A Method for Estimating Numbers of Eggs of Meloidogyne spp. in Soil[1]

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Abstract: A procedure for extracting eggs of *Meloidogyne* spp. from soil was developed by modifying and combining certain existing techniques. Egg masses were elutriated from the soil, gelatinous matrices of the egg masses were dissolved, and the dispersed eggs were stained to facilitate counting. Data on egg population densities thus obtained facilitate the study of population dynamics of *Meloidogyne* spp. and the analysis of root-knot epidemics. *Key Words:* egg extraction, population dynamics, root knot.

Studies of population dynamics of root-knot nematodes, *Meloidogyne* spp., have been hindered by the lack of a suitable technique for extracting eggs from the soil (5). Population density estimates based upon extraction of free-living larvae may be inaccurate if a large proportion of the individuals is in the egg stage. Dickson and Struble (3) developed a sieving and staining technique for extracting egg masses of *M. incognita* from soil. We used this technique with limited success on numerous samples taken from *Meloidogyne*-infested fields in North Carolina. In general, the procedure was too time-consuming to satisfy our needs.

In 1970, we undertook the development of a procedure for extracting *Meloidogyne* eggs from soil by modifying and combining certain existing techniques. It is based upon the premise that the egg masses remain intact in the soil, either free or attached to host roots or root fragments (2). Much attention was given to the evaluation and improvement of each step as our studies progressed. The major steps are as follows.

A. *Extraction of egg masses.*

1. A soil sample is mixed with a 15 X 25 cm sample splitter with 12-mm separator slots (Model SS 50, W. S. Tyler Co., Mentor, Ohio). The divided sample is then composited. The splitter is cleaned after each sample by a jet of compressed air.

2. Egg masses, either attached to root fragments or free in the soil, are extracted from a 500-cc aliquant of the soil sample either by an elutriator (Fig. 1A) or by decantation and

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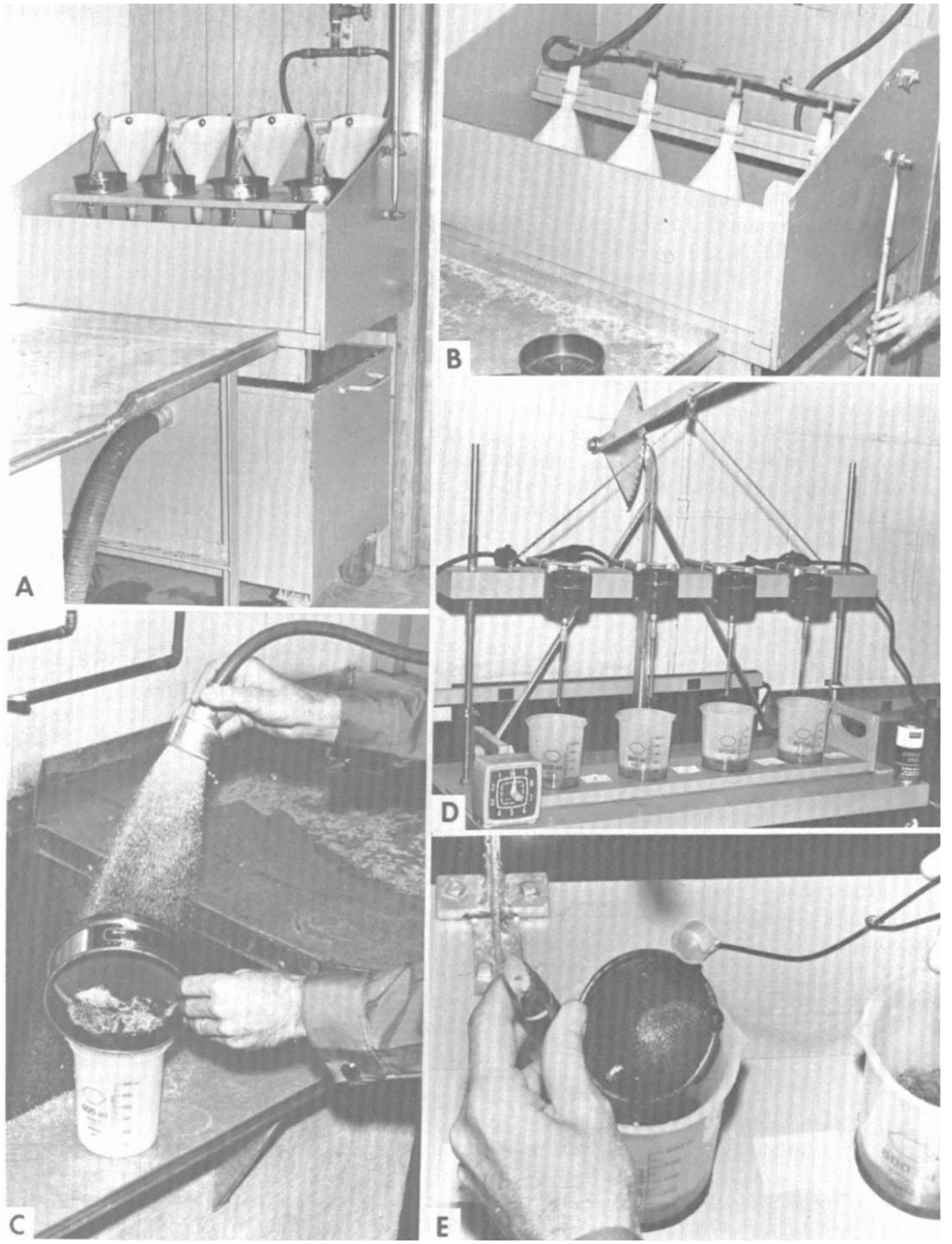

FIG. 1. *Meloidogyne* egg extraction procedure. Elutriator in **A.** operating position; **B.** in flushing position. **C.** Organic material being washed from 40-mesh (420-μ) sieve into beaker. **D.** Assembly of four stirrers on which suspensions of organic material in NaOCl solution are processed for 10 min. **E.** Taking the aliquot from the suspension.

sieving. The former is faster than the latter, gives comparable results and is generally preferred unless the effluent is to be collected for assay of vermiform nematodes.

The elutriator consists of a bank of four 24-cm polyethylene funnels above a mobile soil trap and drain (Fig. 1A, B). Each funnel is supplied with a constant water flow delivered upward through the stem, and has a spout which directs the overflow through a 15-cm 40-mesh (420-μ) sieve supported on a shelf attached to the funnel assembly. During extraction the assembly is fixed in an upright position (Fig. 1A). A soil sample is placed in each funnel with the water running, and the flow is adjusted to a rate of approximately 350 ml/sec so as to float root fragments and egg masses over the spout while the heavier soil particles remain. Smaller soil particles are also washed over the funnel spout, but pass through the sieve on which the root fragments, egg masses and organic debris are caught. The extraction time is approximately 2 min. After extraction the assembly is inverted to permit flushing (Fig. 1B).

Extraction by decantation and sieving involves conventional procedures. The soil sample is placed in a 10-liter plastic bucket, and 6 liters of water are added. The resulting slurry is swirled vigorously for about 30 sec, and the supernatant liquid containing suspended material is poured through a 20-cm 40-mesh (420-μ) sieve. Water is again added to the soil in the bucket and the swirling and pouring are repeated once.

B. *Dissolution of egg mass matrices and dispersal of eggs.*

1. The material caught on the 40-mesh (420-μ) sieve is washed into a 600-ml beaker by a water spray (Fig. 1C), the volume made up to 200 ml, and 20 ml of 5.25% NaOCl (Clorox®, Clorox Co., Oakland, Calif.) are added to the suspension. Dissolution of the gelatinous matrices of the egg masses by NaOCl (1, 4, 6) and recovery of eggs is favored by a solution temperature of 30 C.

2. The suspension is stirred at 1600 rpm for 10 min to facilitate the dissolution of the egg mass matrices and uniformly disperse the suspended eggs. At the commencement of stirring, the contents of each beaker are sprayed momentarily with a silicone aerosol (Antifoam A Spray®, Dow Corning Corp., Midland, Mich.) to prevent foaming. Foam hinders uniform egg dispersal and subsequent operations. For efficiency of operation, we use two assemblies of four stirrers each (Fig. 1D) so that eight samples can be processed simultaneously. This allows the operator sufficient time to complete the subsequent step with eight samples while the next lot is being stirred.

C. *Sampling and staining the eggs.*

1. The stirrers are raised and rinsed into the beakers with a washbottle jet of about 10 ml water. An aliquot is taken from the now 230-ml suspension of eggs and organic material to determine the number of eggs present. A pipette may be used, but we prefer to place a 20-mesh (1.3 mm) household sieve in the top of the suspension to keep away large particles of debris and to take the aliquot with a dipper of required size--2 ml or 5ml (Fig. 1E), thus giving multiplication factors of 115 and 46, respectively, for the number of eggs in the original 500-cc soil sample. The dipper with the aliquot is plunged into a 150-ml beaker containing about 100 ml water. This dilutes the NaOCl and prevents it from disintegrating the eggs. The sieve and dipper are washed under running water before the next beaker is sampled. The stirring and sampling operation is best conducted under a hood to avoid operator discomfort due to Cl fumes.

2. The contents of the 150-ml beaker are swirled and poured through a 7.5 cm 500-mesh (25 μ) sieve to collect the eggs. An additional 50 ml of water is poured over the sieve to wash off any remaining NaOCl which would interfere with egg staining. The eggs are washed into a clean 150-ml beaker by a washbottle jet or fine spray and suspended in about 25 ml water. To facilitate counting, the eggs are stained by adding two drops of a 0.35% acid fuchsin solution in 25% lactic acid to each beaker, and boiled for 1 min.

3. Examination of the eggs by a stereoscopic microscope at 30 X magnification is facilitated by tilting the substage mirror to a nearly horizontal position, directing a diffuse light through the counting dish. This provides excellent contrast between the bright red color of the eggs and nonstained debris.

DISCUSSION

The procedure herein described was used to determine egg numbers in over 6000 samples from various sources during 1971. Experience thus far indicates that variations due to sampling are greater than those due to processing. In one evaluation trial, an 8-liter soil

sample taken from a field on 19 November was mixed and divided into 16 aliquots. From eight of them, egg masses were extracted by the elutriator; from the other eight, by decanting and sieving. All 16 aliquots then were processed as previously described and the following mean egg counts were obtained: by elutriator, 12,255 ± 787; by decanting and sieving, 12,259 ± 864. However, greater variability than this was found when duplicate samples taken 6 September from four different parts of a field approximately 1 hectare in size were compared. The mean egg numbers for each pair of samples were 15,134 ± 3,082; 8,832 ± 1,012; 19,228 ± 6,532; and 2,668 ± 736. These values show considerable variation in egg mass density, not only within the field but also within each area. Moreover, it appears that the greater the mean, the greater is the variation. Because of the relatively small size of the samples used, a difference of only a few egg masses between two samples taken from the same field can result in a difference of several thousand eggs/500 cc of soil. We feel that improvement and standardization of sampling technique would contribute much to the usefulness of assays of egg numbers.

The egg assay procedure provides an important additional tool in studying population dynamics of *Meloidogyne* spp. and perhaps of other forms whose eggs are aggregated in gelatinous matrices. When used in combination with larval assays, bioassays, root disease indices and crop performance data, the prospects of analyzing root-knot epidemics are enhanced.

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