

## **NIPM Item #7.1**

### **SAMPLING PROCEDURES AND TECHNIQUES FOR DETECTION OF NEMATODES BY LABORATORY EXAMINATION**

Sampling requirements and procedures for detection of nematode infestations in field, container, flat, frame grown or bare-root nursery stock are provided in Section 3055.5 of the California Code of regulations. Sampling procedures for the detection of nematodes using approved laboratory methods are as follows:

#### **1. Field grown nursery stock - generally**

Collect root samples on a 40 foot by 40 foot grid interval throughout the planting. Samples should include a small amount of the soil adhering to or along the feeder roots collected at each point. Samples may be composited on an acre or nursery stock variety basis.

#### **2. Field grown nursery stock - special case**

At the discretion of the Department, root samples may be collected on an 80 foot by 80 foot grid interval and composited on a two acre basis when both of the following conditions have been met:

- A. The planting site has been treated at the product labeled rate for the kind of nursery stock being produced.
- B. No nematodes have been found by laboratory methods in the previous two successive nursery crops on the growing site.

#### **3. Container, flat, and frame grown nursery stock**

A composite sample is to be obtained by collecting roots from one plant in every 100 square feet of bench or frame space. Each sample should be kept to logical and practical sizes.

#### **4. Delimitation**

When an original sample is positive for nematodes, delimitation sampling may be performed on a 20 foot by 20 foot grid interval with samples composited on a 1/4 acre basis. All delimitation shall be done at the Department's discretion. Any nursery stock represented by a delimitation sample in which a nematode is detected is considered infested. Any nursery stock represented by a delimitation sample that is free of nematode shall be considered commercially clean. Container, flat, and frame grown lots should not be delimited. The latter should be unnecessary where initial sampling was of reasonable lot sizes.

#### **5. Bare root sampling procedures**

Each kind or variety should be sampled separately. The lot and sample sizes should be determined individually for each kind and variety. Root samples should be carefully protected from heat and desiccation. The sample should be taken from roots that are moist and in fresh condition. The inspector should look for visible symptoms of nematode infestation. Include any roots with galls in the composite sample taken from the lot. Mark the "GALLS" box on the Pest and Damage Record form if any are observed.

**Sampling Table for Examination of Bare-Root Nursery Stock for Nematodes**

<b>Number of plants, bundles or boxes in lot</b>	<b>Sample Size</b>
2 - 8	2
9 - 15	3
16 - 25	5
26 - 40	7
41- 65	10
66 - 110	15
111 - 180	25
181 - 300	35
301 - 500	50
501 - 800	75
801 - 1,300	110
1,301 – 3,200	150
3,201 – 8,000	225
8,001 – 22,000	300
22,001 – 110,000	450
110,001 – 550,000	750
550,001 - Over	1,500

**Table Use Directions:**

**Step 1.** Find the total number of plants in the lot in the left column. The sample size in the right column is the number of plants to sample.

**Step 2.** Find the total number of bundles, boxes or containers in the lot, in the left column. The sample size in the right column is the number of units to take samples from.

**Step 3.** Divide the number from Step 1 by the number from Step 2. That is the number of plants to sample in each of the units from Step 2.

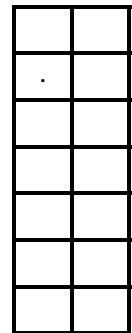
**Nematode Sampling Grids**

The number of sub-samples collected when doing nematode sampling may seem confusing. These grids show how the sampling numbers are calculated. Two acre samples consist of 14 sub-samples. One acre samples consist of 28 sub-samples. One quarter acre samples consist of 28 sub-samples.

**Two Acre Nematode 80 ft. X 80 ft. sampling grid**

One nematode sample is taken per two acres of nursery stock. That one sample is made up of 14 sub-samples that are taken on a 80 foot by 80 foot grid. There are 87,120 sq. ft. in two acres. The 14 sub-samples are computed by dividing 87120 by 80, and dividing the result by 80. A sample is taken every 26 steps at a space of 80 feet. A 80 foot by 80 foot grid actually yields 6,400 sq. ft. When multiplied by the 14 samples, it would yield 89,600, but that is close enough.

$$\begin{array}{r} 1089 \\ 80 \overline{)87120} \end{array} \quad \begin{array}{r} 13.61 \\ 80 \overline{)1089} \end{array}$$

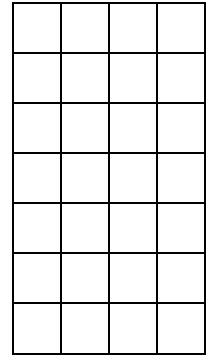


### One Acre Nematode 40 ft. X 40 ft. sampling grid

One nematode sample is taken per acre of nursery stock. That one sample is made up of 28 sub-samples that are taken on a 40 foot by 40 foot grid. There are 43,560 sq. ft. per acre. The 28 sub-samples are computed by dividing 43,560 by 40, and dividing the result by 40. A sample is taken every 13 steps at a space of 40 feet. A 40 foot by 40 foot grid actually yields 1600 sq. ft. When multiplied by the 28 samples, it would yield 44,800, but that is close enough.

$$40 \overline{)43560} \begin{array}{r} 1089 \\ \end{array}$$

$$40 \overline{)1089} \begin{array}{r} 27.23 \\ \end{array}$$

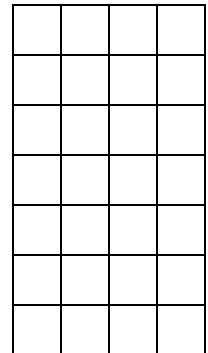


### 1/4 Acre Nematode 20 ft. X 20 ft. sampling grid

One nematode sample is taken per 1/4 acre to delimit an infestation. That sample is taken on a 20 ft. by 20 ft. grid. That works out to 28 sub-samples per 1/4 acre. There are 10,890 sq. ft. in a 1/4 acre. The 28 sub-samples are computed as follows: 10,890 is divided by 20 and the result is divided by 20. A sample is taken every 6 steps at a space of 20 feet. A 20 foot by 20 foot grid would yield 400 sq. ft. When multiplied by the 28 samples, that would yield 11,200 sq. ft. per 1/4 acre or 44,800 sq. ft. .

$$20 \overline{)10890} \begin{array}{r} 545 \\ \end{array}$$

$$20 \overline{)545} \begin{array}{r} 27.25 \\ \end{array}$$



### Paperwork, Tools, Steps, Tips and Notes for Nematode Sampling

#### Filling in PDR's

1. Give complete information on county code, situation code, activity code.
2. Write legibly
3. Use ink
4. Give complete and correct information
5. Mark one of Plant pathology, entomology, nematology
6. Use supplement form for up to seven samples.

#### Tools

1. Trowel, Shovel or sampling tube
2. Disinfectant solution
3. Map, or paper to draw map
4. Plastic Bags, twist ties to seal samples, marker to mark bags
5. Cooler, Blue ice to keep samples cool

#### Collecting the Sample – Steps and Tips

1. Make a map of the area to sample to indicate sites where samples are taken. Be organized and far-sighted. It may be necessary to resample to delimit the area if an infestation is found.
2. Develop a number system for samples
3. Collect up to one quart of roots and adhering soil from plants.
4. Sample plant varieties separately
5. Most nematodes are in the top one foot of soil.
6. Do not moisten sample by enclosing a moist paper towel in sample bag.
7. Clean tools with five parts water one part household bleach in a bucket.

8. Put sample in durable plastic bag or double bag sample.
9. Sample should be neither wet or dry.
10. Handle samples with care. Nematodes may be killed if the sample is dropped from a height.
11. Label sample bag with sharpie pen that will not smear or rub off.
12. Place PDR outside of plastic bag in shipping box.
13. Keep samples cool (50-55°F). During collection, put samples in a cooler. Use blue ice wrapped in paper to avoid freezer burn to sample. Do not forget samples in places that could get hot.

### **Sample Areas and Sizes**

Container, Flat, and Frame Grown Nursery Stock - sample from every 100 square feet of bench space.

Field Grown Nursery Stock

1. 40 by 40 foot one acre samples
2. 80 by 80 foot two acre samples
3. Delimited 20 by 20 foot  $\frac{1}{4}$  acre samples

Bare root samples - refer to sampling table