PATHOLOGICAL RESPONSES OF STEMS TO ROOT KNOT NEMATODE INFECTION[1]

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(Accepted June 22, 1970)

Abstract

Root knot nematodes artificially inoculated on tomato and Coleus stems induced galls on the latters. Development of the stem galls involves giant cell formation, hyperplasia, abnormal trachael differention and disappearance of starch reserves from the infected tissues. Only those nematodes having their heads associated with vascular parenchyma developed to maturity. The pathogens oriented either acropetally or horizontally with respect to the stem axis. Tumour cells in the immediate vicinity of egg matrix collapsed and the egg mass is deposited in the pocket thus formed. A few layers of wound cambia developed around the pocket at a later stage. Large number of the eggs hatched in a tumour and mass migration of the larvae rendered considerable cell destructions. Relatively small number of the larvae successfully established secondary infections in a gall. They lead to the formation of multiple galls. Compared to root galls, the stem tumours are considered better materials for biochemical studies of tumorgenesis because they are larger and carry much less surface contaminants.

I. Introduction

Species of the genus *Meloidogyne* are well known root endoparasites of plants. They typically induce galls on the infected roots, hence acquired the common name "root knot nematodes".

Linford (1941) was able to induce galls on the stems, buds and leaves of various plants, when the organs were artificially inoculated with root knot nematodes. The same author also reported that metaxylem was the primary feeding site and giant cells formed as the result of feeding in stems. No other tissue responses was reported for the aerial infections.

The identity of the pathogen Linford worked with is not known, inasmuch

1. Paper No. 100 of the Seicntific Journal Series, Institute of Botany, Academia Sinica. Supported in part by National Science Council, Republic of China.

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as the work was done before Chitwood made the root knot nematode speciation possible in 1949 (Southey 1965).

Physiological studies of nematode-induced gall formation have been made so far only with root materials (Balasubramanian and Rangaswami 1962, Bird and Millerd 1962, Owens and Novotny 1960, Owens and Battino 1966, Owens and Rubinstein 1966, Owens and Specht 1966, Yu and Viglierchio 1964 and Viglierchio and Yu 1968). With the root galls, however, we have experienced difficulties in the biochemical studies. 1) It is very difficult, if not impossible, to free the galls from surface contaminants, including soil particulates and presumably microorganisms. 2) Root galls, particularly those over one month old, are susceptible to soft rot pathogens. 3) It is laborious to secure pure root gall tissues in a large quantity because of their relatively small sizes.

Stem galls offer advantages in all these respects. In order to further evaluate the feasibility of the aerial galls as experimental materials, we have studied in detail the pathological changes involved in the stem gall formation.

II. Materials and methods

A) *Method of inoculation*

Second stage larvae of *Meloidogyne incognita*, hatched in 1 ppm streptomycin solution, were inoculated on the stems of *Lycopersicon esculentum* and *Coleus blumei* in the following manner: Black sand, screened through a 100-mesh standard sieve and sterilized in an oven at 160 C for one hr., was wrapped around a stem with an aluminum foil in such a way to form a cylindrical package with the stem in the central axis. A package contains approximately 5 ml of dry sand and covers about 5 cm of stem. The foil helps maintain moisture which is required for nematode locamotion and the sand provides bases for nematode penetration into the stems. Lower end of the cylinder was sealed to prevent leakage and 1.5 ml of nematode suspension cotaining about 1000 larvae was then pippetted from the upper end onto the sand. Additional water was added just enough to saturate the sand. The inoculated plants were then kept under white fluorescent light (about 400-ft-candle) in a room where temperature fluctuated between 20–25 C.

After 24 hr incubation, the sand together with unpenetrated larvae were removed and the stem thoroughly rinsed with water. The process ensures that all the infections take place within 24 hr. The plants were then kept in green house. Galls became visible five days after inoculation.

Preliminary tests indicated that mechanical wounds were not necessary for successful inoculation in the region between shoot apex and downward 5th internodes. Much less nematodes penetrated successfully in the basal

portion of a stem if fresh mechanical wound was not provided before inoculation. Only upper portions of a stem, therefore, was chosen for gall induction without mechanical wounding. Both the tomato and Coleus inoculated in this manner resulted in gall formation (Figs. 1 & 2).

B) *Histopathology and histochemistry*

Galls for histological studies were removed and fixed immediately with FAA (Formalin 10 ml+Glacial acetic acid 5 ml+95% Ethyl Alcohol 35 ml+ H_2O 35 ml) for 24 hr. Initial period of the fixation was carried out under reduced pressure to help penetration of the fixative. The fixed materials were then dehydrated and embedded with paraffin according to Jensen's TBA series (Jensen 1962). Serial sections of 8μ thick were made and stained with safranin-fast green (Jensen 1962) for observation.

Fresh sections were employed for histochemical studies. IKI (Jensen 1962) staining coupled with salivary—amylase treatment were employed to identify starch.

III. Results

1) *Nematode feeding sites.*

In order to determine the sites on which the nematodes feed, serial sections of galls induced 10 days after nematode penetration were made and the location of nematode heads as well as host tissue responses determined. Since successful feeding results in the formation of giant cells and the growth of parasite, these two phenomena were used as criteria for feeding activity. Ten days old galls were chosen because at this stage giant cells as well as the enlargement of nematode become evident. The enlargement signifies the growth of nematode from its second stage. As will be discussed in a later section, abnormal vascular elements develope in the older galls as the result of infection.

The abnormal vascular elements are of secondary origin. However, in the initial stages of development, they may be easily confused with the normal vascular tissues. For this reason, galls of more than three weeks old are unsuitable for the evaluation of feeding sites.

As shown in Table 1, successful feeding took place only in vascular parenchyma (Figs. 3 & 4). Nematodes found in the tissues other than vascular were not associated with giant cells and in all cases showed no morphological indication of development (Fig. 5). These parasites, therefore, were considered as transitory and no successful feeding took place up to the time of sampling.

Most of the nematodes oriented acropetally with heads toward the apex or horizontally with the heads toward the pith. No polar orientation was detec-

Table 1. Locations and developmental conditions of *Meloidogyne incognita* in stem galls ten days after inoculation.

No. of nema found in / Conditions of parasite and host	L. esculemtum[1]					C. blumei[2]				
	Cortex	Pith	Phloem	Xylem	Cambium	Cortex	Pith	Phloem	Xylem	Cambium
Enlarged nema, heads associated with host giant cells.	0	0	17	13	2	0	0	8	11	0
Nema not enlarged, heads not associated with host giant cells	2	5	0	0	0	6	1	0	0	0

1. Counts from four galls.
2. Counts from three galls.

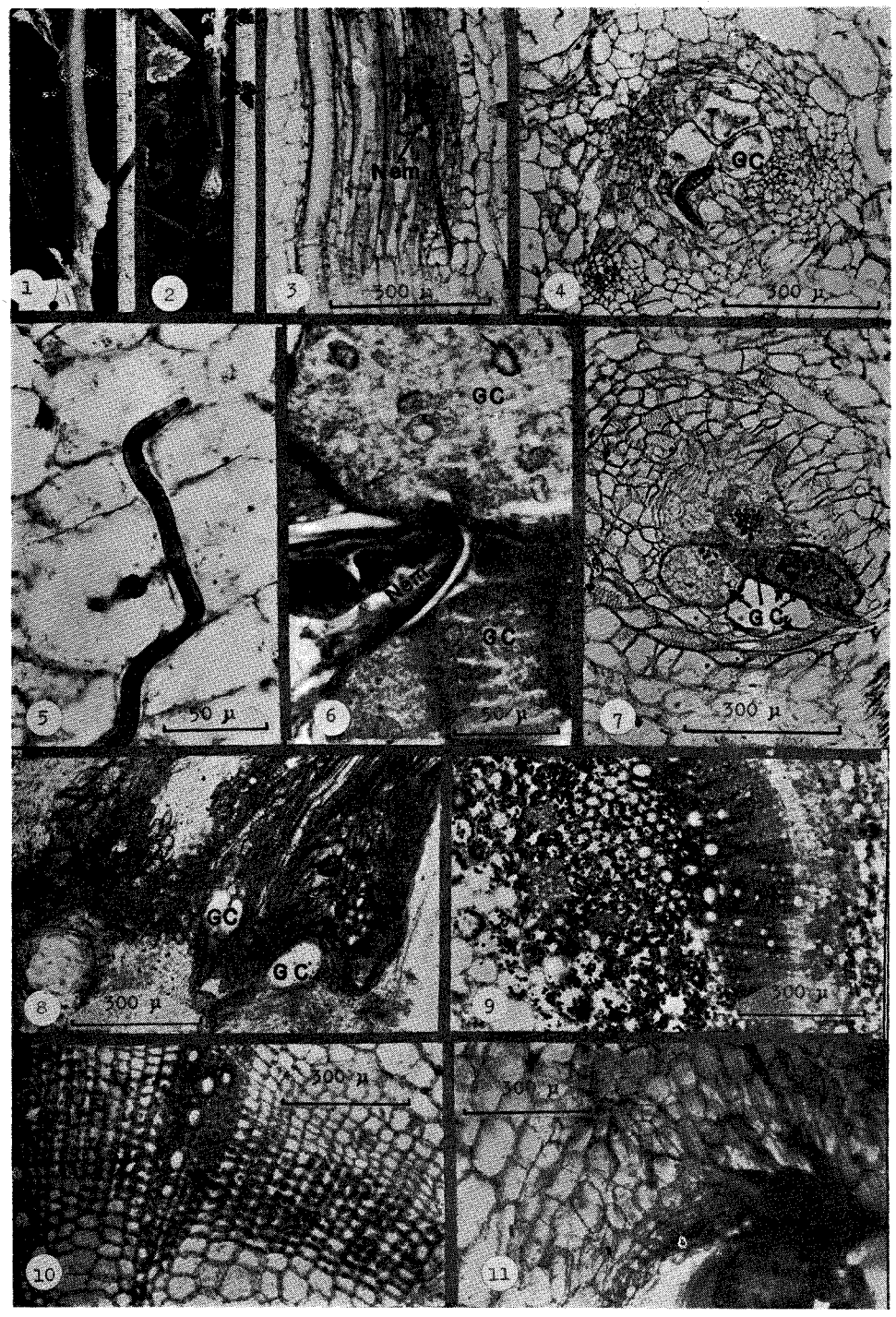
ted for those larvae hatched in a gall and establishing their secondary infections.

2) *Giant cells and hyperplasia*

Multinucleated giant cells were formed around the heads of nematodes, presumably in response to feeding (Fig. 6). However, no actual penetration of the feeding aparatus, the stylet, has been observed in the giant cells. All the giant cells were associated with vascular tissues (Figs. 3 & 4).

Subsequent to giant cell formation, tissues around it became hyperplastic (Fig. 4). Successful infections invariably developed this condition, even in the elder stem where mitosis rarely occured, if at all, without nematode infections.

Figs. 1 & 2. Respectively galls induced on the stems of tomato and *Coleus blumei* two months after inoculated with *Meloidogyne incognita*. 3. Longitudinal section of a tomato stem one week after inoculated with *M. incognita*. Note that the nematode head (Nem) is associated with vascular parenchyma. 4. Cross section of a tomato stem inoculated with *M. incognita*. Giant cells (GC) are developed from vascular parenchyma. Giant cell formation, hyperplasia and swelling of the nematode indicate that feeding did take place. 5. A second stage larvae of *M. incognita* migrates intra-and intercellularly in tomato stem cortex. Note that there is no giant cells or hyperplasia and the nematode remains slender. The nematode therefore is transitory and has not established its feeding site. 6. Section of a *C. blumei* stem gall. Note that the head region of a young ♀ *M. incognita* (Nem) is closely associated with giant cells (GC). 7. Section of a *C. blumei* stem gall one month after infection. Irregular xylem vessels develope around giant cells (GC). 8. Fresh section of a three months old tomato stem gall infected with *M. incognita*. Prominent abnormal xylem vessels developed around the point of infection (GC). 9. Fresh section of a normal tomato stem, stained with IKI. Note the accumulation of starch grains. 10. Fresh section of a normal *C. blumei* stem stained with IKI. Note the starch grain accumulation, particularly in the intervasicular cambium layers. 11. Fresh section of a *C. blumei* stem gall, stained with IKI. No starch grain is detectable in the cells around the nematode (Nem). The gall was induced from a stem of similar age to that of Fig. 10.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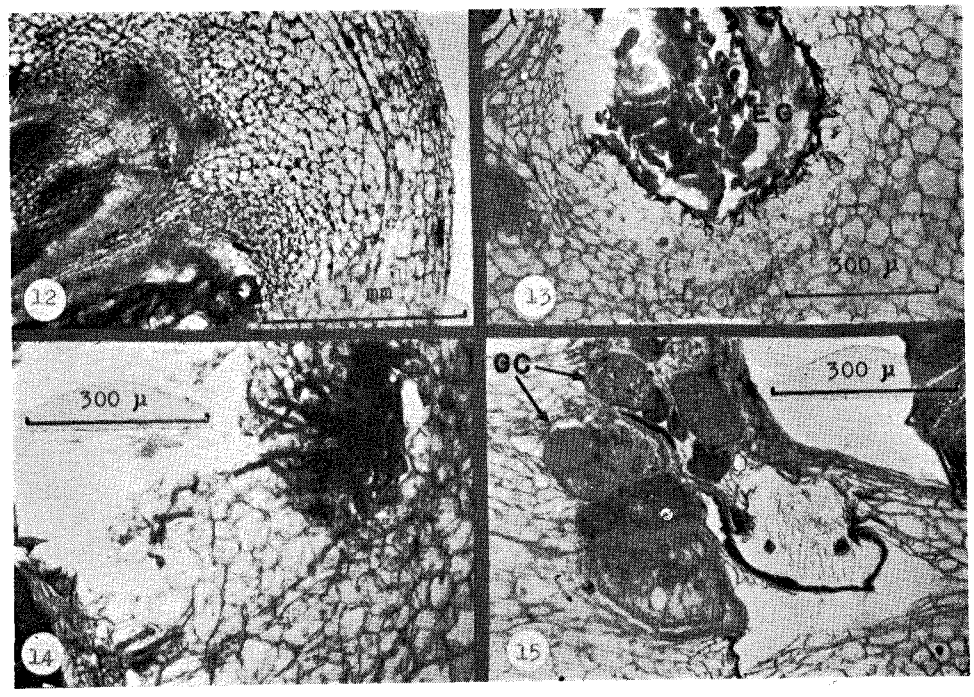

Figs. 12. Fresh section of a tomato stem gall, stained with IKI. No starch grain is detectable in the entire gall tissue. The gall was induced from a stem of similar age to that of Fig. 9. 13. Paraffin section of a *Coleus blumei* stem gall. The nematode egg mass (EG) is embedded in the gall tissue. Note that the host cells in contact with egg matrix lysed and resulted in the formation of a pocket. A few layers of wound cambium-like tissues formed around the pocket. 14. Mass of second stage larvae hatched in a *C. blumei* stem gall. Extensive tissue damage is incurred by the nematodes. Portion of the parent nema is visible at lower left hand corner. 15. A second stage larvae, hatched in a *C. blumei* stem gall, associated itself with the giant cells (GC) induced by its parent.

3) *Abnormal tracheal differention*

About three weeks after infection, abnormal tracheal elements developed around giant cells. The process began with the irregular wall thickenings of the hyperplastic cells (Fig. 7). Mass of the abnormal, disorganized tracheal elements subsequently developed around the giant cells and head region of the parasite (Fig. 8). Whether the abnormal xylem elements serve any function to the parasite is not determined.

4) *Effects of infections on host starch reserves.*

Normal stems of tomato (three-month-old) and Coleus (various ages) were respectively examined for their starch reserves. Their IKI-treated fresh sections revealed that, except the apical portions, all the parenchyma cells in the stems are richly deposited with iodin-positive grains (Figs. 9 & 10). No characteristic deep blue iodine reactions occured if the sections were treated with salivary amylase. The reserves therefore are identified as starch grains.

No starch grain was observed in giant cells and the surrounding hyperplastic tissues (Figs. 11 & 12), regardless of the age of the stems on which the galls were induced. Starch deposition in the tissues adjacent to a gall proper, however, were indistinguishable from that in an uninoculated stem. The disappearance of starch grains in an infected area became detectable about five days after inoculation, when giant cell formations were evident.

Without establishment of its feeding site, a nematode obviously did not render any appreciable effect on starch grains (Fig. 5). Thus dipletion of starch grain was associated with giant cell formations and hyperplasia, and therefore feeding activities of the nematode.

5) *Nematode reproduction and secondary infections in galls.*

Root knot nematodes generally lay their eggs in close proximity to epidermis of root galls. Most of the egg masses, therefore, protrude through the epidermis and expose themselves into rhizosphere (Christie 1936). This was not the case, however, in stem galls. Pronounced hyperplasia perpetuate to the extend where mature females were deeply embedded in gall tissues. The egg masses therefore were also enclosed in the tumours.

Tumour cells in the immediate vicinity of egg matrix collapsed resulting in a pocket which accomodated the egg mass in the gall (Fig. 13). A few layers of cells resembling wound cambium were formed around the pocket in a later stage (Fig. 13).

The larvae hatched in a gall and sometimes migrated out of the pocket in a large mass. Considerable damages in the gall tissues resulted from such mass migrations (Fig. 14). Only a few of the larvae hatched cause secondary infections in a gall. Apparently the wound cambium cells around the egg

mass as well as the abnormal vascular tissues are not suitable sites for feeding. Those few which rendered secondary infections were found either with preexisting giant cells (Fig. 15) or with vascular tissues further away from the parent-nematode. In the latter case, they induced new giant cells much like their parent did.

IV. Discussion

Responses of aerial stem tissues to root knot nematode infections are essentially similar to those of root and rhizome tissues. Giant cell formations, cellular hypertrophy and abnormal vascular differentiations found in the stem gall in this study have also been reported for root galls (Christie 1936, Dropkin and Nelson 1960, Eversmeyer and Dickerson 1966, Krusberg and Nielsen 1958, Owens and Specht 1964), and ginger rhizomes (Huang 1966) infected with root knot nematodes.

Linford (1941) reported that the root knot nematodes exhibited strict acropetal orientation in stem tissues. Our result, however, could not confirm such an observation. The primary inoculum orientated from acropetal to horizontal and no polar characteristic at all for the secondary inocula.

To the best of our knowledge, this is the first report of root knot nematode effect on starch grains. Whether the rapid decrease in starch content in the cells around the nematode feeding sites is due to hydrolyses of existing starch grains, inhibition of starch biosynthesis, or both, is not known. Whatever is the case, the striking phenomenon certainly deserves further investigations.

For biochemical analysis, we have found it difficult to obtain sufficient root galls completely free from soil contaminants, including soil particles and soil microbes. In addition, larger root galls are often susceptible to soft rot bacteria (unpublished observation). Artificially induced aerial stem galls are free from these interferences and therefore should be a much better material for biochemical studies.

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