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# Use of a cellulose acetate electrophoresis system for the simultaneous characterisation of individual root-knot (*Meloidogyne* spp.) females using three isozymes

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**Summary** – A cellulose acetate electrophoresis system is described which can be used for the identification of root-knot nematodes on a routine basis. The results of staining for several isozymes (EST, PGI, MDH & PGM) is reported. It was possible, with large healthy females of root-knot nematodes, to analyse up to three different isozymes per individual female. The technique is briefly discussed in comparison to DNA based approaches for root-knot identification.

**Résumé** – ***Système d'électrophorèse sur acétate de cellulose permettant la caractérisation d'un seul individu de* Meloidogyne *en utilisant trois isoenzymes*** – Description est donnée d'un système d'électrophorèse sur acétate de cellulose utilisable pour l'identification routinière des *Meloidogyne*. Les résultats concernent la coloration de plusieurs isoenzymes (EST, PGI, MDH et PGM). Il est possible, en utilisant des femelles de *Meloidogyne* de grande taille et en bon état, d'analyser jusqu'à trois isoenzymes sur le même individu. L'utilisation de cette technique pour l'identification des *Meloidogyne* est discutée en la comparant aux techniques fondées sur l'ADN.

**Key-words :** *Meloidogyne,* allozyme electrophoresis.

Isozyme electrophoresis has been widely used in studies of taxonomy, systematics and population genetics (Ayala, 1976; Oxford & Rollinson, 1983) and it has proved a very useful technique in the identification of species of *Meloidogyne* (Dalmasso & Bergé, 1983; Esbenshade & Triantaphyllou, 1985). At Rothamsted we have a large collection of root-knot nematodes in culture in our glasshouses and the identities of our populations are routinely checked using esterase isozymes with a polyacrylamide support medium for the electrophoretic separation of proteins (Ibrahim & Perry, 1992). Cellulose acetate has recently been used for allozyme studies involving small insects (Wynne *et al.*, 1991) and it is described here for use with root-knot nematodes.

## Materials and methods

### Electrophoresis equipment

The system was supplied by Helena Laboratories (P.O. Box 752, Beaumont, TX 77704, USA) and was convenient and relatively cheap. It is made up of an electrophoresis chamber (U.K. Cat. No. 1283; Zip Zone Chamber) and an applicator kit (U.K. Cat. No. 4093; Super Z-12 Applicator Kit) which consists of an applicator with twelve micro-tips, an aligning base and two sample plates with twelve wells each. The cellulose acetate plates were produced by Helena (Titan III, U.K. Cat. No. 3033) and ready for use.

### Sample preparation, loading and electrophoresis

Adult females of *Meloidogyne arenaria, M. incognita* and *M. javanica,* were individually dissected from the galled roots of either tomato *(Lycopersicon esculentum)* cv. Pixie, or aubergine *(Solanum melongena)* cv. Blackbell, which had been maintained in a glasshouse at 25 °C.

Females (one to five) were homogenised in 8 µl of extraction buffer (15 % (w/v) sucrose, 0.5 % (v/v) Triton X-100 in 50 mM tris/HC1, pH 7.1) in the wells of the sample plate using the end of a heat sealed Pasteur pipette. The cellulose acetate plates were carefully immersed vertically in running buffer (either 25 mM Tris-glycine, pH 8.5 or 50 mM Tris-citrate, pH 7.8; Richardson *et al.*, 1986) and left to soak for at least 10 min. Once soaked, the cellulose acetate plates were removed, blotted using Whatman No. 1 filter paper and placed on the aligning base. The samples were then loaded using the applicator. One or two cellulose acetate plates were placed on wicks soaked in running buffer in the electrophoresis chamber. Ice was placed in the central section of the chamber to cool the apparatus. Electrophoresis was performed at constant voltage (200 V) for 20 min. Experience has shown that Tris-glycine is suitable for esterase and glucose-phosphate isomerase while phosphoglucomutase and malate dehydrogenase are best run in Tris-citrate.

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VISUALISATION

The following stock solutions were used : nicotinamide adenine dinucleotide (NAD) (Sigma Cat. No. N-7004) 25 mg/mL; nicotinamide adenine dinucleotide phosphate (NADP) 20 mg/mL; 1M $MgCl_2$ 95 mg/mL; methyl-thiazolyl blue (MTT) (Sigma Cat. No. M-2128) 6 mg/mL; phenazine methosulphate (PMS) (Sigma Cat. No. P-9625) 2 mg/mL; malic acid 50 mg/mL pH 8.0). Four staining systems that have been found useful for root-knot nematodes are presented below. Stains should be prepared with the addition of reagents in the order described in the recipes. Richardson *et al.* (1986) provide details for other suitable visualisation systems for use with cellulose acetate plates.

*Esterases* (EST EC 3.1.1.1) : 10 mg Fast Blue RR salt (Sigma Cat. No. F-0500) dissolved in 9.6 mL 0.2 M Tris-HC1 ph 6.6, 8 mg α-napthyl acetate (Sigma Cat. No. N-8505) dissolved in 0.4 ml acetone.

*Glucose-phosphate isomerase* (GPI EC 5.3.1.9) : 5 mg fructose-6-phosphate (Sigma Cat. No. F-3627), 2.0 mL 0.1 M Tris-HC1 pH 8.0, 0.1 mL each of NADP, 1M $MgCl_2$, MTT, PMS and 2 I.U. glucose-6-phosphate dehydrogenase (Sigma G-7750) linking enzyme.

*Malate dehydrogenase* (MDH EC 1.1.1.37) : 2 mL Tris-HCl pH 8.0, 0.2 mL malic acid, and 0.1 mL of each of NAD, MTT and PMS.

*Phosphoglucomutase* (PGM EC 2.7.5.1) : 10 mg glucose-1-phosphate, 2 mL 0.1M Tris-HC1 pH 8.0, 0.1 mL of each of NADP, 1M $MgCl_2$, MTT, PMS.

After electrophoresis the cellulose acetate plates were stained using the agar overlay technique (Easteal & Boussy, 1985). To 2-3 mL of the above staining mixtures an equal volume of molten agar (360 mg/25 ml maintained at 60 °C) was added and mixed before being poured onto the surface of the cellulose acetate plate. After cooling and when the agar had set the plates were placed in a dark incubator at 37 °C until the enzyme bands became visible (15-60 min). After staining, the cellulose acetate plates were washed with water to remove the staining mixture and fixed with 10 % (v/v) acetic acid.

## Results and discussion

The results of staining for EST, MDH and GPI can be seen in Figs 1-3. It is possible, with large healthy females of root-knot nematodes, to analyse up to three different isozymes per individual female. Glucose phosphate isomerase was by far the most sensitive and bands could be visualised with just one transfer of the sample to the plate using the applicator; the other three isozymes required four or five transfers of sample. The Helena cellulose acetate system is quick, simple and reliable, taking 20 min to run two separate plates. Because of the small amount of current drawn from the power pack it is possible to run several systems from one power pack. Loading of samples is accurate and rapid, and several plates can be run together allowing each plate to be stained for different isozymes. Compared to other electrophoresis systems the cellulose acetate plates

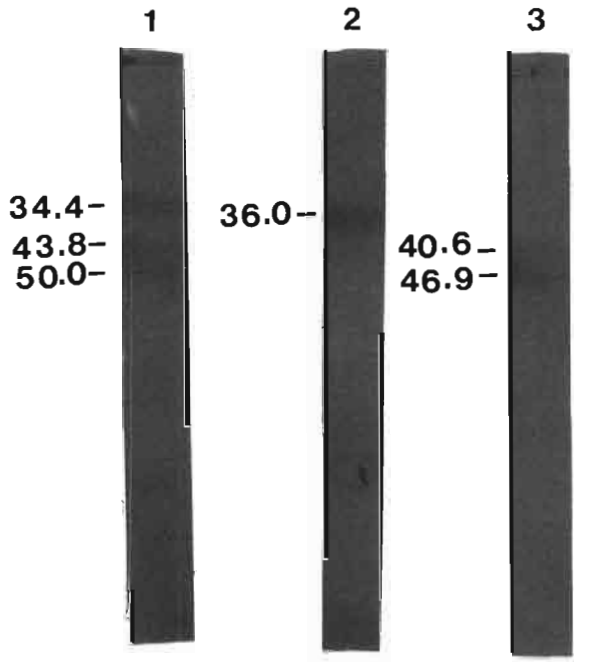


***Fig. 1.*** *Visualisation of non-specific esterase bands of single females of* Meloidogyne javanica *(lane 1),* M. incognita *(lane 2) and* M. arenaria *(lane 3).*

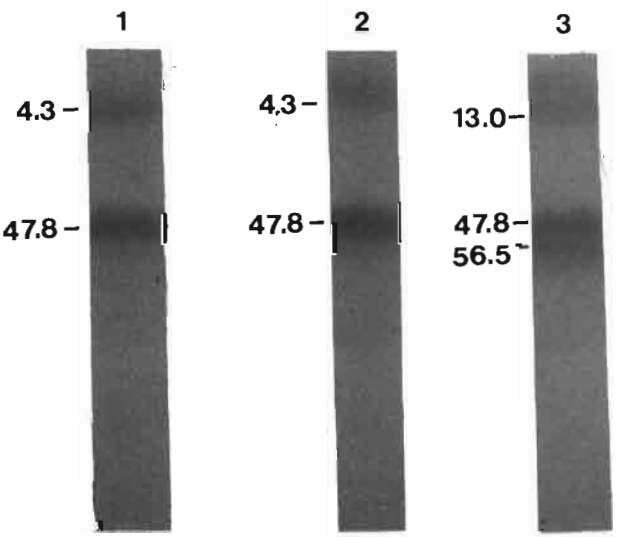


***Fig. 2.*** *Visualisation of malate dehydrogenase bands of single females of* Meloidogyne javanica *(lane 1),* M. incognita *(lane 2) and* M. arenaria *(lane 3).*

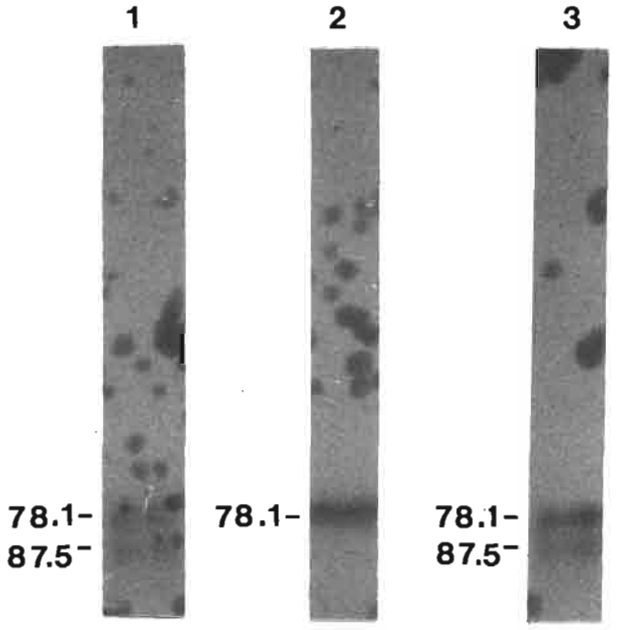


***Fig. 3.*** *Visualisation of bands of glucose phosphate isomerase of single females of* Meloidogyne javanica *(lane 1),* M. incognita *(lane 2) and* M. arenaria *(lane 3).*

are relatively expensive (approx. £ 1.00 per plate). However, this is more than compensated for by the fact that they are easy to use, non-toxic and have a long shelf life. Richardson *et al.* (1986) draw attention to the potential problem that molecular migration through the matrix of cellulose acetate systems, unlike starch or polyacrylamide, is not at a constant rate and may therefore lead to discrepancies between different runs. However, this problem can be overcome by the use of a standard sample on each plate.

Attention has been given to the use of PCR based techniques for the identification of the major root-knot nematodes using a carefully selected primer to mitochondria(mt)-DNA followed by endonuclease digestion (Powers & Harris, 1993). However, recently it has been shown that there is a high degree of correlation between mt-DNA analysis and esterase phenotypes (Hugall *et al.*, 1994) in Australian populations of root-knot nematodes. If phylogenies based on mt-DNA are similar to those based on esterase, the simple technique reported here would have a large advantage over PCR based techniques, which involve several steps requiring careful management.

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