SEROLOGICAL IDENTIFICATION AND QUANTIFICATION OF 
HETERODERA AVENAE FROM PROCESSED SOIL SAMPLES

BY


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Monoclonal antibodies (MAbs) and polyclonal antibodies (PCs) were produced to antigens of an Australian population of cereal cyst nematode (CCN), Heterodera avenae. MAb IACR CCNj-49.2 recognised an antigen with a molecular weight of approximately 200 kDa, which was immunolocalised apparently in granules in the nematode gut. A procedure to extract CCN antigens from soil samples, under laboratory conditions was devised, and 50 g samples of soil containing 5 CCN cysts were processed by two sets of flotation techniques to recover the nematodes. Milling using Ballotini glass beads was then used to release the antigens from the CCN cysts recovered in the float. A double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) was the better ELISA format used to identify and quantify the CCN population from the processed soil samples containing up to 20% of organic matter. The threshold limit of detection was estimated by serial dilutions of the soil extracts. It was approximately equivalent to 0.5 eggs or 3.6 eggs per g of soil in a DAS-ELISA, when using respectively the PC or the MAb as the trapping antibody. The assay could be made more sensitive in soils with lower contents of organic matter.

Keywords: Cereal cyst nematode, monoclonal antibody, nematode quantification.

The cereal cyst nematode (CCN), Heterodera avenae (Wollenweber) is regarded as the most important pathogen of cereals in the southern wheatbelt of Australia. More than 2 million hectares in Victoria and South Australia are infested, and annual losses in grain yield in wheat alone are great (Brown, 1987). In Australia, yield losses can exceed 50% of the production potential (Rivoal & Cook, 1993). Failure to apply a nematicide on a heavily infested field may result in costly losses, but the unnecessary use of a nematicide may be just as expensive. The Sironem bioassay was developed in 1978 to provide an estimate of whether the population density of CCN exceeded the damage threshold. The test takes eight weeks to complete and is commercially available to growers. It rates the CCN infestation on a scale of 0 (not infested) to 5 (heavily infested).

Modern methods of diagnosis can help to increase the efficacy of control measures by early detection, identification and quantification of pre-planting nematode populations. Monoclonal antibodies (MAbs) are epitope specific, so they can differentiate closely related organisms. Amongst the diagnostic test formats
in which MAbs can be used, enzyme linked immunosorbent assay (ELISA) is the most common, reliable, simple and inexpensive. It can be extremely sensitive and used quantitatively to detect specific nematode protein from soil samples or plant tissue. A great advantage of ELISA is the potential for testing very large numbers of samples in a short time in a process which can be fully automated and is user friendly.

Much research has already been done on the application of MAbs to the differentiation and quantification of potato cyst nematodes (PCN). Species-specific MAbs which allow nematode quantification are available (Schots et al., 1992; Robinson et al., 1993). Quantification direct from soil has still to be achieved but using extraction procedures as a preliminary step in the assay has allowed quantification of PCN and root-knot nematodes to be achieved from soil samples under laboratory conditions (Davies & Carter, 1995; Evans et al., 1995; Davies et al., 1996).

This paper describes the production of CCN specific MAbs to an Australian population of *H. avenae* and the development of an immunoassay to identify and quantify the population densities of CCN in soil samples. An extraction procedure to recover the nematodes and release the CCN antigens from the soil samples was also devised, and the procedure proved suitable to process soil with varying amounts of organic matter.

**MATERIALS AND METHODS**

**Nematode material**

The nematode populations used were: *H. avenae* (Australian population obtained from South Australian Research and Development Institute), *H. trifolii* (Goffart) from the Rothamsted cyst collection, *Globodera rostochiensis* (Wollenweber) pathotype Ro1 from Woburn, *G. pallida* (Stone) pathotype Pa2/3 from Cadishead, *Meloidogyne incognita* (Kofoid & White) race 1 NCSU #78 and *M. javanica* (Treub) Chitwood. Egg masses were dissected from glasshouse grown aubergine (*Solanum melongena* L.cv. Blackwell) roots and juveniles (J2) were collected in water. *Anguina tritici* (Steinbuch) was obtained from infected wheat seeds held at Rothamsted. The seeds were soaked in water overnight and cut in half to release J2. *Pratylenchus neglectus* (Rensch) ex barley and *P. thornei* (Sher & Allen) ex wheat were obtained from cultures maintained at Rothamsted. To obtain CCN J2 for cryosections, cysts were soaked for 1 day in glass distilled water and then transferred to cereal root diffusate at 10°C or, alternatively, soaked cysts were cut in half to release eggs and J2.