Acta Phytopathologica et Entomologica Hungarica 38 (1–2), pp. 209–215 (2003)

Efficiency of the System of Nematode Cyst Extraction from Soil Samples Used in Slovenia

G. UREK and S. ŠIRCA

Agricultural Institute of Slovenia, 1000 Ljubljana, Slovenia

The paper describes the technique of nematode cysts extracted from soil samples used at the Agricultural Institute of Slovenia. The efficiency of the procedure described was tested, and the AIS modified method for separation of nematode cysts from organic debris was compared with acetone and ethanol flotation method. The average recovery of cysts from soil samples of different soil texture was 91 ± 3%.

Keywords: Soil sample processing, cyst extraction.

Due to the worldwide economic impact of some species of cyst formed nematodes (sugar beet nematode, soybean nematode and potato cyst nematode), the information about their presence and their dispersal in a certain area is very important. The accuracy of estimating the population density of certain nematode species depends equally on sampling and extracting procedure. Most techniques of the cyst formed nematodes extraction are based on the characteristics of the waxy covering of cysts which results in cyst floating in a liquid medium. The cysts are first extracted from the soil by gravity method, which can be either flotation or centrifugation. The cysts must then be separated from the debris directly or after drying. They can also be floated away from thoroughly dried debris by ethanol or glycero-ethanol mixtures (Fortuner, 1991). Many more or less effective methods for cyst extraction from soil samples were developed in different nematological laboratories.

A very useful and effective method for separating nematode cysts from soil, which was developed in 1977 (Hržič, 1980), has been successfully used at the Agricultural Institute of Slovenia for many years. The first part (separating organic debris from the soil samples) is a modification of Fenwick can. The second part (separation of nematode cysts from organic debris) was developed by Hržič (1980).

In order to simplify our work without influencing the efficiency of our extraction system we have modified and partly automated the system for separating organic debris from soil samples. With regard to very different soil samples which are treated, our extraction system is programmed to define duration of extraction and doze of used water. In this way the system enables us to compare the results of extraction of different soil samples.

Materials and Methods

The central part of the equipment (developed by the Agricultural Institute of Slovenia = AIS) for separating nematode cysts from soil is the flotation tank (cylinder with 20 cm diameter) with its overflow spout. The flotation tank is set to the drainage basin and through electrically regulated system connected to the water supply. The funnel is placed on the top of the flotation tank, and on the funnel a 1 mm sieve is placed.

Basis for electronically managed system used for separating nematode cysts from soil samples

The electronics (Hace, 2000) of the flotation system for extracting nematode cysts from soil samples was constructed exclusively for the need of nematological laboratory of Agricultural Institute of Slovenia. The water stream introduced through the pipe with a perforated tip at the bottom of the flotation tank is regulated (the quantity of water and the duration of extraction) electronically.

In order to obtain a precise measuring of water flow we use a measuring turbine with the capacity of 1–18 l/min and 300 impulse/l. The operating temperature of the measuring turbine is 0–65 ºC; the power voltage is 5 V – 24 V DC. The water flow is regulated by electromagnetic valve with the operating alternating tension of 24 V. The water filter was built in to protect the turbine against sweepings and to assure undisturbed operating of turbine and electromagnetic valve. Electromagnetic valve is regulated by electronics. The safety protocols require the use of an electrovalve with the power voltage of 24 V AC. A special toroidal transformer is used for valve driving so that the power supply for microcontroller is separated by the remaining part of electronics. The device for electronically managed water flow is directly connected to the flotation tank through a tube.

Description of the nematode cyst extraction procedure

The dry soil sample of 200 ml is placed on a 1 mm sieve and then washed into the flotation tank via a plastic funnel, using a nozzle delivering a suitable quantity (about 3.5 l/minute) of water. The outlet of the funnel stem is closed with a rubber cork and, at the spot where soil enters the tank, four 6 mm holes are made in the stem of the funnel so that the soil does not fall down the column too quickly. The time and quantity of water needed for this part of the process depends on the structure of a soil sample.

At the same time, when the soil is washed in through the sieve and the funnel, a stream of water enters the bottom of tank (the diameter of the tank is 20 cm) through a pipe with a perforated tip, roils the soil and carries the floating cysts and other material to the overflow spout. A gentle flow of water carries the floating material from the overflow spout to a 250 µm sieve. Heavier particles settle at the bottom of the tank.

The pressure of the water stream used for extraction of nematode cysts from soil samples is regulated by the quantity of water used and by the duration of extraction, and it influences the intensity of water whirling in the floating tank. The quantity of the required water and the duration of the extraction is defined by electronics described above. The system is switched off automatically when the separation of nematode cysts in one sample is finished. Then the floating tank is cleaned manually and the system is ready for another soil sample. The parameters defined, once they are programmed, are the same all the time. Thus the extraction conditions are the same for all the soil samples.

AIS method used for the separation of nematode cysts from organic debris

When the sample is washed from the floating tank through the overflow spout into the sieve, the water and fine soil particles pass through the sieve, and only very fine soil particles and organic debris with the cysts are collected on the sieve. The material from the sieve which contains nematode cysts is rinsed into the funnel (18 cm diameter at the top) with the flow end stoppered with a conical rubber plug to which a hook is attached for easy removal. The funnel is filled with the water which is whirled before the material from the sieve is rinsed into it. Most of the remaining soil particles are settled at the bottom of the funnel, and the floating material remains on the surface of the water. By opening the tube, the content – without the floating material – is poured out of the funnel. Before all the water is poured out of the funnel completely, a 500 mm beaker is used. A suspension of water and floating material containing nematode cysts is rinsed into the beaker.

Afterwards the suspension of water and floating material is rinsed from the beaker into the funnel (13 cm diameter) with the tube bent at an angle of about 80 ºC. The flow end of the tube is stoppered with a conical rubber plug to which a hook is attached for easy removal. Beneath the bent funnel tube a smaller funnel (10 cm diameter) is placed and a circular filter paper (520 A of Schleisser and Schuell) is put into it before rinsing the water with the floating material from the 13 cm funnel. The floating minor portion of the debris containing the cysts moves outwards to the filter surface. This procedure may be speeded up by using a droplet of detergent which is dropped into the centre of water. After the floating fraction has reached the filter, a small hole is made in the centre of filter paper, and the water is poured out of the funnel very slowly. The floating fraction containing the cysts remains as a thin ring on the filter paper. An examination is made under the dissecting microscope with a magnification of 10 to 40 times by rotating the round plate on which a filter paper is placed. Most of the cysts are collected in the floating debris line near the edge of the filter paper.

Comparative testing of the efficiency of AIS modified method used for the separation of nematode cysts from organic debris, acetone and ethanol flotation

As a result of personal communication with de Nijs, when a certain doubt concerning the efficiency of the AIS modified method used for the separation of nematode cysts from organic debris was expressed with regard to making a small hole in the centre of filter paper in the last phase of the procedure, where some losses could exist, we decided to compare the efficiency of our method with that of acetone (Seinhorst, 1964) and ethanol flotation techniques (Seinhorst, 1970).

Soil samples used for the estimation of the efficiency of different methods of extraction of *Heterodera* cysts from soil were taken from different hop fields near Celje in Savinjska dolina (as known from some previous analyses, many cysts of hop cyst nematode *Heterodera humuli* were present there). The soil was placed in paper bags and allowed to air dry for one week at room temperature. After one week, the soil was additionally dried for 24 hours at 30 °C. The dry soil usually contains aggregates of various size and consistency. Because of that, the soil was comminuted using rubber hammer, sifted through a 5 mm sieve and thoroughly mixed. After that, a 200-g sub-sample of soil was prepared for testing the different extraction methods (AIS method, ethanol and acetone flotation method).

Efficiency of electronically managed system used for separating nematode cysts from soil samples

We studied the efficiency of electronically managed system (AIS) for separating nematode cysts from soil samples used in our Nematology Laboratory. Our objective was to determine the efficiency of cyst extraction from different soil types with different infestation level. Water containing 5 or 25 cysts was added to 200 g portions of air dried soil of each of three soil types, and the soil was allowed to air dry. The soil types were sand (93.9% sand, 5.8% silt, 0.3% clay), sandy loam (49.1% sand, 32.8% silt, 18.1% clay) and sandy loam with added organic material (50.6% sand, 30.7% silt, 18.7% clay).

Results

A: Comparative efficiency of different methods used for the separation of nematode cysts (Heterodera humuli) *from organic debris*

Results of comparative efficiency testing of AIS, acetone, and ethanol flotation methods are reported as means of replicate samples. The best results were achieved with the AIS extraction method. Comparative efficiency was analysed with Fisher's least significant difference (LSD = 95%) procedure. Using this method we determined no statistically significant differences between the AIS and the acetone flotation method while the ethanol flotation method had statistically significant differences *(Fig. 1).*

B: Efficiency of electronically managed system used for separating nematode cysts from soil samples

The efficiency of AIS method for separating nematode cysts from the samples used in our Nematology Laboratory was tested. According to the results neither soil type nor inoculum (5 or 25 cysts per 200 g soil) influenced the recovery of cysts from soil samples *(Table 1).* The average recovery of cysts for all soil types and cyst population densities was 91 ± 3% (Multiple Range Test, LSD=95%).

Discussion

Fundamental details for extraction of *Heterodera, Globodera* and *Punctodera* cysts from soil samples were derived from some older techniques which were founded on the

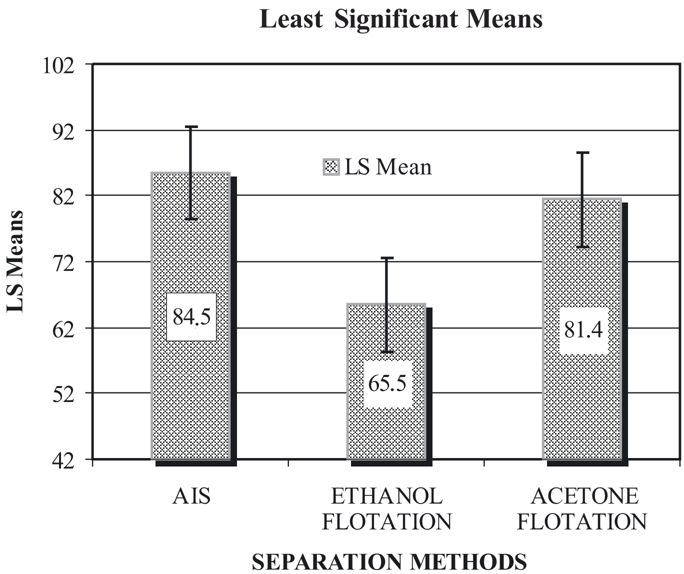

Fig. 1. Comparative efficiency of different methods used for the separation of nematode cysts from organic debris

Table 1

Efficiency of electronically managed system used for separating nematode cysts from different soil samples (%)

	Sandy loam	Sandy loam with added organic material	Sand
Low inoculum: 5 cysts / 200 g soil	91	88	91
High inoculum: 25 cysts / 200 g soil	91.2	92.4	91.4

principles of washing soil in water, its decanting and sieving with the help of different sieves, on the principle of flotation with the help of a constant stream of water and on the principle of soil drying for the collection of cysts. On the basis of these principles many methods have been developed or modified in different laboratories in the past and they have been used more or less successfully since then in the research, advisory and survey work. Most of these methods rely on the principle that, under suitable conditions, cysts will float in a liquid medium due to waxy covering of a mature cyst. In order to obtain an optimum cyst recovery, the soil must be fully air dried and subsequently crushed or passed through a 4 mm sieve to break down clods and remove stones (Turner, 1998). There is no single method or device available for which we could say that it is the most effective for separating nematode cysts from soil samples.

The simplest method of detecting nematode cysts is the elutriation of a soil sample in a white dish; the cysts will float along the edges (Decker, 1969). A widely used and many times modified apparatus for cyst extraction was described by Fenwick (1940). It is efficient when extracting a soil sample up to 300 g dry weight (Turner, 1998). In 1954, a glass funnel with an etched surface was used for separating the cysts from soil samples by Kirchner (Decker, 1969). Seinhorst (1964) separated of cysts from organic debris after previous extraction in a cyst elutriator (Andersson, 1970). In 1964, Seinhorst described a specially constructed elutriator for the processing of moist soil samples. Elutriator which combines the elutriation with sieving was described by Oostenbrink (1960). Hietbrink and Ritter (1982) described a semi-automatic flotation method for extracting cysts from soil samples which was developed by Schuiling. The advantages of this system are speed, reduced manpower demand, more pleasant working conditions, reduced water requirements and a cleaner residue for examination than given by the Fenwick method; the system is not suitable for peaty or other organic soils (Shepherd, 1986).

Extraction procedures vary in reliability, consistency and efficiency. They should be evaluated to test their appropriateness and efficiency, and to allow correction of population estimates to absolute terms.

Fenwick (1940) reported a cyst extraction efficiency for the flotation can when sandy soil containing 2.2 cysts per gram of soil was processed. Seinhorst (1964) reported a cyst extraction efficiency for soil naturally infested with *Heterodera rostochiensis* and *H. trifolii* with a range of 62–99.5% with the maximum recovery occurring at a flow rate of 3.5 litres/minute. Caswell et al. (1985) reported a cyst extraction efficiency to vary depending on the age structure and the condition of cyst population used. Older cysts tend to contain more air and float better than younger cysts. They used the modified Fenwick flotation can and ethanol-glycerine flotation, and found out that the average recovery of cysts from seeded soil samples of differing soil texture was 82.7 ± 2.1%.

The problems concerning the cyst formed nematodes have been studied in Slovenia for a long time. We found out very early that a good extraction system is very important for that kind of studies. As a result of this, our own extraction system was developed in 1977 (Hržič, 1980). Its efficiency was compared with that of the Schuiling centrifuge extraction system; it was found to be a much more efficient system than the Schuiling one (Urek and Hržič, 1997). In order to simplify our work without influencing the efficiency of our extraction system, we have recently modified and partly automated the AIS extraction system in the way that duration of extraction and doze of used water could be programmed.

As some doubts concerning the efficiency of the AIS modified method for separation of nematode cysts from organic debris were expressed due to making a small hole in the centre of filter paper in the last phase of the procedure where some losses could appear, we have decided to find out if this was true. The results of comparative efficiency testing of AIS, acetone and ethanol flotation methods showed that our method was comparable with the acetone flotation method and that it was much better than ethanol flotation method. Beside this, the efficiency testing of AIS system showed a very high average efficiency (91 ± 3) which is in close agreement with that of Fenwick (1940), Seinhorst (1970) and Caswell et al. (1985).

Literature

Andersson, S. (1970): A method for the separation of *Heterodera* cysts from organic debris. Nematologica 16, 222–226.

Caswell, E. P., Thomason, I. J. and McKinney, H. E. (1985): Extraction of cysts and eggs of *Heterodera schachtii* from soil with an assessment of extraction efficiency. Journal of Nematology 17, 337–340.

Decker, H. (1969): Phytonematologie – Biologie und Bekämpfung Pflanzenparasitärer Nematoden. VEB Deutscher Landwirtschaftsverlag, Berlin, 526.

Fenwick, D. W. (1940): Methods for the recovery and counting of cysts of *Heterodera schachtii* from soil. Journal of Helminthology 15, 155–172.

Fortuner, R. (1991): Methods for collection and preparation of nematodes. In: Nickle, R. W. (ed.): Manual of Agricultural Nematology, Marcel Dekker, Inc., New York, Basel, Hong Kong, pp. 75–87.

Hace, F. (2000): Elektronika vse bolj prodira na področje bioloških ved. Svet elektronike, Ljubljana, 68, 40–43.

Hietbrink, H. and Ritter, C. E. (1982): Separating cysts from dried soil samoles by a new centrifugation and a flotation method. Abstracts, 16th International Nematology Symposium, European Society of Nematologists, St. Andrews, 28–29.

Hržič, A. (1980): Raziskava korelacij med anatomsko zgradbo in morfologijo distalne regije cist nematod vrst *Heterodera.* Doktorska disertacija, BF Univ. v Ljubljani, Ljubljana, 129.

Oostenbrink, M. (1960): Estimating nematode populations by some selected methods. In: Saser, J. N., Jenkins, W. R. (eds): Nematology. University of North Carolina Press, Chapel Hill, 85–102.

Shepherd, A. M. (1986): Extraction and estimation of cyst nematodes. In: Southey, J. F. (ed.): Laboratory Methods for Work with Plant and Soil Nematodes. Reference book 402, Her Majesty Stationery Office, London, pp. 31–49.

Seinhorst, J. W. (1964): Methods for the extraction of *Heterodera* cysts from not previously dried soil samples. Nematologica 10, 87–94.

Seinhorst, J. W. (1970): Separation of *Heterodera* cysts from organic debris in ethanol 96%. Nematologica 16, 330.

Turner, S. (1998): Sample preparation, soil extraction and laboratory facilities for the detection of potato cyst nematodes. In: Marks, R. J., Brodie, B. B. (eds): Potato Cyst Nematodes; Biology, Distribution and Control. CAB International, Wallingford, pp. 75–90.

Urek, G. and Hržič, A. (1997): Izločanje cist iz talnih vzorcev in pregled rezultatov analiz geografske razprostranjenosti cistotvornih ogorčic za obdobje 1992–1996. Zbornik predavanj in referatov s 3. slovenskega posvetovanja o varstvu rastlin, Portorož, 1997, pp. 233–239.