RESEARCH NOTES

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Encapsulation of *Meloidogyne incognita* Eggs in Carrageenan[1]

GEORGE FASSULIOTIS[2] AND W. J. CONNICK, JR.[3]

Nematologists need a method to maintain single species populations of *Meloidogyne* spp. for prolonged periods without significant loss of viability. As a standard practice, isolates of *Meloidogyne* populations are identified, characterized, and maintained on the same host plant species. Extreme precautions are necessary to maintain them free of contamination from other nematode populations. Sterile root culture techniques also are used for culturing and maintaining single species populations (2), but both systems require periodic subculturing which after many years can select for certain characters that are uncommon in the original isolated population.

Hydrocolloids extracted from seaweeds are being used to encapsulate various chemicals and biological organisms. Sodium alginate has been used (via gelation with Ca^{++}) to formulate sustained-release herbicides (1) and mycoherbicides (8) and to encapsulate somatic plant embryos (5) and entomogenous nematodes (4). In our laboratory, eggs of *Meloidogyne incognita* (Kofoid & White) Chitwood and *Filipjevimermis leipsandra* Poinar & Welch encapsulated in calcium alginate gels were kept viable for about 2 months (Fassuliotis, unpubl.). Some calcium alginate gels, however, have an undesirable property of extreme syneresis after capsule preparation. After the excess water is removed, the relative humidity must be controlled to prevent desiccation of the capsule during storage.

Kappa carrageenan is a hydrocolloid, often used in food preparations, which produces strong gels with potassium salts (7). It does not have the severe syneresis property of alginate. Gelcarin CIC (FMC Marine Colloids Division, Springfield, NJ 07081), a kappa carrageenan developed especially for microbial immobilization, at a concentration of 2.5 (w/v), produces well-formed beads when gelled with K^+. This material provides a superior matrix for encapsulating root-knot nematode eggs.

All procedures were done under sterile conditions. *M. incognita* eggs were extracted from infected lima bean (*Phaseolus limensis* Macf. cv. Jackson Wonder) roots in a 0.25% sodium hypochlorite solution for 4 minutes, collected on a 26-μm-pore sieve, rinsed with sterile purified water, and concentrated in a known quantity of water (3).

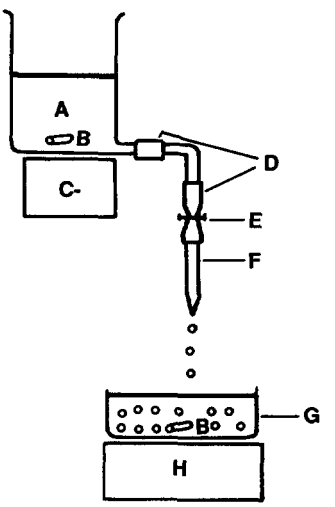

FIG. 1. Apparatus used to encapsulate root-knot nematode eggs in carrageenan gel. A) Beaker with lower hose connection. B) Stir bar. C) Heater-magnetic stirrer. D) Rubber tubing. E) Screw clamp. F) Disposable Pasteur pipet. G) Dish containing gellant solution. H) Magnetic stirrer.

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[2] Supervisory Research Nematologist, USDA ARS, U.S. Vegetable Laboratory, Charleston, SC 29414.

[3] Research Chemist, Southern Regional Research Center, New Orleans, LA 70179.

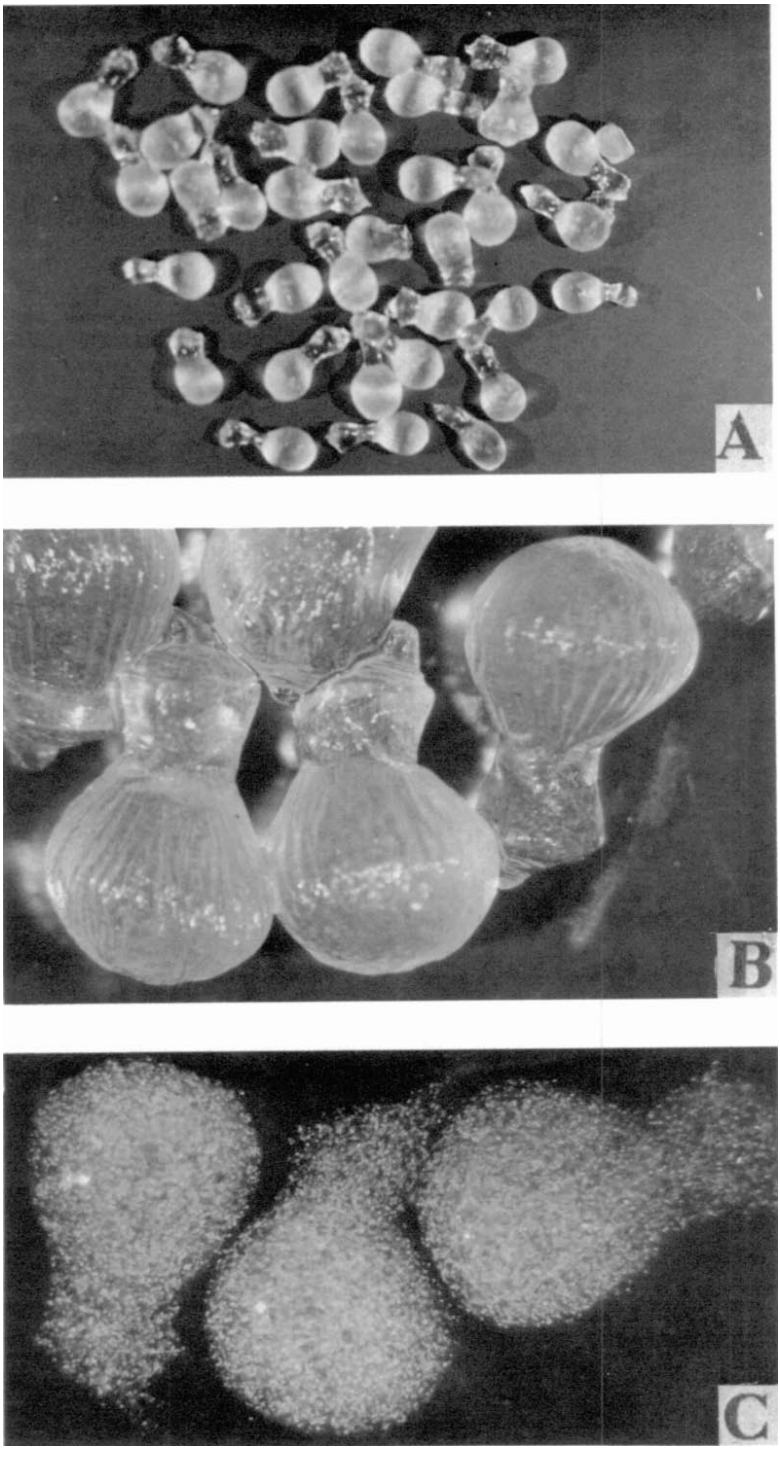

FIG. 2. Photomicrographs of K^+-gelled root-knot nematode eggs in carrageenan beads. A, B) Surface-dried beads showing shape and surface texture. C) Hydrated beads displaying contents with ca. 4,000 *Meloidogyne incognita* eggs. A = 1.5×; B, C = 10×.

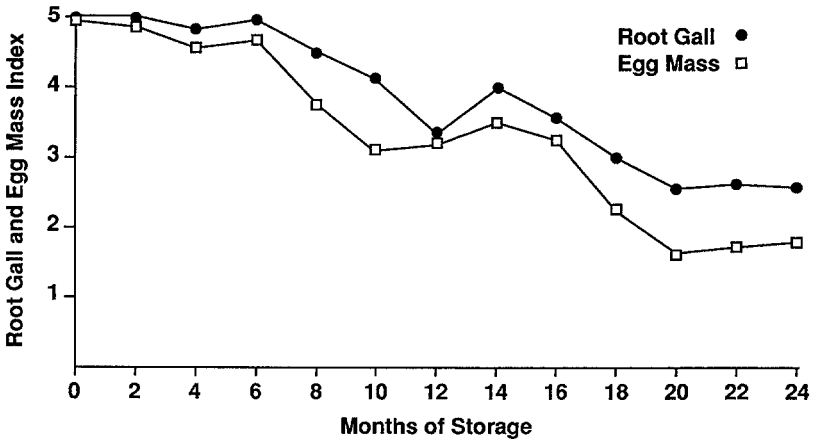

FIG. 3. Survival of *Meloidogyne incognita* eggs encapsulated in carrageenan over a 24-month period of storage at 15 C.

The eggs were encapsulated in the carrageenan by a modification of the method described by Connick (1) in a 5% (w/v) solution of Gelcarin CIC diluted with an equal volume of *M. incognita* egg suspension in the apparatus shown in Figure 1. The suspension was maintained at 28 C to produce a constant viscosity and was slowly stirred to keep the eggs uniformly suspended. The number of eggs incorporated into a capsule was determined by collecting individual drops of the carrageenan mixture on a glass slide (without gelation) and counting the number of eggs with the aid of a dissecting microscope. The suspension was dispensed at a flow rate of about 80 drops per minute into a 200-ml reservoir of 0.3 M KCl which was agitated constantly to obtain discrete beads. After 1–5 minutes, the beads were collected on a 600-μm-pore sieve, rinsed thoroughly with sterile water, and surface dried on paper toweling for 10 minutes.

Beads of the carrageenan–egg mixture, gelled with K^+, formed uniform pyriform gelatinous capsules approximately 5 mm in diameter (Fig. 2.). They were packaged in ziplock plastic bags or in 20-ml scintillation vials and stored at 15 C because preliminary studies indicated that encapsulated nematode eggs lost viability at 5 and 25 C within 2 months (unpubl.).

Two batches of encapsulated eggs, prepared in March 1984 and in November 1986, were checked bimonthly for infectivity on tomato (*Lycopersicon esculentum* Mill. cv. Homestead) seedlings planted in 500-ml styrofoam cups in the greenhouse. Ten capsules, each containing ca. 300 eggs, were added to soil just before planting. Tomato plants were also inoculated with a suspension of 3,000 freshly extracted eggs to serve as controls. After 50–60 days, the roots were washed free of soil and stained with a solution of phloxine B (0.015 g/L) for 15 minutes to enhance visibility of the nematode egg masses. Root systems were rated for galling with Taylor and Sasser's (6) gall and egg mass index (GI, RI). Mechanical failure of the incubation chamber in which the capsules were stored concluded both experiments after 24 months.

Figure 3 shows the gall and egg mass index of batch 2 of encapsulated *M. incognita* eggs that developed on tomato roots in each bimonthly test. Infectivity declined 6 months after encapsulation, but enough eggs retained viability after 24 months to regenerate the population on host plants. An estimate of egg reproduction (3) made after the 24-month test verified the reduced viability of encapsulated eggs. The number of eggs recovered from the capsules was one fifth the number recovered from roots inoculated with freshly extracted eggs (5,269 vs. 26,500).

The encapsulation method is simple, requiring only equipment usually found in a

laboratory. Encapsulation in carrageenan offers a means of maintaining eggs for at least 24 months without the fear of contamination with other *Meloidogyne* species. It can provide the means to store excess inoculum in a minimum amount of space until test plants are ready for inoculation, provide the propagule for inoculating test plants, and provide a means for maintaining a collection of single species populations that nematologists can utilize for systematic and morphological studies. It is an excellent medium in which to ship populations to other scientists with proper certification.

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