INTRODUCTION
Diagnosis of nematode damage and management on an as-needed basis requires assessing nematode soil infestation levels in representative soil samples collected from the target field or a section of the field. Inclusion of roots in the collected soil samples is advantageous and might be necessary in the case of sedentary and migratory endoparasites including the root-knot, cyst and lesion nematodes that complete part or their entire lifecycle in host tissue. However, the accuracy of diagnosing the problem nematode(s) involved and especially determining the level of its infestation will depend largely on the thoroughness of the sampling method, time of sampling, handling and storage of the samples as well as on the biology of nematode species involved.

Plant-parasitic nematodes are unevenly distributed in infested fields and particularly during the early years after introduction (Fig. 1). Their distribution has been best described by a negative binomial model where the sample variance (variability between samples) is greater than the sample mean (sample average). This clumping distribution is complicated further in several nematode species such as the root-knot and cyst nematodes where they deposit large numbers of eggs in an egg sac or a cyst (dead female body), respectively. All plant-parasitic nematodes are associated with plant roots (Fig. 2), which also contribute to the observed clumping distribution. Furthermore, plant-parasitic nematodes are highly sensitive to high temperature, freezing, and drying thus proper handling and storage of the samples is necessary.

TIMING OF SAMPLING
For most plant-parasitic nematodes, the soil populations vary greatly throughout the growing season. Generally, they are lowest in the spring and highest at harvest time and shortly after. Soil population assessment conducted in the spring and close to planting time is most useful, as the population densities can be related to crop performance and potential yield losses. However, it is most convenient to sample at harvest or few weeks later as the nematodes are at their highest numbers and the availability of time to devote for extensive sampling and processing of samples. Sampling should be timed to when the soil is close to field capacity, and should be avoided when the soil is dry or too wet.

INTENSITY AND DEPTH OF SAMPLING
To overcome the uneven horizontal distribution of nematodes, it is critical to take several composite soil samples from each field or production unit. Obviously, the higher number of soil samples taken per field, the higher accuracy will be obtained in assessing the nematode infestation levels and the better infestation map is generated for potential spot (precision treatment) management. If a section of the field exhibits differential plant growth or consists of a different soil type, it will be best to collect separate soil samples from these sections. A minimum of 4 composite samples is suggested per production unit, preferably < 2 to 3 acres per sample.

Each composite soil sample should consist of >15 sub-samples and again the more is the better. Follow a predetermined sampling design for collecting the sub-samples (V, X or Z transect) (Fig. 3). The highest number of plant-parasitic nematodes is found around or in fibrous roots of host plants. For vegetables and field crops, the populations will be highest in the plowed layer/top soil, generally 6 to 10 inches deep. However, roots of trees and ornamental crops grow much deeper, thus a deeper sampling depth is required.

Figure 1. Patchy and uneven soil nematode distribution resulted in patchy uneven onion maturity.

Figure 2. Organic “muck” soil and onion roots collected from an field at harvest.
If sampling is conducted before harvest of row crops, then the sub-samples should be collected in the rows where most of the roots will be. Similarly, in-row sampling is suggested in reduced tillage production systems. Sub-samples from established trees and ornamental crops should be obtained from the drip-line of the foliage.

Using a trowel, narrow-blade shovel or a soil sampling probe; a small volume of soil should be collected from the plowed layer at each sub-sample location and placed in the collection bucket/container. The top inch of the soil should be discarded before collecting the soil, if the soil was without a surface cover or dry. The soil in the bucket/container collected from all the sub-sample sites is then carefully and thoroughly mixed and approximately a 1 to 2 quart-size portion is placed in a plastic bag and labeled appropriately to represent one composite sample.

**Handling and Storage of Samples**

It is extremely critical to preserve the nematodes collected in the soil or root samples in as close a physiological active state as possible until they are processed for nematode extraction and/or bioassay with a susceptible host plant. Soil bioassays depend on the ability of the nematodes in the sample to move toward roots, penetrate appropriate root tissues, and cause the diagnostic symptoms and/or damage. A number of the extraction methods also depend on the mobility of nematode to move through filters or soil as well as on their normal size and/or density, thus the need for preserving them in an active state.

It is important to prevent exposure of the collected samples to direct sunlight or to heat by placing them in the shade or better in an insulated cool container. If the sample are to be stored for days or weeks, they are best placed in a cold room (about 40 to 50°F) or in an unexposed cool place at room temperature.