

# A GUIDE TO SOIL AND CROP SAMPLING FOR NEMATODES

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## WHY TEST FOR NEMATODES?

Plant parasitic nematodes (or eelworms) are microscopic worms found in soil which feed on plants, reducing crop growth and yields. They commonly attack the roots producing symptoms of general root insufficiency: unthrifty growth, leaf yellowing and wilting. All horticultural crops are attacked by one or more nematodes but particular nematodes attack only certain kinds of crops, and crop varieties can vary from highly susceptible to highly resistant. Root-knot Nematode is one type of nematode which attacks a wide range of crops including vegetables, grapevines, fruit and nut trees, ornamentals, and some field crops, plus many weeds. Plant parasitic nematodes cause estimated losses to world agriculture of \$127 billion per year. Other nematodes attacking horticultural crops include Citrus, Cyst, Dagger, Pin, Ring, Root-lesion, Spiral, Stem and Bulb, Stubby-root, and Stunt Nematodes. Losses from nematodes are often underestimated because they are hidden in the soil. Testing for nematodes is needed to diagnose nematode-related problems, and is also useful before planting to help decide whether resistant varieties or rootstocks, soil fumigation or treatment with a nematicide may be beneficial.

## THE NEMATODE TEST

Soil and/or plant samples are tested for nematodes at the Plant Research Centre. The living nematodes are extracted from the samples over a period of up to seven days. The plant parasitic nematodes are then counted using a microscope, and the results are sent to the grower. Some nematodes occur mainly in the soil around roots, while others occur both in the soil and on or inside roots, so it is best to test both soil and roots.

**Soil only:** When only soil (which can include small roots) is submitted, only plant parasitic or free living nematodes can be counted quantitatively. Indicate on the specimen form whether you also want a count of the total free-living (or saprophytic) nematodes. These nematodes do not damage plants but are important in nutrient recycling and in interactions with other soil animals; some growers want an estimate of their numbers as an indication of overall soil health.

**Root tests:** For an effective examination of the roots, a complete (or as near as possible) root ball should be provided. A qualitative test (visual examination) can be made of roots etc. to determine the presence of galls or nematode egg masses, females or cysts. Quantitative root tests use different extraction methods than soil to determine numbers of nematodes per unit (fresh) weight of roots.

**Plants:** Quantitative tests can also be used on stem, bulb, tuber or leaf tissue for nematodes that infect these parts of plants and there are specific extraction methods that are specific for particular stages of these nematodes.

**Soil treatments:** It is important to advise if the soil has been treated with nematicide or fumigant, as this can impact significantly on the results.

## WHEN TO SAMPLE

Growers should take soil samples regularly for nematode testing, particularly before planting and at harvest, and should keep records of population levels in different blocks to assess the effectiveness of management programs. Nematodes are often found in highest numbers in the root zone of the crop towards the end of the growing season or in late summer to mid-winter. For Root-knot Nematode on grapevines, juveniles were found in one study to peak in soil in winter, and then to decline in spring as they entered roots. Numbers decline in weed-free,

fallow ground. Soil should preferably be moist but not wet at the time of sampling; nematode detection is most difficult in dry, fallow soil. Serial samples are best collected at an equivalent stage in the irrigation cycle or when soil moisture levels are similar.

Before planting, sample after cultivation but allowing sufficient time (at least 2-4 weeks) to both test the samples and to make arrangements for soil treatment etc. To determine the effectiveness of treatment, soil can be re-sampled 2-3 weeks after fumigation or 4-6 weeks after application of a non-fumigant nematicide (e.g. Nematicur<sup>(R)</sup>). A repeat sampling at mid-season may further help to determine the effectiveness of a nematicide. It is a good idea to take another sample at harvest or at the end of the growing season to determine the likely carry-over into the next crop and to help plan rotations including use of break crops, resistant varieties and fallows.

Where diagnosis is required for unthrifty growth, plant and soil samples may be collected at any time during the growing season. Samples should be collected from near the edge of declining patches and, for comparison, from areas of healthy plants. Plants that have already died are best avoided since nematode populations in their vicinity may already have declined. It is far easier to detect nematodes before ripping out a crop than afterwards in dry, fallow soil so plan ahead if possible.

*Whole plants, roots or tubers should be included to assist diagnosis.*

## **HOW TO SAMPLE**

Nematodes are usually unevenly distributed in a field and soil sampling procedures need to take this into account to obtain a truly representative sample. Very low or patchy populations are difficult to detect. The reliability of nematode counts is only as good as the sampling procedure used. Particular attention should be paid to the following:

### **Sampling Tools**

A soil tube or auger is useful for taking many sub-samples (cores) while reducing the total amount of soil collected. A spade can be used if most of the soil is removed by hand from along the vertical edge of the blade, and is useful for collecting roots and associated, relatively undisturbed soil. Whatever tool is used, it should be cleaned before sampling a separate plot or field, and soil disturbance should be minimised to avoid abrasion damage to the nematodes.

### **Depth of sampling**

Nematodes are most abundant within the root zone and, for shallow-rooted crops, samples can be taken to a depth of about 20 cm, but few nematodes are present in the first few cm of dry, sun-exposed soil so this can be omitted. Some nematodes are more abundant at greater depth in fallow soils during summer, and a second sample may be taken pre-planting from depth 20 to 40 cm or more, especially where deep-rooted crops, e.g. trees, vines, lucerne, were previously growing. In established crops, sample in the row from the root zone, near the plant stems or inside the dripline, collecting both soil and roots. In grapevines, a sampling depth of 0 to 30 cm is often used but some species reach highest populations at 30 to 60 cm in deep soils.

### **Number of sub-samples/samples**

For plots up to 100 square metres in area, at least 20-30 sub-samples (cores) should be taken per sample. For uniform areas up to 1 ha, at least 50 sub-samples should be taken. Sub-samples should be collected in a bucket or bag while walking up and down the field or orchard; various sampling schemes such as collecting a sub-sample at regular intervals along a "W" or "zig-zag pattern" have been used. Sub-samples that are too large make it difficult to obtain a representative composite sample of manageable size. Fields should be

divided into areas of 1 ha or less for sampling and separate samples should be taken from areas with different cropping histories, soil types/treatments applied, or crop health.

### **Handling and storage of samples**

With samples, fresher is better. Soil sub-samples should be gently mixed before placing at least 500 g in a plastic bag; do not use cloth or paper bags. For a root test at least 20 g of fine feeder roots will be needed, especially include roots showing symptoms such as galling, thickening, bunching, soil adherence etc. Seal bags to prevent drying and protect samples from heat; perishable plant samples especially need to be sent promptly &/or be refrigerated. Soil does not need to be refrigerated unless liable to be exposed to high temperatures or if it cannot be despatched promptly. Label the bag as appropriate and complete a *Specimen Form* with the test required, block/sample numbers, depth, crop type, name, address, phone/fax number etc. Do not put paper labels inside the bags with the soil. **Send the samples promptly to the laboratory.**

To ensure samples are promptly processed, pre-book tests and preferably collect and despatch samples early in the week.

### **Quarantine restrictions (samples collected interstate)**

Soil/ plant samples collected interstate need quarantine clearance: entry is prohibited if collected from areas where regulated pests/diseases such as Phylloxera, Potato Cyst Nematode, Green Snail or *Fusarium oxysporum* race 3 are known to occur. These restrictions protect our valuable agricultural industries, and breaches are subject to substantial penalties. Documentation is required by law; declaration forms/plant health certificates require time to organize, so plan ahead. Samples arriving without quarantine clearance/documentation are liable to be destroyed.

Inquiries can be made to Biosecurity SA phone (08) 8207 7820.  
<http://www.pir.sa.gov.au/biosecuritysa/planthealth>

If you suspect an outbreak of an exotic pest or disease notify your quarantine authority.

### **Suspected outbreaks of quarantine-status pests/diseases**

SARDI strongly encourages reporting of suspected outbreaks of exotic or quarantine-status pests and diseases; the earlier an outbreak is detected the greater the likelihood of successful containment and eradication. If a pest is allowed time to establish it may become impossible or impractical to eradicate: early reporting is in the interests of all agricultural industries. If tests confirm the presence of quarantine-status pests/diseases, re-imbursment of diagnostic charges if levied may be made once positive identification has occurred.

If you see unusual pests or diseases on plants, report it to the exotic plant pest hotline on: 1800 084 881. Visit [www.planthealthaustralia.com.au](http://www.planthealthaustralia.com.au) for further information.