Changes in the Ultrastructure of the Gelatinous Matrix of Meloidogyne javanica During Dehydration

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Abstract: The fine structure of the gelatinous matrix of *Meloidogyne javanica* in both moist and dry states was studied by means of chemical fixation and thin sectioning techniques and the freeze-etch method. The matrix consists of an irregular meshwork when hydrated and a uniform granular mass of much greater density when dehydrated. The spaces in the hydrated meshwork are presumed to contain water. The change from a hydrated to a dehydrated state is accompanied by an overall shrinkage and hardening of the egg mass with a change in color from yellow to reddish-orange. The possible functions of this unusual glycoprotein are discussed. *Key words:* freeze-etch, shrinkage, glycoprotein, electron microscope.

The gelatinous matrix of *Meloidogyne* is a glycoprotein substance produced by six large rectal gland cells (2, 4). The matrix covers the eggs and is thought to help prevent water loss from them (3, 7). It shrinks on drying, and does not appear to have any water retention properties (7). The hydrated gelatinous matrix appeared structureless and homogeneous when viewed with the light microscope (1, 2). However, when viewed under the electron microscope, it appears to consist of an irregular meshwork (2).

In this paper, observations of the changes that take place in the ultrastructure of the gelatinous matrix during dehydration are described and discussed.

MATERIALS AND METHODS

Egg masses of *Meloidogyne javanica* (Treub.) Chitwood were dissected from the roots of infected tomato plants; cleaned, with the aid of fine forceps, of adhering debris; and placed in shallow distilled water in a petri dish until weighed and observed during drying.

Weighing. Material to be weighed was touched to filter paper to remove excess moisture, placed on the pan of a Cahn electrobalance (sensitivity ± 5 μg), and weighed at 1- to 2-min intervals until three readings remained constant. During these weighings, both temperature and relative humidity were recorded.

Observation of shrinkage. A small piece of gelatinous matrix was cut from an egg mass and placed in a drop of distilled water on a microscope slide where it was photographed as shrinkage took place during drying.

Fixation and embedding. One-half of a gelatinous matrix was fixed immediately, and the other half was fixed after dehydration at 20 C and 60% relative humidity.

Matrix was fixed 1 hr in 4% formaldehyde at 5 C and 1 hr in 2% osmium tetroxide in 0.05 M phosphate buffer at pH 7.2 at 5 C with a brief rinse in distilled water between steps. Dehydration was in a successive series of 50%, 70%, 80%, 90% and absolute ethanol, each for 1 hr at 20 C. The material was left overnight in a 1:1 mixture of absolute ethanol and Spurr's low viscosity epoxy resin embedding medium, then embedded *in vacuo* in Spurr's standard medium overnight at 60 C (6).

Microtomy and staining (conventional method). Sections were cut with glass knives using an LKB ultramicrotome, collected on collodion-coated grids, and stained successively with 5% uranyl acetate (90 min) and lead citrate (15 min) at 20 C with a brief rinse in distilled water in between.

Freeze-etching. The only difference in treatment of the dehydrated and hydrated egg masses was that the former were placed in pure glycerol before being frozen. In both cases, egg masses were frozen in liquid Freon-22 and stored in liquid nitrogen. Frozen egg masses were placed on the cold stage of a Balzers freeze-etch apparatus and etched at −100 C for 2 min, then coated with carbon and platinum (5). The carbon-platinum replicas were cleaned

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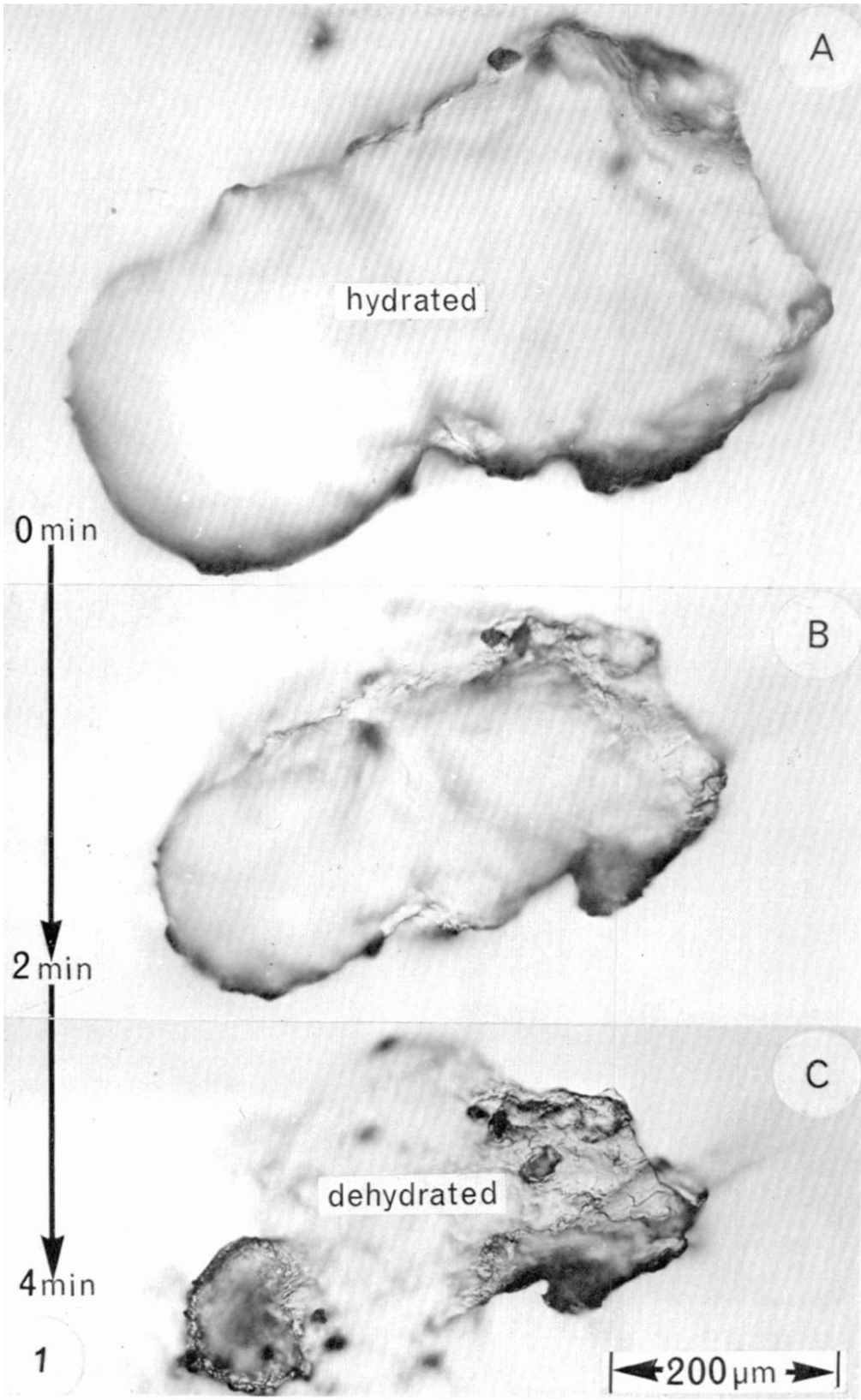
FIG. 1. Shrinkage of a piece of gelatinous matrix at 20 C and 60% relative humidity at **A.** 0, **B.** 2, and **C.** 4 min.


A
hydrated
0 min
B
2 min
C
dehydrated
4 min
1
200 µm

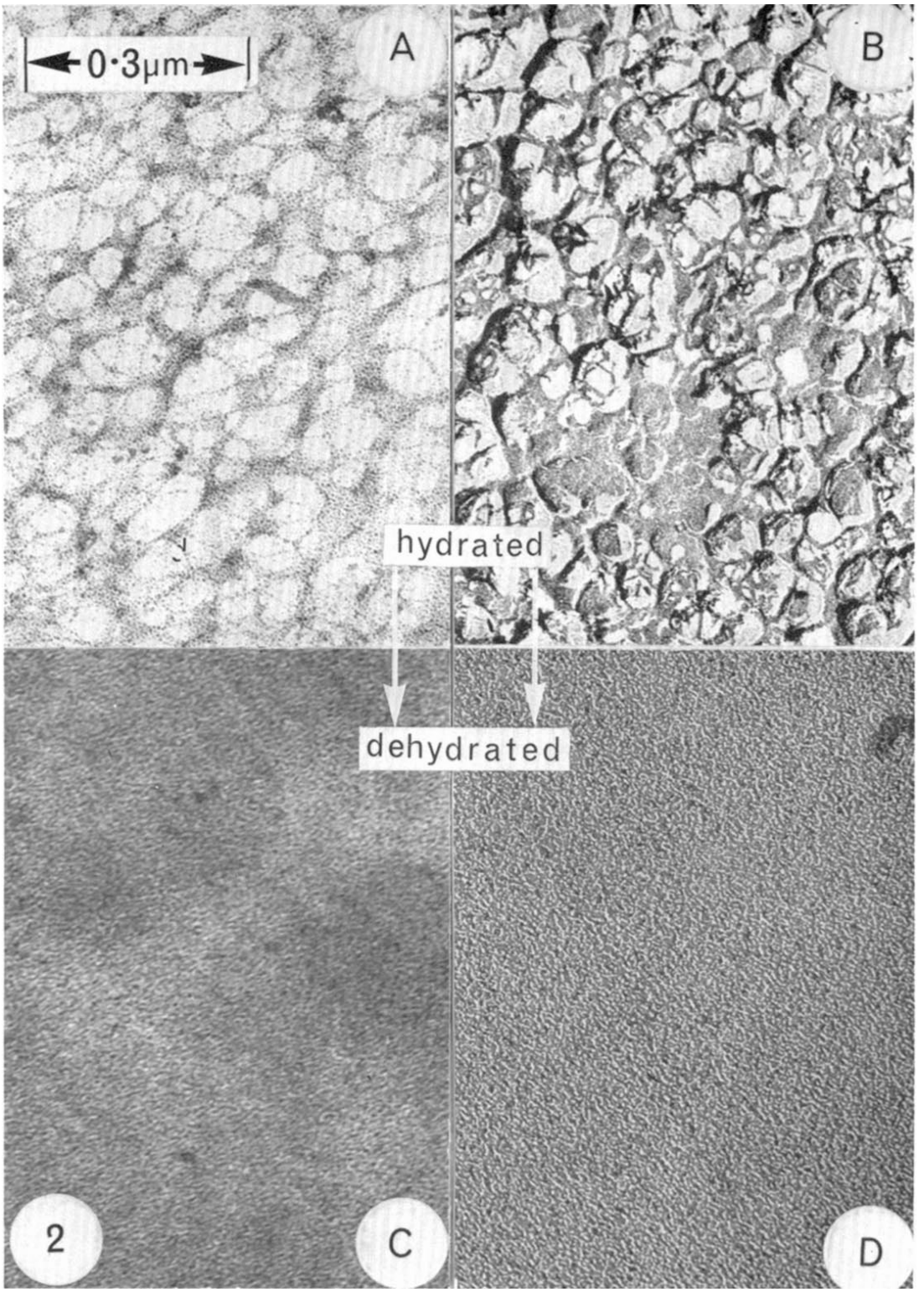


FIG. 2. Electron micrographs of gelatinous matrix; **A.** hydrated (conventional methods); **B.** hydrated (freeze-etched); **C.** dehydrated (conventional methods); **D.** dehydrated (freeze-etched).

by placement in sodium hypochlorite solution (5% available chlorine) overnight; we then washed them in distilled water. The hypochlorite solution breaks down and dissolves the egg mass (1) so that acids and caustics are not needed. The cleaned replicas were picked up on copper grids and examined with a Siemens Elmiskop 1A electron microscope operated at 80 kv.

RESULTS

Hydrated egg masses selected at random and weighed on a Cahn electrobalance ranged from 60 to 240 μg. After drying, weight loss varied from 85% for an egg mass with few eggs to 45% for an egg mass consisting largely of eggs. An egg mass weighing 146 μg (medium size) in an atmosphere at 20 C and 60% relative humidity lost moisture until it attained a constant weight after 18 min. A drop of water of similar dimensions evaporated within 13 min. A small piece of gelatinous matrix became dehydrated within 4 min (Fig. 1). Egg masses shrink, change color from yellow to reddish-orange, and become very hard when dried.

Egg masses dehydrated at 20 C and 60% relative humidity became hydrated and swollen again when placed in distilled water. Furthermore, the eggs hatched and the larvae infected and developed within host plants.

Previously (1, 2) and in this study, the hydrated and dehydrated gelatinous matrix appeared homogeneous when observed with the light microscope. The electron microscope reveals, however, that a considerable change in the fine structure of the matrix takes place during dehydration (Fig. 2). Electron micrographs of both conventionally sectioned (Fig. 2-A) and freeze-etch material (Fig. 2-B) show that the hydrated gelatinous matrix has an irregular, mesh-like appearance. The spaces or pores in this mesh work range from about 50 to 100 nm in width at their widest point. The dehydrated material appears as a uniformly dense mat (Fig. 2-C, D).

DISCUSSION

The structure of the gelatinous matrix obtained by two completely different methods is so similar that the structural configuration appears to be natural and not brought about by preparative artifacts. The spaces of the hydrated material probably contain water which evaporates during dehydration, and the glycoprotein meshwork comes together to form a mat. This may serve to inhibit water loss from the eggs, as suggested by Chitwood and Chitwood (3) and Wallace (7). It is also possible that the dehydrated egg mass exerts mechanical pressure on the egg shells and inhibits hatching by preventing distortion of the egg shell which takes place prior to hatching (7).

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