D. P. TAYLOR [1]): *Histopathology of* Meloidogyne*-induced galls on the stems of roselle,* Hibiscus sabdariffa.

Although species of *Meloidogyne* typically produce root galls on susceptible plants, galls are produced occasionally on above-ground parts. Histopathology of such stem galls have been reported on naturally infected beans by Fassuliotis &

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Deakin (1973) and artificially infected beans and tomato by Wong & Willetts (1969).

Roselle, *Hibiscus sabdariffa* L., is a host for *Meloidogyne* sp. (Bessey, 1911); however, it is more resistant to *M. incognita* (Kofoid & White) than the related plant *H. cannabinus* L. (Wilson & Summers, 1966).

Laboratory experiments were made to determine the reaction of roselle to a population of *Meloidogyne* from Cambérène, Sénégal, which consisted of a mixture of *M. incognita, M. javanica* (Treub), *M. arenaria* (Neal), and other unidentifiable forms. Egg masses were removed from infected tomato roots and placed in lots of ten in distilled water.

Thirty pots, 6 cm in diameter, were filled with autoclaved sandy soil. A small depression was made in the center of each pot into which were placed three seeds of *H. sabdariffa*, variety THS-22, supplied by l'Institut de Recherches du Coton et des Textiles Exotiques. The inoculum, ten egg masses per pot, was poured into the depression at the time of planting and the depression filled with additional soil. Plants were maintained at 25-28° and their stems cut and fixed 10, 15, 20, 25, and 40 days after planting.

Stem tissues were fixed for 48 hours or longer in FAA. Galled tissue was embedded in paraffin, sectioned at 15-18 μm and stained with safranin and fast-green.

In another experiment designed to determine the time and place of penetration of *Meloidogyne* juveniles, the same inoculation procedure was used. However, germinating seedlings before and after emergence were harvested and stained in cold cotton blue-lactophenol (de Guiran, 1967).

General observations. Ten days after planting small swellings were visible on the stems of infected *H. sabdariffa* seedlings. As the plants became older, these swellings could be classified into two types: small localized pustule-like swellings and large general swellings encompassing most or all of the stem diameter near the base. The smaller galls occurred to a height of 6 cm above the soil line but were never observed above the cotyledons; after 40 days they measured 1.0 $\times$ 0.8 mm and were raised about 0.5 mm. Histological examination of these smaller galls revealed the presence of one, very rarely two, *Meloidogyne*. The larger basal swellings were up to 5 mm in diameter after 40 days, whereas noninoculated seedlings had a stem diameter of 2.5 mm or less. Sections through such galls contained several *Meloidogyne* (Fig. 1). Galls of both types were a paler green than adjacent healthy tissue. After 40 days egg masses had ruptured the epidermis of some of the galls; however, in most cases the epidermis remained intact.

Histology. Ten days after planting, sections through infected stems already contained multinucleate giant cells. These were clustered around the anterior end of juveniles (Fig. 2), contained densely staining cytoplasm and had several to many enlarged nuclei with prominent nucleoli. Thirty nuclei were counted in serial sections through one giant cell. Walls surrounding the giant cells were thin. Most giant cells were located in the region of phloem development although a few were

present in the xylem and the cortex. Hypertrophy and hyperplasia were commonly observed at most infection sites. Juvenile development had commenced as indicated by an increase in body diameter visible in some sections.

After 15 days, giant cells were markedly enlarged and their walls were thickened (Fig. 3). Much more hyperplasia was observed in the phloem and adjacent cortex. Juvenile development had reached the late second, or "sausage", stage (Note spikate tail of juvenile in Fig. 4).

After 20 days, infection had progressed further with prominent disruptions in the vascular system caused by the increased size of giant cells, and some showed deterioration of the cytoplasm and an increase in size of vacuoles. One giant cell measured 470 μm in length. Some juveniles had increased considerably in diameter suggesting development to the young female stage. One longitudinal section showed an unusual orientation of nematodes in which the anterior end of one was directed toward the stem apex while another was directed toward the root (Fig. 5).

After 25 days, the infections were further developed with a larger amount of tissue involved in giant cell development, hyperplasia and hypertrophy. Walls of certain giant cells were conspicuously thickened (Fig. 6). Sections through developing females showed gonadal development and the presence of at least one shed cuticle indicating that the adult stage had been reached (Fig. 7).

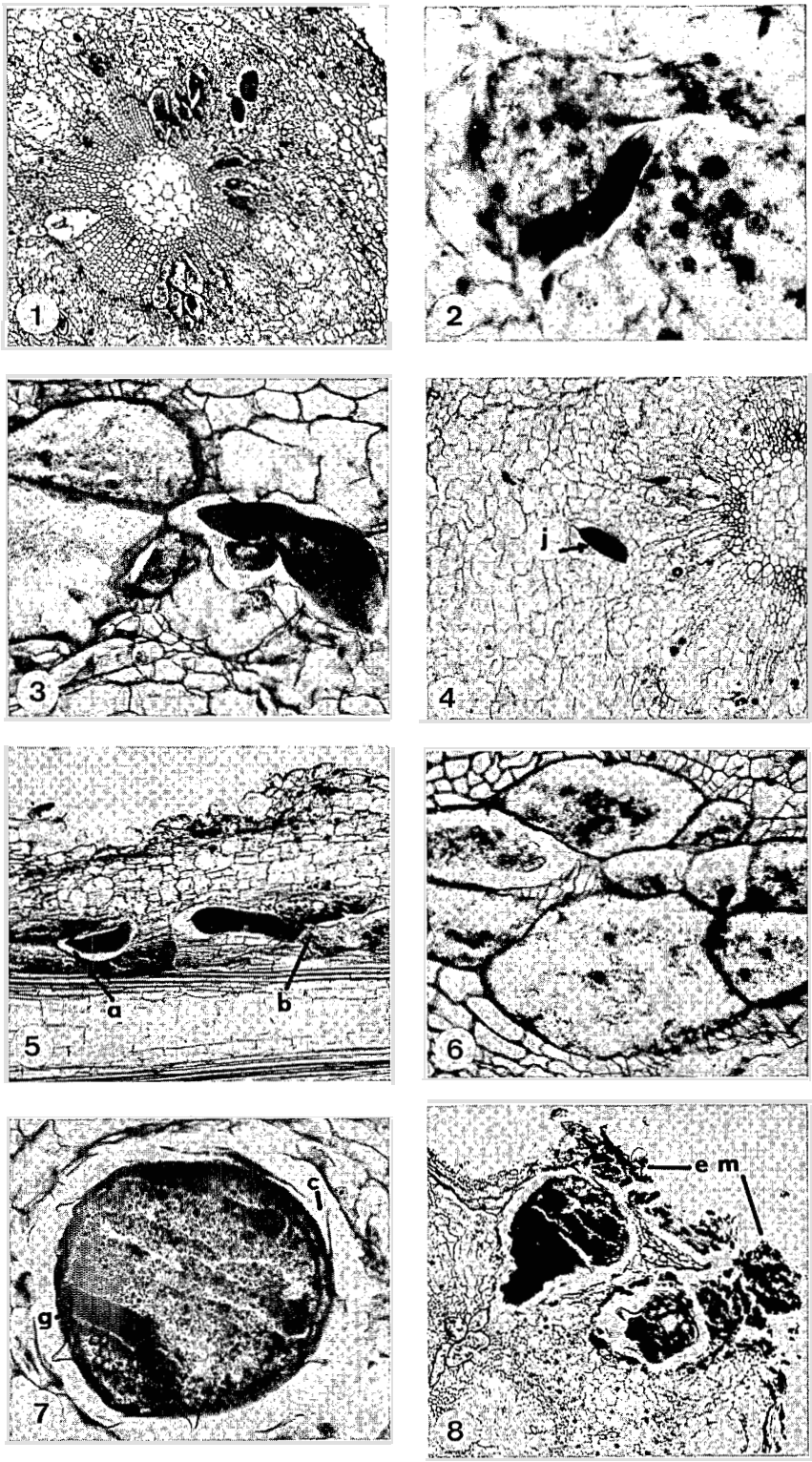
After 40 days numerous females were observed and most had deposited large numbers of eggs (Fig. 8). Young, vermiform juveniles were observed in the cortex, presumably as a result of reinfection.

Mode of infection of juveniles. Examination of germinating seedlings prior to emergence consistently showed the presence of second-stage juveniles within hypocotyl tissue.

Species identification. Mature females were dissected from five infected stems of *H. sabdariffa.* A total of 50 perineal patterns were made (Taylor & Netscher, 1974), and all conformed to the description of *M. incognita.*

Inoculation with clones of M. incognita *and* M. javanica. To determine if *H. sabdariffa* stem tissue could be utilized to select *M. incognita* from a mixed population, a supplemental experiment was made in which the experiment previously described was repeated except that five pots were infested with egg masses from a clone of *M. incognita* derived from a single egg mass and an additional five pots were infested with egg masses from a clone of *M. javanica* derived from a single egg mass. Stem galls, as described above, were produced in this experiment on all plants of *H. sabdariffa* regardless of the species of *Meloidogyne* used. No differences were noted between galls induced by the two species.

Discussion. On the basis of the observations of second-stage juveniles of *Meloidogyne* within hypocotyl tissue of *H. sabdariffa* prior to seedling emergence, it is concluded that penetration occurs while the hypocotyl is still below the soil level. This supports the statement of Linford (1941) that juveniles enter during germination and that of Fassuliotis & Deakin (1973) who maintain that juveniles invade bean hypocotyls during emergence. These juveniles are then



Figs. 1-8, sections through *Meloidogyne*-induced stem galls on *Hibiscus sabdariffa*: 1 — General view 25 days after inoculation; 2 — Multinucleate giant cells surrounding anterior end of juvenile 10 days after inoculation; 3 — Giant cells with thickened walls after 15 days; 4 — Late 2nd-stage juvenile (j) with spikate tail after 15 days; 5 — Longitudinal section with two juveniles, (a) with anterior end directed toward root and (b) toward shoot apex; 6 — Giant cells after 25 days; 7 — Cross-section through female showing shed cuticle (c) and gonad (g); 8 — Egg masses (em) produced by two females 40 days after inoculation.

carried passively as the aerial parts of the developing seedling emerge and expand. There is no evidence that the infection occurred after seedling emergence except for reinfection by second generation juveniles. Once within the stem juveniles were presumably protected from desiccation and radiant energy by the surrounding plant tissues and were able to establish a typical host-parasite relationship. Giant cell development appeared typical of *Meloidogyne* infections of roots. This same conclusion was reached by Wong & Willetts (1969) in infected stem tissues artificially inoculated with *M. javanica*. Although Linford (1941) and Wong & Willetts (1969) reported that juveniles in stem tissues always had the anterior end directed toward the stem apex, observations in this study established that this is not always the case. However, the majority of juveniles observed in longitudinal sections did exhibit such polarity.

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