Seasonal Population Dynamics of Selected Plant-parasitic Nematodes as Measured by Three Extraction Procedures[1]

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Abstract: Seasonal fluctuations in field populations of *Meloidogyne* spp. (*M. incognita* and *M. hapla*), *Pratylenchus zeae, Criconemoides ornatum, Tylenchorhynchus claytoni, Belonolaimus longicaudatus,* and *Helicotylenchus dihystera* were determined monthly for 1 year by three extraction procedures. Baermann funnel method (BF) gave highest recoveries of *Meloidogyne* spp. and *P. zeae* during summer and fall, but centrifugal-flotation (CF) and sugar-flotation-sieving (SFS) usually yielded higher numbers of these nematodes during winter and spring. CF was the only effective method for recovery of *C. ornatum* with maximum numbers occurring in September. Recoveries of *T. claytoni* were similar with all methods in summer and fall. However, BF gave low numbers in winter and spring, whereas population peaks with the flotation methods occurred in January and February. All methods gave similar recoveries of *B. longicaudatus* with highest numbers occurring in November and December. This species declined drastically in late winter and spring. Yields of *H. dihystera* were similar for all three methods with CF consistently higher and the major peaks occurring in August.

Reliable extraction procedures are essential for accurate estimation of seasonal fluctuations of nematode populations in soil. Seinhorst (14), and others (1, 4, 10) evaluated various extraction procedures at a single sampling time. Ayala *et al.* (1) showed that soil type may affect the results obtained with certain assay techniques. Rainfall, season and soil depth also influence nematode populations (5, 8, 12, 13). Little work has been done, however, on the quantitative estimation of nematode population densities by various extraction procedures as affected by season. Powell and Nusbaum (12) showed the effectiveness of the Baermann funnel and sieving-funnel methods to be influenced more by the date of sampling than was a bioassay procedure in which tomato was used as the indicator host. These procedures, however, depend on the viability and motility of the nematodes, and the sampling was limited to a period of January through April.

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The present investigation was designed to compare the efficiency of two sugar-flotation procedures, which are independent of nematode motility, and the Baermann funnel method, which is motility-dependent, in measuring seasonal population shifts of certain endo- and ectoparasitic nematodes.

MATERIALS AND METHODS

COLLECTION AND PREPARATION OF SOIL SAMPLES: Six pre-selected fields in Eastern North Carolina were sampled to a depth of 15 cm at monthly intervals for 1 year. All samples were taken in the row except for field 6 which was in a solid stand of Johnson grass, *Sorghum halepense* (L.) Pers., and field 5 where samples were collected between the rows. Four borings taken from a 5 × 5 m area with a 7-cm sampling tube comprised a given soil sample (about 2300 cc). Four such composite samples were collected from each field each month. Samples were placed in plastic bags and delivered to the laboratory in insulated chests.

Each sample was sieved through a screen with 6-mm openings and then thoroughly mixed by passing it through a sample divider four times. Samples were processed within 24 hr after collection. The soil type and

TABLE 1. Soil type, cropping history and cultural practices of fields sampled.

Field No.[a]	Soil Type	Cropping sequence[b] 1965	1966	1967
1	Norfolk sandy loam	Corn	Peanut	–
2	Norfolk sandy loam	Corn	Cotton	–
3	Norfolk sandy loam	Peanut	Watermelon	–
4	Norfolk sandy loam	Corn	Corn	Corn
5	Appling loamy sand	Corn	Tobacco	Barley
6	Appling loamy sand	Johnson grass	Johnson grass	Johnson grass

[a] Fields were plowed or disked as indicated below during the monthly sampling periods of June, 1966 through May, 1967: #1 and 2—February, March and May; #3—October, November, February and May; #4—February and May; #5—November; and #6—August.

[b] Scientific names of crops as follows: corn—*Zea mays* L.; peanut—*Arachis hypogaea* L.; cotton—*Gossypium hirsutum* L.; watermelon—*Citrullus vulgaris* Schrad; tobacco—*Nicotiana tabacum* L.; barley—*Hordeum vulgare* L. and Johnson grass—*Sorghum halepense* (L.) Pers.

cropping history of the six fields, which were sampled from June, 1966 through May, 1967, are given in Table 1.

NEMATODE EXTRACTION METHODS: An aliquot from each of the four composite soil samples was processed by each of three extraction methods: centrifugal-flotation (6); sugar-flotation-sieving (3); and by modified Baermann funnel (12). Detailed procedures followed for each method of extraction are given below. All counts were converted to numbers of nematodes per 500 cc of soil.

Baermann Funnel Technique (BF).—Approximately 25 cc of soil of a given sample were placed on Scotties® facial tissue, supported by plastic screens in two 10-cm glass funnels. An aqueous solution containing 2 ppm Separan® 2610 (Dow Chemical Company, Midland, Michigan) and 2 ppm methylene blue was added to each funnel to barely cover the soil (12). All funnels were located in an air-conditioned laboratory (24 ± 2 C). Nematodes were collected from the funnels after 3 days.

Centrifugal-flotation Technique (CF).—A swinging bucket International Centrifuge (Model K, Size 2) with a head holding sixteen 50-ml tubes was used for this method as modified by Jenkins (6). Fifty cc of soil were stirred for 20 sec in 500 ml tap water with a motorized stirrer. After allowing the soil particles to settle for approximately 1 min, the nematode suspension was decanted onto a 40-mesh sieve over a 325-mesh sieve; the material collected from the 325-mesh sieve was centrifuged in 50-ml tubes at approximately 420 G (Max.) for 5 min. After decantation, the pellet was resuspended in 50 ml of sucrose solution (454 g/L) with a Vortex Jr. mixer. After centrifugation at 420 G for about 1 min, the nematode suspension was decanted onto a 325 or 400-mesh sieve and rinsed into 150-ml beakers, and the nematodes counted.

Sugar-flotation-sieving Technique (SFS).—Fifty cc of a given soil sample were stirred in 350 ml total volume of 1.0 M sucrose solution containing 12.5 ppm Separan (3). Separan causes soil colloids to flocculate and eliminates the need for centrifugation. After 2–5 min settling time, the sugar solution-nematode suspension was poured onto a 40-

mesh sieve over a 325-mesh sieve. Each sample was again washed onto and recovered from a 325 or 400-mesh sieve before counting.

NEMATODES STUDIED: All counts were made with a stereoscopic microscope. Fresh formalin (3%) mounts as well as glycerine mounts (16) were used in identification of species. Population densities of all plant-parasitic nematodes present were determined, but data for only six of the more important species are presented here. These nematodes and their predominance in the fields sampled (Table 1) were as follows: *Criconemoides ornatum* Raski—fields 1 and 3; *Tylenchorhynchus claytoni* Steiner—fields 1, 2, 3, 4 and 6; *Belonolaimus longicaudatus* Rau—fields 1 and 2; *Helicotylenchus dihystera* (Cobb) Sher—fields 5 and 6; *Meloidogyne incognita* (Kofoid & White) Chitwood—fields 1, 2, 3 and 5 (fields 1, 2 and 3 had mixed populations of *M. incognita* and *M. hapla* Chitwood); and *Pratylenchus zeae* Graham—fields 4, 5 and 6.

STATISTICAL ANALYSIS: In the first analysis of variance of nematode recoveries (numbers per 500 cc of soil), data from all fields, extraction methods and times of extractions were combined. Sampling error was estimated from sets of four samples *within fields*, and the *fields* × *extraction procedure* interaction provided a measure of experimental error. Because the results from the combined analysis indicated a *field* × *extraction procedure* interaction in certain cases, a second type of analysis of variance (independent) was applied to each nematode *extraction technique within* each *field*. Fields having similar patterns of response were combined (*means* over *similar fields* and *replicates within fields*). Such combined monthly plots of means for *Meloidogyne* spp., *C. ornatum* and *T. claytoni* are graphed in Figs. 1 and 2. Typical means from single fields for *P. zeae* (field 4), *B. longicaudatus* (field 2) and *H. dihystera* (field 6) are also given in these figures (crop or "field" effect was apparently greater with these nematodes).

RESULTS

ENDOPARASITES: Combined data from fields 1, 2, 3 & 5 with *Meloidogyne* spp. showed highly significant interactions between each *extraction procedure* and *time* (Fig. 1-A). Low numbers of larvae were found during the months of June through September. BF yielded the highest numbers in October and November, showed a decline in January, an increase in February, and a decline again in March and throughout the spring. Both flotation methods yielded lower numbers of *Meloidogyne* larvae in the fall than did BF. Larval densities dropped in January with SFS and CF but greatly increased in February. Numbers of larvae recovered in March to May by these methods declined but to a lesser degree than with BF.

With *P. zeae*, there were no significant interactions between *extraction procedures* and *time* when data from the three *fields* were combined in the analysis. However, BF yielded much higher numbers in late summer and fall than the flotation methods. The latter techniques usually gave higher yields in winter and early spring than BF. When data from individual fields were analyzed independently, significant differences in numbers of *P. zeae* recovered vs. *time* were detected for each of the *extraction procedures*. This was true for most of the nematode species in all fields except field 5 in which no significant differences were detected. In field 4, both flotation procedures gave significantly higher yields of *P. zeae* in the fall and in certain winter and spring months than in the summer (Fig. 1-B). The highest numbers of this species were recovered by BF in the fall. However, BF yielded about one-half as many *P. zeae* in February, March and April as the flotation methods.

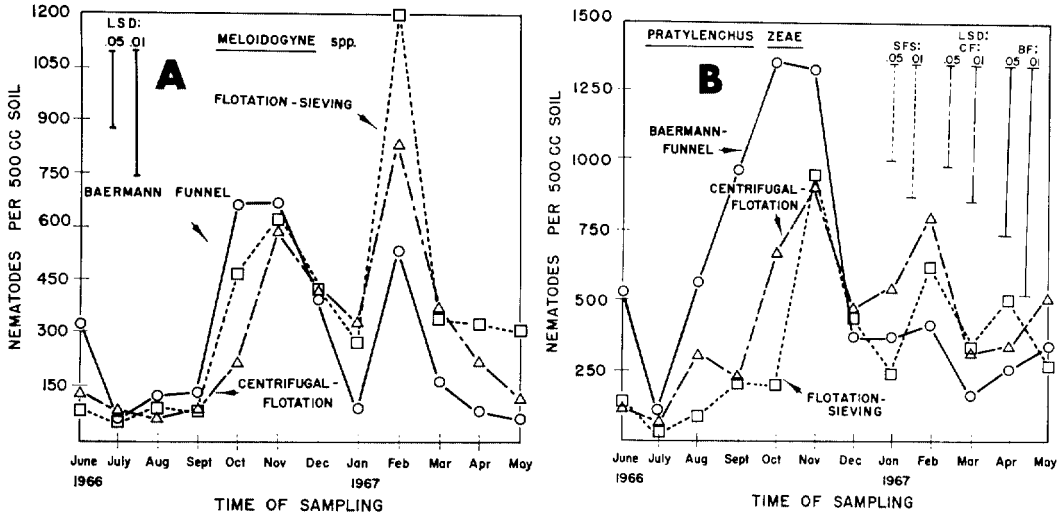

FIG. 1. Seasonal fluctuations of two endoparasitic nematodes as determined by three extraction methods. (A) Curves for *Meloidogyne* spp. (*M. incognita* and *M. hapla*) were obtained by plotting averages of four replicates over fields #1, 2, 3 and 5; LSD values from combined analysis, *methods* × *sampling times* × *fields*, can be used for comparing differences in either direction. (B) Curves for *P. zeae* were obtained from plotting means of four replicates from field #4; LSD values from independent analysis, *method* × *sampling time within* a *field*, can be used only for comparing different recoveries with time for the respective methods (BF = Baermann funnel method; SFS = Sugar-flotation-sieving technique; and CF = Centrifugal-flotation procedure).

ECTOPARASITES: CF was the only procedure that showed striking seasonal changes in population levels of *C. ornatum* (Fig. 2-A). Maximum numbers were recovered in the fall followed by a second peak in February. Although the numbers of *C. ornatum* recovered by SFS and BF were very low compared to CF (Fig. 2-A), both SFS and BF gave significantly different recoveries with *time* when data were analyzed independently by *method* within each *field*.

With *T. claytoni*, all three methods of extracting nematodes gave similar recoveries in the summer and early fall (Fig. 2-B). However, the means from these fields 1, 2, 3, 4 and 6 for both flotation procedures were significantly higher in early winter, than in the summer and fall, whereas those of BF were significantly lower. This trend continued through the winter and spring. With a few exceptions, similar results between *method* and *time* were also found when data were analyzed independently for each *method* and *field*.

There was no significant interaction between extraction *methods* and sampling *time* for *B. longicaudatus* in the combined analysis of fields 1 & 2. However, all three methods gave high recoveries in the fall and early winter and very low recoveries in the spring and summer. Independent analysis for *each method* showed highly significant differences in numbers of nematodes recovered by both *flotation methods* with *time* in field 2 (Fig. 2-C). There were no significant differences in nematode recovery with BF with *time* in this field, but a highly significant difference was detected in field 1.

The three extraction procedures gave similar yields of *H. dihystera* in fields 5 and 6. Although nonsignificant in the initial combined analysis, all three methods gave maxi-

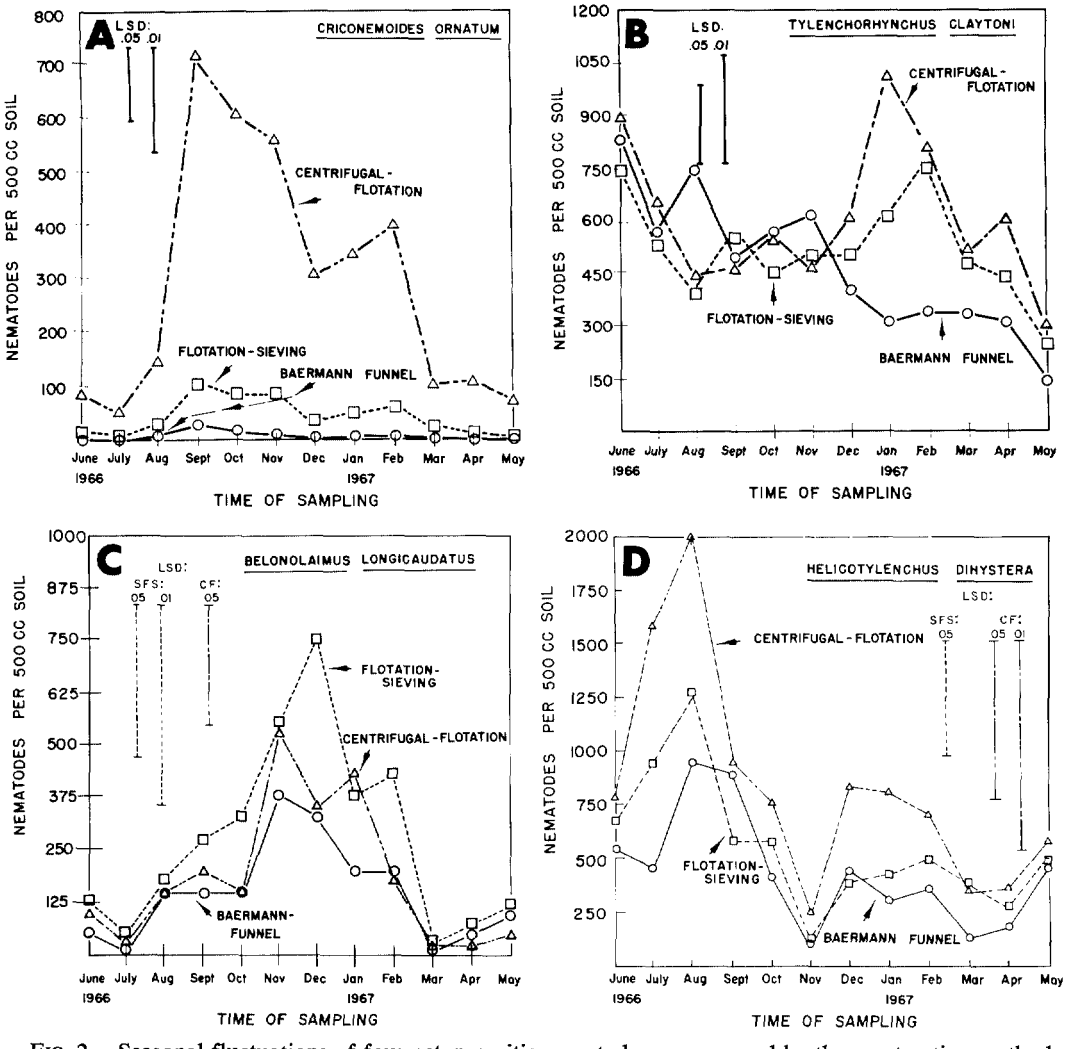

FIG. 2. Seasonal fluctuations of four ectoparasitic nematodes as measured by three extraction methods. Means for *C. ornatum* (2-A) and *T. claytoni* (2-B) are averaged over four replicates per field from fields #1 and 3 and fields #1, 2, 3, 4 and 6, respectively; LSD values from combined analysis, *methods* × *sampling times* × *fields*, can be used for comparing differences in either direction. Means for *B. longicaudatus* (2-C) and *H. dihystera* (2-D) are averaged over four replicates from fields #2 and #6, respectively; LSD values from independent analysis, *method* × *sampling time within a field*, can be used only for comparing different recoveries with time for the respective methods for the latter two species (BF= Baermann funnel method; SFS = Sugar-flotation-sieving technique; and CF = Centrifugal-flotation procedure).

mum recoveries in early fall, with BF yielding fewer nematodes in the winter and spring months. In the analysis for each *method* within each *field*, SFS and CF gave significant and highly significant differences in recovery in field 6 with *time*, respectively (Fig. 2-D). Both methods yielded maximum numbers in July and August. BF also yielded the maximum number of *H. dihystera* from this field in August, but this was not signifi-

cantly different from the other months. No significant differences were detected in field 5.

DISCUSSION

The method of extracting endoparasitic nematodes from soil greatly influenced the numbers recovered during certain periods of the year. High yields of *Meloidogyne* spp. and *P. zeae* recovered from BF in September and October is not surprising since this technique is dependent on motility of the nematodes which is favored in warm soils. The peaks for *P. zeae* in January and for *Meloidogyne* spp. in February from the flotation methods were unexpected, however. Recent work (2) showed that freezing soil before extracting nematodes by the sugar-flotation techniques increases recovery. This phenomenon may be involved in extracting nematodes from frozen soils collected during winter. Accumulation of inactive larvae probably is involved also. The plowing of some of these fields in the fall and winter (Table 1) probably increased the death rates of the nematodes. However, the excessive moisture present in January may have resulted in an immediate migration of *P. zeae* from root fragments, whereas eggs of *Meloidogyne* spp. were induced to hatch and were detected in February. The low recoveries by BF during the winter may reflect decreased nematode motility.

The population peaks of *Meloidogyne* are not fully in agreement with similar investigations. Sasser and Nusbaum (13) also found the maximum population of *M. incognita* to occur in November, but showed that this can be influenced by crop. They failed to obtain the bimodal curves indicated by our data. However, the results of Szczygiel (15) with *M. hapla* on strawberry were similar to ours. Many additional factors such as crop, climate, etc. also may affect the recovery of *Meloidogyne* spp. and *Pratylenchus* spp. Ferris & Bernard (5) and Szczygiel (15), obtained maximum populations of certain *Pratylenchus* species in early summer, whereas Olthof (9) and Koen (8) reported results similar to ours. Kable and Mai (7) suggested that seasonal fluctuations of *Pratylenchus penetrans* (Cobb) Chitwood and Oteifa may be largely due to variations in soil moisture.

The apparent shifts in population densities of three of the four ectoparasites also varied widely with the different extraction procedures. The most striking differences among the extraction methods occurred with *C. ornatum*. CF was the only effective method for measuring population levels of this species as reported by Ayala *et al.* (1). The lack of sufficient movement by these nematodes is apparently responsible for the poor results with BF. In the case of SFS, many of these small nematodes are apparently trapped in the flocculating soil colloids and debris during the extraction process. The decline in numbers of *C. ornatum* during the winter months was more drastic than indicated by Potter (11), possibly reflecting a different extraction procedure.

The extraction method may greatly affect apparent shifts in population densities of *T. claytoni*. Results obtained with BF, for comparable test periods, agree closely with those reported for other *Tylenchorhynchus* species (5). The apparent increase in recoveries in early winter by the flotation methods may be due to an accumulation of immobile nematodes or due to increased recovery rates resulting from freezing as previously discussed.

All methods gave similar results with *B. longicaudatus*. Although adults of this species may overwinter in the field as found by Potter (11), all three extraction procedures indicate that numbers of this nematode decline rapidly during the spring months. Both fields that were infested with this species were plowed in February, March and May (Table

1) which could partially account for this decline.

Ferris and Bernard (5) found the seasonal changes of *Helicotylenchus erythrinae* (Zimmermann) Golden and other nematode species brought about by cropping systems, rainfall and other local conditions could not be predicted in most cases. The *field-to-field* variation that occurred in our results with *H. dihystera* and *P. zeae*, especially, tends to support their conclusion. The disking of field 6 (Table 1) in August may be responsible for the striking decline in the density of *H. dihystera* (Fig. 2-D) during the fall.

In addition to extraction method, rainfall, season, crop and cultural practices that influence apparent shifts in population densities of nematodes, the interaction of *extraction method* and *time of sampling* may often be operative as indicated in this study. Even though Oostenbrink (10) has stated "there is no single method or apparatus available for effective extraction of all kinds of nematodes from soil and plant tissues," many nematologists have assumed that any given extraction method measures the relative population density and inoculum potential of nematodes in given soils. The data presented herein, however, show that results of population studies obtained with different extraction procedures cannot be compared directly. One method may primarily reflect nematode motility, whereas another may reflect total numbers of nematodes regardless of their condition. This is complicated further by the feeding habits of endoparasites vs. ectoparasites. The flotation methods are unsuitable for estimating endoparasitic nematode populations when they are primarily in plant roots. No method of extraction gives a simple difference between hatching and death rates of given species since many environmental and physiological factors may affect the activity of nematodes and thereby influence their recoverability by various techniques. Van Gundy *et al.* (17) have shown that environmental conditions may alter the "physiological age" as well as the motility of certain nematodes. The infectivity and reproductive potential of nematodes recovered by various techniques is another area that needs attention.

Literature Cited

1. Ayala, A., J. Román, and A. C. Tarjan. 1963. Comparison of four methods for isolating nematodes from soil samples. J. Agr., Univ. P. R. 47:219–225.
2. Barker, K. R., C. J. Nusbaum, and W. F. Congleton. 1968. Assays of natural populations of certain plant-parasitic nematodes as influenced by storage conditions and extraction procedures. Nematologica 14:3. (Abstr.)
3. Byrd, D. W., Jr., C. J. Nusbaum, and K. R. Barker. 1966. A rapid flotation-sieving technique for extracting nematodes from soil. Pl. Dis. Rep. 50:954–957.
4. Cairns, E. J. 1960. Methods in Nematology: A review. p. 33–84. *In* J. N. Sasser and W. R. Jenkins (Eds.) Nematology: Fundamentals and Recent Advances with Emphasis on Plant Parasitic and Soil Forms. University North Carolina Press, Chapel Hill, North Carolina.
5. Ferris, Virginia R., and R. L. Bernard. 1961. Seasonal variations of nematode populations in soybean field soil. Pl. Dis. Rep. 45:789–793.
6. Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Pl. Dis. Rep. 48:692.
7. Kable, P. F., and W. F. Mai. 1968. Influence of soil moisture on *Pratylenchus penetrans*. Nematologica 14:101–122.
8. Koen, H. 1967. Notes on the host range, ecology and population dynamics of *Pratylenchus brachyurus*. Nematologica 13:118–124.
9. Olthof, Th. H. A. 1967. Seasonal fluctuations in soil populations of *Pratylenchus penetrans* under tobacco in Ontario. Nematologica 13:148. (Abstr.)
10. Oostenbrink, M. 1960. Estimating nematode populations by some selected methods. p. 85–102. *In* J. N. Sasser and W. R. Jenkins (Eds.) Nematology: Fundamentals and Recent Advances with Emphasis on Plant Parasitic and Soil Forms. University North Carolina Press, Chapel Hill, North Carolina.
11. Potter, J. W. 1967. Vertical distribution and overwintering of sting, ring, and stunt nematodes in a Norfolk sandy loam soil

following peanuts. Nematologica 13:150. (Abstr.)
12. POWELL, W. M., and C. J. NUSBAUM. 1963. Investigations on the estimation of plant parasitic nematode populations for advisory purposes. N. C. Agr. Expt. Sta. Tech. Bull. 156. 21 p.
13. SASSER, J. N., and C. J. NUSBAUM. 1955. Seasonal fluctuations and host specificity of root-knot nematode populations in two-year tobacco rotation plots. Phytopathology 45:540–545.
14. SEINHORST, J. W. 1956. The quantitative extraction of nematodes from soil. Nematologica 1:249–267.
15. SZCZYGIEL, A. 1966. Studies on the fauna and population dynamics of nematodes occurring on strawberry plantations. Ekol. Pol., Ser. A, 14:651–709.
16. THORNE, G. 1961. Principles of Nematology. McGraw-Hill. New York. 553 p.
17. VAN GUNDY, S. D., A. F. BIRD, and H. R. WALLACE. 1967. Aging and starvation in larvae of *Meloidogyne javanica* and *Tylenchulus semipenetrans*. Phytopathology 57: 559–571.