ARE PLANT-PARASITIC NEMATODES PRESENT IN THE GUT SYSTEM AND CASTINGS OF EARTHWORMS IN COTTON FIELDS?

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Abstract

In row crops, earthworms are beneficial soil invertebrates because they aerate the soil and some species produce surface castings which can reallocate nutrients in the soil. However, in addition to nutrients, do surface castings transport nematodes? In this study, we investigated if surface castings contained nematodes and, if so, can nematodes survive the journey passing through the earthworms digestive system? Selected fields in Alabama infested with the reniform nematode (*Rotylenchulus reniformis*) were sampled for earthworms and earthworm castings. These fields have high populations of nematodes and populations of earthworms. Earthworms and castings were collected randomly throughout the field. Casting volume ranged from 2.5 to 7.5 cm^3 and was standardized to a sample of 5 cm^3 per sample. All (100%) of the castings from E.V. Smith contained reniform nematodes with populations ranging from 1 to 7 vermiform reniform life stages per 5 cm^3 sample. Free-living nematodes were also found in the castings with a population range of 77 to 772 per sample. At Tennessee Valley Research and Extension Center, again all the castings contained reniform nematodes with populations ranging from 2 to 37 and free-living nematodes ranging from 9 to 236 per 5 cm^3 sample, respectively. The gut system of *Lumbricus spp*. collected from the cotton fields contained 0 to 102 reniform nematodes and 0 to 309 free-living nematodes. Greenhouse trials with *Lumbricus terrestris* earthworms acquired an average of 7 reniform nematodes, 5 free-living, and 124 nematode eggs from soil after 5 days in nematode infested pots. All nematodes in the guts were alive and viable.

Introduction

The reniform nematode, *Rotylenchulus reniformis* Linford & Oliveira, and the root-knot nematode, *Meloidogyne incognita* Kofoid & White, are the most damaging nematode pathogens of cotton in Alabama (Gazaway *et.al*., 2003). Nematodes are aquatic animals that live in the moisture film on soil particles (Stanton and Stirling, 1997). Nematodes are motile, can move on their own, as well as being transported across a vast distances by farm equipment, vehicles and animals that collect the soil as they travel through the area.

Earthworms have been studied in forest ecology and in peat meadows. These studies have found a negative relationship between the earthworms and nematodes. Studies in peat meadows have shown that when *Lumbricus rubellus* consume nematodes they were found alive in the esophagus and pharynx but not in the crop and gizzard. They concluded the earthworms may have digested the nematodes (Ilieva-Makulec and Makulec, 2002). Studies that were performed in forest leaf litter showed a decrease in nematode populations in the presence of earthworms (Raty and Huhta, 2003). When earthworms feed on organic matter and roots they may be unintentionally obtaining plant-parasitic nematodes and their eggs transporting them to different plants in the field. Thus earthworm may have an indirect mutualistic relationship with soil microorganisms (Swift et. al., 1979).

Conservation tillage practices have increased and are used in approximately 38 % of all U.S. crop lands (CTIC, 2012). Worms are becoming more common in no-till fields; this may be due to the abundance of organic material that is in the soil. Possibly conventional tillage may disrupt the worms and reduce their populations. Earth worms are known for tunneling deep in the earth as well as traveling across the surface of the soil. They do this either to find new burrowing areas due to unfavorable conditions, search for organic matter, or to elude predators (Mather and Christensen, 1988) or to avoid soil-applied pesticides (Christensen and Mather, 2004). This means they move horizontally, vertically, and laterally in the soil profile. As earthworm’s tunnel, they ingest soil and organic matter which can be expelled on the soil surface as castings. Earthworm’s cast on the surface to maintain clean living burrows but casting behavior is not universal among all earthworm species. *Lumbricus terresteris*, a common species in Alabama (Reynolds 1994), is a copious producer of surface castings (Scullion and Ramshaw, 1988).

The interaction between nematodes and earthworms are being explored to distinguish their ability of having a mutualistic relationship. Earthworms feed on leaf litter as well as dead and living roots (Lavelle, 1988). Trials were conducted to determine the ability that earthworms (*Lumbricus spp.*) may have to transport reniform nematode in cotton fields.

Materials and Methods

Earthworm castings were collected from two cotton fields in Alabama that have a history of infestation with reniform nematodes. The field at the Tennessee Valley Research and Extension Center (TVREC) near Belle Mina, Alabama is an irrigated, no-till Decatur silt loam soil (28% sand-49% silt-24 % clay). The second field, E.V. Smith Research and Extension Center (EVS) near Shorter, AL, is an irrigated, conventional tillage Wickham fine sandy loam soil (18% sand-16% silt-70% clay). These fields were sampled in a random pattern collecting the castings that were visible on the soil surface. Twenty samples of five earthworm castings were collected from each field. Along with cast, earthworms were also collected and transported back to the lab where they were dissected and examined for plant-parasitic nematodes in the gut system. These worms were collected by digging root systems of cotton plants and collecting the earthworm that were in the roots. Once the earthworms were collected they were placed into a plastic bag with a moist paper towel so they would not desiccate. There was no soil transferred into the bags to ensure there was no outside contamination of nematodes. When back in the lab cast were then collected from these plastic bags (Lab castings). Each casting was dissolved in water and nematodes were extracted from the cast by sucrose centrifugation. Extractions from the castings were examined using the inverted Nikon TSX-100 microscope at 40 X magnifications.

To determine if the nematodes are found internally inside earthworms, (10) earthworms collected from the field were dissected. Earthworms were dissected under a Nikon SMZ 800 light microscope and gut systems removed. Guts were then extracted for nematodes using the sucrose centrifugation method, and examined as species and number of nematodes counted. Field collected earthworms were placed in plastic bags with a moist paper towel without any soil to keep the earthworm from desiccating for 24 hours. During this time, the earthworms produced castings in the bags. These casts were also extracted and nematodes were counted. This extraction will confirm the presence of the nematodes that were passing through the gut system of the earthworm.

Greenhouse trials were conducted to confirm nematodes were being consumed by the earthworms as they travel through the soil. Cotton plants for this test were grown in 15 cm. pots and inoculated with either 10,000 reniform nematodes per 500 cm^3 of soil or no nematodes, replicated 5 times and allowed to grown for 45 days. Four, 25 count containers of *Lumbricus terrestris* were purchased from four different distributors Redman bait co. Phenix City, AL., Buford Bait, Cordele, GA. DMF Bait Co. Waterford, MI., and C&J Bait Purcell, OK) marketed through local retailers around Auburn, AL. The guts of 10 worms from each container (40 worms total per source) were dissected an extracted as previously described. The nematodes present were counted and identified to order for free-living and to genera if they were plant-parasitic. Only earthworms from containers that were free nematodes were introduced to each pot. Twenty earthworms were allowed 5 days to colonize the pots then the pots were destructively sampled and the earthworms were collected. The earthworms were dissected and the gut systems were examined for nematodes. A 150 cm^3 soil sample from each pot was also collected, extracted, and examined to determine the nematode population in each pot. The average population of reniform nematodes in each pot was 1416 nematodes per 150cc of soil.

All the data that were collected were exact numbers. Once the numbers were collected they were all calculated to 1 cm^3 so that they could be easily compared.

Results

The earthworms collected from the TVREC were identified as *Lumbricus spp*. When examining casting for reniform nematodes, the castings from the TVREC averaged 2.68 reniform nematodes per cm^3 of soil. All castings (100%) from this no till location contained reniform nematodes. The population of reniform in this field averaged 12 reniform per cm^3 of soil (Figure 1). At EVS, there was an average of 0.64 reniform nematodes per cm^3 of castings (Figure 3). The soil population of reniform here averaged 2.4 reniform per cm^3 of soil. Again 100 % of the castings contained reniform nematodes.

Free-living nematodes were also observed in the worm castings. The EVS field averages were 62.8 free-living nematodes per cm^3 of castings (Figure 3). On average at TVREC, there were 13.2 free-living nematodes per cm^3 of castings (Figure 2). In the conventional till field at EVS, it was more difficult to distinguish earthworm castings from field soil. The field at TVREC was a conservation no-tillage field and the earthworm cast could be found with little effort.

The TVREC no-tillage field yielded earthworms whereas; none were found in the conventional field at EV S. At the TVREC, 7.12 reniform nematodes per cm^3 of soil were found in the gut system of the worms. Free-living nematodes were also counted and an average of 10.5 was found in the worm gut system. All nematode numbers were calculated to 1 cm^3 of soil in the results as well as the field populations to compare soil populations to those found in castings.

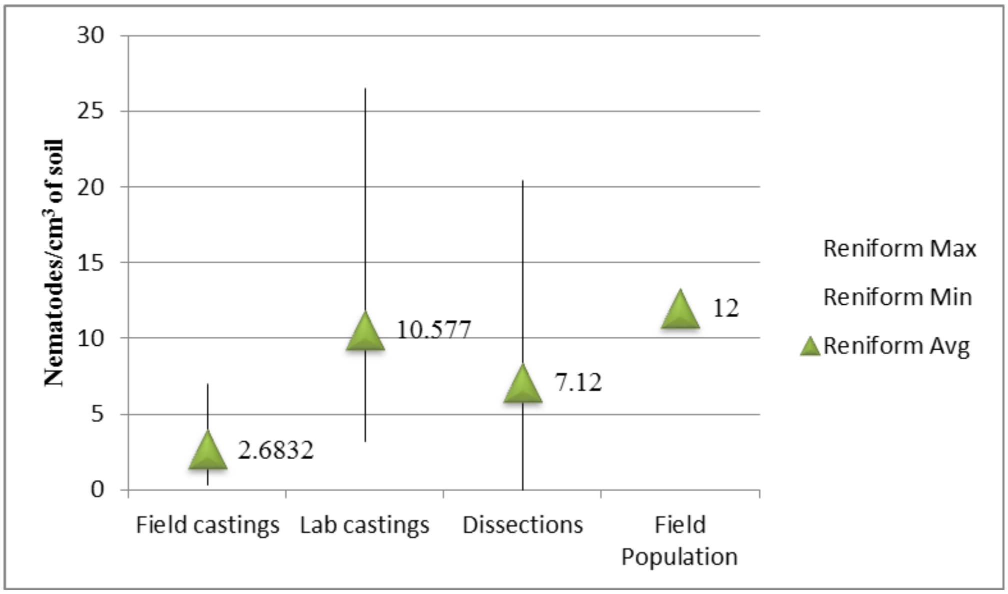

Figure 1. Reniform nematode numbers (ranged from 0-26 with an average of 7) found in a no-till cotton field from worm casting, lab castings, gut systems, and the field soil at Tennessee Valley Research and Education Center (TVREC).

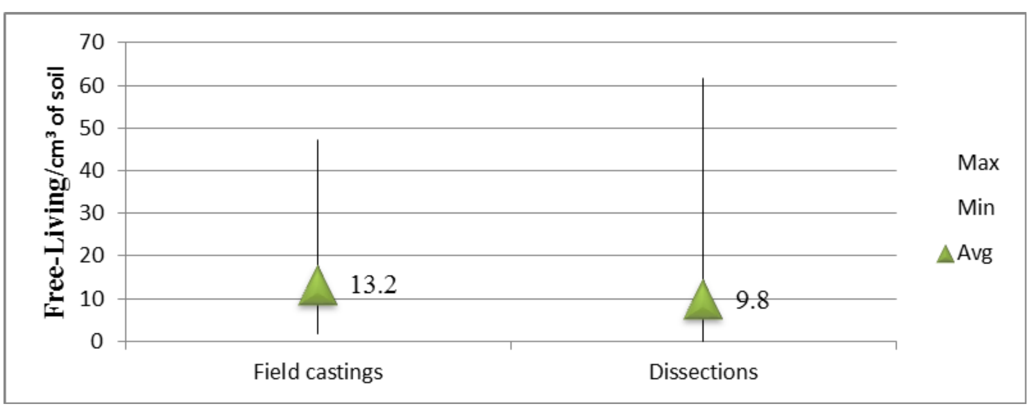

Figure 2. Numbers (ranged from 0 to 62 with an average of 12) of free-living nematode found in field castings and dissected *Lumbricus* spp. collected from a no-till cotton field at the Tennessee Valley Research and Education Center (TVREC).

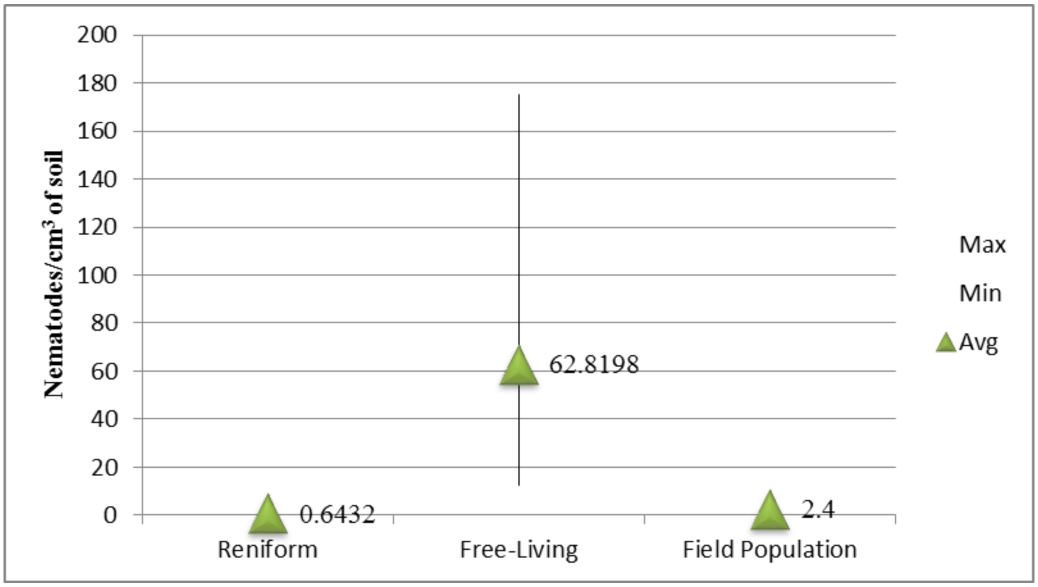

Figure 3. Numbers (ranged from 0.18 to 1.3 with an average of 0.64 for reniform and a range of 12 to 175 with an average of 63 free-living nematodes) of reniform and free-living nematodes found in worm castings from a conventional tilled cotton field at E.V. Smith Research Center (EVS).

While trying to find earthworms for greenhouse experiments, we found nematode in fishing bait container from two distributors. None of the earthworms had plant-parasitic nematodes but we did find live, free-living nematodes in the gut systems. The two distributor brands of worms that did not contain any nematodes were used in the greenhouse study. Earthworms recovered from pots inoculated with nematodes contained an average of 7.15 reniform nematodes and 4.9 free-living nematodes in each cm^3 of earthworms gut. An average of 123 nematode eggs were present in each cm^3 of earthworms gut.

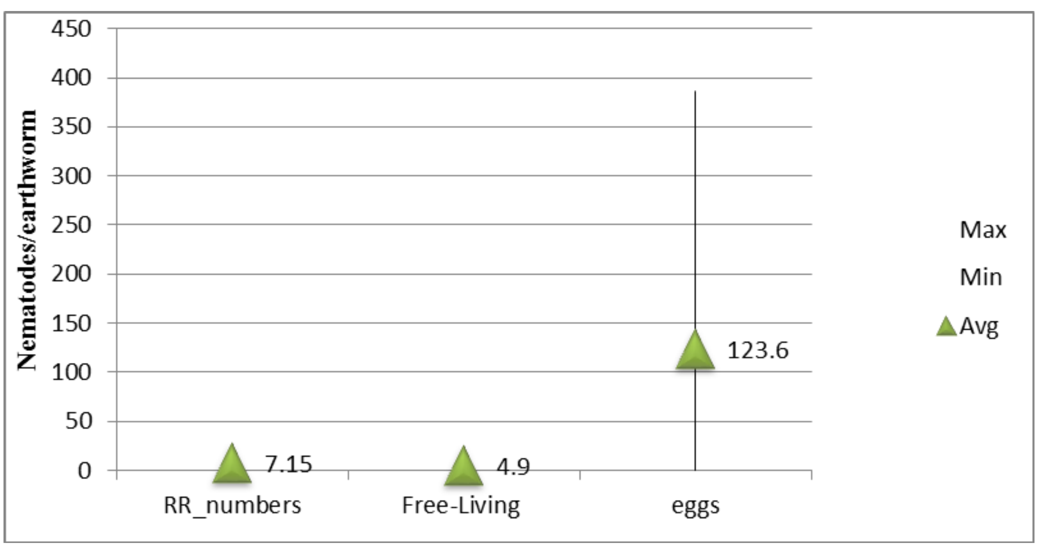

Figure 4. Numbers (ranged from 2 to 17 with an average of 7.15 for reniform and a range of 0 to 16 with an average of 4.9 free-living nematodes, and eggs ranged from 0 to 386 averaging 123.6) of nematodes found in earthworms after tunneling through nematode inoculated soil in the greenhouse.

Summary

Our finding suggests that earthworms obtain plant-parasitic nematodes as they feed on roots, organic material, and travel through the soil. Nematodes were found inside the earthworm as well as in the earthworm castings suggesting a mutualistic relationship where nematodes are transported by the movement of the earthworm. The nematodes found in the gut system and castings were actively moving signifying that passing through the earthworm does not injure the nematodes. Previous studies showed that there were not any nematodes found in the hind gut of *L. terrestris* which deemed that the earthworm had digested the nematodes (Ilieva-Makulec and Makulec, 2002). This is the opposite effect that was found in the study just preformed, nematodes were alive and mobile in the gut systems of *L. terrestris*. In a forest ecology study it was suggested that nematode populations are negatively correlated to the earthworm population indicating the earthworms may be consuming the nematodes and lowering the nematode population (Raty and Huhta, 2003). This study in cotton fields with two soil types found the earthworm populations were transporting and casting out nematodes which were live and active. The number of nematodes that were found in the cast and dissections were lower than the populations that were found in the field. This could be due to the fact that when the earthworms cast, the digested organic matter dries out, as it dries out the nematodes that are found within these castings may migrate back into the soil in search of moisture. As we dissected the gut systems of these nematodes they may have already casted therefore, there would not have as many nematodes in their gut systems as populations found in the field. But, when we examined the earthworm castings in the lab we found that those populations of nematodes were similar to those of the infested fields. This research could help explain how nematodes tend to migrate through fields when they are found in one area of the field and over a few years will colonize the entire field.

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