Two Semi-automatic Elutriators for Extracting Nematodes and Certain Fungi from Soil[1]

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Abstract: Two efficient, semi-automatic elutriators for assaying soil samples for nematodes are described. The first apparatus is a four-unit elutriator which combines conventional extraction methods with the following major features: automatic mixing of 500- to 1,500-cm^3 soil samples with water (± air); "turbinate" sample splitters from which fractions of 1/15 or greater are passed onto 26- or 38-μm sieves for collection of larvae and adult nematodes; the capacity for collecting roots, intact egg masses, and cysts on 250-425-μm sieves; and a variable speed motorized sieve-shaker. Nematodes, after being collected on 38-μm sieves, are separated from debris by centrifugation or by Baermann trays. Secondary features include: air cylinders, solenoid valves, and time clock for automatic dumping residual soil and water; relay-controlled coarse spray nozzles activated for 5 sec every 30 sec for washing nematodes through 250-425-μm sieves; adjustable rates of water and air flow, and timing. The second type of elutriator operates on similar principles but costs less to construct. It requires somewhat more operator participation; sieve spraying is carried out by the operator, and elutriators are dumped manually. Both elutriators also show promise for monitoring populations of certain other soil microorganisms.
Key Words: population dynamics, techniques.

Much progress has been made in developing efficient procedures for extracting nematodes from soil, but investigations of nematode numbers as related to crop damage and other studies dealing with population dynamics are frequently of limited value because of unmanageable variation in sampling and extraction. Many techniques used currently, including the Oostenbrink elutriator (11), the Seinhorst elutriator (13), and sugar-flotation extraction procedures (1, 4, 6, 7, 8), are based to varying degrees, on the flotation and sieving principles developed originally by Cobb (5).

A major problem with all extraction procedures is obtaining a representative subsample of larger soil samples collected from plots or fields. In North Carolina, for example, we have used three sample splitters (The W. S. Tyler Co., Mentor, Ohio) mounted on a reciprocal shaker to obtain a representative sub-sample of 50-100 cm^3 of soil from larger sample of 1,000-1,500 cm^3. The magnitude of the problem in obtaining reliable sub-samples was illustrated by Proctor and Marks (10). They used a modified Baermann-pan technique (14) to extract nematodes from two 25-gm subsamples from single core samples and from five 50-gm subsamples taken from 20- or 40-core samples. Because of the variation encountered, they estimated that at least 7 h of field sampling and laboratory analysis were required to estimate the population of a 0.01-ha plot within 20% of the true mean with 95% confidence limits.

Since soil mixing prior to sub-sampling and current extraction procedures involve considerable labor, efforts were invested in developing two types of semi-automatic elutriators. These incorporate many advantages of current and new approaches. In addition to extraction of larvae and adults of most nematode species, egg masses of *Meloidogyne* spp., roots containing *Pratylenchus* spp. and cysts of *Heterodera* spp., certain fungal spores and sclerotia also can be collected.

Description of apparatus: Two types of apparatus were developed. One is more complex and requires more components, whereas the second apparatus, which is a modification of the apparatus developed by Byrd et al. (3), can be constructed with minimal costs. Brief descriptions of each apparatus are presented. The first is referred to as the "North Carolina elutriator (NC-El)," and the second as the "California

Received for publication 16 January 1976.
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elutriator (CA-El)" because they were developed primarily at these respective locations.

The NC-El consists of four, modified Oostenbrink elutriators (11) supported by a steel frame (Fig. 1-5). Soil samples are mixed, and nematodes are floated out of soil in the stainless steel elutriators by flow of water or water-air mixtures (Fig. 1-A) similar to those of Coolen and d'Herde (6) and Gooris and d'Herde (7). Nematodes, debris, and root fragments fall onto 15-cm diam, 425-μm sieves (Fig. 1-B), where debris and roots are trapped. Nematodes pass through a 20.3-cm diam stainless steel funnel (Fig. 1-C) and are directed uniformly onto a conical surface of the sample splitter (Fig. 1-D, 5-E). The splitter is a turbinate-shaped stainless steel pan (25.4-cm diam) with 15 equally spaced outlets (7.9 mm

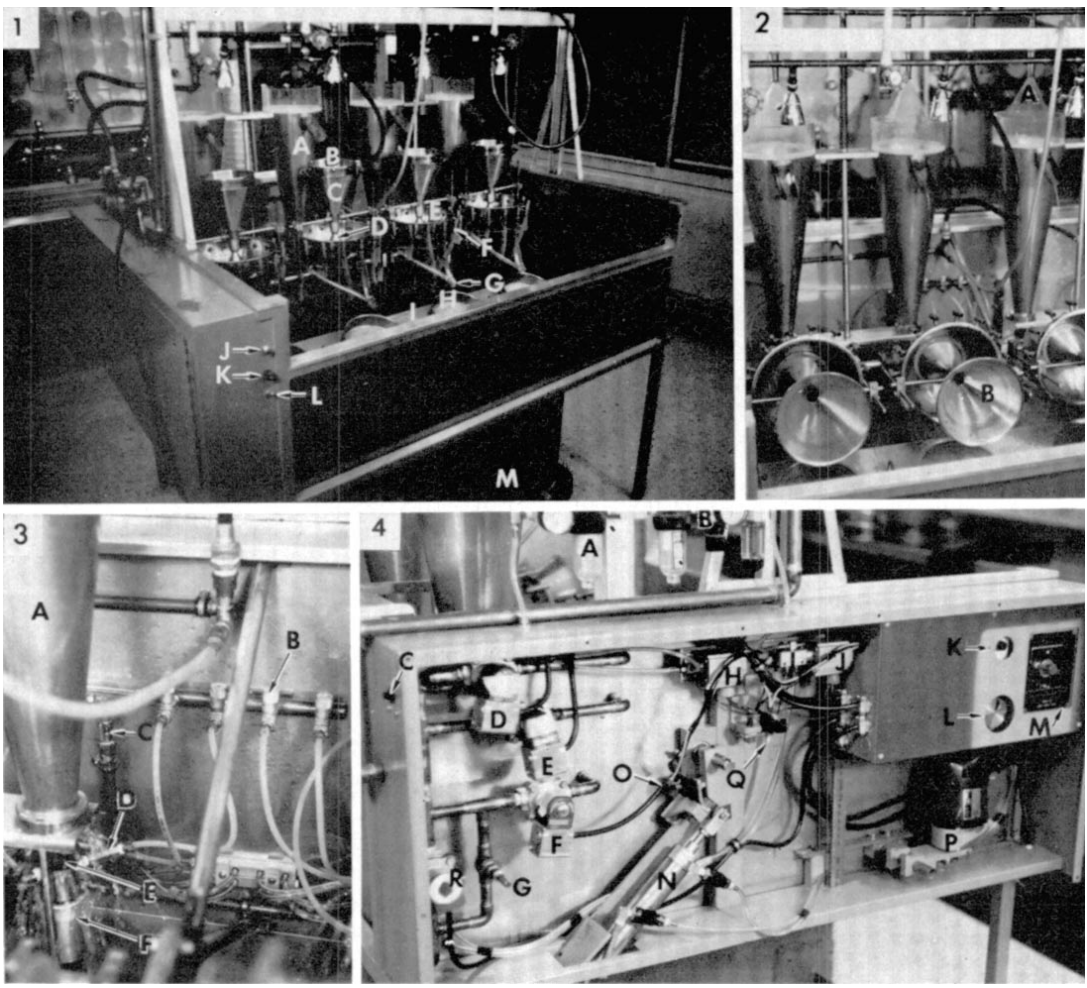

FIG. 1-4. N. C. elutriator for extracting nematodes. *1)* General view of machine. A) Elutriator funnel. B) Sieve (425-μm). C) Receiving funnel of sample-splitter. D) Inner, conical surface of turbinate sample-splitter. E) Sample-splitter, with 15 outlets. F) Anti-siphoning device. G) Tubes which direct flow of suspensions. H) Sieve (38-μm). I) Motorized-sieve shaker. J) Switch for dumping sample-splitters. K) Safety fuse. L) Primary switch. M) Soil trap. *2)* Close-up view of elutriator. A) Fine spray which washes funnels. B) Sample-splitters in dump position. *3)* Bottom view of A) Elutriator, with B) Air, and C) Water controls. D) One-way air valve. E) Elutriator valve with water and air inlets. F) Air cylinder. *4)* Controls of elutriator. A) Air filter and gauge for elutriators. B) Air filter and gauge for air cylinders. C) Switch which turns on timer. D) Intermittent, water-spray solenoid valve. E) Water-solenoid valve for filling sample-splitters. F) Water-solenoid valve for washing elutriators. G) Valve for secondary water supply to elutriator valves. H) Air-solenoid valve for elutriators (for air-water mixture). I & J) Four-way, air-solenoid valve for air cylinders of elutriators. K) Variable-time relay. L) Two-lobe cam clock motor. M) Time clock. N) Double-action air cylinder. O) Microswitch controlling valve (F). P) Variable-speed drill motor. Q) Microswitch which controls valve (E). R) Solenoid valve for primary water flow to elutriators (see text for further details).

FIG. 5. N. C. elutriator in operation. A) Fine-spray nozzle. B) Coarse-spray nozzle. C) Elutriator. D) Sieve (425-μm). E) Sample-splitter. F) Sieve (38-μm). G) Sieve shaker.

I.D.) at the same level above the base (Fig. 1-F). A representative aliquant (1/5) is directed onto 38-μm sieve by tubes attached to three outlets 120° apart (Fig. 1-G). The remaining outlets are allowed to drain into a soil trap. Other aliquant fractions may be directed onto the same sieves or another sieve simultaneously. Nematodes and certain fungal spores are collected on the 38-μm sieves (Fig. 1-H, 5-F).

There are several automated features in NC-El that are not present in CA-El. Air cylinders (Fig. 3-F), which dump residual soil and water from the elutriators and sample splitter, are controlled by solenoid valves wired to a time clock (Fig. 4). An air-water mixing unit [with 15 holes (2-mm diam; 45.5-mm^2 total area/funnel) and a rubber stopper], which is attached to an air cylinder (Fig. 3-E), is supplied with a secondary water flow to prevent clogging as the primary water flow is stopped when the residual soil is dumped. The primary water flow through four tubes of 8-mm I.D. is 60-90 ml/elutriator/sec. Air-flow to elutriator is regulated as needed with a filter-regulator-lubricator unit (Fig. 4-A) and with individual valves for each elutriator (Fig. 3-B). Air pressure for the air cylinders is 4-6 kg/cm^2; air and water flows must be adjusted according to the material to be extracted. For example, with fine soil, no air is needed to recover nematodes of limited size, such as larvae of *Meloidogyne* species, whereas a high rate of air (50-cm^3/sec) is necessary for recovery of root fractions. A variable-speed, motor-powered shaker [which shakes the sieve shelf (Fig. 1-I, 5-G) to prevent clogging of the sieves] is wired into the time clock and runs only when primary water and air are flowing. An adjustable, coarse-spray nozzle (shower head) over each receiving funnel (Fig. 5-B) is activated for 5 sec every 30 sec during soil extraction (by a microswitch and cam) for rinsing nematodes or spores through the 425-μm sieve. A fine-spray nozzle (garden hose) is attached over each elutriator (Fig. 5-A) and is activated for washing elutriators as residual soil is discarded. The sample-splitters are rinsed by hand with a fine, high-pressure nozzle after air cylinders move them into the "dump" position (Fig. 2). Timing and rate of water flow and air flow for each operation are adjusted as needed. After elutriation for 2-8 min (usually 3 min), the nematodes and root or fungal fractions collected on sieves are further processed by hand.

The CA-El operates on similar principles, but the construction is different (Fig. 6-7). The elutriator portion is composed of four polyethylene funnels (25 cm diam) modified to form spouts at the top (Fig. 6-A). The basic frame is composed of exterior plywood. An air-water mixture is introduced through the stem of each funnel (Fig. 7-A). Air is channeled, through a copper tube manifold lying along the lower portion of the funnel, to achieve constant mixing of soil during extraction (Fig. 7-B) and to prevent soil from settling without mixing. Air inflow is governed by a pressure regulator and set at 1.0 kg/cm^3 routinely. The use of air achieves flotation with minimum water flow. Water flow is regulated and standardized by a mercury manometer.

Water flowing over each funnel spout passes through a 250- or 425-μm sieve, supported on a mesh shelf, to remove organic material, egg-masses, cysts, or fungal spores (Fig. 6-B) and into a 20.2 x 22.8 x 4.5 cm sample-splitting tray (Fig. 6-C). Each sample-splitting tray has 10 outlets (1.1 cm

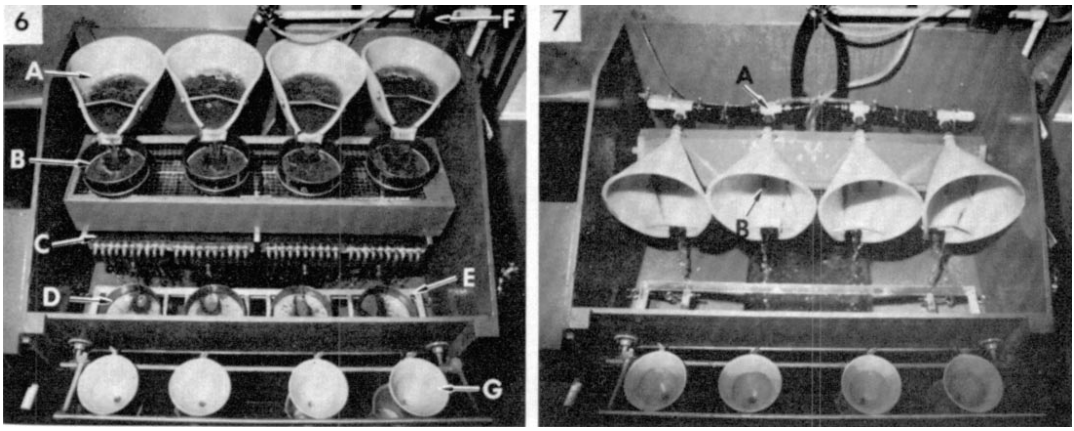

FIG. 6-7. California elutriator. *6)* Machine in operation. A) Elutriator funnel. B) Sieve (425-μm). C) Sample-splitter with 10 outlets. D) Sieve (38-μm). E) Motorized-sieve shaker. F) Manometer for metering water flow. G) Funnel assembly for washing samples from 38-μm sieves. *7)* Elutriator in dump position. A) Water and air inflow manifolds. B) Copper-tube manifold in elutriator for air dispersal.

I.D.) at one end, any number of which can be used to fractionate the sample before directing a portion onto a 45- or 38-μm sieve (Fig. 6-D). Sample subdivision is necessary to reduce the amount of fine soil falling on sieves. The 38-μm sieves are supported on an aluminum-angle shelf (Fig. 6-E) which is shaken as is the one in the NC-El. The unit can be operated without the shaker, but it requires greater operator supervision. Use of the shaker becomes more necessary as greater aliquant fractions are collected from the sample splitter. After a standard elutriation time, depending on soil type, of 2.5-4 min, sieves are removed; the elutriators, shelf, and dividing trays are pivoted on their supporting axis; and the contents dumped into a soil trap below the unit (Fig. 7). A two-way valve reduces water flow in the elutriators and operates sprays which flush the sample-splitting trays as they are dumped. The elutriator funnels are self-rinsed by the continued water flow. An alternate set of sieves is used to start the elutriation of the next set of samples while the previous samples are collected from the sieves by rinsing them through funnels attached to the front of the unit into beakers (Fig. 6-G). A fine, high-pressure spray nozzle attached to the sieves is used to rinse the sieves.

Automatic controls may also be added to the basic CA-El. A rheostat-controlled, motorized cam currently operates microswitches which activate various functions of the unit. The rheostat setting regulates the length of extraction time and is varied according to soil type. Microswitches activate the sieve shaker, banks of sprays over the elutriator funnels (Fig. 6-A), and 250- or 425-μm sieves (Fig. 6-B). Sprays over the elutriators are activated for four 15-sec periods during the cycle to wash debris from the edge of the funnels. Those over the sieves are activated for the last 20 sec of the extraction cycle to rinse nematodes adhering to the organic debris into the sample splitter. Manual over-ride switches are incorporated in each circuit to allow operator intervention at any time during the extraction cycle. Other microswitches operated by the cam activate a warning light and buzzer at the end of the extraction cycle and a counter which tallies the number of cycles completed. The automatic controls insure consistency of procedure among batches of samples, reduce operator-supervision time, and allow his participation in subsequent stages of the process.

Efficiency of apparatus as compared with other techniques: Highest recoveries, in comparison with conventional centrifugal flotation (CF), Baermann funnel (BF), sugar-flotation-sieving (SFS), and NC-El + BF (Table 1), of most nematodes were obtained from soil collected in June with the NC-El without air and centrifugal flotation. NC-El + CF and CF alone yielded greatest numbers of most nematode species counted. Only CF and NC-El + CF were suitable

TABLE 1. Comparative efficiency of methods of extracting nematodes.[a]

Method	Number of nematodes/500 cm³ of soil								
	Meloidogyne sp.	*Pratylenchus* sp.	*Tylenchorhynchus claytoni*	*Helicotylenchus dihystera*	*Paratrichodorus christiei*	*Criconemoides* sp.	*Belonolaimus longicaudatus*	*Hoplolaimus galeatus*	Total
CF	80	110	210	64	40	256	21	26	809
BF	30	104	84	10	24	8	0	15	225
SFS	22	73	131	67	33	28	20	18	384
NC-El + CF									
Air	76	70	208	113	18	228	21	30	659
No Air	67	123	215	92	38	301	11	30	1001
NC-El + BF									
Air	66	58	118	44	31	15	15	24	350
No Air	73	66	146	78	30	13	14	23	434
LSD (P=0.01)	46	44	65	55	NS	66	20	17	228

[a]Soil collected from several crops in microplots at Clayton, N. C., June 1975. (CF = centrifugal flotation; BF = Baermann funnel; SFS = sugar-flotation-sieving and NC-El = North Carolina elutriator).

for extracting *Criconemoides ornatus.* In comparison with water only, the use of air-water mixtures with NC-El depressed the recovery of some species, but differences for most taxa were insignificant. Although the numbers of *Meloidogyne* spp. recovered by NC-El + CF and NC-El + BF were similar in early summer (Table 1), either elutriator (NC-El or CA-El) + BF gave greater recoveries than when these machines were combined with CF in the fall (Table 2). Utilizing a 38-μm sieve in lieu of 45-μm sieves greatly increased nematode recovery. The speed of shaking sieves and the angle on sieves had little influence on nematode recovery. The NC-El + BF or CF gave numbers similar to the CA-El + BF or CA-El + SFS. The relative efficiency of the NC and CA elutriators was tested extensively. The former recovered greater numbers of nematodes in some tests, but differences were not always significant. Shipping infested soil from California to North Carolina, however, caused some species, especially *Tylenchulus semipenetrans,* to coil. This change apparently increased the number of larvae trapped on a 38 or 45-μm sieve.

TABLE 2. Influence of sieve-size on relative efficiency of extraction procedures.

Method and Sieve opening[a]	Number nematodes extracted (per 500 cm³ soil)	
	Tylenchulus semipenetrans	*Meloidogyne incognita*
SFS 45-μm	4,200	480
SFS 38-μm	8,411	606
CF 45-μm	9,363	721
CF 38-μm	14,278	871
BF 45-μm	—	898
BF 38-μm	—	1,146
Sieve Means:		
45-μm	6,782	700
38-μm	11,344**[b]	874**
Method Means:		
SFS	6,306	543
CF	11,821**	796**
BF	—	1,022**

[a]Residue from NC elutriator extracted further by method indicated (SFS = sugar-flotation-sieving; CF = centrifugal flotation; BF = Baermann funnel). Soil collected in California and mailed by air to N. C., November, 1975. CA-El gave similar recoveries, but data not included.

[b]Asterisks (**) indicate a significant difference as compared to first mean (P = 0.01).

Rates of nematode recovery from artificially infested soil varied greatly with nematode species. NC-El + CF gave higher recoveries of *C. xenoplax* (79%) than conventional centrifugal flotation (62%). CF, however, gave higher recoveries of *B. longicaudatus* (65% vs 41% for NC-El + CF) and *M. javanica* (54% vs 35% for NC-El + CF).

These elutriators have the built-in capacity for extracting nematodes from soil and collecting root fragments infected with endoparasitic nematodes which can be extracted by mist or shaker (2, 12). The number of *Pratylenchus brachyurus* extracted from fragments of peanut roots (collected from soil samples) in a mist chamber in August was 6- to 23-fold greater than those in the same volume of soil. For the soil fraction, yields/500 cm of soil for various methods were: sugar-flotation-sieving—333; centrifugal flotation—1,320; and NC-El + BF — 1,398.

Possible use of the elutriators in extracting fungi: In observations of nematodes recovered with these techniques, high numbers of chlamydospores of fungi such as *Glomus* and related genera were seen. The NC-El has been used to collect roots of soybean that are infected with *Glomus* spores. When combined with blending of roots, this technique provides a very effective means of recovering such spores for experimental use. The CA-El is very effective in extracting *Glomus* chlamydopsores from citrus soil.

DISCUSSION

Both semi-automatic elutriators combine the advantages of conventional elutriators with several new features. The semi-automatic, time-controlled modifications reduce labor input 30-40% because no premixing of soil samples is necessary. The use of 500-1,000-cm^3 samples in these elutriators reduces the variability encountered by nematologists. The coefficient of variability within similar samples can still be around 20-30%. The combination of the sample-splitter with the elutriator makes it possible, in assaying large soil samples, to reduce variation and increase efficiency in nematode recovery. This basic problem of variation has been strikingly demonstrated by Proctor and Marks (10). The addition of a motorized sieve-shaker increases the capacity of the apparatus to handle a wide range of soil types and reduces labor input. For sandy soils, the shaker can be run at a low speed, whereas with clay soils, it is necessary to use a higher speed. The motorized shaker on the NC-El is so constructed that two subsamples can be collected simultaneously from a given soil sample (capacity of eight sieves—only four shown in figures), and these may be processed further by techniques such as centrifugal flotation (8) and Baermann trays (14).

The features combined in both elutriators have wider applications than any previous extraction system. They can be used for extracting vermiform nematodes in soil, collecting roots for extraction of endoparasitic species in mist chambers (12), and collecting eggs and cysts of certain nematode species as well as spores and sclerotia of certain fungi. The application of these apparatus for extraction of microsclerotia of *Cylindrocladium* spp. and reproductive structures of other fungi from soil are being investigated by Phipps et al. (9). The recovery of high numbers of spores of endomycorrhizal fungi with nematodes indicates that these machines show promise for assaying populations of some fungi.

Both elutriators still have disadvantages which are inherent in any extraction procedure in which sieves are used. With *Meloidogyne* spp., for example, only 20-40% of second-stage larvae are recovered from soil because a high percentage of these small nematodes are washed through the 26-45-μm sieves. Use of the Baermann funnel in lieu of CF or SFS in mid to late summer resulted in greater yields of this species, partly because some eggs hatched in the funnels. Much of the loss of small nematodes, such as *Meloidogyne* larvae, was due to repeated sieving in the centrifugation process. Approximately 5-10% of *M. incognita* larvae were lost with each sieving on a 500-mesh (26-μm) screen. Much of this loss can be prevented by allowing nematodes to settle and then decanting in the several steps of centrifugal flotation. Another problem that may be encountered with fine sandy loam soils involves the excessive amounts of soil washed through the system onto the 38-μm sieves when a high rate of

air flow is used to recover large root fragments for extracting eggs of *Meloidogyne* spp. or for recovering roots for other purposes. Greater amounts of silt lowered the recovery of nematodes by CF or SFS.

In spite of some minor problems with these elutriators, their capacity to handle large soil samples with no premixing and their potential for recovering nematodes and reproductive structures of certain fungi can improve precision in experiments with these organisms. Both elutriators should prove useful in nematode advisory programs.

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