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Effect of Controlled Cold Storage on Recovery of *Rotylenchulus reniformis* from Naturally Infested Soil

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Abstract: Rotylenchulus reniformis is rapidly becoming the most economically important pest associated with cotton in the southeastern United States. Incentive programs have been implemented to support sampling of production fields to determine the presence and abundance of *R. reniformis*. These sampling programs have dramatically increased the number of soils samples submitted to nematology laboratories during autumn. The large numbers of samples overwhelm most labs and require placement in cold storage until extraction. Therefore, the objective of this study was to examine the length of time soils infested with *R. reniformis* can be stored before nematode extraction without compromising the accuracy of estimates of population densities. A sandy loam and a silty loam were the two cotton production soils used in this study. *Rotylenchulus reniformis* numbers decreased 61% during the first 180 days of storage in both soils. *Rotylenchulus reniformis* numbers from the initial sampling through 180 days decreased as a linear function. The decline of *R. reniformis* numbers during storage was estimated as 0.28% of the population lost daily from the maximum population through 180 days. The diminution of nematode numbers from 180 through 1,080 days in storage continued, but at a slower rate. Numbers of *R. reniformis* declined to less than 89%, 93%, and 99% of the initial population within 360, 720, and 1,080 days, respectively, of storage. The reduction of *R. reniformis* numbers over 180 days can be adjusted, allowing a more accurate estimation of *R. reniformis* levels in soil samples stored at 4 °C.

Key words: Rotylenchulus reniformis, soil storage, population density.

The reniform nematode *Rotylenchulus reniformis* is rapidly becoming the most economically important pest associated with cotton production in the southeastern United States. This nematode has increased from a relative unknown nematode pest to a major production constraint, replacing the root knot nematode (*Meloidogyne incognita*) as the most economically important nematode in Alabama, Louisiana, and Mississippi. In these states 46% to 32.4% of the cotton-producing acres are infested with *R. reniformis,* and it appears to be spreading rapidly (Gazaway and McLean, 2003; Lawrence and McLean, 1999; McLean and Lawrence, 2000; and Overstreet, 1999).

Agriculture crop protection companies have recognized the impact of *R. reniformis* on cotton production and have implemented programs to support sampling production fields to determine *R. reniformis* presence and population levels. These programs have dramatically increased the number of soil samples submitted to the nematology laboratories. The result is samples are placed in cold storage until the laboratory can process them. Little information is available on the recovery efficiency of samples placed in storage over time. Literature has been focused primarily on the survival of the nematode over time. Several authors have reported the effects of time on the survival and pathogenicity of various plant pathogenic nematodes in the absence of a host. *Ditylenchus dipsaci* was found to survive for 242 days at 15 °C and 21 °C; however, the recovered nematodes were not necessarily infective when placed on a host plant (Miyagawa and Lear, 1970). Slack et al. (1972) found *Heterodera glycines* juveniles survived for 90 months in soil maintained at field capacity. Barker et al. (1969) stored soil samples at seven temperatures ranging from −15 °C to 36 °C for up to 16 weeks and concluded the optimum storage temperature for *M. incognita, Belonolaimus longicaudatus,* and *Tylenchorhynchus claytoni* was 13 °C. Birchfield and Martin (1967) reported *R. reniformis* nematodes could survive and remain infective in air-dried soil for 7 months when stored at 20 °C to 25 °C. They concluded this nematode could survive in fallow soil for long periods, thus suggesting how easily it could be spread. Apt (1976) reported 23% of a population of *R. reniformis* survived after 1 month of storage in moist soil placed in polyethylene bags at 20 °C to 25 °C. The populations declined rapidly, with few nematodes surviving longer than 15 months. Apt (1976) concluded a fallow period of 12 to 15 months would be effective in reducing *R. reniformis;* however, the 1 to 4-month fallow period common to pineapple production would not successfully reduce *R. reniformis* populations. Further investigations by Tsai and Apt (1979) suggested anhydrobiosis of *R. reniformis* was not a survival mechanism in that coiling of the nematode did not increase over time or with reduced soil moisture.

The development of effective soil storage strategies directly related to the rate of decline of *R. reniformis* populations is needed for accurate population estimates. The objective of this study was to estimate the decline function during storage of soil for *R. reniformis* populations in two common cotton production soils.

Materials and Methods

Soil was collected from two fields naturally infested with *R. reniformis* and continuously cropped in cotton.

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The soil MS from Washington County, Mississippi was silty loam (38% sand, 52% silt, 9.2% clay, pH 6.2, 25% moisture), and the Escambia County, Alabama soil (AL) was a sandy loam (65% sand, 25% silt, 9.75% clay, pH 6.0, 23% moisture). Soil cores, 2-cm-diam. × 20-cm-deep, were collected from each field and throughly mixed. Composite soil samples were sealed in plastic bags, labeled by location, and placed at a constant temperature of 4 °C in a dark incubator. Every 30 days for 540 days and then every 180 days until 1,080 days, *R. reniformis* was extracted from four 150-cm^3 sub-samples per location. Nematodes were extracted by combined gravity screening and sucrose centrifugal-flotation (specific gravity = 1.13), and *R. reniformis* vermiform juveniles and adult life stages were enumerated using a stereo microscope.

Viability of the extracted vermiform *R. reniformis* juveniles and adults was determined in the greenhouse. The replications of the extracted *R. reniformis* from each time period were combined keeping soil types separate and were evenly distributed into four 10-cm-diam. polystyrene pots containing autoclaved soil. The appropriate nematode suspension was pipeted into two depressions in each pot and covered with soil to prevent dehydration of the inoculum. Plots were planted with Paymaster 1218 B/RR cotton seeds and were grown in the greenhouse for 60 days. Plants were fertilized weekly with a balanced water-soluble fertilizer. In all viability tests, treatments were arranged in a completely randomized design with four replications. At harvest, vermiform *R. reniforms* juveniles and adults were extracted from the soil and enumerated as previously described.

Data from viability tests were collected on numbers of *R. reniformis* juveniles and vermiform adults. A reproductive factor (RF) was calculated by dividing the final *R. reniformis* population density by the initial density. For statistical analysis, mean *R. reniformis* counts for each sample day were converted to a percentage by dividing the counts for a given day and soil type by the initial *R. reniformis* count at the beginning of the experiment and multiplying by 100. The decline in nematode numbers was then modeled as a hyberbolic response in the form of what Schabenberger and Pierce (2002) called "a three-parameter extended Langmuir model" using the NLIN procedure in SAS (SAS Institute, Cary, NC) where Y represents the percentage survival, α the maximum survival (= 100%), β the inflection point, days are the number of days a sample was stored, and γ the rate of decline:

$$Y = \alpha \frac{\beta^{*}\text{days}^{\gamma}}{1+\beta^{*}\text{ days}^{\gamma}}$$

Because the initial decline (up to 180 days) was reasonably linear, we modeled this decline in linear-linear space using SAS Proc Reg.

Results and Discussion

Initial *R. reniformis* populations were 3,772 and 3,386 vermiform nematodes/150 cm^3 for Alabama and Mis-

Table 1. Least squares means for *Rotylenchulus reniformis* populations after storage, *R. reniformis* regenerated populations, and reproductive factor (RF) values. Counts were log transformed for analysis; LCL and UCL are lower and upper 95% confidence limit.

	Alabama			Mississippi		
Days	*R. reniformis*	LCL	UCL	*R. reniformis*	LCL	UCL
			Vermiform per 150 cm^3 directly out of storage			
0	3,772	3,191	4,353	3,386	2,771	4,000
30	2,034	1,453	2,615	3,178	2,559	3,787
180	991	410	1,572	1,731	1,117	2,345
360	193	–387	774	572	–41.2	1187
720	309	–272	890	199	–414	813
1080	12	–568	593	12.8	–601	627
			Vermiform per 500 cm^3 after regeneration on cotton for 60 days			
0	87,100	73,831	100,368	121,476	111,893	131,059
30	88,548	75,279	101,817	132,098	122,514	141,681
180	3,881	–9,386	17,150	9,193	–390	18,776
360	3,328	–9,940	16,597	296	–9,286	9,879
720	1,004	12,264	14,273	405	–9,177	9,988
1080	19	–13,249	13,288	6.5	–9,576	9,589
			Reproductive factor			
0	23.0	9.4	36.6	50.2	46.5	54.0
30	43.5	29.9	57.1	39.0	35.2	42.7
180	14.0	0.42	27.6	5.2	1.4	9.0
360	17.3	3.6	30.8	1.8	–2.0	5.5
720	3.0	–10.6	16.6	2.0	–1.8	5.8
1080	0.8	–12.8	14.3	0.3	–3.5	4.0

sissippi, respectively (Table 1). These population declined within 360 days to 193 and 572 vermiform nematodes/150 cm^3 for the Alabama and Mississippi soils. Numbers of recovered *R. reniformis* remained ($P \leq 0.05$) similar for the first 150 and 180 days for Alabama and Mississippi soils, respectively. In both soil types, *R. reniformis* numbers decreased after 180 days ($P \leq 0.05$) and the loss of numbers that were recorded over the duration of the storage time continued to decline at a slower rate. Numbers of *R. reniformis* declined to less than 61%, 89%, 93%, and 99% of the initial population within 180, 360, 720, and 1,080 days of storage, respectively. However, the *R. reniformis* nematode numbers never reached zero. Apt (1976) found 23%, 21%, and 1% of the *R. reniformis* population in a red oxisol soil survived for 30, 120, and 360 days, respectively, when stored at 20 °C to 25 °C. Thus storage of soil at 4 °C in this study increased *R. reniformis* longevity. Barker et al. (1969), however, found no differences in the number of *M. incognita* recovered from soil samples stored at −2 °C, 13 °C, 19 °C, 24 °C, and 30 °C when extracted monthly over 16 weeks of storage. We selected 4 °C, which is the standard setting for most refrigerated cold storage rooms for plant and soil diagnostic labs, and extended the monthly extractions over 18 months. The reduced temperature apparently reduced the activity and(or) metabolism of *R. reniformis,* resulting in higher recovery over a longer period of time in this study.

The RF value for both soils average 36.5 from the initial extraction, indicating *R. reniformis* was viable when the soil samples were collected. The viability of the nematode decreased with increased storage time. The mean RF values fell to 9.6, 1.6, 2.0, and 0.6 for the 180, 360, 720, and 1,080-days of storage, respectively. *Rotylenchulus reniformis* was recovered in stored soil for the 1,080-day time period. This nematode remained viable and was able to increase in numbers even after 720 days in storage.

The decrease in *R. reniformis* numbers assessed as a percentage of the maximum population density in the soil was negatively related to storage time duration (Fig. 1). The non-linear 3-parameter Langmuir function best described the recovery of *R. reniformis* numbers in the soil over the 1,080-day sample period. The hyperbolic curve illustrates the retrieval of *R. reniformis* over the time gradient. The dominant slope of the lines from the initial counts to 180 days indicates the greatest loss of *R. reniformis* numbers; however, the slope levels to a gradual decline from 180 through 1,080 days, never reaching zero.

The effect of storage on nematode density from the initial collection through 180 days was a linear function (Y = 70 – 0.28 days) (Fig. 2). The decline of *R. reniformis* numbers while in storage can be predicted by the percent of the maximum initial population counts. The coefficient of determination (r^2) = 0.7729 and probability ($P \leq 0.003$) indicated that the model was accurate for both soils, which behaved similarly with parallel slopes. The liner regression equation indicated a mean survival of 70% of the maximum population. The data indicated 0.28% of the maximum population was lost daily through 180 days; therefore, 28% of the population is lost within 100 days of storage. This reduction of the nematode numbers over time can be adjusted allowing a more accurate estimate of *R. reniformis* level densities in soil samples stored at 4 °C for up to 180 days. This relationship between recovery and storage time can be used for a more accurate estimate of *R. reniformis* population densities in soil samples stored at 4 °C for up to 180 days.

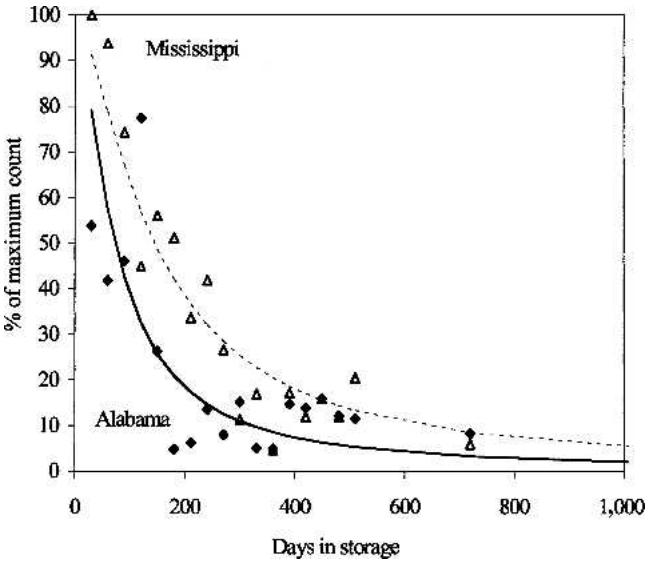

FIG. 1. Effect of storage at 4 °C on *Rotylenchulus reniformis* nematode numbers. Lines were fitted using a 3-parameter Langmuir function.

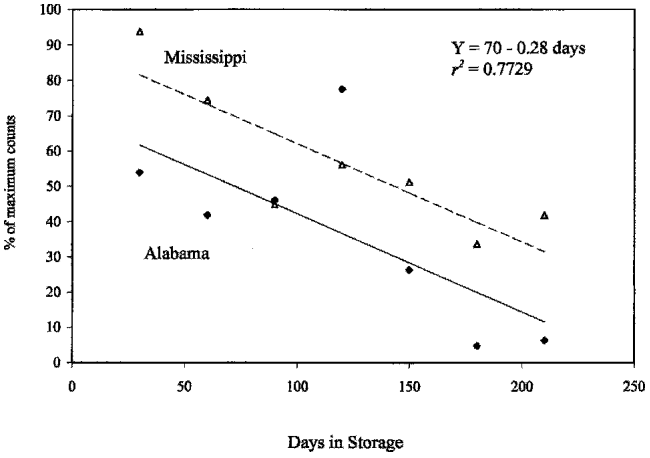

FIG. 2. Effect of 180 days of storage at 4 °C on *Rotylenchulus reniformis* nematode numbers. Lines were fitted using a 3-parameter Langmuir function.

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