Document 9

SAC

REPORT ON POTATO CYST NEMATODE ASSESSMENTS

PPG (Premier Property Group) Gogarbank Road Field

On behalf of tie Ltd.

Dr Andy Evans
SAC

28th March 2007

Summary

This report regards the sampling and assessment of Gogarbank Road Field belonging to PPG (Premier Property Group) for Potato Cyst Nematode (PCN) at the request of tie Ltd.

Standard methods for sampling of PCN and soil washing to determine the presence of any PCN were followed.

PCN cysts were found in 2 of the 15 areas sampled, and one of these cysts contained viable PCN eggs or juvenile nematodes which equates to a PCN egg/juvenile population of <1 per g of soil.

Methods

The field to be sampled for PCN was segregated into 15 blocks (see Map 1). The field was sampled on 29th February 2007.

The methodology for sampling of PCN follows the protocol recommended in the proposal for the new European Union PCN Directive which aims to standardise sampling methods for PCN throughout the EU.

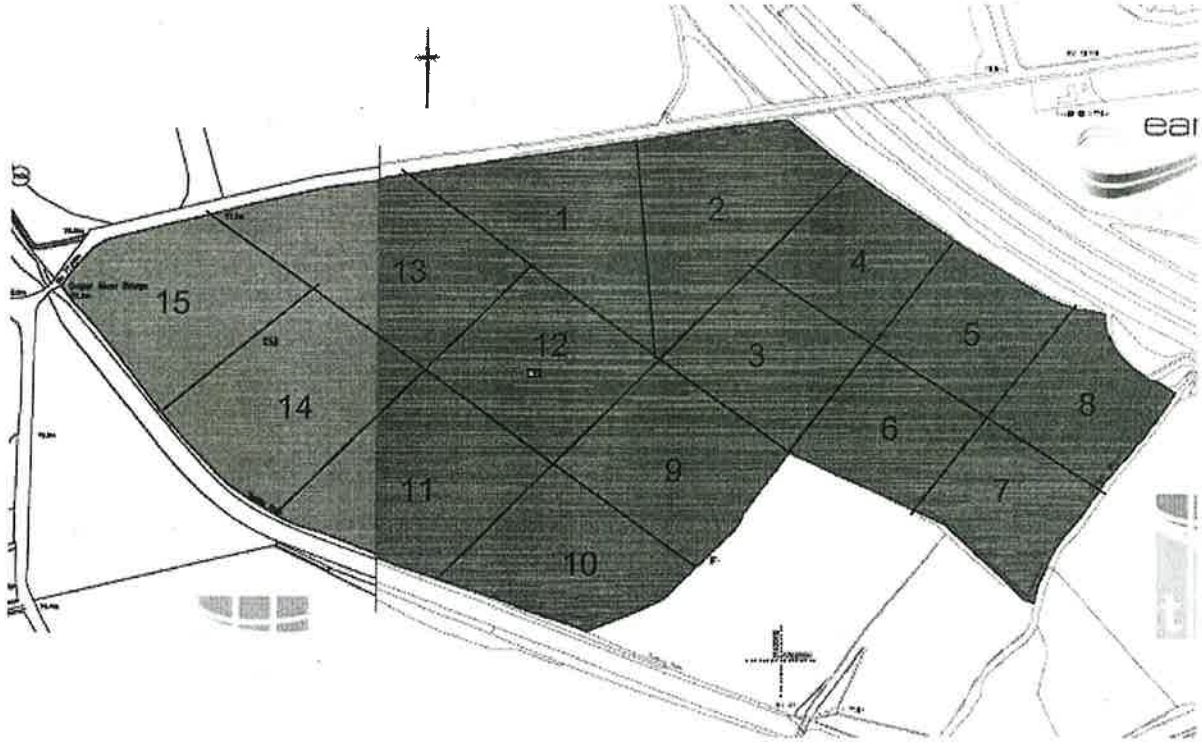
A soil sample with a standard size of approximately 1000-1500 ml soil/ha was collected from at least 100 cores/ha in a rectangular grid covering the entire block. Soil samples were taken using a corer that takes approx. 15ml of soil from a depth of approx. 5cm with each sample. The soil samples were bagged, labelled and taken back to SAC.

The SAC Standard Operating Procedure for PCN soil sample analysis (see Appendix 1 for full procedure) was strictly followed and is based on the European Plant Protection Organisation (EPPO) standards for Phytosanitary procedures for *Globodera pallida* and *Globodera rostochiensis*: Soil sampling methods (PM 3/30 (1) (see Appendix 2) (http://archives.eppo.org/EPPOStandards/PM3_PROCEDURES/pm3-30-e.doc).

In summary, soil samples are dried, crushed and washed through a Fenwick can which has a 850µm sieve in the funnel, and a 250 µm sieve at the outlet of the funnel. The 250µm collection sieve is removed and its contents washed into a filter paper in a filter funnel. Any PCN cysts are collected in the filter paper. Cysts are counted and removed from the filter paper and broken open whilst viewed under a microscope to determine whether there are any live eggs or juvenile nematodes present.

Results

The results for the dry weight of soil processed, total No. of PCN cysts found, No. of viable cysts, No. of eggs or juvenile nematodes and No. of other (non-PCN) cysts are given in Table 1.



Map 1. Field plan of area sampling for PCN

PCN cysts were found in areas 8 and 12 (Table 1, Map 1). The cyst in area 8 was non-viable, whereas the cyst in area 12 contained viable eggs and juvenile PCN, which equates to a PCN population of <1 eggs/juveniles/g of soil.
Other nematode cysts were also present – these are non-pathogenic to potatoes.

The fact that PCN cysts were found is indicative of potatoes being grown in the field in the past. PCN cysts are very resilient, and can remain viable for more than 25 years in the soil in the absence of any potatoes. The fact that 1 viable cyst was present suggests that potatoes were grown in the field within the last 25 years.

In summary, PCN cysts were detected in the field belonging to PPG, and one viable cyst was present.

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Table 1. Presence of PCN cysts and viability

Block No.	Dry weight (g)	Total No. cysts	No. viable	No. eggs/juveniles per g of soil	Other cysts
1	913	0	0	0	0
2	798	0	0	0	0
3	786	0	0	0	0
4	810	0	0	0	0
5	763	0	0	0	0
6	896	0	0	0	0
7	920	0	0	0	1
8	1001	1	0	0	1
9	763	0	0	0	0
10	703	0	0	0	2
11	770	0	0	0	0
12	771	1	1	<1	0
13	820	0	0	0	0
14	947	0	0	0	0
15	824	0	0	0	0

APPENDIX 1

Scottish Agricultural College
CROP & SOIL RESEARCH GROUP (CROP SECTOR)
STANDARD OPERATING PROCEDURE
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Potato Cyst Nematode (PCN) Soil Analysis

SOP Number:CS/POT/035/01	Date Of Issue: Dec 06	Written By: K Kasperek
Supersedes: New	Revision Date: Dec 09	Approved By: A Evans

Objective

Testing Soil for presence of Potato Cyst Nematodes (PCN)

Field of Application: Soils

Responsibility: Trained staff

Documents required: SAC data sheets or log books as required

Check Divisional Risk Assessments before carrying out this procedure.

Procedure

1. On arrival

1.1 Samples are sent to the Crop Clinic to be logged in and given a unique number.

1.2 Samples are secured by checking seals on bags and placing in a box labelled with samples' reference numbers, ready for transferring to the soil wash facilities.

2. Soil Preparation

2.1 At all times during the analysis, equipment and work surfaces must be cleaned immediately before and after use, to avoid cross contamination.

2.2 Empty the sample from the bag onto a tray. Keep the bag on the tray with the sample.

2.3 Place the tray in a rack and allow the sample to air dry in a warm, ventilated room. (Drying the soil aids cyst recovery).

2.4 Transfer the dry soil onto a large metal tray and, with a metal rolling pin, break down the soil cores and remove large stones.

2.5 Mix the soil and return the sample to the bag.

3. Extraction of cysts from soil

3.1 Extract the cysts from the prepared sample by washing the soil through the Fenwick can apparatus. Use a 850μm sieve in the funnel, with a 250μm sieve placed below the collar outlet, to collect the floating debris.

3.2 Fill the can with water and wet the sieves.

3.2 Write the sample reference on a small paper label and place it in the collecting sieve. This label will follow the sample through the extraction and examination process.

3.3 Record the weight of the sample onto the worksheet and if more than 700g take a 500g sub sample. Otherwise, use the whole sample.

3.4 Place the soil in the 850μm sieve and wash through into the can, with a strong jet of water from above. Organic material and some soil will overflow rapidly into the collar and pass down into the 250μm collecting sieve.

3.5 When the water from the can runs clear, turn off the water jet. This allows organic material, which may have been trapped by the downward force, a chance to float up. After a minute, turn the jet back on for a few more minutes.

3.6 Remove the collection sieve and empty the can.

3.7 Take a 30cm diameter circular filter paper, pre-printed with concentric rings 4mm apart, and fold in half and half again. Open out to form a cone of concentric circles and place in a 15cm diameter filter funnel. Moisten the paper.

3.8 Wash the contents of the collection sieve into the filter paper and fill with water to just below the rim of the paper.

3.9 Add a drop of detergent to the centre of the floating debris. This breaks the surface tension, so that the debris moves towards the paper. As the water drains away, the cysts and debris are left in a band around the top of the filter paper.

3.10 Remove the filter paper from the funnel and open out onto a support plate which is placed on a revolving turntable, for examination under a stereo microscope (x 25mag). Ensure the label is still with the sample.

3.11 Using the lines on the filter paper as a guide, systematically search through the debris using a fine forceps. Count and record the number of cysts onto the worksheet.

4. Cyst viability assessment

4.1 Transfer cysts from the debris on the filter paper to a drop of water on a glass microscope slide. Where large numbers of cysts are present, take a percentage of the total as a representative sub-sample but do not use less then 50 cysts.

4.2 Allow the cysts to soak for 24 hours by placing the slide in a damp chamber.

4.3 Using a stereo microscope (x 25mag), with forceps burst open each individual cyst to determine if the content is viable. Record the number of viable cysts on the worksheet.

5. Estimation of cyst, egg and larval content

5.1 After determining cyst viability, use a glass crusher to break up the cysts on the slide to release the contents.

5.2 Wash the debris on the slide and crusher into a 50ml measuring cylinder and make up to 25ml with water.

5.3 Agitate the contents of the cylinder by bubbling air through the liquid, to give a homogenous suspension, and remove a 1ml aliquot and dipense it into a gridded counting chamber. Repeat and dispense a further two aliquots.

5.4 Using a stereo microscope (x 25mag) count the number of viable eggs and larvae in each 1ml. Use the mean of the three counts to calculate the number of eggs and larvae per gram of soil. This figure is used to assess the risk of damage by PCN to a potato crop.

$$\text{No. of eggs and larvae/gram of soil} = \frac{\text{Mean no. of eggs and larvae per ml}}{\text{weight of soil used in test}} \times \frac{25}{\text{\% of cysts sub-sampled}} \times 100$$

APPENDIX 2

◆ EPPO Standards ◆

PHYTOSANITARY PROCEDURES

GLOBODERA PALLIDA & G.ROSTOCHIENSIS

SOIL SAMPLING METHODS

PM 3/30(1) English

Organisation Européenne et Méditerranéenne pour la Protection des Plantes
1, rue Le Nôtre, 75016 Paris, France

APPROVAL

EPPO Standards are approved by EPPO Council. The date of approval appears in each individual standard.

REVIEW

EPPO Standards are subject to periodic review and amendment. The next review date for this set of EPPO Standards is decided by the EPPO Working Party on Phytosanitary Regulations.

AMENDMENT RECORD

Amendments will be issued as necessary, numbered and dated. The dates of amendment appear in each individual standard (as appropriate).

DISTRIBUTION

EPPO Standards are distributed by the EPPO Secretariat to all EPPO member governments. Copies are available to any interested person under particular conditions upon request to the EPPO Secretariat.

SCOPE

EPPO Phytosanitary Procedures are intended to be used by National Plant Protection Organizations, in their capacity as bodies responsible for the inspection, testing and treatment of plants and plant products moving in trade, or for the implementation of surveys against quarantine pests.

REFERENCES

OEPP/EPPO (1996) Glossary of Phytosanitary Terms. *EPPO Technical Documents* no. 1026.
CABI/EPPO (1997) Quarantine Pests for Europe, 2nd edition (Ed. by Smith, I.M.; McNamara, D.G.; Scott, P.R.; Holderness, M.), CAB International, Wallingford, UK.
OEPP/EPPO (in preparation) Specific Quarantine Requirements. Available as electronic documents from the EPPO Web Site.

DEFINITIONS

Phytosanitary procedure: Any officially prescribed method for performing inspections, tests, surveys or treatments in connection with plant quarantine.

Inspection: Official visual examination of plants, plant products or other regulated articles to determine if pests are present and/or to determine compliance with phytosanitary regulations.
Survey: An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species occur in an area.
Test: Official examination, other than visual, to determine if pests are present or to identify pests.
Treatment: An officially authorized procedure for the killing, removal or rendering infertile of pests.

OUTLINE OF REQUIREMENTS

EPPO Phytosanitary Procedures describe the methods to be followed for performing inspections, tests, or treatments of commodities moving in trade, or surveys against quarantine pests. For many quarantine pests, a reference to the relevant EPPO Phytosanitary Procedure is made in the corresponding EPPO Specific Quarantine Requirements. The development of EPPO phytosanitary procedures started many years ago, and these methods have been published in the Bulletin OEPP/EPPO Bulletin under several titles: 'Fumigation standards', 'Quarantine Inspection Procedures' and 'Quarantine Procedures'. All of them are now appearing under the title 'EPPO Phytosanitary Procedures' and are being edited into EPPO Standard format. The numbering of these procedures will continue to follow the sequence described in the Bulletin OEPP/EPPO Bulletin 20(2), 229-233, which corresponds approximately to the chronological order of appearance of the Phytosanitary Procedures.

european and mediterranean plant protection organization
organisation europeenne et mediterraneenne pour la protection des plantes

PM 3/30(1) **English**

Phytosanitary procedure

GLOBODERA PALLIDA & G. ROSTOCHIENSIS

SOIL SAMPLING METHODS

Specific scope

This standard describes the soil sampling methods for *Globodera pallida & G. rostochiensis*, to satisfy the requirements of EPPO Standards PM 2/124(2) and PM 2/125(2).

Specific approval and amendment

First approved in September 1990.

Edited as EPPO Standard in 1998.

Introduction

Globodera pallida and G. *rostochiensis* are A2 quarantine organisms and details about their biology, distribution and economic importance can be found in Data sheets nos 124 and 125 (OEPP/EPPO, 1978, 1981). The EPPO specific quarantine requirements (OEPP/EPPO, 1990) for these nematodes require that the field in which seed potatoes or rooted plants were grown was inspected by taking soil samples according to an EPPO-recommended method and found free from viable cysts of *G. pallida* and *G. rostochiensis*. The sampling must have been performed after the harvest and removal of the previous potato crop.

Methods

Two methods of sampling are possible: (1) taking soil samples and processing them in the laboratory; (2) lifting plants and examining their roots for females or cysts. The latter method is not widely used in the EPPO region for statutory purposes but has been used in New Zealand for several seasons to detect low populations of potato cyst nematodes which, it is claimed, would mostly have been undetectable by European soil-sampling methods (Wood *et al.*, 1983). The New Zealand method is extremely labour-intensive in the field, involving lifting 150-170 plants ha^{-1}. For each plant lifted, the effective sample size is stated to be about 2000 ml, i.e. at least 300 1 ha^{-1}. The large effective sample size should theoretically make possible correspondingly lower average detection levels, and therefore earlier detection. Laboratory work is eliminated or minimized and species identification (based on colour of females in *Globodera*) is done in the field. Within-field distribution of infestation density can be noted. Plant sampling is, however, extremely labour-intensive and timing is critical. The efficiency of the method is severely affected by soil type and the weather conditions at the time of sampling; it is impracticable, for example, with wet soils having appreciable clay content.

The basic requirements of soil sampling to detect or estimate potato cyst nematodes in the soil are that: (1) the final sample examined in the laboratory is large enough to achieve the required accuracy and/or sensitivity; (2) the sample is derived from sufficient points to ensure that it is representative of the area sampled, i.e. as far as possible heterogeneity (patchiness) in the nematode distribution is overcome; (3) the laboratory processing procedures are as efficient and free from operator error as possible, so as to give accurate and consistent results. The problems of sampling errors in this work were reviewed by Southey (1974) and discussed in more detail by Barker & Campbell (1981) and Seinhorst (1982). EPPO's earlier recommendations on soil sampling (OEPP/EPPO, 1955) are replaced by the present quarantine procedure.

The most commonly used techniques for extracting cysts from soil samples use the principles of flotation, elutriation or centrifugation. The flotation methods have the disadvantage that soil must be dried before processing but they are inexpensive and easy to use. The Fenwick can is the most widely used of these and, although it is subject to some operator error, it is robust and familiar to most nematologists. The elutriators can be used for soil without drying. It has been estimated that the Wye washer can extract approximately

99%, of cysts in a sample (compared to 97%) by the Fenwick can) but it uses a large amount of water (100 l per sample) and is expensive to buy. The Schuiling centrifuge is becoming more widely used and is more rapid and produces a cleaner sample than the other methods. It is virtually free from operator error but is considerably more expensive to purchase.

See Appendix I for details of the methods.

APPENDIX I

Lifting and examination of potato roots in the field

Entire plants are lifted carefully with a fork, the adhering soil is gently shaken from the roots and the root system is examined carefully, with a hand-lens where necessary. Pieces of rootlet bearing suspected females or cysts may be taken back to the laboratory for checking. Inspection should proceed until infestation is discovered or 20 well-distributed plants have been examined per unit area (e.g. 1 ha). Extra attention should be paid to poor patches, field entrances and clamp sites.

Soil sampling

The probability that a soil sample of any given size will contain one or more cysts depends on the average population density of cysts in the area sampled. Conversely, the probability that a population of given average density will be detected depends on the quantity of soil examined and, to a lesser degree, on the number of points from which the sample is derived and the area over which the sample points are distributed. For practical purposes, to detect relatively low populations, as large a volume of soil as possible should be taken from the field which can be processed completely for extraction of cysts in the laboratory; there should be a sufficient number of field sample points to account for heterogeneity of distribution. The area to be sampled can be of any size: the only limitation is that the whole area should have a similar cropping history in relation to potato growing.

A suitable sampling procedure (Southey, 1986) is to take 100 cores (borings) of 4-5 ml of soil with a half-cylindrical sampling tool, from not deeper than 5 cm in the soil. These sub-samples are distributed on a grid pattern throughout the plot and collected in a polyethylene bag to provide a sample of 400 ml (500 g) which is processed completely in the laboratory.

Plant protection services in different countries adopt different standards for the size of sampled area. In some countries, one sample is taken from each area for which a separate decision on presence/absence of cysts is to be made, irrespective of the size of the area, but up to a maximum of the usual field size in that country (e.g. 4 ha). In this case, the probability of detecting a given infestation level will be the same whatever the area sampled. In other countries a standard number of samples per ha is required (e.g. 4 samples per ha, 1 sample being the minimum requirement for areas less than ¼ ha). This method ensures consistency by the inspectors in sample-taking. It should be noted, however, that from a statistical point of view larger areas are sampled more intensively by this procedure than smaller areas and therefore smaller areas are less likely to be rejected because of the detection of cysts.

Laboratory processing of soil samples

- **Flotation methods**

These include the oldest methods for recovering heteroderid cysts from soil. All depend on the fact that, when soil has been air-dried, nematode cysts present in the sample will float in water and can be picked from the surface or separated by filtration. They are mostly well tried and reliable but disadvantages include the additional time required for drying and the fact that the latter affects the viability of some cyst nematodes (not usually *Globodera* spp.).

Flask and paper-strip methods

These simple methods are useful for qualitative tests on very small mineral soil samples, e.g. taken from imported plant material. They extract cysts from air-dry soil.

Flask method. A sample of soil is placed in a 2 litre conical flask and approximately 500 ml water is added and shaken in order to wet the soil thoroughly. The flask is then almost filled with water by means of a strong jet so that the soil suspension is well mixed. The filling of the flask is completed slowly. When the suspension has settled, the floating organic matter (including cysts) at the neck of the flask is carefully poured onto two sieves, one with an aperture of 840 µm above a 250 µm-aperture sieve. During pouring, the flask is rotated to ensure that all the organic matter is transferred to the sieves. The sieves are washed so that the cysts are collected on the 250 µm sieve.

Paper strip method. A strip of filter paper is placed around the inside, and just below the rim, of a 2-litre plastic beaker. The beaker has a closable outlet near the base. The soil sample is placed in the beaker, water is added and the suspension is thoroughly mixed. Water is added until the level reaches the filter paper. The floating organic debris and cysts will adhere to the filter paper when water is allowed to drain slowly from the outlet at the base of the beaker. The paper strip can be examined directly under a binocular microscope.

Fenwick can

Air-dry samples of up to 300 g can be used. The apparatus is a brass container, tapering towards the top, with a sloping collar around the outside of the rim which collects overflow and directs it towards an outlet (Fig. 1). The can has a sloping internal base with a drain plug at its lowest point. The can is first filled with water and the soil sample is added by washing through a 1-mm sieve supported on a long-stemmed funnel above the can. The organic matter will immediately rise and overflow onto the collar and be collected on two sieves of 840 µm and 250 µm. Most of the cysts in the soil sample will be collected at this stage. The funnel above the can is then removed and the soil at the base of the can is elutriated by means of water flowing rapidly through a long glass or metal tube. The tube is inserted deep into the can to stir the sediment and release any trapped cysts; this is continued for approximately 1 min. The cysts are collected on the 250-µm sieve.

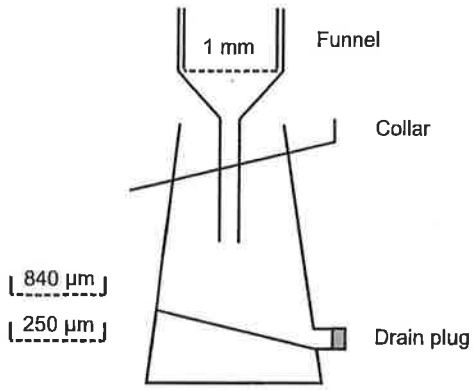

Fig. 1. Vertical-section diagram of Fenwick can.

- **Elutriation method**

Soil particles in the sample are allowed to sediment against an upward current of water so that the less dense particles (including nematode cysts) are carried upwards and directed via an overlow lip or tube onto a receiving sieve or sieves. Some elutriators may be used for either cyst-forming or vermiform nematodes; others are designed for one or other categories. The soil need not be dried before processing.

Wye washer

The apparatus (Winfield *et al.*, 1987) is constructed of a 50-cm length of 15-cm diameter clear acrylic tube which, at its lower end, is held inside two tight-fitting concentric PVC sleeves (Fig. 2). Water enters through an inlet pipe on the outer sleeve and is caused to swirl by means of an arrangement of grooves and angled holes on the inner sleeve and the acrylic tube. At the top of the tube is a spout which directs overflow onto sieves of similar size to those used with the Fenwick can (i.e. 840 µm and 250 µm). A soil sample, up to 1 kg, is added to a small quantity of water in the Wye washer. More water is added as rapidly as possible, to break up the soil. until the rim is reached, whereupon the flow is briefly stopped and then increased gradually to about 10 l min^{-1} for 10 min. The overflow carries: (1) small soil particles, which will pass through both sieves; (2) large organic debris which will be retained by the upper larger sieve; (3) Cysts and similar sized organic particles, collected on the 250 µm sieve.

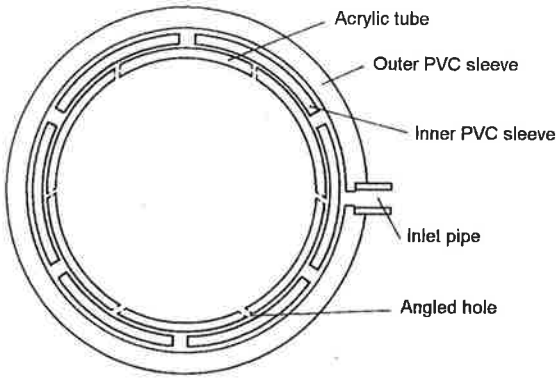

Fig. 2. Cross section of the base of the Wye washer.

- **Centrifugal method**

Schuiling centrifuge

This is a semi-automatic extraction method for soil samples developed by J. Schuiling of The Netherlands Inspection Service for Field Seeds and Seed Potatoes (NAK) and now marketed commercially in The Netherlands (Hietbrink & Ritter, 1982). The air-dried, 200 ml soil sample is added to a transparent cylindrical container half-filled with water. The contents are swirled with a rotating two-pronged fork at 450-500 rev min^{-1}, creating a vortex and causing cysts and similar-sized floating particles to be forced to the centre through a wire-mesh cylinder (1.5 mm aperture). The mesh cylinder is fixed above a tube of the same diameter leading down to an outlet. While swirling, more water is added around the inside of the main container washing off any adhering debris and cysts which are channelled to the outlet with the rest. The apparatus cleans itself after each sample processing.

Further separation of cysts is by a special cleaning process involving the so-called Schuiling can and special sieves. The apparatus requires only about 6 l of water per sample and, as used in The Netherlands, enables 30 samples per h to be processed; one person can operate three Schuiling centrifuges at a time.

In some laboratories the Schuiling units have been modified to suit different soils and conditions: the modifications include additional spinning and cleaning time, larger collecting sieves, an improved plastic cleaning 'can' for reducing the amount of debris, and removal of the electrical parts from the apparatus to the wall above for safety reasons.

References

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