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Sampling Error in Relation to Cyst Nematode Population Density Estimation in Small Field Plots

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Abstract: Cyst nematodes are serious plant-parasitic pests which could cause severe yield losses and extensive damage. Since there is still very little information about error of population density estimation in small field plots, this study contributes to the broad issue of population density assessment. It was shown that there was no significant difference between cyst counts of five or seven bulk samples taken per each 1-m^2 plot, if average cyst count per examined plot exceeds 75 cysts per 100 g of soil. Goodness of fit of data to probability distribution tested with χ^2 test confirmed a negative binomial distribution of cyst counts for 21 out of 23 plots. The recommended measure of sampling precision of 17% expressed through coefficient of variation (*cv*) was achieved if the plots of 1 m^2 contaminated with more than 90 cysts per 100 g of soil were sampled with 10-core bulk samples taken in five repetitions. If plots were contaminated with less than 75 cysts per 100 g of soil, 10-core bulk samples taken in seven repetitions gave *cv* higher than 23%. This study indicates that more attention should be paid on estimation of sampling error in experimental field plots to ensure more reliable estimation of population density of cyst nematodes.

Key words: cyst nematodes, detection, *Heterodera schachtii*, population density, small field plots.

Cyst nematodes are serious plant-parasitic pests which could cause severe yield losses and extensive damage. Statutory sampling methods introduced by the EPPO (European and Mediterranean Plant Protection Organization) were withdrawn as the probability of detecting a standard focus was 12% (Schomaker and Been, 2006). As a result, many efforts have been made to improve sampling techniques on potato production areas to increase the probability of detecting small infestations to 90% (Been and Schomaker, 2000). However, there is still very little information about soil sampling error in field experiments conducted in small field plots. These experiments are often related to resistance/tolerance testing and development of control measures which need higher precision for estimating population densities than are required for nematode management (Francl, 1986).

The level of inaccuracy of cyst nematode population density estimation in a particular field plot is called "sampling error" and is usually expressed in terms of variance or standard deviation (van Bezooijen, 2006). According to the EPA (U.S. Environmental Protection Agency) soil sampling contributes to random and systematic error of data assessment. Random error is frequently described quantitatively by the variance of random error and derives from the sampling process, preparation dissimilarities, subsampling problems, and analytical discrepancies. Random error (precision) of nematode density estimation is also affected by the type of cyst nematode distribution. On the other hand, systematic error (bias) causes the mean value of the sample data to be either consistently higher or consistently lower than the "true" mean value as a result of faults in sampling design, sampling procedure, or analytical procedure.

Estimation of sampling error is of great importance for keeping observations in field experiments within certain limits of confidence (Seinhorst, 1986). Knowing that each category of cyst nematode distribution (very small-, small-, medium, and large-scale distribution) has its own standard deviation (Seinhorst, 1986), the quality of population density estimation has to be adapted to the required distinctive power of the experiments (Schomaker and Been, 2006). According to Seinhorst (1986), the predictability of very small and small-scale variability provides with knowledge of sampling error and the possibility for proper planning of field experiments regarding sample size and number of replicates. If there is no predictable sampling error, which depends on knowledge of a minimum area in which the distribution of cysts and eggs per cyst is always (more or less) the same, no field experiment can be planned properly. As a result, assessment of the sampling error, which includes investigation of samples from a sufficiently large number of plots, should be taken before conducting field experiments.

The coefficient of variation, as a measure of sampling precision, is usually used to indicate sampling error of cyst counts over large area to make an advisory system more efficient; however, estimation of sampling error for cyst counts of small field plots is rarely provided (Seinhorst, 1986; Heijbroek et al., 2002). Small plots are usually used for conducting experiments dealing with resistance/tolerance testing and nematode control measures. Many of these include correlation assessment of nematode density and yield loss, but yield loss can be overestimated or underestimated if correlation is made with respect to mean value of nematode population density (Ferris, 1984; Noe and Barker, 1985; Campbell and Benson, 1994). If yield loss is correlated

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with mean density of cyst nematode population it could be overestimated as much as 300% when population density is highly aggregated (Campbell and Benson, 1994). As a consequence, testing the goodness of fit of data to a probability distribution, and using indices of aggregation for analysis of unmapped data are of great importance for good interpretation of experimental results. Since there is very little information on the relation of small-scale distribution of cyst nematodes to soil sampling error, this study contributes to the broad issue of population density assessment.

Materials and Methods

Sampling: The soil samples were collected from 23 plots of 1 m^2 at the locality of Senta (Vojvodina province, Serbia) from sugar beet fields infected with *Heterodera schachtii* (Schmidt, 1871) as a dominant population. Since we wanted to estimate sampling error of small plots contaminated with different average population densities (from 40 to 100 or more cysts per 100 g of soil) the selection of experimental plots was made on the basis of the previous arbitrary knowledge on the range of contamination level across the sugar beet production area. The presampling of the area was made using a method which is prescribed for advisory purposes to allow the estimation of sampling error of 1-m^2 plots. Soil samples were collected randomly in a "zigzag" pattern, in the direction of cultivation, within each 1-m^2 plot. The zigzag pattern was a modification of the rectangular grid which increases the probability of including individuals form every area within the plot to the population density estimation and facilitates sampling of two rows in one passage over the sampling area. Plots of 1 m^2 were sampled to the depth of 30 cm, with 10 cores per bulk sample taken in five or seven repetitions depending on previously determined contamination level using an auger (Ø 1.8 cm). Each bulk sample weighed about 500 g. Five bulk samples (5×10 cores) resulted in 2.5 kg of soil per 1-m^2 plot, whereas seven bulk samples (7×10 cores) resulted in 3.5 kg of soil per 1-m^2 plot. Sequential samplings were taken on the same date after sugar beet harvest. Bulk samples were thoroughly mixed and three subsamples of 100 g (triplets) were processed per each bulk sample using a Fenwick can. Depending on whether five or seven bulks were taken per 1-m^2 plot, statistical differences in cyst counts within and between five and seven triplets were analyzed. Cysts were collected on a 160 μm aperture sieve and examined using stereomicroscope at ×25 magnification. Cyst counts of triplets were expressed in number of cysts per 100 g of soil.

Sampling error estimation: The precision of soil sampling per each 1 m^2 was measured by the *cv* (standard deviation per mean value of cysts). According to Schomaker and Been (1999) sampling error for 1-m^2 plot, expressed as *cv*, should be less than 17%. If experiments are conducted to predict yield losses in the presence of cyst nematodes, *cv* should be 15% implying departure from true density in the range ± 30% (Seinhorst, 1986; Schomaker and Been, 2006). To determine the optimal number of cores which would give *cv* lower than the prescribed 17 % or even stricter 15%, cyst counts of triplets were compared in all possible combinations to simulate a multiple-core bulk sampling per each 1-m^2 plot. Thus, cyst counts of triplets taken per 2, 3, 4, 5, 6, or 7 bulks were compared in all combinations in order to estimate the range of *cv* for 20, 30, 40, 50, 60, and 70 core sampling.

Statistical analysis: Statistical differences in cyst counts within and between triplets of each 1-m^2 plot were estimated using location equivalence tests. The statistical software Wolfram Mathematica 8.0 was used for all analyses.

Fitting a probability distribution: There are two methods to quantify the degree to which organisms are aggregated. The first considers using indices of aggregation, whereas the second is more preferable and considers fitting the theoretical distribution to experimental data. The preference of using a χ^2 test over the indices of aggregation is based on the fact that the Poisson distribution is only one of many distributions that provide a variance/mean ratio of 1.0 and only the χ^2 test can make a distinction between random and nonrandom distribution Hurlbert (1990). As a result, probability distribution of the data for each 1-m^2 plot was initially tested by using the index of dispersion ($s^2/\bar{x}$) as a suggestion of data aggregation (if ID$>$ 1) and not as a measure of probability distribution. Afterwards, a χ^2 test was performed for testing the goodness of fit of cyst counts to the probability distribution. If cyst counts did not depart from those predicted by the negative binomial distribution, the exponent *k* was calculated as an index of aggregation. According to Schomaker and Been (2006), *k* indicates the degree of clumping of the population and should be calculated as follows:

$$k = \frac{\bar{x}^2}{s^2 - \bar{x}}$$

Results

Variation of cyst counts per plot: To investigate whether there was a difference between triplets analyzed per each 1-m^2 plot, statistical tests on the means were employed. It was shown that triplets were not significantly different ($P < 0.05$) if the average cyst count per examined 1-m^2 plot exceeded 75 cysts per 100 g of soil. However, if the average cyst counts per 1 m^2 took the range between 40 and 75 cysts per 100 g of soil, significant differences between triplets were found (Fig. 1). Average cyst counts per 1-m^2 plots are presented in Table 1.

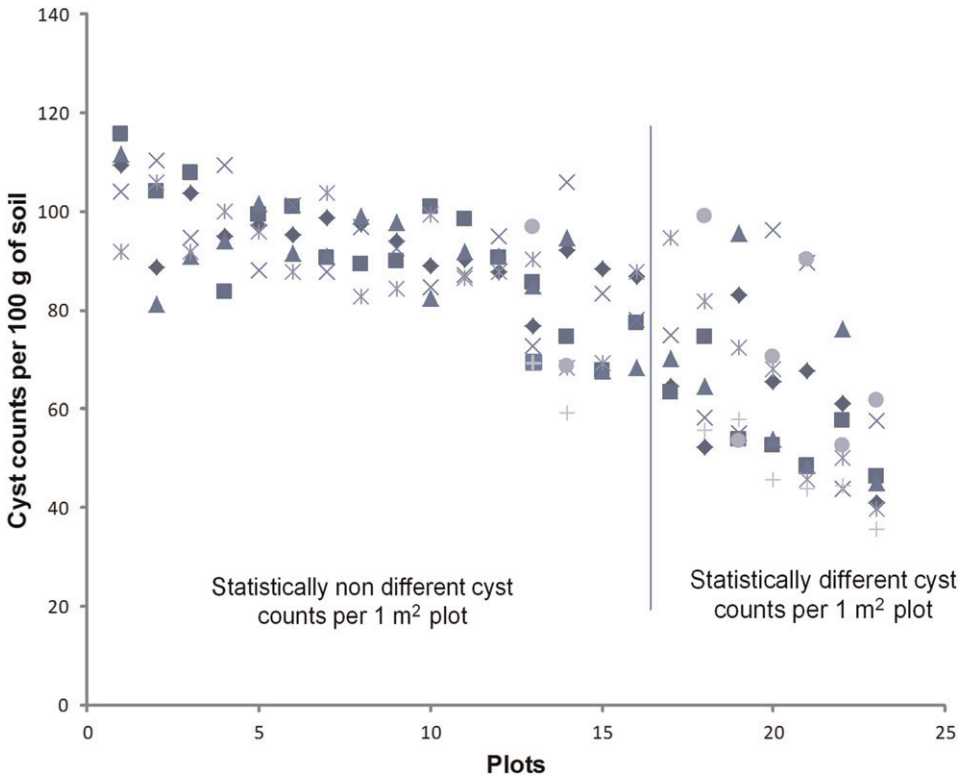

FIG. 1. Statistical difference between triplets analyzed per each 1-m^2 plot.

Population density of H. schachtii in small field plots: To investigate the probability distribution of *H. schachtii* in small field plots, indices of dispersion of quadrat counts and χ^2 goodness of fit to the negative binomial and Neyman type A distribution were applied. The Neyman type A distribution is generated mathematically by compounding two Poisson distributions and describes a dispersion that is less aggregated than the negative binomial distribution. Variances of cyst counts per 1-m^2 plots were higher than mean values of cyst counts for

TABLE 1. Estimates of cyst counts, precision of sampling and aggregation level of *Heterodera schachtii* in small field plots.

Plot (1 m^2)	Population density per 1-m^2 plot (Mean value) cysts/100 g soil	Dispersion of data (Standard deviation) cysts/100 g soil	Variances	Coefficient of variation (*cv*)	Coefficient of aggregation (*k*)
1	101.7	13.3	176.89	0.13	137.56
2	98	17.1	292.41	0.17	49.40
3	97.7	13.8	190.44	0.14	102.93
4	96.4	10.4	108.16	0.11	790.21
5	96.4	14.4	207.36	0.15	83.75
6	95.3	8.2	67.24	0.07	-
7	94.3	15.6	243.36	0.17	59.66
8	93	11.8	139.24	0.13	187.05
9	91.7	15.6	243.36	0.17	55.45
10	91.3	13.2	174.24	0.14	100.50
11	91.1	8.8	77.44	0.09	-
12	90.7	11.3	127.69	0.12	222.39
13	82.33	13.3	177.03	0.16	71.58
14	80.52	18.8	355.26	0.23	23.6
15	79.6	11.5	302.76	0.14	120.34
16	75.3	12.8	164.78	0.17	63.29
17	73.5	17.4	132.25	0.24	23.56
18	69.48	18.1	328.66	0.26	18.62
19	67.4	16.7	280.35	0.25	21.30
20	64.6	20.0	400.85	0.31	12.42
21	62.0	21.5	462.35	0.35	9.62
22	55.04	15.3	233.85	0.28	16.95
23	46.7	10.6	113.01	0.23	32.91

21 out of 23 plots which indicated an aggregated distribution of the nematode population. In addition, density classes within 1-m^2 plots were positively skewed, which is characteristic of a negative binomial distribution. To determine the goodness of fit of data to the probability distribution, a χ^2 test was performed and the negative binomial distribution of cyst counts was confirmed for all 1-m^2 plots except for two plots (6 and 11) where a Neyman type A was confirmed.

The coefficient of aggregation (k) was estimated for plots where the frequency distribution of data fit the negative binomial distribution. Coefficients of aggregation were lower than 33 for all 1-m^2 plots contaminated with 40 to 75 cysts per 100 g soil. Lower k values indicate higher aggregation. Aggregation coefficients ranged from 23.6 to 790.2 for all the other plots contaminated with more than 75 cysts per 100 g of soil except for the two plots where the Neyman type A distribution was confirmed (Table 1).

Soil sampling error: To determine quality (precision) of the population density estimation for each 1-m^2 plot, the *cv* was calculated using cyst counts of all subsamples per each 1-m^2 plot (Table 1). In our study, *cv* of cyst counts per 1-m^2 plots were equal or less than 17% only if bulk samples of 10 cores were taken five times from the plots contaminated with more than 90 cysts per 100 g of soil. Taking bulk samples of 10 cores in 7 repetitions from the plots contaminated with less than 75 cysts per 100 g of soil was not enough to reach *cv* of 17%. The *cv* for these plots were higher than 23% showing a tendency of increment when cyst numbers per 100 g soil decreased.

To determine the optimal number of cores which would give *cv* lower than the 15 %, the multiple-core bulk sampling per each 1-m^2 plot was performed. Sampling the plots contaminated with more than 90 cysts per 100 g of soil with 20 cores (10 core bulks taken in 2 repetitions) resulted in *cv* in the range of 5% to 23%, which indicates low sampling precision. With increasing numbers of cores, the range of *cv* became narrower. For example, if bulk samples were taken with 50 cores (10 core bulks taken in 5 repetitions), *cv* reached 15% for 66.6% of plots contaminated with (90–100) cysts per 100 g of soil. Sampling plots contaminated with less than 90 cysts per 100 g of soil with 20 cores resulted in *cv* in the range of 7% to 43%. The precision of sampling was higher than 23% for all plots contaminated with less than 75 cysts per 100 g of soil and sampled with 70 cores (Fig. 2).

Discussion

An accurate estimation of cyst nematode density is one of the most important steps in nematological studies conducted under field conditions. The present study contributes to the broader issue of density assessment of cyst nematode in trial field plots.

As cyst distribution per 1 m^2 was aggregated (indicated by higher values for variance of cyst counts to a mean values), the goodness of fit of actual data to a negative binomial distribution was tested using the χ^2 test. A negative binomial distribution of cyst counts was confirmed for 21 out of 23 plots. For two plots, the goodness of fit tests to the negative binomial distribution could not be performed as variances were less than mean values, indicating a noncontiguous distribution. This distribution was not significantly ($P < 0.05$) different from the Neyman type A distribution, according to the χ^2 test.

The degree of aggregation of cyst nematode populations, described by k, differed from plot to plot and was lower than 33 for all plots contaminated with less than 75 cysts per 100 g of soil (indicating high aggregation). Coefficients of aggregation reached a value of 790.2 for the plot contaminated with 96.4 cysts per 100 g of soil (Table 1). The negative binomial distribution

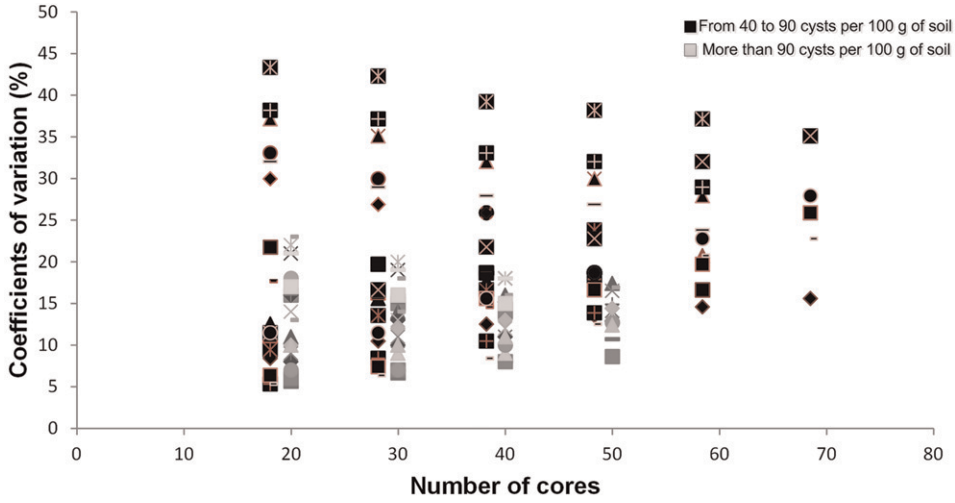

Fig. 2. Coefficients of variation related to multiple-core bulk sampling.

can describe a random distribution if *k* is large which indicates that cyst counts of highly contaminated plots could fit more than one discrete distribution. Schomaker and Been (2006) reported the range of coefficients of aggregation (from 35 to 550) after sampling of 456 (1 m^2) plots with 1.5 kg of soil for the presence of *H. schachtii*. Those coefficients of aggregation were in accordance with the range of *k* obtained in our study.

The accuracy of the estimates of population density which should be achieved is associated with purpose of sampling. The precision of cyst nematode population density estimation per 1-m^2 plots was determined using *cv*. These were less than 17% when 50 cores were taken from the plots contaminated with more than 90 cysts per 100 g of soil. Schomaker and Been (1999) reported that sampling errors for 1-m^2 plots, expressed as the *cv*, should be equal or less than 17%. However, it was also reported that *cv* should be less than 15% when experiments are conducted to predict yield losses in the presence of cyst nematodes (Seinhorst, 1986; Schomaker and Been, 2006). Field experiments concerned with yield loss estimations are usually conducted using plots of approximately 20 m^2 or less. The number of cores taken per plot was not uniform through different studies, although it was reported that using simple arithmetic mean for estimation of cyst nematode population density may result in overestimation or underestimation of crop losses (Campbell and Benson, 1994). As a result, the investigation of relationships between the level of contamination and the number of cores that should be taken per plot is of a great concern. In our study, cyst counts of subsamples were analyzed in all possible combinations to simulate a multiple-core bulk sampling and to determine the optimal number of cores which would give *cv* lower than 15%. Sampling the plots of 1 m^2 with bulks of 10 cores taken in 7 repetitions resulted in *cv* of 23% or higher if the plots were contaminated with 40 to 75 cysts per 100 g of soil. Seinhorst (1973) reported that the relation between average population density of nematodes and yield depends on the frequency distribution of nematode density in the field, and this result points out that highly aggregated cyst nematode population should be sampled with more than 70 cores per 1 m^2 to obtain recommended sampling precision. According to Barker and Campbell (1981) the overestimation of yield losses can be minimized by suitable stratification of the field, so that each stratum represents an area of uniform population density of the parasitic species of interest.

Schomaker and Been (1998) recommended to count at least 200 cysts of the genus *Globodera* per 1.8 to 2.5 kg soil sample taken from 1 m^2 to keep the *cv* under 14%. Although it was also noted that the number of examined subsamples determines the accuracy of population density estimation, it was better to take more samples than to analyze more subsamples of one sample. According to Lane and Trudgill (1999) and van Bezooijen (2006), taking larger and higher number of samples, especially from plots with small population densities, is more preferable than analyzing many subsamples of one sample. It was suggested that more soil must be collected at small nematode densities than at higher ones to keep the same sampling error (Schomaker and Been, 1998). In this study, multiple-core sampling was simulated, and it was shown that the *cv* will reach the value of 17% only if bulks of 10 cores taken in 5 repetitions are taken from plots contaminated with more than 90 cysts per 100 g of soil. To investigate whether soil sampling with higher number of cores will give prescribed *cv*, 1-m^2 plots contaminated with 40 to 75 cysts per 100 g of soil were sampled with bulks of 10 cores taken in 7 repetitions. Obtained *cv* values were still higher than 23% for all plots contaminated with less than 75 cysts per 100 g of soil. These data indicate that the number of cores which should be taken for sampling depends on the density of the cyst nematode population, and taking 70 cores (3.5 kg of soil) per 1 m^2 from the plots contaminated with less than 75 cysts per 100 g of soil is not enough for estimation of cyst nematode population density with precision 15% to 17%.

Aggregated distribution of cyst nematodes in small field plots have great influence on the error in estimating population density, indicating that the number of cores that should be taken to sample experimental plots is highly dependent on the type of cyst nematode distribution. Taking bulks of 10 cores in 7 repetitions was not enough to provide sampling precision lower than 23% for highly aggregated populations within 1-m^2 plots. Knowing that field experiments are usually conducted on plots which are larger than 1 m^2, and that sampling is commonly made with less than 70 cores per plot, this study indicates that more attention should be paid to estimation of sampling error in order to make more reliable estimation of population density.

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