SHORT COMMUNICATION

R. J. HOLLAND1) & K. L. WILLIAMS1,2): A new technique for obtaining eggs of known age from excised females of Meloidogyne javanica.

Adult female *Meloidogyne javanica* (Treub) Chitwood live parasitically within the roots of host plants (Eisenback & Triantaphyllou, 1991). The female remains protected by root tissue and extrudes eggs and gelatinous matrix to form an egg mass which is vulnerable to attack by pathogens. Interest in the development of biological control of plant parasites has increased due to growing concerns over the use of chemical pesticides (Cook, 1993). The nematode parasitic fungus, *Paecilomyces lilacinus* (Thom) Samson, is undergoing development for use as a bionematicide against *Meloidogyne* spp. on a variety of crops. Since *P. lilacinus* is considered to be mainly an egg parasite (Jatala, 1986), research has focussed on the interactions between this fungus and *Meloidogyne* eggs.

There is a need for clean eggs to be readily available for experimental microscopy. Traditionally, *Meloidogyne* eggs have been released from the gelatinous matrix with dilute bleach (e.g. McClure et al., 1973). While this does not adversely effect the hatching process (McClure et al., 1973), it does reduce galling by treated *M. javanica* juveniles (Kanwar et al., 1991). The use of bleach may affect the ability of *P. lilacinus* to recognise a suitable egg to infect. Eggshell surfaces may have proteins which change with the maturity of the egg, and which are recognised by the fungus *P. lilacinus*. Some changes have been noted in the structure of the egg membranes during the development of the egg, in that the lipid layer breaks down prior to hatching (Bird & McClure, 1976). However, changes in egg membranes have not been recorded during the early stages of egg development. Also, it is more meaningful to study eggs with some associated gelatinous matrix in order not to eliminate its important role.

The egg mass contains all stages of embryological development as eggs are laid over several weeks. Since *P. lilacinus* is considered to be mainly a parasite

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of eggs in the early embryological stages (Jatala, 1986) a method of obtaining eggs of known age is of benefit to enable further research to be done on eggs of different ages.

Presented here is a method for obtaining clean eggs of known age from adult females of *M. javanica*. The method was developed after the chance observation that some adult females, after being dissected from root tissue and placed onto water agar plates, remained alive and continued to lay eggs.

Adult females of *M. javanica* were obtained from roots of tomato plants, 10 to 12 weeks after infection with six egg masses. Root pieces with single galls and a small egg mass, were placed into sterile salt solution (NaCl, 0.6 g/l; KCl, 0.75 g/l; CaCl₂·2H₂O, 0.4 g/l; autoclaved). Using fine forceps, and keeping the tissue immersed in salt solution, eggs and plant material were gently cleared away until the female was attached only by the head. Then forceps were used to ease her away from the root. Damaged females were discarded and intact females were placed initially into fresh salt solution, then onto approximately 2 x 3 mm pieces of sterile dialysis membrane on water agar plates (agar 20 g/l; dihydrostreptomycin sulphate 0.05 g/l; autoclaved). Females were positioned preferably such that eggs were laid directly onto the dialysis membrane, covered and incubated at 26 ± 1°C for a known period, usually 24 h. (Note that placing females in water resulted in lysis of the body contents of the females, usually within an hour, whereas they could survive for at least several days in the salt solution.) Most females laid eggs during this time, and females were then removed, leaving groups of eggs of known age on the membrane for further treatment or development. If eggs adhered to the female they were dislodged with a fine sterile needle and if eggs remained in a clump, they could be gently teased apart with two fine, sterile needles to leave a single layer of eggs on the membrane. This was especially useful for light microscopy and SEM. Most eggs developed normally to hatching and all stages of embryonic development could be followed, from one-cell through to hatched juveniles.

Excised *M. javanica* females continued to lay eggs for up to two weeks, after which they looked transparent. After excision and incubation for 24 h there was considerable variation in the total number of eggs laid per female (0-85, average 36, from 100 examined). Initially about one half of the females did not survive excision, with body contents deposited on the dialysis membrane, but experience with the technique led to improvement, with about 80% surviving. Excision in a laminar flow cabinet minimised the occurrence of contaminants on the agar plates.

Most excised females produced gelatinous matrix after they were placed onto the dialysis membrane, but where females were removed from the initial batch