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MORE ABOUT THE DROP BY DROP DISTRIBUTION OF A NEMATODE SUSPENSION

Georges Reversat *

Samples containing exactly equivalent numbers of small nematodes such as infective juveniles of zoo- and phyto-parasitic species, are often prepared by distributing a homogeneous suspension of nematodes into samples of equivalent volumes. When the required volumes of suspension are pipetted at one time, the error variance observed on their nematode content should be as a rule, more or less closely equivalent to the mean count, displaying a Poisson distribution (Peters, 1941). In practice however, the observed variance may be higher (Moriarty, 1963) and in some recent papers authors have proposed a modified procedure to improve reproducibility. Wilson (1976) made a complete sample with two partial pipettings, arguing, «... thus any tendency for the pipetting procedure to select water rather than larvae was randomized. » Castro and Fairbairn (1969) made ten partial pipetings and obtained 1.4 to 2.6 % coefficient of variation. Reversat (1976) using a drop by drop distribution made 300 partial samplings to achieve 3 % accuracy.

New facts are presented about the reliability of the last procedure. In the apparatus used (Fig. 1), the suspension of nematodes, maintained homogeneous by gentle air bubbling or by stirring, flows out by gravity through a narrow plastic tubing and is delivered drop by drop (100 drops = 3.25 ml) at its end.

In a first experiment, five 250 ml suspensions of freshly hatched juveniles of *Heterodera oryzae*, ranging from 2,000 to 10,000 individuals per ml, mixed by air bubbling were treated successively. After 50 ml were withdrawn, 24 consecutive drops were collected separately and their nematode content was determined (Fig. 2). The relationship between variance and the mean agreed with the Poisson law, with diluted suspensions, but diverged with increased nematode density. Lellouch (1964), discussing a similar relationship between variance and the mean obtained for red cells in blood counts, attributed this effect to the volume of particles, which is not negligible compared with the sample volume. Adapting his argument to the present case, we can consider the maximum number of nematodes in a drop (n), equal to the ratio between the volume of the drop (32.5 μl) and the volume occupied by one nematode (a).

If volume a has a probability p to contain a nematode, the average number of nematodes in a drop is $\mu = np$ and the variance equals $\sigma^2 = npq$ with $p + q = 1$, wich leads to the formula $\sigma^2 = \mu(1 - \frac{\mu}{n})$. By using the estimations m and s^2 of μ and σ^2, n can be calculated from the formula $n = m^2/(m - s^2)$. The mean numerical value for n, calculated with this formula from the four highest values (given on Fig. 2) of m is 491. From this, a curve giving the relationship between m and s^2 can be traced according ot a second formula, wich is the reciprocal of the first : $s^2 = m - m^2/n$, which fits the experimental results (dotted line in Fig. 2). Further, if we consider a drop (32.5 μl), filled with 491 spherical particles arranged in the densest state, the void ratio, i.e. ratio of volume of voids to volume of particles, equals 0.35 (Means & Parcher, 1964). From this, the volume of the particle can be calculated by the relation : $(32.5\ \mu l - 49a)/491a = 0.35$. The result is $a = 0.049\ \mu l$, wich corresponds to a spherical particle with a diameter of 0.45 mm, while the length of the *Heterodera oryzae* juvenile has a

* *Laboratoire de Nématologie, ORSTOM, BP 1386, Dakar, Sénégal.*

mean of 0.44 mm (Luc & Taylor, 1977). This suggests that, until this density of 491 juveniles by drop (15,000 juveniles per ml), juveniles moving in a tumbling motion in the agitated suspension act like spherical particles with a diameter equal to their length.

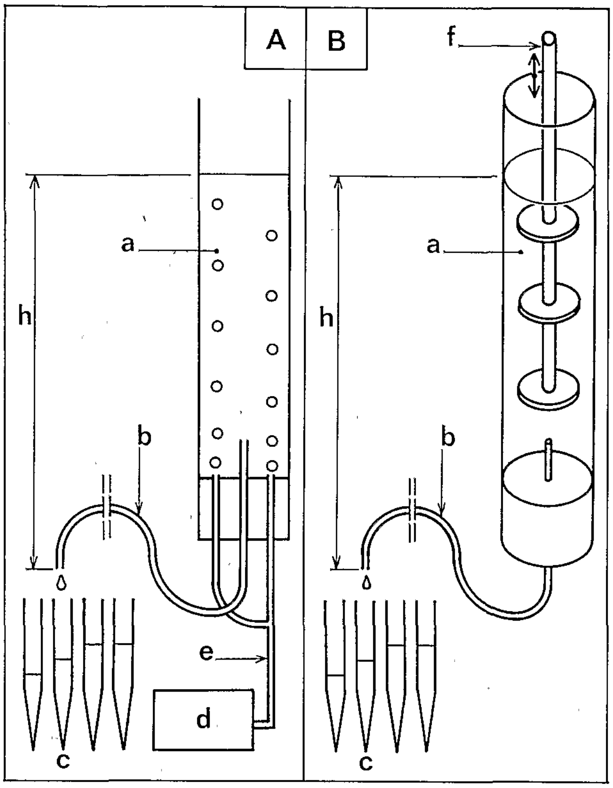

Fig. 1. Apparatus for the drop by drop distribution of a nematode suspension. A : Mixing by air bubbling. B : Mixing by vertical alternative stirring. a : 250 ml nematode suspension ; b : plastic capillary tubing diameter 1 mm, length 60 cm ; c : sample tubes ; d : aquarium pump ; e : pipe for air bubbling ; f : stirrer with vertical alternative movement ; h : constant perpendicular, empirically adjusted for obtaining a distribution rate of 80 to 100 drops per minute.

In a second experiment a 250 ml diluted suspension of *H. oryzae* juveniles was introduced into the apparatus and, mixed by air bubbling, flowed out continuously. When the effluent volume was 0, 50, 100 and 150 ml, series of 24 consecutive drops were collected separately and counted (Tabl. 1). The density of the nematode suspension remained constant throughout the operation, demonstrating the convenience of air bubbling for mixing the suspension. Mixing by air bubbling was also efficient for a suspension containing a mixture of all developmental stages of *Aphelenchus avenae*. To the contrary, when air bubbling a suspension of *Meloidogyne javanica* juveniles the numbers of juveniles in a drop increased with the volume of effluent. This appeared to be the result of juveniles floating on the water surface. Thus, juveniles of *M. javanica* used bubbles of air as a lift and accumulated in the top of the liquid column. With this species, air bubbling must be replaced by mechanical stirring with an adapted stirrer (Fig. 1B), powered by a vibratory stirrer (Southey, 1970) or any kind of electrical apparatus giving a vertical alternative movement.

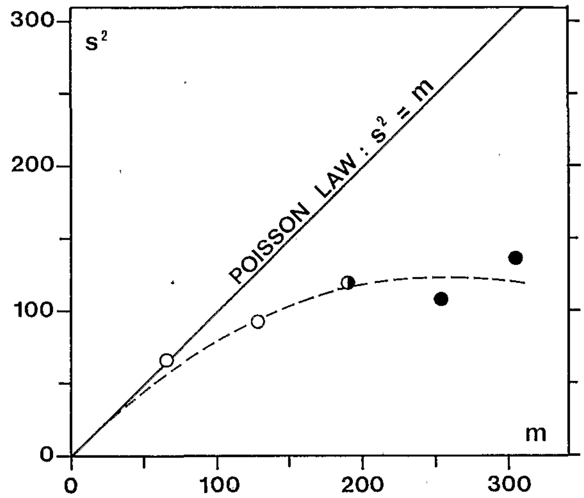

Fig. 2. Drop by drop distribution of suspensions of *Heterodera oryzae* juveniles : relationship between mean (m) and variance (S^2) of the juvenile number in a drop for five suspension densities (24 countings for each density). Experimental points and theoretical Poisson distribution were compared with χ^2 tests according to Peters (1952) and Lellouch (1964) : Black circles : differing significantly at the 0.01 level. Half black circles : differing significantly at the 0.025 level. White circles : not differing significantly. Dotted line curve traced from the formula : $S^2 = m - m^2/491$.

Table 1

Drop by drop distribution of a suspension of *Heterodera oryzae* juveniles : effect of effluent volume on the juvenile content of a drop.

Effluent volume (ml)	*Counting of 24 drops for each effluent volume*	
	Mean	Variance
0	88.3	98
50	90.2	90
100	85.9	121
150	86.2	71

Analysis of variance : F = 1, with a F limit of 4.

These results affect the pratice of the drop by drop distribution technique in two ways. First, the use of very dense suspension, in the range of 10,000 to 15,000 nematodes per ml is the most convenient : the variability between drop content is small (Fig. 2) and the time required for collecting the samples is shorter. Secondly, the efficiency of the method used to obtain the mixing of the nematode suspension must be checked for each species.

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NAGELUS ABALOSI (DOUCET, 1978) NOV. COMB. ET *N. VIRGINALIS* (DOUCET, 1978) NOV. COMB.

Marcelo E. Doucet*

Siddiqi (1979) a redéfini les genres réunis dans la sous-famille des Merliniinae Siddiqi, 1970. Deux de ces genres, *Amplimerlinius* Siddiqi, 1976 et *Nagelus* Thorne & Malek, 1968, sont caractérisés par une région labiale non divisée longitudinalement et comportant donc des anneaux continus, alors que les trois autres, *Scutylenchus* Jairajpuri, 1971, *Geocenamus* Thorne & Malek, 1968 et *Merlinius* Siddiqi, 1970, ont une région labiale partagée en six secteurs.

L'observation en vue de face de *Merlinius abalosi* Doucet, 1978 et *M. virginalis* Doucet, 1978 nous a montré que leur région labiale n'était pas subdivisée. Nous considérons qu'ils doivent être inclus dans le genre *Nagelus*, tel que redéfini par Siddiqi (1979); leur dénomination doit donc devenir : *Nagelus abalosi* (Doucet, 1978) nov. comb. et *Nagelus virginalis* (Doucet, 1978) nov. comb.

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* *INRA, Station de Recherches sur les Nematodes, 123, Bd Francis Meilland, 06602 Antibes, France.*