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Utilizing management zones for *Rotylenchulus reniformis* in cotton: Effects on nematode levels, crop damage, and *Pasteuria* sp.

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ABSTRACT

Nematode management zones (MZs) based on soil electrical conductivity (EC, a proxy for soil texture) have not been published for *Rotylenchulus reniformis*. We tested 1) whether *R. reniformis* levels and the amount of damage caused to cotton differed among MZs, 2) if the relative effectiveness of nematicides differed among MZs, and 3) whether the prevalence of *Pasteuria* sp. on *R. reniformis* differed among MZs and nematicide treatments. A field was divided into three MZs where MZ3 had sandier soil than MZ1 or MZ2, which were the same, and MZ2 had higher elevation than MZ1 or MZ3, which were the same. Levels of *R. reniformis* near planting in plots not receiving nematicide averaged 1342 (per 150 cm^3 soil) in 2008, 610 in 2009, and 869 in 2010. Both soil texture and elevation influenced *R. reniformis* population levels with greater reproduction in finer-textured soil and reduced *R. reniformis* levels at higher elevation. Treatment effects on *R. reniformis* levels were the same in all MZs (no MZ × treatment interactions). The effects of texture and elevation on yield were similar to the effects on nematode levels. We observed endospores of *Pasteuria* sp., a bacterial parasite of nematodes, on *R. reniformis* at the field site used for this study. *Pasteuria* sp. generally had greater spore attachment to juvenile *R. reniformis* than to adults with no differences among MZs in percentage of nematodes with endospores, but the number of spores per nematode was lower in MZ3, which had the greatest sand content. The percentage of *R. reniformis* with endospores and the number of attached endospores were reduced by 1,3-dichloropropene + aldicarb. We documented that *R. reniformis* levels are affected by modest differences in soil texture and elevation, but levels of *R. reniformis* were above the action threshold in all MZs, therefore a uniform rate of nematicide would have been recommended and there would have been no cost savings from utilizing MZs in this field.

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1. Introduction

Management of plant-parasitic nematodes in cotton in the US has been primarily through application of nematicides (Herring et al., 2010; Koenning et al., 2004; Wheeler et al., 1999). Nematicide application is a significant input cost, so it is recommended only when nematode levels are above specified action thresholds, which may vary among states. Despite the cost, nematicides are typically applied to entire fields even though nematode levels are rarely uniform throughout the field and there may be areas where there will be no economic benefit (Evans et al., 2002). An approach known as site-specific management is an improvement where a field is subdivided into portions, and nematicides are applied only where the mean nematode levels within a subdivision are above the action threshold (Monfort et al., 2007; Mueller et al., 2010; Ortiz et al., 2012; Starr et al., 2007). The limiting factor for site-specific management has been identifying areas of the field with damaging levels of nematodes in a cost-effective manner.

The population levels of soil-borne, plant-parasitic nematodes are often affected by soil texture (Koenning et al., 1996; Monfort et al., 2007; Wyse-Pester et al., 2002). Population levels of *Meloidogyne incognita* and *Hoplolaimus columbus*, both significant pathogens of cotton, are typically greater in sandier (coarsely textured)

Abbreviations: EC, electrical conductivity; MZ, management zone.

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soils than in soils with more silt or clay (finely textured) (Khalilian et al., 2001; Koenning et al., 1996; Monfort et al., 2007; Ortiz et al., 2012), and this has been the primary basis upon which management zones (MZs) for site-specific nematode management in cotton have been delineated. The reniform nematode, *Rotylenchulus reniformis*, also is a very damaging pathogen of cotton and is affected by soil texture (Herring et al., 2010; Koenning et al., 1996; Robinson et al., 1987). In contrast to *M. incognita*, *R. reniformis* population levels are typically greatest when the silt plus clay fraction is approximately 28% and the levels decline as the texture becomes either coarser or finer (Koenning et al., 1996). Management zones based on soil texture are created such that the variation in soil texture and other edaphic factors within a zone is minimized and the variation among zones is maximized (Mueller et al., 2010; Ortiz et al., 2012). Each zone can then be sampled independently and unique management decisions can be made for each zone.

Crop growth and yield are affected by soil texture in part because soil texture affects the water and nutrient holding capacity of the soil (Monfort et al., 2007; Mueller et al., 2010). Consequently, soil texture can affect root growth, so the relationship between nematode population levels and crop production could be altered (Monfort et al., 2007). Soil texture may also indirectly influence nematicide efficacy due to differences in water-holding capacity. Plant growth, crop yield, nematode population levels, and nematicide efficacy could all potentially be influenced by soil texture and differ among nematode MZs.

We observed endospores of *Pasteuria* sp. on vermiform stages of *R. reniformis* at the field site used for this study. *Pasteuria* spp. are endospore-forming bacteria that are parasites of nematodes. These bacteria are obligate in nature, only reproducing within the body of their hosts, and often show a high degree of host specificity (Chen and Dickson, 1998). Endospores of *Pasteuria* spp. attach to the cuticle of host nematodes as they migrate through soil. The bacterium forms a germ tube through the cuticle, and begins to grow vegetatively within the pseudocoelom, eventually forming mature endospores that are released into the soil upon rupture of the nematode cuticle (Sayre and Wergin, 1977). A species of *Pasteuria* from *R. reniformis* has been recently characterized and described (Schmidt et al., 2010). This species of *Pasteuria* can complete its lifecycle within the vermiform juveniles, females, and males of *R. reniformis*; whether it can also complete its lifecycle in the mature sedentary female is not known. In greenhouse experiments, the reniform-parasitic species of *Pasteuria* suppressed numbers of *R. reniformis* on cotton when applied to the seed or soil surface (Schmidt et al., 2010).

The concept of using nematode MZs in cotton based on soil texture has been applied primarily to *M. incognita* and *H. columbus* (Monfort et al., 2007; Mueller et al., 2010; Ortiz et al., 2012), but the concept should apply just as well for *R. reniformis*. Our goal was to evaluate the usefulness of nematode MZs based on soil texture and other edaphic factors in managing *R. reniformis* in cotton. Our specific objectives were to determine: 1) whether *R. reniformis* levels and the amount of damage caused by the nematode to cotton differed among MZs, 2) if the relative effectiveness of nematicides differed among MZs, and 3) whether the prevalence of *Pasteuria* sp. on *R. reniformis* differed among MZs and nematicide treatments.

2. Materials and methods

2.1. Field experiment

A study was conducted from 2008 to 2010 in a 16-ha section of an irrigated field near Cochran, GA (Bleckley County). The field had been planted to cotton every year and had been infested with *R. reniformis* for more than 20 years. Soil survey maps of the county indicated that soil types in the field were Dothan loamy sand and Nankin loamy sand, both with 2–5% slope.

A Veris 3100 soil electrical conductivity (EC) meter from Veris Technologies (Lund et al., 1999) was used to map soil texture in the field in December 2007. The Veris unit was linked to a GPS system and mapped EC measurements, which were used as a proxy for soil texture, at two depths (0–30 cm deep = EC_s; 0–90 cm = EC_d) (Mueller et al., 2010). Elevation and EC data were collected at the same time using a real-time kinematic GPS, and slopes of the terrain were calculated from changes in elevation (Ortiz et al., 2011). Management Zone Analyst (MZA) software (Fridgen et al., 2004) was used to identify three distinct regions of the field based on EC_s, EC_d, elevation, and slope that maximized uniformity of soil edaphic factors within each region. The Mahalanobis Distance Method was selected along with a fuzziness threshold of 1.3, as suggested by Fridgen et al. (2004) for soil surfaces. The clustering software, MZA, generates two performance indices as a metric of the organization gained with each additional cluster. Performance indices indicated that the study site was best organized into three zones. The three regions, or MZs, were labeled MZ1, MZ2, and MZ3.

Five soil cores (7.5 cm diam. and 90 cm deep) were collected from each MZ in January 2010. Cores were divided into four segments (0–15 cm, 15–30 cm, 30–60 cm, and 60–90 cm) and soil textural analysis was run on each segment to determine the percentages of sand, silt, and clay. Percentages for the 0–15 cm and 15–30 cm sections for each core were averaged to correspond to the 0–30 cm depth used in measuring EC_s, and percentages for all sections for each core were averaged to correspond to the 0–90 cm depth used in measuring EC_d. Multiple regression analysis was conducted to relate the soil Ec_s or EC_d readings collected in 2007 to the actual soil texture values (percentages of sand, silt, and clay) determined in 2010.

Five replications of five nematicide treatments were randomly assigned to plots within each of the three MZs for a total of 75 plots. For simplicity of application, the nematicide treatments were applied to randomized and replicated eight-row-wide strips through the field, but data were collected only from the small plots within each strip. Each plot was eight-rows wide and 15-m long, and data were collected only from the middle four rows. The treatments were 1) no nematicide (non-treated control), 2) aldicarb (Temik 15G) at 0.6 kg a.i./ha, 3) aldicarb at 1.0 kg a.i./ha, 4) aldicarb at 0.6 kg a.i./ha plus 1,3-dichloropropene (1,3-D; Telone II [97.5% 1,3-D by weight]) at 28 l/ha, and 5) aldicarb at 1.0 kg a.i./ha plus 1,3-D at 56 l/ha. Aldicarb was applied in-furrow at planting, and 1,3-D was applied prior to planting. All seed was treated with thiamethoxam (Cruiser) insecticidal seed treatment for thrips control. Plants from approximately 3 m of row were removed by hand at the ends of each plot three weeks after planting to create distinct plot boundaries.

The nematicide treatment plots were arranged in a completely randomized design within each MZ. Twenty five plots within each zone were initially established in 2008, and the precise location of each plot was recorded using a GPS unit with accuracy to within a few centimeters. Plot locations were selected to minimize variability in edaphic factors among plots within a MZ and the same small-plot locations were used in all years of the study. Treatments were re-randomized within each MZ for each year of the study, except that plots that had received the high rate of 1,3-D were kept in the same place because of a high potential for a carry-over effect. The high rate of 1,3-D is twice the rate typically recommended to growers and was intended as a positive control treatment to minimize damage from nematodes rather than to evaluate the effectiveness of that treatment.

Prior to planting, all plots received strip-tillage, which consisted of a single sub-soil chisel per row with shallow (approximately