0 – 15 cm depth, 2008 irrigated trial.

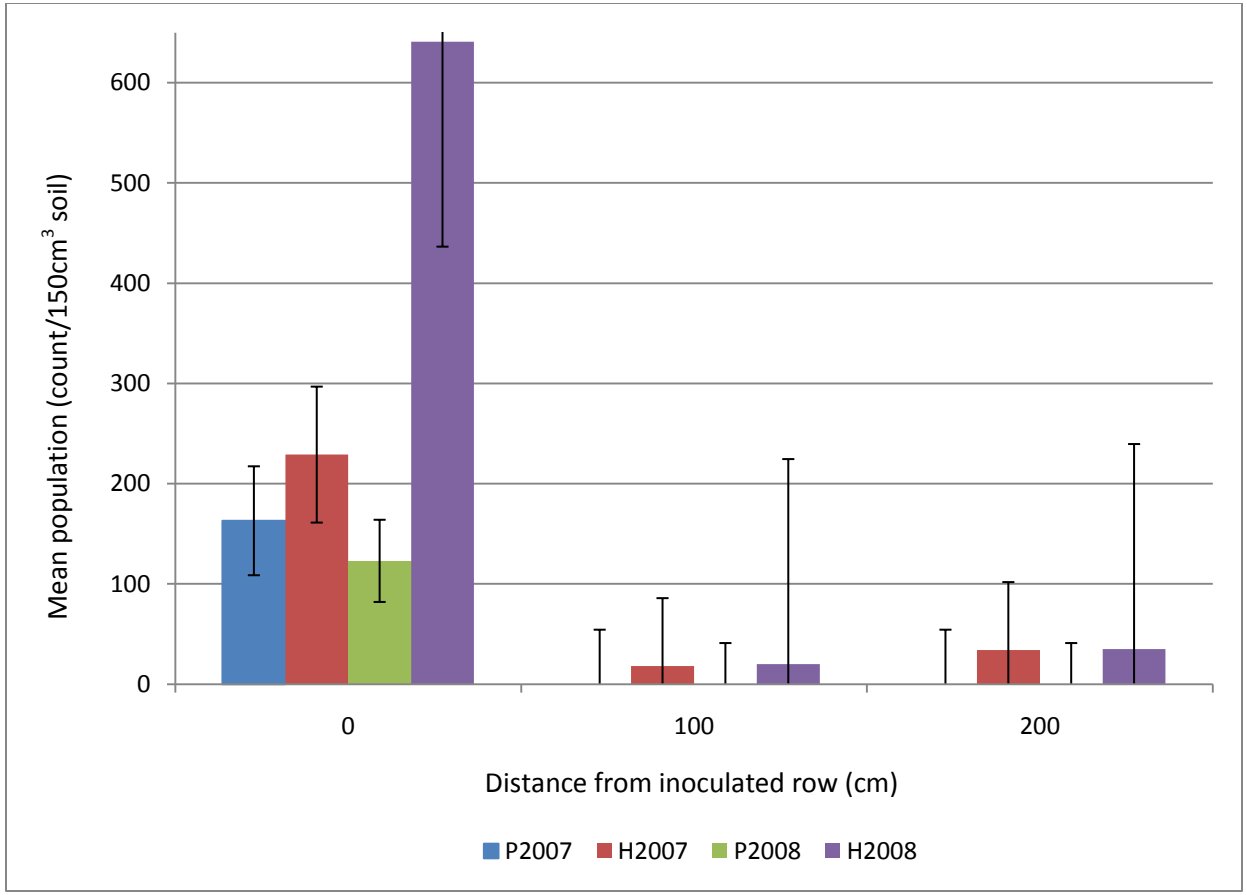


Figure 2.12. Irrigated trial mean populations of *R. reniformis* per 150cm^3 of soil at 2007 planting (P2007), 2007 harvest (H2007), 2008 planting (P2008) and 2008 harvest (H2008) within the inoculated row (0cm), one row from the inoculated row (100cm) and two rows from the inoculated row (200cm) at the 0 – 15 cm depth.

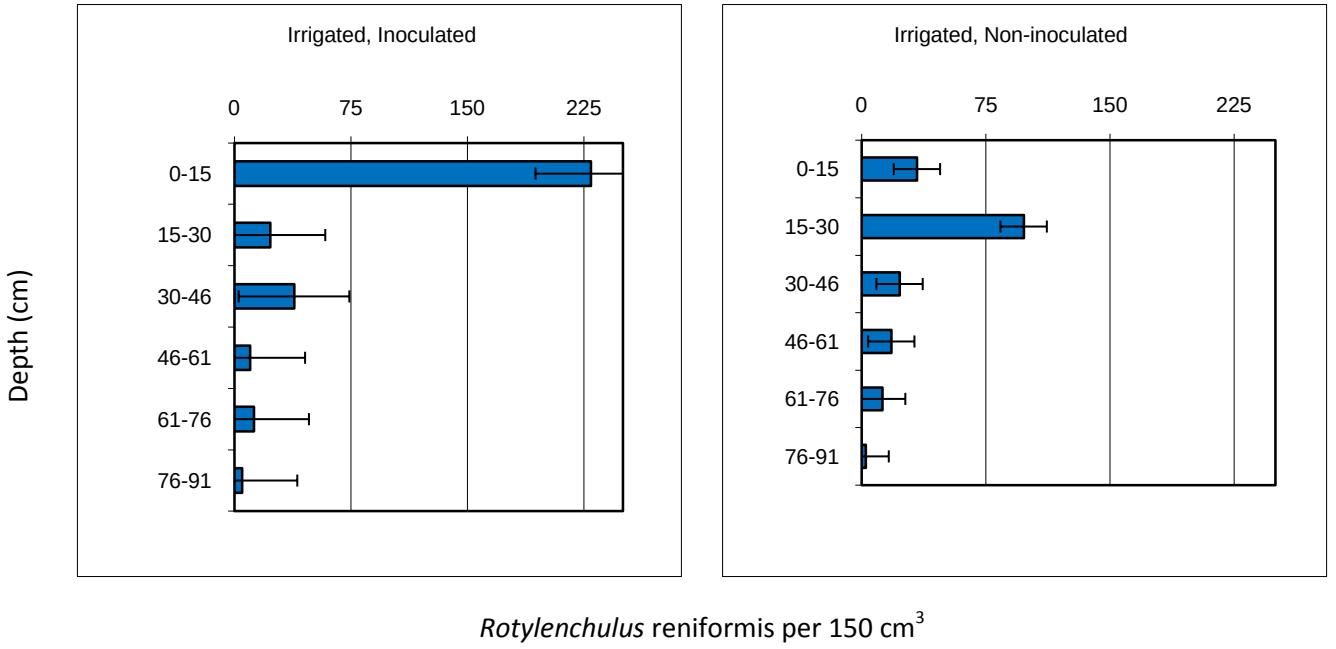


Figure 2.13. *Rotylenchulus reniformis* populations by depth in the irrigated trial subsequent to harvest, 2007. A comparison of the originally inoculated rows vs. non-inoculated rows 200cm from the originally inoculated row.

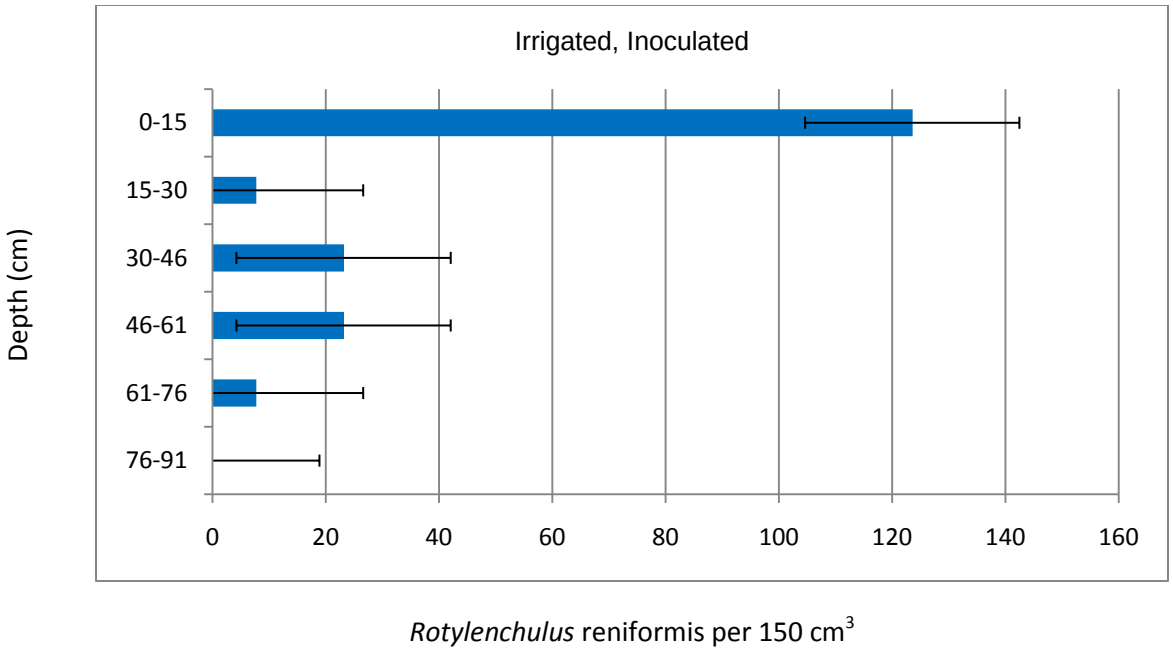


Figure 2.14. *Rotylenchulus reniformis* populations by depth in the irrigated trial at planting, 2008: Inoculated rows.

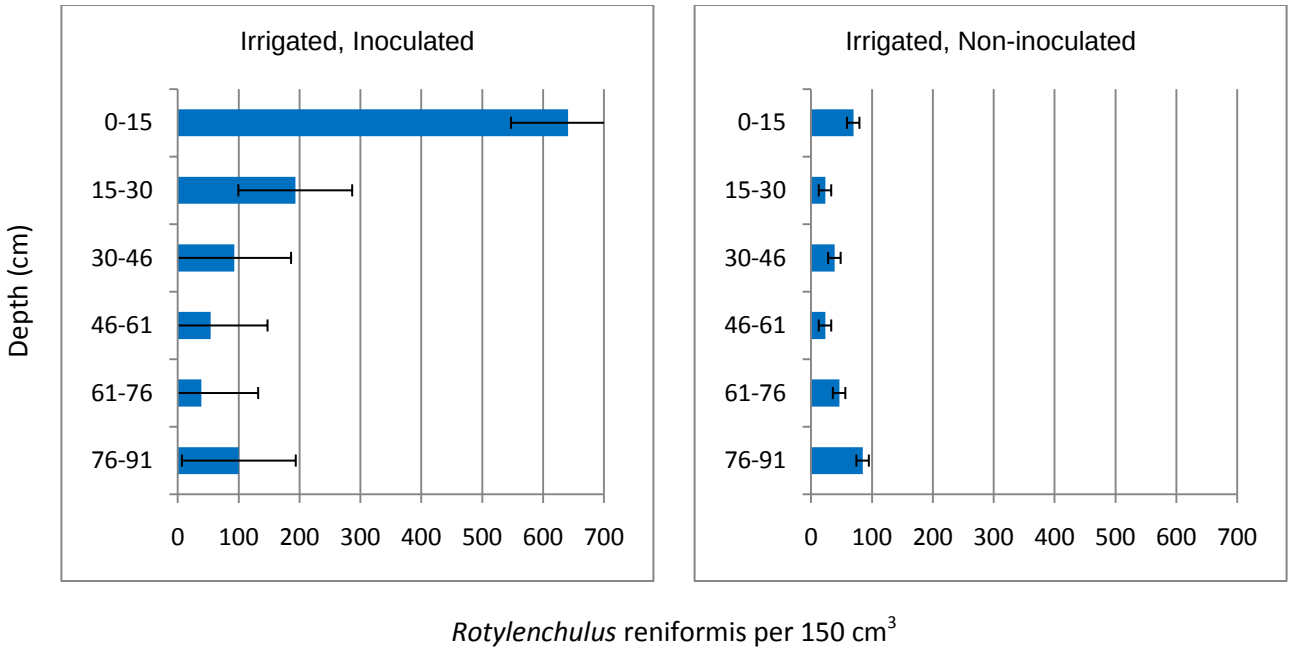


Figure 2.15. *Rotylenchulus reniformis* populations by depth in the irrigated trial subsequent to harvest, 2008. A comparison of the originally inoculated rows vs. non-inoculated rows 200cm from the originally inoculated row.

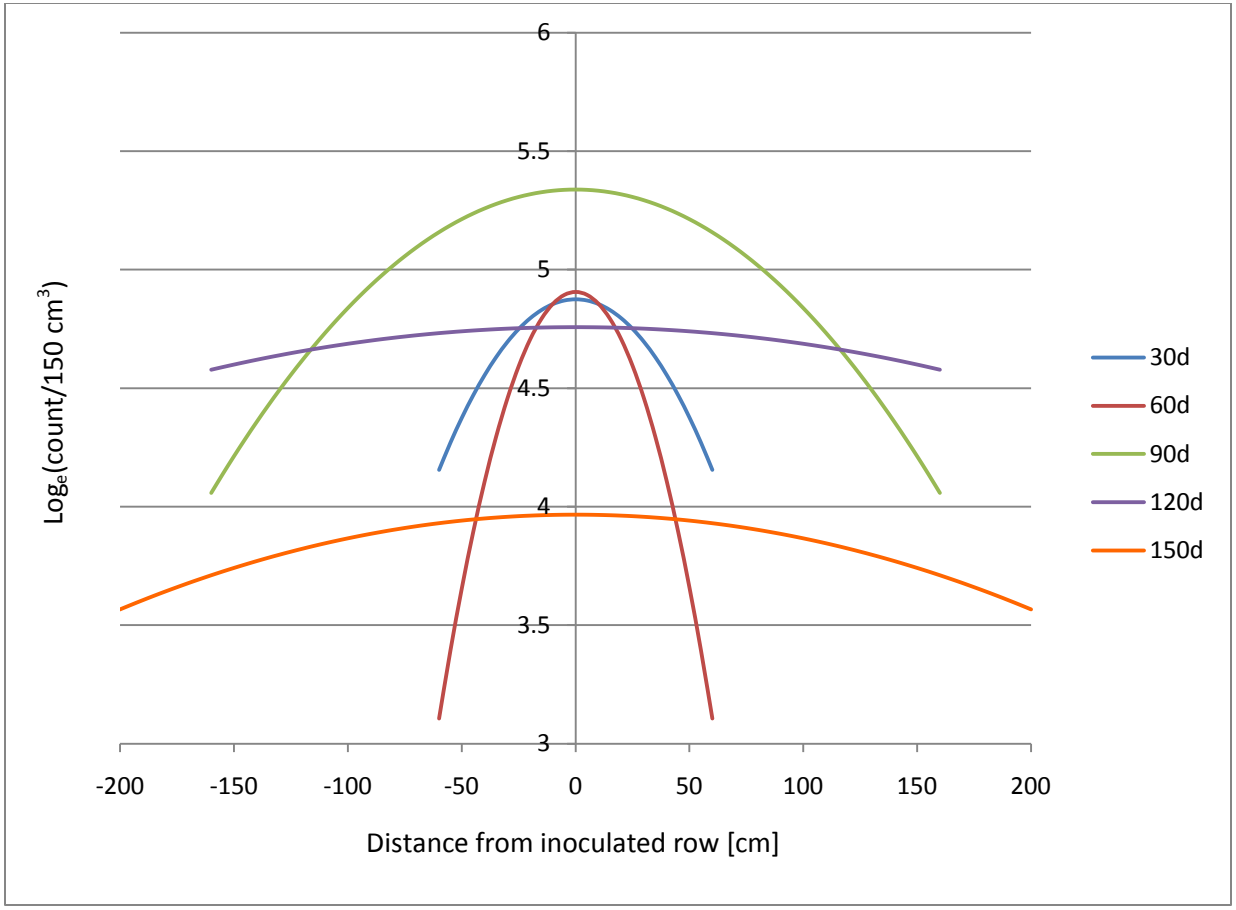


Figure 2.16. 2007 Non-irrigated Trial: *Rotylenchulus reniformis* vermiform females and juveniles Log$_e$ count per 150cm^3 of soil at the 0 – 15 cm depth for each sampling date, 30d, 60d, 90d, 120d, and 150d days after planting as measured at set distances (cm) from the originally inoculated row (0).

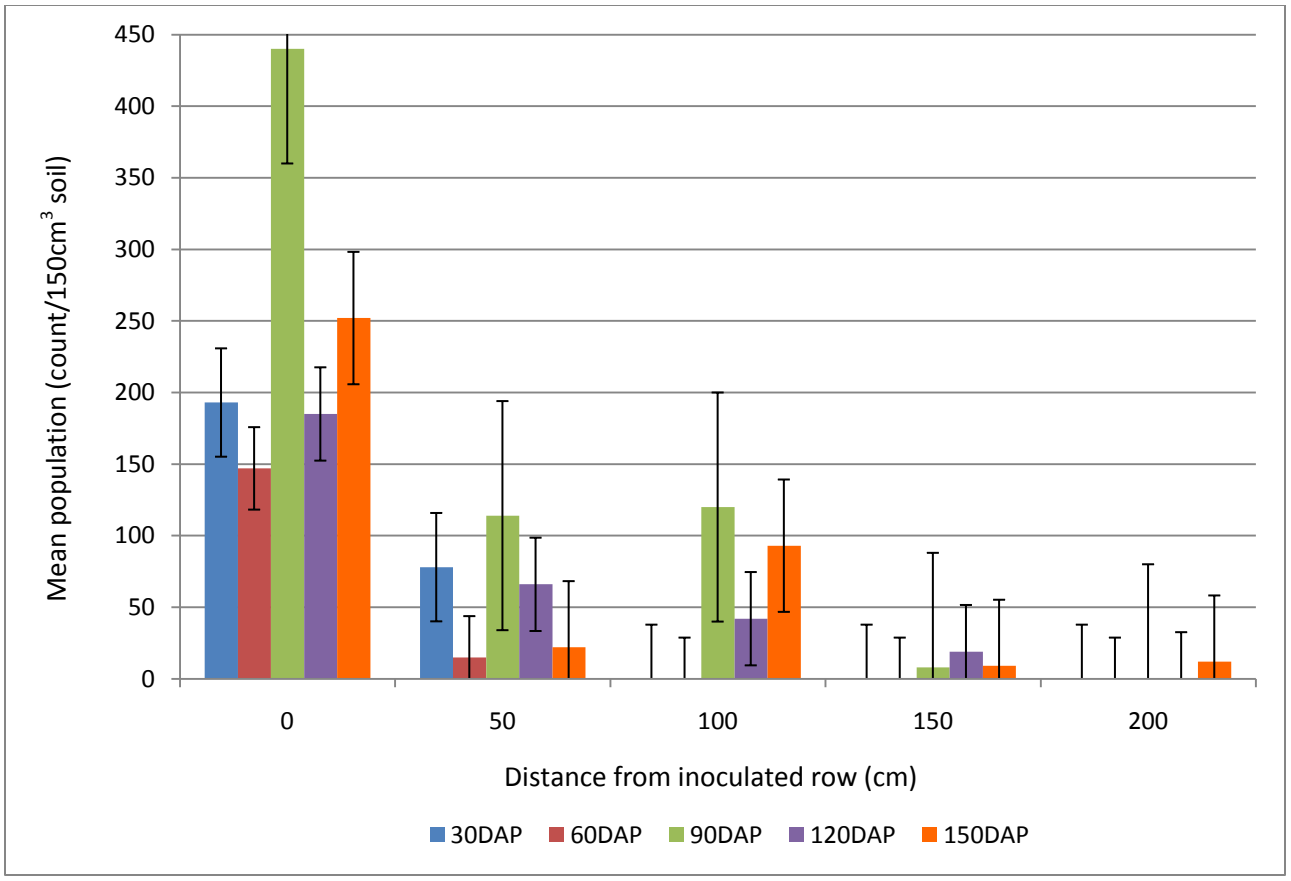


Figure 2.17. 2007 mean populations of *R. reniformis* vermiform females and juveniles per 150cm^3 of soil at the 0 – 15 cm depth at 30, 60, 90, 120 and 150 days after planting by distance (cm) from the inoculated row.

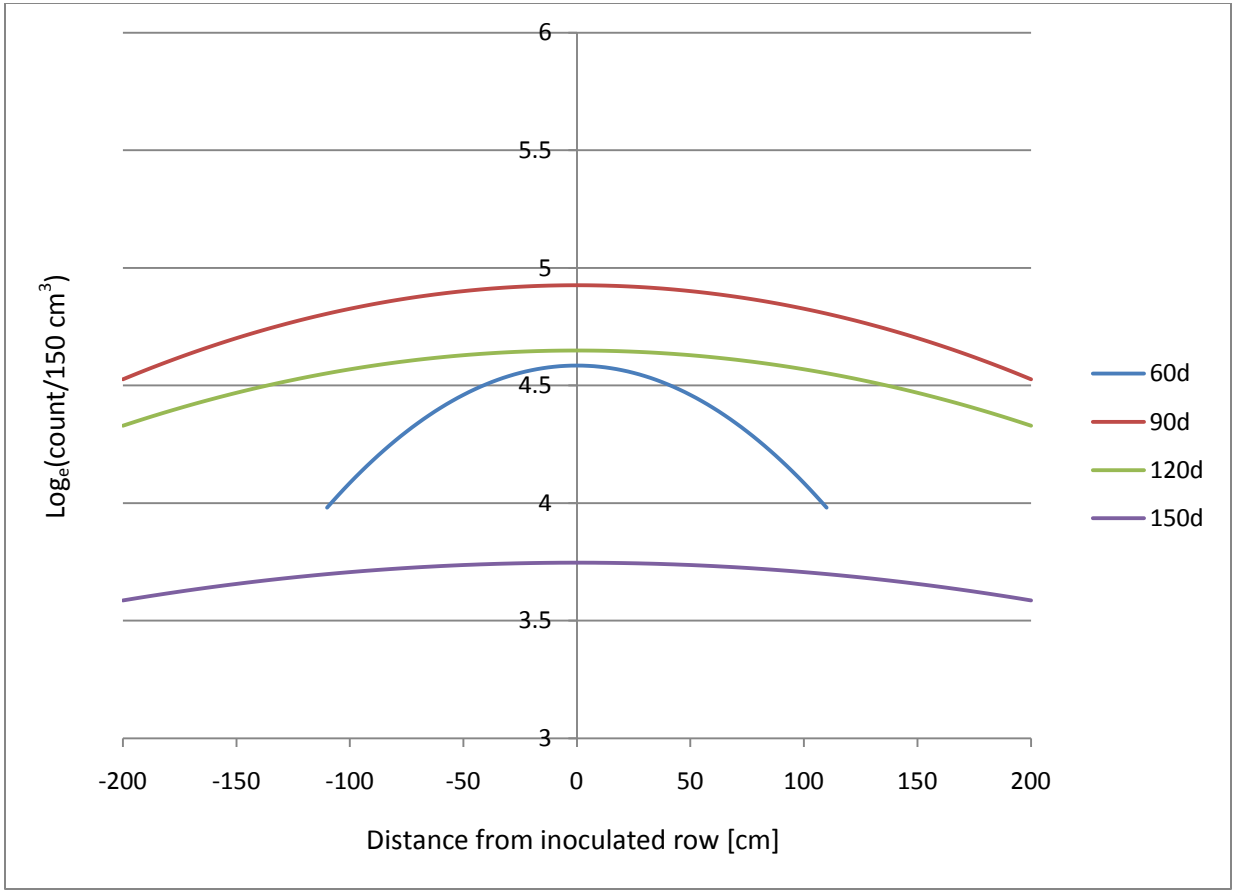


Figure 2.18. 2007 Non-irrigated Trial: *Rotylenchulus reniformis* males Log$_e$ count per 150cm^3 of soil at the 0 – 15 cm depth for each sampling date, 60d, 90d, 120d and 150d days after planting as measured at set distances (cm) from the originally inoculated row (0).

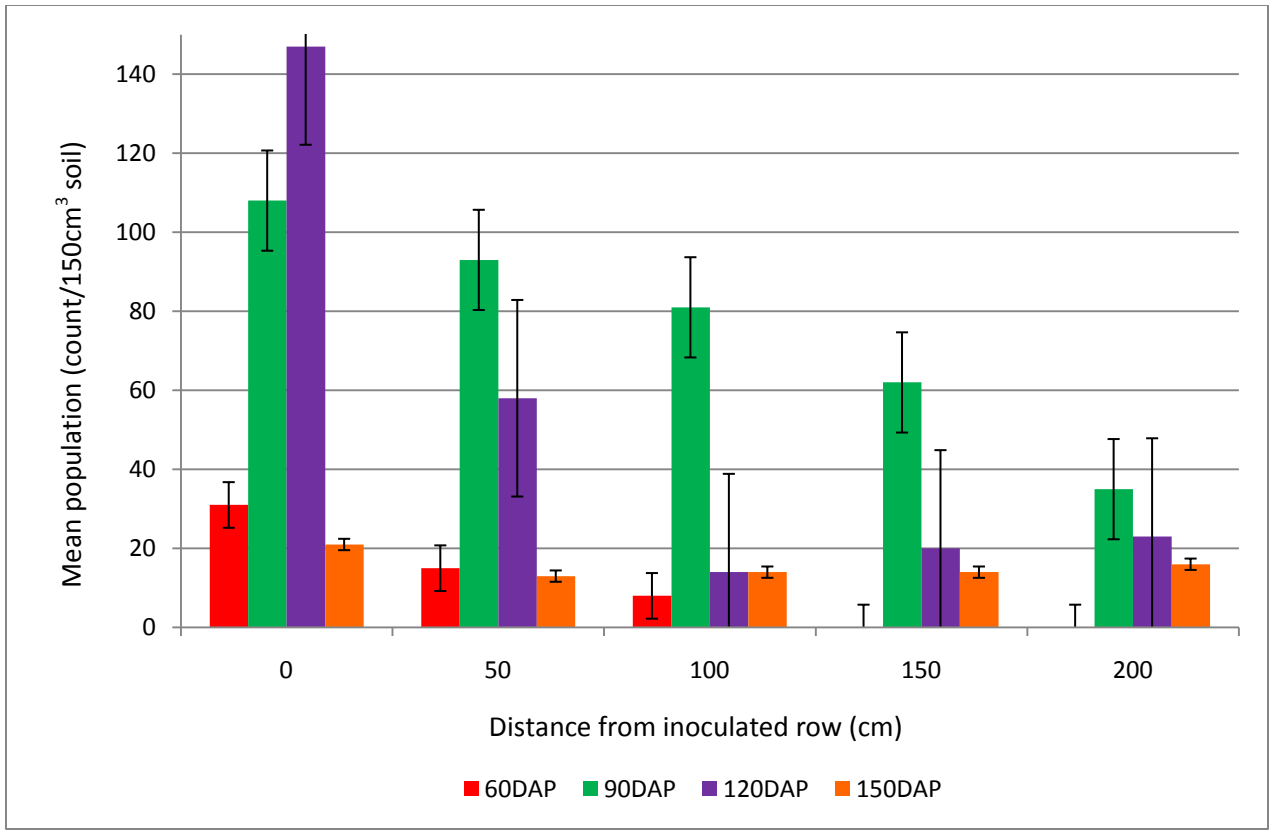


Figure 2.19. 2007 mean populations of *R. reniformis* males per 150cm^3 of soil at the 0 – 15 cm depth at 60, 90, 120 and 150 days after planting by distance (cm) from the inoculated row.

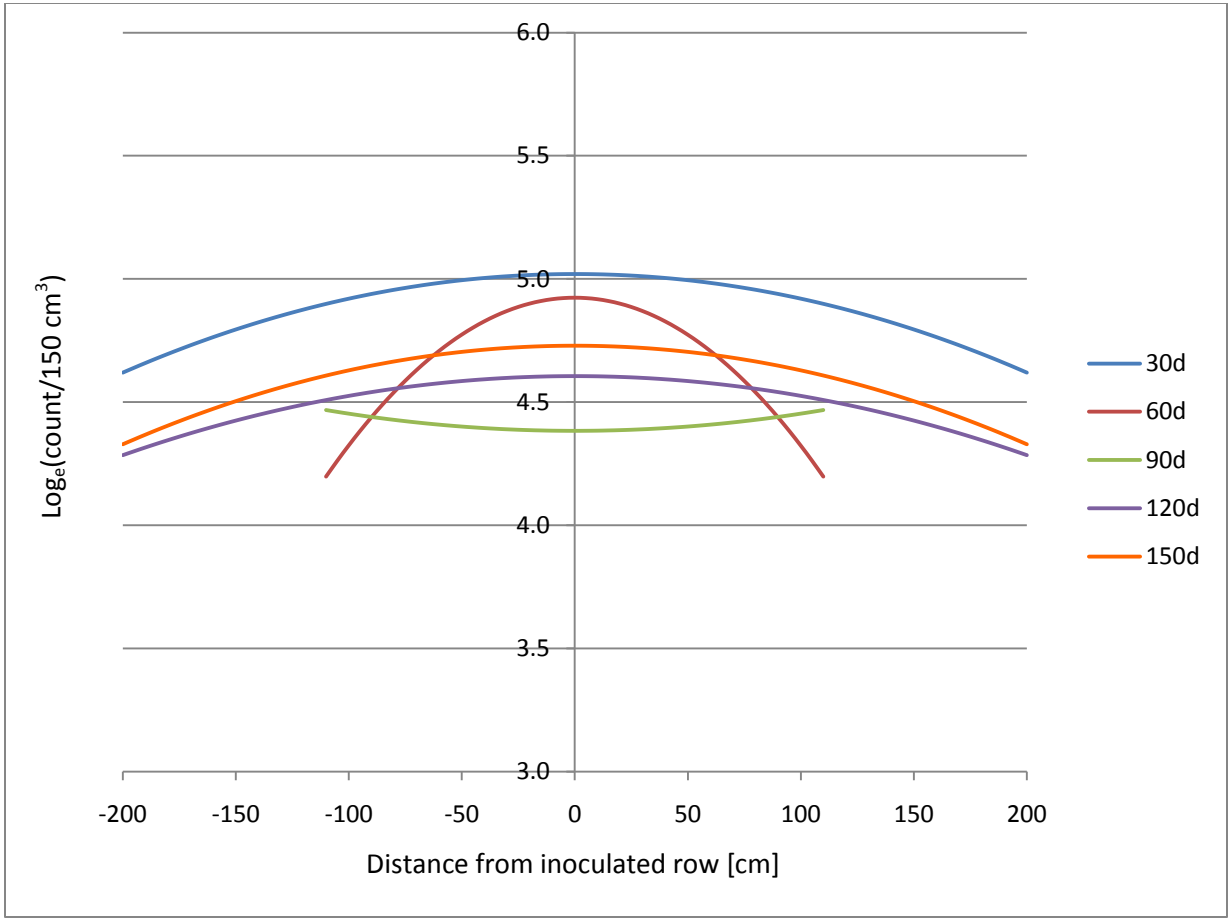


Figure 2.20. 2008 Non-irrigated Trial: *Rotylenchulus reniformis* vermiform females and juveniles Log$_e$ count per 150cm^3 of soil at the 0 – 15 cm depth for each sampling date, 30d, 60d, 90d, 120d, and 150d days after planting as measured at set distances (cm) from the originally inoculated row (0).

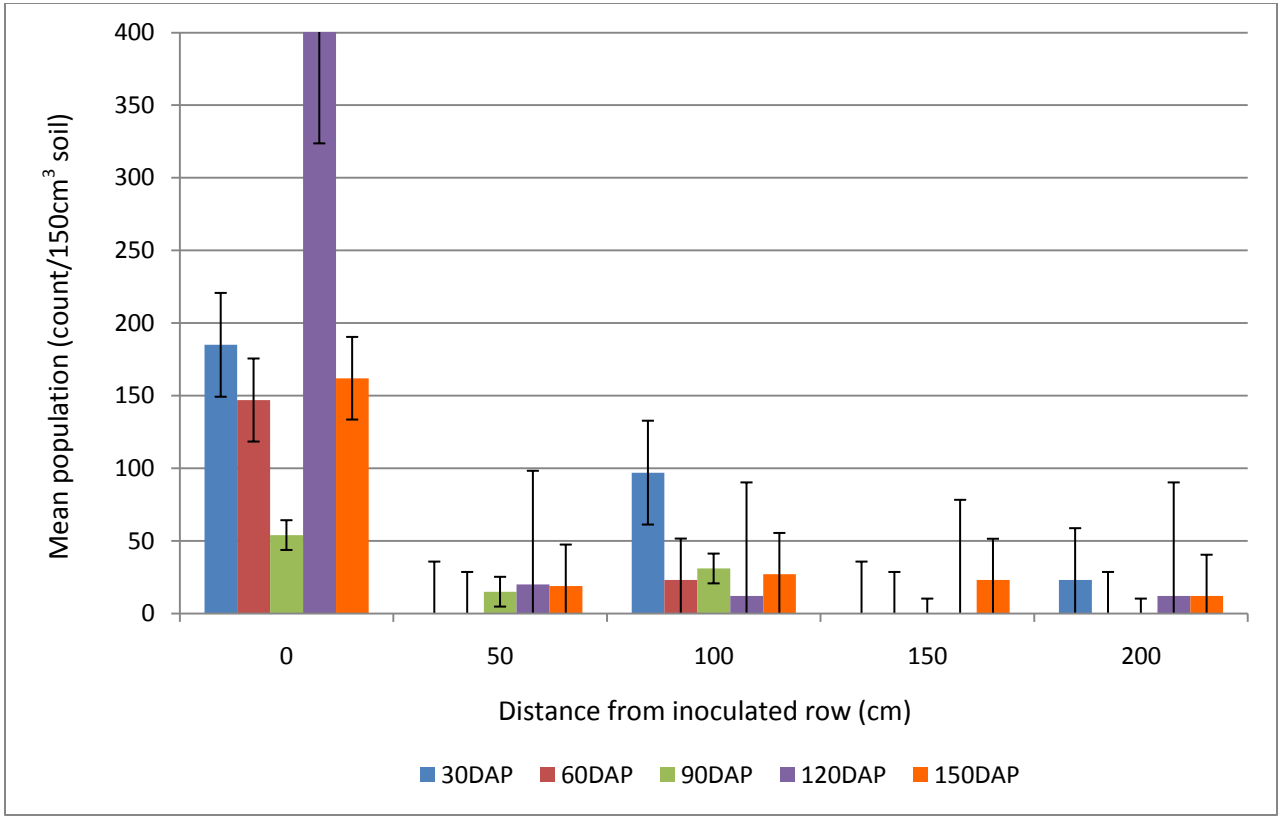


Figure 2.21. 2008 mean populations of *R. reniformis* vermiform females and juveniles per 150cm^3 of soil at the 0 – 15 cm depth at 30, 60, 90, 120 and 150 days after planting by distance (cm) from the inoculated row.

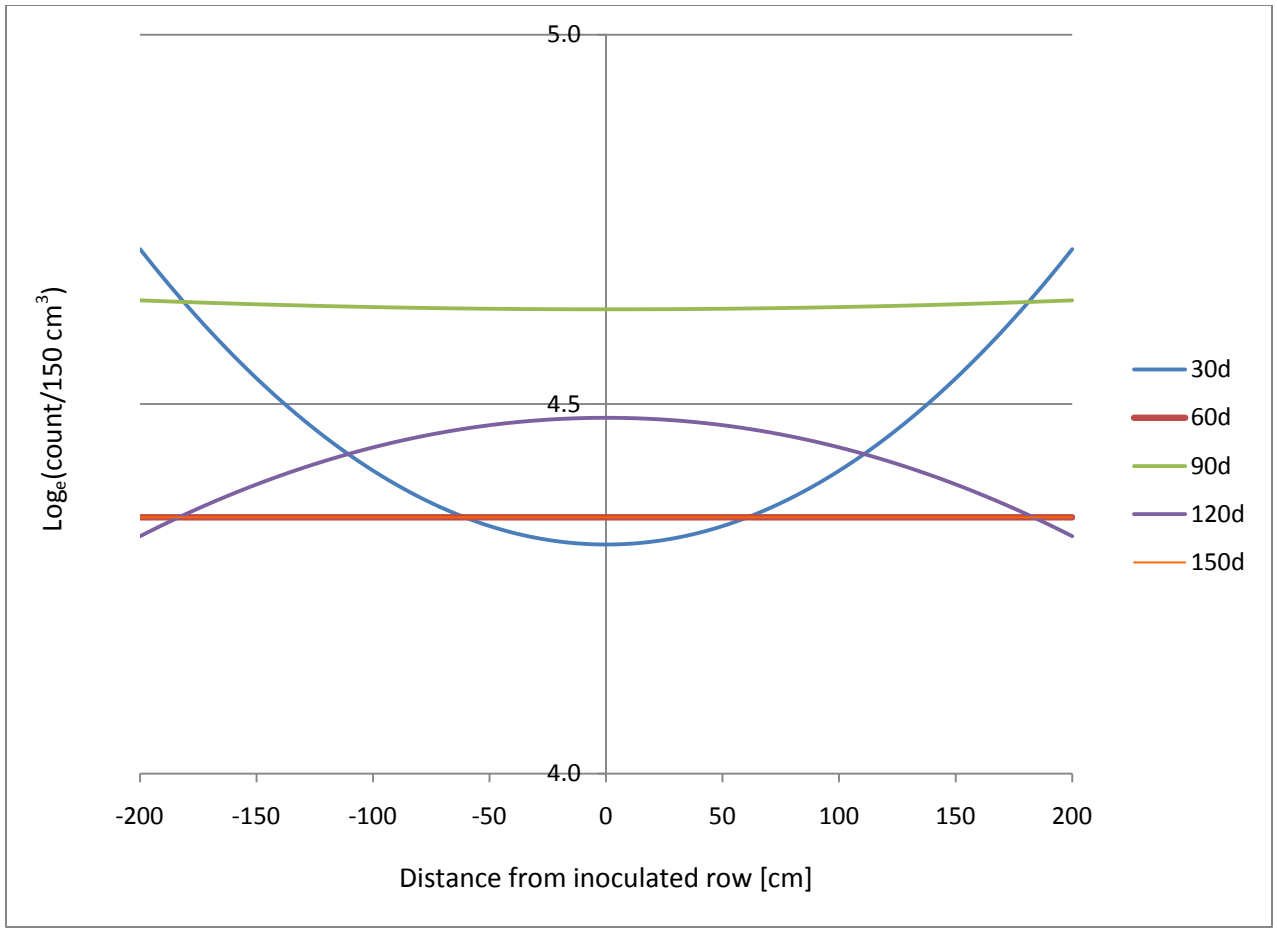


Figure 2.22. 2008 Non-irrigated Trial: *Rotylenchulus reniformis* males Log$_e$ count per 150cm^3 of soil at the 0 – 15 cm depth for each sampling date, 30d, 60d, 90d, 120d, and 150d days after planting as measured at set distances (cm) from the originally inoculated row (0).

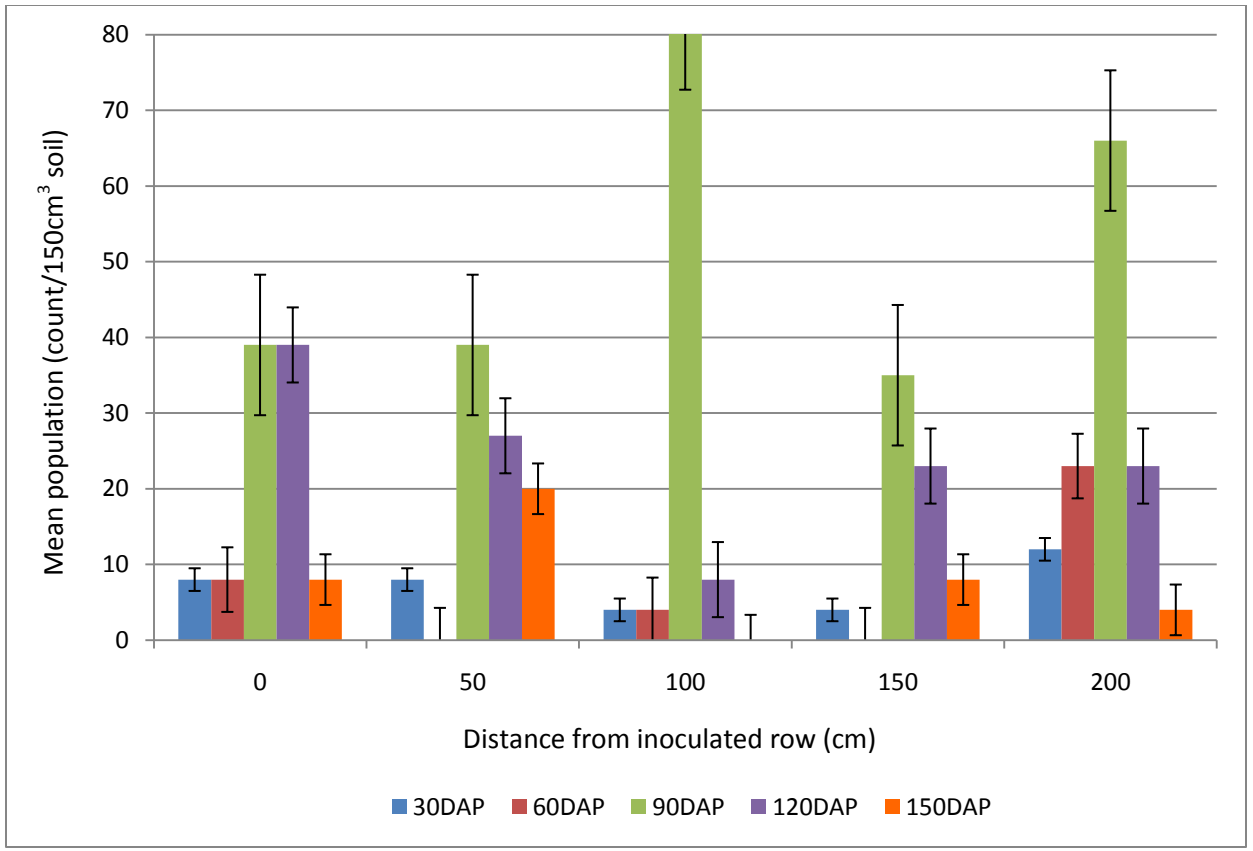


Figure 2.23. 2008 mean populations of *R. reniformis* males per 150cm^3 of soil at the 0 – 15 cm depth at 30, 60, 90, 120 and 150 days after planting by distance (cm) from the inoculated row.

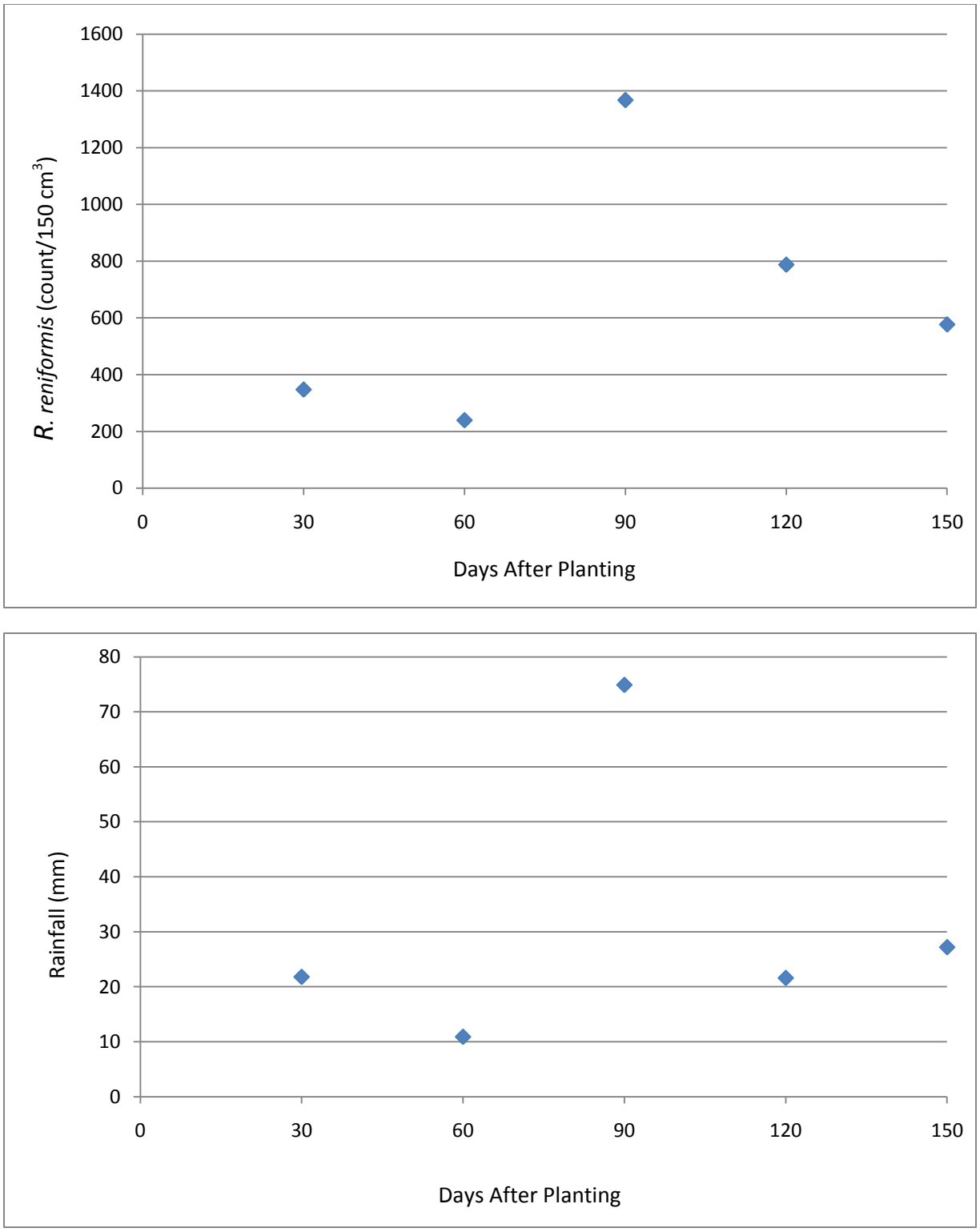
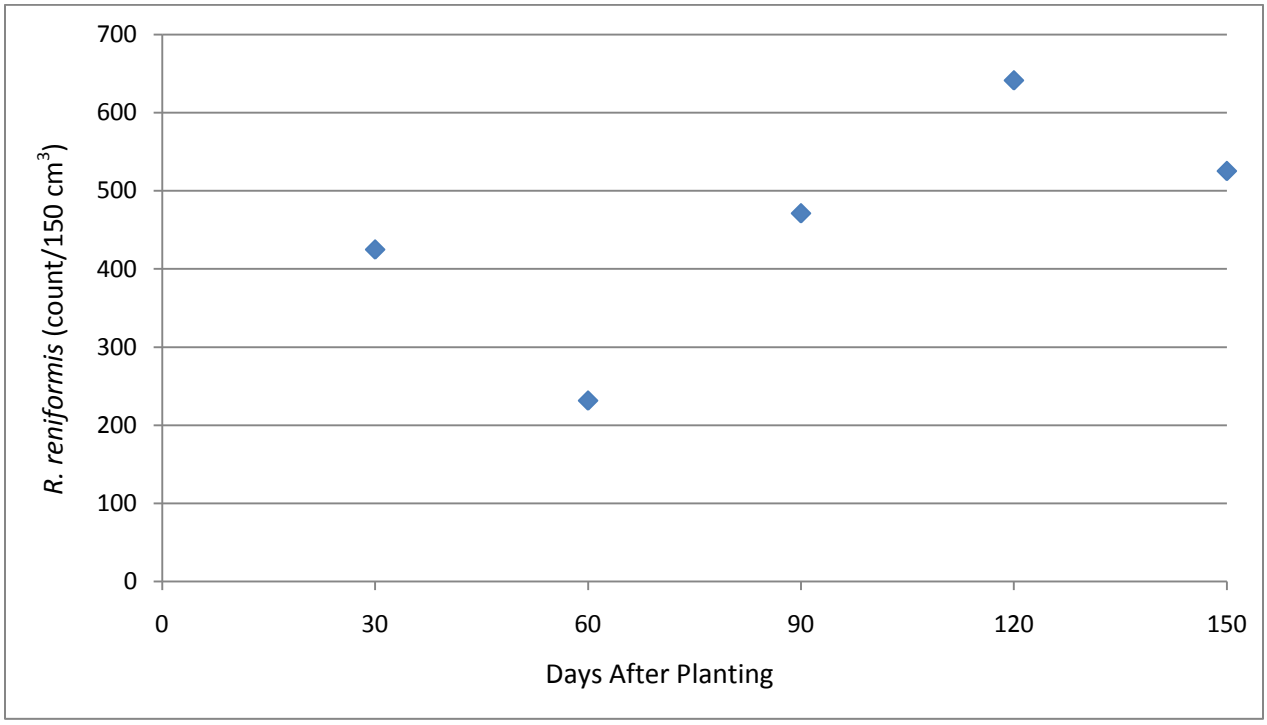


Figure 2.24. *Rotylenchulus reniformis* populations at the 0 – 15 cm depth (top) by sampling date compared to rainfall amounts (bottom) between sampling dates; 2007 non-irrigated trial.



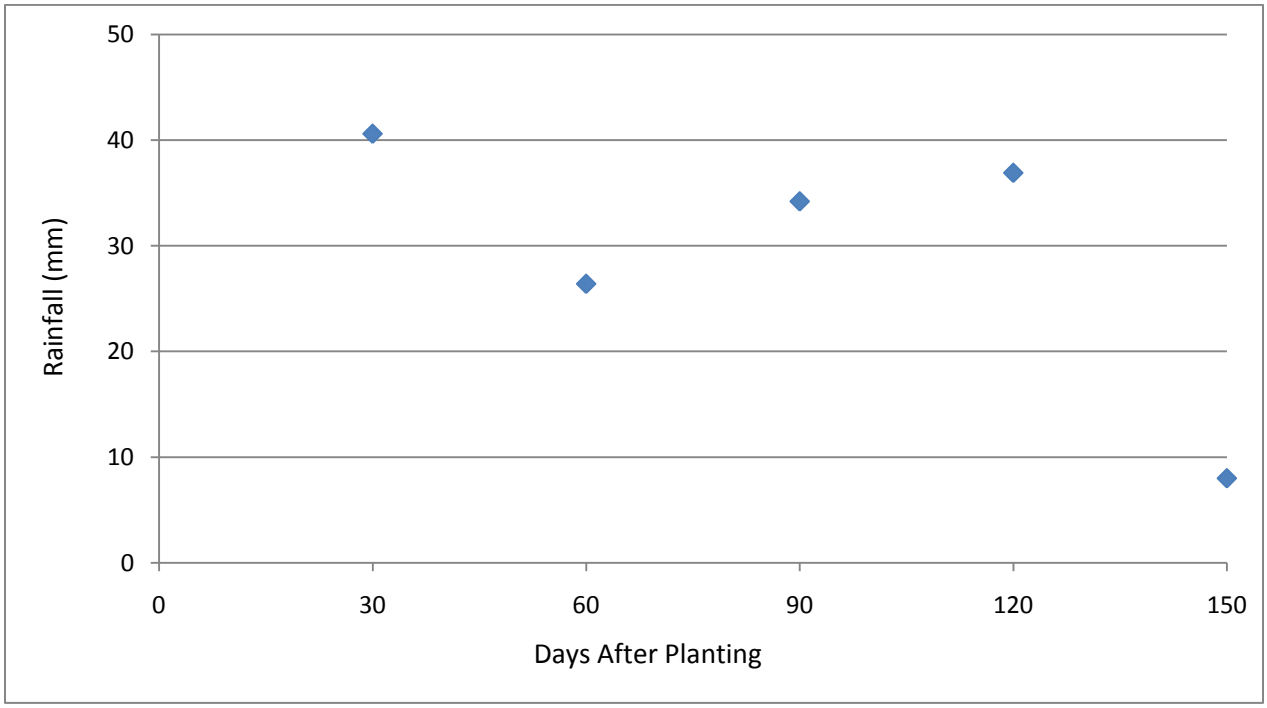


Figure 2.25. *Rotylenchulus reniformis* populations at the 0 – 15 cm depth (top) by sampling date compared to rainfall amounts (bottom) between sampling dates 2008; non-irrigated trial.

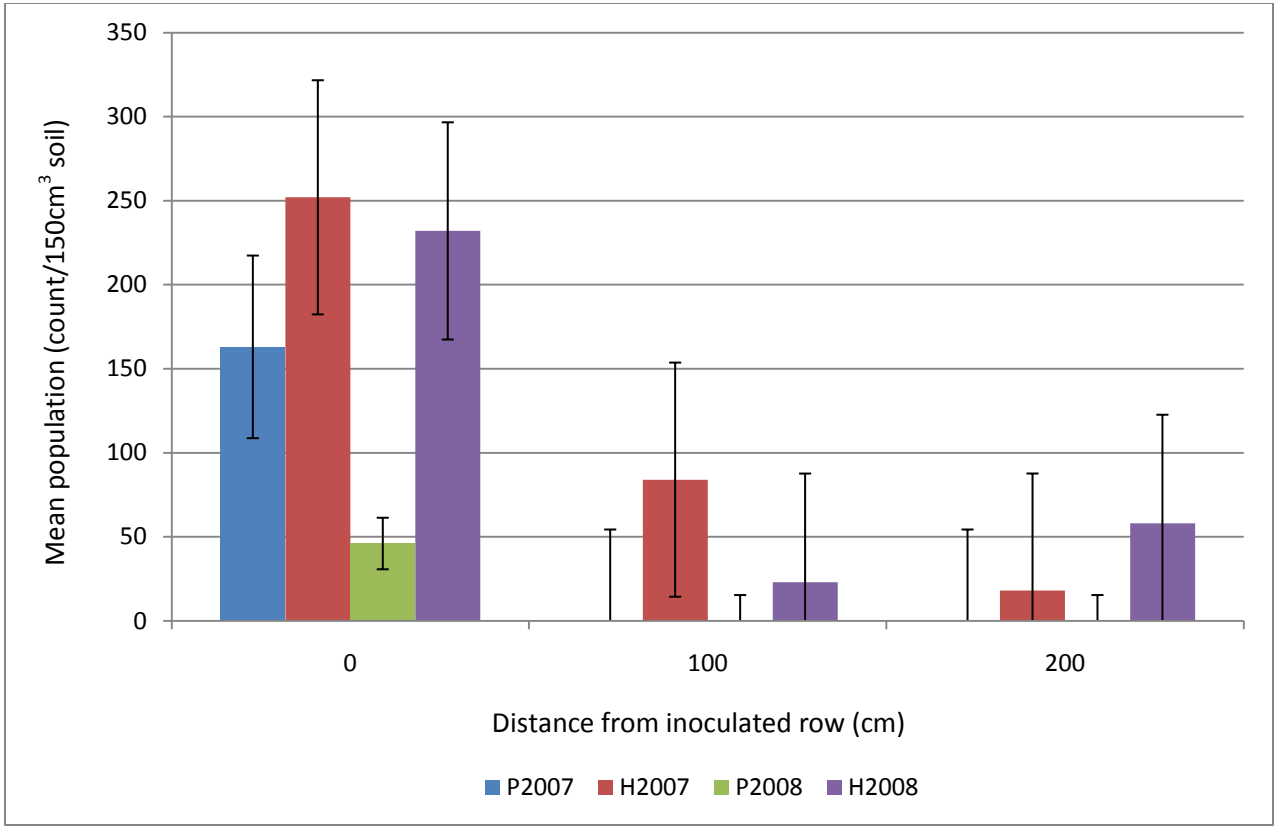


Figure 2.26. Non-irrigated trial mean populations of *R. reniformis* per 150cm^3 of soil at 2007 planting (P2007), 2007 harvest (H2007), 2008 planting (P2008) and 2008 harvest (H2008) within the inoculated row (0cm), one row from the inoculated row (100cm) and two rows from the inoculated row (200cm) at the 0 – 15 cm depth.

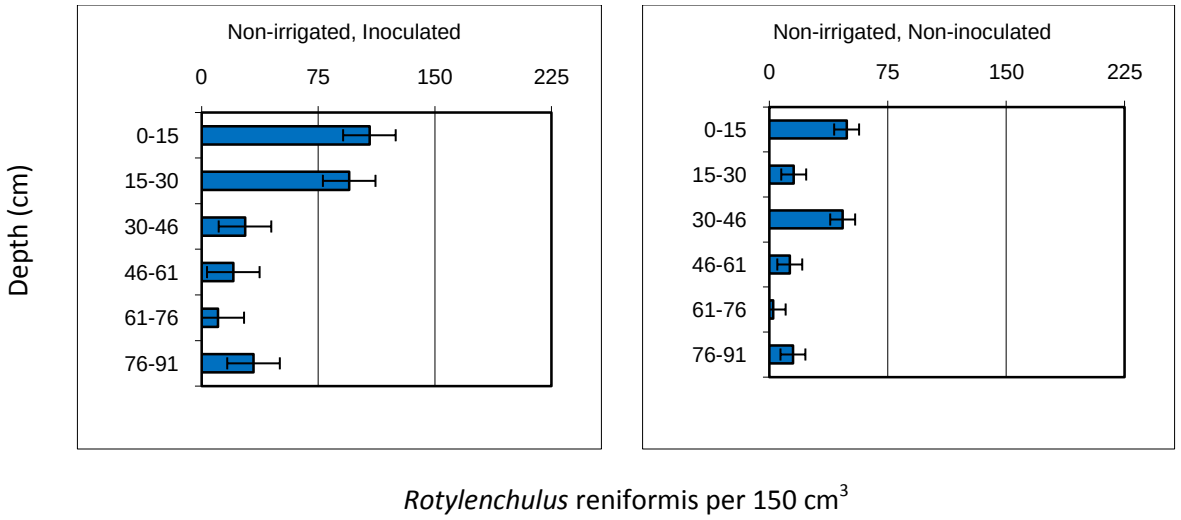


Figure 2.27. *Rotylenchulus reniformis* populations by depth in the non-irrigated trial subsequent to harvest, 2007. A comparison of the originally inoculated rows vs. non-inoculated rows 200cm from the originally inoculated row.

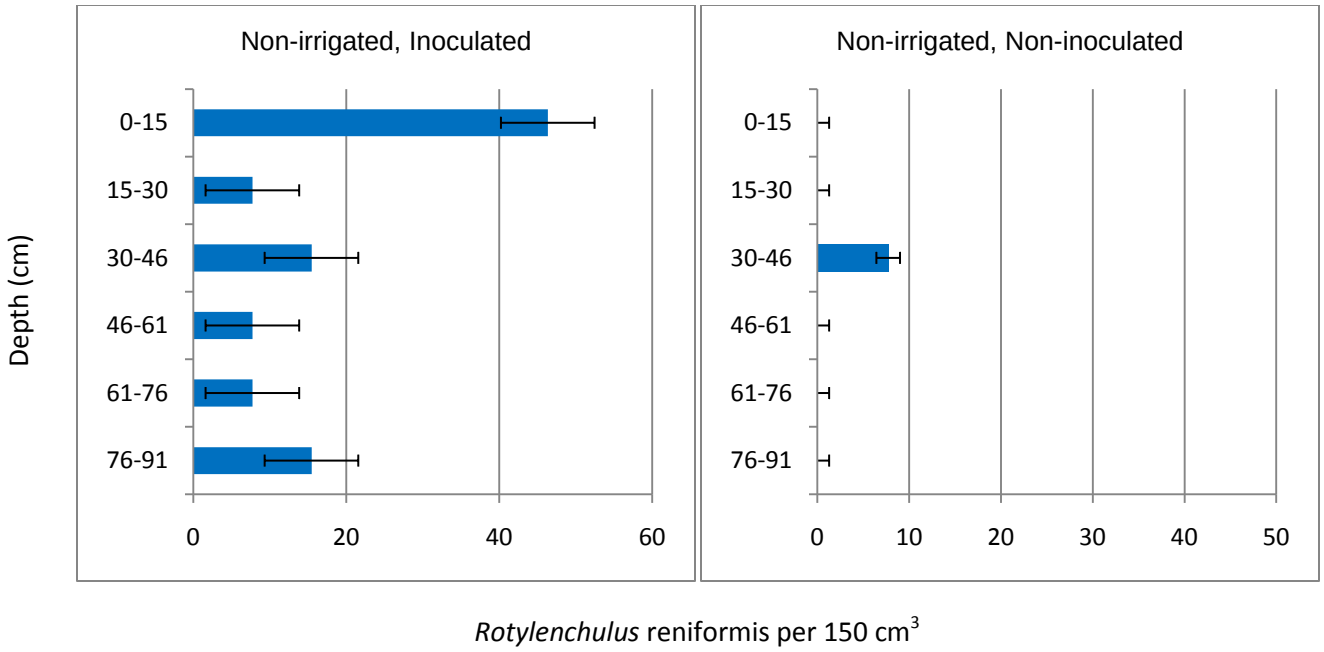


Figure 2.28. *Rotylenchulus reniformis* populations by depth in the non-irrigated trial at planting in 2008. A comparison of the originally inoculated rows vs. non-inoculated rows 200cm from the originally inoculated row.

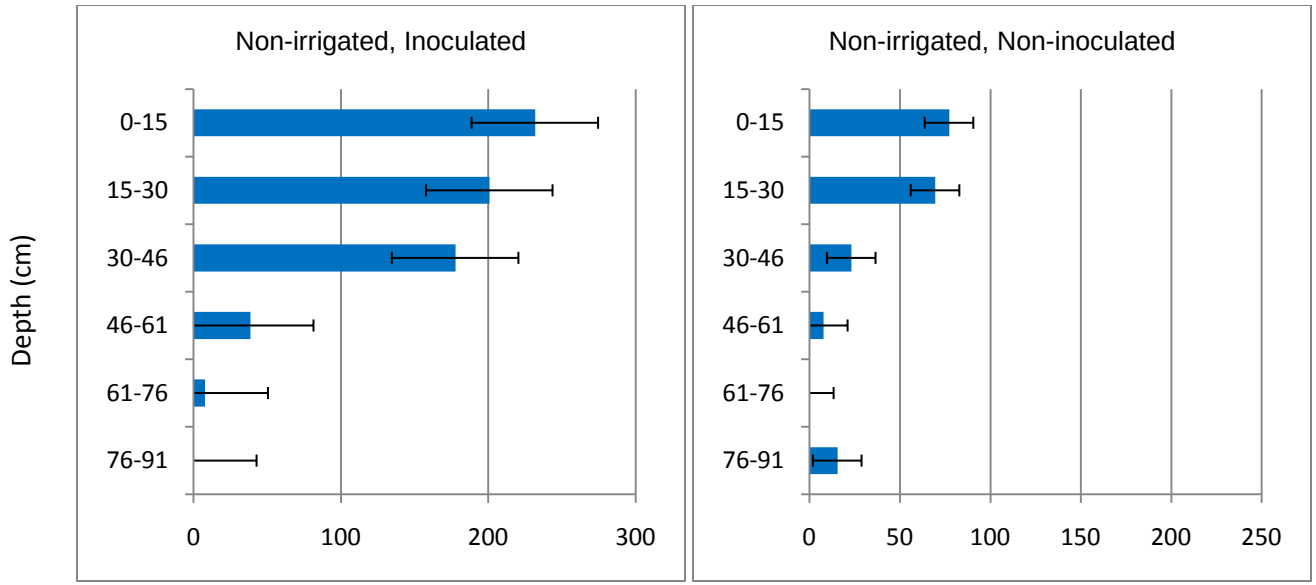


Figure 2.29. *Rotylenchulus reniformis* populations by depth in the non-irrigated trial subsequent to harvest, 2008. A comparison of the originally inoculated rows vs. non-inoculated rows 200cm from the originally inoculated row.

Downward Migration of *Rotylenchulus reniformis* Through the Soil Profile as Influenced by Rainfall and Root Growth

Abstract

The presence of *Rotylenchulus reniformis* at depths of greater than 1.5 m can have negative effects on cotton health. Two trials were established in 7.62 cm diameter by 75 cm deep soil cores to determine 1) the effect of water infiltration on vertical translocation of *R. reniformis*, and 2) the role of root growth in the downward migration of *R. reniformis*. The water infiltration study consisted of three treatments of simulated rainfall amounts, 25.4 mm, 76.2 mm, and 127 mm, and no rainfall. Water infiltration minimally affected the movement of *R. reniformis* through the soil profile. The 25.4 mm rainfall treatment enabled the nematodes to reach a depth of 30 cm. Rainfall of 76.2 mm was required to reach a depth of 45 cm and 127 mm of rainfall was needed to surpass 45 cm. To determine the effect of root growth on *R. reniformis,* the nematodes were monitored as a cotton root system developed over time. Cotton roots reached the maximum depth of 75 cm at 60 days after planting (DAP). Vermiform life stages reached 75 cm at 45 DAP. Females colonized roots at the maximum sampling depth of 75 cm by 90 DAP. Root growth increase at 15 day intervals was higher when *R. reniformis* population increases were the lowest. In contrast, root growth increase was the lowest when *R. reniformis* population increases were the highest. *Rotylenchulus reniformis* colonized down through the profile as a food source became available.

Rotylenchulus reniformis (Linford and Oliveira) has become the most economically damaging pathogen in Alabama, causing an average of 7% yield loss equaling nearly $126 million over the past decade (Blasingame *et al*., 2009). Yield losses can reach levels as high as 40% and are caused by females feeding on the roots, restricting the flow of water and nutrients to the plant (Robinson, 2007). When introduced into the upper 15 cm of a cotton field, *R. reniformis* can colonize the root zone to depths below the plow layer within the first growing season and survive until the following year (Moore *et al*., 2007 and 2008). Survival of *R. reniformis* at depths well below the plow layer can directly affect cotton yields (Robinson *et al*., 2005) and enable rapid population resurgence into the upper horizons subsequent to rotation or nematicide application (Lee *et al*., 2002; Newman and Stebbins, 2002). Although the existence and consequences of deep populations of *R. reniformis* are well documented, the factors that influence these populations are unknown. The aim of this research is to explore two possible factors that influence downward migration of *R. reniformis*: water infiltration and root growth.

MATERIALS AND METHODS

Two trials to monitor the migration of *R. reniformis* through the soil profile, the first as affected by water infiltration and the second as affected by root growth, were established at the Auburn University Plant Science Research Center in Auburn, AL. The trials were conducted in soil cores, 7.62 cm in diameter and 75 cm deep, collected at the Tennessee Valley Research and Extension Center, near Belle Mina, AL using a #5-UV4 Model GSRPSUV4G (Giddings Machine Company, Windsor, CO). The soil is classified as a Decatur silt loam (fine, kaolinitic, thermic, Rhodic Paleudults: 23%, 49%, 28%, S-S-C, 1% OM, CEC of 9 – 10, pH 6.2) and was tested to confirm the absence of *R. reniformis* at all depths. The soil cores were supported in wooden racks and nylon wicks were attached to the bottom of each to facilitate water drainage. A week

prior to initiation of the trials, the soil cores moisture was brought to field capacity and allowed to acclimate to their environment.

The *R. reniformis* population was increased from stock cultures grown on cotton (*Gossypium hirsutum* L.), cv. Delta and Pine Land (DPL) 555 BGRR at Auburn University Plant Science Research Center located on the Auburn University campus. The nematodes were increased in 10 cm diameter polystyrene pots containing 500 cm^3 of a loamy sand soil (72.5% sand, 25% silt, 2.5% clay, OM 1%, pH 6.4). The soil was autoclaved at 121˚ C and 103.4 kPa for two hours on two successive days for sterilization. Nematode inoculum consisted of *R. reniformis* vermiform life stages extracted from the soil using combined gravity screening and sucrose centrifugal flotation. The nematodes were enumerated using a Nikon TS100 inverted microscope at 40x magnification and populations were standardized by volume for treatments.

Water Infiltration:

Rotylenchulus reniformis was added to the top 2.54 cm of soil by pipetting in 50,000 vermiform life forms in 2 mL of water and were allowed to acclimate for 12 hours. The cores were then subjected to one of three different rainfall amounts: 25.4 mm, 76.2 mm and 127 mm using a drip rainfall simulator, or no rainfall. The trial was arranged in a randomized complete block design with six replicates. Forty-eight hours after the simulated rain event cores were separated into 15 cm sections and the nematodes extracted and enumerated as previously described.

Root Growth:

One cotton plant, (DPL 555 BGRR) was planted in each soil core and *R. reniformis* was added to the top 2.54 cm of soil by pipetting in 10,000 vermiform life forms in 2 mL of water.

The cotton was then allowed to grow until termination 15, 30, 45, 60, 75, or 90 days after planting. Soil cores were arranged in a randomized complete block design and replicated three times. At each respective termination date, the cores were divided into 15 cm sections for analysis. The cotton roots were removed from each section, weighed, and stained with acid fuchsin as described in Byrd *et al*., (1983). The stained roots were evaluated for numbers of embedded females using a Nikon SMZ800 stereo microscope at 63x magnification. The soil from each section was evaluated for number of vermiform life stages that were extracted and enumerated as previously described.

All tests were repeated twice, and data collected was analyzed by SAS (SAS Institute, Inc) using generalized linear models. The best fit models of interactions were determined using the Mallow's Cp and adjusted R-squared methods. Least squares means of fixed effects were used to determine significance ($P \leq 0.10$) by depth.

RESULTS

Water Infiltration:

Water infiltration had no significant effect on the short-term downward movement of *R. reniformis* in our trial. One hundred percent of the applied population of *Rotylenchulus reniformis* was recovered in the top 15 cm of the 0 mm rainfall treatment and thus the nematodes exhibited no significant migration on their own within 48 hours of application (Fig. 3.1). The 25.4 mm rainfall treatment transported 1.6% of the applied population to the 15 – 30 cm sampling depth with 98.4% remaining in the upper 15 cm. Similarly, the 76.2 mm rainfall treatment transported 1.8% of the applied population to the 15 – 30 cm depth, and an additional 0.2% to the 30 – 45 cm depth. Thus with 76.2 mm of rainfall, 98% of the population of nematodes remained in the top 15 cm of the soil profile. The 127 mm treatment transported

small portions of the applied *R. reniformis* population throughout the entire soil core depth. The population dispersed with 94.25% remaining in the top 15 cm, 1.32% in the 15 – 30 cm depth, 2.27% in the 30 – 45 cm depth, 0.89% in the 45 – 60 cm depth and 1.28% in the 60 – 75 cm depth. A large amount of rainfall in a short period of time is necessary to transport *R. reniformis* deeper than 30 cm. The 25.4 mm treatment enabled the nematodes to reach a 30 cm depth; however, 76.2 mm was required to reach a 45 cm depth and 127 mm of rainfall to surpass 45 cm.

Root Growth:

Populations of *R. reniformis* females embedded in the roots and vermiform life stages found in the soil were significantly affected by root mass. Average cotton taproot lengths increased linearly (r^2 = 0.96) until reaching the maximum possible length of 75 cm at 60 DAP (Fig. 3.2). Root elongation averaged 1.3 cm per day for the first 60 days. Subsequently, root mass and nematode numbers increased at each depth throughout the trial. Embedded females were detected to a depth of 30 cm at 15 and 30 DAP, to a depth of 45 cm at 45, 60, and 75 DAP and through the maximum depth of 75 cm at 90 DAP. Vermiform life stages of *R. reniformis* were detected to a depth of 30 cm at 15 DAP, a depth of 60 cm at 30 DAP, and reached the maximum depth of 75 cm at 45 DAP.

Root mass accounted for 83% of the variation in populations of *R. reniformis* females embedded in the roots (r^2 = 0.83) (Fig. 3.3) following the line y = - 22.656 + 464.33x, where y is the number of embedded females and x is the root mass. At 15 DAP, the root mass was significantly higher ($P \leq 0.10$) in the top 15 cm of soil than at all other soil core depths (Fig. 3.4). Root mass was higher ($P \leq 0.10$) at a depth of 15 – 30 cm compared to at 30 – 75 cm. No

differences in root mass ($P \leq 0.10$) were present at depths of 30 – 75 cm at 15 DAP. Females within the roots at 15 DAP were higher ($P \leq 0.10$) in the top 15 cm than all other depths. No differences were observed at 15 - 75 cm. At 30 DAP, both root mass and number of females embedded in the roots were significantly higher ($P \leq 0.10$) in the top 15 cm, but no differences were observed for either at 15 – 75 cm (Fig. 2.4). Root mass at 45 DAP was significantly greater ($P \leq 0.10$) within the top 15 cm, but no differences were observed at 15 – 75 cm (Fig. 3.5). However, the numbers of females embedded in the roots were not significantly different at any depth. The upper 15 cm contained a higher root mass ($P \leq 0.10$) compared with the 30 – 75 cm depths at 60 DAP, but not compared with the 15 – 30 cm depth (Fig. 3.5). Numbers of females embedded in the roots were significantly higher ($P \leq 0.10$) in the top 15 cm than all other depths. At 75 DAP, both root mass and females embedded in roots were significantly higher ($P \leq 0.10$) in the top 15 cm than all other depths (Fig. 2.6). No differences were observed at 15 – 75 cm for either root mass or numbers of embedded females. At the final sampling date (90 DAP), root masses within the top 15 cm were higher ($P \leq 0.10$) than the horizons at 15 -75 cm (Fig. 3.6). However numbers of embedded females in the top 15 cm were higher ($P \leq 0.10$) than only the 45 – 75 cm horizons. The population in the 15 – 30 cm horizon was increasing and was not significantly less ($P \leq 0.10$) than the population at 0 - 15 cm nor was it significantly higher ($P \leq 0.10$) than at 30 – 75 cm.

Populations of *R. reniformis* vermiform life stages in the soil were also closely related to root mass (Fig. 3.3). The variation in root mass accounted for 79% of the variation in *R. reniformis* populations ($r^2 = 0.79$) following the line $y = -2086.2 + 9592.9x$, where y is the number of vermiform life stages in the soil and x is the root mass. Root mass at 15 DAP was significantly higher ($P \leq 0.10$) in the top 15 cm compared with all other depths, and higher ($P \leq 0.10$) at 15 – 30 cm compared to all horizons at 30 – 75 cm, which did not differ (Fig. 3.4). Populations of

vermiform life stages were significantly higher in the top 15 cm compared with all other depths. The populations of vermiform life stages at 30 DAP were significantly higher ($P \leq 0.10$) in the top 15 cm compared to at 45 – 75 cm. At 45 DAP, root mass and populations of vermiform life stages in the top 15 cm were significant ($P \leq 0.10$) over all other depths (Fig. 3.5). Root mass at 60 DAP was higher ($P \leq 0.10$) in the top 15 cm compared to the horizons at 15 – 75 cm (Fig. 3.5). Populations of vermiform life stages were significantly higher ($P \leq 0.10$) in the top 45 cm compared to at 45 – 75 cm. The root mass at 75 DAP followed the same pattern of separation as 60 DAP (Fig. 3.6). Vermiform life stages populations were higher ($P \leq 0.10$) in the top 15 cm of the soil compared with the horizons at 30 – 75 cm. Root mass at 90 DAP was higher in the top 30 cm compared to all other depths (Fig. 3.6). Populations of vermiform life stages were significantly higher ($P \leq 0.10$) in the top 15 cm of the profile compared to at 30 – 75 cm. Populations in the 15 – 30 cm horizon were higher ($P \leq 0.10$) compared to at 45 – 75 cm.

Root growth and number of *R. reniformis* populations in the roots or in the soil followed a pattern of inverse 15 day intervals throughout the trial (Fig. 3.7). Root mass increased throughout the profile by an average of 290% between 15 and 30 DAP, 132.6% between 30 and 45 DAP, 264.5% between 45 and 60 DAP, 136% between 60 and 75 DAP and a final increase of 584.8% between 75 and 90 DAP. Numbers of *R. reniformis* females embedded in the roots increased 115.6% between 15 and 30 DAP, 406.6% between 30 and 45 DAP, 89% between 45 and 60 DAP, 402.8% between 60 and 75 DAP and 212.8% between 75 and 90 DAP. Populations of vermiform life stages in the soil mirrored the wave pattern of the numbers found in the roots. Numbers of R. reniformis increased an average of 173.7% between 15 and 30 DAP, 500% between 30 and 45 DAP, 94.8% between 45 and 60 DAP, and 382.4% between 60 and 75 DAP. Between 75 and 90 DAP vermiform life stages in the soil did break the pattern by increasing 775.9%.

The patterns produced by plotting the increases in root mass and nematode populations exhibit the percentage increases of populations of both embedded females and vermiform life stages in the soil rise while the percentage of increase in root mass declines. Once the females entered the roots, the percentage increase of the roots again increased while the populations of *R. reniformis* embedded in the roots and vermiform life stages in the soil declined. As each nematode generation hatched and began entering the roots, the percent increase in root growth would again begin to decline. The contrasting pattern of cotton root growth and *R. reniformis* populations is an excellent example of the cotton root/nematode relationship that occurs through the growing season.

DISCUSSION

The availability of a food source is the apparent drive behind colonization of the soil horizon by *R. reniformis.* As the cotton roots elongated and extended into the soil, so too did the nematode populations. Newman and Stebbins (2002) and Lee *et al*., (2002) both observed a late season resurgence of *R. reniformis* populations from the lower horizons to the upper horizons following either an early season nematicide treatment or rotation to a non-host, such as corn. In these trials, the food source in the upper horizons was diminished or deprived for a period, but once a food source was again available, *R. reniformis* populations were again able to colonize the upper soil horizons.

The influence of water infiltration on the downward movement of *R. reniformis* was, numerically, insignificant in our trial. A large amount of rainfall was required to transport even small amounts of *R. reniformis* downward from the top 15 cm of the profile. However the significance of even small populations of *R. reniformis* in the lower horizons, where root mass is less but critical to plant health, can be great. *Rotylenchulus reniformis* introduced later in the

season, possibly on contaminated equipment during a side dress application of a fertilizer or nematicide, could find roots in the lower horizons and thus could become a significant yield-limiting factor. Robinson *et al*., (2005) found that populations of *R. reniformis* below the plow layer can suppress root growth and decrease the ability to supply moisture to the plant. This can be an even greater factor in dry years when moisture is unavailable in the upper horizons. Furthermore, control of *R. reniformis* at these depths is only experimental and expensive. As such, the introduction of *Rotylenchulus reniformis* into the surface of a cotton field can have much deeper consequences.

The analysis of root growth rates and *R. reniformis* population fluctuations produced interestingly inverse wave patterns. These inverse wave patterns grant further insight to the nematode/root relationship that occurs during the growing season. A closer study of this relationship could be used to predict management opportunities, especially for mid-season nematicide applications. Additional benefits of the knowledge of these patterns could be gained in the form of strategic timing of irrigation or fertilizer applications to aid the cotton plant when root growth restriction is at its greatest. The management of *R. reniformis* is costly, and many times inefficient due to a large variety of factors. More knowledge of the interactions of *R. reniformis* and cotton root growth can be utilized to allow for more efficient management of this pathogen.

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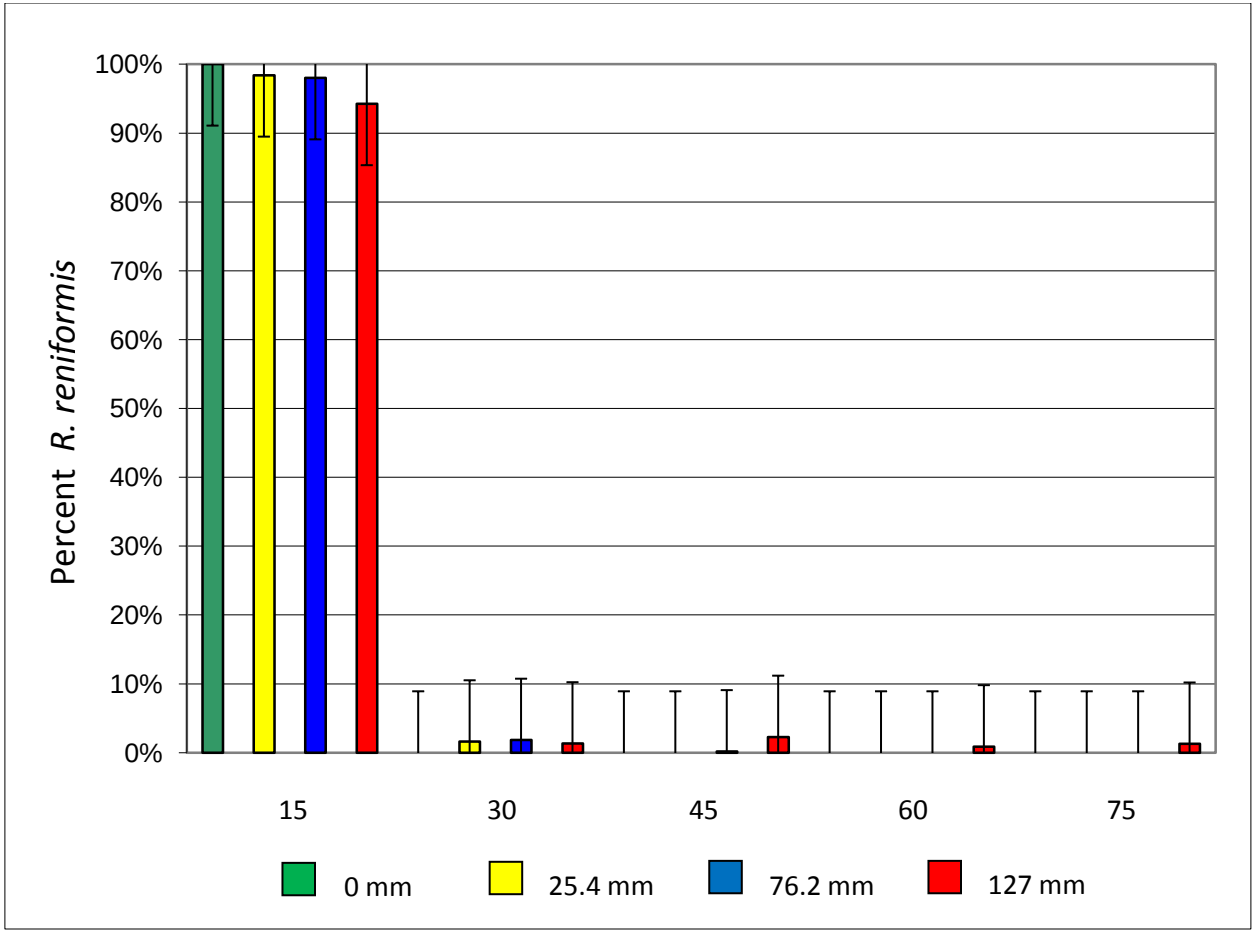

Figure 3.1. Percent of total *R. reniformis* recovered by depth for each rainfall amount.

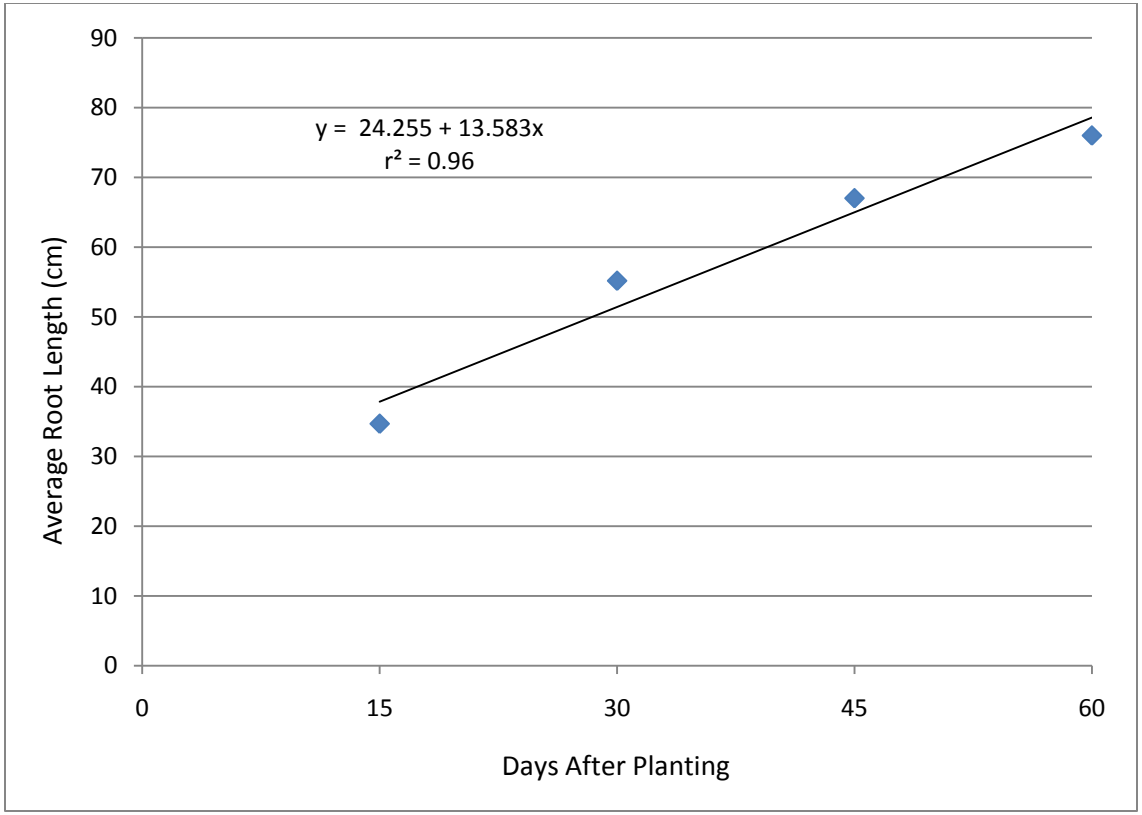

Figure 3.2. Average cotton root length (cm) at 15, 30, 45 and 60 days after planting.

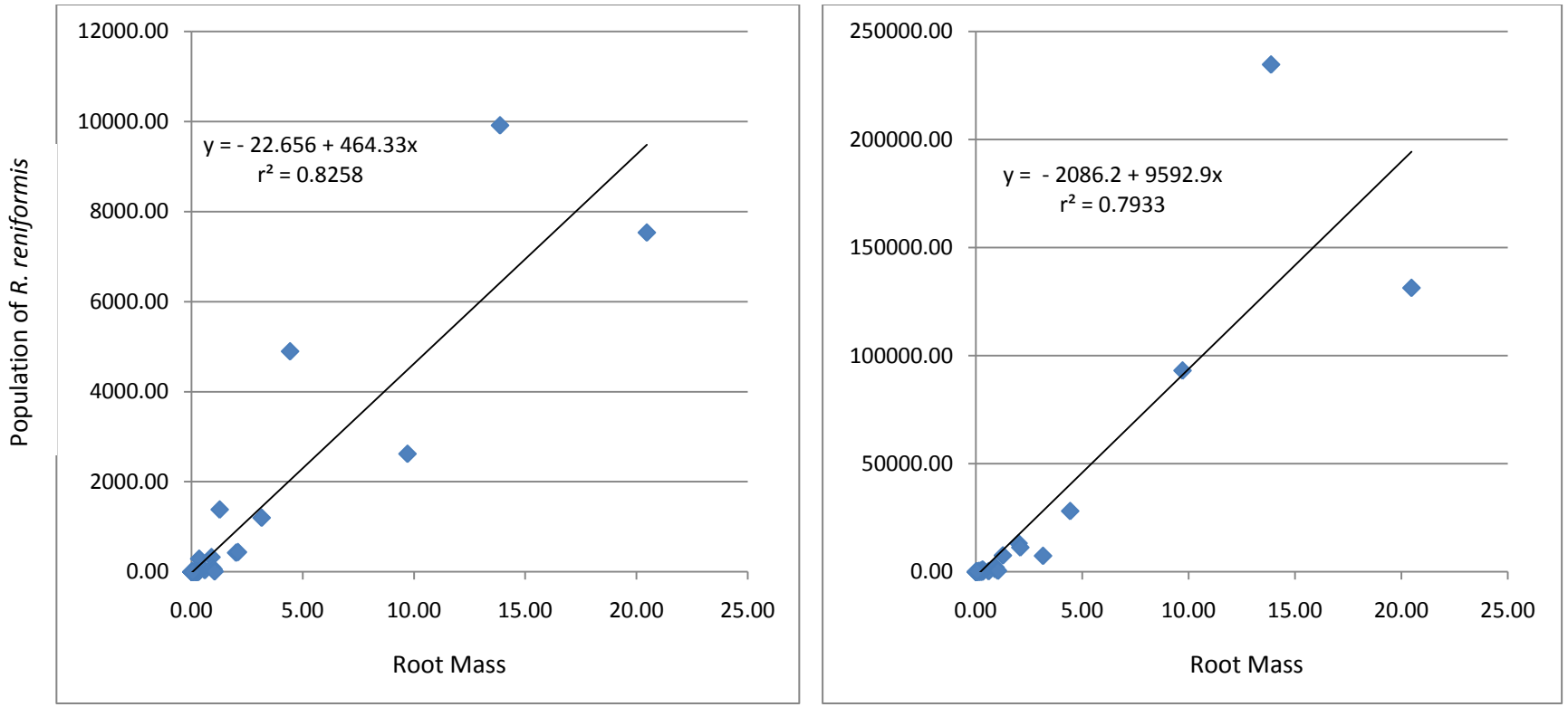

Figure 3.3: Fit plots for the number of *R. reniformis* females embedded in the cotton roots (left) and populations of vermiform life stages in the soil (right) by depth.

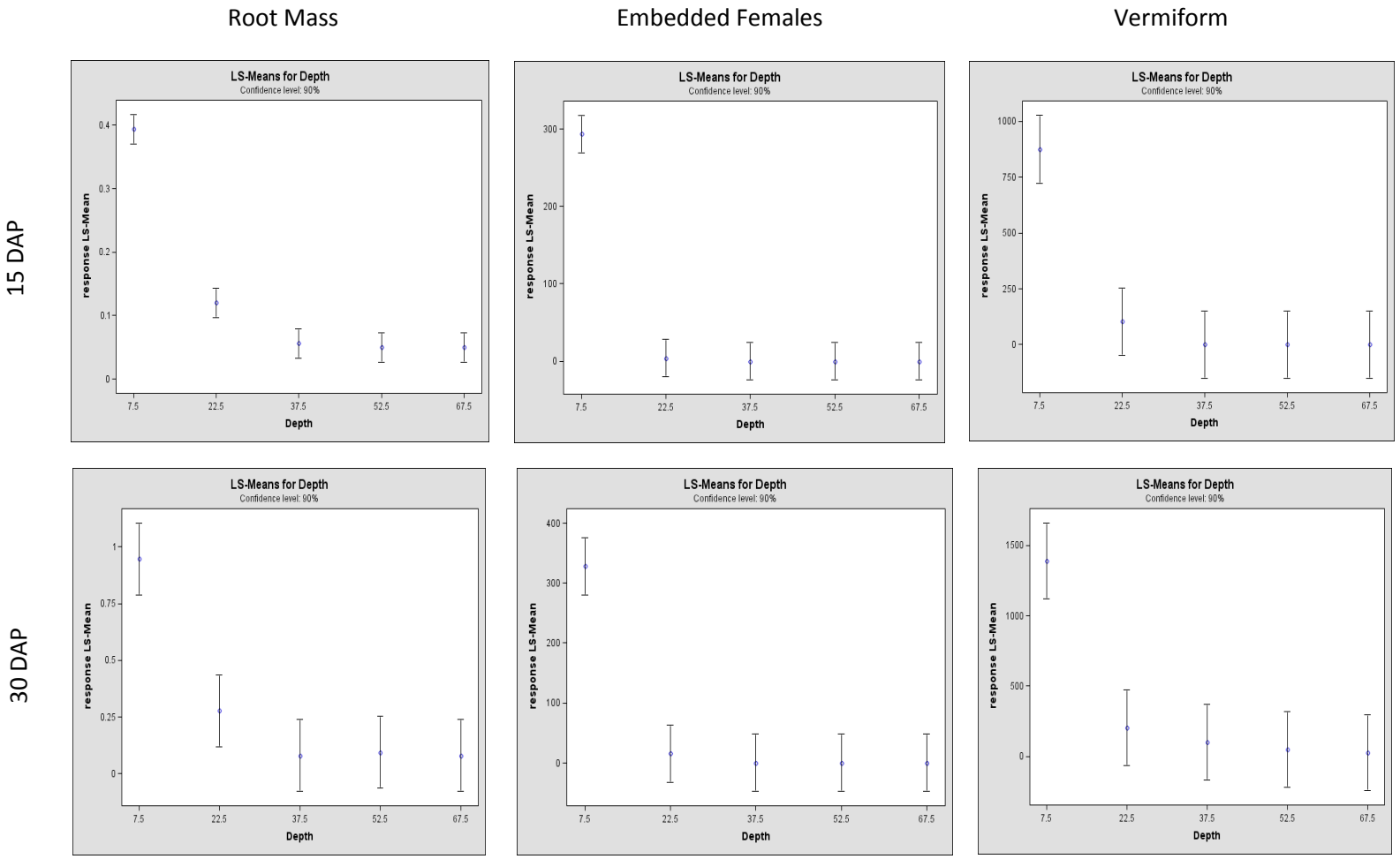

Figure 3.4: Least squares means comparison for root mass (left), *R. reniformis* females embedded in the cotton roots (center) and *R. reniformis* vermiform life stages in the soil (right) by depth at 15 (top) and 30 (bottom) days after planting.

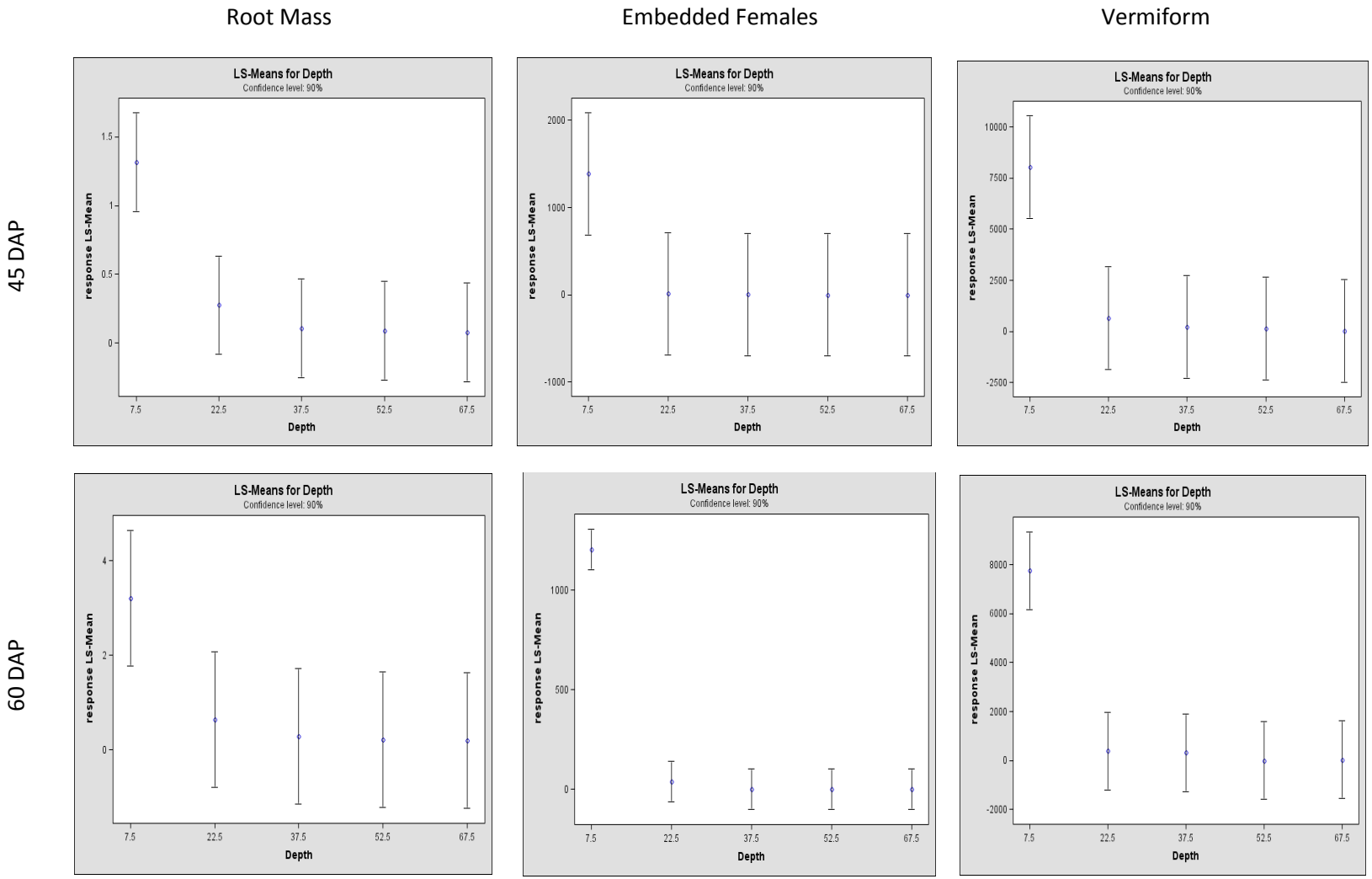

Figure 3.5: Least squares means comparison for root mass (left), *R. reniformis* females embedded in the cotton roots (center) and *R. reniformis* vermiform life stages in the soil (right) by depth at 45 (top) and 60 (bottom) days after planting.

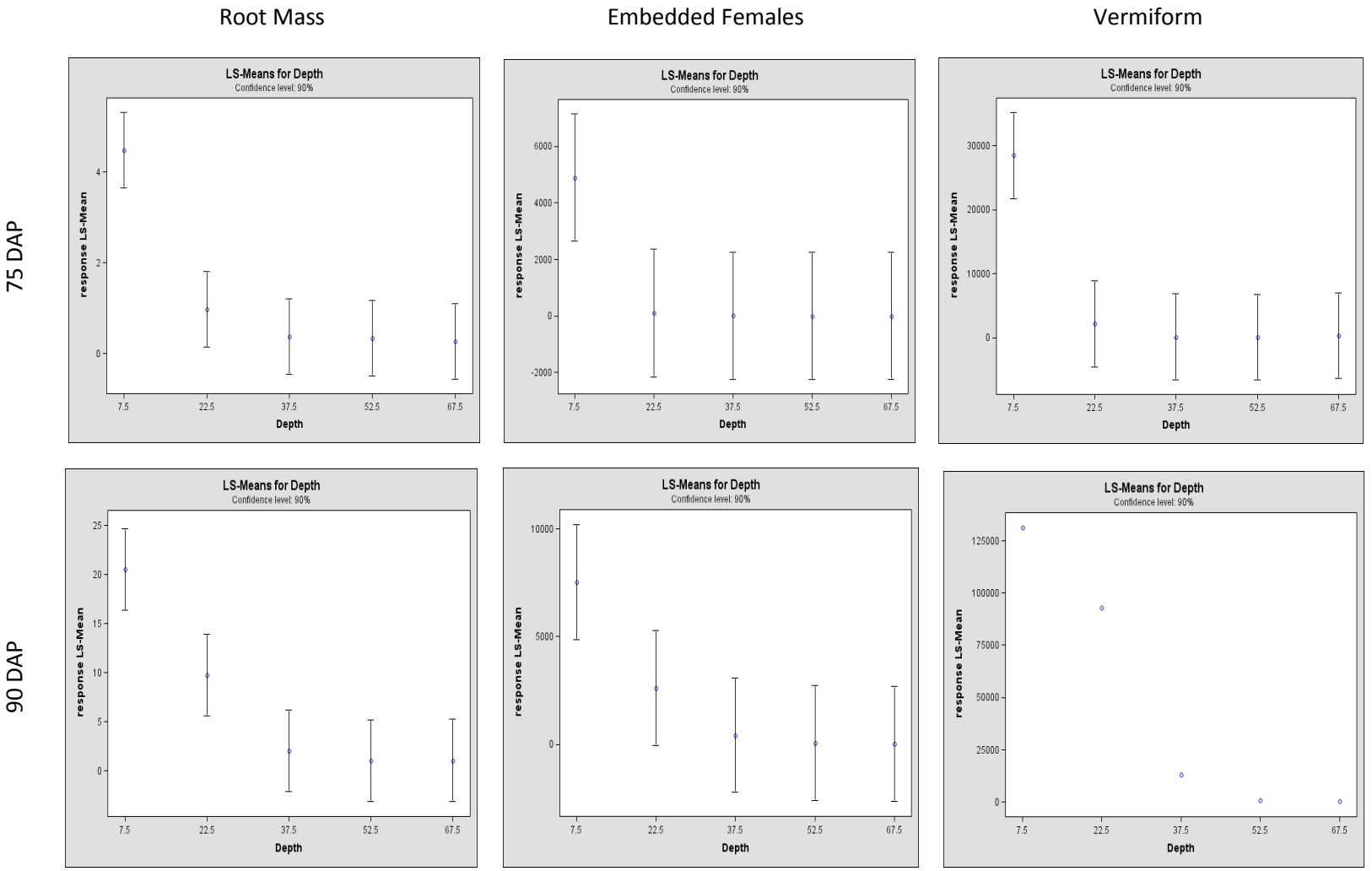

Figure 3.6: Least squares means comparison for root mass (left), *R. reniformis* females embedded in the cotton roots (center) and *R. reniformis* vermiform life stages in the soil (right) by depth at 75 (top) and 90 (bottom) days after planting.

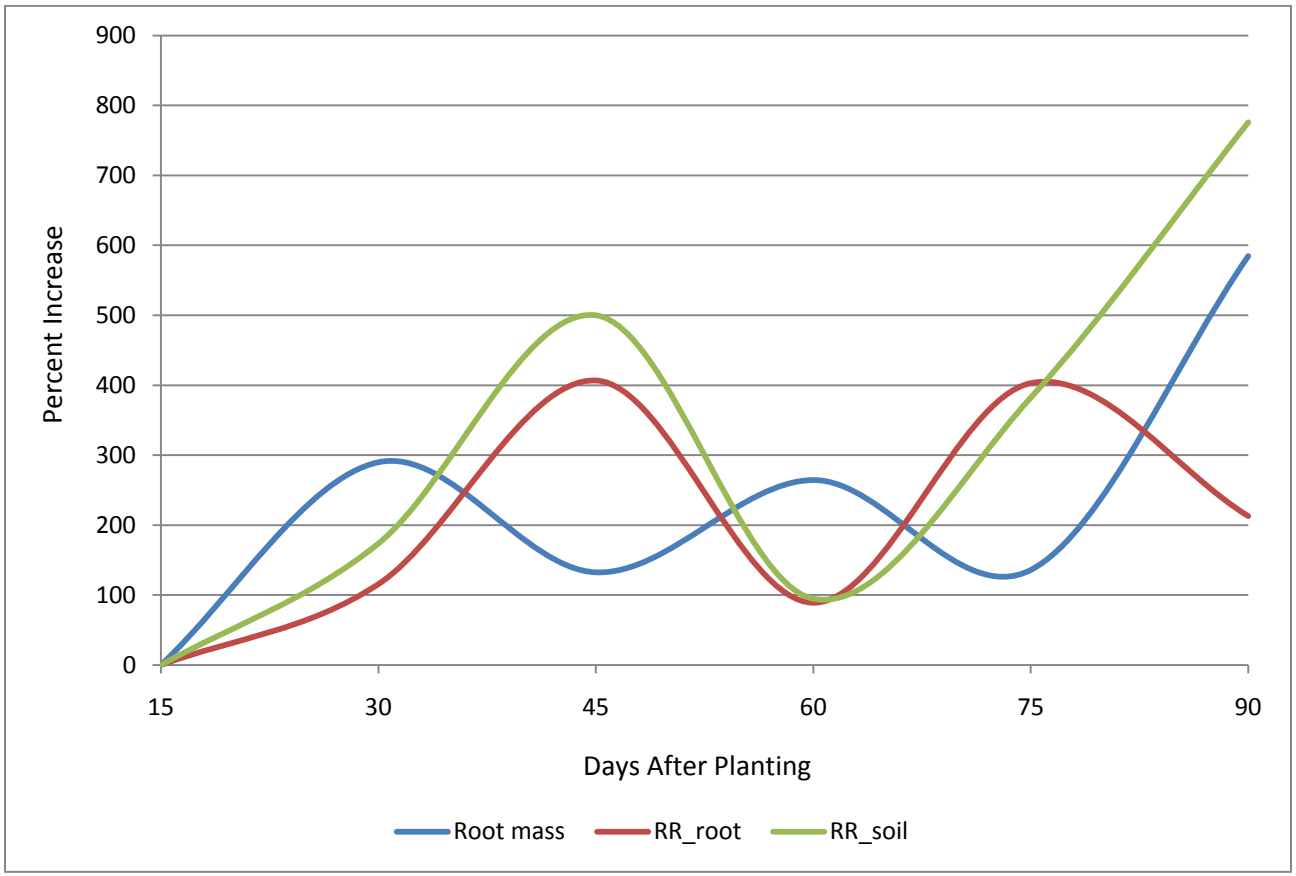

Figure 3.7: Percent increase between sampling dates of the average root mass, *R. reniformis* females embedded in cotton root (RR_root) and *R. reniformis* vermiform life stages in the soil (RR_soil) by sampling date.