problèmes de l'espèce dans le règne animal », tome III. *Mém. Soc. Zool. France.*, nº 40 : 427-452.

MAMIYA, Y. & ENDA, N. (1979). *Bursaphelenchus mucronatus* n. sp. (Nematoda : Aphelenchoididae) from pine wood and its biology and pathogenicity to pine trees. *Nematologica*, 25 : 353-361.

NICKLE, W. R., GOLDEN, A. M., MAMIYA, Y. & WERGIN, W. P. (1981). On the taxonomy and morphology of the pine wood nematode, *Bursaphelenchus xylophilus* (Steiner & Buhrer, 1934) Nickle, 1970. *J. Nematol.*, 13 : 385-292.

ORSTEIN, L. & DAVIS, B. J. (1964). Disc electrophoresis. *Annls N. Y. Acad. Sci.*, 121 : 321-349, 404-127.

WINGFIELD, M. J., BLANCHETTE, A. & KONDO, E. (1983). Comparison of the pine wood nematode, *Bursaphelenchus xylophilus*, from pine and balsam fir. *Euro. J. Forest Pathol.*, 13, 360-373.

Accepté pour publication le 17 décembre 1984.

A MICROPLOT METHOD FOR RECOVERY OF ENTIRE PLANT ROOT SYSTEMS AND THEIR ASSOCIATED ENDOPARASITIC AND SEMI-ENDOPARASITIC NEMATODES (1)

Edward P. CASWELL*, Charles E. NELSEN and Ivan J. THOMASON***

Microplots are enclosures that allow plant growth in limited volumes of field soil which is physically isolated from ambient soil. By definition, microplot systems are implemented under field or near-field conditions. Dimensions should be large enough to allow root growth similar (not identical) to that which occurs under natural conditions. They facilitate study of interactions between soil-borne pathogens and their plant hosts under biotic and environmental conditions which are more realistic than greenhouse conditions (Jones, 1956a, 1956b; Olthof & Potter, 1972; Barker, Shoemaker & Nelson, 1976; Benson, Barker & Aycock, 1976; Abawi & Mai, 1980; Martin, Riedel & Rowe, 1982; Weingartner, Shumaker & Smart, 1983). Greenhouse studies using small pots permit the recovery of entire plant root systems, but do not allow accurate determination of the effect of pathogens on root growth due to the confining nature of small pots.

Although several methods for establishing microplots in the field are published (Jones, 1956 b; Olthof & Potter, 1972; Barker, Daughtry & Corbett, 1979; Johnson, Rich & Boatright, 1981; Martin, Riedel & Rowe, 1982), we have several criticisms of these. Our first concerns the shallow depth to which the microplots are placed in the soil, 30-60 cm. Even the deepest of these, 60 cm, is not adequate to contain the root system of many crop plants, such as maize *(Zea mays)*, sugarbeet *(Beta vulgaris)*, tomato *(Lycopersicon esculentum)*, and potato *(Solanum tuberosum)* (Weaver, 1926; Weaver & Bruner, 1927). This is important because the soil bed into which the microplots are placed is often fumigated prior to use (Benson, Barker & Aycock, 1976; Abawi & Mai, 1980; Martin, Riedel & Rowe, 1982) and inoculated soil is subsequently added to the microplot. Using this technique the inoculum potential is confined to only a portion of the soil volume eventually occupied by the root system. Because the distal end of the microplot is open, growing roots leave the microplot and move into nonfumigated soil, effectively escaping further infection. It is possible that nematodes from deeper soil strata moving upwards towards the plant root system will contaminate the microplot (Weingartner, Shumaker & Smart, 1983). This situation is not desirable in experiments putatively designed to assess the impact of nematode population density on crop growth. Another criticism of these methods is that they do not allow recovery of the entire plant root system with its associated nematodes. A microplot system which allows such recovery is necessary for quantitative studies addressing the biological interactions between plant growth and the population dynamics of endoparasitic or semi-endoparasitic nematodes within the root system.

In this paper we describe a microplot technique for establishing individual plants under near-field conditions using a limited soil volume which allows recovery of entire plant root systems and the endoparasitic or semi-endoparasitic nematodes associated with them. This system allows us to obtain quantitative information

(1) *A portion of the Ph. D. dissertation of the senior author.*

* *Department of Nematology, University of California, Riverside, CA 92521, U.S.A.*

** *Plant Genetics, 1930 Fifth St., Davis, CA 95616, U.S.A.*

on root growth and temporal modifications of root penetration through the soil column as affected by nematodes. In addition, root recovery leaves the majority of endoparasitic or semi-endoparasitic nematodes intact in the root system. None of the available microplot techniques (Olthof & Potter, 1972; Barker, Daughtry & Corbett, 1979; Martin, Riedel & Rowe, 1982) has these specific advantages.

We grow plants in schedule 40 (12.65 kg/cm^2 at 23°) polyvinyl chloride (P.V.C.) tubes which are 15.24 cm in diameter, and 1.52 m in length (Fig. 1). Polyethylene tubing (20 cm i.d., 0.1-0.2 mm thickness) is cut to 1.8 m lengths, sealed at one end, and used to line each tube. Within the tube, the sealed end (bottom) of each polyethylene bag is supported by a wooden block 1.3 cm thick which is held in place by wire supports anchored through holes in the side wall of the tube.

Soil is prepared prior to filling the tube. Nematode inoculum, fertilizer, and/or mycorrhizal spores may be mixed into the soil as desired using a cement mixer, twin-shell blender, or other mechanical device. If the nematode to be used in the study will not tolerate mechanical distribution throughout the soil, small holes can be drilled in the side of the tube and inoculation made via a syringe equipped with a cannula. However,

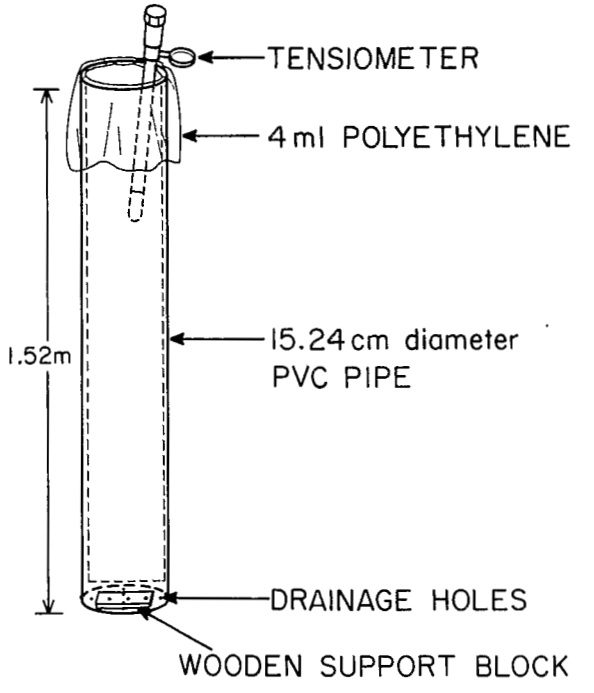

Fig. 1. P.V.C. tube with polyethylene liner and wooden basal support block.

mixing the nematodes into the soil should work well, and such a technique has been used with *Pratylenchus penetrans* (Cobb) successfully (Martin, Riedel & Rowe, 1982).

The polyethylene bag is filled with a small amount of soil and lowered into the tube. The remainder of the tube is then filled with soil, and the tube is shaken gently to settle the soil within the tube. If the soil has a high gravel content it should be passed through a 3.1 mm screen prior to use to remove the larger gravel particles. Such soils are not desirable for use as gravel particles caught in the root system make it more difficult to clean the roots for observation. Tensiometers may be placed in the tube during filling. These permit irrigation to be based on plant water consumption. After the tube is filled with soil, drainage holes may be punched through the bag at the bottom end of the tube via the holes drilled for the wire supports.

The tubes are placed in the ground for insulation by preparing holes of the appropriate diameter using a soil auger. Additional soil may be placed around the tube within the hole to assure that the temperature exchange between the tube and the ambient soil is adequate.

Tubes are removed from the ground for plant harvest using a simple hand operated cable hoist. The polyethylene bag containing the soil core is gently slid from the tube onto a set of screens supported on a wooden frame (Fig. 2). If the bag does not slide easily out of the tube, the tube can be cut lengthways with a circular saw equipped with a carborundum blade. The blade is adjusted so that it only cuts through the P.V.C. After removal from the tube the polyethylene bag is cut lengthways and disposed of. At this point soil samples for analysis of pathogen population densities may be taken from the soil column at different depths using an oakfield tube or other suitable sampling device.

To remove the soil surrounding the root system the soil column is gently misted with water using five misting nozzles (Fig. 2). Each misting nozzle has three orifices yielding a total flow rate of 1.9 l/min per nozzle (water pressure of 21.1 kg/cm^2). Using this design the soil column is gently rinsed for 2-3 hours to remove the soil, and the entire root system can be recovered intact from the screen. The washing process does not damage the roots, and many associated organisms such as semi-endoparasitic nematodes are not dislodged.

The diameter of the tubes limits lateral growth of roots, which may be disadvantageous for some studies, and larger diameter tubes may then be required. This system has been used successfully by the authors to examine the impact of *Heterodera schachtii* Schmidt population densities on sugarbeet fibrous root growth, and to screen wheat cultivars for drought tolerance. In both instances the authors were interested in the dynamics of root growth through the soil column. The vertical distribution of sugarbeet fibrous roots obtained from tubes compares favorably with those we have extracted from the field.

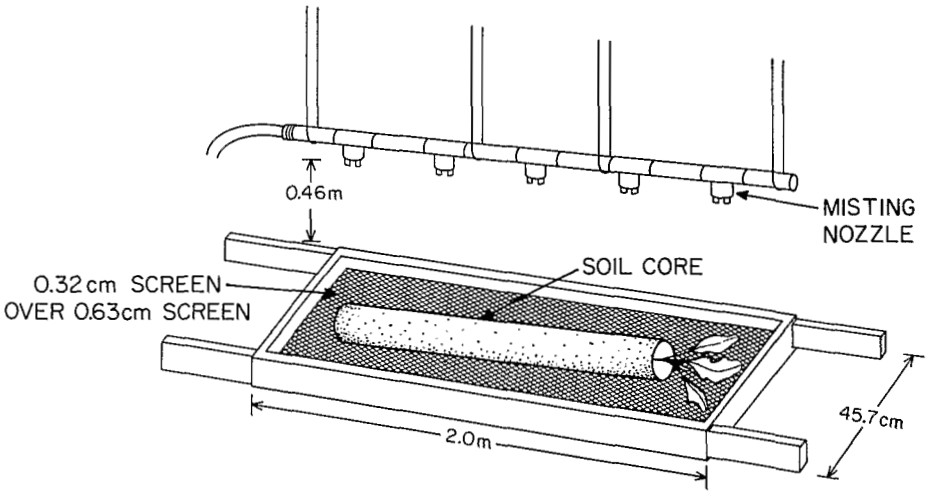

Fig. 2. Misting apparatus and supporting screens for removing soil from around plant root system.

ACKNOWLEDGMENTS

We thank J. G. Baldwin, D. W. Freckman and R. M. Ohta for helpful comments and suggestions on this manuscript.

REFERENCES

ABAWI, G. S. & MAI, W. F. (1980). Effects of initial population densities of *Heterodera schachtii* on yield of cabbage and table beets in New York state. *Phytopathology,* 70 : 481-485.

BARKER, K. R., SHOEMAKER, P. B. & NELSON, L. A. (1976). Relationships of initial population densities of *Meloidogyne incognita* and *M. hapla* to yield of tomato. *J. Nematol.,* 8 : 232-238.

BARKER, K. R., DAUGHTRY, B. I. & CORBETT, D. W. (1979). Equipment and techniques for establishing field microplots for the study of soil-borne pathogens. *J. Nematol.,* 11 : 106-108.

BENSON, D. M., BARKER, K. R. & AYCOCK, R. (1976). Effects of density of *Helicotylenchus dihystera* and *Pratylenchus vulnus* on American boxwood growing in microplots. *J. Nematol.,* 8 : 322-326.

JOHNSON, J. T., RICH, J. R. & BOATRIGHT, A. W. (1981). A technique for establishing microplots in the field. *J. Nematol.,* 13 : 233-235.

JONES, F. G. W. (1956 a). Soil population studies using microplots. *Nematologica,* 1 : 109-110.

JONES, F. G. W. (1956 b). Soil populations of beet eelworms (*Heterodera schachtii* Schm.) in relation to cropping. II. Microplot and field plot results. *Ann. appl. Biol.,* 44 : 25-56.

MARTIN, M. J., RIEDEL, R. M. & ROWE, R. C. (1982). *Verticillium dahliae* and *Pratylenchus penetrans* : interactions in the early dying complex of potato in Ohio. *Phytopathology,* 72 : 640-644.

OLTHOF, T. H. A. & POTTER, J. W. (1972). Relationship between population densities of *Meloidogyne hapla* and crop losses in summer-maturing vegetables in Ontario. *Phytopathology,* 62 : 981-986.

WEAVER, J. E. (1926). *Root development of field crops.* New York : McGraw-Hill, 291 p.

WEAVER, J. E. & BRUNER, W. E. (1927). *Root development of vegetable crops.* New York, McGraw-Hill, 351 p.

WEINGARTNER, D. P., SHUMAKER, J. R. & SMART, G. C. (1983). Why soil fumigation fails to control potato corky ringspot disease in Florida. *Pl. Dis.,* 67 : 130-134.

Accepté pour publication le 1er février 1985.