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

R.F. Davis[a,*], S.K. Aryal[b], C.D. Perry[c], D.G. Sullivan[d], P. Timper[a], B.V. Ortiz[e], K.L. Stevenson[b], G. Vellidis[c], G. Hawkins[c]

[a] Crop Protection and Management Research Unit, USDA-ARS, P.O. Box 748, Tifton, GA 31793, USA
[b] Dept. of Plant Pathology, University of Georgia, 2360 Rainwater Road, Tifton, GA 31793-5766, USA
[c] Crop and Soil Sciences Dept., University of Georgia, 2360 Rainwater Road, Tifton, GA 31793-5766, USA
[d] TurfScout, Inc., 1015 Carolina St, Greensboro, NC 27401, USA
[e] Dept. of Agronomy and Soils, 204 Extension Hall, Auburn University, Auburn, AL 36849, USA

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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 $\times$ 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.

* Corresponding author. Tel.: +1 229 387 2341.
E-mail address: Richard.Davis@ars.usda.gov (R.F. Davis).

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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

10 cm) disking and rolling that left a smooth seed bed 20-cm wide. Applications of 1,3-D were made behind the sub-soil shank during tillage on 24 April 2008, 2 June 2009, and 13 May 2010. Actual placement of 1,3-D was measured at 41 cm below the soil surface. Delta and Pine Land cotton cv. DP 555 BR was grown in all years of the study. Aldicarb was applied at planting on 12 May 2008, 9 June 2009, and 20 May 2010. Herbicides, irrigation, and all other management inputs were applied equally to all plots as needed following recommendations by the Georgia Cooperative Extension Service.

Soil samples to assess nematode population levels were collected on 9 May, 7 July, 4 September, and 21 October 2008; on 25 June, 26 August, and 18 November 2009; and on 1 June, 22 July, and 19 October 2010. Samples consisted of approximately 20 soil cores collected from the four middle rows of the 15-m-long plots. Nematodes were extracted and counted from 150 cm^3 of soil from each plot (Jenkins, 1964). Cotton was machine harvested on 5 November 2008, 1 December 2009, and 2 November 2010. Cotton stalks were mowed within three weeks following harvest, and a winter cover crop of rye was grown to minimize soil erosion. Rye was killed with herbicides 2–3 weeks prior to planting in the spring.

2.2. *Pasteuria* sp. quantification

The prevalence of *Pasteuria* endospores on *R. reniformis* was determined from the control, the high rate of aldicarb, and the high rate of 1,3-D + aldicarb plots. On three sampling dates (18 November 2009, 1 June 2010, and 19 October 2010), 25–35 vermiform stages of *R. reniformis* per plot, selected at random, were examined at 400× for *Pasteuria* endospores using an inverted microscope. The stage of each nematode examined was recorded as juvenile, immature female (presence of vulva), or male (presence of spicule). If the adults still had the retained juvenile cuticle, then the individual was recorded as a juvenile because the adult cuticle was shielded from endospore attachment. Both the average endospores per stage and the proportion of each stage with at least one endospore on the cuticle were determined for each plot.

2.3. Data analyses

Nematode population density, yield data, and prevalence of *Pasteuria* sp. on *R. reniformis* were analyzed by analysis of variance; MZ were treated as separate locations and were analyzed both independently and also pooled for a 2-way factorial ANOVA with 3 MZ and 5 nematicide treatments. Means were separated by Fisher's protected LSD ($\alpha = 0.05$). Regression analysis was used to determine the relationship between the proportion of reniform nematodes (juveniles and females) with endospores and the mean number of reniform nematodes in the sample.

3. Results

3.1. Field experiment

Mean EC_s, EC_d, and elevation values for plots within each MZ differed among zones (Table 1). MZ1 and MZ2 had similar mean values for both EC_s and EC_d, and both zones had greater values than MZ3, thereby indicating that MZ3 had coarser, sandier soil than MZ1 or MZ2. Plots in MZ2 had higher elevation than plots in MZ1 or MZ3, but plots in MZ1 and MZ3 had similar elevations. After the treatments had been randomized within each MZ, analysis of variance indicated that mean EC_s, EC_d, and elevation values did not differ among treatments within a MZ in 2008, 2009, or 2010 ($P > 0.10$; data not presented).

Table 1
Soil electrical conductivity (EC) readings from small plots within in three management zones based on distinct combinations of soil texture, elevation, and slope.

Zone[a]	EC_s (0–30 cm deep)[b]	EC_d (0–90 cm deep)[b]	Elevation (m)[c]
MZ1	6.94 a[d]	10.74 a	110.4 b
MZ2	7.24 a	10.39 a	114.6 a
MZ3	4.10 b	5.67 b	110.0 b

[a] Zones created to minimize variability of EC_s, EC_d, elevation, and slope within an entire zone and to maximize the differences among the zones.
[b] EC_s and EC_d were used as proxies for soil texture. Values reported are means for the 25 small plots within each zone.
[c] Elevation is m above sea level.
[d] Means within a column followed by the same letter are not significantly different according to Fisher's Protected LSD ($P > 0.05$).

Regression analysis to relate actual soil texture to EC_s and EC_d was based on five deep soil cores randomly collected in each MZ and the EC readings from those five locations rather from each plot. Mean values from MZ1 were $EC_s = 7.71$, % $sand_s = 74.7$, % $silt_s = 9.9$, % $clay_s = 15.4$, $EC_d = 12.55$, % $sand_d = 68.0$, % $silt_d = 8.7$, and % $clay_d = 23.2$. Mean values from MZ2 were $EC_s = 7.61$, % $sand_s = 80.1$, % $silt_s = 7.8$, % $clay_s = 12.2$, $EC_d = 12.85$, % $sand_d = 69.9$, % $silt_d = 7.6$, and % $clay_d = 22.5$. Mean values from MZ3 were $EC_s = 4.35$, % $sand_s = 81.8$, % $silt_s = 10.9$, % $clay_s = 7.2$, $EC_d = 5.38$, % $sand_d = 73.2$, % $silt_d = 11.2$, and % $clay_d = 15.5$. The regression equation relating EC_s to the percentage sand, silt, and clay was not significant ($P > 0.10$). However, EC_d was related to the percentage sand, silt, and clay by the equation $EC_d = 4105.75 - (40.95 \times \%\ sand) - (40.62 \times \%\ silt) - (41.77 \times \%\ clay)$ ($P = 0.014$; $R^2 = 0.61$).

In 2008, mean nematode population density (averaged across all treatments within a MZ) differed among the MZs on all sampling dates (Table 2). MZ2 had lower mean population density than MZ1 or MZ3 early in the season and lower population density than MZ1 throughout the season. On the final sampling date, MZ1 had higher mean population density than the other zones. Nematode population density differed among the treatments in all MZ on all sampling dates in 2008 except in MZ2 on 9 July and in MZ3 on the final sampling date (Table 2). The effect of the nematicide treatments on nematode population density was consistent among the three MZs (no MZ × treatment interaction) on all sampling dates. Mean cotton yield averaged across all treatments did not differ among the MZs in 2008 (Table 2). The nematicide treatments had similar effects in all MZs (no MZ × treatment interaction), but statistical analysis indicated differences in yield among nematicide treatments only in MZ1.

Mean nematode population density in 2009 differed among the MZs on 25 June and 26 August when MZ1 had the highest population density, but levels were similar in all MZs on 18 November, the final sampling date (Table 3). Nematode population density differed among the nematicide treatments in all MZs on all sampling dates in 2009 (Table 3). The effect of the nematicide treatments on nematode population density was consistent among the three MZs (no MZ × treatment interaction) on all sampling dates. Mean cotton yield averaged across all treatments differed among the MZs in 2009 with yields being greater in MZ2 than in MZ1 or MZ3 (Table 3). The nematicide treatments had similar effects on yield in all MZs (no MZ × treatment interaction), but statistical analysis indicated differences in yield only in MZ1 and MZ2.

In 2010, mean nematode population density differed among the MZs on all sampling dates with MZ2 having lower population density than MZ3 in mid to late season and lower population density than MZ1 on all sampling dates (Table 4). On the final sampling date, MZ1 had higher population density than the other zones. Nematode population density differed among the treatments in all MZs on all sampling dates in 2010 (Table 4). The effect

Table 2
Rotylenchulus reniformis population density and cotton yield in 2008 in three management zones based on distinct combinations of soil texture, elevation, and slope.

Zone[a]	Treatment	9 May	9 July	4 Sept	21 Oct	Yield (kg/ha)[b]
MZ1	Non-treated	1844 a[c]	986 a	3532 a	1918 a	904 b
	Aldicarb (0.6 kg a.i./ha)	1208 ab	296 b	1814 bc	1480 ab	991 ab
	Aldicarb (1.0 kg a.i./ha)	1446 a	440 b	2390 ab	1650 a	917 b
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	476 bc	264 b	922 c	1522 ab	1125 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	204 c	176 b	802 c	794 b	1075 ab
	MZ1 mean	**1036 A**	**432 A**	**1892 A**	**1473 A**	**1003 A**
MZ2	Non-treated	542 b	258 a	1206 ab	992 ab	994 a
	Aldicarb (0.6 kg a.i./ha)	938 a	172 a	1388 ab	1054 ab	854 a
	Aldicarb (1.0 kg a.i./ha)	620 ab	184 a	1846 a	1492 a	894 a
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	138 c	222 a	750 b	536 b	971 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	166 c	76 a	570 b	948 ab	1019 a
	MZ2 mean	**481 B**	**182 B**	**1152 B**	**1004 B**	**946 A**
MZ3	Non-treated	1640 ab	466 ab	1228 ab	1314 a	931 a
	Aldicarb (0.6 kg a.i./ha)	1854 a	584 a	1308 ab	1262 a	941 a
	Aldicarb (1.0 kg a.i./ha)	992 bc	414 ab	1420 a	1046 a	884 a
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	334 c	244 ab	622 b	710 a	1053 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	204 c	133 b	1062 ab	1042 a	1036 a
	MZ3 mean	**1047 A**	**378 A**	**1128 B**	**1075 B**	**969 A**
Pooled[d]	Non-treated	1342 a	570 a	1989 a	1408 a	943 ab
	Aldicarb (0.6 kg a.i./ha)	1333 a	351 b	1503 a	1265 ab	928 b
	Aldicarb (1.0 kg a.i./ha)	1019 a	346 b	1885 a	1396 a	898 b
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	316 b	243 bc	765 b	923 b	1050 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	205 b	128 c	811 b	928 b	1044 a

[a] Zones created to minimize variability of EC_s, EC_d, elevation, and slope within an entire zone and to maximize the differences among the zones.
[b] Lint yield estimated as 40% of seed cotton yield.
[c] Nematodes/150 cm^3 soil. Within a column, treatment means within a zone (lower case letters) or zone means (capital letters) followed by the same letter are not significantly different according to Fisher's Protected LSD ($P > 0.05$).
[d] Data pooled and analyzed as a 3 MZ × 5 treatment ANOVA (no MZ × treatment interaction).

of the nematicide treatments on nematode population density was consistent among the three MZs (no MZ × treatment interaction) on all sampling dates. Mean cotton yield averaged across all treatments did not differ among the MZs in 2010 (Table 4). There were differences in yield among treatments in all MZs, but a significant MZ × treatment interaction ($P = 0.03$) indicated that the nematicide treatments did not have the same effect in each MZ. This interaction was caused by high yields in plots receiving the low rate of 1,3-D plus aldicarb in MZ2, and the interaction was not significant if yields for those plots were excluded from the analysis.

3.2. *Pasteuria* sp. quantification

Fewer attached endospores of *Pasteuria* sp. were observed on males than on females and juveniles of *R. reniformis* on all sampling dates (Fig. 1). Males averaged less than 1 spore per individual, whereas juveniles averaged 2–4 spores per individual. Juveniles also had greater endospore attachment than females in November 2009 and October 2010, but not in June 2010. We observed only a few juveniles and females, and no males containing mature endospores.

Table 3
Reniform nematode population density and cotton yield in 2009 in three management zones based on distinct combinations of soil texture, elevation, and slope.

Zone[a]	Treatment	25 June	26 Aug	18 Nov	Yield (kg/ha)[b]
MZ1	Non-treated	814 ab[c]	2312 ab	3484 a	1252 ab
	Aldicarb (0.6 kg a.i./ha)	1034 a	3348 a	2806 ab	971 c
	Aldicarb (1.0 kg a.i./ha)	448 bc	1820 abc	1695 abc	1402 a
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	112 c	478 bc	906 bc	1191 b
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	223 c	160 c	420 c	1150 b
	MZ1 mean	**543 A**	**1679 A**	**1932 A**	**1186 B**
MZ2	Non-treated	634 a	2098 a	2914 a	1411 b
	Aldicarb (0.6 kg a.i./ha)	248 b	600 ab	1800 ab	1414 b
	Aldicarb (1.0 kg a.i./ha)	324 ab	788 ab	2758 a	1673 a
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	42 b	72 b	670 b	1304 b
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	130 b	966 ab	710 b	1635 a
	MZ2 mean	**276 B**	**905 B**	**1770 A**	**1488 A**
MZ3	Non-treated	382 ab	962 a	1432 b	1185 a
	Aldicarb (0.6 kg a.i./ha)	620 a	1212 a	4824 a	1089 a
	Aldicarb (1.0 kg a.i./ha)	814 a	1298 a	3146 ab	1284 a
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	58 b	344 b	1608 b	1118 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	24 b	128 b	1018 b	1305 a
	MZ3 mean	**380 AB**	**789 B**	**2406 A**	**1196 B**
Pooled[d]	Non-treated	610 a	1791 a	2610 a	1283 bc
	Aldicarb (0.6 kg a.i./ha)	634 a	1720 a	3143 a	1158 d
	Aldicarb (1.0 kg a.i./ha)	534 a	1265 a	2593 a	1457 a
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	71 b	298 b	1061 b	1204 cd
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	119 b	436 b	737 b	1379 ab

[a] Zones created to minimize variability of EC_s, EC_d, elevation, and slope within an entire zone and to maximize the differences among the zones.
[b] Lint yield estimated as 40% of seed cotton yield.
[c] Nematodes/150 cm^3 soil. Within a column, treatment means within a zone (lower case letters) or zone means (capital letters) followed by the same letter are not significantly different according to Fisher's Protected LSD ($P > 0.05$).
[d] Data pooled and analyzed as a 3 MZ × 5 treatment ANOVA (no MZ × treatment interaction).

Table 4
Reniform nematode population density and cotton yield in 2010 in three management zones based on distinct combinations of soil texture, elevation, and slope.

Zone[a]	Treatment	1 June	22 July	19 Oct	Yield (kg/ha)[b]
MZ1	Non-treated	1522 a[c]	4180 a	9244 a	1280 a
	Aldicarb (0.6 kg a.i./ha)	478 b	3568 ab	5804 ab	1059 b
	Aldicarb (1.0 kg a.i./ha)	620 b	3934 ab	6646 a	1396 a
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	64 b	2018 bc	7482 a	1309 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	21 b	744 c	1578 b	1275 a
	MZ1 mean	**541 A**	**2889 A**	**6152 A**	**1264 A**
MZ2	Non-treated	248 ab	3266 a	2188 ab	1121 b
	Aldicarb (0.6 kg a.i./ha)	245 ab	3098 a	3772 a	1309 b
	Aldicarb (1.0 kg a.i./ha)	412 a	1642 b	3002 a	1191 b
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	14 b	319 c	1084 b	1600 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i//ha)	17 b	133 c	884 b	1307 b
	MZ2 mean	**187 B**	**1692 B**	**2186 C**	**1306 A**
MZ3	Non-treated	836 a	3802 a	4998 a	1269 ab
	Aldicarb (0.6 kg a.i./ha)	532 ab	3056 ab	3830 abc	1234 b
	Aldicarb (1.0 kg a.i./ha)	392 bc	3060 ab	4194 ab	1293 ab
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	46 c	1918 bc	3190 bc	1381 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	59 c	1008 c	2256 c	1395 a
	MZ3 mean	**373 AB**	**2569 A**	**3694 B**	**1264 A**
Pooled[d]	Non-treated	869 a	3749 a	5477 a	1224 bc
	Aldicarb (0.6 kg a.i./ha)	418 b	3241 a	4469 a	1200 c
	Aldicarb (1.0 kg a.i./ha)	475 b	2879 a	4614 a	1293 bc
	1,3-D (28 l/ha) + Aldicarb (0.6 kg a.i./ha)	41 c	1418 b	3919 a	1430 a
	1,3-D (56 l/ha) + Aldicarb (1.0 kg a.i./ha)	32 c	628 b	1573 b	1326 ab

[a] Zones created to minimize variability of EC_s, EC_d, elevation, and slope within an entire zone and to maximize the differences among the zones.
[b] Lint yield estimated as 40% of seed cotton yield.
[c] Nematodes/150 cm^3 soil. Within a column, treatment means within a zone (lower case letters) or zone means (capital letters) followed by the same letter are not significantly different according to Fisher's Protected LSD ($P > 0.05$).
[d] Data pooled and analyzed as a 3 MZ × 5 treatment ANOVA (no MZ × treatment interaction).

For analysis of endospore prevalence in the different zones and nematicide treatments, only the average from juveniles and females was used. The percentage of *R. reniformis* with attached endospores differed among sample dates, with the greatest percent in June 2010 and the least in October 2010 (Table 5). The number of spores per nematode was also lower in November 2010 than on the other sample dates. The average number of endospores per nematode differed among MZs, but the percentage of nematodes with endospores was similar among MZs. Consistent among all sample dates, MZ3 had the lowest spores per nematode compared to the other zones (Table 5). Application of the high rate of 1,3-D + aldicarb reduced the percentage of nematodes with spores as well as the spores per nematode compared to the control and application of aldicarb only. There was no MZ × nematicide interaction; however, there was a sample date × nematicide interaction for both the percentage with endospores ($P = 0.003$) and the number of endospores per nematode ($P = 0.01$). The number of spores per nematode was numerically lower in the 1,3-D treatment on all sample dates, but the magnitude of the reduction differed among sample dates. For the percentage of nematodes with spores, the effect of nematicide was inconsistent among sample dates.

There was a negative linear relationship ($P < 0.0001$; $r = -0.60$) between the number of *R. reniformis* in soil and the log-transformed proportion with endospores (Fig. 2). The 1,3-D + aldicarb plots were not included in this analysis because the low nematode numbers in these plots may have masked the suppressive effect of *Pasteuria* sp. The uniformly low numbers of *R. reniformis* on the June 2010 sample date may have unduly influenced the relationship. The low numbers of nematodes at this time may have been due to seasonal population changes or to the high prevalence of *Pasteuria* sp. Nevertheless, when the data were analyzed without the June sample date, there was still an inverse relationship ($P = 0.003$; $r = -0.16$) between nematode numbers and prevalence of *Pasteuria* sp., though the relationship was not as strong.

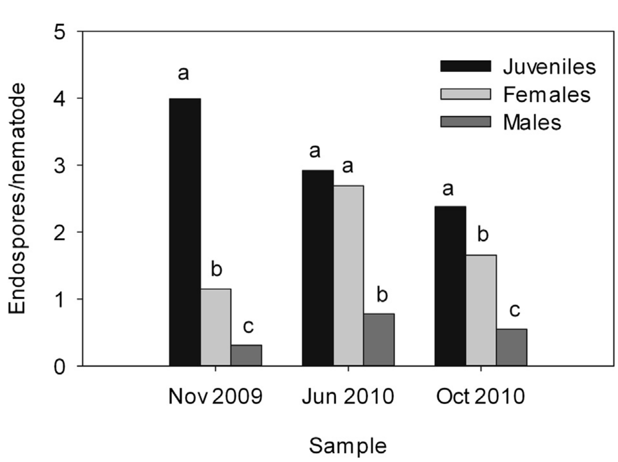

Fig. 1. Endospores per nematode. Average number of *Pasteuria* sp. endospores per stage of *Rotylenchulus reniformis* over three sample dates. Letters above bars indicate significant differences within a sample date.

4. Discussion and conclusions

Differences in soil texture among the MZs, as indicated by differences in soil EC readings, appeared to influence *R. reniformis* population levels in this study. The primary difference between MZ1 and MZ3 was lower mean soil EC readings in MZ3, meaning that MZ3 had coarser, sandier soil. Mid- to late-season nematode levels were often greater in MZ1 than in MZ3, but early-season population densities were not statistically different in any year, and population density in MZ3 was never statistically greater than the density in MZ1. This suggests greater nematode reproduction in the finer-textured soil of MZ1 than in MZ3. This is consistent with previous findings (Koenning et al., 1996). Even though the differences in EC readings, and therefore texture, were relatively small in this study they were large enough to affect population levels of *R. reniformis*.

The effect of soil texture was anticipated, but elevation appears to have also had a measurable effect on *R. reniformis* levels.

Table 5
Effect of sampling date, management zone and nematicide treatment on the percentage of *Rotylenchulus reniformis* with endospores of *Pasteuria* sp. and the number of endospores per nematode.

Factor	Nemas with spores (%)	Spores/nema	Zone/nematicide/sampling date	Nemas with spores (%)	Spores/nema
	P-value	*P*-value			
Sampling date	<0.0001	0.002	November 2009	66.0 b	2.6 a
			June 2010	75.0 a	2.8 a
			October 2010	60.7 c	2.0 b
Management zone	0.87	0.004	1	67.2	2.7 a
			2	68.0	2.7 a
			3	66.3	2.0 b
Nematicide[a]	0.009	<0.0001	Control	68.4 a	2.8 a
			Aldicarb	70.1 a	2.7 a
			1,3-D + aldicarb	62.3 b	1.9 b

[a] Sampling date × nematicide interaction for both percentage nematodes with spores ($P = 0.003$) and spores per nematode ($P = 0.01$). For the percentage of nematodes with spores, soil treated with 1,3-D (56 l/ha) + aldicarb (1.0 kg a.i./ha) was only lower than the other two treatments in June 2010. For the spores per nematode, 1,3-D + aldicarb was numerically lower than the other treatments in all years, but the magnitude of the reduction differed among years.

Throughout the growing season in all three years, MZ1 generally had greater *R. reniformis* levels than MZ2. MZ1 and MZ2 had similar EC readings and therefore had similar soil textures, but the zones differed in that MZ2 had a higher mean elevation than MZ1. The difference in elevation was the most obvious difference between the two zones and was a significant factor contributing to the lower nematode population density in MZ2, possibly because higher elevations should drain better and stay drier than lower elevations. Previous research found greater *R. reniformis* levels in non-irrigated microplots than in irrigated microplots (Herring et al., 2010), which seems to contradict our findings, but we are unable to accurately compare the previous study and the current one because soil volumetric water content was not measured in our study.

In one of our zones, texture and elevation provided opposing influences on *R. reniformis* levels. MZ2 differed from MZ3 by having both greater mean EC readings, which would tend to increase *R. reniformis* levels, and greater elevation, which would tend to decrease nematode levels. These conflicting influences resulted in mid- to late-season *R. reniformis* levels in MZ2 that were either similar to or less than the levels in MZ3. This is evidence that the differences in elevation had a similar or greater effect on *R. reniformis* levels than did the differences in soil texture for the MZs in this study. In fields with greater differences in soil texture or elevation, the relative effects could be different.

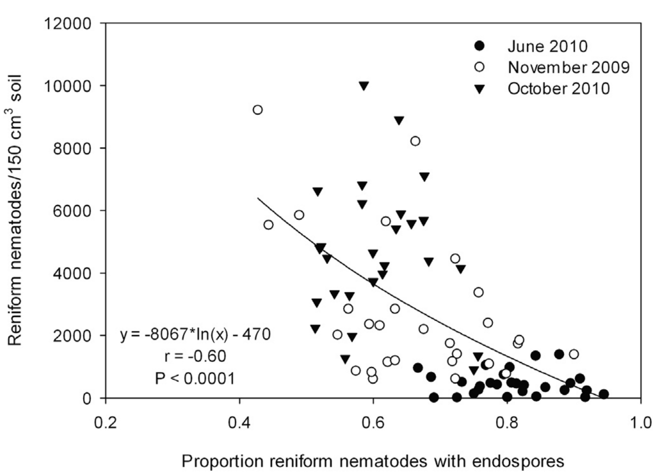

Fig. 2. Endospores per nematode. Relationship between number of reniform nematode (*Rotylenchulus reniformis*) and proportion of the nematode with at least one endospore of *Pasteuria* sp. attached to the cuticle. The proportion with spores was determined by examining 20–30 females and juveniles of *R. reniformis* (males were not included). Data points are from the control and aldicarb plots only; 1,3-D + aldicarb was excluded because numbers of *R. reniformis* were very low due to the efficacy of 1,3-D.

The purpose of having various nematicide treatments was not to compare treatments but to determine whether the relative effectiveness of the treatments differed among the MZs, and the range of soil textures and elevations in this field did not influence the relative effects of the nematicide treatments on *R. reniformis* levels. There were no MZ × treatment interactions affecting nematode levels in any year, which means that the treatment effects were the same in all MZs. The effects of texture and elevation on yield were similar, but not identical, to the effects on nematode levels. The effects of the treatments on yield were the same in all MZ (no MZ × treatment interaction) in 2008 and 2009, but there was an interaction in 2010 due to very high yields in plots treated with aldicarb at 0.6 kg a.i./ha plus 1,3-D at 28 l/ha in MZ2; there was no interaction when that treatment in MZ2 was excluded from the analysis. If the high yields in those plots were due to the effectiveness of the treatment, then plots in MZ2 receiving aldicarb at 1.0 kg a.i./ha plus 1,3-D at 56 l/ha should also have had notably high yields, but they did not. Therefore, the interaction seems unlikely to be reproducible, and we conclude that differences in texture and elevation in this study did not influence the effect of nematicides on yield.

Despite significant effects of texture and elevation on *R. reniformis* levels, cotton yield was not affected by the differences among the MZs in two of three years. Mean yields, averaged across the five nematicide treatments, did not differ among the MZs in 2008 or 2010. However, in 2009, mean yields were greater in MZ2 than in the other MZs. Rainfall during this study was highly field specific, and we do not have rainfall records for this field, but we observed that 2009 had greater rainfall and there were more periods during the 2009 growing season when MZ1 was noticeably wetter than MZ2. We speculate that the better drainage in MZ2 resulting from higher elevation benefited this zone during wet periods and that the wetter soil conditions in MZ1 were detrimental to cotton yield because saturated soil can reduce root growth (Tharp, 1960).

Soil texture is not uniform throughout the soil profile, but soil EC readings provide a single value for the whole profile and may give similar EC readings for profiles with differing combinations or positions of texture within the profile. Therefore, EC readings may not provide all of the information needed to judge how the soil will affect plant growth or nematode reproduction. This is a likely source of variability for yield and nematode levels in this study, and it also could be why the regression equations relating EC readings to the percentage sand, silt, and clay in our study explained less variability than expected. Despite this limitation, using EC readings is a convenient and cost-effective way to divide a field into MZs.

The prevalence of *Pasteuria* sp. differed among the stages of *R. reniformis*, with greater spore attachment to juveniles than to females on two sample dates and to males on all sample dates. The lower spore attachment to females on the November and October sample dates may have been due to molting and inactivity. Many females may have shed their endospores along with their juvenile cuticle and then did not acquire new spores because of cooler soil temperatures. Males had fewer endospores attached to their cuticle than either females or juveniles on all sample dates. It is unlikely that males acquired fewer spores because they were inactive and therefore did not come in contact with as many spores. Moore et al. (2010) showed that horizontal movement of males was greater than females or juveniles in an irrigated field and similar to these other stages in a non-irrigated field. Males may have fewer receptors for endospore attachment than females and juveniles.

In the June sample, a greater percentage of *R. reniformis* (juveniles + females) had attached endospores than in the November and October samples. This was likely due to warmer soil temperatures in June and greater activity of the nematodes. Soil temperatures at 20 cm averaged 27 °C in June 2009, 17 °C in November 2009, and 22 °C in October 2010 (Georgia Automated Environmental Monitoring Network). Although there was no difference in percentage of *R. reniformis* with endospores, the number of spores per nematode was lower in MZ3 than in the other two zones, which may be related to sand content since MZ3 had a higher sand content than MZ1 or MZ2. Leaching of *Pasteuria penetrans* endospores was shown to be greatest in sandy soil and to decline with increasing clay content (Dabire and Mateille, 2004).

The application of aldicarb (1.0 kg a.i./ha) did not reduce the prevalence of *Pasteuria* sp., but the combination of 1,3-D (56 l/ha) + aldicarb (1.0 kg a.i./ha) reduced both the percentage of *R. reniformis* with endospores and the number of attached endospores. Similarly, Kariuki and Dickson (2007) found the abundance of *P. penetrans* endospores on *Meloidogyne arenaria* juveniles was lower in plots treated with 1,3-D than in non-fumigated plots. Mankau and Prasad (1972) reported that neither aldicarb nor 1,3-D had a noticeable effect on *Pasteuria* sp. endospores. However, 1,3-D may have an indirect effect on *Pasteuria* spp. by reducing the number of hosts for the bacterium. The high rate of 1,3-D + aldicarb was considerably more effective than aldicarb in reducing populations of *R. reniformis* in the current study. For a density-dependent parasite such as *Pasteuria* spp. (Ciancio, 1995), a reduction in the number of hosts that can become infected leads to decreased endospore production.

Recently, *in vitro*-produced endospores of *Pasteuria* sp. were shown to reduce populations of *R. reniformis* in greenhouse pots (Schmidt et al., 2010). Our results indicate that a naturally-occurring population of this bacterium also suppressed populations of *R. reniformis* in a cotton field. However, even with 60–70% of females and juveniles with attached endospores, population densities of *R. reniformis* were still quite high. It is possible that not all the attached endospores are able to infect. Heavy rains may also leach spores below the root zone, thus preventing maintenance of high spore densities.

Although we found that both *R. reniformis* and *Pasteuria* sp. levels often differed among MZs, nematode levels in all MZs were sufficiently high to warrant treatment. Furthermore, within the range of soil textures in this study, the effects of the nematicide treatments did not differ among MZs, so the choice of treatment would have been the same in each MZ. In this field, a uniform rate of nematicide would have been recommended and there would have been no cost savings from utilizing nematode MZs. However, because nematode levels can differ significantly among MZ, it seems likely that recommendations could differ among MZ in some fields, especially in fields with greater differences in texture and elevation among zones. Therefore, we believe that utilizing MZs based largely on soil texture and elevation as a method of subdividing a large field into smaller units will be useful in some fields even though it will not provide cost savings in all fields.

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