

Practical 15-16

Extraction of plant-parasitic nematodes from plant material and insect vectors

In general, only migratory and sedentary endoparasites are recovered from plant material. Nevertheless, few ectoparasites might also be extracted, such as specimens still feeding on epidermal cells or nematodes trapped under collapsed root tissue. The extraction of motile nematodes requires different methods than extraction of sedentary immotile nematode stages. In addition, the plant tissue also affects the extraction procedure as roots, bulbs, wood or seeds require different methods to release the nematodes. Unfortunately, there is no single method suitable for all purposes. For quantitative analysis, it also needs to be considered that a significant part of the nematode population within plant tissue is in the form of eggs that will hatch over time. If those numbers are of interest, samples need to be incubated for 3–4 weeks allowing nematodes to hatch.

Direct examination

Plant material and insect vectors can be examined directly for motile and immotile nematodes under a dissecting or inverse microscope at magnifications of 10–50× or 50–400×, respectively, using transmitted and/or incident light.

Materials

- Petri dish;
- Pair of forceps, dissecting needle, scalpel;
- Handling needle for picking nematodes;
- Dissecting, inverse or compound microscope;
- Counting slide;
- Microscope slide.

Procedure

- Wash roots, bulbs or tubers to remove soil debris; seeds, foliage and wood chips can be processed directly;

- Place plant tissue in water in a Petri dish;
- Tear apart plant tissue with forceps, dissecting needle or scalpel to release nematodes;
- Examine nematodes within a counting slide at 25–40× magnification using a dissecting or inverse microscope or transfer nematodes with a handling needle to a microscope slide for inspection at higher magnification in a compound microscope;
- For insects carrying nematodes, decapitate and transfer the insect into a glass dish with water or 1% saline (NaCl) solution and then cut the insect in pieces with a scalpel to release the nematodes from the insect body.

Advantage

- Simple, fast and cheap;
- Small amount of water.

Disadvantage

- Laborious;
- Suspension is usually dirty due to plant or insect debris;
- Only suitable for small samples

Remarks

Motile nematodes will be released from the tissue within few minutes. However, resting stages of *Ditylenchus dipsaci* and *D. destructor*, such as in flower bulbs and infected seeds (*D. dipsaci* only), first have to rehydrate to become active which can take 2–4 h. Too long an exposure of nematodes in suspension should be avoided as plant secondary metabolites and decomposition products can kill nematodes.

2.2 Baermann funnel/Oostenbrink dish

This method for the extraction of motile nematodes was introduced by Baermann (**1917**) using a funnel. In its original version, the sample was wrapped in a tissue cloth and almost fully incubated in water resulting in very low nematode recovery. Modified versions use a wire basket plus filter to spread the sample over a larger area. In addition, the sample is only immersed half-way into the

water. Oostenbrink (**1954**) replaced the funnel by a dish. Since then, several modifications have been published such as by Whitehead & Hemming (**1965**), Rodríguez-Kábana (**1981**) and others.

Materials

- Knife, pair of scissors or blender;
- Cotton-wool milk filter or equivalent (e.g. cheesecloth, filter paper, paper towel);
- Funnel made of glass with a piece of soft polyethylene tube attached to the stem and closed with a spring or screw clip (Fig. [1](#)). Recommended slope of funnel is approx. 30°. For the Oostenbrink dish method plastic or stainless steel dishes (pie pan) are used (Fig. [2](#));
- Stand to hold the funnel;
- Support, such as plastic sieve or wire basket of large enough aperture to allow nematode passage (i.e. 250 µm);
- 20 or 25 µm aperture sieve;
- 100 mL glass beaker;
- Dissecting, inverse or compound microscope;
- Counting slide;
- Microscope slide.



Figure 1

[Open in figure viewer](#)[PowerPoint](#)

Modified Baermann funnel for extracting nematodes from plant material or soil (Photo: JKI, Germany).



Figure 2

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Oostenbrink dish. Left: Set-up showing plastic dish, supporting sieve made of 250 μm polyamide gauze and cotton-wool milk filter (Photo: JKI, Germany). Right: Set-up consisting of plastic dish, plastic basket and cotton-wool milk filter for extracting *Bursaphelenchus xylophilus* from wood chips (Photo: Vladimir Gaar, Diag. Lab. Prague, Czech Rep.).

Procedure

- Peel and chop plant tissue such as wood chips (for preparation of wood samples for *Bursaphelenchus xylophilus* see Appendix 3 of PM 9/1), leaves, roots, bulbs or tubers in ± 1 cm pieces or macerate in a blender for up to 1 min depending on source of plant material; seeds remain intact or can be split longitudinally to facilitate nematode removal (e.g. *Aphelenchoides besseyi*/rice, Hoshino & Togashi, 1999);
- Place plant material on the cotton-wool milk filter placed within a support (sieve);
- Submerge support with sample gently in the water of the funnel/dish;
- Nematodes leave the plant tissue, pass through the cotton-wool milk filter and sink to the bottom of the funnel stem or dish, respectively;
- Collect nematodes after 24–72 h by opening the spring or screw clip on the funnel stem or by collecting the nematodes of the dish in a glass beaker;
- Let the nematodes settle in the glass beaker and remove the supernatant, or pass suspension in the beaker over a 20 or 25 μm sieve to reduce the volume of water;
- Examine nematodes within a counting slide at 25–40 \times magnification using a dissecting or inverse microscope or transfer nematodes with a handling needle to a microscope slide for inspection at higher magnification in a compound microscope.

Advantages

- Simple and inexpensive;
- Small amount of water;
- Final suspension is clean;
- Good recovery of motile nematodes from small samples.

Disadvantages

- Only suited for samples up to 50 g;
- Lack of aeration in the water reduces nematode movement;
- Rapid bacterial growth for some plant materials (e.g. bulbs), especially after maceration;
- Poor recovery of relatively immotile nematodes (e.g. *Xiphinema*, *Hemicycliophora*, *Criconemoides*);
- Poor recovery from large samples.

Remarks

Alternatively, funnels made of plastic or stainless steel and/or using silicone tubes can be used. However, regarding the latter, diffusion of oxygen into water is lower than for polyethylene (Stoller, **1957**) which could slowly lead to asphyxiation. Depending on the plant tissue, most (50–80%) of the motile nematodes present will be recovered within 24 h; however, samples can be left on the funnel for up to 72 h or for wood chips even up to 14 days to increase recovery rate. For longer extraction periods regular tapping and adding of fresh water increases nematode motility and therefore recovery rate. Similar results can be achieved by using a solution of 0.15% H₂O₂ instead of water, or by placing the Baermann funnel/Oostenbrink dish in a mistifier. In general, extraction can be performed at room temperature (20°C). The diameter of the funnel or dish should be chosen so that sample layer will not be more than 1–2 mm. For larger sample sizes use aliquots or divide the sample over several funnels/dishes. The filter paper should retain remaining soil particles and plant debris but allow easy passage of nematodes at the same time. Milk filters made of cotton wool or fleece are commonly used. Usually, one or two layers work well. However, users should be aware that nematode passage can vary highly depending on filter material and thickness of filter. If in doubt, efficacy tests should be performed.

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