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Seasonal Fluctuations of *Globodera tabacum solanacearum* as Estimated by Two Soil Extraction Techniques[1]

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Abstract: Two soil extraction methods were compared to determine their efficiency in recovering cysts and juveniles of a tobacco cyst nematode, *Globodera tabacum solanacearum.* The methods were equally efficient when extracting nematodes from soil samples seeded in the laboratory; however, there was a significant extraction method × month interaction when the methods were used to estimate field soil populations over 2 years. The centrifugal sugar flotation method recovered greater numbers of cysts when densities were near 400 cysts/100 cm^3 soil and greater numbers of juveniles in all samples. The sugar flotation method recovered greater numbers of cysts during months when densities were less than 400 cysts/100 cm^3 soil. Numbers of cysts and juveniles were lowest in June and July following land tillage in May. A soil freeze in January 1982 may have been responsible for unusually high numbers of recovered cysts in February and March 1982, a pattern that did not occur in 1983.

Key words: tobacco cyst nematode, *Nicotiana tabacum,* extraction methods, population dynamics.

Knowledge of seasonal fluctuations of nematode populations is required to determine the proper time to apply nematicides and to take soil samples for estimating nematode densities necessary for predicting potential crop losses. The population density of a specific nematode may vary considerably with soil temperature (6), soil moisture (5) and soil type (1). There is usually a positive correlation between soil temperature and population density and an inverse relationship between soil moisture and density, as was shown for *Meloidogyne incognita* (Kofoid and White) Chitwood (6).

Estimates of nematode populations may vary with the soil extraction method used (7,10). Extraction method and time of sampling often interact so that no one method yields the highest recovery of all nematodes throughout the year (3). Therefore, studies of soil populations of nematodes where different extraction procedures were used are not usually comparable.

Density flotation techniques were shown to extract nematodes of different genera with different efficiency, and solute density and osmotic activity were found to be of paramount importance to extracting nematodes from soils (1). All the techniques tested were less than 50% efficient. The conclusion was that all density flotation methods were inaccurate and should be used only for comparing recovery of the same nematode species from very similar samples.

Our study compares, over time, two techniques regularly used in our plant clinic to extract a tobacco cyst nematode, *Globodera tabacum solanacearum* Miller and Gray, 1972, Behrens, 1975, Stone, 1983 = *Globodera solanacearum* Miller and Gray, 1972, Behrens, 1975 (8) from soil. The objectives were to determine the best time of year to collect soil samples to detect nematodes, to apply chemicals, and to determine if extraction methods interact with

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TABLE 1. Efficiency of two procedures to extract cysts and juveniles of *Globodera tabacum solanacearum* from 100 cm³ soil.

Procedure*	Cysts (20 incorporated) No. recovered	Cysts (20 incorporated) % efficiency	Juveniles (120 incorporated) No. recovered	Juveniles (120 incorporated) % efficiency
CSF	20	100	95	79
	20	100	90	75
	10	50	115	96
	15	75	105	87
SF	19	95	105	87
	16	80	105	87
	17	85	118	98
	14	70	116	97
	$F = 0.009$, NS†		$F = 0.64$, NS	

* CSF = centrifugal sugar flotation. SF = sugar flotation.
† F = F test not significant at $P = 0.05$.

TABLE 2. Month × extraction method interactions from the population dynamics data for cysts and juveniles of *Globodera tabacum solanacearum* per 100 cm³ soil.*

Month	Cysts SF	Cysts CSF	Juveniles SF	Juveniles CSF
January	56	99	17	49
February	222	322	26	58
March	221	312	51	65
April	161	173	26	77
May	161	120	30	60
June	79	57	14	17
July	109	71	32	54
August	240	204	178	241
September	281	440	114	168
October	154	196	31	17
November	213	199	17	36
December	162	91	29	5
LSD 0.05†	103		50.8	

* SF = sugar flotation. CSF = centrifugal sugar flotation.
† Use LSDs to compare mean numbers of cysts or juveniles from SF and CSF methods within the same month.

the time of year that the samples are collected.

MATERIALS AND METHODS

A tobacco field naturally infested with *Globodera tabacum solanacearum* was chosen for this study. The soil was a Wedowee sandy-clay loam (26% silt, 5.4% clay, 68.6% sand) with 0.6% organic matter and a pH of 6.3. Plots were marked permanently for relocation each year. Two cultivars of flue-cured tobacco (*Nicotiana tabacum* L. cvs. McNair 944 and Coker 319), both susceptible to this nematode, were planted in May 1982 and May 1983. Spacing was 1.2 m between rows and 51 cm between plants within rows. Plots consisting of four rows of 13 plants each were replicated four times. Soil cores (2.0 cm d × 15–20 cm deep) were taken from the center two rows 7.5 cm from the base of plants. Each sample consisted of a composite of 15 cores per plot.

In fall 1981 and 1982, a winter wheat cover crop was planted; on day 15 of each winter month, soil samples were taken in the area covered by the center two rows of the previous tobacco crop. Samples were stored at 10 C until processed, usually within a week.

The land was prepared for tobacco by plowing under the wheat in March and disking in early May just before transplanting in mid-May. The tobacco stalks were plowed up after the last leaf harvest which occurred after the 15 September soil samples were collected. Two weeks after plowing, the field was disked and planted to wheat.

Soil extraction methods: 1) In the centrifugal-sugar flotation method (CSF), 250 cm³ soil was first passed through an elutriator equipped with 710-, 250-, and 38-μm-pore sieves to collect debris, cysts, and juveniles, respectively. Eluate representing 100 cm³ soil was collected from 6 of the 15 exit tubes of the elutriator. Cysts were washed directly from the 250-μm-pore sieve into glass vials and refrigerated until counted. Juveniles were washed from the 38-μm-pore sieve into 50-ml centrifuge tubes and centrifuged at 400 *g* for 4 minutes. The supernatant was discarded and the precipitate resuspended in sucrose solution (specific gravity 1.14), the suspension centrifuged at 400 *g* for 2 minutes, the supernatant poured onto a 38-μm-pore sieve, and the nematodes washed from the sieve into glass vials and refrigerated until counted;

2) In the sugar flotation method (SF), 400 ml sucrose solution (sp. gr. 1.37) was added to 100 cm³ soil in a 1-liter beaker. The soil–sucrose mixture was stirred gently by hand for 1 minute and then allowed to stand for 10 minutes to settle the denser particles. The supernatant was poured over

TABLE 3. Year × month interaction from the population dynamics data for cysts of *Globodera tabacum solanacearum* per 100 cm³ soil.

Month	Year 1 (1981–82)	Year 2 (1982–83)
September	439	281
October	174	176
November	197	214
December	106	147
January	No sample	154
February	420	124
March	393	139
April	215	118
May	163	118
June	99	36
July	104	75
August	359	84
LSD 0.05*	104	

* Use LSD to compare monthly means of year 1 to the same monthly means in year 2.

a series of sieves of 710-, 250-, and 38-μm-pore sizes situated on a wrist-action shaker attached to an elutriator. The contents of the sieves were rinsed to remove excess sucrose. Cysts were washed directly from the 710-μm-pore sieve and stored in vials. Juveniles were washed from the 38-μm-pore sieve with sucrose solution (sp. gr. 1.14) into 50-ml centrifuge tubes and allowed to stand for 10 minutes. The supernatant was poured onto a 38-μm-pore sieve and rinsed; the nematodes were stored in vials.

The relative efficiency of the two methods was estimated by mixing noninfested soil of the same composition as that in the test plots with known numbers of juveniles and cysts which were then extracted by these methods. Four artificially infested soil samples were extracted by each method.

A split plot design was utilized with years as main plots, months as subplots, varieties as sub-subplots, and extraction methods as sub-sub-subplots. The data were combined over years and tested by an analysis of variance. Least significant differences and correlation coefficients were computed when appropriate.

RESULTS AND DISCUSSION

The results from extracting soil artificially infested with known numbers of cysts and juveniles showed that both methods were very efficient (CSF—81% of cysts, 84% of juveniles recovered; SF—82% of cysts, 92% of juveniles recovered) and not different (Table 1). Although this experiment showed the two soil extraction methods to be equally efficient for nematodes, manipulation of the soil during incorporation of nematodes may have disrupted natural soil factors that influence extraction efficiency and extraction method × month interactions.

Quantitative estimates of nematode populations are influenced by the extraction procedure and by season (3). There was a significant extraction method × month of the year interaction in both years, indicating that the methods differed in their ability to extract cysts depending on the month. In year 1, the CSF method recovered higher numbers of cysts than did the SF method in months when the counts were approximately 400 cysts/100 cm³ soil. During other months the SF method estimated higher numbers of cysts. There was no month × extraction method interaction for juveniles in year 1. In year 2, the CSF method again estimated higher numbers of cysts in September than did the SF method; this interaction was significant also for cysts in the combined analysis (Table 2). There was a significant month × extraction method interaction for juveniles in the combined analysis; the CSF method estimated higher juvenile numbers than did the SF method in August and September (Table 2). Cyst counts were significantly higher in the first year of the test than in the second (Table 3); juvenile numbers were similar in both years. Cyst numbers were approximately 14% higher on McNair 944 than on Coker 319, confirming previous population dynamics data (unpubl.); however, the difference was not significant.

There was no year × extraction method interaction for cysts, which indicated that the methods acted similarly in both years of the test; however, there was a significant year × month interaction for cysts (Table 2), probably because of the large peak in February and March of 1982 that did not occur in 1983. The peak in 1982 was not related to variations in soil temperature or moisture, as it was in 1983. The average February–March soil temperatures for 1982 and 1983 were within 1 C of each

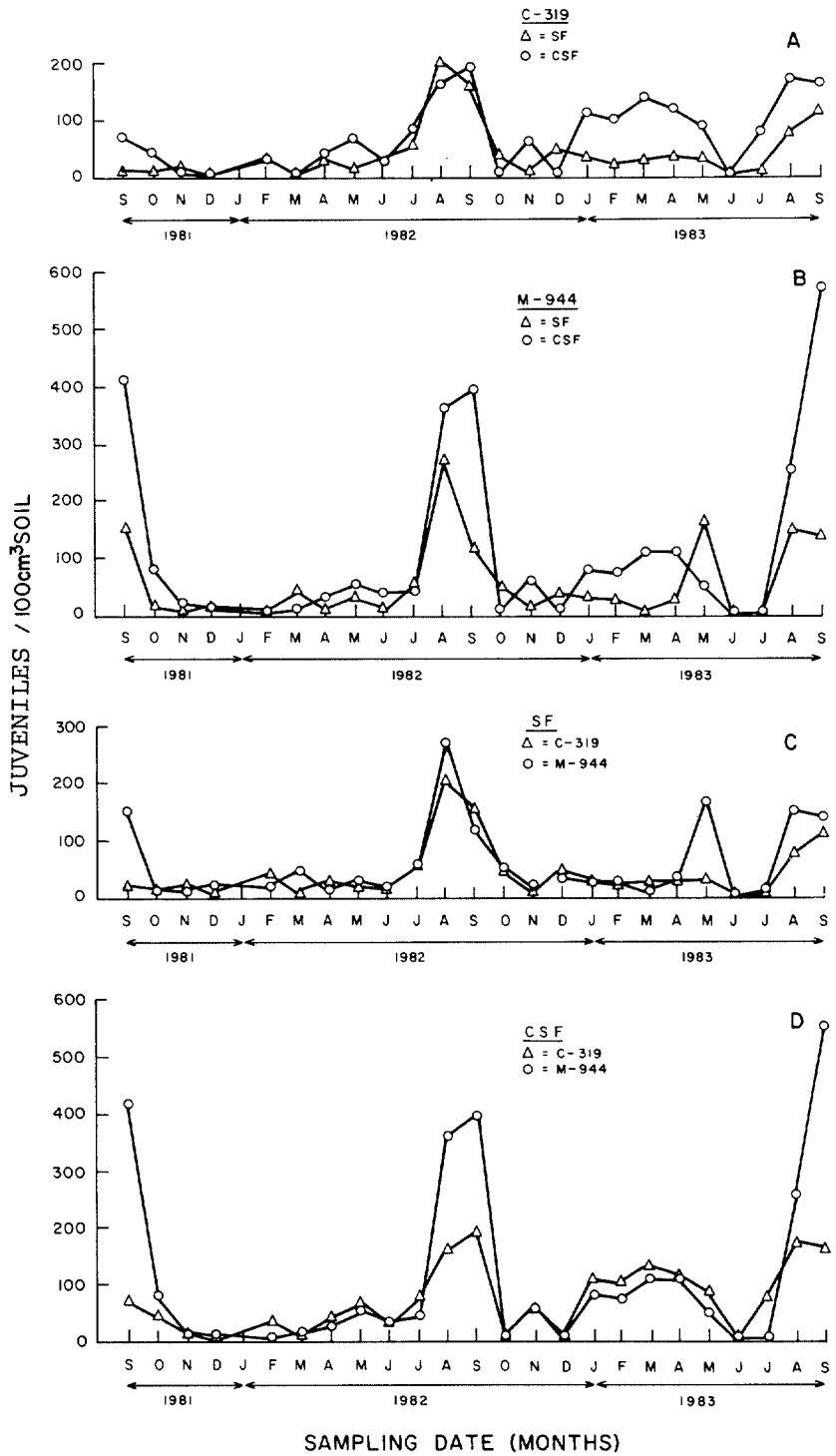

FIG. 1. Seasonal fluctuations in soil populations of juveniles of *Globodera tabacum solanacearum* averaged over four replicates. C-319 and M-944 = cultivars Coker 319 and McNair 944 flue-cured tobacco, respectively. SF = sugar flotation, CSF = centrifugal sugar flotation extraction methods. Graphs A and B depict methods within cultivars, C and D depict cultivars within methods.

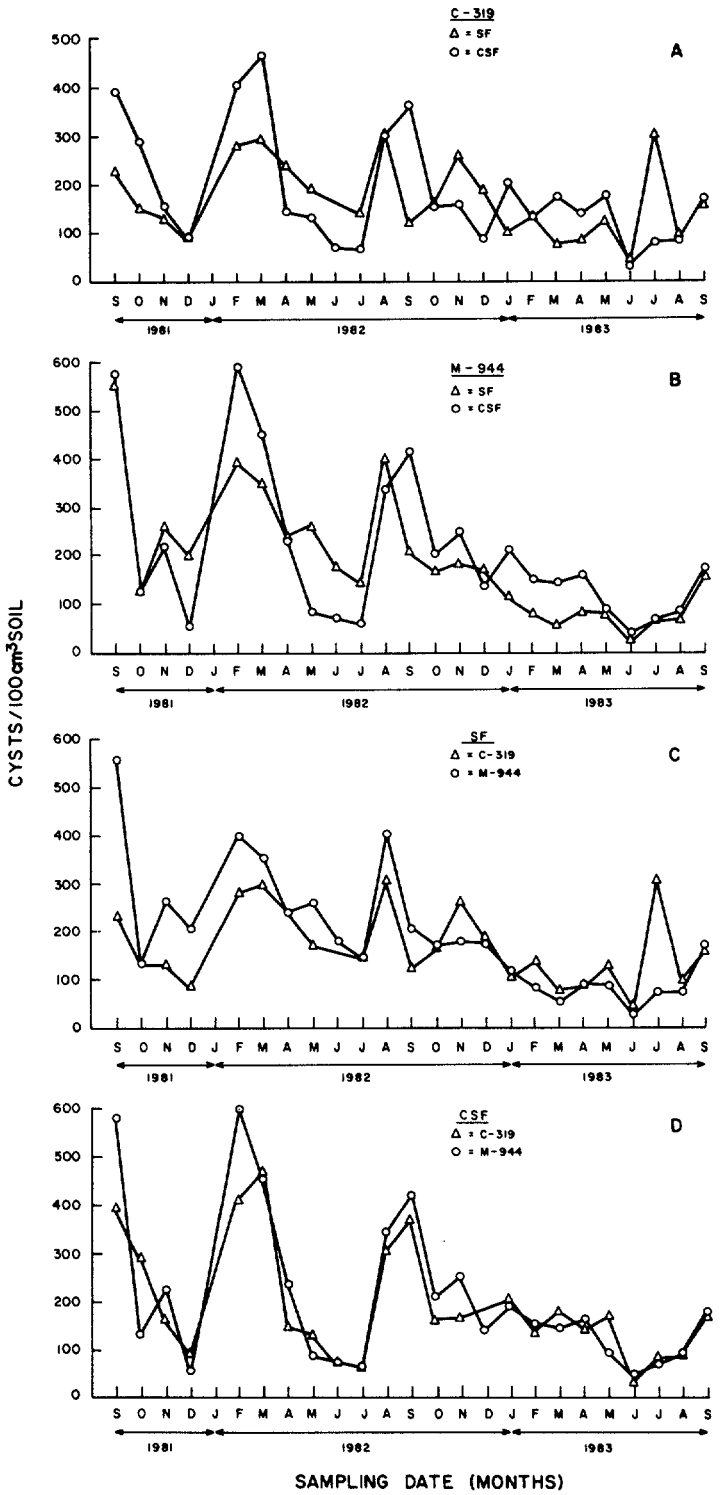

FIG. 2. Seasonal fluctuations in soil populations of cysts of *Globodera tabacum solanacearum* averaged over four replicates. C-319 and M-944 = cultivars Coker 319 and McNair 944 flue-cured tobacco, respectively. SF = sugar flotation, CSF = centrifugal sugar flotation extraction methods. Graphs A and B depict cultivars within methods, C and D depict methods within cultivars.

other. Also, the average soil temperature for the 3 days before sampling in February and March of 1982 were within 2 C of the temperatures during the same days in 1983. The freeze in January 1982 may account for the increase in cysts extracted during February and March 1982. This was the only time in the 2 years of the study when the soil was frozen, and samples were not collected. Freezing soil before extracting cysts by sugar flotation techniques may increase recovery (2), which may account for the year × month interaction for cysts. However, the January 1982 freeze did not appear to stimulate egg hatch during February and March, because juvenile numbers did not increase and no year × month interaction was noted for juveniles. No correlation existed between average monthly precipitation and numbers of cysts or juveniles.

Juvenile numbers were very low from October through July of both years (Fig. 1). The rapid decline in juveniles in October may be caused by tilling the field after harvest in late September. The fewest juveniles were recovered in June, again reflecting the adverse effects of land preparation. The sharp increase in juveniles in July was certainly due to egg hatch. A hatching factor from tobacco has been demonstrated for this nematode (4).

The immediate decline in cyst numbers from September to October (Fig. 2) reflected the influence of tilling which buries some cysts below the upper 15 cm soil where samples were collected. The gradual decline from November to April indicates that both extraction methods were more efficient during cold months than warm months or that there was a decline in actual cyst numbers due to biodegradation.

Estimates of soil populations of several endoparasitic nematodes followed a bimodal curve, with peaks in September–October and January–February, in a study of Barker et al. (3). Populations were followed for only 12 months, so the winter peak may not be a constant feature of those populations; instead, it may be caused by the freeze–thaw phenomenon mentioned. A similar bimodal curve for populations of *Meloidogyne hapla* on strawberries has been reported (9).

Nematode population densities were highest before tilling in September and October; therefore, this should be the best time of year to collect soil samples for nematode assay. Because of warm soil temperatures, early fall is also the best time to fumigate the soil. Temperatures above 13 C help volatilize fumigants and increase nematode activity, resulting in increased nematicidal action. The lowest numbers of cysts from both extraction methods (12–35% of the peak September population) were recovered from May through July. Cysts may go undetected in fields with low nematode populations if samples are collected after tilling because the number of cysts recovered drops sharply.

We conclude that both CSF and SF extraction methods give good recovery of cysts and juveniles of *G. tabacum solanacearum*, but the SF method recovers somewhat more cysts than does CSF when samples are collected during periods of low cyst counts. An advantage of the SF method is that an elutriator is not required. However, if soil samples are collected during September–October when nematode populations are high, the CSF method recovers more nematodes and requires less processing time per sample than does the SF method.

Literature Cited

1. Ayala, A. J., and A. C. Tarjan. 1963. Comparison of four methods for isolating nematodes from soil samples. The Journal of Agriculture of the University of Puerto Rico 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. Barker, K. R., C. J. Nusbaum, and L. A. Nelson. 1969. Seasonal population dynamics of selected plant-parasitic nematodes as measured by three extraction procedures. Journal of Nematology 1:232–239.

4. Fox, J. A., and A. J. Webber. 1970. The hatching response of Osborne's cyst nematode to root leachates from germ-free tobacco plants. Journal of Parasitology 56:105 (Abstr.).

5. Kable, P. F., and W. F. Mai. 1968. Influence of soil moisture on *Pratylenchus penetrans*. Nematologica 14:101–122.

6. Rickard, D. A., and K. R. Barker. 1982. Nematode assays and advisory services. Pp. 8–20 *in* R. D. Riggs, ed. Nematology in the southern region of the United States. University of Arkansas, Southern Cooperative Series Bulletin 276.

7. Seinhorst, J. W. 1956. The quantitative extraction of nematodes from soil. Nematologica 1:249–267.

8. Stone, A. R. 1983. Three approaches to the status of a species complex, with a revision of some species of *Globodera* (Nematoda: Heteroderidae). Pp. 221–233 *in* A. R. Stone, H. M. Platt, and L. F. Khalil, eds. Concepts in nematode systematics. New York: Academic Press.

9. Szczygiel, A. 1966. Studies on the fauna and population dynamics of nematodes occurring on strawberry plantations. Ekologia Polska Series A, 14: 651–709.

10. Viglierchio, D. R., and R. V. Schmitt. 1983. On the methodology of nematode extraction from field samples: Comparison of methods for soil extraction. Journal of Nematology 15:450–454.