6.2 Sampling

Storage temperature of soil sample, 4℃, is too low to keep plant parasitic nematodes alive, while 10-15 ℃ is more suitable (Barker and Nusbaum, 1971). This is possibly true for free living nematodes, because the nematodes include some species (e.g., Aphelenchoididae, Anguinidae) taxonomically very close to plant parasitic ones. The fact also supports 10-15℃ as the storage temperature, that *C. elegans* researchers usually choose this temperature range rather than 4 ℃, to maintain the worms for long.

6.3 Extraction

Oostenbrink elutriator is expensive and difficult to set up in some laboratories. In addition, the equipment is commercially produced only by one company in the world, which may cause unstable supply. Such equipment should not be designated in an international standard procedure.

Bearmann funnel/tray extraction without prior soil elutriation, is still a popular method worldwide (Forge and Simard, 2001; Fu, et al., 2000; Hanel, 2001; Hoschitz and Kaufmann, 2004; Okada, et al., 2004; Ruess, et al., 2001; Sohlenius, 2002; Yeates, et al., 1999). Usually free living nematodes do not have cysts or inactive stage, so that Bearmann funnel/tray extraction is effective to obtain the nematodes, although the extraction efficiency may differ between nematode taxa, as is often the case with any type of extraction method (McSorley and Frederick, 2004). One of the advantages of Bearmann extraction is that the nematode suspension obtained includes few or no debris which will hinder nematode identification seriously. More importantly, the method is simple, inexpensive and less laborious to operate, which makes it available worldwide. Such a method is suitable as an international standard. Soil elutriation prior to the extraction should not be designated obligatory, but optional.

Cotton-wool filters are not available in some countries. Cotton-linter filters, or paper wipers such as Kim Wiper (Kimberly-Clark) are more available world wide.

6.4 Counting

In counting nematodes, the general procedure is acceptable, but the size of bottle (100 ml) to keep nematode suspension, and the volume of suspension for counting (10 ml) should be optional. This is because almost all nematodes from the sample soil should be counted if the nematode density is too low, while only 1% of suspension may includes too many nematode individuals if the original density in soil is very high. Thus, degree of dilution or concentration of the original suspension, and % of suspension to be

examined should be changed depending on the nematode density.

Nematodes just fixed in formalin may lack key characteristics for generic level identification. Thus, formalin fixation should be just minimum requirement. Transfer of nematodes into anhydrous glycerol by Seinhorst's rapid method (Seinhorst, 1959) should be recommended for more promising identification.

References

Barker and Nusbaum (1971) Diagnostic and advisory programs. In Plant parasitic nematodes. Vo. 1 Zuckerman, B.M. & R.A. Rohde, eds., New York, Academic Press, 281-301.

Forge, T. A. Simard, S. W. (2001) Structure of nematode communities in forest soils of southern British Columbia: relationships to nitrogen mineralization and effects of clearcut harvesting and fertilization. Biology and Fertility of Soils. 34: 3, 170-178.

Fu ShengLei Coleman, D. C. Hendrix, P. F. Crossley, D. A., Jr. (2000) Responses of trophic groups of soil nematodes to residue application under conventional tillage and no-till regimes. oil Biology & Biochemistry. 32: 11/12, 1731-1741.

Hanel, L. (2001) Succession of soil nematodes in pine forests on coal-mining sands near Cottbus, Germany. Applied Soil Ecology. 16: 1, 23-34

Hoschitz, M. Kaufmann, R. (2004) Nematode community composition in five alpine habitats. Nematology. 6: 5, 737-747

McSorley, R. Frederick, J. J. (2004) Effect of extraction method on perceived composition of the soil nematode community. Applied Soil Ecology. 27: 1, 55-63.

Okada, H. Harada, H. Kadota, I. (2004) Application of diversity indices and ecological indices to evaluate nematode community changes after soil fumigation. Japanese Journal of Nematology. 34: 2, 89-98,

Ruess, L. Schmidt, I. K. Michelsen, A. Jonasson, S. (2001) Manipulations of a microbial based soil food web at two arctic sites - evidence of species redundancy among the nematode fauna? Applied Soil Ecology. 17: 1, 19-30

Seinhorst, J.W., (1959). A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica 14, 67-69.

Sohlenius, B. (2002) Influence of clear-cutting and forest age on the nematode fauna in a Swedish pine forest soil. Applied Soil Ecology. 19: 3, 261-277

Yeates, G. W. Wardle, D. A. Watson, R. N. (1999) Responses of soil nematode populations, community structure, diversity and temporal variability to agricultural intensification over a seven-year period. Soil Biology & Biochemistry. 31: 12, 1721-1733