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Standard Guide for Field Evaluation of Nematode Control Agents—Determination of Nematode Population Responses to Control Agents¹

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INTRODUCTION

Chemical control agents are necessary for the efficient production of many crops. If nematicides are to be tested and used properly, there must be a consensus of informed opinion on reliable, standardized sampling procedures and extraction methods. In selecting extraction procedures, special consideration must be given to target species and soil type. Regardless of methods used in evaluating nematode responses to nematicides, results are no better than the weakest links of the experiments. For nematode population data to be of value, soil samples must be collected at the proper time in an appropriate probing pattern. Samples also must be handled properly after collection, and the appropriate extraction procedure must be utilized.

No one method of collecting samples for nematode assay or method for extracting nematodes to evaluate nematicides can be used in all situations. Therefore, the procedures presented in this standard are presented only as a guide. However, specific procedures for sampling and extracting nematodes are recommended for various combinations of different types of crops, soil textures, sampling periods, and nematodes. If any of these procedures are to be utilized reliably in evaluating responses to nematode control agents, investigators must be relatively familiar with the population dynamics of the target nematode on the particular crop and the specific geographic region involved. Procedures in the various methods for extracting nematodes are given in Sections 9 through 59 and in Tables 1 and 2.

In evaluating the efficacy of nematode control agents, characterization of population responses of the target nematode species is as important as determining plant responses. Most nematicides exert their maximum effect shortly after application, but long-term changes in soil biology often occur. For example, the proportions of species in nematode communities often shift after chemical treatments of soil. In addition, some nematicides stimulate plant growth in the absence of nematodes (1)²; others affect insect, fungal, or bacterial populations, or a combination of these (2). See Task-Force report on “Evaluation of plant responses”, C. C. Orr, Chairman; and “Interactions with other pathogens and other pesticides”, G. A. Bergeson, Chairman.

The most important considerations in determining nematode population responses to nematode control agents are the collection of representative soil or root samples, or both, from each plot or field, adequate mixing of these samples, and extraction of nematodes by the procedure best suited for a given experiment, target species, and soil type. Procedures used depend on the kinds and numbers of nematodes present and their characteristics and the nature and condition of the samples, including soil texture and the time of collection. If nematode numbers are below a detectable level, an appropriate bioassay may also be used.

1. Scope

1.1 This guide covers soil sampling techniques, numbers of soil cores, root samples, and related procedures for soil collection for nematode population studies.

1.2 This guide also covers methods used to separate nematodes from soil or roots, counting techniques, rating of

tests, and reporting of results.

1.3 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* For more specific precautionary statements see 4.6.2.1, 5.3.1, Section 7, and 39.6.1.

¹ This guide is under the jurisdiction of ASTM Committee E-35 on Pesticides, and is the direct responsibility of Subcommittee E35.16 on Nematode Control Agents.

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² The boldface numbers in parentheses refer to the list of references appended to this guide.

2. Terminology

2.1 Definitions:

2.1.1 *bioassay*—the utilization of appropriate hosts to determine relative population levels (has been used primarily

TABLE 1 Extraction Procedures for Various Combinations of Nematode and Soil Types^A

Type of Soil	Types of Nematodes				
	Sedentary Forms			Migratory Forms	
	<i>Meloidogyne</i> and "Related" Types ^B	<i>Heterodera</i> or <i>Globodera</i> spp. ^C	<i>Cricone-mella</i> spp.	Endoparasites ^D	Ectoparasites ^E
Sandy	CF, BT, SAE-CF, SBT, SAE-BT, SFS, EE	CF, BT, SAE-CF, SBT, FC, SAE-BT, CFHS	CF, SAE-CF	CF, BT, SBT, SAE-BT, SAE-CF, SFS, MC, SK	CF, BT, SBT, SFS, SAE-BT, SAE-CF
Clay	CF, BT, SBT, SAE-CF, EE, SAE-BT	CF, SAE-CF, BT, SAE-BT, FC, CFHS	CF, SAE-CF	CF, BT, SAE-CF, SBT, SAE-BT, MC, SK, BBT	CF, SAE-CF, BT, SBT, SAE-BT
Organic	CF, BT, EE	CF, BT, CFHS	CF	CF, BT, MC, SK, BBT	CF, BT

^A See Table 2 for selection of specific techniques for given sampling time. See the Annex for abbreviations.

^B Also for other nematodes—*Nacobbus*, *Rotylenchulus*, and *Tylenchulus* spp.

^C If a hatching factor is required, use FC only.

^D *Pratylenchus*, *Radopholus*, *Hoplolaimus*; for some plants, *Helicotylenchus*, *Tylenchorhynchus*, *Ditylenchus*, and *Aphelenchoides*.

^E *Belonolaimus*, *Dolichodorus*, *Helicotylenchus*, *Hemicylophora*, *Longidorus*, *Paratylenchus*, *Rotylenchus*, *Scutellonoma*, *Paratrichodorus*, *Trichodorus*, *Tylenchorhynchus*, and *Xiphinema*. (SFS is most efficient for large forms such as *Belonolaimus* and *Xiphinema*.)

TABLE 2 Extraction Procedures for Use at Various Sampling Times for Nematodes on Annual and Perennial Crops^A

Sampling Time	Types of Nematodes				
	Sedentary Forms			Migratory Forms	
	<i>Meloidogyne</i> and "Related" spp. ^B	<i>Heterodera</i> or <i>Globodera</i> spp. ^C	<i>Cricone-mella</i> ^D	Endoparasites ^E	Ectoparasites ^F
Annuals:					
P_i	CF, BT, SAE-CF, SBT, SFS, SAE-BT	BT, CFHS, SBT, FC, SAE-CF or BT	CF, SAE-CF	CF, BT, SBT, SFS, SAE-BT	CF, BT, SAE-CF, SBT, SFS, SAE-BT
P_m	BT, SBT, SAE-BT	BT, SBT, SAE-BT	CF (+VS) ^D	BT, SBT, SAE-BT	BT, SBT, SAE-BT
P_m	CF, BT, SAE-CF, SBT + EE, SAE-BT	CF, BT, SBT, SAE-BT	CF, SAE-CF (+VS) ^D	CF, BT, SBT, SAE-BT, MC, SK	CF, BT, SBT, SAE-BT
P_i	CF, SBT + EE + GI	CF, SAE-CF, SBT, FC, SAE-BT, CFHS	CF	CF, SBT, SAE-CF, SFS, SAE-BT, MC, SK	CF, SAE-CF, BT, SBT, SFS, SAE-BT
Perennials:					
P_i	CF, SAE-CF, SBT + EE + GI, SAE-BT	...	CF, SAE-CF	CF, SAE-CF, BT, SAE-BT, SBT, SFS, MC, SK	CF, SAE-CF, BT, SBT, SFS, SAE-BT
P_m	BT, SBT, SAE-BT	...	CF, (+VS) ^D	BT, SBT, SAE-BT, MC, SK	BT, SBT, SAE-BT
P_m and P_i	(Same as with Annuals)				

^A See Table 1 for consideration of soil type; see Table 3 for data required for sampling times; see the Annex for abbreviations.

^B Also for other nematodes—*Nacobbus*, *Rotylenchulus*, and *Tylenchulus* spp.

^C If a hatching factor is required, use FC only.

^D Need vital stain to identify living specimens for P_m and sometimes P_m samplings.

^E *Pratylenchus*, *Radopholus*, *Hoplolaimus*; for some plants, *Helicotylenchus*, *Tylenchorhynchus*, *Ditylenchus*, and *Aphelenchoides*.

^F *Belonolaimus*, *Dolichodorus*, *Helicotylenchus*, *Hemicylophora*, *Longidorus*, *Paratylenchus*, *Rotylenchus*, *Scutellonoma*, *Paratrichodorus*, *Trichodorus*, *Tylenchorhynchus*, and *Xiphinema*. (SFS is most efficient for large forms such as *Belonolaimus* and *Xiphinema*.)

for endoparasites such as root-knot and cyst nematodes, but can be used for any nematode under suitable propagation conditions; usually done in greenhouse).

2.1.2 *detection level*—the minimum population density of a nematode species that can be routinely identified from a soil or plant tissue sample (will vary with each sampling and extraction technique).

2.1.3 *economic threshold density*—the minimum number of nematodes per unit volume of soil (or weight of plant tissue) required to cause an economically significant loss in crop production.

2.1.4 *fumigant nematicide*—a gas, volatile liquid, or solid that diffuses through the soil pore spaces as a vapor (or gas, which is partially water-soluble).

2.1.5 *grid or systematic sampling*—collection of the specified number of borings over entire plot area (one for each square metre, etc.) for P_i ; if crops are established, collection of borings from root zone (in the rows for row crops)

(sometimes referred to as *stratified sampling*). (See also P_i .)

2.1.6 *horizontal head*—for centrifuge, head so structured that individual tubes (swinging bucket) are in horizontal position when centrifuge is operating.

2.1.7 *molar solution*—a 1-g molecular weight in sufficient water to make 1 L of solution.

2.1.8 *multipurpose chemicals*—these are of three basic types:

2.1.8.1 *fumigant*—a volatile chemical that disperses throughout the soil and controls nematodes, fungi, bacteria, insects, and weeds.

2.1.8.2 *contact nematicide*—a nonvolatile chemical that does not disperse readily but which controls nematodes (and in some cases soil insects) that come in contact with the chemical.

2.1.8.3 *systemic nematicide*—a chemical that is absorbed by a plant and translocated throughout the plant and controls nematodes.

TABLE 3 Nematode Population-Density Data Required from Standardized-Volume Soil Samples from Annual and Perennial Crops^A

Sampling	Types of Nematodes				
	Sedentary Forms		Migratory Forms		
	<i>Meloidogyne</i> and "Related" spp.	<i>Heterodera</i> or <i>Globodera</i> spp.	<i>Cricone-mella</i>	Endoparasites	Ectoparasites
Annuals:					
P_i	Juveniles in soil	Cysts and juveniles in soil	Nemas in soil	Nemas in soil	Nemas in soil
P_{ie}	Living juveniles in soil	Living juveniles	Living nemas in soil	Living nemas in soil	Nemas living in soil
P_m	Juveniles and eggs (Soil and roots)	Juveniles and cysts	Living nemas in soil	Living nemas in soil and roots	Nemas living in soil
P_r	Above + GI	As above	Nemas in soil	Nemas in soil and roots	Nemas in soil
Perennials:					
P_i	Juveniles and eggs (Soil and roots)	...	Nemas in soil	Nemas in soil and roots	Nemas in soil
P_{ie}	Living juveniles	...	Living nemas in soil	Living nemas in soil and roots	Living nemas in soil
P_m and P_r	Same as for annuals				

^A See Tables 1 and 2 for specific extraction procedures for specific combinations of types of nematodes and soil.

TABLE 4 Nomograph of Root-Knot Galling Indexes

Galling Index Systems ^A				Total Root System Galled, %
0 to 4	0 to 5	1 to 6 ^B	0 to 10	
0	0	1	{ 0	0
	1	2	{ 1	10
			2	20
1	2	{ 3	{ 3	30
			4	40
2		{ 4	{ 5	50
	3		6	60
			7	70
3		{ 5	{ 8	80
	4		9	90
4	5	6	10	100

^A Roots are scored for the degree of galling using any one of several root-galling indexes, all of which are comparable and relatively interchangeable. A minimum of ten root systems should be evaluated per treatment.

^B This scheme is recommended for use in evaluating nematicides (see Fig. 2 for examples that are similar to those of Zeck (3)).

2.1.9 *nonfumigant nematicide*—a nematicide that has little or no volatility and which must be mixed thoroughly in soil as a granular formulation or applied in water (water-soluble formulation).

2.1.10 P_f —final population density (per 100 to 500 cm³ of soil near or at harvest or end of growing season).

2.1.11 P_i —pretreatment initial population density (per 100 to 500 cm³ of soil as determined within 1 week prior to treatment).

2.1.12 P_{ie} —intermediate (2 to 4 weeks), post-treatment population density (per 100 to 500 cm³ of soil).

2.1.13 P_m —midseason population density (per 100 to 500 cm³ of soil).

2.1.14 *ppm*—parts per million (1 mg/L of H₂O equals 1 ppm; 1 lb/120 000 gal H₂O equals 1 ppm) and with water is equivalent to µg/mL (W/V) or µg/g (W/W). It is recommended that µg/mL or µg/g be used in place of ppm. The solvent should be specified where non-water solutions are utilized.

2.1.15 *population density*—number of nematodes per unit volume of soil or weight of plant tissue.

2.1.16 *random sampling*—points for collecting borings determined by random chance (not usually suitable for nematodes such as *Meloidogyne* species, which occurs in aggregates).

2.1.17 *relative g force*—for centrifuge, force (minimum, average, maximum) times that of gravity:

$$\text{relative } g \text{ force} = 1.11 \times 10^{-5} R \times \text{rpm}^2$$

where:

R = radius, mm.

2.1.18 *sampling tube*—a metal (all or part) tool for collecting soil cores of constant diameter and volume. Major types are:

2.1.18.1 *can or bucket auger*—steel auger with 50 to 75-mm diameter by 150-mm bucket fitted with cutting edges at the base (useful for obtaining large soil samples and roots to depths of 900 to 1000 mm).

2.1.18.2 *cone-shaped sampling tube*—useful for routine sampling of loose sandy soils (4).

2.1.18.3 *Oakfield soil-sampling tube*—20-mm inner diameter, open side, 380 mm long (good for routine sampling to depth of 200 mm).

2.1.18.4 *Viemeyer soil-sampling tube*—25-mm inner diameter, 1200 mm long (with reinforced head. The tube can be driven into the soil to a depth of 900 mm).

2.1.19 *sieve-mesh number*—number of wires (filaments) per inch (running in each direction). It is recommended that the size of the openings be given in micrometres and that the mesh number be given in parentheses.

2.1.20 *soil sterilant*—a chemical (methyl bromide, etc.) that kills all soil organisms.

2.1.21 *tolerance limit*—minimum number of nematodes that inhibit plant growth (number per unit volume of soil or weight of plant tissue). The tolerance limit may not be of economic significance.

2.2 Abbreviations and Symbols:

2.2.1 The following abbreviations and symbols appear throughout this guide:

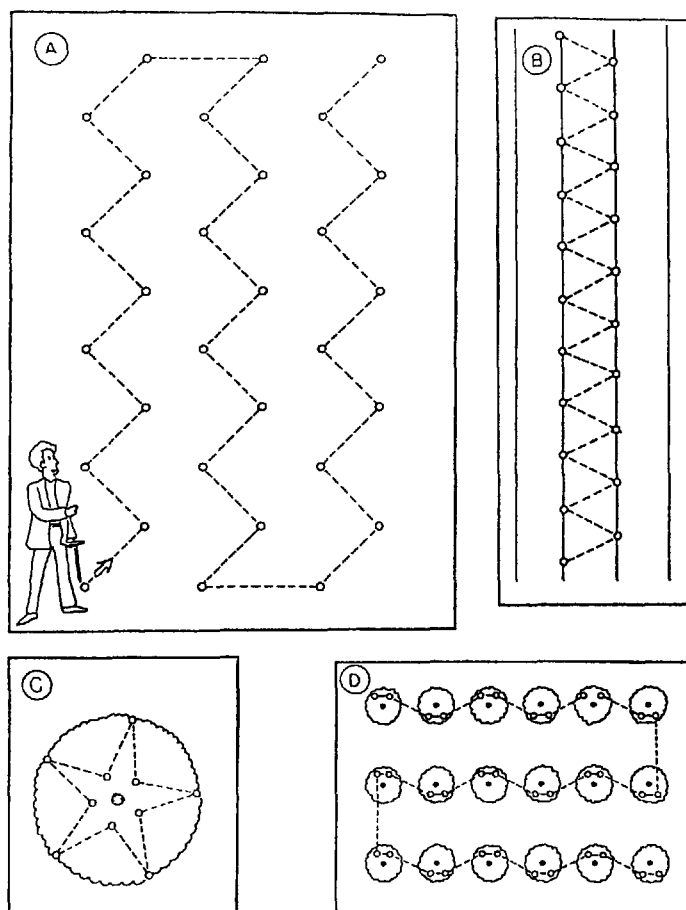


FIG. 1 Diagrams for Collecting Samples

- (a) Recommended pattern for collecting minimum of 20 to 30 cores for pretreatment nematode assay of a large test field or plot.
 (b) Sampling pattern for collecting soil in the two center rows in a four-row plot (data taken only from these two rows).
 (c) Procedures for sampling in feeder-root zone of established perennials. (For small plots, additional cores should be collected from each plant.)

BBT	= blender-Baermann tray combination
BF	= Baermann funnels
BT	= Baermann trays
CDS	= Cobb's decanting-sieving method
CF	= centrifugal flotation
CFHS	= centrifugal flotation with "heavy sugar"
EE	= elutriation-dissolution of gelatinous matrices of egg masses and staining
FC	= fenwick can
GI	= gall index
MC	= mist chamber
P_f	= final nematode density
P_i	= initial nematode density
P_{te}	= post-treatment nematode density
P_m	= midseason nematode density
SAE	= semi-automatic elutriator
SAE-BT	= combination of semi-automatic elutriator with Baermann trays
SAE-CF	= combination of semi-automatic elutriator with centrifugal flotation
SBT	= sieving-Baermann trays
SFS	= sugar-flotation-sieving
SK	= shaker extraction
VS	= vital stain

3. Summary of Guide

3.1 For nematode population data to be useful, soil cores must be collected at the proper time and in an appropriate

probing pattern to provide representative samples. A minimum number of soil cores should be collected in a systematic procedure to cover each plot, depending on the size: small plots (1 to 2 m²),—10 or more cores; medium size plots (5 to 100 m²)—20 or more cores; large plots (>100 m²)—30 or more cores. On established row crops, sampling should be done in the row (50 to 120 mm from bases of plants). For ornamentals or other perennials, cores should be collected in the drip line. The biology and population dynamics of nematode species must be considered in selecting appropriate extraction methods for each sampling time. Nematode populations may consist of relatively motile to quiescent forms, cysts, individual eggs, eggs in masses, or various combinations of these. Soil and root samples are best utilized for most endoparasites and semi-endoparasites. Actively-moving juveniles or adults (in roots or soil) can be extracted by (1) Baermann trays or other modifications, (2) a combination of sieving and Baermann methods (flotation or sieving and pans, etc.), and (3) flotation (or elutriation) and cottonwool method. Methods adapted to both motile and nonmotile forms include: sieving, elutriation, centrifugal-flotation, and sugar-flotation-sieving. Nematodes in roots