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COMPARISON OF THREE EXTRACTION TECHNIQUES FOR THE RENIFORM NEMATODES IN THE LIGHT TO MIXED SOIL

Fanny X. Liu and Gabe L. Sciumbato
Delta Research and Extension Center
Mississippi State University
Stoneville, MS
Lawrence D. Young
USDA-ARS
Stoneville, MS

Abstract

The three most commonly used techniques for the recovery, identification, and enumeration of plant parasitic nematodes from soil were evaluated for efficiency and consistency for the extraction of the reniform (*Rotylenchulus reniformis*) nematode in light to mixed texture soils. The results indicated that there were significant differences in nematode extraction numbers among the three methods, and that the elutriation-centrifugation method gave the highest recovery numbers and the lowest variation among the sub-samples.

Introduction

Reports of losses by the Beltwide Cotton Disease Loss Committee for the reniform nematode have increased dramatically in several Southern cotton growing states in recent years. The reniform nematode has become a major pest of cotton in Mississippi according to reports of the Mississippi State Extension Nematology Laboratory.

In order to conduct research, it was necessary to determine the best technique for recovering, identifying, and enumerating the reniform nematode. The Baermann technique of extracting nematodes only recovers live and active nematodes. This method involves less laboratory equipment and labor. The flotation-centrifugation technique is a passive method and extracts live and dead nematodes depending upon their density, the gradient of the extraction solution, the g force of the centrifuge, the pore size of the sieves and, most importantly, the consistency of the laboratory procedures. The third method, the elutriation-centrifugation technique, is similar to the flotation-centrifugation technique. The major difference between the two techniques is that the elutriation-centrifugation technique is semi-automated, and four samples can be run at one time while the flotation-centrifugation is manual.

This experiment was conducted to determine the extraction method that would be the most efficient, give the highest recovery rate, and have the lowest variation among sub-samples for the reniform nematode.

Materials and Methods

Soil was collected in August 2000 at 0-30 centimeter depth from reniform infested fields which had been continuously cropped to cotton . A total of 200 soil cores from four fields were combined and homogenized by tumbling for one hour. Eight sub-samples per replication were run for each of the three methods. Three replications were extracted and counted within two weeks. Nematodes were identified under the dissecting microscope, counted, and converted to the number of reniform nematodes per pint of soil.

Baermann-Funnel Method

Double layers of facial tissue without lotion were placed on a screen in a funnel. The tissue was moistened with a solution of deionized water supplemented with 0.5 gram $CaCl_2$ dihydrate per liter. One hundred milliliters by volume of soil to be extracted were placed on the tissue and water was added until the soil was submerged, and the funnel was covered to minimize evaporation. After five days, the water + nematodes were collected and passed through a 400 mesh sieve to concentrate the nematodes.

Flotation-Centrifugation Method

One hundred milliliters by volume of soil to be extracted were placed in a plastic container and covered with 600 ml of water. The mixture was stirred vigorously for 40 seconds, allowed to settle for 60 seconds and the water was decanted through two sieves (top #60 and bottom # 400). The sieves were washed with water and the contents of the bottom sieve were collected in

a 50 ml centrifuge tube, and centrifuged at 370 g force for 5 minutes. The supernatant was decanted and a 50% sucrose solution was added to the soil-nematode pellet, and the pellet was resuspended and centrifuged for one minute. The supernatant was poured through a #400 sieve, and the nematodes were collected and enumerated.

Elutriation-Centrifugation Method

Two hundred milliliters by volume of soil were placed in the elutriator, washed and collected onto a #400 sieve. After this step, the samples in water and sucrose solution were processed by centrifugation as occurred in the flotation-centrifugation method.

Results and Discussion

There were significant differences in reniform nematode extracted by the three methods (Table 1).

Figure 1 shows the reniform nematode extracted with each of the three methods. As expected, the Baermann-funnel method gave the lowest nematode counts (1,500 to 5,940) because this method counts only the active nematodes. It is a way to estimate quickly nematode populations, but there is not enough data to relate extracted nematodes to the total population. Figure 2 shows the distribution of 24 sub-samples for each method. The elutriation-centrifugation method gave the highest numbers of extracted nematodes (9,525 to 18,300) and gave the lowest variation among the sub-samples. It speeds up the first wash step and limits individual of handling samples compared with the flotation-centrifugation method, but it requires a elutriator. The flotation-centrifugation method gave the second highest (2,640 to 16,830) nematode extraction number and had second highest variation among the sub-samples. The high variation could be caused by inexperience of laboratory workers. It is hard to eliminate the differences among the people. The elutriation-centrifugation technique used more volume of soil than other two techniques which might increase consistency of extraction.

There were no significantly difference among the sub-samples within the replications. The wide range of numbers of the nematode extracted among the replications indicated that the consistency of extraction needs to be improved.

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Table 1. Means of extraction reniform nematodes number in three techniques at three replications.

Extraction Method	Nematode / Pint [1] Overall 24 means	Nematode / Pint [2] Replication 1.	Nematode / Pint [2] Replication 2.	Nematode / Pint [2] Replication 3.	C. V.
Elutr.	13081 a	15741 a	11100 a	12403 a	14.0
Flota.	7954 b	11567 b	6510 b	5775 b	23.4
Funnel	3065 c	2370 c	4268 c	2558 c	26.1
M.S.D.	1264.4	2259.5	1459.7	1361.6	
C.V.	30.5	23.4	20.4	20.3	
F Value	100.4	69.7	43.8	102.7	

1. Means in the same column followed by the same letter are not significantly different according to Waller- Duncan t test (K ratio = 100).
2. Means of 8 sub-samples in the replication. Means in the same column followed by the same letter are not significantly different according to Waller-Duncan t test (K ratio = 100).

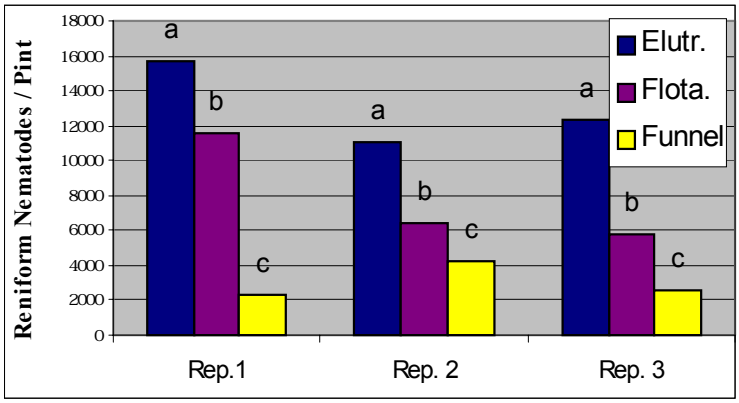

Figure.1 Comparison extraction reniform nematodes in three methods using three techniques.

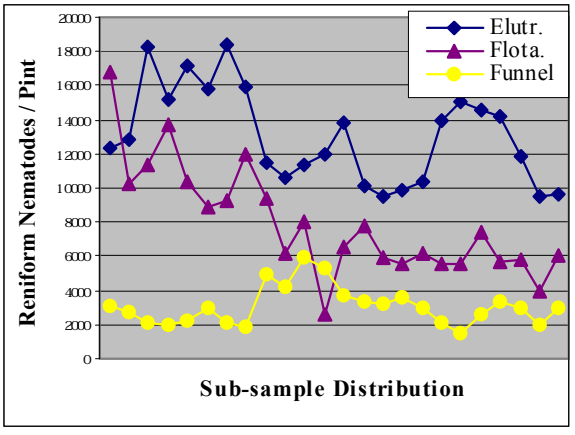

Figure. 2 Distribution of all sub-samples in all three replications for each techniques.