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Rapid and non-destructive assessment of the number of eggs in cysts of potato cyst nematodes by weighing

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Summary – Dry cysts of potato cyst nematodes, derived from greenhouse rearing, were weighed and afterwards the number of eggs was determined by crushing the cysts and counting the eggs. A high correlation (r = 0.96) was found between the number of eggs per dry cyst of potato cyst nematodes and cyst weight, measured with a supermicrobalance. The number of eggs per cyst can be assessed rapidly and in a non-destructive way by weighing.

Résumé – ***Estimation rapide et non destructrice par pesage du nombre d'œufs contenus dans les kystes des* Globodera *parasites de la pomme de terre*** – Des kystes secs de *Globodera* parasites de la pomme de terre élevés en serre sont pesés puis le nombre d'œufs contenus déterminé par écrasement des kystes et comptage de ces œufs. Une corrélation élevée (r = 0,96) a été observée entre le nombre d'œufs contenu dans un kyste sec et son poids déterminé à l'aide d'une supermicrobalance. Cette technique de pesage permet ainsi une estimation rapide et non destructrice du nombre d'œufs par kyste.

Key-words : potato cyst nematodes, *Globodera rostochiensis, G. pallida,* number of eggs, weighing.

For the assessment of the density in soil of potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida,* cysts are recovered by the flotation method (Fenwick, 1943), and the number of cysts in the debris is counted (van der Wal & Vinke, 1982). In addition, the number of eggs per cyst may be assessed by crushing cysts in water and counting the number of eggs in samples of the resulting egg suspension. This method was first described by Bijloo (1954) and later modified by Seinhorst and Den Ouden (1966). The number of eggs per cyst can vary considerably (Hesling, 1959; Stelter & Gaur, 1969).

This method for determining the number of eggs per cysts is, however, destructive to the cysts and labour-intensive. A method for non-destructive assessment of the cyst content was described by Stelter and Gaur (1969). They found a good relationship between volume of cysts, as assessed by measuring their diameter, and the number of eggs in these cysts. Fenwick and Reid (1951) used the weight of cysts, but only with the aim of obtaining uniformly sized batches of cysts for experiments.

The work reported here was carried out to investigate whether weighing cysts could be a fast and accurate method for the assessment of the number of eggs in dry cysts.

Materials and methods

Samples of air-dry cysts of various PCN populations of differing species, virulence group and place of origin, were used in three experiments. The populations had been reared in different years in a greenhouse on various susceptible cultivars, with the inoculum spread freely in the soil. Per sample, the number of eggs was assessed by crushing the cysts in water (Seinhorst & Den Ouden, 1966) and counting the number of eggs in subsamples of the egg suspension.

EXPERIMENT 1

A sample of 50 cysts of each of seventeen *G. rostochiensis* and thirteen *G. pallida* populations was taken. Moreover, from eight *G. rostochiensis* populations and seven *G. pallida* populations, a sample with 50 small cysts and a sample with 50 large cysts, selected using a microscope, were prepared. All samples were weighed three times on a Sartorius R160D electronic microbalance with sensitivity of 0.01 mg and an error of maximal 0.1 mg, as indicated by the manufacturer. Afterwards, the number of eggs per sample was assessed by crushing the cysts in 50 ml water and counting the number of eggs in three subsamples of 1 ml each.

EXPERIMENT 2

A sample of cysts of eleven *G. rostochiensis* and nine *G. pallida* populations each was used. For each of the 20 samples, the number of cysts was randomly chosen between 10 and 250. In addition, twenty samples were prepared in the same way with small or large cysts. All samples were weighed once on a Sartorius R160D electronic microbalance and the total number of eggs was assessed by crushing the cysts in 25 to 200 ml water,

depending on the size of the sample, and by counting three subsamples of 1 ml each.

EXPERIMENT 3

Ten individual dry cysts with different size of each of five *G. rostochiensis* and six *G. pallida* populations were chosen. The mean diameter of these cysts was measured with a microscope and the cysts were weighed twice on a Sartorius S-4 electronic supermicrobalance with sensitivity 0.1 μg and an error of maximal 1 μg (manufacturer's specification). Cyst volume was calculated, using the formula $(4/3)*\pi*(1/2)d)^3$, with d being the diameter. Afterwards, the number of eggs was assessed by crushing the cysts in a small volume of water and counting the total number of eggs.

REGRESSION ANALYSIS

Data were analysed with geometric mean regression (Sokal & Rohlf, 1968).

Results

EXPERIMENT 1

The correlation between cyst weight and number of eggs per cyst in samples, selected for size, was very high ($r = 0.92$; $P < 0.01$). The variance between repeated weight measurements of samples accounted for less than 1 % of the total variance of cyst weight. Therefore, one weight measurement appears to be sufficient. With random samples, differing less in cyst content, the correlation between cyst weight and number of eggs per cyst was still $r = 0.77$ ($P < 0.01$).

EXPERIMENT 2

The correlation between weight of cyst samples and the number of cysts was very high, both for samples of cysts, selected for size, and for random samples, not selected for size (Table 1). For samples of cysts, selected for size, the correlation of weight with number of eggs was considerably and significantly ($P < 0.01$) higher than the correlation of weight with number of cysts (Table 1).

EXPERIMENT 3

Again, the variance between repeated weight measurements of samples accounted for less than 1 % of the total variance of cyst weight. The relationship between number of eggs per cyst and cyst weight, using a supermicrobalance, is shown in Figure 1. A few cysts contained less eggs than expected from their cyst weight, which may be due to the fact that the newly formed cysts at rearing may have been mixed with some largely empty cysts of the inoculum. Despite this, a very high correlation was found between the number of eggs per cyst and cyst weight (Table 2). The correlation between cyst volume and number of eggs per cyst was considerably and significantly ($P < 0.01$) lower (Table 2).

REGRESSION ANALYSIS

All samples, either selected or not selected for size, were used for linear regression analysis. In each of the experiments, the observed value of the constant did not differ significantly from zero. Therefore, a simplified regression analysis was preferred, in which the constant was omitted. Thus, a regression line $y = b*x$ was used, in which y is the number of eggs, x is sample weight (μg), and b is the regression coefficient. The value of b did not differ significantly between Experiments 1, 2 and 3 (Table 3).

Table 1. *Correlations between number of cysts, total number of eggs and sample weight, with and without selection of cysts for size, in Experiment 2.*

	Number of eggs	Sample weight
NO SELECTION FOR SIZE		
Sample weight	0.93 *	
Number of cysts	0.79 *	0.89 *
SELECTION FOR SIZE		
Sample weight	0.93 *	
Number of cysts	0.60 *	0.67 *

* = significant ($P < 0.01$)

Table 2. *Correlations between volume of cysts, as calculated from the cyst diameter, cyst weight and number of eggs per cyst, in Experiment 3.*

	Cyst weight	Number of eggs
Number of eggs	0.96 *	
Cyst volume	0.89 *	0.78 *

* = significant ($P < 0.01$)

Table 3. *Values and confidence interval (P = 0.05) of the regression coefficient* b *of the regression line* y = b*x, *with* y *being the number of eggs per sample and* x *being weight of the sample (μg), and the percentage explained variance (r^2) by regression analysis, in Experiments 1, 2 and 3, determined by geometric mean regression.*

Experiment	b	Confidence interval	Explained variance (%)
1	8.60	8.11 < b < 9.09	77
2	8.15	7.63 < b < 8.67	86
3	8.10	7.84 < b < 8.36	90

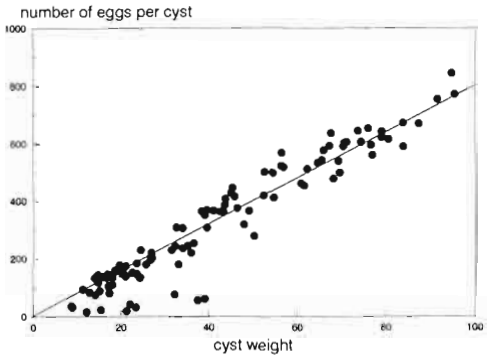

Fig. 1. *Relationship between total number of eggs per cyst and cyst weight (µg), using a supermicrobalance, in Experiment 3.*

Discussion and conclusions

The high correlations between cyst weight and number of eggs show that cyst weight provides a good assessment of the average number of eggs per cyst. Weighing cysts is much less labour-intensive than crushing and counting the number of eggs. It has to be noted that the results have been obtained with clean samples of cysts, obtained from greenhouse rearing. For weighing cysts, a supermicrobalance is preferable, as it can be used for weighing of even individual cysts (Experiment 3), and has the highest accuracy. When the cyst content of samples with ten cysts or more has to be assessed, weighing with a microbalance, which is less sensitive, may be sufficient (Experiments 1 and 2). In experiments, usually cysts from various replicates of the treatments will be weighed separately, and the treatment mean number of eggs thus will be assessed with increased accuracy.

The weight of cysts not only includes the weight of eggs, but also the weight of other cyst contents and the cyst walls. Despite this, the explained variances of regression of number of eggs on weight were very high (Table 3), and the relationship found proved to be very useful. However, the actual weight of eggs cannot be estimated in this way.

In our experiment, a significantly lower correlation of cyst volume with number of eggs was found than the correlation between cyst weight with number of eggs. However, Stelter and Gaur (1969) found a very high correlation ($r > 0.95$) between cyst volume and number of eggs. Their assessment of volume differed from ours, as they took into account both the smallest diameter and the largest diameter for calculation of the volume. Diameter measurements are, however, very labour-intensive, unless they can be done by image analysis.

The method for assessment of the number of eggs, presented here, has the advantage that it is not destructive to the cysts, as compared to fast methods for counting larvae (Been *et al.,* 1990; Robinson *et al.,* 1992) or possibly quantitative ELISA for detection of cysts (Schots *et al.,* 1992). It can be useful to assess final PCN densities rapidly in pot trials and afterwards, the cysts can be used for further research. Moreover, it can be very useful to prepare uniform samples of PCN inoculum.

Weighing may therefore be regarded as a simple and effective method of assessing the number of eggs in cysts.

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