A cost-efficient sampling process to reliably estimate nematode populations in the field.

Sampling for nematodes

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The nematodes important in crop production are microscopic roundworms that spend all or at least a part of their life cycle in the soil. They can significantly reduce crop yields by an estimated 5 to 15 percent regionally or nationally. Losses may be much greater in local situations, including areas marginal for the host crop or favorable for the parasitic nematodes, or both. Under such conditions, a crop may undergo considerable moisture or nutrient stress and be unable to compensate for the additional biological stress of nematode parasitism.

A few plant-parasitic nematodes cause distinct symptoms on plant roots, which can be used to detect and determine the amount of infection. Most species have a general debilitating effect on the root system and reduce its effectiveness in uptake and transport of moisture and nutrients. Aboveground symptoms of nematode infection are unthriftiness of the plant and general indications of root damage. Plants wilt under hot, dry conditions, because the roots cannot take up enough water to replace that lost from the leaves. In wet soils, plants become waterlogged, because impaired root systems are unable to facilitate removal of excess water by transpiration.

Considerable research is now being conducted on crop losses to be expected under various nematode population densities. Such information will be useful in making pest management decisions, which involve cultural, biological, or chemical manipulations, alone or in combination, as and where they are necessary. Sampling the nematode populations in a field makes it possible to determine the types of nematodes present and, therefore, to assess susceptibility of a proposed crop. Knowing infestation levels aids in predicting damage to the crop.

Sampling may also detect a pattern of nematode distribution in the field, which may permit partial treatment, reducing the economic and environmental impacts of the method(s) used. Any pest management decision process, for nematodes or other pests, is only as good as the reliability of the sample upon which population measurements are based.

The sampling problem

Because of the nematodes' size and habitat, a two-step process is required to estimate population densities and identify the nematode types present. Unlike estimation of aboveground pest populations in the field, determining nematode numbers involves a costly process of collecting soil samples and extracting the nematodes for microscopic examination in a laboratory.

In quantifying any population, cost and practicality limit the precision of the estimate. One could determine the exact number of nematodes in a field by removing all of the soil and extracting all of the nematodes. A compromise is to take samples from the field, determine the population in the samples, and assume this represents the field as a whole. The greater the proportion of the field included in the sample and the more efficient the sampling process, the greater the probability that the sample estimate represents the field situation.

We are faced with a cost and benefit tradeoff. If it costs far more to determine the nematode population in the field than to use any of the possible control or management alternatives, the best management decision would be to employ one of those alternatives as insurance without measuring the nematode community. Such an approach would be contrary to general pest management philosophy. The sampling pattern, therefore, must be streamlined to the most cost-efficient process of reliably estimating the nematode population in the field.

Plant-parasitic nematodes are classified into two major groups according to their feeding habits—that is, as ectoparasites or endoparasites. Ectoparasitic nematodes spend their entire life cycle outside the roots and feed by inserting a stylet into cell layers at various depths below the root surface. Most ectoparasites remain motile throughout their life cycle and can move to other feeding sites as food diminishes or ecological conditions become unfavorable. They may, however, be vulnerable to biological and chemical antagonists in the soil.

Endoparasites spend at least a portion of their life cycle within the root. They may remain migratory and move from one section of the root system to another as conditions become unfavorable, or may set up specialized feeding sites and become immobile in the root. In the latter case, a strong physiological relationship is usually established between the parasite and its host. Since different procedures are required to extract nematodes from soil than from root tissue, it is useful to know which nematode species are potentially important to the established or anticipated crop.

Nematode life cycles and feeding habits must be considered in relation to the time of sampling. Time of year the population is sampled is important when interpreting nematode counts as potential causes of crop damage in perennials. Environmental and climatic conditions determine the rates at which nematodes progress through their life cycle.

In a study of nematode populations in a vineyard (fig. 1), samples were taken at monthly intervals for a year. Root-knot nematodes reached a population peak in September, but were at a very low level in the soil in June and July, when most were in the roots and entering a reproductive phase.

For annual crops, preplant sampling is most convenient in making nematode pest management decisions. If the preplant population is being measured at the end of a previous crop, then it is important to consider rates of nematode population decline in soil and root tissue. Actual counts of nematodes during the winter do not indicate their potential to infect roots when they become active in the spring. Nematode survival and infectivity are related to the amount of time since the previous crop and environmental conditions during this period. Further research in this area is needed, since it is impossible for every field to be sampled on the same date.

The most important factor affecting nematode distribution in soil is their food source—the host plant. Nematodes aggregate within the root zone of a plant they are feeding on or within an area influenced by the root zone of a previous host crop. Since the preferred feeding site of many nematodes is the actively growing root tips, the distribution of root tips is important in determining nematode microdistribution within a root system.

Nematode biology also influences microdistribution. Some nematodes, such as the endoparasitic root-knot nematodes, deposit their eggs in a mass, and the developing larvae and adults become distributed in a region centered on the original egg mass. Other nematodes, such as the migrating endoparasitic lesion nematode, deposit their eggs individually, leading to a more uniform distribution of larvae and adults within the root system.

Vertical distribution is affected by similar factors and also by the nematode's soil-moisture, oxygen, and temperature requirements. In the vineyard population study, vertical distribution of *Meloidogyne* spp. and *Xiphinema americanum* (fig. 1) varied with depth, apparently because of their differing oxygen requirements and ability to withstand the fluctuating environmental conditions of the upper soil regions.

Distribution of plant-parasitic nematodes across a field is influenced by many factors, both ecological and cultural. Soil texture is important: some species are better adapted to coarse-textured soils, whereas others flourish in a fine-textured soil. In a sampling study of an alfalfa field with a fine-textured streak across the generally sandy field (fig 2), *Helicotylenchus* nematodes were closely associated with the finer soil, whereas the root-knot nematodes (*Meloidogyne*) were generally distributed across the field with higher numbers in the sandy regions. Other conditions that affect nematode distribution across a field include the drainage and irrigation pattern, and leveling operations.

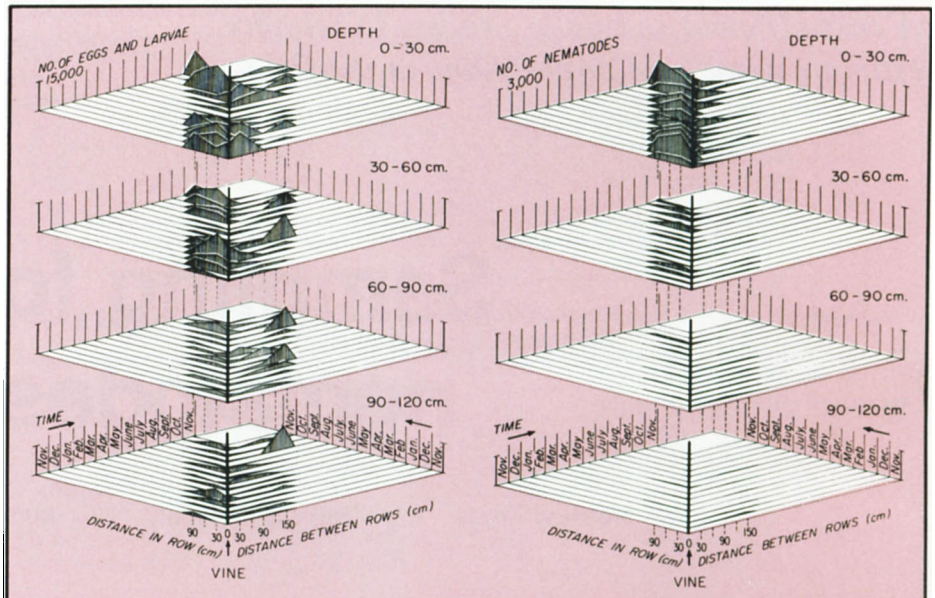

Fig. 1. *Meloidogyne* spp. (left), *Xiphinema americanum* (right) distribution on grapevine roots. Closest set of axes is first sampling date; farthest set, the last.

In a field previously subdivided and used for two different crops, it is probable that two different nematode communities exist. Since root distribution influences nematodes, their distribution in a field is related to the spacing of current or previous crops. An orchard causes major aggregations uniformly distributed across the field with low population densities between aggregations. A row crop results in aggregations between rows and within the rows, depending on plant spacing.

Solving the problem

Reliability of a nematode sampling scheme is indicated by the variability among repeated estimates of the field population. If repeated samples from the same field, at the same time, give radically different estimates of the nematode populations, the sampling plan is not a reliable basis for a pest-management decision.

One approach for improving efficiency in estimating nematode population densities is to stratify—to divide the field into regions of probable difference in nematode population densities. Such differences may result from variations in cropping history or in soil or climatic conditions, which can often be determined by grower observations. Each stratum is represented by at least one sample. Ideally, variability between population estimates from each stratum would be maximized, and variability within each stratum minimized.

If the field in figure 2B were stratified perpendicularly to the fine-textured soil region, every sample would include soil from both the fine- and coarse-textured areas, and the effect of this soil variation would be diluted. If the field were stratified parallel to the fine-textured streak, at least one stratum would represent this region and would maximize the variability in population estimation of *Helicotylenchus* between strata. Nematode distribution within the field thus can be mapped so that treatment of individual strata, resulting in a lower pest-management cost, may be considered.

Much of the time and effort in evaluating nematode populations is spent extracting, identifying and counting nematodes from soil samples. Consequently, each sample usually represents as large an area of the field as possible. The field is stratified and a sample collected as a series of soil cores chosen throughout each uniform stratum and bulked together.

The number of cores that can be bulked into a sample is limited. If it is too large, it may require mixing and subsampling, which introduces another potential source of variability. However, subsampling may cost less than increasing the number of samples.

Since the defined strategy for measuring the nematode community in a field is to minimize the variability within a stratum, the necessity for a sampling pattern for an established crop is evident. One pattern is to sample from the region of the root zone of the plants. For orchard crops, two cores are taken in the region of the drip line from each tree site sampled. In row crops, cores are taken from the root zones of the plants sam-

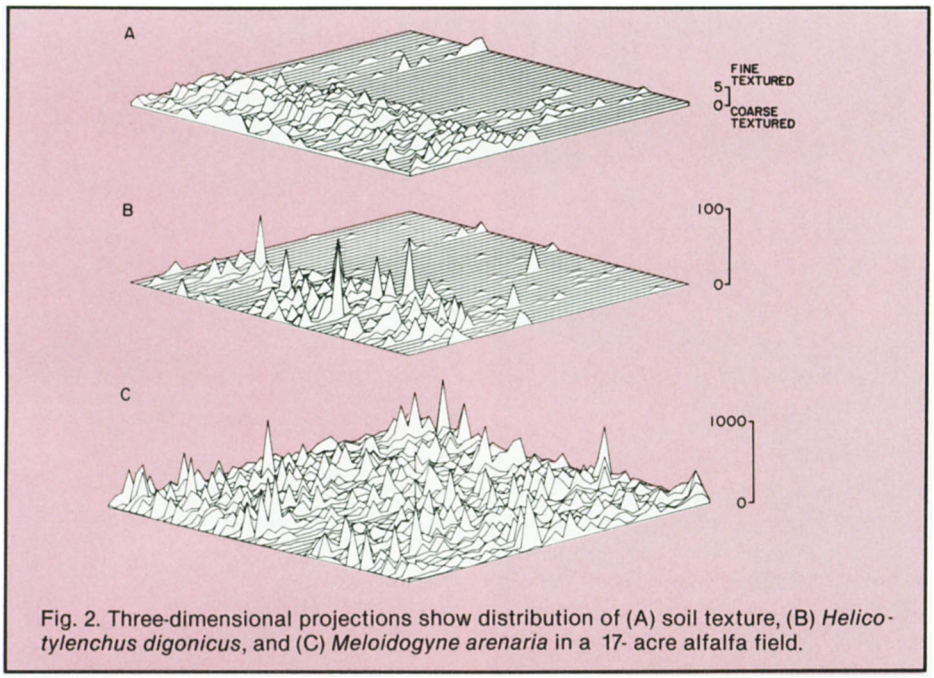

Fig. 2. Three-dimensional projections show distribution of (A) soil texture, (B) *Helicotylenchus digonicus*, and (C) *Meloidogyne arenaria* in a 17- acre alfalfa field.

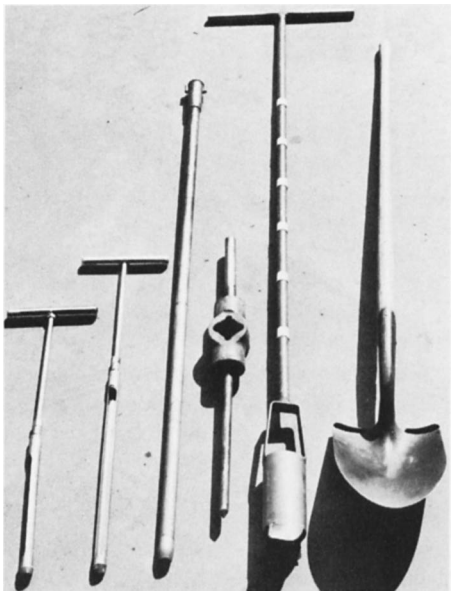

Fig. 4. Sampling tools, from left: 12- and 18-inch (1-inch diameter) soil tubes, Veihmeyer tube, hammer, auger, and shovel.

pled. In a fallow field, it is usually impossible to determine where the root systems of previous crops were, and cores constituting each sample are usually taken across the entire stratum in a zigzag pattern.

As the number of samples representing a stratum or field increases so does the cost. If the number of cores constituting each sample is increased, the cost increase is much less, because the sample number does not increase. Increasing the cost by increasing the number of cores or samples, or both, is weighed against the benefit it provides in reliability, as measured by the variation among repeated estimates when additional cores or samples are incorporated in the field population estimate.

This approach was used in studying nematode distribution in an alfalfa field. The variability among samples is very high at low sample and core numbers and decreases rapidly as sample and core numbers increase. The rate of decrease in variation becomes gradually less, and a point is reached at which further increases in number of samples or cores give little benefit (fig 3). For this 20-acre field, reliability improved little beyond five samples of about 12 cores. The study supported empirically derived recommendations that one sample of about 12 to 20 cores should be used to represent no more than 5 acres of uniform soil texture.

As more data are generated on the relationship between precision and sample size, it should be possible to analyze costs to determine the sample and core number giving maximum return per unit cost. It is difficult to generalize here, because the figures used in such analyses will depend on individual field situations and on sampling and extraction techniques used.

Sampling cost may depend not only on pattern and areas to be covered, but also on the depth of sample required. If a field is being prepared for an orchard, information may be needed on nematode densities and distribution to depths of 3 to 5 feet. The sample will not be taken with a standard 12- or 18-inch long Oakfield-type tube, but perhaps a Veihmeyer tube or an auger (fig. 4), requiring much more effort. Again, there is a trade-off between cost and benefit from improved reliability of the estimate with increased core or sample numbers. In an orchard, where the investment is large and the objective is to prolong productivity and longevity of the trees, the higher sampling cost can be justified. Further research is necessary to allow cost analyses for row crops and orchard crops.

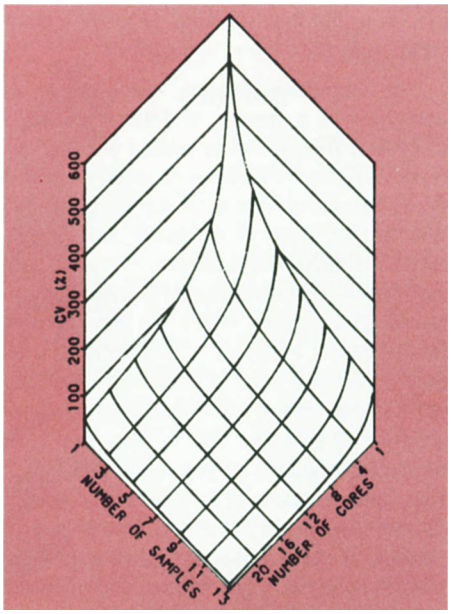

Fig. 3. Effect of sample number and size on coefficient of variation (CV%) among repeated samples from an alfalfa field.

Many of the extraction techniques used to evaluate nematode densities in the soil require that the nematodes be alive and able to move out of either root material or soil samples. Even when the extraction is by a passive flotation mechanism, identification of the nematodes in the sample is considerably easier and more certain when they are alive. Nematodes are easily killed by high temperatures and mishandling of soil samples. After considerable investment in achieving a reliable soil sample, it is of utmost importance that the soil samples be handled with care so that the nematodes will remain as active and infective as the populations in the soil that they represent. If soil samples are left in the field or in a car so that temperatures in the bags kill the nematodes or reduce their activity, the reliability of the population estimate will be decreased. The transportation, handling, and storage of soil samples for estimation of nematode populations are extremely vulnerable steps in estimating nematode densities.

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