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C. HEIP [1]), *A rapid method to evaluate nematode density*

The centrifugal-flotation technique described by Jenkins (1964) to separate nematodes from soil consists basically of two centrifugations of the material at 1750 rpm, the first for 5 min in water, the second for about one minute in a saccharose solution of 450 g/l ($\delta = 1.18$). We used some modifications of this method to separate nematodes of a lentic, brackish water habitat in northern Belgium from the detritus obtained after washing the sediment with tap water, as described by Barnett (1968). In our investigation, the first centrifugation is redundant as all the material is, of course, heavier than water.

To test the method, the number of nematodes appearing in the supernatant after each centrifugation of a given sample, was counted. These numbers formed a geometric series which allows a rapid and accurate calculation of the number of nematodes N_t in the sample.

A geometric series can be written as :

$$1 + a + a^2 + \ldots + a^n = S \tag{1}$$

Each term in the series is obtained from the previous one by multiplication with a constant ratio. The sum of the series is given by :

$$S = \frac{a^{n+1} - 1}{a - 1} \tag{2}$$

As $a^n = N_1$, the number of nematodes in the supernatant after the first centrifugation, the total number of nematodes in the sample, $N_t = S$ is given by :

$$N_t = \frac{N_1 a - 1}{a - 1} \tag{3}$$

The ratio a of this series can be obtained experimentally with a series of centrifugations. We found a to be constant when the procedure is standardized; a is a function of the volume of the sample,

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the concentration of saccharose and the centrifugal force. As these parameters will vary in each specific investigation we will not consider this matter in detail and only mention that we obtained values of a between 2 and 4. The important thing is that a can be determined in a precise way and that it is a constant for a given set of operational parameters. Once this constant is determined the total number of nematodes in the sample can be calculated after only one centrifugation using(3). When however the constancy of the prodecure is not trusted, two centrifugations should provide for the total number in the sample; as $a = N_1/N_2$, (3) becomes :

$$N_t = \frac{N_1^2/N_2 - 1}{N_1/N_2 - 1} \tag{4}$$

In (1) it can be seen that the series is finite and stops at one. Seinhorst (pers. comm.) drew my attention to the infinite series obtained by continuing to add numbers as they become smaller than one. This may yield a slightly better estimate of density, which is a ratio, but a slightly worse estimate of numbers in the sample (there are not less than one nematode in the sample).

The infinite series is obtained when considering the number after each centrifugation as a constant proportion of the number present. This yields $N_1 = a'N_t$. After the removal of the N_1 individuals there remain $(1 - a')N^t$ individuals in the sample from which $N_2 = a'(1 - a')N_t$ are obtained after the second centrifugation; there remain $(1 - a')^2N_t$ in the sample. It is easily seen that $1 - a' = N_2/N_1$ and $N_t = N_1/a'$. As $a' = (a - 1)/a$, (3) and (4) modify to :

$$N_t = \frac{N_1 a}{a - 1} \tag{3'}$$

$$N_t = \frac{N_1^2/N_2}{N_1/N_2 - 1} \tag{4'}$$

TABLE I

The number of nematodes in the supernatant

1*a*						
N_1	N_2	N_3	N_4	S	S'	S''
481	96	29	15	621	701	601
86	38	17	22	178	154	153
214	52	17	4	287	291	282
184	118	30	16	348	331	376
1*b*						
N_1	N_2	N_3	rest	S	S'	S''
151	67	30	27	275	271	271
73	33	14	11	131	131	132
21	9	4	2	36	36	37
43	18	6	2	69	69	73
61	30	15	13	119	119	119

The difference between (3, 4) and (3', 4') is in most cases extremely small, less than one nematode for a larger than 2. In Table Ia the number of nematodes in the supernatant after each of four centrifugations of a given sample is given and their sum S is compared with the calculated number according to (3) S' and according to (4) S''. In Table Ib S is obtained after carefully counting the nematodes remaining in the detritus after three centrifugations. The conformity between calculated numbers and numbers observed is in most cases extremely good. Only once is this conformity slightly less (153 calculated against 178 found). This could be due to improper handling as this sample was processed by two persons, but the error is nevertheless far smaller than it would have been without centrifugation.

As to the composition of the nematodes collected after each centrifugation, we did not identify species but there is certainly no bias for sizes or weights; some specimens which could be easily recognized showed no marked quantitative difference towards their presence in subsequent centrifugations. This indicates the possibility that the first supernatant is a representative sample of the whole population and would allow estimates of fauna composition and biomass as well as estimates of density.

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D. P. TAYLOR & C. NETSCHER [1]) : *An improved technique for preparing perineal patterns of* Meloidogyne *spp.*

Species in the genus *Meloidogyne* Goeldi are identified most commonly on the basis of characteristics of the perineal pattern, as originally proposed by Chitwood (1949). Specialized techniques are required in order to observe the details of the pattern clearly. Sasser (1954), Taylor, Dropkin & Martin (1955), and Triantaphyllou & Sasser (1960) used a rather crude technique in which the posterior third of the female was mounted in lactophenol with no attempt made to remove body tissues. Photographs of perineal patterns in these publications are frequently unclear because of extraneous material beneath or attached to the cuticle. J. B. Goodey (1957) and Whitehead (1968) recommended that inner or body tissues be removed and that the cuticle containing the pattern be trimmed to a small size prior to mounting. Complete removal of these tissues is not easily accomplished, and many of Whitehead's (1968) photographs of perineal patterns are unsatisfactory because diagnostic details are obscured by unsuccessful or incomplete removal of adhering tissues (eg. Whitehead's Figs 75 and 77).

During cytological examination of *Meloidogyne* females, using the technique of Triantaphyllou & Hirschmann (1966), we observed that perineal patterns in these preparations were exceptionally clear and free of body tissues. Laboratory tests showed that immersion of cuticles of *Meloidogyne* females in a 45 % aqueous solution of acetic acid (V/V) permitted the easy removal of all adhering tissues; however, the acetic acid was a difficult medium in which to work. Additional acids were tested, and it was found that 45 % lactic acid produced excellent results without any of the difficulties

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