Cell lysis activity of *Meloidogyne* gelatinous matrix

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SUMMARY

The root-knot nematode, *Meloidogyne incognita,* was monoxenically cultured on excised tomato roots. Fifteen to 30 days after inoculation galls were sampled at 3-day intervals, fixed and processed in the conventional paraffin method for histological observations. Some three weeks after inoculation, gelatinous matrix (GM) secretion became evident. A small cavity bordering the female posterior portion and fragments of cell walls engulfed by the GM were observed. Twenty-four days after inoculation the cavity became larger in size to form a canal leading to the gall surface. Twenty-seven days after inoculation eggs deposited by the nematode female were embedded in the GM and pushed through the canal to form the egg mass on the gall surface. The present study suggests a cellulytic enzymatic activity of the GM.

RÉSUMÉ

Action cellulolytique de la sécrétion gélatineuse de Meloidogyne

Le nématode *Meloidogyne incognita* a été élevé sur racines excisées de tomate. Quinze à trente jours après inoculation, les galles ont été prélevées à trois jours d'intervalle, fixées puis traitées suivant la méthode conventionnelle à la paraffine pour les observations histologiques. La sécrétion gélatineuse est perceptible trois semaines environ après l'inoculation et l'on observe une petite cavité entourant la portion postérieure de la femelle ainsi que des fragments de la membrane cellulaire dissoute par la sécrétion gélatineuse. Vingt-quatre jours après l'inoculation, la cavité s'élargit et forme un canal dirigé vers la surface de la galle. Vingt-sept jours après inoculation, la sécrétion gélatineuse contient des œufs qui sont poussés dans le canal pour former la masse d'œufs à la surface de la galle. La présente étude suggère une action enzymatique cellulolytique de la sécrétion gélatineuse de *Meloidogyne incognita.*

The root-knot nematode species (*Meloidogyne* spp.) are among the most intensively studied plant-parasitic nematodes. It is surprising, therefore, that the gelatinous matrix (GM) which is secreted by the nematode female in voluminous amounts has been so little investigated. Maggenti and Allen (1960), reported in great detail that the GM is synthetised in the six rectal gland cells (RGC) and is excreted through the anus just before and during egg-laying. Dropkin and Bird (1978) studied stimulation of GM secretion by compounds extracted from plant roots. Studies on the histochemistry and ulstrastructure of the RGCs and of the GM they produce (Bird & Rogers, 1965; Bird & Soeffky, 1972) have shown that the matrix is an irregular mesh containing proteins, carbohydrates and certain enzymes which are transformed into a uniform granular mass of much greater density when dehydrated. Wallace (1968) suggested that the GM of the egg sac appears to maintain a high moisture level and provides a barrier to water loss from eggs. Bird (1979) considered the GM to have great survival value but there has been no experimental evidence to indicate the functions of the GM.

After viewing scores of histological sections of *Meloidogyne* spp. - induced galls, it was found that the egg mass protruded outside the gall surface through a clean-cut canal. Examination of fleshy roots, like banana or ginger infested with root-knot nematodes, revealed that the egg mass was located in a spherical cavity adjacent to the nematode posterior end. These observations suggested that the GM acquires cellulytic properties explored in the present work.

Materials and methods

Root-knot nematodes *(Meloidogyne incognita)* were cultured on excised tomato (*Lycopersicon esculentum* cv. Roma) roots on a chemically defined medium as previously described (Orion, Wergin & Endo, 1980). The cultures were kept in the dark at a temperature of 25°. Galled roots were sampled six times at 3-day intervals, beginning on the 15th day after inoculation. The galls were fixed in FAA, dehydrated and paraffin-embedded, following the conventional procedure. Ten µm thick sections were made and stained either with Mallory's

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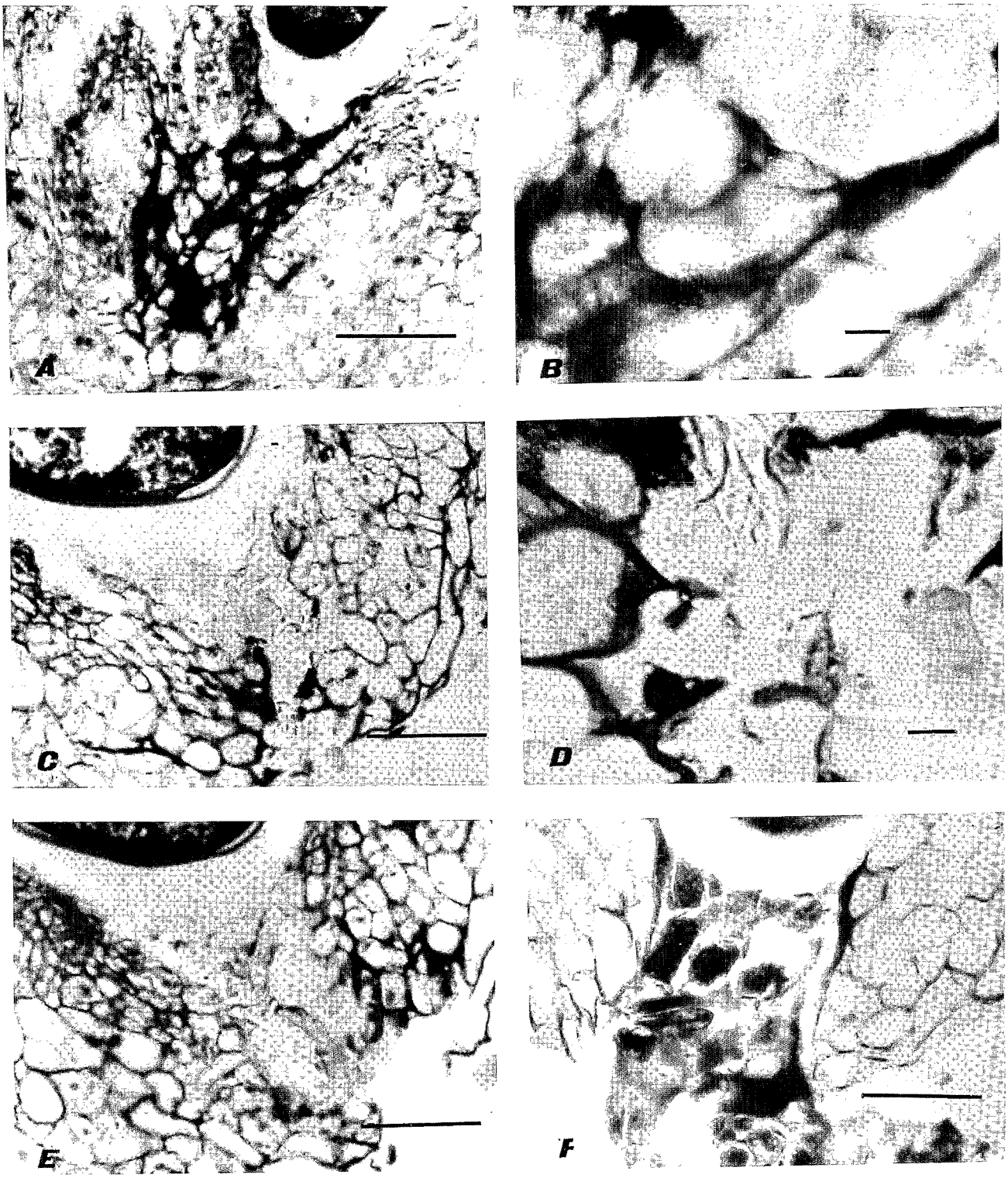

Fig. A : Section trough *M. incognita*-induced gall 21 days after inoculation. A small cavity is formed near the nematode posterior end. Note the dark-stained cells behind the nematode; B : High magnification of the contact area of the GM and the plant cells (of Fig. A). Breakdown of cell walls and flow of GM into dark-stained cells are seen; C : Section of *M. incognita*-induced gall 24 days after inoculation. Note the flowing appearence of the GM and the canal formation leading from the nematode posterior portion to the gall surface; D : High magnification of Fig. D, cell wall break-down is shown; E : Section of *M. incognita*-induced gall 24 days after inoculation, the final stages of the canal formation; F : Section in *M. incognita*-induced galls 30 days after inoculation. An egg mass is protruding through the canal to the gall surface. The inner wall of the canal is coated with dark-stained material. *(Bars = 100 µm for A, C, E and F; = 10 µm for B and D).*

triple stain (Barbosa, 1974) or toluidine blue and acid fuchsin (O'Brien & McCully, 1981). Observations were directed at the host tissues adjacent to the posterior end of the nematode female.

Results

In the galls sampled 15 and 18 days after inoculation, the nematodes were at a young female stage but no GM secretion could be detected. Twenty-one days after inoculation a small cavity within the gall parenchymal tissue bordering the nematode posterior portion was observed. The cavity contained material with a semi-transparent or viscuous fluid appearance which apparently was the GM (Fig. 1, A). At the contact area of the GM and the plant cells, a breakdown of cell walls was evident and a flow of GM, or its fractions could be observed in neighbouring cells (Fig. 1, B). The dark staining of the plant cells beginning at the contact area of the GM and the plant cells spreading some 200 µm suggests diffusion of GM materials within the gall tissues (Fig. 1, A). Twenty-four days after inoculation a large cavity, stretching up to the gall surface, was observed. The semi-fluid pattern of the GM and cell walls at various stages of lysis are shown in Figure 1, C and Figure 1, D. Further dissolution of host cells forming almost a complete canal, leading from the nematode posterior portion to the external environment and cells along this cavity in advanced stages of lysis, are presented in Figure 1, E. Twenty seven and 30 days after inoculation eggs were deposited in the GM and pushed through a clean-cut canal to form the egg mass (Fig. 1, F). At this stage no further cell lysis could be observed and the walls of the canal are coated with a dark stained layer (Fig. 1, F).

Discussion

The present study provides evidence of the GM cellulytic activity enabling the depositing of the eggs by the root-knot nematode female to reach the gall surface. This is an essential stage in the root-knot nematode dissemination process, allowing the short period active second stage larvae to migrate in the soil and to find new feeding sites immediately upon hatching. In extremely large galls or in fleshy roots infested with root-knot nematodes, the cavity in which the egg mass is located is formed also by the GM cell lysis activity, though, under these circumstances most of the hatching 2nd stage larvae stay within the host tissues. Bird and Rogers (1965) analyzed the GM and reported positive histochemical tests for the enzymes polyphenol oxydase and phosphatase, which could be associated with cell lysis processes. The presence of high amount of cellulase in *M. javanica* induced galls was demonstrated by Bird, Downtown and Hawker (1975), part of, could originate from the GM. However, a thorough enzyme analysis of the GM should be undertaken to identify the enzymes involved in this process. The fact that the canal or the spherical cavity formed by the GM is limited in diameter suggests that the GM cellulytic fractions are active for a limited period of time only; in later stages the GM probably fulfils other functions.

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