Effects of Storage Temperature and Extraction Procedure on Recovery of Plant-parasitic Nematodes from Field Soils[1]

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Abstract: Storage of nematodes in soil at −15 C for 1 to 16 weeks greatly increased nematode recovery by a sugar-flotation-sieving procedure. One week of exposure to −15 C killed all nematodes except *Pratylenchus zeae* and *Tylenchorhynchus claytoni* which were recoverable in decreasing numbers up to 10 weeks by the Baermann funnel method. Optimum storage temperature for survival of most nematode species was 13 C. The numbers of *Meloidogyne incognita, T. claytoni, Belonolaimus longicaudatus,* and *P. zeae* recoverable by either extraction method remained constant or increased when stored at 13–24 C for 16 weeks. This was also true for *Helicotylenchus dihystera* and *Xiphinema americanum* extracted by the Baermann funnel technique, whereas the numbers retrieved by the sugar-flotation-sieving method decreased slightly. All species except *T. claytoni* decreased appreciably in soil stored at 36 C.

It is often necessary to store soil samples to be assayed for plant parasitic nematodes for varying lengths of time before laboratory processing. Such storage may affect the results of the assay. Nematode recovery may decrease if many of the individuals die during storage, or it may increase if sufficient emergence from root fragments or egg-hatching occurs. In experimental programs, standardization of soil storage and processing procedures is an important consideration.

Many plant-parasitic nematodes will survive for weeks or months in soil stored in plastic bags. Harrison and Hooper (7) showed *Longidorus elongatus* (de Man) Thorne and Swanger and certain other plant-parasitic nematodes could survive up to 29 months in moist soil. Oostenbrink (10) reported increased numbers of *Pratylenchus pratensis* (de Man) Filipjev in soil stored 18 weeks in plastic bags at 4–6 C, whereas *Tylenchorhynchus dubius* (Bütschli) Filipjev decreased during this storage time. Numbers of both species declined when soil was placed in paper bags. The recoveries of some species such as *Xiphinema americanum* Cobb have been shown to increase with storage at certain temperatures (6).

This investigation was undertaken with the following objectives: (i) to determine the effects of storage temperature and duration on survival of plant-parasitic nematodes in soil and (ii) to compare the effectiveness of the Baermann funnel method and the sugar-flotation-sieving method in extracting nematodes from samples exposed to various storage conditions.

MATERIALS AND METHODS

Soil samples were collected in October, 1966 from three locations in the Coastal Plain region of North Carolina. These soils, which were collected with a shovel to a depth of 15–20 cm, provided many different plant-parasitic nematodes. The soil types and 1966 crops were: (i) Appling loamy sand planted to tobacco (*Nicotiana tabacum* L.); (ii) Appling loamy sand supporting Johnson grass [*Sorghum halepense* (L.) Pers.]; and (iii) Norfolk sandy loam planted to cotton (*Gossipium hirsutum* L.).

Each soil was passed through a 6 mm sieve and thoroughly mixed in a concrete

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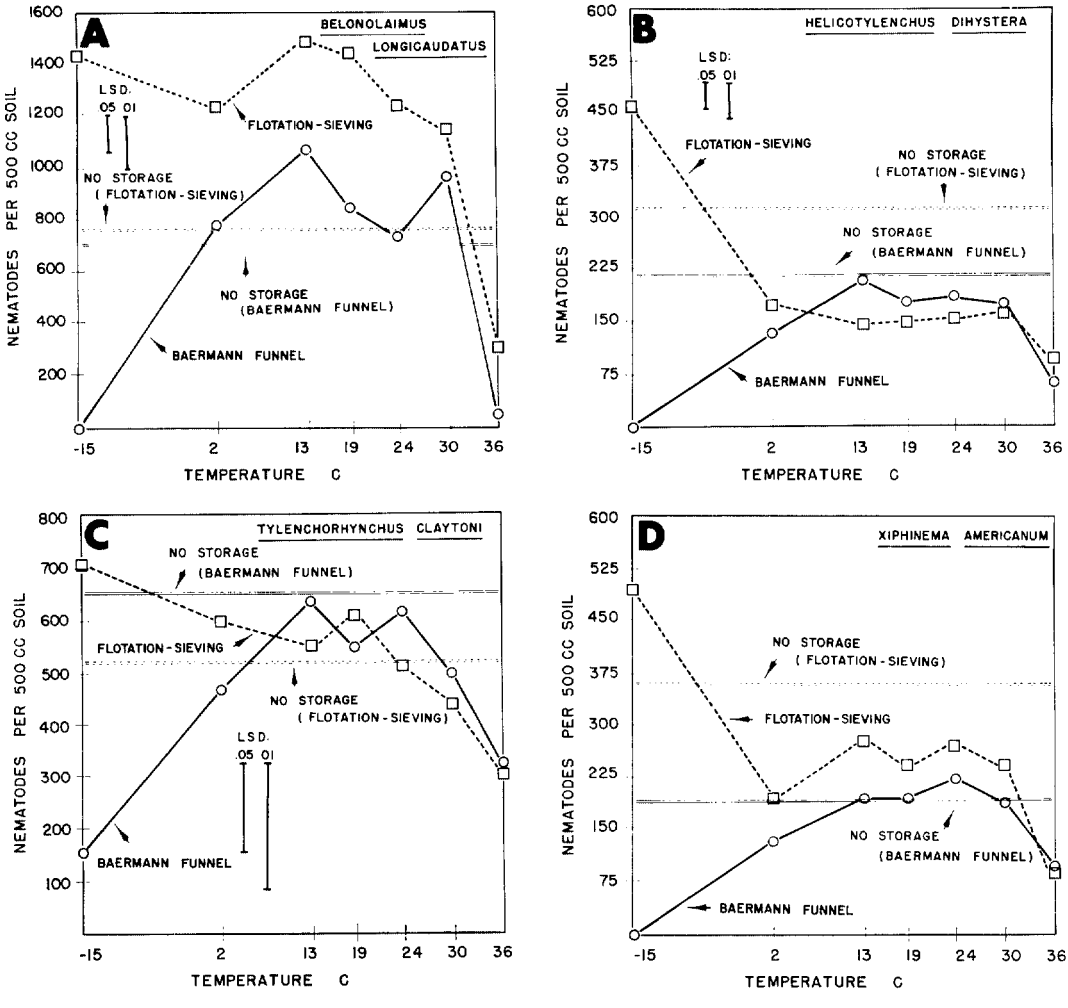

FIG. 1. Efficiency of sugar-flotation-sieving (SFS) and Baermann funnel (BF) methods for extracting ectoparasitic nematodes. A, B, C, D—Effects of storage temperature on nematode recovery. All temperature data are means of 10 extractions made during 16 weeks (excludes initial recoveries). LSD values can be used for comparing differences in either direction.

mixer within 24 hr after collection. Each bulk soil lot was subdivided into 250-cc units which were placed in plastic bags (500-cc capacity) for storage. Sufficient 250-cc lots of each soil were provided for all factorial combinations of seven temperatures, 11 extraction periods, including the no storage treatment, and three replications per temperature-evaluation period. All small bags of a soil to be subjected to a given temperature were placed in a large plastic bag to minimize moisture loss. The seven storage temperatures were: –15, 2, 13, 19, 24, 30, and 36 C.

Nematodes were extracted by two methods: Baermann funnel (BF) (11) and sugar-flotation-sieving (SFS) as described by Byrd *et al.* (4) and adapted from Caveness

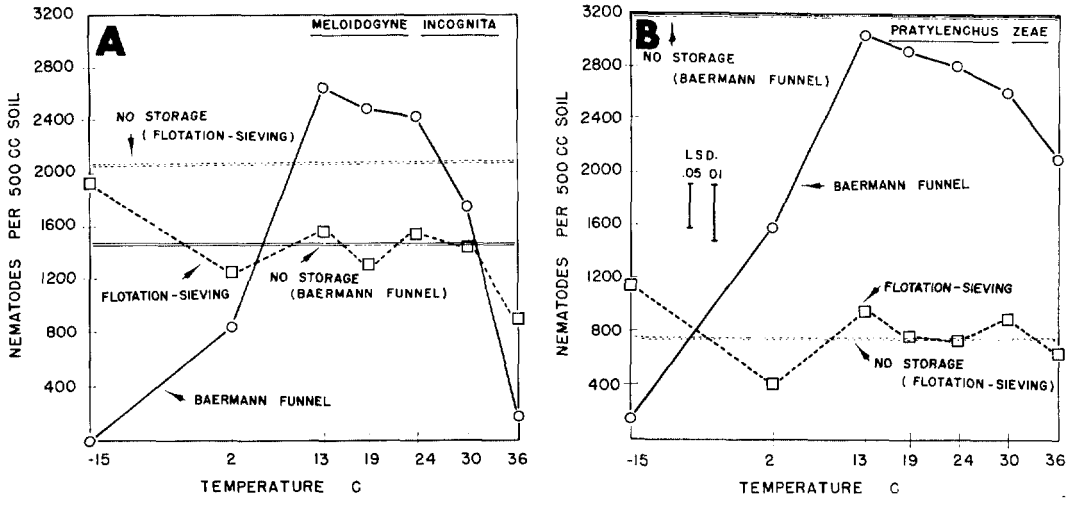

FIG. 2. Recovery of two endoparasitic nematodes by sugar-flotation-sieving (SFS) and the Baermann funnel (BF) methods. A, B—Effect of storage temperature on nematode recovery. All temperature data are means of 10 extractions made during 16 weeks (excludes initial recoveries). LSD values can be used for comparing differences in either direction.

and Jensen (5) and Jenkins (8). To compare these two methods, two sub-samples were taken from each of the three replications of a given treatment: 25 cc for BF and 50 cc of soil for SFS. An aqueous solution of 2 ppm Separan® 2610 (Dow Chemical Company, Midland, Michigan) and 2 ppm methylene blue was used in BF. Nematodes were collected after 3 days with this method (24 ± 2 C). Separan was also used in SFS where it induces soil colloids to settle rapidly (4).

Extractions by each method were made weekly for 4 weeks and bi-weekly thereafter for 12 additional weeks. Stereoscopic microscopes were used for all counts which were converted to numbers per 500 cc soil.

All data from both methods for a given nematode were combined in an initial analysis of variance to detect possible interactions between *storage temperature, time,* and *extraction method.* Based on these results, the data for given soil-nematode-method combinations were analyzed independently to determine *time* trends within *extraction methods.* Because a large number of soil-nematode-method combinations exist, results will be reported only for certain key combinations typical of the types of patterns which occur in these data. Nematode species-soil combinations selected for inclusion in this report were as follows: *Belonolaimus longicaudatus* Rau—soil #3; *Helicotylenchus dihystera* (Cobb) Sher—soil #1, 2 & 3; *Pratylenchus zeae* Graham—soil #2; *Meloidogyne incognita* (Kofoid & White) Chitwood—soil #1 & 3 (soil #3 had a few specimens of *M. hapla* Chitwood); *Tylenchorhynchus claytoni* Steiner—soil #2 & 3; and *Xiphinema americanum*—soil #2 & 3.

RESULTS

INTERACTION OF STORAGE TEMPERATURE AND EXTRACTION METHOD: Of the storage temperatures tested, –15 C gave the most striking results. Freezing the soil prior to extraction greatly increased the recovery of the ectoparasites, *B. longicaudatus, H. dihystera, T. claytoni,* and *X. americanum,* by SFS as compared to no storage. (Fig. 1). Less striking increases were usually obtained

TABLE 1. Changes in soil moisture during 16 weeks storage.

Storage Temperature (C)	Moisture content of each soil[a]		
	Soil #1	Soil #2	Soil #3
No storage[b]	7.87	12.25	8.27
– 15	7.80	12.77	7.32
2	8.00	13.00	7.39
13	7.42	13.25	7.22
19	8.16	13.13	7.89
24	6.94	12.56	7.48
30	5.52	12.30	4.72
36	4.27	9.89	4.95

[a] Expressed as a percentage of oven-dry weight of soil at end of experiment unless indicated otherwise.
[b] Moisture content at beginning of experiment.

with the endoparasites, *M. incognita* and *P. zeae* (Fig. 2). Although there were generally no significant interactions between *extraction method* and *temperature* for *X. americanum* (Fig. 1-D) and *M. incognita* (Fig. 2-A), the differences in recovery by BF and SFS at –15 C were significant at the 1% level. As expected, BF gave low yields in this treatment because most of the nematodes were killed. One week of exposure to –15 C killed all species except *P. zeae* and *T. claytoni* which survived in decreasing numbers for 10 weeks. Storage at 2 C also resulted in low recoveries of all nematodes when extracted by BF (Figs. 1 & 2). This was especially striking with the endoparasites, *M. incognita* and *P. zeae* (Fig. 2).

Maximum recoveries for all species studied, excluding –15 C for SFS, were obtained when samples were stored at 13 C. Population densities of all species, as measured by BF, remained about constant with storage time or increased in soil stored at 13 C (Figs. 1 & 2). However, the numbers of *H. dihystera* and *X. americanum* decreased under such conditions when assayed by SFS as compared to initial recoveries (no storage). Numbers of each species recovered from samples stored at 19, 24 and 30 C were similar to those for 13 C or slightly lower. Storage at 30 C resulted in moderate decreases of most nematodes species with time compared to no storage as will be shown later.

Exposure to 36 C markedly reduced recoveries with both extraction techniques. This treatment resulted in high moisture losses from the soil (Table 1). *Belonolaimus longicaudatus* (Fig. 1-A) and *M. incognita* (Fig. 2-A) were especially sensitive to this temperature, whereas the other species were less affected.

INTERACTION OF STORAGE TEMPERATURE AND TIME WITH TWO EXTRACTION METHODS: Data showing effects of *storage temperature* × *time* × *extraction method* on nematode recovery are presented only for *B. longicaudatus* and *P. zeae*. There were no significant interactions between these three factors in the combined statistical analyses for the other four species. Separate or independent analyses were also carried out for each nematode-soil-method combination. Even within these separate analyses, there was a tendency for *time* and *temperature* to interact, especially at the extreme temperature ranges. Therefore, results are based primarily upon these individual analyses for each soil-method-nematode combination. Interactions between *temperature* and *storage time* in the individual analyses were detected for the other species in some cases, but only data from the independent analyses for the two species indicated above are presented. (Tables 2 and 3).

Increases in the recovery of *B. longicaudatus* as compared to no storage were most striking with SFS at 13 C after 8 to 16 weeks storage (Table 2). High yields were usually obtained from soil stored at –15 C by this method regardless of storage time, whereas the recovery from this soil with the BF was nil. Storage at 36 C resulted in a rapid decrease in numbers of this species as measured by both extraction procedures. The greatest increase in nematode recovery with

TABLE 2. Effects of *storage time, temperature* and *extraction method* on numbers of *Belonolaimus longicaudatus* recovered from soil samples.

Storage Time	Mean number nematodes per storage temperature (C)[a]						
(Weeks)	−15	2	13	19	24	30	36
Sugar-Flotation-Sieving (Initial recovery = 757):							
1	1558**	1133	917	1192	1333*	1258	1225
2	1200	1500**	1408*	1342*	1192	1475*	867
3	1550**	1725**	1208	1608**	1283	992	558
4	1233	1333*	1142	1308*	992	950	250
6	658	916	1300	1075	1167	667	108*
8	1600**	1275	1817**	1283	1283	1300	0**
10	1817**	1175	1567**	1875**	1192	1500	8**
12	2092**	1350*	1608**	1567**	1033	1208	0**
14	1475*	850	2217**	1567**	1142	1017	0**
16	1100	1017	1667**	1600**	1675**	1050	0**
LSD (for comparing above means horizontally or vertically) .05 = 548†							
.01 = 721†							
Baermann Funnel (Initial recovery = 700):							
1	17*	1167*	1133	583	967	1250*	167*
2	0*	1000	1167*	917	817	1450*	167*
3	0*	867	1133	1083	983	1217*	33*
4	0*	633	983	500	833	883	33*
6	0*	633	767	783	533	1150*	33*
8	0*	933	967	717	383	650	0*
10	0*	817	1033	833	550	717	0*
12	0*	383	1133	1367*	483	583	67*
14	17*	850	1050	817	783	633	0*
16	0*	417	1317*	867	917	1067	0*
LSD (for comparing above means horizontally or vertically) .05 = 441†							

[a] Mean number nematodes per 500 cc of soil.
* Difference from no storage (initial recovery) significant at .05 level.
** Difference from no storage (initial recovery) significant at .01 level.
† LSD values for comparing *storage time* × *temperature* × *extraction method* means (independent analysis for each extraction method).

BF occurred after 1 to 3 weeks storage at 30 C. The harmful effects of storage at 2 C on this nematode as determined by BF were not pronounced, but increased with time. Generally, the numbers of *B. longicaudatus* recovered by SFS increased with storage time at 13 and 19 C, whereas the recovery by BF remained fairly constant (the low recoveries at 6 weeks were apparently due to the use of a faulty sieve).

The most consistent and highest recoveries of *P. zeae* were obtained by BF from soil stored at 13 to 24 C (Table 3). The detrimental effects of exposure to −15 and 2 C, as indicated by this method, increased with time. The number of nematodes recovered by BF dropped to 2% of the initial number after 16 weeks storage at 2 C in contrast to little change at 13 to 24 C. Storage at 30 or 36 C eventually resulted in decreases in numbers of *P. zeae* as measured by this technique. SFS yielded much lower numbers of this nematode than did BF. Significant differences in nematode recovery at the various *storage temperatures* with *time* for SFS were detectable only when data for the two methods were analyzed independently (Table 3). The highest and most consistent yields by this method were from soil stored at 13 C for 12 to 16 weeks.

TABLE 3. Effects of *storage time, temperature* and *extraction method* on numbers of *Pratylenchus zeae* recovered from soil samples.

Storage Time	Mean number nematodes per storage temperature (C)[a]						
(Weeks)	−15	2	13	19	24	30	36
Sugar-Flotation-Sieving	(Initial recovery = 750):						
1	1917**	583	825	883	625	667	592
2	850	317	725	450	408	367	475
3	1467**	342*	758	658	633	758	567
4	1025	358	533	525	550	633	550
6	858	475	775	550	650	1025	775
8	867	617	1033	583	775	1233*	875
10	1158**	292*	867	908	950	1083	733
12	1533**	292*	1392**	1183*	825	950	675
14	717	408	1200*	1167*	908	858	700
16	1092	250*	1242*	825	908	1142	317
LSD (for comparing above means horizontally or vertically)				.05 = 441†			
				.01 = 526†			
Baermann Funnel (Initial recovery = 3230):							
1	133**	2567	2683	3367	2450	4517	1967
2	233**	1800*	3383	1767	4400	3317	3683
3	467**	1283**	2483	3317	2767	4367	2417
4	350**	2617	3533	3033	3433	1867	4433
6	283**	1067**	3000	2650	3833	1967	2883
8	17**	1033**	3067	2950	4083	2417	1633*
10	83**	750**	3117	2883	1383	2417	1933
12	0**	433**	2733	2900	2467	2000	783**
14	0**	250**	3683	3100	3200	1250**	1167**
16	0**	67**	2633	4017	2900	2683	233**
LSD (for comparing above means horizontally or vertically)				.05 = 1419†			
				.01 = 1865†			

[a] Mean number nematodes per 500 cc of soil.
* Difference from no storage (initial recovery) significant at .05 level.
** Difference from no storage (initial recovery) significant at .01 level.
† LSD values for comparing *storage time* × *temperature* × *extraction method* means (independent analysis for each extraction method).

DISCUSSION

The finding that the sugar-flotation-sieving method (SFS) (4) is more efficient for most ectoparasitic species than the Baermann funnel method (BF) is not surprising. Ayala *et al.* (1), in comparing four methods, found the basic sugar-flotation technique to be superior for nematode recovery from most soils. Although the results with the BF were more variable, it gave much greater recoveries of the endoparasite, *P. zeae*, than SFS. The effectiveness of both methods may also be greatly altered by the time of the year in which the samples are collected (2).

The marked increase in the recovery of nematodes by SFS following storage at −15 C was unexpected. The mechanism involved has not been illucidated. However, freezing is known to result in partial dehydration of cells (13) and may also increase their permeability. Sayre (12) observed that exposure of *M. incognita* larvae to −8 C caused the cuticle to rupture. It appears likely that nematodes are injured in this manner and would become plasmolyzed readily when placed in sugar solutions. Both phenomena could be involved and result in increased buoyancy of the nematodes, thus accounting

for the increased efficiency of SFS in assaying frozen soils. Since the susceptibility of nematode species to freezing varies (12), recoveries of various species, especially *Meloidogyne* sp., from frozen soil may also vary with this extraction technique.

The results, indicating 13 C to be near the optimum storage temperature for survival of the nematodes investigated, agree closely with the findings of other researchers. Bergeson (3) showed that larvae of *M. incognita* survived up to 12 months at 10 C and only 1 month at 38 C. Kerr and Vythilingam using BF (9) found that storage at 25 C resulted in higher yields of *Pratylenchus loosi* Loof than when samples were stored at higher temperatures. These high recoveries from storage temperatures ranging from 10 to 25 C are apparently the result of reducing the activity and metabolism of the nematodes and keeping them physiologically young as suggested by Van Gundy *et al.* (16).

The low recoveries by BF of all species from soil stored at 2 C may result from chilling injury of the nematodes as concluded by Sayre (12) who studied *M. incognita* exposed to 4 C. Thomason *et al.* (14), Van Gundy (15), and Van Gundy *et al.* (16) obtained similar results with *Meloidogyne javanica* (Treub) Chitwood and *Tylenchulus semipenetrans* Cobb in relation to storage temperature and nematode motility. They found 5 C decreased motility and infectivity. Reduced motility could explain the low recoveries by BF from soil stored at 2 C as compared to SFS which does not depend on nematode movement.

The reduced nematode yields from soil samples stored at 30–36 C may reflect the loss of soil moisture (Table 1) which may require an increased utilization of energy for survival as suggested by Van Gundy *et al.* (16). Their finding that the body contents are used up rapidly at high temperatures helps explain the rapid declines at 30 and 36 C. Moreover, nematodes that succumb at these temperatures may collapse and lose their identity.

The maintenance of fairly consistent population densities of most species over a 16-week period at optimum storage temperatures indicates that soil samples can be stored for many purposes. Storage may be especially helpful in programs where assays of nematode populations are desired. The longevity of most species stored at 13 C as compared to the detrimental effect of storage at 2 or 30–36 C serves to illustrate the importance of maintaining the proper temperature.

The results of this study demonstrate how various storage temperatures may affect the efficiency of nematode extraction procedures. If sugar-flotation-sieving methods of extraction are employed, soil samples may be stored at –15 or 13 C. Although the individual specimens from –15 C are probably not as well preserved as with the freezing technique described by Zuckerman (17), they can be readily identified. If methods that depend on nematode motility, such as the Baermann funnel procedure, are used, samples may be stored at 13 C. Since freezing the soil at –15 C kills most nematodes very quickly, this storage temperature is not suitable when the Baermann extraction method is employed.

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