Sample Optimization for Five Plant-Parasitic Nematodes in an Alfalfa Field¹

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Abstract: A data base representing nematode counts and soil weight from 1,936 individual soil cores taken from a 7-ha alfalfa field was used to investigate sample optimization for five plant-parasitic nematodes: Meloidogyne arenaria, Pratylenchus minyus, Merlinius brevidens, Helicotylenchus digonicus, and Paratrichodorus minor. Sample plans were evaluated by the accuracy and reliability of their estimation of the population and by the cost of collecting, processing, and counting the samples. Interactive FORTRAN programs were constructed to simulate four collecting patterns: random; division of the field into square sub-units (cells); and division of the field into rectangular sub-units (strips) running in two directions. Depending on the pattern, sample numbers varied from 1 to 25 with each sample representing from 1 to 50 cores. Each pattern, sample, and core combination was replicated 50 times. Strip stratification north/south was the most optimal sampling pattern in this field because it isolated a streak of fine-textured soil. The mathematical optimum was not found because of data range limitations. When practical economic time constraints (5 hr to collect, process, and count nematode samples) are placed on the optimization process, all species estimates deviate no more than 25 % from the true mean. If accuracy constraints are placed on the process (no more than 15% deviation from true field mean), all species except Merlinius required less than 5 hr to complete the sample process. Key words: sampling, advisory services, economics.

The optimization of sampling plans, including those for nematode advisory purposes, involves a compromise between the level of precision of the estimate for a particular management decision and the cost of obtaining such information. Nematode population sampling studies have concentrated mainly on species in the Heteroderinae (2,6,8,11). Proctor and Marks (16), investigating sampling optimization of *Pratylenchus penetrans* in small plots, found the time required to achieve high precision (estimates within 20% of the true mean with 95% confidence) was unacceptable for advisory purposes. A plan which provides accurate and reliable information is of little value if it is too expensive to implement. The value of a field estimate for plant-parasitic nematodes depends on many factors, including the cost of sampling, the cash value of the state of development of

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economic threshold information, and the cost of treatments, if necessary. If the cost of sampling and advising is high relative to the cost of treating and unreliable at the same time, a grower may be inclined to treat without sampling. With the low cost of nematicides, this situation has occurred frequently.

Most sampling optimization studies in nematology have been conducted by collecting samples composed of various numbers of cores in prescribed patterns and making comparisons between the results of these samplings (1,5,14,16,19). Sampling studies in other disciplines have approached the optimization problem by sampling the field intensively in a systematic manner and using the data as a base on which many sample strategies can later be tested (3,17) or by computer simulation based on some knowledge of the population distribution in the field (12). The objectives were to investigate various sampling plans for nematode advisory purposes and evaluate them with respect to accuracy, reliability, and cost.

A sample plan consists of a collecting pattern, the number of samples comprising that pattern, the number of composite cores in the samples (= size), and the cost of collecting, processing, and counting the nematodes.

MATERIALS AND METHODS

The data base used in this study was established from a previous nematode distribution study (9) in which 1,936 soil cores were systematically collected from a 7-ha alfalfa field. The study site (Fig. 1) had a streak of fine-textured soil running in a north-to-south direction which influenced the distribution of some of the nematode species. Plant-parasitic species present were *Meloidogyne arenaria* (Neal) Chitwood, *Pratylenchus minyus* Sher and Allen, *Merlinius brevidens* (Allen) Siddiqui, *Helicotylenchus digonicus* Perry, and *Paratrichodorus minor* (Colbran) Raski.

Optimal sampling pattern: Interactive FORTRAN programs were written to simulate four collecting patterns: i) random collection of cores throughout the field; ii)

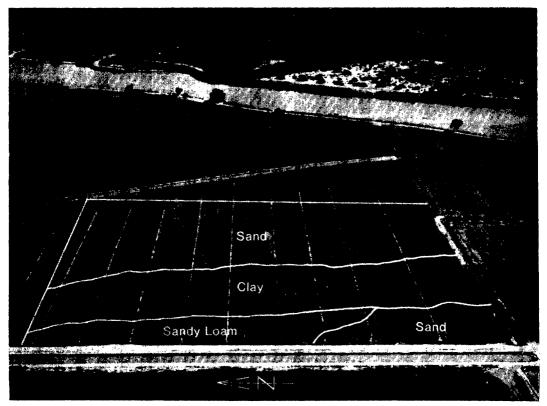


Fig. 1. Aerial view of the 7-ha alfalfa study site with soil texture areas delineated.

division of the field into a number of equalsized squares with cores randomly collected from each square (= cell, = sample); iii) division of the field into strips running north to south with the cores collected randomly in a zig-zag manner from each strip (= sample); and iv) division of the field into strips running east to west. For each collecting pattern, samples were collected ranging in number from 1 to 25 and ranging in size from 1 to 50 cores. Each strategy was replicated 50 times.

The parameters used to evaluate sampling plans were accuracy, reliability, and cost. Accuracy is the absolute deviation (DEV) of the estimate from the true mean of the field population, expressed as a percentage of the true mean. Reliability is the coefficient of variation (CV) between repeated field estimates, determined by the standard deviation between replications of the same collecting pattern divided by the mean of the field estimates for all replications. Functions were developed to describe the relationships for both DEV and CV with increasing numbers and size of samples.

Optimal sample size and number: Once the optimal sampling pattern was found, the cost of the entire sampling process including collecting, processing, and counting—was calculated. Cost is expressed as the number of hours required to execute the sample plan. The most cost-effective sample plan is the one which provides the required level of information at the minimum cost. The three major activities were partitioned into their components (Table I) and each component was given a time value. The sum of the components results in the cost function:

Table 1. Contributions of various activities to the cost of sampling nematode populations in a 7-ha alfalfa field.

Activity	Component	Minutes
Collect	Set up field	15
	Remove core from soil	.5/core
	Tag, bag, bulk, subsample	5/sample
Process	Log in sample, record results	2/sample
	Weigh	2/sample
	Set up extraction	30 .
	Extract	5/sample
Count	Count sample	10/sample

y = A + 24S + .5C + 1.1
$$\sqrt{C^2 + (16)(S^2)}$$

(i)

where:

- y = time required in minutes for that sample plan
- A = set-up time (45 min. in this case)
- S = number of samples
- \mathbf{C} = number of cores

The final radical in equation (i) represents the time required to walk this field in a zig-zag fashion. The equation is based upon ideal conditions (level field, adequate soil moisture) and on the use of a semi-automatic elutriator. It assumes easy recognition and identification of the nematodes. Overhead and cost of travel to the field are not included.

To determine the optimal sample and core combination with regard to cost, relative efficiency is introduced. Relative efficiency (RE) is defined as precision divided by cost; it provides a measure of the amount of information per unit effort. Precision is the inverse of DEV.

$$RE = 1/(DEV)(cost)$$
 (ii)

A mathematical approach was attempted using the partial derivatives of DEV and cost with respect to samples and cores. The relationship was expected to reach some maximum RE value and then decline as the increased cost of further samples and/or cores reduces the relative efficiency of the estimate. This however did not occur within the range of our data, and a descriptive approach is taken.

RESULTS

Optimal sampling pattern: No sampling pattern was outstanding in providing accurate and reliable estimates for the field. For all species, there was an inverse relationship between the sample size and numbers and the DEV and CV. Negative exponential response surfaces were fitted in each case using the equation:

$$y = (k)(S^{j})(C^{i})$$
(iii)

where:

y = DEV or CV

						DEV (%)				
					Sample Strategy					
Species	Pattern*	k	j	i	Small 1 sample (S) 1 core (C)	Medium 6 samples (S) 8 cores (C)	Large 12 samples (S) 16 cores (C)			
Meloidogyne	Random	123	497	530	123	17	8			
02	Cells	145	586	566	145	17	8			
	Strip N/S	113	492	552	113	15	7			
	Strip E/W	112	530	527	112	14	7			
Pratylenchus	Random	148	562		148	20	10			
	Cells	135		551	135	18	9			
	Strip N/S	164	515	610	164	18	8			
	Strip E/W	111		464	111	17	8			
Merlinius	Random	434	469	437	434	75	40			
	Cells	524		537	524	70	34			
	Strip N/S	564	535	500	564	76	37			
	Strip E/W	262	290	339	262	78	50			
Helicotylenchus	Random	330	503	495	330	48	24			
	Cells	357	476	591	357	45	21			
	Strip N/S	322	598		322	45	22			
	Strip E/W	564	625	678	564	45	18			
Paratrichodorus	Random	234	560	578	234	26	12			
	Cells	206	482		206	25	12			
	Strip N/S	210	540	561	210	25	12			
	Strip E/W	173		545	173	22	11			

Table 2. Constants for equation, $y = kS^{j}C^{i}$, where y is the deviation (DEV) of the nematode population estimate from the true mean. DEV was calculated using the appropriate constants for small, medium, and large sample strategies in four patterns.

*See text for pattern descriptions.

			j		CV (%) Sample Strategy				
Species	Pattern*	k		i	Small 1 sample (S) 1 core (C)	Medium 6 samples (S) 8 cores (C)	Large 12 samples (S) 16 cores (C)		
Meloidogyne	Random	170	562	564	170	19	9		
	Cells	178	579	591	178	18	8		
	Strip N/S	153	533	559	153	18	9		
	Strip E/W	164	577		164	18	8		
Pratylenchus	Random	217	606	507	217	26	12		
	Cells	267	629	626	267	24	10		
	Strip N/S	211	517	625	211	23	10		
	Strip E/W	167	567	528	167	20	9		
Merlinius	Random	824	589	533	824	93	43		
	Cells	836	556	579	836	93	42		
	Strip N/S	975	—. 57 7	610	975	97	43		
	Strip E/W	1045	618	630	1045	93	39		
Helicotylenchus	Random	568	589	589	568	58	26		
	Cells	577	534	634	577	59	26		
	Strip N/S	516	640	490	516	65	29		
	Strip E/W	711	578	736	711	55	22		
Paratrichodorus	Random	329		606	329	33	14		
	Cells	310	565	621	310	34	15		
	Strip N/S	290	557	591	290	31	14		
	Strip E/W	249	538	595	249	28	13		

Table 3. Constants for equation, $y = kS^{3}C^{1}$, where y is the coefficient of variation (CV) between repeated estimates of the same sampling strategy. CV was calculated using the appropriate constants for small, medium, and large sampling strategies in four patterns.

*See text for pattern descriptions.

- **S** = number of samples
- C = number of cores

The data fit the equation at P < .001 in all cases.

As sample size and number increased, accuracy and reliability increased (Fig. 2, Tables 2, 3). Although some patterns initially provided lower estimates and reduced DEV and CV rates faster than others, all patterns using more than eight samples of eight cores provided similar levels of accuracy and reliability for a particular species (Fig. 2). Substantial differences among the species occurred in the accuracy and reliability of the field estimates as well as in the rate of decline of DEV and CV, due to distribution and population density differences (Tables 2, 3).

Because all patterns were similarly efficient in reducing DEV and CV over the entire range of core/sample combinations (Tables 2, 3), the quickest one to implement

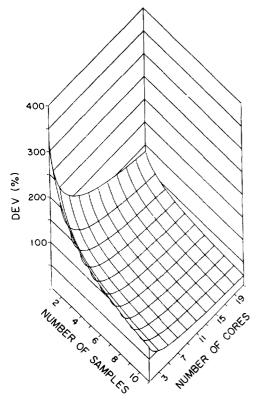


Fig. 2. Influence of increasing number of samples and cores on the percent deviation of the population estimates from the true field mean of *Helicotylenchus digonicus* from equation (iii), DEV=(322.)(S^{-.598})(C^{-.435}).

in the field would be the least expensive. Division of the field by strips is the quickest to implement because of the common border of all strips. The establishment of strips involves partitioning one edge of the field appropriately. The sampler begins at this edge, walks the length of the strip in the recommended zig-zag fashion (4,6,13, 18), randomly collects half the number of cores required for a strip on the way out to the opposite border and collects the remainder of the cores on the return trip to the starting border. All the samples are thereby deposited at one edge of the field for easy collection. Subdividing the field in this way is a form of stratified random sampling and supplies more information for the effort invested than other sampling techniques, since within-field distribution of the nematode populations may be discerned, as in the association of Helicotylenchus and Merlinius with the fine-textured soil (9). Optimally, the stratification should maximize the variance between strata and minimize the variance within a stratum (7,10). In this particular field the north-south pattern of strips was more efficient than eastwest because it isolated the main edaphic influence, the streak of fine-textured soil.

Random sampling requires walking the field many times to collect the cores from the random positions. Subdivision by cells involves additional time to establish the cells since the internal cells have no existing border and must be established by survey. Only the results of stratified random sampling by north-south strips in this field will be discussed.

Optimal sample size and number: Values of DEV were generated using equation (iii) for 1-11 samples and 1-100 cores and substituted into equation (ii) to yield RE. Since the previous regression used data points to a maximum of only 50 cores, it was necessary to collect data from the strip stratification program with the strips running north-south to a maximum of 100 cores for 1-11 samples, replicated 25 times. This was significantly fitted to the same negative exponential curve at P < .001. The strip stratification algorithm proved to be too costly to go beyond 11 samples of 100 cores. The relationship of RE to samples and cores was similar for all species but differed

Table 4. Relative efficiency (RE) of various sample-core combinations for five plant-parasitic nematodes. Values above the bold line represent sample plans requiring less than 5 hr.

							Cores						
Samples	4	12	20	28	36	44	52	60	6 8	76	84	92	10
						M	eloidogy	ne					
1	1.28	1.91	2.14	2.24	2.28	2.29	2.27	2.25	2.22	2.18	2.15	2.11	2.0
2	1.46	2.28	2.65	2.85	2.95	3.00	3.0 2	3.02	3.00	2.98	2.95	2.92	2.8
3 4	1.49 1.47	2.39 2.40	2.85 2.91	3.11 3.2 2	3.27 3.42	3.36 3.55	3.42 3.64	3.44 3.70	3.45 3.73	$3.45 \\ 3.75$	$3.44 \\ 3.75$	3.42 3.75	3.4 3.7
4 5	1.44	2.38	2.91	3.25	3.49	3.66	3.77	3.85	3.91	3.94	3.96	3.97	3.9
5 6	1.44	2.38	2.88	3.25	3.51	3.70	3.84	3.94	4.02	4.07	4.11	4.13	4.
7	1.36	2.28	2.80 2. 8 4	3.22	3.50	3.71	3.87	3.99	4.08	4.15	4.20	4.24	4.
8	1.33	2.23	2.79	3.18	3.48	3.70	3.88	4.01	4.12	4.20	4.26	4.31	4.
8 9	1.30	2.18	2.73	3.14	3.44	3.6 8	3.87	4.01	4.13	4.22	4.30	4.36	4.
9 10	1.30	2.18	2.69	3.09	3.40	3.65	3.84	4.00	4.13	4.23	4.32	4.39	4.
11	1.24	2.09	2.64	3.04	3.36	3.61	3.81	3.98	4.12	4.23	4.33	4.40	4.
		4					atylencl						
•	1.02	1.57	1.80	1.90	1.95	1.97	1.97	1.95	1.94	1.91	1.89	1.86	1.
1 2	1.02	1.97	2.25	2.44	2.55	2.61	2.64	2.66	2.65	2.64	2.63	2.61	2.
3	1.21	2.01	2.43	2.69	2.85	2.95	3.01	3.05	3.07	3.08	3.08	3.07	3.
4	1.20	2.03	2.50	2.80	3.00	3.13	3.23	3.29	3.34	3.36	3.38	3.39	3.
5	1.18	2.01	2.51	2.84	3.07	3.23	3.36	3.44	3.51	3.55	3.58	3.60	3.
6	1.15	1.98	2.49	2.84	3.10	3.29	3.43	3.53	3.62	3.68	3.72	3.76	3.
7	1.12	1.95	2.46	2.83	3.10	3.30	3.46	3.59	3.68	3.76	3.82	3.86	3.
8	1.10	1.91	2.42	2.80	3.08	3.30	3.48	3.61	3.72	3.81	3.88	3.94	3.
9	1.07	1.87	2.38	2.76	3.06	3.29	3.47	3.62	3.74	3.84	3.93	3.99	4.
10	1.05	1.83	2.34	2.73	3.03	3.27	3.46	3.62	3.75	3.86	3.95	4.03	4.
11	1.02	1.80	2.30	2.69	2,99	3.24	3.44	3.61	3.75	3.86	3.96	4.05	4.
							Merliniı						
1	0.25	0.37	0.42	0.44	0.44	0.44	0.44	0.43	0.43	0.42	0.41	0.41	0. 0.
2 3	0.29 0.30	$0.45 \\ 0.48$	0.52 0.57	0.56 0.62	$\begin{array}{c} 0.58 \\ 0.65 \end{array}$	0.59 0.67	0.59 0.68	0.59 0.68	0.59 0.68	0.58 0.68	0.58 0.68	$0.57 \\ 0.67$	0.
3 4	0.30	0.48	0.57	0.64	0.68	0.71	0.03	0.08	0.03	0.74	0.75	0.74	0
5	0.29	0.48	0.58	0.65	0.70	0.73	0.75	0.77	0.78	0.79	0.79	0.79	-0
6	0.29	0.47	0.58	0.65	0.71	0.74	0.77	0.79	0.81	0.82	0.82	0.83	0.
7	0.28	0.46	0.57	0.65	0.71	0.75	0.78	0.80	0.82	0.83	0.84	0.85	0
8	0.27	0.45	0.57	0.65	0.70	0.75	0.78	0.81	0.83	0.85	0.86	0.87	0.
9	0.27	0.45	0.56	0.64	0.70	0.75	0.78	0.81	0.84	0.85	0.87	0.88	0.
10	0.26	0.44	0.55	0.63	0.69	0.74	0.78	0.81	0.84	0.86	0.88	0.89	0
11	0.26	0.43	0.54	0.62	0.68	0.74	0.78	0.81	0.84	0.86	0.88	0.89	0
						Hel	icotylen	chus	_				
1	0.38	0.58	0.67	0.71	0.73	0.74	0.74	0.73	0.73	0.72	0.71	0.70	0
2	0.44	0.72	0.86	0.93	0.98	1.00	1.01	1.02	1.02	1.02	1.01	1.00	0
3	0.46	0.77	0.94	1.04	1.10	1.14	1.17	1.18	1.19	1.20	1.20	1.20	1
4	0.46	0.79	0.97	1.09	1.17	1.22	1.26	1.29	1.31	1.32	1.33	1.33	1
5	0.46	0.79	0.98	1.11	1.21	1.27	1.32	1.36	1.38	1.40	1.41	1.42	1
6	0.45	0.78	0.98	1.12	1.22	1.30	1.36	1.40	1.43	1.46	1.48	1.49	1
7	0.44	0.77	0.97	1.12	1.23	1.31	1.38	1.43	1.47	1.50	1.52	1.54	1
8	0.43	0.75	0.96	1.11	1.23	1.32	1.39	1.44	1.49	1.53	1.56	1.58	1
0	0.42	0.74	0.95	1.10	1.22	1.32	1.39	1.45	1.50	1.54	1.58	1.61	1
9 10	0.41	0.73	0.94	1.09	1.21	1.31	1.39	I.46	1.51	1.56	1.59	1.62	1

Samples	Cores												
	4	12	20	28	36	44	52	60	68	76	84	92	100
						Par	atrichod	lorus					
1	0.68	1.08	1.24	1.33	1.37	1.39	1.39	1.39	1,38	1.36	1.35	1.33	1.32
2	0.79	1.32	1.58	1.72	1.81	1.86	1.89	1.91	1,91	1.91	1.90	1.89	1.88
3	0.82	1.40	1.71	1.91	2.03	2.11	2.17	2.20	2.23	2.23	2.24	2.24	2.24
4	0.82	1.42	1.77	1.99	2.15	2.26	2.34	2.39	2.43	2.45	2.47	2.48	2.49
5	0.81	1.41	1.78	2.03	2.21	2.34	2.44	2.51	2.56	2.60	2.63	2.65	2.66
6	0.79	1.40	1.78	2.04	2.24	2.38	2.50	2.58	2.65	2.70	2.74	2.77	2.79
7	0.77	1.38	1.76	2.04	2.24	2.40	2.53	2.63	2.71	2.77	2.82	2.86	2.89
8	0.76	1.35	1.74	2.02	2.24	2.41	2.54	2.65	2.74	2.81	2.87	2.92	2.96
9	0.74	1.33	1.71	2.00	2.22	2.40	2.55	2.67	2.76	2.84	2.91	2.97	3.01
10	0.73	1.30	1.69	1.98	2.20	2.39	2.54	2.67	2.77	2.86	2.93	3.00	3.05
11	0.71	1.28	1.66	1.95	2.18	2.37	2.53	2.66	2.77	2.87	2.95	3.01	3.07

Table 4. (Continued)

in the level of precision (Fig. 3). The order of decreasing precision of population estimates was Meloidogyne, Pratylenchus, Paratrichodorus, Helicotylenchus, and Merlinius. In all species, the maximum RE within a 5-hr time constraint was obtained by taking six samples of 68 cores. Beyond 68 cores, the RE leveled off asymptotically at an upper level, except for Helicotylenchus and Paratrichodorus which oscillated slightly about their maxima (Table 4, Fig. 3). We concluded that the RE did not peak above 60 cores because of the limitations placed by the number of cores that were taken. If more cores could be taken, the flattened portion of the curve might begin to decrease. This plateau could be explained by

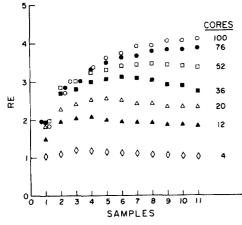


Fig. 3. Influence of increasing number of samples and cores on the relative efficiency (RE) in estimating the population of *Pratylenchus minyus*.

the nature of the cost function, equation (i), which allows increasing number of cores to be taken at very little increased cost (Fig. 4).

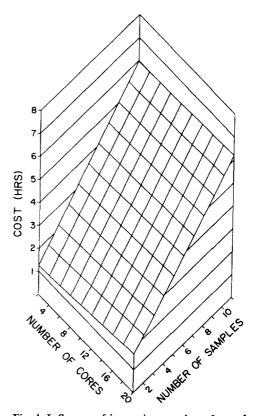


Fig. 4. Influence of increasing number of samples and cores on the cost of estimating the population of nematodes in an alfalfa field. Cost function includes collecting, processing, and counting the samples.

DISCUSSION

No sampling process has unlimited resources (15). Economic constraints, which can be considered fixed at some upper limit, are the most important factors in the design of sample programs in commercial advisory services. For the purpose of discussion, 5 hr is set as the upper limit for sampling, processing, and counting the nematodes in this 7-ha alfalfa field.

There are many sample and core combinations which fall below the 5-hr limit (Fig. 4). Table 4 is divided into two parts by a bold line which represents the 5-hr cutoff; the RE values above the line are achieved in less than 5 hr, below the line in more than 5 hr. The greatest maximum RE at 5 hr or less occurs at 6 samples of 68 cores (Table 4). This is true for all five species. The population estimate of three nematode species (Meloidogyne, Pratylenchus, and Paratrichodorus) fall below 10%, while Helicotylenchus and Merlinius are within 15% and 25% of the true mean. These latter levels are misleading since they represent the mean for the field, averaged over all strips. In reality, each strip mean would be known, providing valuable information in locating areas of nematode concentration. Such areas then might be differentially treated according to the infestation level.

If it is assumed that pest management decisions can be made with population estimates within 15% of the true mean, then extra effort was expended for some species while for one (Merlinius) the 15% level of precision was not reached. Another way to consider optimization might be by reaching the required precision level for management decisions at minimum effort. The population densities of four nematode species in this field can be estimated within the 15% precision limits and remain within the practical cost constraints of 5 hr. However *Merlinius* would require 8 hr to reach 15% of the true field mean.

Current recommendations (4,13,18) suggest that a sample represent an area no larger than about 2 ha and be composed of no less than 20 cores. In this 7-ha field, this would be four samples of 20 cores and would result in population estimates within 15% of the true mean for two species

(Meloidogyne and Pratylenchus) and an estimate of Paratrichodorus within 20%. The remaining two species would have estimates within 38% for Helicotylenchus and within 60% for Merlinius.

Since this study site was sampled at only one point in time, recommendations cannot be generalized from this study because the variation between fields and dates is unknown. However, the main components of a sample which influence the resulting estimator are emphasized by this investigation (Fig. 5). The foremost consideration is the biological reality of the species being sampled. This reality is described in part by the horizontal distribution which varies greatly among the various species in this field (9). Other components of the estimator should be tailored to this reality of the pest's biology. For example, the objectives of a sampling may be for quantifying *Meloidogyne* rather than Merlinius for pest management reasons. Precision levels of 50% may be acceptable for Merlinius, while the Meloidogyne estimate must be kept within 20% of the true mean.

An important consideration in sample design from an advisory viewpoint is the economics of sampling. This will have a great influence on confidence levels, overall sample design, and possibly on the objectives of the sample. Increasing sample size is much less costly than increasing the number of samples (Fig. 4).

Other components of sample design include the survey technique (simple random, stratified random, cluster), sample pattern (cell, strip), number of samples, and number of cores. All these components are interconnected with each other (Fig. 5) and one cannot be changed without influencing the others.

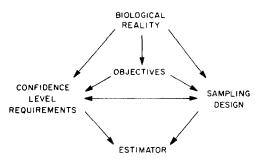


Fig. 5. Major components of sampling which influence the population estimate.

The preceding approach to the sampling problem allows a systematic consideration of the cost/precision compromise among species. In field situations, most of the information required for this systematic approach is not available, suggesting the importance of preliminary sampling and perhaps longer term contracts between the advisory services and their clients to build this type of data base. Such an approach may add to the cost of sampling, but the resulting information will be more valuable for pest management decisions.

This investigation has demonstrated the importance of having some knowledge of the biology of the nematode pest of interest when designing a sample plan to provide the appropriate level of precision at minimal costs. Samples should consist of as many cores as possible (more than 20) to improve precision while not substantially increasing the cost.

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