HISTOPATHOLOGICAL STUDIES ON ROOT-KNOT NEMATODE GALLS ON CERTAIN VEGETABLE CROP PLANTS

By

M. P. GOVINDANKUTTY

THESIS

submitted in partial fulfilment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

BOTANY

BIRLA INSTITUTE OF TECHNOLOGY & SCIENCE

PILANI (INDIA)

1974

DEPARTMENT OF BIOLOGICAL SCIENCES

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE

SUPERVISOR'S NOTE

The thesis entitled "Histopathological Studies on Root-Knot Nematode Galls on certain Vegetable Crop Plants" submitted by Shri M.P. Govindankutty, M.Sc., for the Degree of Doctor of Philosophy embodies the results of investigations done under my supervision, and I certify that the work is original.

May, 1974
Pilani.

(S.K. PILLAI)
Professor of Botany

ACKNOWLEDGEMENTS

I am indebted to my thesis supervisor, Professor S.K. Pillai, who offered guidance and encouragement throughout this investigation.

I wish to thank Dr. Alan F. Bird, Principal Nematologist, C.S.I.R.O., Australia, for his stimulating counsel on several aspects of plant-nematode interrelationships. I am grateful to Dr. Gopal Swarup and Dr. C.L. Sethi, Nematologists, I.A.R.I., New Delhi, for the confirmatory identification of the nematode species.

I owe a good deal to Mrs. Virginia Harrington of the U.S.D.A., Beltsville, U.S.A., who provided me with much literature, which otherwise would have been difficult to obtain.

Thanks are due to Prof. John B. Jepson, Courtland Institute of Biochemistry, London, and Messrs. I.C.I., London, and E. Merck, Darmstadt for the samples of indole derivatives.

I am thankful to Prof. A.K. Dutta Gupta, Dean, Faculty of Science and Dr. H.L. Kundu, Head, Department of Biological Sciences for facilities.

My thanks are also due to Dr. S.N. Mathur and Dr. C.G.P. Rao, Department of Biological Sciences; Dr. B.K. Razdan and Dr. S.K. Banerji, Department of Pharmacy; Dr. A.D. Taneja and

Dr. Rup Dutta, Department of Chemistry; Mr. T.N.R.K. Kurup, Department of Physics; Mr. Jacob George, Department of Languages (all of B.I.T.S., Pilani); and Dr. (Mrs.) A. Pillai, Department of Botany, University of Rajasthan, for their help, and the interest they showed in the present work.

My stay here and completion of this work was chiefly due to the invaluable help I received from many of my friends. Among them I must mention, Drs. K.T. Sebastian, P.S. Raghavan, Joseph Mathew, N.V. Gopinath, K. Balachandran, Arun Kumar, Nemi Chand, A.S. Reddy; Messrs. R. Divakaran, Viney Seth, T.K. Kaul, R.K. Dhand, A.O.N. Panikkar, Baby Chacko, M.C. Arunan, S.C. Taneja and John V. George - to whom I extend my sincere thanks.

My thanks are due to Mr. P.L. Mehta for the personal interest and care with which he typed this thesis.

The financial help from U.G.C., New Delhi, and B.I.T.S., Pilani, in the form of doctoral fellowships is gratefully acknowledged.

Finally, I want to express my deep gratitude to my parents, brothers and sisters for all sorts of help and timely encouragement.

(M.P. Govindankutty)

TABLE OF CONTENTS

SUPERVISOR'S NOTE ... ... (i)

ACKNOWLEDGEMENTS ... ... (ii)

TABLE OF CONTENTS ... ... (iv)

LIST OF TABLES ... ... (viii)

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE 1

CHAPTER 2

MATERIALS AND METHODS 19

2.1 HOST PLANTS 19

2.2 PREPARATION OF INOCULUM 20

2.3 INOCULATION OF PLANTS 22

2.4 BIOLOGY AND DEVELOPMENT OF THE PARASITE ... 22

2.5 HISTOLOGICAL PREPARATIONS 23

2.6 HISTOCHEMICAL METHODS 25

2.7 CHEMICAL ANALYSES 25

2.7.1 Determination of Nucleic Acids and Proteins 25

2.7.2 Extraction of Amino Acids, Sugars and Indole Compounds 27

2.7.3 Thin Layer Chromatography 29

2.7.4 Column Chromatography of Indolic Substances 32

2.7.5 Spectrophotometric Determination of Indolic Substances 33

2.7.6 Bioassay of Indolic Substances ... 33

CHAPTER 3

OBSERVATIONS 35

3.1 BEHAVIOUR OF THE PARASITE AND INFECTION ... 35

3.1.1 The Infective Stage of the Parasite 36

3.1.2 Larval Orientation and Movements ... 36

3.1.3 Sites of Penetration 37

3.1.4 Stylet Action and Penetration Process 38

3.1.5 Movements Inside Host Roots 40

3.2 NEMATODE INFECTIVITY IN INTACT AND DEFOLIATED PLANTS AND EXCISED ROOTS ... 41

3.2.1 Infectivity in Intact Plants ... 41

3.2.2 Effect of Defoliation and Root Excision 42

3.3 GALL FORMATION AND GENERAL HISTOLOGICAL ALTERATIONS 48

3.3.1 General Morphology of Galls 48

3.3.2 Host Tissue Deformations Along Nematode Path 49

3.3.3 Sites of Nematode Lodging and Feeding 51

3.3.4 Damage to Cell Walls 53

3.3.5 Stimulation of Cell Division... ... 54

3.3.6 Giant Cell Formation 55

3.3.7 Tissue Changes Around Giant Cells ... 61

3.3.8 Variation in Giant Cell Complexes and Hyperplastic Cells Among Different Hosts 63

3.4 ROOT-KNOT HISTOGENESIS IN *SOLANUM MELONGENA* 68

3.4.1 5-Days After Infection 69

3.4.2 10-Days After Infection 70

3.4.3 15-Days After Infection 72

3.4.4 20-Days After Infection 73

3.4.5 30-40-Days After Infection 75

3.5 RESPONSES OF GIANT CELLS TO CERTAIN HISTOCHEMICAL STAINS 78

3.6 CHANGES IN CHEMICAL CONSTITUENTS DUE TO INFECTION 80

3.6.1 Nucleic Acids 80

3.6.2 Proteins 82

3.6.3 Amino Acids 82

3.6.4 Sugars 85

3.6.5 Indole Compounds 87

3.7 DEVELOPMENT OF *MELOIDOGYNE INCOGNITA ACRITA* IN *SOLANUM MELONGENA* 94

CHAPTER 4

DISCUSSION 98

4.1 NEMATODE BEHAVIOUR, INFECTION AND INITIAL HOST RESPONSES 99

4.2 GALL FORMATION AND HISTOPATHOLOGY 108

4.3 CHANGES IN CHEMICAL CONSTITUENTS 127

4.4 DEVELOPMENT OF THE PARASITE 142

SUMMARY 144

APPENDIX 1 - A NEW CHROMOGENIC REAGENT AND MODIFICATIONS IN THE SPECTROPHOTOMETRIC DETERMINATION OF INDOLE ACETIC ACID ... 149

ABBREVIATIONS USED IN FIGURES 154

FIGURES AND LEGENDS 155

LITERATURE CITED 181

LIST OF TABLES

Table		Page
1	Concentrations of dehydration and infiltration mixtures	24
2	Infectivity of M. incognita acrita in S. melongena	45
3	Infectivity of M. incognita acrita in A. esculentus	46
4	Comparative infectivity of M. incognita acrita in intact and defoliated plants and excised roots	47
5	Variations in giant cell complexes and hyperplastic cells among different host plants	67
6	Concentrations of nucleic acids in normal and M. incognita acrita infected roots	81
7	Protein concentrations in normal and M. incognita acrita infected roots ...	83
8	Free amino acids and amides in normal and M. incognita acrita infected roots..	84
9	Soluble sugars in normal and M. incognita acrita infected roots	86
10	Chromatographic and chromogenic reactions of indolic compounds from normal and M. incognita acrita infected root tissues	89

List of Tables (Contd.)

11	Concentrations of indolic compounds in normal and M. incognita acrita infected roots	92
12	Estimation of indole acetic acid with Gordon and Weber and ferric citrate reagents	151

CHAPTER 1

INTRODUCTION AND REVIEW OF LITERATURE

1 INTRODUCTION AND REVIEW OF LITERATURE

A remarkable feature, characteristic of all higher animals and plants, is the precise manner in which all their functional parts fall into a coherent pattern, which is species specific. Also, a well regulated and specifically oriented sequence of metabolic functions synchronised to bring about morphogenetic differentiation at all levels of growth is all pervading. These regulated processes are so integrated to give a determinative proportion to any organism.

Steward (1968) evaluated growth in terms of multiplication of self-duplicating cell components like nuclei, nucleoli, plastids, mitochondria, golgi bodies or membranes. All these phases of growth are accompanied by the synthesis of cell proteins. In addition to this type of growth, even in the absence of any morphological differentiation, cell enlargement and resultant increase in chemical constituents form the second phase of growth. Both these phases are essential, and obviously it is their occurrence in sequence which directs a set growth pattern. The second phase of growth succeeds the first and gradually merges into the other, but each phase has quite distinct controlling

mechanisms.

Any interference with the dynamic state of equilibrium of normal metabolism of a plant, preventing full utilisation of the available resources of the environment, may be often ascribed to a disease. The agency causing such a physiological deviation is a pathogen, even if it is a nonliving entity. The influence of the pathogen and its progressive association with the host plant often alters the usual mode of growth, development and differentiation and results in the concomittant production of anomalous structures. Galls or tumors caused by a variety of disease producing organisms are typical examples of localised overgrowths where the normal growth pattern is altered and cells of the host induced to excessive multiplication.

Plant galls are essentially neoplastic outgrowths, or growth abnormalities exhibiting a pronounced departure from the general morphogenetic restraints of the particular organ of the plant. They arise mostly by hypertrophy (cell overgrowth) and hyperplasia (cell proliferation) under the influence of parasitic organisms like viruses, bacteria, fungi, nematodes, mites and insects. Some tumors or galls represent the growth reaction of plants to the attack of the parasite and are in some way related to the biology of the parasite (cf. Mani, 1964).

It has also been observed that where a biological association exists between a host and a parasite as a

regular phenomenon, galls are induced on the host by the parasite. Galls have a striking characteristic in that they are new abnormal growths, discrete and, at least to some extent, independent of their adjoining structures. Mani (1964) described such a biological association between the gall-inducing parasite and the plant, as one in which the former apparently derives all the benefits and the plant suffers from loss of substance, deviations from normal course of growth, disturbances in sap flow, premature decay, increase of non-essential parts at the cost of essential ones and other injuries. Cook (1923) believed that the formation of a gall is probably the resultant of efforts on the part of the host plant to protect itself from lethal injuries. However, gall development involves factors that release a cell or a group of cells from the normal decisive and coordinating morphogenetic influences of the host.

Gall induction involves a complex series of interactions of the host plant cells and the inducer. The tissues of the gall essentially arise as a result of morphogenetic processes not much different from the normal ones. But, herein the processes are in an assorted order and do not obey any synchronised pattern of the normal organism. Although individual histogenetic processes in morphogenesis or cecidogenesis are identical with those in normal organogenesis, some of these processes arise either earlier or later, so that there is a localised

imbalance. Mani (1964) considered a wide array of situations like hypoplasy, redifferentiation and retrograde differentiation, form anomaly, hyperplasy, infiltration, anomalies of cell division, tissue stretching and tissue rupture, cell or tissue fusion, tissue degeneration, necrosis, cytolysis and dedifferentiation as significant processes in gall formation.

Amidst the various organisms harbouring the soil in the rhizosphere of plants, nematodes form a chief taxon, and they influence greatly plant growth and crop production. The fact that there is hardly any cultivated plant spared from the attack of these phytophagous parasites is often unnoticed or overlooked. Maladies caused by most nematodes are insidious and chronic in nature. They invade host organs for deriving nutrients and in the process of penetrating, feeding and multiplying, damage host tissues to varying degrees by moving about in the host interior. They pierce the cells and remove their contents, thereby inducing cell lysis and necrosis by mechanical and chemical injuries. Many nematodes induce malformations or galls which are the products of a modified metabolism of host structures favouring parasite growth and development. In addition, nematode penetration of host organs may pave the way for secondary infections of other pathogens and establishment of disease complexes.

Even though there are hundreds of phytoparasitic nematodes inhabiting the soil, only nematodes belonging to

the genera Anguina Scopoli, 1777; Heterodera Schmidt, 1871; Meloidogyne Goeldi, 1887; Tylenchulus Cobb, 1913; Xiphinema Cobb, 1913; Ditylenchus Filipjev, 1936; Nacobbus Thorne and Allen, 1944 and Radopholus Thorne, 1949 are known to be gall inducers (Krusberg, 1963; Dropkin, 1969). They are endo and ectoparasites belonging to 3 taxonomic groups, viz., Tylenchidae, Dorylaimidae and Aphelenchoidae. Even though all are essentially capable of inducing galls of some kind, root-knots caused by the obligate endoparasite Meloidogyne is of the most complex type. The present review chiefly includes studies made on Meloidogyne and Heterodera infections.

Root-knot disease is a very serious infection in many cultivated crops and ornamentals, whose pathogenecity is evidenced by the root galls incited by the females in host organs. Several studies have brought out that nematode infections and feeding by the pathogen cause general crop decline due to depletion of available nutrients, impairing of water and mineral uptake and conduction. Distortion of root system, cessation of root growth in length, excessive root branching, necrosis and even mineral deficiency symptoms are concomittant with nematode infestations (cf. Webster, 1972).

The host-parasite relationships and associated aspects of nematode infections have been reviewed by Chitwood and Oteifa (1952), Fielding (1959), Seinhorst (1961), Krusberg (1963), Dropkin (1969) and Webster (1969). Two recent

treatises on plant parasitic nematodes have also given considerable information regarding plant-nematode interactions (Zuckerman et al., 1971; Webster, 1972).

The root-knot nematodes were among the first plant nematodes to be recognised. They were first described by Berkeley in 1855 followed by Goeldi in 1887 (cf. Thorne, 1961). According to Thorne's (1961) descriptions, earlier they were known as Heterodera radicicola and H. marioni. Then Chitwood (1949) revised their taxonomy. Earlier workers designated these as Anguillula marioni Cornu, 1879; A. arenaria Neal, 1889; A. vialae Lavergne, 1901; H. javanica Treub, 1885; Tylenchus arenarius Cobb, 1890; Meloidogyne exigus Goeldi, 1892; Oxyurus incognita Kofoid and White, 1919; Caconema radicicola Cobb, 1924, and Berkeley (1855) called them "Vibrios" (cf. Thorne, 1961). Chitwood's (1949) detailed investigations on their morphology established the genus Meloidogyne with 5 species and 9 sub-species. Since then the number of known species has risen upto 35 (Franklin, 1971). These parasites are known to have a wide host range of over 2000 plants belonging to different and unrelated plant families.

Most root-knot nematodes exhibit 6 stages in their life cycle. A gravid female lays eggs that embryonate and moult into larvae. Egg-hatching releases the infective second stage larva which on successful parasitisation of a host, moults three times more to become an adult . Plant

nematodes possess a stylet for penetrating the host cells, a set of oesophageal glands, and a pump in between the stylet and the intestine. Many of them induce host cells to provide specific feeding sites or syncytia. In addition to changes in these cells, there frequently arise varied effects mediated by chemicals secreted or activated by nematodes at other centres (Dropkin, 1969).

Linford (1937, 1939) first provided evidence for the fact that larvae of the root-knot nematodes are attracted to host roots. Wallace (1958, 1960) found 3 species of Heterodera - H. major, H. schachtii and H. rostochiensis - attracted by seedlings of hosts. In contrast, a theory of random movements and location of hosts was suggested by Bergman and Van Duuren (1959). Kühn (1959) and Weischer (1959) noticed little attraction of H. schachtii and H. rostochiensis to isolated host root diffusates. The root attractiveness was considered to be due to a negative redox potential of root exudates (Bird, 1959b) and CO_2 (Klingler, 1959).

According to Godfrey and Oliveira (1932) and Peacock (1959), Meloidogyne larvae get localised at a site behind the root apical meristem of pineapple and tomato respectively. Linford (1939) and Huang (1966) attached importance to the zone of root elongation. Wieser (1955) described the apical 2 mm distance of excised tomato roots as repellant and the succeeding 6 mm behind the root tip as

attractive sites. A proximal region upto 16 mm was considered neutral or repellant. It was also reported that roots of eggplant (Solanum melongena) and Soybean (Glycine max) are neutral to M. hapla (Wieser, 1956). Smith and Mai (1965) found the larvae always within 1200 μ of the root tip in onion but they never were in or below the region of apical initials.

Meloidogyne larvae took 6 hr to penetrate plants like pineapple and cowpea (Godfrey and Oliveira, 1932) whereas an average of 3-4 hr was recorded by Loewenberg et al. (1960). The movement into host roots is reported to be aided by effecting cell separation along middle lamellae using nematode spears (Linford, 1942). One to three days were found necessary for penetration of Radopholus similis into citrus roots (Du Charme, 1959). Thus, our present knowledge regarding nematode larval infective processes shows several disimilar situations. Linford (1937, 1939) studied the feeding process of Heterodera marioni and distinguished two phases: (1) puncturing of host cell wall with the buccal spear and extrusion of digestive secretions, and (2) withdrawal of cell contents. Bird (1959a) believed that the root-knot nematodes do not feed during ecdysis and development, whereas Mankau and Linford (1960) have observed feeding by third and fourth stage H. trifolii larvae.

Histopathological studies on host parasite interactions in heteroderid nematode infections have revealed the

production of giant cells or syncytia at the feeding sites. The presence of a group of large multinucleate nurse cells around the head of the sedentary feeding female nematode was first mentioned by Treub (1887) but was overlooked by subsequent workers. From India, Saran (1934) provided a brief description of the deformities in Hibiscus esculentus caused by Heterodera radicicola. Since Christie (1936) compiled a review on the development of root-knot nematode (Heterodera marioni) galls, several workers have interpreted their origin and development differently. They attributed the development of these tumorous cells as a result of mitosis, amitosis, dissolution and coalescence of cell walls and resultant pooling of nuclei.

Dropkin and Nelsen (1960), Bird (1961), Crittenden (1962) and Birchfield (1964) held that the giant cells are formed by the cell wall dissolution and subsequent fusion of protoplasm. Bird (1961) used the terms 'syncytia' and 'coenocyte' and Crittenden (1962) called them 'lysigenoma', to denote cells fused together and formed into multinucleate structures.

Myuge (1956) suggested a different mechanism and reasoned that repeated 'endomitosis' devoid of subsequent cell wall formation, to be the cause for production of giant cells. Workers like Dropkin (1965), Littrell (1966), Owens and Novotny (1960), Smith and Mai (1965) and Huang and Maggenti (1969a) reported synchronous nuclear divisions without cytokinesis, and dissolution of cell walls.

Christie (1936) reported intercellular larval migration, and subsequent settling at plerome, hypertrophy of cells and nuclei in cells around larval head, stimulation of cell division and syncytia formation. He also noted the proliferous production of lateral roots from galls. In his review Christie referred to Muller (1884), who noted cell proliferation outside the vascular ring of Musa rosacea but failed to notice the giant cells. Frank (1885) noted abnormal divisions of cells and development of xylem near the point of invasion in lettuce, beet and clover root-knots, and the cells near the point of invasion dividing for sometime and differentiating into xylem. Treub (1887) did not notice any significant root injury and reported production of lateral roots near the galls but did not attach any significence.

Atkinson (1890) studied the effects of H. marioni in cotton, tomato, potato and parsnip and reported that eelworms do not confine themselves to any particular tissue system. In his observations all the tissue elements of the diseased root underwent hypertrophy and some cells were subjected to special changes in form and direction of growth. Queva (1894) found the parasites localised near the periphery of the primary vascular system. According to him in some galls, secondary vascular bundles and formation of giant cells from tissues in between the primary and secondary bundles are occurred. A general reduction in the amount of phloem tissues, presence of crushed xylem

elements and disintegration of giant cells were reported by Beille (1898) in Papaya gracilis. Cessation of meristematic activity and production of lateral roots were observed by Molliard (1900) who discussed the formation of giant cells in supplying food to the parasite. Nemec (1910) held that larvae in the periblem of roots do not bring about any changes, but induce multinucleate giant cells on reaching the plerome. He described that the giant cell nuclei undergo mitosis, coalesce and form clumps which will ultimately reduce the number of nuclei per cell. Nuclear divisions without accompanying cell division during the initial stages and later coalescence were described by Kostoff and Kendall (1930) who worked on Nicotiana hybrids. Both Nemec (1910) and Kostoff and Kendall (1930) felt that salivary secretions from the invader increase cell permeability and bring about exosmosis and accumulation of food in giant cells. They believed that this localisation increases growth rate and deprives other regions of nutrients, thus inhibiting growth.

Krusberg and Nielsen (1958) found in root-knots of M. incognita acrita in sweet potato, 4-9 giant cells containing 50 to several hundred nuclei. They wrote of cork cells that developed around giant cells, which were described as a wound healing response. Crittenden (1958) noticed numerous large giant cells with enlarged nuclei and dense cytoplasm in susceptible soybeans, whereas few small giant cells with a small number of nuclei and sparse

cytoplasm only arose in resistant soybean varieties. Dropkin and Nelson (1960) followed the giant cell development in 19 varieties of soybeans infected by 2 species of root-knot nematodes, and on the basis of cytological details, classified giant cells into four morphological types.

Davis and Jenkins (1960) inoculated gardenia (Gardenia jasminoides) with M. incognita and M. incognita acrita, but could not visualise any difference in histopathological effects. They found the young giant cells numbering 2-5 to 10-12, generally vacuolated though they had 30-40 nuclei within. These workers have enumerated fewer nuclei in older roots indicating the possibility of nuclear coalescing. Meloidogyne infection in roots and fleshy rhizomes of ginger produced internal lesions in addition to other abnormalities (Huang, 1966). Numerous interconnected tracheidal elements were reported to surround the female nematodes and giant cells in peony (Paeonia albiflora) roots. Eversmeyer and Dickerson (1966) found these elements reconnected with the main stele. Swamy and Krishnamurthy (1971) detailed the formation of atypical xylem, suppression of phloem differentiation and suppression of cork cambial activity in M. javanica-induced galls of Basella alba.

Monocotyledons were generally regarded as unsuitable to root-knot nematode development. But Zea mays could harbour M. incognita that produced the usual histopathological effects in maize (Baldwin and Barker, 1970).

Again Siddiqui Islam and Taylor (1970) and Siddiqui (1971) could successfully inoculate M. naasi into the roots of wheat and oats respectively, in which galls containing 2-8 giant cells arose. Contrastingly, a closely related heteroderid H. graminophila though, could complete its life cycle in Echinochloa colonum, Oryza sativa or Sorghum halapense, failed to produce giant cells or galls (Birchfield, 1973). The first report on the histopathology of root-knots on a pine was provided by Riffle (1973) whose descriptions corresponded with all the classical attributes to Meloidogyne infection.

Ultrastructure and histochemistry of giant cells were dealt with by Bird (1961) whose extended work revealed certain details regarding the inducement of giant cells (Bird, 1962). According to this worker, the giant cells which are formed by the fusion of several cells, are interconnected at their extremities, contain many mitochondria, plastids, golgi bodies and endoplasmic reticulum, and irregularly thickened walls projecting into the cytoplasm. Working with the same host galls, Paulson and Webster (1970) reported certain fine structural observations such as proliferation of cytoplasm and organelles, lobing of nuclei and the development of irregularly thickened cell walls. Cell walls 5-10 times thicker than those of surrounding cells, with numerous projections jutting out into cytoplasm showing anastomosing trabeculae of secondary wall material, were reported for V.faba giant

cells by Huang and Maggenti (1969b), though no cell wall breakdown has been mentioned.

Cell wall dissolution and coalescence of protoplasm have been reported in a number of works (Christie, 1936; Krusberg and Nielson, 1958; Dropkin and Nelson, 1960; Bird, 1961; Owens and Specht, 1964, and Littrell, 1966). Jones and Northcote (1972) recently published that cell wall dissolution was not observable but wall gaps arose between giant cells, produced as a result of breakage during cell expansion. Knob-like projections from the cell walls into the cell interior were referred to by Endo (1971) and were considered significant by Jones and Northcote (1972). Perforation in walls and wall protrusions into cytoplasm were recently revealed through electron microscopic investigations on susceptible soybeans (Gipson, Kim and Riggs, 1971) and on resistant ones (Riggs, Kim and Gipson, 1973). Tests of Dropkin and Nelson (1960) on syncytial walls gave positive reactions for cellulose and pectin but were negative for lignin, suberin, starch or ninhydrin-positive substances. But analysis of Bird (1961) revealed all normal polysaccharides in cell walls.

Some workers are in agreement with Nemec (1910) and speculated that the number of nuclei in a given giant cell represents the number of cells that contributed to syncytia formation (Owens and Specht, 1964). Nuclear multiplication was attributed to repeated endomitosis and lack of cytokinesis by Huang and Maggenti (1969a) who counted 64 n

chromosomes in giant cells of Vicia faba (2n = 12). Dropkin (1965) also located synchronous nuclear divisions and situations containing more than 100 chromosomes in syncytia of Vicia villosa (2n = 14). Contrastingly Mankau and Linford (1960) and Sembdner (1963) argued that there occurred a continuous disintegration of nuclei in giant cells of clover-knots, while Endo (1964) also could not find any evidence of mitosis in syncytia.

Histochemical and autoradiographic analyses of giant cell cytoplasm have evidenced that the infection site is very rich in synthetic proteins, enzymes and nucleic acids (Bird, 1961; Rubinstein and Owens, 1964; Endo and Veech, 1969; Veech and Endo, 1969, 1970; and Endo, 1971). Dense cytoplasm and vacuolar systems are identified in syncytia (Christie, 1936). Bird (1961) has compared giant cell cytoplasm to that in meristematic cells and found the former to have abundant mitochondria, plastids, dictyosomes, golgi bodies and endoplasmic reticulum.

Even though both cyst and root-knot nematodes induce giant cells (syncytia) in susceptible host roots, they are demarcated on the basis of gall induction property. Gall formation is distinct from giant cell development; for, not all root-knot parasitic species are capable of gall formation, and galls induced on different hosts show considerable differences. Galls arise by hypertrophy and hyperplasia of tissues surrounding the nematode, and its

feeding site. Even surface feeding without actual entry into the roots was found sufficient to induce galls (Loewenberg, Sullivan and Schuster, 1960). Kostoff and Kendall (1930) suggested that salivary secretions of nematodes were responsible for giant cell formation in galls of Meloidogyne. Goodey (1948) suggested the possible involvement of plant growth substances based on similarities to auxin induced deformations. Ustinov's (1951) experiments with α-Naphthalene acetic acid (NAA) applications brought about an increased and intensified galling effect. Sayre (quoted by Mountain, 1960) suggested an Indole acetic acid (IAA)-involving mechanism of gall formation exhibited by Meloidogyne, following experiments of Nusbaum (1958) with an inhibitor, Maleic hydrazide (MH), which could reduce galling. But Myuge (1957) found that even application of a growth promoter inhibited gall production in tomato.

Balasubramanian and Rangaswami (1962) extracted certain indole compounds from root-knots of Abelmoschus esculentus infected by Meloidogyne javanica. Yu and Viglierchio (1964), Cutler and Krusberg (1968), Vigilierchio and Yu (1968) extended this type of investigations and obtained positive results for the involvement of exaggerated growth substances. Recent reviews by Krusberg (1971) and Viglierchio (1971) detailed these studies.

Chemical analyses of normal and galled organs have been attempted only by a few workers. Their results have indicated that metabolic activities and rates are strikingly different in these two tissues. It was shown that amino acids accumulate in giant cells of galls owing to nematode secretions and they increase the buffering capacity from 1.7 to 3.2 times (Myuge, 1958). Myuge (1956), Owens and Novotny (1960) and Owens and Specht (1966) observed that normal and galled tissues of tomato roots had considerable variations in concentration of carbohydrates, proteins, amino acids, lipids, nucleic acids and mineral constituents.

All these studies indicate that the giant cells or syncytial system and the hyperplastic gall tissue contributing to the bulk of root-knots, show vast differences in metabolism. It is likely that stimulants for giant cell formation and gall development are different. Bird's (1962) findings demonstrating the inevitable dependence of the parasite on the host for its nutrients and that of the giant cells on the parasite for maintenance, and subsequent collapse of giant cells upon cessation of stimulus from the parasite pointed out the complex and intricate mechanisms involved in root-knot nematode parasitism. The influence of different parasite species on a host and the reaction of a parasite species on different hosts further the complex nature of host-parasite relations involved here. This indicates that plants within

the same family and of different families must show marked differences in susceptibility to attack by gall forming nematodes.

The purpose of the present study was to investigate (1) the infective processes and development of the root-knot nematode, Meloidogyne incognita acrita, (2) the histological alterations in certain vegetable crops belonging to different families and (3) the changes in chemical constituents in host plants due to root-knot nematode infection.

CHAPTER 2

MATERIALS AND METHODS

. -ollowed by repeated

washings in sterile water. For studying the larval behaviour, infective processes and infectivity plants were raised on vermiculite, autoclaved at 15 lb p.s.i. for 20 min, and

distributed in petridishes of 15 cm diameter. Only seeds that germinated upto a week after sowing were utilised in infectivity studies. Seedlings were supplied with 50 ml half strength Hoagland's solution and sterile water intermittently.

The plants used in histological and chemical determinations were grown in clay pots filled with a soil mixture (river sand, loamy garden soil and farm yard manure in equal proportions). The soil-filled pots were autoclaved. Further, a basal dressing with NPK mixture (2:1:1), at the rate of 50 mg/kg soil, was given. These pots were placed in the glass house or in open shady areas. This method could obviate the presence of nematodes and fungi already present in the soil and thereby reduce interference of other pathogens. Samples were also collected from plants grown in the Botanical Garden of B.I.T.S., where natural infestation of the root-knot nematode was quite significant.

2.2 PREPARATION OF INOCULUM

The root-knot nematode, Meloidogyne incognita acrita Chit., used in the present studies was originally picked from eggplant grown in the garden. The eggplant roots had two types of egg masses - pale white and brownish ones. A single pale white egg mass was isolated and used as a progenitor of M. incognita acrita population. Confirmatory identification of the parasite species was obtained from the Division of Nematology, I.A.R.I., New Delhi.

A continuous culture of the parasite was maintained on eggplants grown in pots as described above. Fresh egg masses were collected after 40-50 days of inoculation and these were disinfected with 0.1% Cetavlon (I.C.I., London) for 7 min. The egg masses were preserved in 0.3 M NaCl in glass vials at refrigerator temperature vide Dropkin, Martin and Johnson (1958). Two or three days prior to inoculation the egg masses were centrifuged in sterile water to remove salt and the recovered egg masses were placed in glass cavity blocks with 1 ml distilled water in each. The cavity blocks were placed in 15 cm petridishes layered with wet filter paper and cotton to prevent drying up of the medium. Rupturing the egg matrix was found to increase the larval hatch number. The hatch of second stage larvae was pipetted out and stored at 4-5°C. Fresh water was added to the glass cavity blocks. The larval suspensions were combined, the larval number determined and used for inoculation. Centrifugation helped to concentrate the larvae.

Heavily infected roots of eggplant produced numerous egg masses by 5-7 weeks after inoculation. Many egg masses protruded out of the root surface. Roots were washed and the egg mass bearing segments chopped, and these were added to fresh pots as inoculum. The second stage larvae were active and virulent and they infected all the plants included in the present studies.

2.3 INOCULATION OF PLANTS

The larval suspensions of known concentrations were pipetted into several equidistant hollows in the vermiculite medium containing the seedlings. Usually a concentration of 200 larvae per seedling through 1 ml suspension was used. When inoculating the seedlings *en masse* the nematodes were concentrated by centrifugation to prevent flooding that could be unfavourable for root growth and nematode activities.

Seedlings were exposed generally for 36 hr and thereafter washed and transferred to glass tubes of size 10×5 cm containing vermiculite and nutrients. Earthen pots were inoculated with egg masses from chopped roots. About 25-30 egg masses were used for pots containing 2 kg of soil. No attempt was made to assess the number of eggs each egg mass contained.

2.4 BIOLOGY AND DEVELOPMENT OF PARASITE

The different stages in the life cycle of *M. incognita acrita* infecting eggplant and its developmental aspects were examined. Individual eggs were collected from fresh egg sacs or those boiled in lactophenol-cotton blue. Second stage larvae and males were picked up with a fine pipette and mounted on double cover slip aluminium slides (Goodey, 1963).

Live females and developing larvae of different stages were teased out of infected roots under stereomicroscope. Nematodes fixed and stained in lactophenol cotton blue were also used. Use of double cover slip aluminium slides helped to observe both the sides as well as use of high magnifications. Watching live nematodes in glass cavity slides helped in studying activities such as movements, stylet action and median bulb pulsations. Moving larvae and males were anesthetized by adding 1 or 2 drops of diethyl ether to the nematode suspension in the cavity slides, which immobilised the worms.

To locate larvae inside roots, roots were plunged into boiling lactophenol cotton blue and subsequently cleared in lactophenol. The roots were dehydrated in glycerine by keeping in a desiccator.

Nematodes were fixed in TAF (triethanolamine:formaline: water; 2:7:91) as recommended by Bird (1959a). Staining was done with 0.02% cotton blue in lactophenol.

2.5 HISTOLOGICAL PREPARATIONS

The root segments from normal and galled plants were collected; fixed in FAA, Alconlic Bouin's and Carnoy's fluids and evacuated during fixation. Tissues were preserved in 70% ethanol.

A modified TBA series considering step by step increase in ethanol-butanol concentration and decrease in water

content was used for dehydration and infiltration (Table 1). Tissues preserved in 70% alcohol were immersed for 4 hr each at every stage and three changes of 2 hr each at stage 6. For infiltration a tube was filled two-third with molten paraffin (52°C). allowed to set and thereupon the tissues along with butanol were transferred to this. The tube was incubated at 56°C and as the concentration of wax in the butanol increased the tissues sank to the bottom. Two more changes of fresh paraffin were given, at a day's gap, and the material was subsequently blocked.

TABLE 1

Concentrations of dehydration and infiltration mixtures

Component	Concentration (%) in each stage					
	1	2	3	4	5	6
Ethanol	60	60	50	35	20	0
t-Butanol	10	20	40	60	80	100
Water	30	20	10	5	0	0
Total alcohol (%)	(70)	(80)	(90)	(95)	(100)	(100)

Serial transverse and longisections were cut at 10 μ thickness. Johansen's safranin-aniline blue, safranin-light green and crystal violet-erythrosin B combinations were used for staining (Johansen, 1940). Crystal violet-erythrosin B was best of the lot for studying nucleoli and

xylem development, for crystal violet stood out very clearly against a red background. Safranin-aniline blue combination was very good to differentiate giant cells, their nuclei and female nematodes.

2.6 HISTOCHEMICAL METHODS

Tissues fixed in F.A.A., A.A. and Carnoy's fluid as recommended by Jensen (1962) were used. The Feulgen method of Gomori (1952) with Schiff's reagent was used for DNA localisation. The methods of Flax and Himes (1952) employing Azure B, and that of Brachet (1953) with methyl green-pyronin were also employed for differential staining of DNA and RNA. The periodic acid - Schiff's (PAS) reaction (Hotchkiss, 1948 and McManus, 1948) was followed for localising insoluble polysaccharides in giant cell walls. All reagents were prepared as described in Jensen (1962).

2.7 CHEMICAL ANALYSES

2.7.1 Determination of Nudeic Acids and Proteins

Comparisons were made of the levels of extractable nudeic acids and soluble proteins from equivalent fresh weights of normal and gall tissues. The method of Shibko et al. (1969) with certain modifications was adopted as the procedure involved the sequential separation of these

2. MATERIALS AND METHODS

2.1 HOST PLANTS

Most of the host plants used in the present studies were propagated from seeds procured from the National Seeds Corporation, New Delhi, and P. Pocha and Sons, Poona. Root-knot histogenesis and histopathology were investigated in eggplant (Solanum melongena Linn. var. Black Beauty). However, general histopathological studies and comparisons of giant cells were made on mature gall tissues of okra (Abelmoschus esculentus Moench. var. Pusa Sawani), ridge gourd (Luffa acutangula Roxb., var. Pusa Chikni), musk melon (Cucumis melo Linn. var. Hearts of Gold), tomato (Lycopersicon esculentum Mill. var. Pusa Rubi), pepper (Capsicum frutescens Linn. var. Local), coriander (Coriandrum sativum Linn. var. Local), and hyacinth bean (Dolichos Lablab Linn. var. Local).

Seeds were surface sterilised by immersing for 20-30 min in Chlorox (5.25% NaOCl, aqueous solution) followed by repeated washings in sterile water. For studying the larval behaviour, infective processes and infectivity plants were raised on vermiculite, autoclaved at 15 lb p.s.i. for 20 min, and

metabolites from the same tissue sample.

Extraction: Three hundred mg of normal or gall tissue was rinsed in distilled water, blotted dry between filter papers and sliced into small pieces. The tissue was thoroughly homogenized in a glass mortar and the homogenate was made upto 10 ml with distilled water. Centrifugation followed by resuspension of the pellet in water was performed thrice and the final volume of the extractive was made upto 40 ml. Ten ml samples were used for the sequential fractionation of individual metabolites.

Fractionation: Proteins were precipitated with full strength perchloric acid (PCA) at 0°C. This was followed by removal of glycogen into two 10 ml fractions of 5% PCA. From the pellet first RNA fraction was extracted into a mixture of 9 ml 0.3N NaOH and 1 ml 60% PCA, during an incubation period of 1 hr at 37°C. From the remaining pellet DNA was extracted in a medium containing 1.5% PCA at 90°C (final volume 10 ml). The supernatant, after centrifugation following 20 min incubation, yielded the DNA fraction. Lipids were extracted out successively in ethanol, chloroform, ether and pet. ether. The residue contained the protein fraction.

Estimation: RNA, DNA and proteins were estimated with a Beckman DU2 spectrophotometer. RNA and DNA were measured by UV absorption at 260 nm, while proteins were estimated

by standardising against bovine serum albumin (Lowry et al., 1951). Colour development was with Folin-Ciocalteu-phenol reagent and the abosrbance was read at 750 nm (Ligget Bailey, 1959). Appropriate blank solutions were used to make corrections in readings. Relative concentrations were calculated by comparing absorbance against separate standard curves for each compound, and expressed in mg/g fresh wt.

2.7.2 Extraction of Amino acids, Sugars and Indole compounds

Amino acids and sugars: For the extraction of free amino acids from normal and galled root tissues 5 g of fresh tissue was used. Tissues were thoroughly cleaned in distilled water and homogenised in 5-10 ml of 80% ethanol in three steps. The extractives were combined, filtered through a G2 scintered glass funnel and defatted by thoroughly extracting with three volumes of chloroform for every part of extractive. Using a separating funnel, the supernatant was retained and later concentrated by evaporating in an oven at 60°C. When the extract was about to dry it was dissolved in 10% isopropanol. Sugars were extracted in boiling 80% ethanol, for hot water/alcohol mixture is known to be a good extractant for free sugars. Sugar residue was taken in pyridine using 1 ml per g of the root material.

Extraction of indole compounds: Extractions were attempted with two solvent mixtures (Larsen, 1955). Mostly water-saturated - diethyl ether was used, for this prevents extraction of auxins from auxin protein complexes. At least

two parts of ether, purified for peroxides, was used for every part of tissue. Tissues were cut into fine pieces, homogenized as much as possible in a glass mortar and was transferred to a glass container. Sufficient ether was poured to submerge the tissues, the container covered with a black paper and was kept in refrigerator for 24-48 hr. The bottle was shaken intermittently, and later filtered through scintered glass funnel.

Ethanol extraction by soxhlation: The method suggested by Larsen (1955) was followed for ethanol extraction. A soxhlet apparatus, of capacity 100 g load of tissue, was used in the extraction, with ethanol made to 95% from absolute alcohol (Bengal Chemicals). The root materials were sliced, crushed in a glass mortar and loaded in chromatographic paper cylinders. A glass tube with several holes was introduced into the cylinder filled with the material, to facilitate better penetration and contact of tissues with the solvent. The glass mortar and pestle were rinsed with ethanol, and the ethanol poured into the cylinder. Three hundred ml of ethanol was taken in the evaporating flask and subsequently boiled by a thermostat electric heater.

Extraction was carried out for 48 hr in a partially darkened room. The extractives were filtered with scintered glass funnel and concentrated by vacuum distillation. The crude residue was stored in a dessicator wrapped with black paper.

The extraction, purification and fractionation programmes are summarised in page 30. Lipoid compounds were eradicated from the extract by careful and slow shaking with n-hexane. After concentrating, the crude remnant was solvent partitioned by shaking three times with sodium bicarbonate solution (pH 8.6) so that only non-acid auxins remained in the ethereal supernatant. The aquous carbonate solution was adjusted to pH 3.75 (with 1 M tartaric acid) and again shaken with 3 instalments of ether, so that all acid auxins partitioned into ether. The fractions were further concentrated and used in the chromatographic or bioassay experiments.

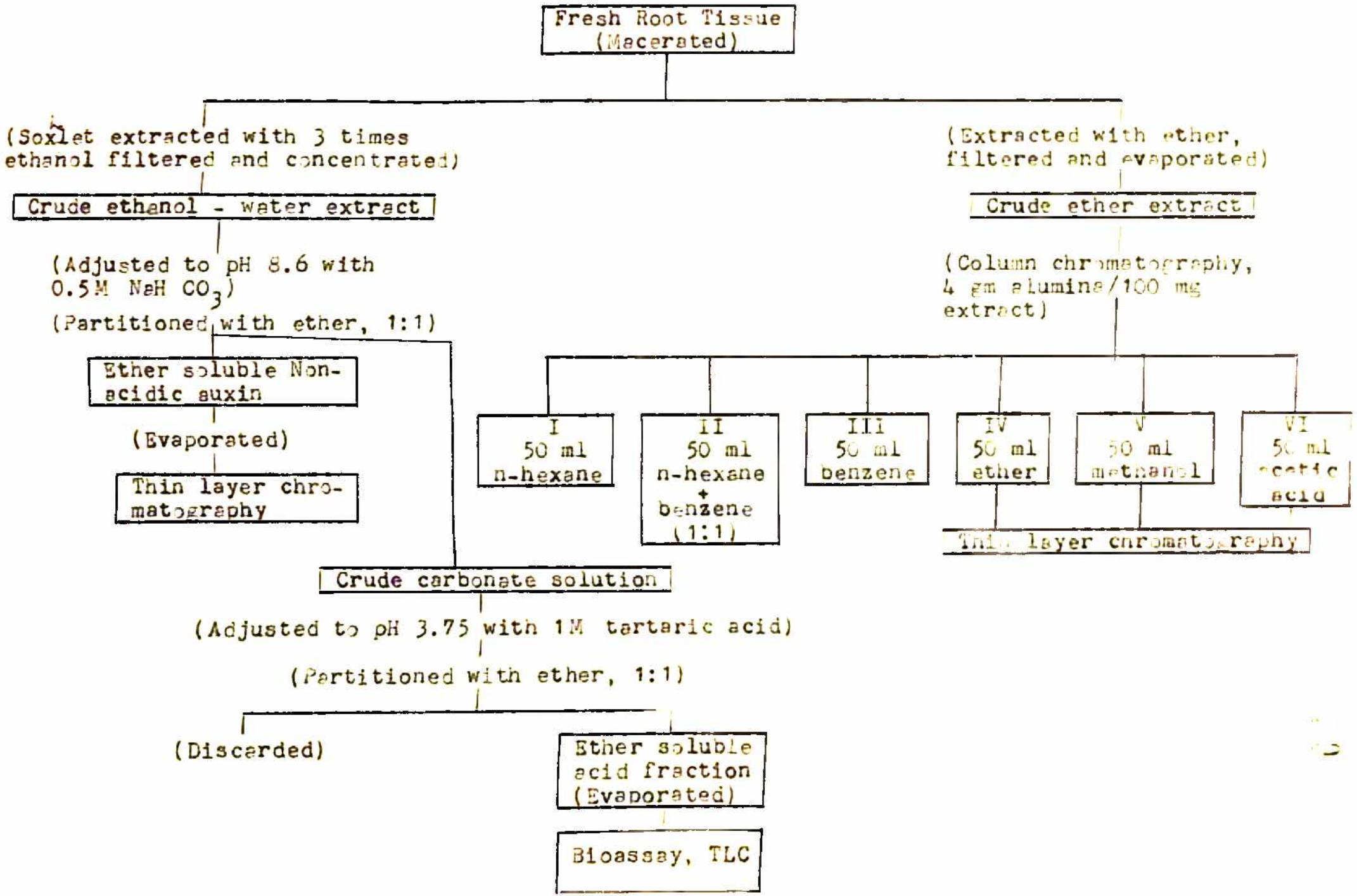
2.7.3 Thin Layer Chromatography

Thin layer chromatography (TLC) was chosen because of its higher resolution over paper. Also the relatively short developing time and distance minimised the decomposition of labile organic components.

Even 250 μ thick layers of silica gel G (E. Merck, Darmstadt) were made on 20×20 cm glass plates. For amino acid chromatography, air dried plates were used whereas layers for sugars and indole compounds were activated for 30 min at 110°C (Stahl, 1965).

Synthetic standard samples were spotted for reference. Compounds were made at a concentration of 1 mg/ml. A micropipette of capacity 5 μl and a hot air blower were of

EXTRACTION AND IDENTIFICATION PROCEDURES FOR INDOLE COMPOUNDS



help in spotting and preventing spread of spot area. All chromatographic runs were done at ambient temperature. For improving chamber saturation, chambers were lined with chromatographic paper, and solvents were poured in at least 1 hr before introducing the chromatogram. All solvent systems were freshly prepared. While chromatographing indole compounds the chamber was covered with black paper except for a window for checking the solvent front.

The following solvent systems were used.

Amino acids: (1) n-Butanol:glacial acetic acid:water (4:1:1)
(2) Phenol:water (3:1)

Sugars: n-Butanol:glacial acetic acid:water (4:1:1)

Indole compounds: (1) n-Hexane:diethyl ether:glacial acetic acid (8:2:0.1)
(2) Chloroform:96% acetic acid (95:5)

The following chromogenic reagents were employed.

Amino acids: 0.3 g ninhydrin in 100 ml n-butanol and mixed with 3 ml glacial acetic acid just before spraying. Plates were then heated to 60°C for 30 min (Stahl, 1965).

Sugars: Anisaldehyde - sulfuric acid:
Fresh solution of 0.5 ml anisaldehyde + 9 ml ethanol (95%) + 0.5 ml conc. H_2SO_4 + 0.1 ml glacial acetic acid. Heated to 110°C for 10 min (Stahl, 1965).

Indole compounds: (1) Salkowski reagent: 100 ml 5% $HClO_4$ + 2 ml 0.5 M Ferric chloride. After spraying the plates were air dried or heat activated at 110°C for 10 min.

(2) Ferric citrate reagent: 100 ml 30% $HClO_4$ + 2 ml 0.2 M Ferric citrate. After spraying the plates were heated to 110°C for 10 min.

The plates with indole compounds were viewed in UV light to note the fluorescence. This was followed by colour development. Exposure to aqua regia improved fluorescence after spraying.

The relative concentrations were graded as 3+, 2+, +, = and the sign - used indicate absence of the compound.

2.7.4 Column Chromatography of Indolic Substances

Column chromatography was based on Cutler and Krusberg (1968). Column dimensions were 27×1.6 cm. Alumina (E. Merck) was used as the adsorbent, which was deactivated by keeping in open for 1-2 hr. Crude residue of tissue extract was dissolved in ether and added to the top of the packed column. Separations were made by a solvent series, involving (i) 50 ml n-hexane; (ii) 55 ml n-hexane + benzene (1+1); (iii) 25 ml benzene; (iv) 50 ml diethyl ether; (v) 50 ml methanol, and (vi) 50 ml glac. acetic acid. Fractions

were collected separately, concentrated and used for TLC or bioassay.

2.7.5 Spectrophotometric Determination of Indolic Substances

Indole extracts after purification were determined spectrophotometrically (Larsen, 1955). The Gordon and Weber (1951) version of the Salkowski reagent and ferric citrate reagent were used for the colour reactions. Colour development was carried out in dark and the pink colour produced was measured at 530 nm after 1 hr.

2.7.6 Bioassay of Indolic Substances

Wheat (var. NP 824) seeds obtained from Cereal Laboratories, IARI, New Delhi, were planted in wet vermiculite in petridishes of 16 cm diameter, in a dark-room at 25°C. When the coleoptile length was 1-1.5 cm they were incised. The initial 3 mm distance was discarded and the next 5 mm segment used. These operations were carried out in dim red light. The primary leaves were removed from the 5 mm segments and the segments were left in sterile distilled water for 12 hr in total darkness.

A set of 5 sections was then floated in a glass vial containing 5 ml of solution or eluate to be tested and 1 ml of fresh sucrose phosphate buffer (pH 5.9). The sections were incubated in total dark for 24 hr and later measured with a micrometer. Controls of 2 vials maintained

with water in place of auxin solutions served for comperison. Four segments were incubated in each tube. Adsorbent silica gel after TLC from which solvents were driven away was taken in centrifuge tubes and eluted in methanol. After drying the methanol residue was dissolved in water and used in bioassay.

The growth increments of wheat coleoptiles in synthetic auxin solutions and eluated from chromatograms were compared with controls.

CHAPTER 3

OBSERVATIONS

3. OBSERVATIONS

3.1 BEHAVIOUR OF THE PARASITE AND INFECTION

Movements, penetration and migration of the infective larvae have been studied in the roots of Solanum melongena and Abelmoschus esculentus seedlings. One week old seedlings were transferred to petri-dishes containing vermiculite saturated with Hoagland's nutrient solution (pH adjusted to 7.0). One ml of a nematode suspension containing about 200 larvae was then pipetted into the medium, approximately 2 cm away from the growing root tips and the roots then sparsely masked with vermiculite which facilitated larval movements to the root vicinity at the same time retaining moisture on the root surface. Care was taken to see that the medium did not obscure complete visibility of the entire root. These cultures were maintained at room temperature which varied from 25-30°C.

Several samples of host roots were partially exposed from adhering particles of the medium for observation of larval behaviour. Almost the entire root surface, save the region adpressed to the container, was accessible to the infective larvae. Larvae were observed through an Olympus stereomicroscope with variable magnifications and

epiillumination.

3.1.1 The Infective Stage of the Parasite

The second-stage larvae emerging out of eggs laid by the females represent the infective stage in the life cycle of all root-knot nematodes. Freshly hatched larvae are vermiform, virile and move about in search of suitable host roots. Such movements when extended in the absence of hosts caused depletion of body reserves and made the larvae sluggish. The larvae are equipped with a buccal stylet, a median bulb and a pair of oesophageal glands, which form the most important structures in the infective and feeding processes. The stylet is a pointed spear attached to cephalic muscles and encloses a narrow lumen and is comparable to a hypodermic needle.

3.1.2 Larval Orientation and Movements

Within an hr after introducing the juveniles into the medium they have been found wriggling about with their heads oriented towards seedling roots. Head orientation and movements in the direction of root tips suggested that the sensory organs in the head of the nematode help in orienting towards attractants and spotting suitable sites for penetration. Many larvae reached the root vicinity within 2-3 hr. Some larvae moved to directions different from that of the roots. The larvae were wriggling along the vermiculite particles, and moving over root surface

before anchoring at some points (fig. 1). The larvae attracted to the roots, showed preference to the root tips though a few of them abrased the surface and other areas of roots.

3.1.3 Sites of Penetration

In S. melongena the root cap region was often the site most preferred for M. incognita acrita attack (figs. 2 and 3) while the region representing the cytogenerative centre also was often used by larvae (fig. 4). Similar penetrations occurred in A. esculentus also, but here more larvae gathered about the meristematic region (figs. 5 and 6). Often several larvae accumulating around the root cap in S. melongena attacked the tip cells of the calyptra and they thrust aside the cap cells in some cases (fig. 7).

The larvae occesionally invaded the roots at points distant from root tips as well. Some were found oriented behind the meristematic region, and the zones of cell elongation and root hair formation were also used (fig. 8). The larvae also attacked the piliferous layer of well differentiated roots (fig. 9) and any surface fissures caused by mechanical injury (fig. 10) and sites of emerging lateral roots were also points of entry. However, compared to the root extremities, these points were relatively less frequented. Presumably, emanations from points of this kind elicited attractive factors. It has

been noted that the larvae that probed mature zones generally moved down to the tender undifferentiated regions. The sites and process of invasion illustrated in the present work give additional evidence for infectivity of M. incognita acrita in the test plants employed. Although penetration did not necessarily establish pathogenicity, the histological alterations produced in the hosts and ovulation by mature females indicated clear evidences of successful parasitisation.

3.1.4 Stylet Action and Penetration Process

Once a penetration site was spotted, the larvae showed great activity of body movements and stylet thrusts. Fig. 11 illustrates several larvae penetrating the root tip and fig. 3 shows a larva coiled inside the root while another is penetrating through the cap. Larvae arched their bodies during their attempt to pierce host cell barriers and gain entry, and one such invader is shown in fig. 7. The stylet movements against cell walls pierced host cells and the exuding plant juice through these breaches subsequently attracted a large number of invaders. Also the presence of a larva at a particular site, and even in the medium, seemed to stimulate others which were drawn towards that point. Larval attacks deranged the root cap and tip cells and thereby caused injuries (figs. 4 and 7).

As the nematode reached root vicinity its movements were accentuated and head oscillations were more frequent.

Larvae, after spotting penetration points, exhibited vigorous stylet darts, ranging from a few initial thrusts followed by repeated activity reaching a maximum of 70-80/min. The larvae at times altered the angle of stylet orientation and this was indicated by the changed direction of the body and stylet thrusts. The larvae were not seen to be pushed back as a probable result of recoil from root surface.

Following repeated stylet action the tender root cells got punctured and the larvae gradually pushed their head into the fissures. After the larvae introduced their head into the host cell, the stylet was seen extruded from the buccal capsule. Feeding at this position was indicated by pulsations of oesophageal bulb which is known to be concommittant with ingestion of cell contents. Larvae were relatively immobile during feeding. Attempts to assess the time taken by second stage larvae of M. incognita acrita to gain entry into the host cells showed varying times ranging from 1-5 hr, in the case of successful ones. Larvae gradually pulled in their body. The tail showed movements during such advancing until only the tail end was left outside. Intermittent probings and withdrawal, as well as feeding before entry seemed to influence the penetration time. Also, the host root texture was likely to vary at different points. Passage of larvae through natural breaks in the host root surface or holes made by pioneering invaders has been quicker.

3.1.5 Movements Inside the Roots

After penetrating the terminal root cells the larvae passed generally in between the cells in a longitudinal or radial direction (figs. 11 and 12). In general intercellular movement was common (fig. 13) and thus quicker for the larvae. However, some of the larvae took an intracellular course of advancement causing slight disruption and damage to the tissues (fig. 14).

The larvae which entered through root cap or root meristem region travelled up and subsequently reached the provascular zone, where they aligned themselves in the longitudinal plane, whereas those penetrating through comparatively mature points were heading towards the stelar region. In case of multiple infections along the root tip, the larvae even occupied the central position corresponding to the plerome of the young roots (fig. 15). Fig. 16 illustrates several larvae lying parallel and migrating along the tissues where xylem elements have already differentiated. Infections with larvae in this position have been noted to produce giant cells from cambial parenchyma, phloem or xylem mother cells.

Larvae placed their heads proximal or distal to the root tip, which is determined by the point of entry. Most of the larvae were oriented to the proximal end of roots (fig.11). Larvae entering from the lateral positions of differentiating or differentiated root zones settled themselves in

a radial direction with their head located in stele. In their migration along the provascular tissues, larvae penetrated the intracellular transverse septa (figs. 17 and 18). Migration path of M. incognita acrita in both the hosts was mainly intercellular, and intracellular movements were very infrequent. Movements separating the cell lamellar cementing did not appear to cause any visible cell damage to the host tissues.

3.2 NEMATODE INFECTIVITY IN INTACT AND DEFOLIATED PLANTS AND EXCISED ROOTS

3.2.1 Infectivity in Intact Plants

To assess the infective potential of the parasite population, inoculations were given to S. melongena and A. esculentus seedlings. When the plants were a week old 5 groups of 6 seedlings each were transferred individually to petridishes containing vermiculite. Their main and lateral roots were covered with the medium saturated with nutrient solution. Each one was then inoculated with 200 larvae through one ml aliquot at 2 cm from the root tip terminals. The seedlings were exposed to 12, 24, 36, 48 and 60 hr durations and removed, washed and plunged into boiling lactophenol-acid fuchsin. On cooling, the roots were cleared in lactephenol, subsequently teased out in a known volume of water and larval counts made. Centrifugation helped to concentrate the larvae along with broken root tissues. The results are given in Tables 2 and 3.

It is evident that in S. melongena and A. esculentus roots exposed for 12 hr the number of larvae present within the root tissues was the smallest. The number of successful penetrations increased with an increase in the exposure period. Maximum larvae accumulated in S. melongena by 48 hr. In A. esculentus a corresponding increase in larval counts has been noted upto the 60th hr. It is likely that larvae required some time to detect attractants and reach the source, travelling through vermiculite particles. It is presumable that the roots of S. melongena were attractive only upto certain stage of development after which no increase in larval infestation took place; for there was no significant difference between 36, 48 and 60 hr treatments with regard to the rate of infestations. Egg-plant roots were comparatively thin and might have accommodated relatively fewer larvae than okra roots in which a regular increase in the number of larvae was encountered. In both the cases differences existed between individual seedlings even within similar exposure duration. The results indicated that A. esculentus was a host preferred more by M. incognita acrita larvae which invaded their roots more rapidly and in greater numbers than S. melongena.

3.2.2 Effect of Defoliation and Root Excision

The question of differential attractiveness of Lycopersicon esculentum Mill. and neutral behaviour of S. melongena raised by Wieser (1955, 1956), was the subject of experimentation. The preceding tests proved that root

attraction exists in S. melongena. Loewenberg et al. (1960) speculated that the use of excised roots in Weiser's experiments might have not been attractive and behaved differently. This area has been reinvestigated and larval reaction to defoliated plants and excised root segments tested.

Seedlings similar to those used in the previous experiments have been employed for these studies too. The cotyledonary leaves of test plants were excised 6 hr before inoculation and subsequently the plants exposed for 48 hr. In another experiment larval reaction to the root tip segments has been studied. Four excised 3 cm segments each washed in sterile water for 6 hr, were laid in each petridish with vermiculite before inoculation. The results in Table 3 for defoliated and intact plants are the mean of 6 replicates, and for excised root tests the total number of larvae in 4 samples. Intact seedlings were used for comparison.

Defoliation reduced attractiveness significantly. However, defoliated seedlings retained some attractiveness, possibly through root exudates in the medium. But when compared to intact seedlings, a smaller number of larvae only got attracted towards these roots. It is worthwhile to recall (from Table 4) that these defoliated seedlings regained attractiveness and larval number increased as the exposure duration was extended. By this time the apical buds grew and the first leaves started developing. The

results indicated that a photosynthetically active and growing seedling is more attractive and it may be speculated that the substances it biosynthesizes and supplies to the roots have some role in root attractiveness.

The excised root clearings did not show any larvae during the initial stages. Washings have removed all the exuded materials from their surfaces. Segments left for longer periods in vermiculite containing larvae were penetrated by a few larvae. This might either be due to penetration of larvae moving at random or leachates might still have been emerging from such excised roots which attracted the few larvae.

The data in Tables 2 and 3 were tested statistically and the results showed that values for infectivity in both the hosts were significant at 10% level. This was also confirmed from the results in Table 4. Results for defoliated and excised roots of A. esculentus were significant at 5% level, whereas for S. melongena these were insignificant even at 10% level.

TABLE 2

Infectivity+ of *M. incognita acrita* in *S. melongena*

Exposure duration (hr)	Number of larvae per seedling						Total No. of larvae in 6 seedlings	Mean No. of larvae per seedling	Percentage of infection for 6 seedlings
	A	B	C	D	E	F			
12	11	17	24	31	4	16	103*	17*	8.58
24	74	81	58	24	62	72	371	62	30.91
36	94	112	93	77	39	71	486	80	40.50
48	75	83	47	125	104	61	495	82	41.25
60	63	104	84	68	97	77	493	82	41.08

+ Two days old larvae, 200 per 5-7 days old seedling.

* Significant at 10% level, with value of $P = 0.10$

TABLE 3

Infectivity[+] of *M. incognita acrita* in *A. esculentus*

Exposure duration (hr)	Number of larvae per seedling						Total No. of larvae in 6 seedlings	Mean No. of larvae per seedling	Percentage of infection for 6 seedlings
	A	B	C	D	E	F			
12	16	2	0	22	6	40	86[*]	14[*]	7.16
24	54	38	98	65	112	26	393	66	32.9
36	62	120	99	114	51	32	488	81	40.5
48	91	50	102	80	60	122	505	84	41.6
60	126	108	38	74	97	83	526	88	43.66

[+] Two days old larvae, 200 per 5-7 days old seedling

[*] Significant at 10% level, with value of $P = 0.10$

TABLE 4

Comparative infectivity[+] of *M. incognita acrita* in intact and defoliated plants and excised roots

Host plant	Exposure duration (hr)	Mean No. of larvae per 6 defoliated plants	Total No. of larvae in 4 excised roots	Mean No. of larvae per 6 intact plants
Solanum melongena	12	11***	0***	14*
	24	9	1	62
	36	17	0	73
	48	53	8	80
Abelmoschus esculentus	12	7**	0*	22**
	24	11	0	61
	36	46	6	75
	48	67	14	89

[+]Inoculated with 200 larvae per seedling or excised root

*Significant at 10% level, with value of P = 0.10

**Significant at 5% level, with value of P = 0.05

***Not significant

3.3 GALL FORMATION AND GENERAL HISTOLOGICAL ALTERATIONS

3.3.1 General Morphology of Galls

Galls were first noticed as spindle shaped protuberances on the root tip. They were round, oval or elliptical when formed on differentiated zone of roots. In tender galls the surface remained smooth and retained the same colour as the tissues of unaffected areas. However, in certain galls the regions close to the nematode body especially the eggmasses, became brownish. Figs. 19, 20 and 21 show galls from different hosts. When multiple infections occurred galls arose in close proximity to each other giving the organ very irregular shapes (figs. 20 and 22). Galls from certain plants grown in the field, that were exposed continually to several generations of second stage larvae, became conspicuously enlarged. They were amorphous and usually produced distorted shapes (fig. 23). This was due to the production of new feeding sites and hyperplasia by secondary invaders in gall tissues already produced by the parent population. Certain portions of such galls degenerated, whereas other regions continued to produce new overgrowths (fig. 24).

Galls occupying the entire root circumference were produced when the invader occupied a position inside the vascular ring, and induced the development of abnormal tissues almost on all sides. Galls were formed on sides of roots when the parasites were not deep seated but invaded

the cells of the cortex. Under field conditions, galls induced in young roots generally belonged to the former type and those in mature differentiated areas were of the second category. Also, secondary infection caused by larvae hatching from egg masses imbedded within root tissues, contributed to the further growth increment of such galls.

In naturally infected materials there was a tendency of increased number of galls and presence of nematode near the points of emergence of lateral roots.

The growth of the females was associated with large giant cells produced from host tissues at the feeding sites. The production of giant cells, the development of extensive proliferation of the parenchyma around the giant cells and increase in the size of the females gradually increased the diameter of root galls.

3.3.2 Host Tissue Deformations along Nematode Path

Infectivity studies (Chapter 3.2) have revealed that the second stage larvae entered the root terminals even as early as 6 hr after inoculation. These larvae advanced into root interior either by moving through or between cells. The larval body was generally straight except for the bends offered by the cell wall orientations (fig. 13).

In general root-knot larval penetrations caused comparatively slight mechanical damage to root tissues.

Larvae penetrated the roots, dissolved or separated the middle lamellae and moved ahead intercellularly (fig. 13). However, those that pierced cross walls caused direct histological damage (figs.18, 19). Also the larvae occasionally left a track as evidence of movement. Some cells on either side along the larval path showed compression and looked different from adjacent rows of cells. At the subsequent stage these cells became enlarged and their nuclei showed increase in size. Their cytoplasm too was dense, but the walls at this stage did not show any increase in thickness, instead they appeared to have been stretched. The cell constituents showed an intense staining of aniline blue indicating changes to those of the normal cells.

In most of the root segments, nematode head was close to the stele indicating larval preference to such a disposition. Larvae further moved to the zone of vascular differentiation and this situation was usual in all the hosts. Larval movements within roots appeared to be easy. Salivary emanations lysed middle lamella and affected cell adhesiveness. Advancing along such directions the larvae get lodged at suitable feeding sites. The head of the nematode in such situations lay proximal to the root (fig. 11).

The movements of the male nematodes caused considerable tissue disruptions, and there frequently arose necrotic cells along their path. Fig. 25 shows a site with a male that

indicated increase in wall thickness and necrosis near its head. The cell walls are stained intense red with safranin. Fig. 26 depicts a male nematode approaching a sedentary female. Necrotic cells usually arose along the path of the male nematode.

3.3.3 Sites of Nematode Lodging and Feeding

Most larvae migrated to the stele where they lodged in tissues near the vascular elements. When penetrations took place along the root cap most of the larvae advanced along the differentiating elements (fig. 16) and settled generally in the pericyclic, cambial or phloem tissues. Larvae that entered through the mature regions proceeded along a radial direction and reached the endodermis. The endodermal thickenings, however, limited whole length larval entry into vascular tissues. The larvae usually punctured the endodermal walls and introduced their head further into the conductive tissues (fig. 27). Cell wall dissolution and hypertrophy of cells and nuclei and occasionally breakage were evident (figs. 28 and 29). This was the initiation stage for the production of abnormally large giant cells.

Occasional feeding by larvae could be visualised during the process of penetration. Extrusion of larval stylet followed by pulsations of oesophageal gland bulb occurred and the larvae lay still during the feeding processes. After settling at a spot the second stage larvae fed very actively initiating synthetic and tissue changes around

feeding sites. After 15 and 20 days of inoculation, larvae dissected out of roots were found enclosed in sloughs of cuticles of the preceding instars and it is not certain whether these larvae were feeding during the developmental stages. However, adult females teased out of roots and observed in water exhibited slight movements of head and pulsations of oesophageal bulb.

After penetration, the larvae moved about in search of suitable feeding sites. In 5 day old infections of S. melongena and A. esculentus larvae were located in the provascular and differentiating zones (figs. 15 and 16). When multiple infections took place along root terminals larvae were heading to the plerome of the root (fig. 11). Some larvae settled near the apical initials itself (fig. 28). In general, following entry the larvae showed a tendency to establish feeding sites. Larval movements inside the roots caused slight disruptions to the root tissues and was insignificant when compared to the heavy damage known to be associated with cyst nematode infections. Certain cells that surrounded nematode head were deeply stained indicating the effects of interactions of salivary secretions and cell contents. Some cells and their nuclei around the nematode head were hypertrophied (figs. 14 and 31). Fig. 32 shows a larva in parenchymatous cells forming a track and nuclei in adjoining cells were enlarged (figs. 30 and 31). All larvae did not settle and start feeding simultaneously and some larvae migrated inside roots for longer periods.

As a result, tissues of successive ages did not always show a corresponding correlation in the stage of disease development.

Th larvae that lodged in cortex had a general tendency to differentiate into males. Around their feeding sites cells enlarged but their walls did not exhibit any thickening when compared to those of the surrounding cells (fig. 32a). Cell wall deformities were less pronounced though signs of multicellular formations were noticed. Vacuolated cells showing a poorer production of cytoplasm were a characteristic of male induced giant cells. Stimulation of hypertrophy and hyperplasia, although rare, was seen near males in hosts like L. acutangula (fig. 33).

3.3.4 Damage to Cell Walls

The movements of nematodes in roots broke cross walls in some cells (fig. 29). In certain roots after 5 days of inoculation a few cells had their walls distended due to hypertrophy and certain wall areas were thinned down. There was a general increase in the staining reaction and cell walls adjoining larvae were deep blue when stained with safranin-aniline blue. Walls of certain cells were broken and protruded into neighbouring tissue. When giant cells were expanding, the pressure caused compression in surrounding tissues, which were subsequently crushed. When the giant cells arose in stele the expansion disrupted the xylem poles and phloem patches and also broke the endodermal

ring.

Giant cells were formed by coalescence of several adjoining cells (fig. 34). The cell walls thinned down at certain points and later dissolved to form coenocytic structures. Significant damage was caused to the surrounding root cells by the gelatinous egg matrix. These damaged cells became necrotic and stained deep red with safranin, whereas adjoining cells absorbed aniline blue.

3.3.5 Stimulation of Cell Division

In roots of root-knot nematode-infected plants, a stimulation of cell division had been noted. Cell enlargement, soon followed by divisions, had been noted to flare along the larval path and the site of feeding. The nuclei in the cells at these sites were larger in comparison to those in unaffected areas, and contained enlarged nucleoli as well. These reactions were very clear in S. melongena, A. esculentus and other hosts. The number of nuceoli in hypertrophied nucleus was found to range from one to several and the nucleoli were more chromophilic. Very often the nucleoli were unusually large, and their size approximated the size of nuclei in normal parenchyma. The nature of alterations in nuclear and nucleolar morphology showed that certain stimulating factor for cell division probably originated from the invading parasite and crossed the cell boundaries, to which the cells reacted readily.

Hyperøplasia originated mainly in the pericyclic and cambial layers. The products of these abnormal divisions were chunks of closely packed parenchyma cells. Often they arose from the surrounding tissues of giant cells also and gave a tiered appearance (fig. 35). Such cells also showed divisions in several planes. These hyperplastic parenchyma damaged the endodermis and extended into the cortex. Abnormal parenchyma also grew over the parasite covering it as the body of the latter increased in size.

3.3.6 Giant Cell Formation

The feeding activity of M. incognita acrita in host tissues produced abnormally large feeding sites (figs. 36 and 37). They showed thick cell walls, dense cytoplasm, many hypertrophied nuclei of varying contours and more than the usual number of nucleoli. Fig. 37 shows two larvae lying opposite to each other amidst several giant cells. Such cells appeared to have originated in response to the feeding activities of nematodes. In the literature, feeding cells induced by heteroderid nematodes are referred to as giant cells, syncytia, coenocyte, lysigenoma etc., indicative of their mode of formation (cf. Chapter 1, page 9).

Each successful nematode had its head included in the centre of the giant cell complex (figs. 38 and 39) which extended more towards the longitudinal axis of the root. Often the giant cell complex was produced as a result of coalescence of more than one type of cells (fig. 40).

Giant cells enlarged and elongated to attain abnormal dimensions (fig. 41) and the variations in their measurements are detailed separately (Table 5). Each giant cell complex consisted of varying number of units morphologically varying very much among themselves. Some have a large number of nuclei whereas in others multiplication of other cell organelles was visible (fig. 42). Generally giant cells had a rich mass of cytoplasm (fig. 41), although many others appeared to be relatively less in cytoplasmic contents. Certain giant cells contained prominent vacuoles (fig. 39). But as the amount of cytoplasm increased these were obliterated. These giant cells became vacuolate and empty again subsequent to nematode death and giant cell deterioration. Reduction in cytoplasm in giant cells was regarded to be due to removal of their inclusions by the nematode (fig. 43).

Giant cells in roots of approximately the same age appeared variable in morphology and giant cell units of a complex showed variations in number. The extent of histological abnormalities appeared to be determined by the origin and location of giant cells. The reactivity of the particular host tissue also must be important.

Usually giant cells were initiated in the young stelar ring (fig. 44) in which case the damage was very severe, or even in vascular parenchyma and ray cells of secondary xylem (fig. 45). These giant cells were multinuclear

(fig. 46) and nuclei had been observed to be polynucleolar in many cases. There was no stage indicative of regular mitotic nuclear divisions and normal cytokinesis even in tissues processed in cytological fixatives. Nuclei of varying shapes and dimensions were a usual feature in these feeding cells (figs. 47 and 48).

The coenocytic condition had been observed to be brought about by dissolution and coalescence of adjacent cells (figs. 34, 37 and 44). A pooling of nuclei from these coalesced cells, their subsequent hypertrophy and amitotic duplications increase the nuclear number in giant cells (fig. 96).

Essentially all the contents of giant cell cytoplasm multiplied and nuclear fragmentation appeared to occur effecting a further increase in number. The giant cell nuclei underwent several morphological changes and produced irregular surfaces and amoeboid lobes (fig. 48). In many cases the nuclear membrane appeared distorted. Nuclei of a varying range of size were common and seemed to be the products of fragmentation (fig. 49).

The nuclei of giant cells generally aggregated in the centre of the dense cytoplasmic mass (figs. 46 and 50). However, occasionally they were found to adhere to the giant cell walls, especially in those giant cells which were degenerating or having sparse cytoplasm. In the giant cells, the nuclei and nucleoli survived longer than the cytoplasm.

The nuclei in affected cells were seen to undergo marked changes in shape. In addition to the usual round and elliptical nuclei as seen in the normal cells, elongated and amoeboid ones were of frequent occurrence in giant cells (figs. 47 and 49). Examination of nuclei from different giant cells and from different hosts revealed that no particular shape is a specific characteristic of root-knot nematode infection. Sickle shaped, elongated and round nuclei existed in hyperplastic cells that contributed to the bulk of gall (fig. 35). Irregularities of nuclear membrane was a common feature and in some cases deep fissures were found on nuclear surface (fig. 46). The giant cell cytoplasm often intruded into such fissures.

Measurements of nuclear diameters showed that size was quite variable even among nuclei of the same giant cell and in different components of the same giant cell complex. Abnormally large nuclei measuring upto 30-48 μ in diameter along the longest plane had been spotted in some of the syncytia. The giant cells had many micronuclei the appearance of which suggested that the smaller type of nuclei could be formed from bigger ones by fragmentation. Such changes were more pronounced in advanced stages of infection. Nuclei in cells around giant cells also hypertrophied but were however, never as big as giant cell nuclei (figs. 39, 49 and 73).

The nuclear envelope was irregular, and wavy in most of the cases. Nuclei contained chromatin materials apparently

stuck to the inner edges of nuclear membrane. These were observed at the nuclear periphery in Feulgen-stained preparations also. However, the nuclear membrane was less chromophilic in old and degenerating giant cells.

A certain number of nuclei at any time were found to be more reactive, emphasizing that nuclear formation and proliferation continued throughout the feeding period of the parasite; for, some nuclei were apparently degenerating whereas others were strongly reacting to staining.

Nucleoli in giant cells were also hypertrophied, but did not show the sort of changes in contour peculiar to nuclei. Round nucleoli of varying sizes, numbering one to several were a usual feature. Nucleolar diameters in many cases corresponded to that of nuclei of parenchyma cells that did not colesce with the giant cells as seen within the same root section. A normal cell usually showed one or two nucleoli per nucleus, whereas a giant cell generally had many nucleoli in each nucleus. Nucleoli even within the same nuclear envelope displayed variation in sizes. Some preparations showed nucleolar vacuoles (fig. 48). Certain nucleoli had glistening globular bodies within. Nucleoli stained vary deeply with histological stains and were very prominent in all infected root sections. They persisted longer than all other cell organelles, for many nucleoli with intact nucleolar envelope and staining properties were encountered in degenerating giant cells.

In the initial stages, the giant cell walls resembled those of parenchyma. The cell walls were rather thinned down in certain cases, perhaps due to stretching as a result of cell enlargement. Soon irregularly thickened areas appeared making the cell walls uneven. Secondary wall materials synthesised in giant cells appeared to be deposited non-uniformly in giant cell walls. Certain thin areas were left out and these might have been acting as sites for cell to cell transfer of materials. Certain cell walls were broken and protruded into neighbouring cells. Such processes increased the area of giant cells, and wall fragments jutting out into other cells were clear indications of the role of wall modifications in giant cell expansion. In advanced stages of giant cell growth certain cells had irregular secondary wall thickenings (figs. 38 and 49). But these thickenings also were restricted and had left areas resembling plasmodesmata.

Wall disintegration happened through other ways too. As the giant cells expanded, the adjoining parenchyma got dissolved and gradually merged into giant cells (fig. 37). Fig. 2o shows damage caused to xylem elements surrounding giant cells. Endodermal and pericyclic cells also got converted into giant cells and xylem walls too merged into these. Wall fragments projected into the cytoplasm of such broken cells.

3.3.7 Tissue Changes around Giant Cells

The expanding giant cells damaged cells mechanically also. When giant cells arose in between xylem poles they crushed the tissues on the sides. Another significant change was deviation in normal meristematic activity. Cambial cells affected by nematode emanations failed to differentiate into xylem or phloem, instead merged with other cells forming the giant cells. As a result, normal stelar tissues were wanting at the affected portions. Those cambial cells that survived produced patches of vascular elements (figs. 49 and 50). Also there was a marked suppression of phloem formation. In no part of the gall additional phloem tissues developed; instead abnormal xylem arose in certain areas. Figs. 51 and 52 show the damage to vascular tissues and the resultant dissected appearance of stele. Occasionally the xylem in infected area got transformed into parenchymatous cells. A transection (fig.53) of the secondary structure revealed many deranged xylem elements and hypertrophied parenchyma.

Along with hypertrophy of cells, proliferative growths occurred around infection court. The hyperplastic tissue originated mostly in the pericyclic and adjoining layers. Although in galls a tiered appearance was usual with such hyperplasia, divisions occurred later in several directions.

Xylem developed in many non-stelar tissues. Around the giant cells there developed groups of abnormal xylem (figs. 54 and 55). As the giant cell development

progressed the number of xylem elements increased, even enclosing the stele during later stages. Xylem elements were also developing towards the direction of the parasite. In large galls there were many areas in the parenchyma getting differentiated into xylem. As the gall matured these xylem strands established connections with the main stele. (fig. 56).

3.3.8 Variations in Giant Cell Complexes and Hyperplastic Cells among Different Hosts

All plants included in the present study though taxonomically unrelated, produced root-knots of varying sizes. A comparison of the histological details of their mature tissues revealed several differences among the hosts, indicating variations in host responses. Data in Table 5 represent the maximum number and size of the giant cell components representative from galls of different sizes. Wall thickness of normal cells could not be measured with the ocular micrometer.

Abelmoschus esculentus

Four to 5 giant cell complexes were usual, each having 3-8 units. They were 225-567 µ in length and 160-225 µ in width. Giant cell walls thickened upto 11.1 µ which had several perforations and breaks. Wall incrustations measured 8-10 µ thick. Upto 83 nuclei were found in giant cell complexes. Their size ranged from 7.4-18.5 µ. These nuclei had upto 5 nucleoli of 2.1-11.1 µ diameter. Nuclei in hyperplastic cells measured only 7.4 µ in diameter.

Luffa acutangula

Ridge gourd was a host highly suitable for the *M. incognita acrita* population used in the present study. Four to 9 giant cell sites were seen that measure 610-945 µ long and 215-470 µ wide. Cytoplasm filled these giant cells and there

was a significant accumulation of lipid globules. Giant cell walls measured about 11 µ. The number of nuclei in relation to the cell size and amount of cytoplasm was small. The highest number counted 52. Contrastingly the nuclear size was unusually big and these exhibited a wide variation in their morphology. Round, oval, elongated, bean shaped and amoeboid nuclei were observed. Nuclear size ranged from 10-45 µ. The range was 1.4-11.1 µ in the case of nucleoli, three times bigger than in normal cell nuclei (2.8 µ). Hyperplastic cell nuclei also enlarged 2-3 times.

Cucumis melo

Musk melon, another cucurbit studied, also maintained a heavy population of M. incognita acrita. Four to 8 giant cells were produced per feeding site, having dimensions of 550-935 µ in length and 350-540 µ in width. Wall thickness was about 7.4 µ. Dense alveolar cytoplasm filled the giant cells which possessed 52-67 nuclei per complex. Elongated nuclei measuring 20 µ long and 9 µ wide were seen in certain cases. The maximum nuclear diameter measured was 30 µ. Such nuclei contained 4 or 5 nucleoli of 1.4-2.1 µ. Nuclei from hyperplastic cells were not much different from those from normal cells where the size averaged 2.8µ in diameter.

Lycopersicon esculentum

The Pusa Ruby variety of tomato showed 3-5 feeding sites in certain sections. Each of these had 5-8 giant cell units.

Giant cell complexes measured upto 730×420 µ. Their walls had thickened over 11 µ. More than 70 nuclei were counted in such sites. Nuclei measuring 7-14.8 µ were common here and their nuclei enlarged upto 7 µ. The cytoplasm was quite dense and occupied almost the entire giant cell area. Nuclei from hyperplastic cells measured 4.9 µ showing the usual enlargement.

Capsicum frutescens

A local variety of pepper produced small knots though in small numbers. Their histology revealed that there was no necrotic reactions. Small but well developed giant cells 95-368 µ × 81-168 µ surrounded the heads of females. The giant cell complexes were made of 5-7 units with dense cytoplasm. Cell wall thickenings upto 14.8 µ were observed. In C. frutescens the nuclei and nucleoli in the cells surrounding the giant cells and female nematode also hypertrophied. Nuclear hypertrophy in such cells was 7-8 fold (26 µ as against 3.5 µ in normal parenchyma cell). In the present study, the biggest nucleoli (14.8 µ) were seen in the giant cells of pepper. The amount of hyperplastic parenchyma however, was comparatively less pronounced. In the absence of excessive proliferation of parenchyma the galls remained small.

Coriandrum sativum

Coriander, the only umbellifer studied, had several small galls. Production of 5-7 giant cells at the feeding sites

that measured 520×235 μ was proof of successful parasitisation. Thickness of the giant cell wall increased only slightly (2.8 μ). Most of the giant cells in fennel had dense cytoplasm. Nuclear hypertrophy was 2-4 fold (from 3.5 μ to 14.8 μ). Such nuclei contained 1-3 nucleoli of about 2.8 μ diameter. Nucleolar hypertrophy was only doubled in giant cells. Even at some distance from the parasite's head cells had hypertrophied (nuclear diameter, 7.5 μ).

Dolichos Lablab

Hyacinth bean grown in garden soil coexisted with root nodule bacteria and produced 3-6 well-developed giant cell units. Several large females were embedded in the nodular tissue and nodular meristem persisted near such sites. Giant cells, 270-325 μ long and 205-230 μ wide occurred. Upto 64 nuclei were present in some of these giant cells. Nuclear dimension increased from 3.5 μ of the normal cells to 14.8 μ in the giant cells. Nucleoli per nuclei varied in number (1-4) and the biggest diameter of such nucleoli was 11.1 μ. Nuclei in hyperplastic parenchyma were 4.9 μ in diameter.

TABLE 5

Variations* in giant cell complexes and hyperplastic cells among different host plants

Host plant	Giant cell complex			Giant cell wall thick-ness (µ)	Giant cell nucleus		Giant cell nucleolus		Hyper plastic cell nucleus diameter (µ)	Normal cell nucleus diameter (µ)
	No. of units	Length (µ)	Width (µ)		No.	Dia. (µ)	No.	Dia. (µ)		
S. melongena	7	840	415	11.1	81	30.0	6	7.4	7.4	4.9
A. esculentus	8	567	225	11.1	83	18.5	5	11.1	11.1	7.4
L. acutangula	9	945	470	11.1	52	45.0	5	7.4	7.4	2.8
C. melo	8	935	540	7.4	67	30.0	3	2.1	2.8	2.8
L. esculentum	8	730	420	11.1	72	17.0	3	2.8	4.9	4.2
C. frutescens	7	338	168	14.8	41	26.0	3	14.8	4.9	3.5
C. sativum	7	520	235	2.8	56	14.8	3	2.8	7.5	3.5
D. Lablab	6	325	230	7.4	64	14.8	4	11.1	4.9	3.5

*From values in 6 readings from 30-40 days old tissues.

3.4 ROOT-KNOT HISTOGENESIS IN SOLANUM MELONGENA

Root tissues and galls were examined during an interval of 5-10 days, upto 2 months. Histological preparations were made to understand the progression of disease and the concomittant histopathological changes.

S. melongena has a profusely branched root system characteristic of dicotyledons. The anatomy of the root was similar to that described by Thiel (1931). Briefly, the stele is diarch and protostelic with patches of protophloem alternating with xylem poles. There are strips of parenchyma separating xylem and phloem. The stele is delimited by a pericycle and endodermis. The cortex consists of loosely arranged parenchyma with starch grains.

Laboratory inoculations had showed that S. melongena attracted M. incognita acrita larvae. The initial infective processes studied revealed that the root cap and meristematic regions were the penetration sites (cf. Chapter 3.1). Fig. 57 shows a club-shaped root removed from vermiculite 2 days after exposure to larvae. The larvae that penetrated moved into root either through or between the cells (figs.13, 14 and 18). Larvae travelled up along the undifferentiated tissue and entered the stele, whereas some others used a route along the cortex (figs. 58 and 59). They reached the zone of tissue differentiation, and started probing the primary vascular tissues (fig. 58). Hypertrophic effects and cell wall damage were caused occasionally (figs.59 and 60). Larvae

generally settled to feed along the primary vascular tissues. Some larvae continued their movements within the root tissues for several days (fig. 12). No attempt was made to determine if these eventually settled and started feeding.

3.4.1 5 Days After Infection

By this time the larvae had already initiated giant cell formation. In a longisection they were located along the stelar region. The cytoplasm was a dense mass filling the expanding giant cell. Nuclei were noticed in clusters, and appeared to be dividing amitotically (fig. 62). These nuclei at later stages proliferated, increasing the nuclear number further. A leading edge of a giant cell component with an aggregation of nuclei is illustrated in fig. 62. The cell walls at this stage had apparently normal thickness. Fig. 63 shows a root T.S. where the giant cell initiation is seen on one side of the primary xylem. The giant cells appear to have originated from phloem cells. The metaxylem elements at later stages merged with the giant cell. At a further distance the giant cell had coalesced with xylem walls (fig. 64). The preparation in fig. 64 had four giant cell units. Phloem patches were included between such giant cells, which had become part of the giant cell as the infection advanced. Compression caused by the expanding giant cell distorted the metaxylem elements (figs. 64 and 65). Giant cell nuclei at this stage showed distinct

hypertrophy, and on an average they measured 7.5 μ in diameter. The cytoplasm was dense and filled these cells.

3.4.2 10 Days After Infection

In a longisection 28-30 larvae with a corresponding number of giant cells were noticed. Such massive invasions caused a total root swelling of 1685-2365 μ almost along the entire root length. A normal root measured only 1100-1200 μ in diameter. Giant cells were produced in stelar and cortical tissues, and in general such giant cells contained 5-7 units. Giant cells at this stage measured 325-420 μ in length and 216-250 μ in width. Their walls were of varying thickness and indicated regions of intercellular coalescence. Also certain cells showed clear evidence of wall disruption possibly due to cell expansion and dissolution of wall material. Walls of giant cells remained thin as in surrounding cells or got thickened from 0.7-6.3 μ. Certain giant cells contained wall incrustations that added to the wall thickness. There was a difference in distance of about 150 μ between the sections shown in figs. 66 and 67. The stele at this portion was of larger diameter as a result of general hypertrophy, though its appearance was more or less normal.

The giant cell cytoplasm was dense and granular. Cytoplasm in some of these giant cells was protruding into the vacuoles, and foldings of plasmalemma were visible in Fig.68. However, certain galls showed giant cells with poorly differentiated cytoplasm. Vacuoles were common in many giant

cells surrounded by a lining of cytoplasm (fig. 69). But some cytoplasm was amassed in the centre with the nuclei clumped together. Nuclear clumping seems to be a regular feature in giant cells (fig. 68).

The giant cell nuclei showed wide variations. Nuclei with almost round to wavy margins in varying numbers and sizes were present in giant cells (figs. 62 and 68). Nuclear number varied from 18-31 per giant cell unit and a giant cell complex contained a total of over 80 nuclei (fig. 68). Nuclear diameters ranged from 5-30 μ (2-3 times larger than nuclei in cortical parenchyma). Nucleolar number ranged from 1-3 per nucleus and the biggest nucleolus measured 5.5 μ in diameter, as against 1.5 μ of normal parenchyma. Certain nuclei did not show the presence of clear nucleoli. Also, many nuclei had micronuclei and certain glistening bodies within. Nucleolar vacuoles were encountered in certain cases.

Hyperplastic tissues were produced from cells in pericyclic and cambial layers. The stimulation for hyperplasia was felt at 405 and 610 μ away from the point of nematode head, in one of the galls. Often, these excessive proliferations occurred around the giant cell sites producing 12-14 layers of cells (175 μ wide) in certain cases. Such growth of closely packed parenchyma contributed to the root swellings (fig. 69). Hyperplastic cells, in general had round nuclei measuring 2-3 μ in diameter. Nucleoli in such cells measured 0.7-1.5 μ.

Initiation of abnormal xylem was noticed in certain roots, at this age. Xylem tracheids had started differentiating around giant cells close to the stelar area and along the direction of the parasite.

In certain cases lateral roots originated by divisions in the pericycle, near the sites of giant cells (fig. 68). The presence of the parasites and the increase in their body size disrupted the cortical and vascular tissues. The giant cell growth caused dislocation of the pericycle and endodermal layers.

3.4.3 15 Days After Infection

An uninfected root transection measured 402 µ in diameter whereas galled root was 605 µ. Many feeding sites or the giant cell complexes were noticed, the biggest one in the specimen studied reaching 540×324 µ. Each giant cell complex had 3-7 units and the biggest one measured 360×175 µ (fig.70). The giant cell units were interconnected by perforations (figs. 71 and 72). They were also coalescing with the adjoining vascular parenchyma.

The giant cells had developed unusually thick walls, uneven at several points. Wall deposits measuring upto 7 µ were noticed. Round, amoeboid and even elongated nuclei (fig. 73) numbering 31, 38 and 41 were distributed in three units of a giant cell complex shown in fig. 74 which were about 240 µ long and 215 µ wide. Another giant cell complex

had a total of 53 (27+17+9) nuclei, the largest one about 15 µ. The biggest nucleus at this stage was 30 µ in diameter. Nucleolar number increased as usual and hypertrophied nucleoli ranged from 1.5-6.3 µ in diameter.

The cytoplasm was dense and granular and almost filled the giant cells (fig. 75). Essentially more cytoplasm is synthesized. This resulted in filling up of vacuoles, which were therefore smaller in number than those in 10 day old infections. However, certain giant cell complexes with 5-6 units looked vacuolate (fig. 76).

Extensive hyperplasia of cambial cells occurred (fig.79). These newly formed cells were longitudinally elongated and storied in appearance. Such tissues contained larger sized nuclei of 5.5-7 µ and bigger than those in normal parenchyma.

Anomalous xylem was extensive and grew around giant cells (figs. 71 and 72). The enlargement of the giant cell pushed apart the vascular elements. Owing to the pressure thus created cells near the endodermis were damaged and stained deeply (fig. 76). The giant cell walls protruded into parenchyma and xylem tissues around it.

3.4.4 20 Days After Infection

The development of the female into a sacciform body and the formation of extensive giant cells compressed the stelar envelope and disrupted the cortical cells. A gall-bearing root at this stage measured 1900 µ in diameter, with

an increase of over 925 μ over the non-galled portion from the same root segment. The giant cell complex was 510×300 μ and the stele including the giant cells occupied 540 μ. On the contrary the normal stele was only 290 μ. In another longitudinal section the diameter difference was between 580 μ in nongalled to 2100 μ in the gall area.

Four to 5 giant cell complexes were usual per transection. In transection a giant cell complex of this age measured 670 μ long and the maximum width was 280 μ. A lysigenous cavity was formed around the head of the female nematode which was 40 μ in diameter (fig. 77). The giant cells extended to either side of the nematode head and in no section the nematode's head was seen protruded into the giant cell.

Wall thickness had continued to increase further. Certain giant cell walls were 10-12 μ thick (fig. 78). In any case the advancing rim of walls remained thin (fig. 79). Cell wall dissolution is a process in giant cell growth and multinucleation is a part of it (figs. 80 and 81). The giant cells were encroaching upon the neighbouring parenchyma whose nuclei could have got pooled into giant cell contents, at later stages. Nuclear size varied from 4-36 μ and the majority of them had 18-22 μ diameter (figs. 81 and 82). Nucleolar number showed a further rise to 5 per nucleus and diameter upto 3.5-7.5 μ (double that of a parenchyma cell nucleus).

Nematodes were most active during this age. The increase in bulk of gall tissue was partly due to the enlargement of the female body and partly to the development of the giant cells and production of hyperplasia (figs. 83 and 84). In such tissues large nuclei of 7.5 μ diameter could be noticed. The cortical parenchyma cells merge with the giant cell complex (fig. 85). Gall parenchyma cells were induced to further changes. Conversion of hyperplastic tissue into xylem near the giant cells and females had taken place and they had considerable lignin deposition. Whether this was a jacketing process of offering additional strength to the expanding giant cell or host-reaction to prevent the spread of infection was uncertain. Xylem also merged with the giant cells, for broken walls were seen projecting into giant cell cytoplasm (figs. 79 and 80). These observations lead to the belief that giant cells could originate from practically any living cell. Their expansion by addition of all types of tissues is a usual feature in root-knot development.

3.4.5 30-40 Days After Infection

By this stage the nematodes had produced a gelatinous eggmatrix and started laying eggs (figs. 86 and 87). The presence of the eggmatrix produced necrotic effects in the surrounding cells. The nematode and eggmatrix lay in a cavity that in some instances measured 840 μ in length and 315 μ in width. The space occupied by the animal in the root measured 580×310 μ. The secondary xylem in the root

section had an anomalous way of growth taking a radial orientation. Each component looked bigger. In a longitudinal section (fig. 88) two females and the associated pathological tissues were clear. These females measured 540×350 μ and 432×230 μ respectively. There was a wedge of parenchymatous cells separating the two nematodes. The cytoplasm was gradually vanishing from such giant cell complexes. However, there was more cytoplasm at the leading edge of giant cells (fig. 89). Nuclei were still present in them. Walls showed several breaks.

Infections caused much of the secondary vascular tissues to be obliterated. There was significant tissue damage (fig. 90). Fig. 91 shows a similar tissue from which a comparison of the damage to xylem could be made out. The giant cell complex in this case, produced from tissues destined to become secondary xylem left a big cavity of size 290×165 μ. Those xylem tissues that were around the cavity were radially oriented, instead of the usual longitudinal manner.

Parenchymatous cells grew over the parasite as gall development proceeded. This characteristic overgrowth above the females situated in the cortex produced lateral bulges (fig. 92). Galls collected beyond these stages showed many dead females and their eggmasses inside the root tissue or protruding out of the root surface. The eggs hatched within and caused secondary infections.

Egg laying masked the completion of the life-cycle. The females did not survive once the eggs were laid. The death of the females caused a breakdown of giant cells whose cytoplasm in histological preparations appeared darkly stained and turbid. Figs. 93 and 94 show a number of giant cells and dead females. In fig. 95 a transection of a heavily damaged root, whose vascular elements were completely destroyed except for patches of abnormal xylem, is shown. The disruptions caused in the conducting elements resulted in wilting of the infected plants.

3.5 RESPONSES OF GIANT CELLS TO CERTAIN HISTOCHEMICAL STAINS

Three methods were used for localising DNA and all of them provided positive results. The Feulgen stain was restricted to nuclei with a negative reaction occurring in the nucleoli. Intense crimson colour indicated high concentrations of DNA in giant cell nuclei (figs. 96 and 97). Normal root parenchyma did not show any perceptible colour. To a certain extent the nuclei of hyperplastic cells were reactive and produced comparatively visible colour. The nuclear periphery showed the most intense staining and there were masses of basophilic bodies in the fissures. Also, there were several deeply stained Feulgen bodies distributed in the interior of the nuclei. Clumping of nuclei and amitotic duplications are indicated in the preparation in fig. 96.

Methyl green-pyronin and Azure B methods were also employed. With the former nuclei were stained greenish-blue. All nucleoli and the dense cytoplasm retained intense red staining (fig. 98). This indicated the occurrence of cytoplasmic RNA of high concentration and increased levels of RNA in nucleoli. Pyronin-positive bodies were distributed throughout the cytoplasm. Also present in the nucleoli were certain glistening bodies that stained deep red (fig. 98). Such bodies appeared negative in the Feulgen preparations. Nematode body was highly pyronin-positive. Sections of female nematodes embedded in root tissues contained portions of ovary

that had methyl green- and pyronin-positive substances.

Azure B method helped to resolve the sites of nucleic acids and incrustations on giant cell walls. The nucleoli and cytoplasm stained blue with Azure B. The nuclei were greenish in colour and the wall incrustations showed a rich blue. Fig. 99 is a photograph of an Azure B stained preparation in phase contrast illumination. Heavy staining of the deposits in walls is clear from fig. 100. Also, in such preparations was an intense staining reaction along the inner walls of giant cells.

The PAS procedure was positive with the walls of giant cells and indicated that walls were rich in insoluble polysaccharides (fig. 101). The deposits also appeared PAS positive. The PAS reagent imparted a crimson colour to starch grains also. The number of starch grains in infected roots was small and those that were present showed a faint staining.

3.6 CHANGES IN CHEMICAL CONSTITUENTS DUE TO INFECTION

3.6.1 Nucleic Acids

Nucleic acids were determined from normal and gall tissues of S. melongena, A. esculentus and L. acutangula. RNA was isolated by alkaline hydrolysis and DNA by perchloric acid extraction. The two nucleic acids were estimated separately by the absorbance of their purine and pyrimidine bases in the UV region (260 nm). The data given in Table 6 and fig. 116 A and B show the relative changes in levels of extractable nucleic acids. Concentrations of both the nucleic acids present in the normal and gall tissues of the three hosts varied. They were high in gall tissues.

L. acutangula galls showed the highest increase in RNA followed by A. esculentus. Giant cells were found to contain dense cytoplasmic masses that were pyronin and Azure B - positive. Giant cell nucleoli of higher numbers and sizes, and the huge mass of cytoplasm have possibly contributed to the high concentration of RNA.

DNA estimation showed significant changes in concentration in gall tissues from that in the normal, the highest being in A. esculentus. In the other 2 hosts the increase was comparable. Increased number of abnormally large nuclei that were positive to Feulgen and other histochemical stains present in giant cells, appeared to contribute to the high amounts of the nucleic acids.

TABLE 6

Concentrations* of nucleic acids in normal and M. incognita acrita infected roots

Component	Host plant	mg/g tissue (fresh wt) Normal	Gall	Increase (%)
RNA	S. melongena	2.27	2.67	17.60
	A. esculentus	1.44	1.88	30.50
	L. acutangula	1.68	2.42	44.04
DNA	S. melongena	0.48	0.84	75.00
	A. esculentus	0.43	0.79	83.70
	L. acutangula	0.73	1.29	76.70

*Estimated by UV absorption at 260 nm with Beckman DU 2 spectrophotometer

It may be stressed here that the actual increase in the concentration of the nucleic acids in giant cells is, in all possibility much more than the values obtained, because of the presence of parenchyma cells around the giant cells in gall which have lesser quantities of nucleic acids.

3.6.2 Proteins

Proteins were extracted from the same tissues used for nucleic acid determination. The protein fraction was separated at the end of the isolation procedure (Chapter 2). Results given in Table 7 and histograms (fig. 116c) established that proteins, as indicated by the colour produced by all the amino acids in the hydrolysed protein isolate increased discernibly in galls of host species. Variations existed between individual estimations, in gall tissues, which could be due to the presence of varying numbers of female nematodes embedded in them. Also the possible difference in the number of giant cell complexes in such galls and the amount of cytoplasm could contribute to the increase and variations.

3.6.3 Amino Acids

A total of 13 amino acids were found to occur in the normal roots of S. melongena (Table 8). An amino acid that occurred in between glutamic acid and valine in the butanol: acetic acid:water system did not correspond to any of the reference samples. The Rf, however, corresponded to Y-amino butyric acid (Stahl, 1965).

TABLE 7

Protein* concentrations in normal and *M. incognita acrita* infected roots

Host plant	mg/g tissue (fresh wt)		Increase (%)
	Normal	Gall	
S. melongena	49.05	76.54	56.04
A. esculentus	31.70	60.31	90.80
L. acutangula	28.33	39.63	28.50

*Estimated with Folin-Ciocalteu reagent at 750 nm with Beckman DU 2 spectrophotometer

TABLE 8

Free amino acids and amides in normal and *M. incognita acrita* infected roots

Amino acid	*S. melongena*		*A. esculentus*	
	Normal	Gall	Normal	Gall
γ-Aminobutyric acid	+	3+	-	-
Arginine	+	2+	+	2+
Alanine	+	3+	+	+
Asparagine	-	-	+	2+
Aspartic acid	2+	3+	2+	3+
Cystine	=	+	=	+
Glutamic acid	2+	3+	+	3+
Glutamine	-	-	=	+
Glycine	=	+	=	+
Histidine	=	+	=	+
Leucine	+	2+	2+	3+
Phenylalanine	-	+	+	2+
Proline	+	3+	-	2+
Threonine	=	+	+	2+
Tryptophan	-	-	-	=
Tyrosine	+	2+	2+	3+
Valine	=	+	+	2+

- absent = trace + moderate

2+ intense 3+ abundant

In A. esculentus 14 free amino acids occured. Two amides, asparagine and glutamine, were present in both normal and gall tissues. Y-Amino butyric acid was absent in okra roots.

There was a marked increase in the levels of all the amino acids due to infection. This is clear from the relative concentrations represented in Table 8. Several amino acids that could not be located or occured in traces gave strong spots in gall extracts. Such increases in free nitrogenous compounds appear to be a common feature in Meloidogyne infection. Significant were the occurrence of proline in A. esculentus and phenylalanine in the galls of S. melongena. Notable were the increases shown by Y-amino butyric acid, alanine, arginine, aspartic acid, glutamic acid and proline. The occurrence of tryptophan in A. esculentus is interesting. This appeared only in the gall tissues.

3.6.4 Sugars

Among soluble sugars glucose, sucrose and fructose only were found to be distributed in the root tissues of the plants studied. L. acutangula contained only sucrose and fructose. These sugars were found in both normal and infected tissues. However, there was notable reduction in their concentrations in gall tissues. In normal roots of S. melongena sucrose and glucose were present in high concentration. In A. esculentus a higher concentration was shown by glucose. Table 9 gives the relative concentrations of soluble sugars and changes due

TABLE 9

Soluble sugars in normal and *M. incognita acrita* infected roots

Sugar	*S. melongena*		*A. esculentus*		*L. acutangula*	
	Normal	Gall	Normal	Gall	Normal	Gall
Sucrose	3+	+	+	+	+	-
Glucose	+	=	2+	=	-	-
Fructose	2+	+	+	-	2+	=

\- absent, = trace, + moderate

2+ intense, 3+ abundant

to infection.

3.6.5 Indole Compounds

Indolic growth substances were analysed in normal and gall tissues of S. melongena, A. esculentus and L. acutangula. L. acutangula extracts were employed only in TLC. Plants grown for 30-40 days after inoculation were used, and their extraction procedure is described in Chapter 2.

Initial chromatographic studies with 1-5 g tissues were not successful, hence 25-100 g starting materials were used in further studies. Concentrated oily extracts from 100 g fresh wt of tissue were shaken with hexane to remove the lipoid substances. The residue was solvent partitioned at different pHs and divided into 2 halves for TLC and bioassay. The acidic portions only were used in bioassay.

Ether extracted residues were purified by column chromatography through alumina. Of the 6 fractions only the ether, methanol and acetic acid fractions were used for TLC.

Thin layer chromatography

Extracts were developed on activated thin layers of silica gel. An acidic solvent system, chloroform : 96% acetic acid (95:5) was used. Identical concentrations of normal and gall tissue extracts were spotted on activated chromatoplates.

Reference compounds of indole (IND), anthranillic acid (AA), indole butyric acid (IBA), indole propionic acid (IPA), indole acetic acid (IAA), indole acetamide (IAM), tryptamine hydrochloride (TNH_2), and tryptophan (TRP) were also chromatographed.

Indole compounds were detected by scanning the chromatograms in UV light and spraying the plates with the Salkowski reagent or the new ferric citrate reagent. The ferric citrate reagent is a new formulation developed during the present study for the chemical determination of IAA. This reagent was quite efficient in the detection of most indole derivatives. The composition and uses are given in Appendix 1. Occasionally the Ehrlich's reagent (1% p-dimethylaminobenzaldehyde in 25% HCl + 96% ethanol) was also employed.

Table 10 describes the results of TLC of identical concentrations of normal and gall tissue extracts. The Rf×100 values and colours produced by extracts and indole compounds with both the reagents are also given.

Acidic fractions from ethanol extracts of gall tissues from S. melongena and A. esculentus showed ash coloured spots in UV light. In the corresponding areas of normal tissue extracts of both S. melongena and A. esculentus faint zones were seen when scanned in UV light. Fractions from acetic acid eluates after column chromatography also showed similar fluorescing compounds in corresponding positions. The plates

TABLE 10

Chromatographic and chromogenic reactions of indolic compounds from normal and M. incognita acrita infected root tissues

Sample	Rf (×100)	Colour produced	
		Salkowski reagent	Ferric citrate reagent
S. melongena			
Normal	ft+26-29 (27)	None	None
Gall	st 24-31 (27)	Pink	Pink
A. esculentus			
Normal	ft 25-26 (26)	None	None
Gall	st 25-28 (26)	Pink	Red pink
L. acutangula			
Normal	st 43-47 (45)	Pale green	Green
Gall	st 43-48 (45)	Green	Deep green
Indole	71	Orange red	Brown
Indole acetonitrile*	(46)	Green	-
Anthranillic acid	37	Yellow	None
Indole butyric acid	34	Red brown	Red pink
Indole propionic acid	32	Brown	Orange
Indole acetic acid	26	Pink	Red pink
Indole acetamide	11	Pink	Deep pink
Tryptamine hydrochloride	00	Brown	Golden yellow
Tryptopham	00	Brown	Brown

+In UV light ---ft = faint colour, st = strong colour

*Not chromatographed, data from Stahl (1965)

were subsequently sprayed with one or the other chromogenic reagent. The gall extracts produced strongly coloured areas, whereas the normal tissues did not give any perceptible colour in visible light.

The compound that fluoresced in UV in the case of S. melongena had an Rf position 24-31 (27). In the case of A. esculentus gall, the compound remained between 25-28 (26). The fluorescing areas of normal S. melongena and A. esculentus marked in UV light showed positions 26-29 and 25-26 respectively. The closest corresponding colours in UV and with the reagent was shown by IAA (Rf 26).

Acidic and non-acidic fractions of L. acutangula extracts did not contain any colour characteristic of auxins employed in the present studies. But the ether extracts cleared with hexane produced a bluish green fluorescence in UV light. With both Salkowski and ferric citrate reagents this compound from both normal and gall tissues produced a green colour. With Ehrlich's reagent it was light yellowish in colour. The colour produced by extracts of gall and normal root were quite strong with ferric citrate reagent. The Rf value was 43-47 (45). Stahl (1965) attributed bluish-green fluorescence and Rf 46 in chloroform:acetic acid system to indole acetonitrile (IAN), a neutral auxin.

Spectrophotometric determination

The amount of the colour produced by acid auxins when sprayed with Salkowski or ferric citrate reagent could be

determined spectrophotometrically also. Extracts corresponding to 100 g tissues of normal and galled roots were taken up in water and mixed with the chromogenic reagent and their absorbance measured.

The Gordon and Weber (1951) version of Salkowski reagent and the new ferric citrate reagents were used. The composition of both and the advantages of the latter are described in Appendix 1. Preparative TLC was carried out to purify the solvent partitioned acidic fraction further. These plates were scanned in UV light and the fluorescing zones along with some surrounding adsorbent were scraped out. The compounds were eluted in methanol by shaking. Adsorbent silica gel was eradicated by centrifugation. Methanol was evaporated and when about to dry, the compounds were dissolved in known volume of distilled water.

Two ml of the reagent + 1 ml of the extract were mixed and kept in dark for 1 hr. The red pink colour developed was measured at 530 nm with a Hitachi Perkin-Elmer spectrophotometer.

Blanks with water in place of auxins or extracts were used to make the necessary corrections in absorbance of the latter. Absorbance was calibrated by referring to standard curves (Appendix 1) and the concentrations of extracts are shown in µg per 100 g (Table 11 and fig.117A).

TABLE 11

Concentrations* of indolic compounds in normal and M. incognita acrita infected roots

Host plant		Gordon and Weber reagent	Ferric citrate reagent
S. melongena			
	Normal	0.08**	1.04
	Gall	4.72	7.81
	Increase	4.64	6.72
A. esculentus			
	Normal	0.58	1.50
	Gall	7.01	10.20
	Increase	6.43	8.70

*Measured at 530 nm with Hitachi Perkin-Elmer spectrophotometer

**µg/100 g fresh tissue

Bioassay

Fig. 117B shows the results of the assay experiment to test the biological activity of wheat coleoptile elongation in normal and M. incognita acrita induced galls. The extracts tried were from the fluorescing zones in thin layer chromatogram, as also from the corresponding acidic fraction of ethanol extracts. Between the normal and gall tissues, growth stimulation was greater and 3-fold in the case of A. esculentus. However, the activity difference was greater in the extract of S. melongena among the galled tissues. The results corresponded with the higher values for gall tissues in spectrophotometric determination also.

It is interesting that normal root tissues of both the hosts possessed certain growth stimulating substance(s) and that hyperauxiny occurred under pathological conditions brought about by root-knot nematode infection.

3.7 DEVELOPMENT OF MELOIDOGYNE INCOGNITA ACRITA IN SOLANUM MELONGENA

Six developmental stages have been reported by Christie and Cobb (1941) in the life cycle of root-knot nematodes. Females of M. incognita acrita laid eggs in a gelatinous matrix (egg sac). As the organism is endoparasitic and sedentary, the eggmasses were produced in situ and they were extruded into the root tissue. Often the egg matrix containing the eggs in groups protruded out on the root surface. The gelatinous matrix inside the host was pale white and mucilagenous, while exposed ones turned brown and accumulated soil particles.

The eggs of M. incognita acrita are elongated with smooth outline. They are slimy and stuck together. Fig. 102 shows several eggs teased out of the egg-matrix and cleared in lactophenol. The eggs are filled with cytoplasm, but in some cases the cytoplasm had withdrawn from both the elongated ends. Such a change is known to precede embryo development. Repeated divisions in the egg produced a gastrula which later get organised into the first stage larva.

The first stage larvae while in the egg showed rotating movements. From the eggmasses many second stage larvae emerged within a day or two, some emerging even on the same day. Egg matrix teased out in water released several larvae showing that some had already hatched and were trapped in

the matrix. These second stage juveniles are vermiform and resembled their adult males except for the shorter body length and the absence of sex organs. These juveniles moved about vigorously. They represent the infective stage and their behavioural details are described in Chapter 3.1.

The larvae from macerates of roots 2 days after inoculation resembled the preparasitic larvae. Figs.103 and 104 show root clearings with the larvae imbedded inside S. melongena roots. Larvae dissected out 5 days after inoculation were thicker (fig. 108). Their body length remained more or less the same but the width increased almost two fold. Such a change was subsequent to the nematode lodging itself at a suitable site within the host and actively feeding, preparatory to the next moult. These larvae bore characteristic tail spikes at the cuticular ends, remniscent of the pointed tail tip of the preparasitic stage. In juveniles observed on the 7th day thebody was sausage-shaped (fig. 109). Here the body, and especially the posterior region, looked thicker. The larvae had entered the third stage and the moult was indicated by the presence of third stage larvae within second stage cuticle (fig. 110). The body mass separated from both the spike and the head ends. In most of the larvae the body showed curves and bends indicative of the position they had occupied in the host tissues (figs. 108 and 109). Fig. 105 shows a cleared root in which several larvae were developing around the stele of a root. Fig. 106 shows one sausage

shaped larva imbedded in root tissue and fig. 109 is of another, teased out from root. The genital primordium was initiated at this stage in the tail end of the larvae.

In a 15-day old specimen, the gonads were present and looked bifurcated indicating differentiation into the female. In another, paired ovaries measuring 1/3 of the body length were noticed. Such animals were encased in additional layers of cuticle. Fig. 111 shows one such dissected out of root tissue. In 20 day- old collections, females with elongated ovaries covering almost 2/3 of the body length were visible.

Along with gonad formation, in the larvae differentiating into females, several rectal glands developed near the valve and these were present in 15-day and 20-day collections. More moultings increased the number of cuticular covers and nematodes enclosed in three layers were often encountered. Females carefully teased out of roots had sloughs of cuticle including that of the second stage with spikes. This observation revealed the fact thst after settling at a point the larvae differentiating into female nematodes do not move away, but become sedentary.

Twenty to 25 days after inoculation females with spherical posterior and coiled ovaries were abundant (fig. 112). Fig. 107 illustrate a female imbedded in host tissues. The pressure created by the growth of the posterior end had caused swelling of the root surface. Between 30-40

days many eggmasses protruded out of root surface. Eggs were sticky and aggregated in the egg matrix which was connected to the posterior end of females. The egg matrix appeared to be secreted first by the animal into which the eggs were laid.

Males were occasionally seen. They were distinguishable right from the 15th day. They developed into elongate forms and were folded several times in second stage larval cuticle (fig. 113). Rotating movements of the sort that first stage larvae made, were exhibited by males also. In a larval cuticle a male was noticed folded 4 times. Males also possessed stylets and possibly they are used to break open the egg and second stage-cuticles.

In the males, females and larvae, the head region is narrow. The cephalic end bears characteristic 'cheek-like' structures. Cuticular annulations are noticed on the anterior ends of males and females. Prominent oesophageal bulbs and their valves and stylets are clear in the females. Stylet and its position are clearly brought out in illustrations (figs. 114 and 115).

CHAPTER 4

DISCUSSION

4. DISCUSSION

The present study shows that the root-knot nematode, M. incognita acrita is successful in infecting all the test plants under artificial inoculation. Also, collections from the field established that natural infestation is quite usual and abundant.

More than 40% of the inoculated larvae could penetrate S. melongena and A. esculentus and produce characteristic feeding sites, with abnormally large giant cells. Galls were produced in the roots of all the hosts in response to the nematode secretions. The parasites could feed, grow in size and lay eggs in abundance within the root tissues, that protruded out of roots later. The second generation of larvae emerging out of egg masses could affect secondary infection in the gall tissues or near-by root segments. The pathological tissues produced sum up the effects of nematode activities within, and the response of the root tissues to the infection. The injection of salivary secretions is toxic to the susceptible host cells, to the extent that metabolism of a few cells falls under the direct control of the invader (Linford, 1937 and 1942) and the existence of such cells depends on the presence of the parasite and continuous stimulus from it (Bird, 1962).

4.1 NEMATODE BEHAVIOUR, INFECTION AND INITIAL HOST RESPONSES

Infective second stage larvae of M. incognita acrita are attracted to the roots of both S. melongena and A. esculentus and it is presumable that root emanations are responsible for this. Bird (1959) and Peacock (1959) have clearly shown that substances produced by living root tissues attract nematode larvae. Lownsberry and Viglierchio (1960) held that the accumulation of M. incognita and M. hapla in tomato is due to dialysable root emanations effective beyond the root surface. Wallace (1960) considered the possibility of random movements and habituation but favoured a theory of orientation towards an attracting chemical exudate. Bird (1960) believed that protoplasm in the surface cells of the roots plays a major role in maintaining the larvae close to the root as well as their penetration. Contrastingly, neither concentrated nor dilute tomato exudates or any sugars could exert significant attraction. In the studies of Peacock (1959) on L. peruvianum root tips were seen to retain resistance to M. incognita even after 27 transfers in culture. This means resistance or attraction is perpetuated by root tissues. Griffin (1969) investigated the attractiveness in D. dipsaci and M. hapla and concluded that larvae are attracted to both susceptible and resistant ones but to a higher degree to the former. Further, as hosts, nonhosts and several rhizosphere microbes attract nematode larvae (cf. Wallace, 1963), the reaction should be one mediated by certain chemical stimuli.

The role of amino acids, plant growth regulators and inorganic salts present in root exudates in attractiveness appears to be worth investigating. Auxins are secreted from plant roots (Vancura, 1964). The agglomeration of many larvae around root tips of eggplant, and okra in all possibility indicates M. incognita acrita attraction in response to root exudates.

Esterases are present in the amphidial pouches of M. hapla and M. javanica (Bird, 1966) and it has been suggested that in the larvae these enzymes have an important role in their orientation towards food sources.

No repelling action was shown by growing roots of S. melongena and A. esculentus, for all the root clearings after inoculation contained nematode larvae. Nor was there any region of repulsion in S. melongena. Infection in S. melongena along the root cap and meristematic zones in high numbers and also along the region of elongation and differentiation at times provides evidence against Wieser's (1955 and 1956) theory of attractive, neutral and repellant zones. Peacock (1959) found no evidence to support Wieser, for in the former's experiments with tomato under aseptic conditions, larvae were found to be attracted to an area closely behind root meristem. Similar observations were made by Christie (1936) for H. marioni and Huang (1966) for M. incognita. These authors could not visualise any special area of infection. Krusberg and Nielson (1958)

found that M. incognita acrita larvae penetrated the root terminals as well as further up the root of sweet potato. They have recorded larval entry even through the periderm.

The present work shows that the second-stage larvae attracted by the roots of S. melongena and A. esculentus are capable of invading the roots breaking a variety of host tissue barriers. Such penetrations are common in tomato, ginger and sweet potato. Heald (1969) showed that M. graminis infecting certain monocotyledons entered the root tip just posterior to the root cap and migrated parallel to the vascular system, and ultimately settled to feed from xylem tissues. According to him more than one larvae were feeding from the same point.

Nematodes of other genera, such as Rotylenchulus uniformis, Tylenchorhynchus dubius, Pratylenchus penetrans, P. crenatus and Hemicycliophora arenaria were inoculated on a variety of plants by Klinkenberg (1963) and most of these showed multiple invasions along a number of sites. Penetration by Pratylenchus occurred anywhere along the roots and in the case of Radopholus similis roots were generally penetrated behind the tips (Birchfield, 1957). T. dubius was attracted to root hairs and epidermis behind root meristem, a condition according to the pioneer worker Linford (1942) not shown by root-knot nematodes. In the present work, larvae of M. incognita acrita, were seen

invading root hair region of A. esculentus. According to Widdowson et al. (1958) the potato golden nematode juveniles (H. rostochinesis) are attracted by the zone of elongation.

Some hosts are highly suitable for a given race of root-knot nematodes. According to Christie (1946) there is no correlation between the suitability of the host and the freedom with which larvae enter its roots, for many non-hosts are also invaded by nematodes. He also showed a contrasting situation by reporting that given an equal opportunity larvae enter some plants, only in very small numbers.

The experiments carried out to assess infectivity of M. incognita acrita in S. melongena and A. esculentus showed that the present population was quite virulent. The noteworthy difference between 12 and 24 hr inoculation in both the hosts indicated increase in larval number with passage of time and a sharp rise in infection rate owing to attractiveness following initial penetrations.

However, the two plants showed differences in that larval attraction ceased in S. melongena by 48 hr whereas they continued to enter A. esculentus seedlings even after the 60th hr. Factors such as pressure due to high nematode number, decrease or loss of attractiveness and production of inhibitors, might have limited the larval number in S. melongena. However, difference between 48 and 60 hr inoculations in A. esculentus was not significant, meaning

that for any given host species, root-knot nematode infectivity is somewhat uniform and that the differences are caused by the host. The differences in the results in Tables 2 and 4, 3 and 4 and individual seedlings must be due to inherent differences in attractiveness, or delay in nematode movement through vermiculite.

Barrons (1939) reported no discrimination among resistant and susceptible seedlings as far as the number of larvae was concerned.

Defoliating the seedlings reduced attractiveness in both the hosts. It is likely that attractiveness depends to a certain extent on substances produced by the seedling foliage. Hale et al. (1973) citedevidences that substances such as auxins sprayed to leaves are excreted through root leachates. Further, the low number of larvae in excised roots, cleared of leachates emphasises the role of leachates and growth activity in attracting larvae.

In the present study, M. incognita acrita generally attacked the root-tip cells of S. melongena. This situation though less marked, was common in A. esculentus also. Root tips are composed of tender and loose parenchyma that are favourable for penetration. Mollenhauer (1967) held that all terrestrial and epiphytic roots have some secretory activity from root cap cells and Moré et al. (1967) have shown that a certain polysaccharide is secreted by root cap cells. Study of such substances and their role in

attractiveness and penetration of M. incognita acrita would explain the reasons for larval preference to such sites.

The incidence of penetration through natural openings and fissures created by pioneering larvae appeared to be easier. Emanations from such sites may behave as attractive agents. Restricted invasion of old roots may be due to reduced attractiveness and cell wall characteristics. Although M. incognita acrita could penetrate almost any point of both S. melongena and A. esculentus roots, maximum number of larvae were drawn towards the root cap and meristematic area; both composed of soft, undifferentiated and metabolically active tissues. According to Bird (personal communication) the larvae may prefer the root tips as they find it easier to induce syncytia in undifferentiated meristematic tissues than in mature differentiated ones. Thus penetration although a specific characteristic, is influenced by the texture of host tissues and points of root exudations.

Loewenberg et al. (1960) felt that the use of excised roots by Wieser (1955 and 1956) might have caused larval repulsion. But Peacock (1959) showed that though excised, a growing root tip in culture is attractive. He also held that this response is lost when apical dominance ceases. Active root tips were found to be more attractive in the present experiments too. Excised roots washed in water for a few hours were not infected equally (did not remain attractive) during the initial hours of exposure. The

leaching away of exudates may have reduced the attractiveness of the root resulting in fewer larval entry.

Infectivity studies showed that M. incognita acrita infection in S. melongena is reduced after 2 days of exposure. Perhaps the roots might have lost attractiveness due to disturbances caused by larvae and possibly subsequent production of inhibitors.

Several larvae clinging to a point through which another has successfully penetrated indicates that a pioneer larva produces a port of entry and attracts others. Bird (1959) has shown that M. hapla and M. javanica larvae attract each other. However, Wallace (1966) held that nematodes probably have little influence on each other during penetration, except at high population levels when inhibition may occur. But larval congregation in certain roots observed under the present experimental conditions indicated that as far as entry is concerned, the labour of a pioneer nematode and its intermittent probings, help others in their search for suitable sites. Also, larval polarisation at a site penetrated once is common, probably because of attractants emanating from such points and the relative ease in entry. Multiple invasions of apical meristems of secondary roots have been observed by Endo (1964) in H. glycines also.

Mankau and Linford (1960) reported counting 50 larvae of H. trifolii, conglomerated around a wound and a few

distributed over other areas. Nematodes could abide in certain hosts studied here, by entering along the breaks of secondary roots. These observations indicate that mechanical injury can intensify infestation. Under field conditions in addition to attack on root tips these sites may also be used by larvae.

Larval movements on the root surface and probings as observed are similar to the reports of Linford (1942) for H. marioni and Zuckermann (1968) for Tetylenchus joctus infecting cranberry roots.

Root-knot nematodes invaded S. melongena and A. esculentus in less than 6 hr although exceptions occurred. Penetration within 15 min was reported for H. trifolii by Mankau and Linford (1960). Endo (1964) showed that penetration by H. glycines took less than 4 hr. P. crenatus larvae probed on Poa annua root for 2 months (Klinkenberg, 1963), while Radopholus similis took 24-72 hr for invading citrus roots (Du Charme, 1959). Thus host penetration by different nematodes shows variations. Physiological variations in larvae and texture of host tissues at infection sites probably influence the time taken for entry.

After entry, migration in S. melongena and A. esculentus, as in most other hosts, is generally along the intercellular spaces although larvae are capable of heading through the cells also. Such migrations through root tips do not kill the apical and subapical tissues though the consequent

macerating action would upset the coordination of tissue differentiation. Linford (1942) explained that H. marioni separates middle lamellae of host roots with the stylet. Entry into root is facilitated by stylet thrusts, and possibly also by enzymatic secretions that may breakdown the hindering cell walls.

M. incognita acrita and other species of Meloidogyne are known to contain a variety of cell wall degrading enzymes like pectinases and celluloses (cf. Dropkin, 1966), which may help in penetration. Intracellular penetrations too occurred but are not characteristic, as are known, for cyst nematodes (Mankau and Linford, 1960).

The arched position which nematodes adopt prior to penetration is known to orientate their stylet at right angles to the root and provide maximum thrusts (Wallace, 1963).

As observed in S. melongena, Russel and Perry (1960) noticed larvae and even adult worms of Trichodorus christiei thrusting aside loose cells of wheat root cap apparently for feeding in root meristem. This trend, they believe, is responsible for severe injury to meristematic cells. Essentially, deranging the growth pattern of meristem and mechanically damaging the tip devitalise the infected roots.

4.2 GALL FORMATION AND HISTOPATHOLOGY

Root-knot nematode attack reduces the growth vigour of the roots, and when infection is severe, destroys the growth-potential of root tips. In soil where a heavy population of nematodes exists, injuries to germinating seeds may be sufficient to prevent them from emerging and establishing. Linford (1942) brought forward evidence for root injury by Heterodera marioni (Meloidogyne sp.) through direct observation. Chitwood et al. (1952) reported considerable injury to peach root stocks by two species of Meloidogyne. According to them, even a species that failed to develop within, caused much damage to young roots.

Massive larval invasions resulting in spindle formation of root tips and inhibition of forward growth may be assigned to cell enlargement subsequent to nematode entry and effect of their emanations. Check of forward growth may be due to the mitostatic action of growth regulators in saliva (Viglierchio, 1971, and Krusberg, 1971). Hypertrophy and cessation of meristematic activity are responses similar to application of growth substances and certain amino acids (Murray and Whitning, 1946). There are indications of nematode larvae exuding such substances into the incubating medium (Krusberg, 1971). It is interesting to recall that tumour induction coupled with polyploidy, giant nuclear formation, and anomolous cell divisions were caused by external application of apiol whose active ingradient is allyl-1-dimethoxy-2-5-methylenedioxy-3-4-benzene. Also,

roots are known to be very sensitive to exogenous supply of auxins (Sheldrake, 1973).

Godfrey and Oliveira (1932) found retardation of elongation within 48 hr, even before the larvae could lodge and start feeding. Christie (1936) reported this to occur within 36 hr. Inhibition of mitosis in root apical meristem appears to be common with Belenolaimus, Hemicycliophora and Trichodorus also (cf. Seinhorst, 1961).

All the host plants studied here produced galls in response to M. incognita acrita infection. Galls produced on the cucurbits, L. acutangula and C. melo, and those on S. melongena, A. esculentus and L. esculentum were relatively large ones, whereas hosts like C. sativum and C. frutescens had small galls. In Dolichos, the nematodes and nodule bacteria occupied the same root tissue. Presence of the nematode makes the nodules abnormally large. Gall sections have shown active nodular meristem, which means that the two organisms can coexist well. Taha (1968) has shown that Rhizobium trifolii and M. javanica and H. trifolii could be grown together. According to him nematode infection did not reduce the number and size of nodules.

Thus, gall size appears to be related to host species and essentially indicates the host response. Also different species of nematodes are known to produce galls of varying sizes. Davis and Jenkins (1960) found variations in the size of galls produced by 3 species of Meloidogyne on gardenia.

It is of interest to recall the report of Steiner and Buhrer (1934) that H. marioni produce galls on Thumbergia grandiflora, Rheum rhaponticum and Begonia sp., reaching a diameter of $1\frac{1}{2}$ feet in about one year. These galls are known to be rugose and hard with lignified cells. Steiner and Buhrer (op. cit.) believed that such galls are produced due to autoinfection by successive generations. The conspicuous and amorphous galls in L. acutangula and S. melongena observed in the present work are also produced by multiple infections and successive invasion by M. incognita acrita larvae.

It may be mentioned in this context that galls are generally absent in Heterodera infections. This is regarded by Mankau and Linford (1960) to be due to the entry of infective larvae at regions considerably more mature than the root tip. Endo and Veech (1969) attributed this to the low number of Heterodera larvae getting established in the proximity of apical or lateral meristems. Both these explanations do not appear sound. However, in certain hosts like tomato, H. rostochiensis produces galling (Sembdner, 1963)

Movements of M. incognita acrita larvae within the hosts show similarities with published works (Christie, 1936; Krusberg and Nielsen, 1958; and Dropkin and Nelson, 1962). Hypertrophy of cell and nuclei along nematode path is regarded as effects due to salivary secretions. There

were no necrotic cells around the larvae or feeding females in S. melongena or A. esculentus. Smith and Mai (1965) noticed occasional necrotic cells but did not consider them significant. The varieties employed in the present study are highly susceptible and hence did not show any hypersensitive reaction as demonstrated by Crittenden (1958) and Dropkin and Nelson (1962). M. incognita caused extensive lesions in storage rhizomes of ginger (Huang, 1966).

In all the hosts the larvae showed a preference for lodging in vascular tissues. Also, the usual tendency of larval head orientation was towards the proximal end of the root, i.e. the morphological base of the plants. A similar situation was noticed by Linford (1941). Such a polar orientation at the time of loding suggests certain relation to translocation through vascular bundles. Perhaps the nematodes lie along the direction of the gradient of food and water. All previous reports show larval preference to vascular elements. Larvae entering from the radial aspects were seen to penetrate the endodermal walls. Bird (1967) reported the mobility of larvae in steler tissues 2 days after penetration.

Second stage larvae seen in root clearings several days after inoculation when others have lodged, and swollen up as a result of feeding, means that they were moving about. Wallace (1961) postulated that lack of nutrients is responsible for movement of A. ritzemabosi in host tissues. This does

not seem to be applicable for S. melongena which is a good host for M. incognita acrita. Veech and Endo (1969) have observed undifferentiated larvae even 21 days after inoculation.

The larvae that settled to feed induced the formation of giant cells. The giant cells represent a unique type of pathological tissue produced around the head of the nematodes, tumorous in nature but nutrient supplying to the incitant nematode, in function. The giant cells are usually initiated in the stele and their formation interrupted the vascular tissues. Substances from the conducting elements in such situations can be drawn into the giant cells, from which the nematode can derive these through stylet feeding. According to Bird (1962) there is no record of a member of the genus Meloidogyne developing in the absence of giant cells. Also, such feeding cells are necessary for the development of the closely related genus, Heterodera. However, H. graminophila did not produce any giant cell or induce galling in Echinochloa colonum, Oryza sativa, Sorghum halapense and Saccharum officinarum, though all of them supoorted the nematode population (Birchfield, 1973). Birchfield believes that the predigestive enzyme necessary for initiating these changes may have been lacking or perhaps failed to form.

The giant cells in most of the plants observed here, and reported by earlier workers, possess abnormally thick

cell walls, and an anomalous number of nuclei of varying contours and dimensions (Christie, 1936; Krusberg and Nielsen, 1958; Bird, 1961; and Dropkin and Nelson, 1962). A large number of organelles have been reported by workers who made ultrastructural studies (Bird, 1961; Paulson and Webster, 1970). Similar feeding sites of comparable morphology are produced by the cyst nematodes (Gipson et al., 1971, and Riggs et al., 1973).

Giant cells in S. melongena, A. esculentus, L. acutangula and other hosts, are large and show proliferation of nuclei and nucleoli. Histochemical staining have revealed intense activity in these cells and chemical determinations also showed active metabolism. Such increases were shown by Bird (1961), Owens and Specht (1964), Rubinstein and Owens (1964), Owens and Rubinstein (1966), and Owens and Specht (1966). Bird (1961) and Paulson and Webster (1970) demonstrated increases in the number of mitochondria, proplastids, dictyosomes and other organelles in giant cells. Most of these workers have compared the giant cells to meristematic cells. But the gross alterations in metabolism brought about by pathological changes are more comparable to metabolic evocation during floral induction. During the transition to flowering there is a rise in general physiological activities, evocation of metabolic processes and mitotic flare (Bernier, 1971).

The giant cells are produced as a result of coalescence of several types of cells. These cells enlarged and incorporated more and more cells. Christie (1936), Krusberg and Nielsen (1958), Bird (1961) and Dropkin and Nelson (1962) believed that giant cells are formed from fusion of pre-existing ones. Endo (1964) reported that syncytia produced by H. glycines follow a similar mechanism. Giant cells arise around the permanent feeding sites of M. incognita acrita and enlarge by dissolution of walls of surrounding cells and incorporation of their contents. Such acquisition happens more towards the edge of the giant cells, away from the head of the parasite.

The appearance of a track along the path of the nematode seems to be due to chemical and structural changes in cells on either side of the larval trail. Larval movements through or between cells could have initiated such changes in surrounding cells. Cells pierced by H. glycines showed cell wall dissolution (Endo, 1964) and M. incognita acrita caused increase in enzymatic activities in such cells (Veech and Endo, 1970). Hypertrophy arising in such cells is regarded to fill up the gaps, a defense mechanism from the host (Endo, 1964).

Broken cell walls are seen along the path of the nematode as well as during the giant cell formation. There is, according to Dropkin and Boone (1966), a brief period of cell wall destruction during the enlargement of syncytia

followed by cell wall synthesis so that the mature giant cell is surrounded by thick secondary walls. Cell wall breaks were observed by Paulson and Webster (1970) also, but they do not regard them as unequivocal means for giant cell development.

These workers proposed an altogether different mechanism. According to them giant cells are initiated in stretchable tissues at a time when cell walls are easily modified. Huang (1968) also noticed cell wall breakdown during M. javanica penetrations but considered these to be limited to the nematode path before they reached the feeding sites. He regarded giant cell formation by the expansion of a single cell. Development of giant cells, which are 300-700 times larger than the adjacent normal cells, by the enlargement in single cells hardly seems probable as evidenced by the present work.

Electron microscopic studies by others have provided different information. During histopathological studies in susceptible (Gipson et al., 1971) and resistant hosts (Riggs et al., 1973), the existence of enzymatic cell wall perforations was proved. These workers have concluded cell wall dissolution as the initial phenomenon leading to syncytial development in Heterodera infections (Riggs et al., 1973). The fact that these changes were noticed as early as 42 hr after inoculation excludes the possibility of wall damage due to other factors. Bird (1972) found a close association

between giant cells and adjacent cells. According to him cell wall breaks and contact of cytoplasm contribute to giant cell expansion rather than enlargement of a single cell. Jones and Northcote (1972) found wall protuberences in giant cells induced by M. arenaria in coleus roots. They regarded the wall gaps to be produced during giant cell expansion. Also most workers have agreed that giant cell walls are very thin towards the leading edge (Christie, 1936; Krusberg and Nielsen, 1958; Dropkin and Nelson, 1962; and Littrell, 1966). If a single cell expanded to such dimensions it should have caused much cell destruction around owing to mechanical damage.

The giant cells were composed of several units that clustered around the head of the nematode. These units also indicate the number of feeding chambers the nematodes have initiated and fed from. Giant cell units varied from 6-9, the highest number occurring in the cucurbits. M. incognita acrita produced upto 8 giant cell units in A. esculentus and S. melongena. Balasubramanian and Rangaswami (1964) reported 5 and 4 units respectively for these 2 hosts infected with M. javanica. Paulson and Webster (1970) recorded upto 8 giant cells* in tomato infected with M. incognita and M. hapla. According to Davis and Jenkins (1960) and Paulson and Webster (1970) host histological responses to different species was similar.

---

*Refers to giant cell units.

But in Pusa Rubi tomato M. incognita acrita could induce upto 8 giant cell units. Thus different nematode species induce varying number of giant cell units in different hosts. Further, the size and number of syncytia* per nematode are determined by the host species and the physiological age of the parasite (Bird, personal communication).

The total area occupied by giant cells corresponded to 945 μ × 470 μ in L. acutangula and 935 μ × 540 μ in C.melo. Pepper (C. frutescens) exhibited the smaller giant cells. The giant cells produced in Pusa Sawani variety of A. esculentus measured 567 μ × 225 μ. Littrell (1966) reported a maximum length of 500 μ and width of 200 μ for giant cells in Clemson Spineless variety infected with the same nematode.

The mature giant cells in A. esculentus contained 83 nuclei. S. melongena and L. esculentum also showed high nuclear numbers (cf. Table 5). Krusberg and Nielsen (1958) recorded several hundred nuclei in giant cells of sweet potato.

Enhancement of nuclear activity seems to be the fundamental process in root-knot formation, for nuclei are stimulated along the nematode path, in giant cells and also in surrounding tissues. Nuclear hypertrophy is regarded as a susceptible reaction in D. dipsaci infection also

*Refers to giant cell units.

(Krusberg, 1961). Large nuclei, abnormally big or multiple nucleoli, chromatin unequally distributed in dense masses, an irregular nuclear surface and a high nucleocytoplasmic ratio are certain properties attributed to all cancer cells (Breton and Moule, 1961).

Oberling and Bernhard (1961) asserted that nuclear hypertrophy occurs in other tumour tissues also, and represents an attempt from the nucleus and cytoplasm to adjust to the abnormal environment. Increase in nuclear and nucleolar size prolong only so long as the nematodes are stimulating the giant cells. After the death of the female there is a decline in nuclear activity (Bird, 1962; and Rubinstein and Owens, 1964). This indicates that nuclear activities are essentially stimulated by the parasite which lasts only so long it feeds on the cells.

It is undoubtable that nuclear volume is altered due to the nematode, for this is usual with other nematode infections also. In T. semipenetrans enlarged nuclei and nucleoli measuring 84-125 times the normal volume are known (cf. Dropkin, 1969). Multinucleate cells and clumping of nuclei were also mentioned by Rebois et al. (1970) for Rotylenchulus reniformis infections.

Larger nuclei measuring upto 45 μ in diameter were observed in L. acutangula. Its normal cell nucleus measured only about 3 μ in diameter. Such enlargements were common in C. melo and S. melongena too, where nuclei of 30 μ size were

measured. Giant nuclei were recorded in Crepis capillaris and L. esculentum which Owens and Specht (1964) believed to have been formed by nuclear fusion. They found 6-fold increase in nuclear diameter that was equivalent to a volume difference of 200 times. Through another work they reported 10 to 12 fold increase as usual (Rubinstein and Owens, 1964). But in the present work no evidence of nuclear fusion was obtained. Nuclear hypertrophy alone is believed to effect such increases in size.

Large nuclei ~~have~~ obviously have extensive nuclear surface. Such increases in membrane surface are known to influence nucleocytoplasmic ratio (Oberling and Bernhard, 1961), and nuclear volume according to them increases in cells with a very active metabolism. Irregular lobbing of nuclear membrane and accumulation of Feulgen bodies around the edges of giant cell nuclei are illustrated in the present investigation. Piegat and Wilski (1963) have encountered similar situations in nuclei of potato infected by H. rostochiensis. Histochemical studies of Bird (1961), Endo and Veech (1969) and Veech and Endo (1970) on root-knots, and Endo and Veech (1970) on cyst nematode infected roots have revealed high localisation of several enzymes as a manifestation of high metabolic processes.

It is worth mentioning that Dropkin (1966) found over 100 chromosomes in giant cells of Vicia villosa where the usual 2n is 14. Also upto 64n nuclei were encountered by

Huang (1968). The fact that the polyploid cells were present in surrounding cells indicates the possibility of their being incorporated into giant cells at later stages. Also, upto 64 nuclei were encountered by Huang (1968) which he regards to be due to accumulation of chromosome sets in the process of repeated divisions in giant cells. His finding is an extension of the hypothesis of repeated endomitosis postulated by Myuge (1956). Workers like Bird (1961), Owens and Specht (1964), Littrell (1966), Smith and Mai (1965), and Siddiqui and Taylor (1971) have encountered occasional mitotic divisions in certain giant cells.

Presence of nuclei of different sizes, in clumps, indicated a likelihood of amitotic duplication. Swamy and Krishnamurthy (1971) also presumed that amitotic nuclear budding could be the method of multinucleation in Enterolobium saman and Basella alba. Feeding by an insect Pachysylla on Celtis produced multinuclear giant cells and Dundon (1962) explained this to be due to cellulolytic activity. The number of nuclei in a giant cell according to him indicated the number of cells merging together. Bird (1961 and 1972) also opined that the acquisition of nuclei from incorporated cells is presumably responsible in increasing the nuclear number, even though he found occasional mitotic figures in giant cells.

Polynuclear as well as polyploid cells arise in other tissues also. As early as 1940 Wipf and Cooper explained

that in bacterial nodules tetraploid cells arise from preexisting ones. Another theory proposed by Bhaskaran and Swaminathan (1958) based on studies in Trifolium alexandrium root-nodule is that the tetraploid cells stem from diploid cortical ones in which the chromosome complement was doubled by endoreduplication due to bacterial stimulus.

Recently Libbenga and Torrey (1973) have shown that cytokinins in the presence of auxin induce 2 rounds of DNA synthesis prior to the first mitosis. Such endoreduplication produced upto 16n conditions in cytokinin-auxin treated cells. Another report of interest is by Mottram (1944) where he related endomitosis and polytene polyploidy and polynuclear conditions to increased stiffness of cytoplasm.

Most nuclei stimulated in the giant cells had several enlarged nucleoli of larger numbers. Nucleoli may reach sizes close to or above that of nuclei in normal cells. Nucleolar hypertrophy is believed to be connected with intensive protein synthesising systems in normal, regenerated and malignant cells (cf. Oberling and Bernhard, 1961).

Nucleolar number increased upto 7 (Table 5). These nucleoli showing different sizes could have arisen by budding. Such a phenomenon was observed in the parenchymatous, meristematic and bacteria induced cells of Wisteria root nodules and such heterotypic variations were not found in normal somatic cells (Jimbo, 1927).

Within the nucleoli vacuoles were located whose importance is not known. Intranucleolar vacuoles are noticed in several pathological situations and Oberling and Bernhard (1961) referred to a number of these.

Another significant feature of the nucleoli was the inclusion and close association of several glistening bodies. These structures were clear in preparations stained with methyl green and pyronin. Littau and Black (1949) have described similar structures in wound tumour cells of Rumex acetosa. Such bodies in Aureogenes magnivena induced cells were pyronin-positive and resembled nucleoli. Littau and Black (1949) held that these glistening bodies are composed essentially of the same materials as nucleoli. As far as known such structures are not reported in earlier works on root-knots. The formation and role of such structures in tumour tissues seem worth investigating.

The walls of giant cells were excessively thickened, except for certain regions were deposition of secondary wall materials did not take place. Bird (1961) has also recognised such a manner of unequal thickening. The knob-like projections observed in the present study were encountered by Krusberg and Nielsen (1958), Owens and Specht (1964) and Littrell (1966) also.

Ultrastructural studies of Huang (1968), Gipson et al. (1971) and Riggs et al. (1973) have revealed certain peculiar membrane formations prior to secondary wall development.

The secondary wall materials are deposited below these 'boundary formations' (Huang , op. cit.) or 'paramural bodies' (Gipson et al. and Riggs et al., op. cit.) in a nonuniform manner. The knob-like projections seen under the light microscope may resemble excessively thickened areas containing 'boundary formations' or 'paramural bodies'.

The richness of giant cell cytoplasm is believed to be due to increase in density. Owens and Specht (1964), Huang (1968) and Paulson and Webster (1970 and 1972) have described the formation of vacuoles in giant cell cytoplasm. In plants like C. melo the cytoplasm looked alveolar which may be due to the presence of enormous small vacuoles. Meloidogyne induced giant cells were shown to be very rich in several enzymes (Endo and Veech, 1969; Veech and Endo, 1970). This is true of cyst-nematode syncytia also where enzyme localisation was very intense (Endo and Veech, 1970). Mankau and Linford (1960) and Dropkin and Nelson (1962) could identify rod-like hyaline structures in giant cells. The dense cytoplasm is also found to have an enormous number of plastids, mitochondria, endoplasmic reticulum, dictyosomes and lipid globules (Bird, 1967; Huang, 1968; Gipson et al., 1971; Paulson and Webster, 1970 and 1972; and Riggs et al., 1973).

Histochemically the nuclei in giant cells were found to be very rich in nucleic acids. Localisation with three staining procedures provided unequivocal evidence for heightened synthesis of both DNA and RNA. Hyperchromaticity

of cancer cells is known since long (Oberling and Bernhard, 1961). In reactive ones DNA concentrations were high and Feulgen-positive bodies were seen distributed on the nuclear envelope and further interior of the nuclei. This is in agreement with the observations of Bird (1961) and Rubinstein and Owens (1964) who found intensely staining giant cell nuclei. The latter workers have also confirmed this with H^3 autoradiography. In contrast, Mankau and Linford (1960) held that nuclei close to the nematode head were weaker in staining and had indistinct nuclear membranes. They also referred to nuclear disintegration, which is observed in the present studies also. Perhaps, the nuclei that stained weakly indicate disintegrating ones. The possibility of nuclear disintegration was considered ~~possibly~~ by Rubinstein and Owens (1964) also.

Intense staining of nucleoli and giant cell cytoplasm in methylgreen-pyronin and Azure B methods is attributed to excessive concentrations of RNA. RNA synthesis is reported to persist in giant cells even after DNA synthesis has ceased (Rubinstein and Owens, 1964).

Disappearance of starch from giant cell areas and other parts of infected roots was usual. Most workers agreed that starch depletion occurs, which indicates depletion of reserve food materials for use in the giant cell, by the parasite or by the weakened plant. Cohn (1965) provided some information regarding starch depletion by T. semipenetrans. Disappearance

of starch is usual with many fungal infected tissues also (Allen, 1954).

Jensen (1962) has held that plant cell wall staining with the PAS reaction is due to their cellulose content. Knob-like incrustations revealed in histological and Azure B staining are similar to projections reported by Krusberg and Nielsen (1958), Owens and Specht (1964) and Owens and Bottino (1966). In okra (H. esculentus) heavy staining of the cell walls was interpreted to be due to hemicellulose depositions (Littrell, 1966).

In root-knot infected roots there is a tendency for parenchyma cells to differentiate into xylem. In root tips where growth in length has stopped, vascular differentiation proceeds to the tip. Pilet and Meylan (1953) has observed that in Lens roots little auxin is present in the tip, most is found about 5 mm behind the tip, where vascularisation takes place. Vascularisation of the retarded roots may be due to an altered auxin concentration.

The gall parenchyma also differentiates into distorted and disorganised xylem. The transverse orientation and absence of xylem components, like rays, speaks of the anomolous nature of such xylem tissues. Except for occasional connections with the main stele, these are scattered in patches around giant cells, or in hyperplastic tissues. Conversion of parenchyma into tracheary or tracheidal elements were reported in earlier works also (Christie, 1936; Krusberg and

Nielsen, 1958; and Swamy and Krishnamurthi, 1971), but no specific reasons were attributed to this.

There is evidence that growth substances like auxins, giberellins, cytokinins and certain carbohydrates are prerequisites to xylogenesis, and the necessity of auxin to initiate the process is unmistakable (Roberts, 1969). Wetmore and Rier (1963) showed that for morphological organisation in a homogeneous mass of callus joint induction of xylem and phloem are necessary. They showed that auxins (IAA or NAA) and sugars (sucrose and glucose) are involved and necessary for complete differentiation of these. Dalessandro (1973) held that in pith parenchyma callus an increase on exogenous auxin level from 1-15 µg/ml in the presence of a constant concentration of cytokinin (0.1 µg/ml) could increase the number of tracheary elements formed.

Bird (1962) has shown that giant cell area is rich in an indole protein-bound substance. In the present work gall tissues are shown to contain more tryptophan and IAA like compounds. Krupsagar and Barker (1966) found high cytokinin levels in root-knots of tomato and Sandstedt and Schuster (1966) held that nematodes interfere with auxin translocation retain it and make available to the plant tissues. High concentrations of these growth substances may lead to xylogenesis in unusual sites. Jacobs (1952) had reported that differentiation of xylem elements both in normal and crown-gall tumour tissues is initiated by stimulation of growth substances.

4.3 CHANGES IN CHEMICAL CONSTITUENTS

Host influences on the parasite and effects of the parasite on different hosts are variable. The differential reactivity and preference of a parasite for a particular host and host suitability are determined on the basis of chemical constitution. Also, the histological abnormalities produced in different hosts and the success in parasite development also seem to be under diverse chemical influences.

The results of the chemical analyses clearly indicated considerable differences between the normal and nematode infected roots. Since all the roots on the same plant or all galls on a root at a particular period were not at the same stage of development, analysis of root samples could not represent uniformity. Also, in studies with tissues infected with obligate parasites it is difficult to say how much of the change in metabolites is in respect of the parasite and how much belongs to the host tissues.

The data regarding nucleic acid analysis are the mean of three determinations and help to show only the relative differences between the 2 types of tissues. Levels of RNA were high in galls produced by *M. incognita acrita* in *S. melongena*, *A. esculentus* and *L. acutangula*.

It is well established that RNA is a dynamic component associated with active metabolism, and there is more RNA in embryonic and tumour tissues (Hawk, Oser and Summerson, 1954).

Increase in RNA concentration occurs prior to and associated with protein synthesis. Owens and Specht (1960) showed that 2 months-old root-knot tissues of tomato and adjacent healthy tissues had considerable differences in the concentration of RNA. They reported that the increase in RNA in gall tissues was 87% which was confirmed through another subsequent study (Rubinstein and Owens, 1966). Owens and Specht (*op. cit.*) have cited certain cases of a similar nature in tumours of animal tissues. In contrast, Arya *et al.* (1962) had shown that normal grape callus tissue contained more RNA than *Phylloxera* induced gall.

There were significant rises in the levels of DNA in all the 3 hosts analysed. Increase in DNA concentration seems to be usual with many tumours. Griffin and Rhein (quoted by Breton and Moule, 1961) found the DNA in liver tumours to have an increased concentration of guanylic acid. Likewise, differences were recorded for cancer tissues of the epithelium in rat. Also changes in physicochemical proportion of nucleic acids like sedimentation characteristics are known to vary in tumour DNA (Polli and Shooter, 1958).

The differences in the concentration of DNA in root-knots, when compared to uninfected tissue may be related to nuclear hypertrophy, and growth flare of giant cell nuclei. An increase of 70% was observed by Owens and Specht (1966) which they correlated with syncytial nuclear size and ploidy. Analysis of DNA by Arya *et al.* (1962) showed that *Phylloxera*

gall tissue yielded more DNA. Marked rise in DNA synthesis is reported in Agrobacterium tumefaciens-induced crown gall, and DNA seems to play an important role in tumorigenesis (Kupila-Ahvenniemi and Therman, 1968). Bopp (1964) even held that DNA synthesised in crown gall tissue is qualitatively different from that in the normal. Inhibitors of DNA synthesis like 5-fluurodeoxyuridine were shown to prevent crown gall formation which could be reversed with supply of DNA precursors like thymidine (Bopp, 1964).

The discovery in recent years that plant growth substances may act largely through regulation of syntnesis of RNA strongly suggests the existence of interactions between growth regulators and genetic material (Nooden and Thimann, 1963). Auxins stimulate the rate of RNA synthesis in sections, isolated nuclei or even chromatin (Hamilton et al., 1965; Roychoudury et al., 1965; and Matthysse and Philips, 1969). Key and Hansen (1961) found 2,4-D and Fan and Machlachlan (1967) IAA, inducing marked increases in total nucleic acids. Also, auxins and cytokinins together, and to some extent, by itself, cause even qualitative difference in rapidly synthesised RNA (Klämbt, 1972). This sort of increase could be prevented by antiauxins which also inhibit auxin-induced growth. The greater RNA synthesis induced by IAA was noticed first in the nucleolus and in later stages in the cytoplasm (Davies et al., 1968). The histochemical tests in the present study have indicated abnormal concentrations of RNA in both nucleolus and cytoplasm of root-knot giant cells. Also, in

the present work increased levels of auxin compounds are found in galls similar to what Bird (1962), Yu and Viglierchio (1964) and Viglierchio and Yu (1968) have reported indicating that the nematodes are capable of modifying the auxin concentrations of particular host plants. Increase in the level of these growth substances might have produced higher nucleic acid occurrence in galls. Increase in RNA levels has further implications too. Niu et al. (1962) reported that treatment of Ehrlich's ascites tumour cells with RNA induced permanent enzyme synthesizing properties. Increase in RNA concentrations in giant cells possibly through IAA may stimulate the production of abnormally high quantities of proteins and enzymes. Endo and Veech (1969) and Veech and Endo (1970) have demonstrated high levels of enzymes in root-knots. It may be pointed out that azauracil and similar inhibitors of RNA synthesis, when applied to clover roots inhibited the development of H. trifolii beyond third larval stage, and that this inhibition could be reversed with the addition of RNA precursor such as uridine (Endo and Schaeffer, 1967).

Protein concentrations were high in root-knot tissues. The variations are thought to be due to the large volume of giant cell cytoplasm produced at the command of the parasite. Bird (1969) has held that histone-like basic proteins in nematode exudates are responsible for maintaining a high level of nucleic acid and protein synthesis in giant cells. A rise in bulk protein was reported by Owens and Specht (1966). Also through electrophoretic studies they showed changes in 8

protein fractions during gall formation in tomato. In a succeeding report Owens and Rubinstein (1966) demonstrated a 3-20 fold increase in syncytial protein. Earlier such increases were cytochemically demonstrated by Bird (1961).

Proteins from certain other tumour tissues are also known to differ in their electrophoretic behaviour (Toennies, 1952). Tumour formation and protein synthesis are related but too little is known of the origin and formation of the altered proteins, to evaluate their significance with respect to tumour formation. Key and Hansen (1961) and Fan and Machlachlan (1967) have shown that auxins like 2,4-D and IAA can promote synthesis of proteins in general.

Myuge (1956) held that root-knot nematode infection causes increases in the amino acid concentrations. It was reported later that the cyst nematodes (Heterodera spp.) do not cause such accumulations (Myuge, 1960). Bird (1961) had shown that the hypertrophied giant cells are rich in both amino acids and proteins. This was confirmed in another cytochemical study that showed 5-14 times increase in amino acid concentration in tomato (Owens and Specht, 1966). Also the chromatographic quantitation by these workers had shown a 300% rise in free amino acids in galls, and the increase ranged from 108% for tyrosine to 720% for proline. Rangaswami and Balasubramanian (1964) have showed the qualitative fluctuations in A. esculentus galls induced by M. javanica. High concentrations of nitrogenous compounds were revealed

in gall tissues through another study (Kannan, 1968). Fourteen, 13 and 9 free amino acids were detected in M. javanica, root-knot and healthy root of jute plant (Corchorus capsularis) respectively (Saxena, 1972). The nematode's contribution to amino acid increases in root-knot is quite thus obvious.

In both the host plants employed a marked increase in concentrations was found in all the free amino acids. Normal roots of S. melongena contained a total of 13 free amino acids. Phenylalanine appeared new in gall extracts. In addition to the 11 amino acids (Rangaswami and Balasubramanian, (1964) normal roots of A. esculentus contained glycine and 2 amides, asparagine and glutamine which were detected by Demetriades (1956) though in iron-deficient okra plants. Proline and tryptophan were noticed as additional amino acids in gall tissues of A. esculentus.

In an earlier study with M. javanica, tryptophan was found to occur in gall tissue, but persisted only for 2-3 weeks (Rangaswami and Balasubramanian, 1964). In A. esculentus infected with M. incognita acrita tryptophan was present even in older tissues. Krusberg (1961) found tryptophan as an addition in D. dipsaci galls. The presence of tryptophan is significant, for it is regarded as a precursor of IAA, the natural plant growth hormone. In plants, a pathway of IAA synthesis seems to be from tryptophan through tryptamine, indole pyruvic acid or indole acetonitrile, though others

are likely to exist (Winter, 1966). Also, tryptophan is known to form from phenylalanine and histidine or by combination of serine with indole (cf. Viglierchio, 1971). Sayre (in Mountain, 1960) proposed that proteolytic activities of nematodes release tryptophan from which IAA is produced. According to Bird (1962) this is an extension of the hypothesis proposed by Ustinov (1951). The high concentration of phenylalanine and histidine in the galls of S. melongena are also significant. Proline was absent in normal roots of A. esculentus but was present in high concentrations in root-knots of both the plants.

Owens and Specht (1966) attached considerable importance to the occurrence of hydroxyproline in protein hydrolysates of root-knots. They pointed out that this is usually found in rapidly growing tissues and tissue cultures. But mention must be made that hydroxyproline is usually found in most nematode hydrolysates (cf. Krusberg, 1971). Also Epstein and Cohn (1971), found a depletion of hydroxyproline in galls induced by Longidorus africanus.

Hanks and Feldman (1966) found an 81% increase in arginine in leaves of grape fruit infected with R. similis. They could show that this was due to nematodes, for in the 'recovered' plants the original concentration was restored. Doney et al. (1970) reported that amino acid concentration in a resistant species of beet, Beta petellaris, was unchanged by H. schachtii while that in B. vulgaris, a susceptible

host, was increased. Epstein and Cohn (1971) detected 184% more free amino acids in galls on Bidens tripartita, induced by L. africanus. They recorded a 1900% rise in the concentration of proline, and also a fall of aspartic acid by 56%.

Changes in amino acids are known for other parasitic diseases too. Fife (1961) showed that in the sugar beet, virus infection causes similar changes. Amino nitrogen concentration increased and several amides accumulated in melon as a pathological repurcussion to Colletotrichium lagenarium (Touze, 1964). Pronounced changes were caused in the xylem sap amino acids of tomato infected with Verticillium albo-atrum (Dixon and Pegg, 1972). The formation of large amounts of amino acids would indicate their availability in the pool to be utilized for quick protein synthesis.

Several workers hold that proteolytic enzymes secreted by nematodes into host, cause breakdown of the host material and a subsequent accumulation of amino acids Myuge (1956) and Mountain (1965). Such hydrolysis must cause a reduction in the levels of total proteins. This is not found to be true for S. melongena, A. esculentus and L. acutangula, where levels of proteins also increased instead. Bird (1961) described an increase of proteins by several times. Owens and Specht (1966) also did not agree that protein degradation could produce the large amounts of amino acids present in galls. They considered 3 possibilities: the amino acids must

be (1) synthesized within the syncytia, (2) translocated from other parts of the plant, and (3) deposited by the parasite. Autoradiographic studies have proved the first possibility of synthesis *in situ* (Owens and Rubinstein, 1966).

Several workers have demonstrated that nematodes like *Meloidogyne*, *Aphelenchoides*, *Ditylenchus* and *Pratylenchus* discharge many amino acids into incubating medium (cf. Krusberg, 1971). Such amino acids are considered as secretory and excretory products. The effects of such substances are unknown. Only further studies would bring out the role of excretory products in gall induction.

Sugars are found to be depleted due to *M. incognita acrita* infection. Total sugar content in infected sugar beet root is known to be decreased by 60% in *M. naasi* infections (Weischer and Steudel, 1972). Reduction was more than half in tomato galls (Owens and Specht, 1966). Kannan (1968) also observed a marked decrease in the amounts of reducing sugars in tomato root-knots. According to Leopold (1955) reduction in carbohydrate fraction followed by rise in amino acid and protein content happens in auxin supplied plants. Thus, reduction in carbohydrate metabolites appears to be a usual feature in root-knot disease.

Krusberg (1961) could not visualise changes in sugar composition of alfalfa infected with *D. dipsaci* and *Aphelenchoides ritzemabosi*. But he did not quantify their distribution which is likely to be different. Zinovev (1964) noticed increases in the levels of both amino acids and

monosaccharides in root-knots in comparison to normal roots. Again, Plasmodiophora brassicae, the club-root fungus, induced in galls a 20-fold increase in trebalose, 4-fold of glucose and 2-fold each of fructose and mannose (Keen and Williams, 1969). At the same time the amounts of galactose and inositol were lower than that in normal tissue. Keen and Williams (op. cit.) also reported that club-root tissues were less efficient in exporting sugars from their sites.

Kannan (1967) had reported increases in the levels of several dehydrogenase enzymes of the TCA cycle in root-knots. He explained that the metabolism of breakdown products, in root-knot tissues would need more activity of these enzymes, which also stimulate respiratory rate. It is likely that in the wilted leaves of root-knot infected plants photosynthetic capacity is reduced and rate of respiration increased, which depletes the carbohydrate reserves. Allen (1954) has referred to such instances. Also, Chinball (1939) believed that increase in respiration, decrease in sugar level and synthesis of glutamine occur during proteolytic process. Owens and Rubinstein (1966) have recorded a slightly lower respiration rate in 40-day old galls, and a higher rate in 70-day old galls than healthy tissues. However, both produced more CO_2 and had consistently higher RQ. They also showed that RQ of the galls increased with increasing concentrations of sugar supply. According to them the higher rate of glycolysis in root-knots is similar to that in other tumours.

Analysis of growth substances from normal and gall tissues of 3 hosts have shown positive results in chromatography, chromogenic reactions and wheat coleoptile bioassay. In the normal and gall tissues of S. melongena a compound was present on chromatogram that produced slightly higher Rf than that in A. esculentus, and synthetic IAA. However, the fluorescence in all the cases was similar. The compound from A. esculentus was identified as IAA. The indolic compound of higher Rf value present in both normal and root-knot tissues of L. acutangula, on the basis of Rf value and chromogenic reaction described by Stahl (1965) is identified as IAN.

Acidic fractions corresponding to spots in TLC from both S. melongena and A. esculentus produced pink colour with both Gordon and Weber and ferric citrate reagents. The pink colour produced with both the chromogenic reagents was considered to be due to IAA-like substances. The ferric citrate reagent was obviously more efficient and with this the gall extracts of S. melongena showed a ca. 6.5-fold increase in colour concentration. The increase was almost 6 times in the case of A. esculentus gall extract.

In the biological assay, extracts from root-knot tissues of both the hosts showed greater activity, compared to the activity in the extracts from the respective normal tissues. Further, gall tissue extracts of S. melongena recorded greater activity difference than the gall tissue extracts of A. esculentus.

Thus infection causes a rise in the levels of indolic growth substances and hyperauxiny must be responsible for the pathological repercussions. It is presumable that inherent characteristics in different host species may be certain factors determining responses to infection.

Christie (1936) did not explain how root-knot infection in tomato caused a suppression of radicular growth in length and simultaneous production of lateral roots from the galled region. It is, however, now well recognised that lateral roots are frequent in M. hapla infections, and that vascularisation occurs upto the tip in suppressed main roots. Such phenomenon is usual with sterile root decapitation which upsets auxin balance. Cytokinins are also known to produce similar effects. Sandstedt and Estes (1965) noted development of buds from Nacobbus batatiformis galls. Dropkin et al. (1967) visualised differentiation of stem buds from green vesicles produced from M. incognita root-knots. Varghese and Kamlesh Kumari (1970) also noted meristem induction in root-knots. It is conceivable that localization of growth stimuli in galls is primarily responsible for initiating meristematic activity.

Considering the analogy between artificially induced tumours due to auxin application and nematode induced galls, Goodey (1948) speculated the involvement of plant growth substances in the latter. Nolte and Kohler (1952) reported that D. dipsaci gall extracts could increase length in sunflower seedlings. Myuge (1956) obtained growth increments

in bean plants applied with tomato root-knot extracts, though his subsequent experiments with a growth promoter itself inhibited gall formation (Myuge, 1957). External supply of growth promoters might have naturally increased the auxin level beyond optimum physiological concentration, which inevitably suppressed development of galls. The present bioassay experiments have brought out that a many fold increase of growth promoters occurs in M. incognita acrita root-knots.

Ustinov (1951) found that application of NAA (0.01-0.02%) could intensify galling. Nusbaum (1953) and Orion and Minz (1968) observed that maleic hydrazide and similar antiauxins could inhibit the extent of gall formation by Meloidogyne.

Loewenberg et al. (1960) and Dropkin and Boone (1966) have observed gall formation even without the entry of nematodes. Also, the cells along the nematode path and permanent feeding sites hypertrophy. Similar observations made several workers doubt the presence of growth substances in nematode secretions and galls.

Indole compounds were detected from root-knots induced by M. javanica (Balasubramanian and Rangaswami, 1962; and Rangaswami and Balasubramanian, 1964). They recorded the presence of IAA in okra, indole pyruvic acid in tobacco and hydroxy-tryptamine in brinjal, which were absent in normal tissues in all the cases. In another investigation with tomato root-knots, where Ehrlich's reagent was used as chromogenetor in chromatography no colours developed (Bird,

1962). However, Bird's (op. cit.) wheat coleoptile experiments yielded growth promoting activity in galls. Lefkowitz (1962) could not detect any Ehrlich's or Salkowski positive compound in tomato or tobacco galls. In addition he held that although normal tomato and tobacco tissues contained certain growth promoting substances, their concentration was similar in gall tissues also. This made him conclude that no differences in the levels of auxins existed between normal and tumour tissues and that IAA is not involved in the formation of root-knots.

Two years later Yu and Viglierchio (1964) analysed the extracts of tomato root-knots, larvae and eggmasses of M. hapla, M. incognita and M. javanica, by paper chromatography and Avena first internode bioassay. They derived positive results for auxins in all the tissues. In healthy tomato roots the presence of auxin was not confirmed, but growth promoting activity was measured. The root-knots of M. hapla, according to them, had IAA, indole acetic acid ethyl ester (IAE) and IAN; M. incognita galls contained IBA and M. javanica galls had IAA and IAN. Viglierchio and Yu (1968) found similar conditions in brussel's sprouts and beet roots also. IAA was demonstrated in the larvae of H. schachtii too (Johnson and Viglierchio, 1969). These workers held that the relative concentrations of auxins present in a nematode is its primary characteristic, but can be modified by the host auxin content. The occurrence of IAA methyl ester ($IAAOCH_3$) in D. dipsaci was established by Cutler and Krusberg (1968).

Setty and Wheeler (1968) also agreed that tomato roots have larger total amounts of bound auxin, more tryptophan and other amino acids than uninfected ones. They believed that hydrolysis of plant proteins by larvae, yield tryptophan that reacts with endogenous phenolic acids and produce excess auxin. Lewis (1973) revealed a growth inhibitor and a growth regulatory enzyme in root-knot resistant cotton. He held that hyperauxiny in gall tissue of susceptible variety is due to uninhibited synthesis.

Thus there is ample evidence that root-knot nematode infection and the concomittant anomalies involve plant growth regulators of the auxin type. The physiological effects of auxins in various metabolic processes have been discussed in the preceding pages. However, according to Bird (personal communication) it is generally agreed that indole compounds are found in nematodes but whether they are exuded through the nematode stylet or not is unknown. Identification of these and related substances in homogenates of Meloidogyne does not necessarily mean that they have any role in syncytial formation or in the swelling of roots.

4.4 DEVELOPMENT OF THE PARASITE

M. incognita acrita developed successfully in the Black Beauty variety of eggplant. Extremes in the climatic conditions of Pilani caused fluctuations in developmental duration. The sequence of nematode development was similar to the descriptions of Bird (1959a) for M. hapla and M. javanica and Triantaphyllou and Hirschmann (1960) for M. incognita, all parasitising tomato.

The larvae, after penetration, induced extensive giant cells and fed on them until the onset of moulting. Not all larvae introduced into the medium succeeded in penetrating the roots. Larval activity was reduced and they became sluggish when incubated in water for more than 2-3 days. This may be due to the depletion of body reserves (Wallace, 1966). The number of giant cells and their histological details was quite variable, which according to Bird (personal communication) is due to differences in the physiological age of the nematode. For, not all eggs are laid at the same time nor do all larvae settle and feed simultaneously.

The details of moulting were not studied. The 3rd and 4th moults took place within the second stage cuticle (Christie and Cobb, 1941; and Bird, 1959). Larvae did not move about after the initiation of moulting and until sex differentiation was over and maturity attained. Most of the larvae having their heads in the root stele got differentiated into saccate females that were sedentary. Van der Linde (1956)

and Dropkin (1959) have reported that sex in Meloidogyne is determined by the environment and the male/female ratio in a population depends on the host.

In eggplant, plenty of eggs were produced that were deposited in eggmasses inside the root tissues or extruded out of root surface. The pale white egg-masses turned brownish, which is believed to be due to tanning.

General absence of any hypersensitive necrotic reaction and the facility with which the giant cells and egg-masses were produced showed that M. incognita acrita-eggplant is a good parasite-host combination.

SUMMARY

5. SUMMARY

The present population of root-knot nematode (*M. incognita acrita*) maintained on eggplant was quite virulent and infected all the test plants under artificial inoculation.

The second stage larvae detected the presence of host roots and migrated towards them. Larvae showed a preference in invading the root tips. In *S. melongena* the root -cap region was preferred most for penetration, and in *A. esculentus* infection was generally along the cytogenerative region. Infrequently penetration through other areas and natural openings occurred. Massive attacks which damaged the root tips were usual. Larvae arched their body during penetration. Stylet movements ranged from 70-80/min. Penetration was effected in 1-5 hr. The juveniles lay still during feeding. Movements inside the roots was generally intercellular in a longitudinal direction and occasionally intracellular. Intercellular movements was by lysis of middle lamellae and intracellular by disruption of intercellular septa.

In 1 week old seedlings of *S. melongena* and *A. esculentus* maximum penetration occurred by 48-60 hr. Infectivity was higher in *A. esculentus*. Defoliation of seedlings reduced attractiveness, until new leaves sprouted. Excised roots did not attract larvae.

Most of the larvae were oriented towards the proximal end of roots. Larvae generally lodged in the plerome. In mature regions they settled in the pericyclic, phloem or cambial cells. Endodermis limited the entry of larvae from radial directions. Larvae that settled in the cortical cells tended to become males.

M. incognita acrita produced large tumorous giant cells around their feeding sites. Giant cells originated chiefly in stelar tissues. Initial hypertrophy in such cells was followed by coalescence with surrounding cells. Giant cells had thick walls except for occasional narrow regions. A giant cell complex had 5-9 units, each often showing morphological variability. They contained large nuclei, nucleoli, dense cytoplasm and vacuoles.

High nuclear number in the giant cells is due to the pooling of nuclei from adjacent cells, and fragmentation of hypertrophied nuclei. Nuclei of different sizes and shapes existed and often aggregated. Nuclear surface was wavy, distorted and produced fissures. Nuclear size increased 10-12 times. Micronuclei were usual and their presence suggested nuclear acquisition and amitotic divisions. Nuclei in surrounding cells also hypertrophied.

The giant cell nuclei were polynucleolar, which also showed hypertrophy. Nucleolar number varied from 1-7 and sizes within the same nucleus also varied. Nucleolar vacuoles and glistening bodies were seen in some nuclei.

Giant cell cytoplasm was dense, granular or alveolar. They contained many lipid globules and vacuoles.

Irregular deposition of secondary wall materials produced nonuniformly thickened giant cell walls. Cell walls near nematode head remained intact, and those towards the leading edge of the giant cells were thin. Cell wall damage was a usual feature.

Cambial activity was suppressed in infected sites. Xylem was produced only in irregular patches and there was a marked suppression of phloem. Cell proliferation occurred around giant cells. Hyperplasia originated in pericycle and cambial cells and produced tiered layers of cells.

Abnormal xylem developed in non-stelar tissues, and around the nematode. Such xylem had a radial orientation. Later some of these xylem strands established connection with the main stele.

In S. melongena giant cells were produced by 5 days after inoculation. Growth of giant cells compressed other stelar elements. By 10th day wall deposits were noticed. Cytoplasm was dense and nuclei were clumping. The stimulus for hyperplasia was noticed, even at a distance from the giant cells. Lateral roots originated near certain giant cells. By 15th day giant cells had excessively thickned walls and several cell units. Abnormal xylem was produced by redifferentiation of parenchyma. Females produced eggs by 30-40 days. The eggmatrix caused necrotic effects. The nuclei and cytoplasm

in giant cells gradually degenerated. Hyperplasia continued to occur around the giant cells and nematodes. Death of the nematode caused the collapse of giant cells.

Histochemically the giant cell nuclei were rich in DNA. Feulgen-positive bodies were distributed in nuclei and around the nuclear membrane. Nuclei in surrounding cells also were positive. Nucleoli and cytoplasm were rich in RNA. Walls were PAS-positive and wall incrustations Azure B-positive. Starch grains disappeared from infected sites.

Chemical analyses showed a rise in RNA concentration in root-knots by ca. 45% , than normal tissues. DNA concentration showed an increase of 83.7%. Total protein in gall tissue showed a rise of over 90%. Concentrations and number of free amino acids were high in gall tissues. Tryptophan was detected in the infected root of A. esculentus. There was a marked reduction in the levels of soluble sugars.

TLC revealed the presence of indolic growth substances in normal and gall tissues. IAA was found in A. esculentus. S. melongena had a similar compound with slightly higher Rf. L. acutangula contained IAN. Concentrations of all these were high (6-6.5 fold) in root-knots. Acidic fraction of gall extracts showed greater growth promoting activity in wheat coleoptile bioassay. Growth promoting activity difference was higher in S. melongena galls.

The parasite developed well on S. melongena. Sausage-shaped larvae were seen by 7th day after inoculation. Sexual

differentiation was apparent by about the 15th day. Egg laying occurred between 30-40 days. Male nematodes were rare.

APPENDIX 1

APPENDIX 1

A NEW CHROMOGENIC REAGENT, AND MODIFICATIONS IN THE SPECTROPHOTOMETRIC DETERMINATION OF INDOLE ACETIC ACID

In the usual Salkowski reaction for the colorimetric determination of IAA, ferric chloride is used as the chromogenic ion in a strong mineral acid medium. This reagent imparts a pink colour to most synthetic acid auxins. There are several modifications of the method (cf. Larsen, 1955). In the present study the Gordon and Weber (1951) version was used, for the estimation of IAA in aqueous aliquots. As this reagent produced almost identical contours with auxins other than IAA a search was made for chelating agents that could differentiate various indole compounds.

It was found that ferric citrate (Fe C_6 O_5 H_7 $5H_2O$), a combination of iron and citric acid could discriminate IPA from IAA and IBA at least. Also ferric citrate reacted producing more intense colour with IAA. So a new reagent was formulated and used in estimating IAA like compounds from normal and root-knot tissues of S. melongena and A. esculentus. The effectiveness of the Gordon and Weber reagent and the new ferric citrate reagent in spectrophotometric determination of IAA was compared. The composition, preparation and spectrophotometric absorption data for IAA with both the reagents are given below.

Composition:

Gordon and Weber reagent

0.5M ferric chloride ($FeCl_3.6H_2O$) - 1 ml

35% perchloric acid ($HClO_4$) - 50 ml

Ferric citrate reagent

0.2M ferric citrate ($FeC_6O_5H_7.5H_2O$) - 1 ml

35% perchloric acid - 50 ml

Preparation:

As hydrate forms were used in the case of both ferric chloride and ferric citrate necessary weight corrections were made. These were dissolved in water. Ferric chloride dissolved well in cold water, but ferric citrate had to be heated. One ml of each was used to prepare the reagent making up 50 ml with perchloric acid diluted to 35%.

The reaction mixture consisted of 1 ml of IAA in water and 2 ml of the reagent. A dilution series of 1.25-60 µg IAA/ml of water was prepared from a concentrated stock of 100 µg/ml. The solutions were mixed in identical test tubes and the colour development was carried out for 1 hr in dark. Absorbance measurements of the individual concentrations for both the reagents were determined at 530 nm with a Hitachi Perkin-Elmer spectrophotometer and are given in Table 12.

TABLE 12

Estimation* of indole acetic acid with Gordon and Weber and Ferric citrate reagents

IAA. concentration (µg)	Absorbance	
	Gordon and Weber reagent	Ferric citrate reagent
1.25	0.010	0.020
2.50	0.020	0.035
3.75	0.025	0.070
5.00	0.060	0.090
7.50	0.130	0.190
10.00	0.180	0.275
15.00	0.325	0.440
20.00	0.495	0.580
30.00	0.770	1.930
40.00	1.070	1.240
50.00	1.360	1.570
60.00	1.700	1.900

*Measured at 530 nm with Hitachi .Perkin-Elmer spectrophotometer

Differences in absorbance are appreciable starting from the low concentrations. Standard curves prepared showed that the Gordon and Weber reagent did not follow Beer and Lambert's law below 4 µg/ml. But ferric citrate reagent could be used with accuracy even at 2 µg/ml concentration.

Use of 1-Nitropropane

1-Nitropropane could be used to further improve the measurement procedure. After serial dilution and reaction with chromogenic reagent IAA was transferred to this solvent. One ml of nitropropane was added to the reaction mixture (3 ml), shaken vigorously and allowed to stand. Two layers get separated and the upper nitropropane had all the colouring matter. This fraction was used for measuring the absorbance at 530 nm itself against a blank of nitropropane.

1-Nitropropane could be advantageously used especially while determinations are made of dilute concentrations of IAA. Theoretically such a transfer should have made a three fold improvement in absorbance but it was found to be only 25-35%.

Usefulness of the modifications

For identical concentrations of IAA and ferric chloride (Gordon and Weber) or ferric citrate reagent, it was found that the latter (FC) produced perceptibly deeper colour.

This is also clear from the absorbance data (Table 12).

The red pink colour produced with ferric citrate was found stable for a relatively longer time when compared to the colour with the Gordon and Weber reagent.

Ferric citrate was equally stable in ambient temperature. However, preservation in brown bottle at refrigerator temperature was practiced, to reduce disintegration.

The hydrate form of ferric citrate is not hygroscopic. It is composed of garnet coloured shiny crystals. Ferric chloride is very hygroscopic and therefore susceptible to unfavourable weight changes and subsequent fluctuations in absorbance when the determination is repeated.

Use of 1-nitropropane is an effective way of improving the absorbance at low concentrations. It could be used also with IAA in ether or alcohols.

Other strong and weak acids were also tried with ferric citrate but perchloric acid produced the best results. Also the concentration of the acid is critical. Lower concentrations produced weaker colour.

ABBREVIATIONS USED IN FIGURES

ax - abnormal xylem
bs - buccal stylet
bw - break in cell wall
cc - coalescing cells
cn - clumping nuclei
co - cortical cell
cx - crushed xylem
cy - cytoplasm
da - degenerating area
dx - differentiating xylem
ec - egg cuticle
ed - endodermis
em - egg mass
gb - glistening body
gc - giant cell
gw - giant cell wall
hp - hyperplasia
ht - hypertrophy
lc - lysigenous cavity
le - leading edge of giant cell
lg - lipid globule
lr - lateral root
mn - male nematode
nb - nematode body
nc - necrotic cell
ne - nematode egg
nf - fissure on nucleus
nh - nematode head
nl - nematode larva
nm - nuclear membrane
no - nucleolus
nt - nematode track
nu - nucleus
nv - nucleolar vacuole
ob - oesophageal bulb
ov - ovary
pc - pericycle
pe - perinneum
ph - phloem
pm - plasma membrane
rc - root cap
rm - root meristem
rk - root-knot
rt - root tip
sc - sloughed cuticle
sg - starch grain
sk - skylet knob
st - stele
ts - tail spike
vc - vacuole
vs - vascular strand
wd - cell wall dissolution
wi - wall incrustation
xd - discontinuous xylem
xp - patch of xylem
xy - xylem

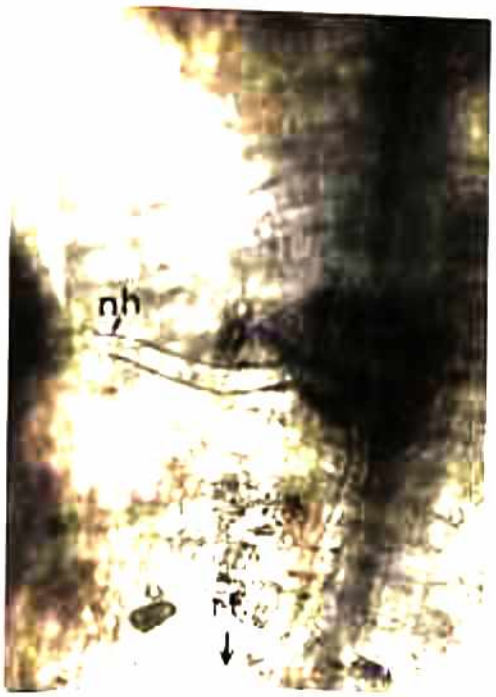
FIGURES AND LEGENDS

Fig. 1 A second stage M. incognita acrita larva moving about on A. esculentus root before penetrating. Arrow indicates the direction of root tip (×225).

Fig. 2 A larva penetrating through the root cap of S. melongena. Phase contrast;(×96).

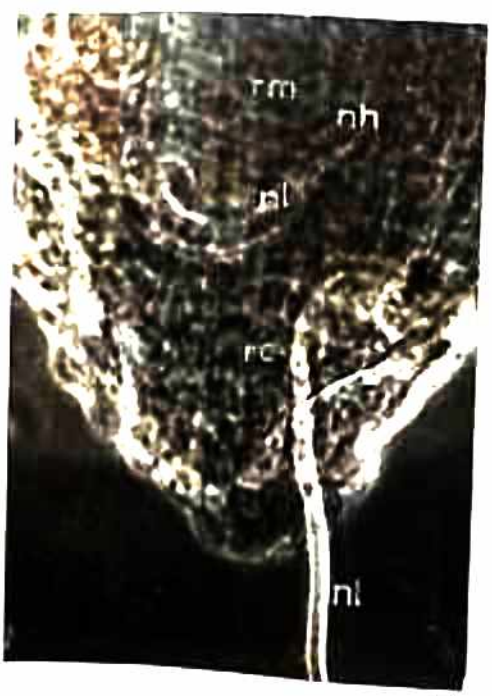
Fig. 3 A larva penetrating through the calyptra of S. melongena; while another is seen in a coiled position within the cleared root. Phase contrast; (×128).

Fig. 4 Nematode penetration through the cyto-generative region of S. melongena root (×165).

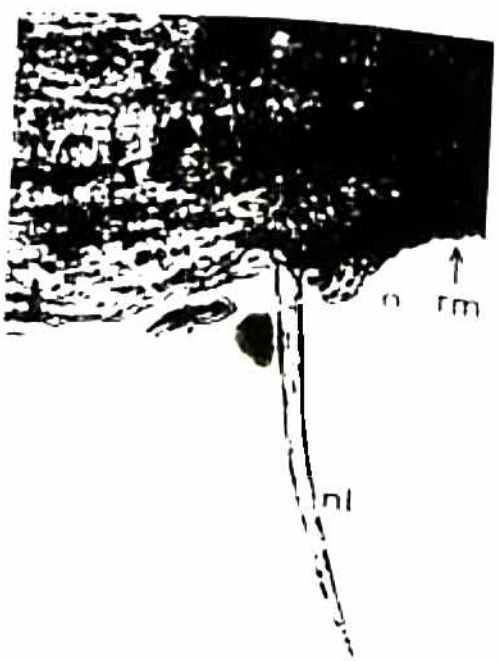


1

2



3



4

Fig. 5 Larval penetration through the root cap of A. esculentus. Phase contrast; (×112).

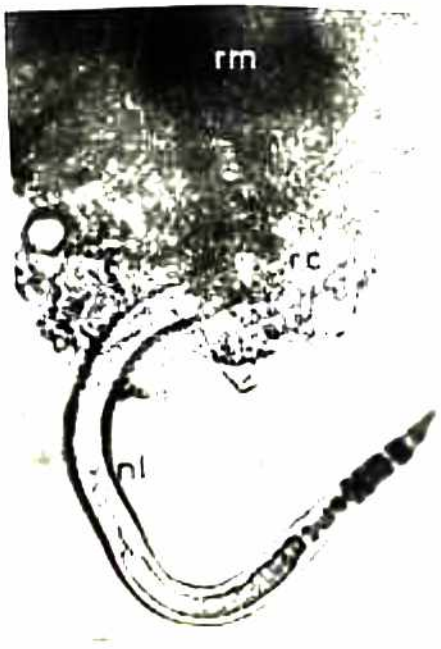
Fig. 6 A juvenile invading the okra root at a point above the root meristem (×165).

Fig. 7 Disruption of S. melongena root cap cells caused by nematode attack. The larva was anesthetised for photography, by the addition of ether (×384).

Fig. 8 A larva entering S. melongena root along the zone of elongation. Phase contrast; (×256).

5

6



7

8

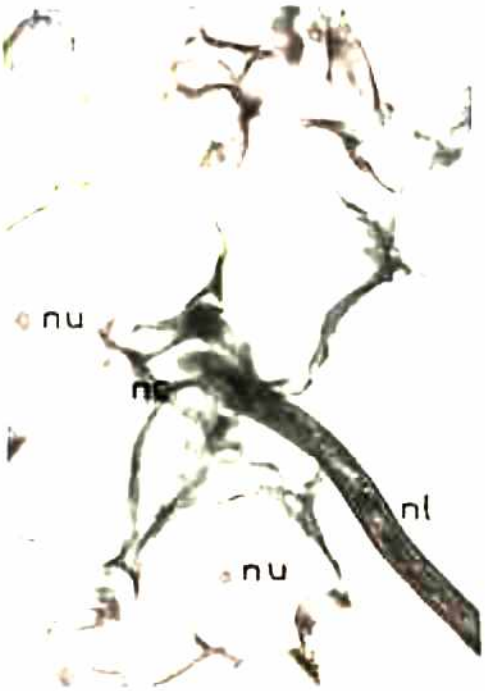
Fig. 9 Nematode invasion in S. melongena along the mature differentiated zone (×400).

Fig. 10 Larval entry through a natural wound on S. melongena root. The transection also shows certain necrotic cells and hypertrophied nuclei (×256).

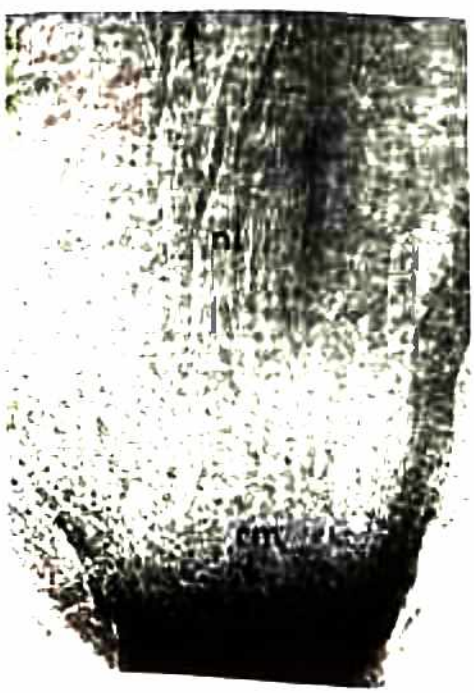
Fig. 11 Massive invasion of root-knot nematodes in S. melongena root. Note the larvae disposed in the plerome region. Arrow indicates the direction of larval movement (proximal end of the root) (×35).

Fig. 12 A second stage juvenile in the cortex of a root, one week after inoculation. Arrow indicates the proximal end of the root (×45).

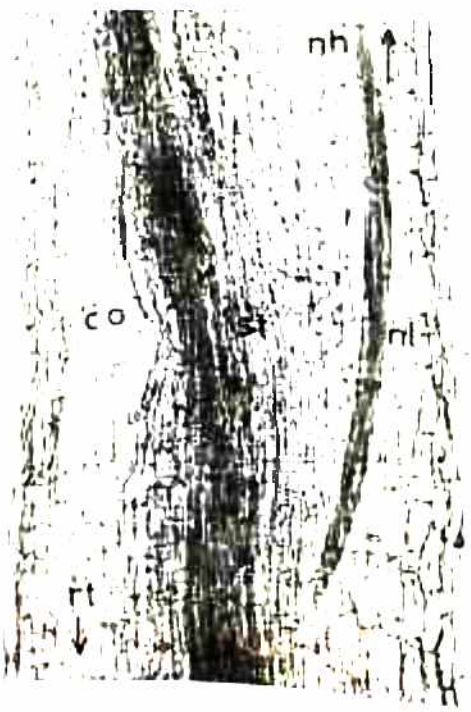
9



10



11



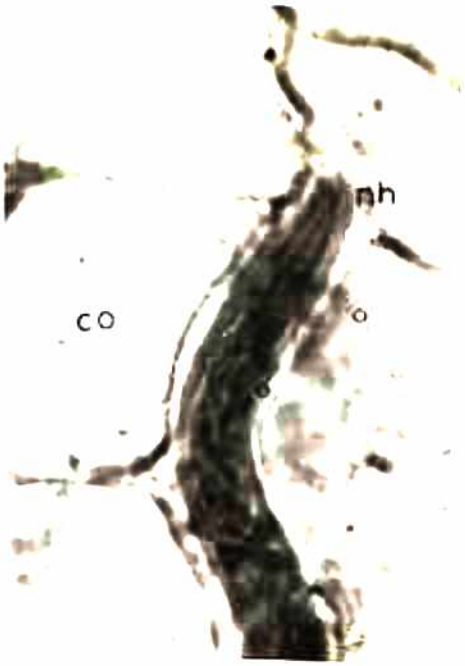
12

Fig. 13 Intercellular migration of a larva in S. melongena. Note the bends in the body due to the orientation of host cells (×256).

Fig. 14 Intracellular migration of a larva in S. melongena. Note the cell wall break and hypertrophy of nucleus (×256).

Fig. 15 A longisection of A. esculentus root showing nematode disposition in the stelar region (×192).

Fig. 16 A cleared root showing several larvae migrating along the zone of vascular differentiation of S. melongena. Note the differentiating xylem element and the direction of nematode movement (×160).



13

14

15

16

Fig. 17 A longisection showing the migration of a nematode in A. esculentus causing cell lysis (×160).

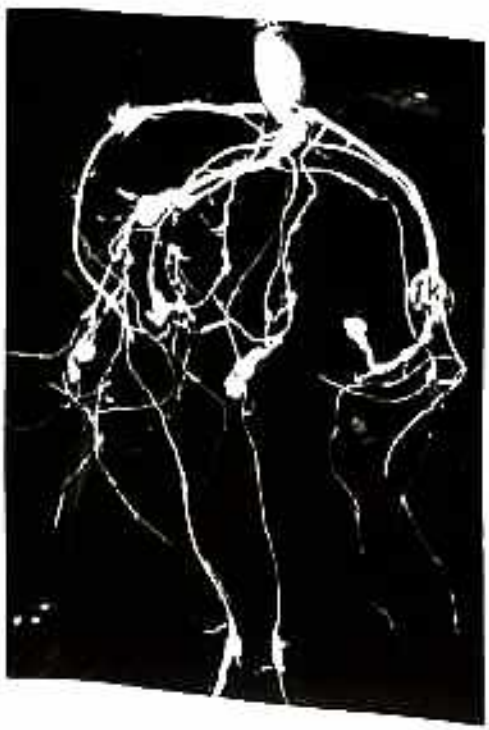
Fig. 18 A transection of A. esculentus root. Note the breaks in cell wall caused by the intracellular movement of the larvae (×360).

Fig. 19 A root-knot infected root system of C. melo showing galls of varying sizes.

Fig. 20 A nematode infected root system of L. acutangula with several galls on the main and lateral roots.

17

18



19

20

Fig. 21 A root system of S. melongena with several galls induced by M. incognita acrita.

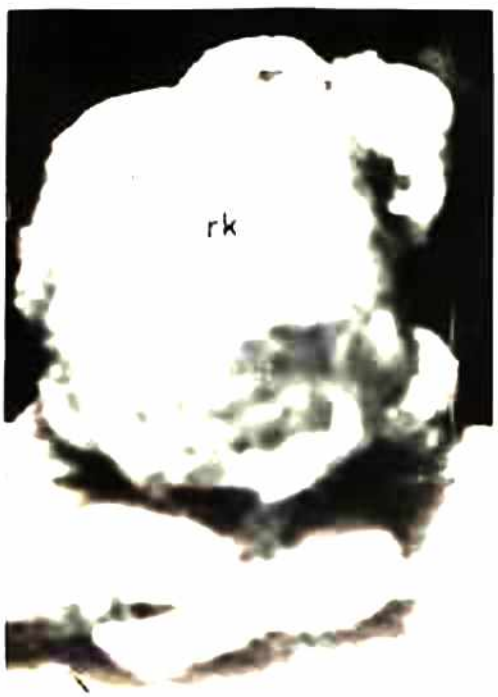
Fig. 22 A heavily galled segment from A. esculentus root. Note the presence of several compound galls (×2).

Fig. 23 A large gall on L. acutangula root showing the amorphous mass tissues (×2.5).

Fig. 24 A heavily galled segment of S. melongena root. Note the presence of several compound galls due to secondary infections, and degenerating areas in old tissues. Compare the size of the gall and uninfected portions (×1.5)

21

22



23

24

Fig. 25 A transection of L. acutangula gall showing necrotic effects in cortical cells around the head of a male nematode (×475)

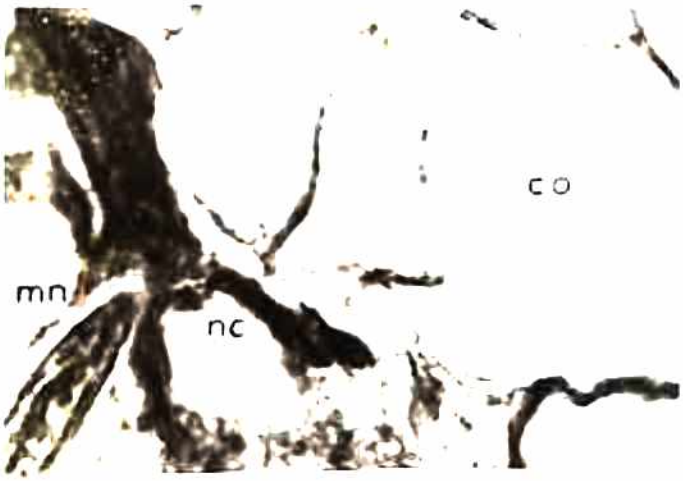
Fig. 26 A transection of L. acutangula gall showing a male nematode near a female. Note the necrosis of cells along the track of the male (×212).

Fig. 27 A longisection of A. esculentus root with the nematode having its head introduced into the stelar tissues (×212).

Fig. 28 A larva located inside the cytogenerative region of A. esculentus root. Note the cellular damage caused (×360).

Fig. 29 A nematode moving about in the root of C. sativum. Breaks in cell wall are clear (×260).

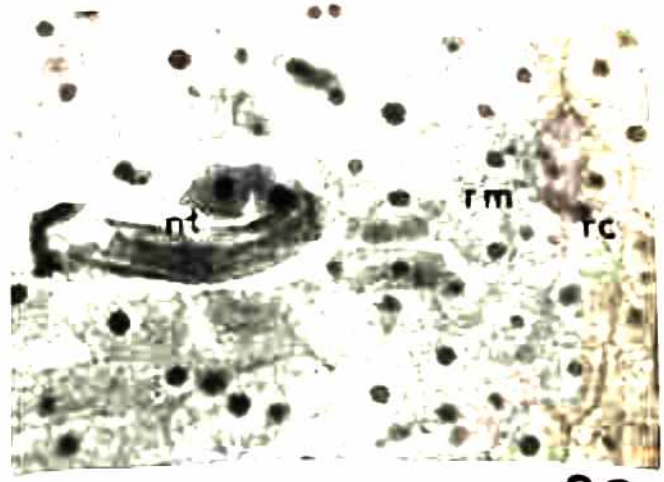
Fig. 30 A larva lodged near the differentiating xylem in A. esculentus root. Note the changes in the cell near nematode head (×215).



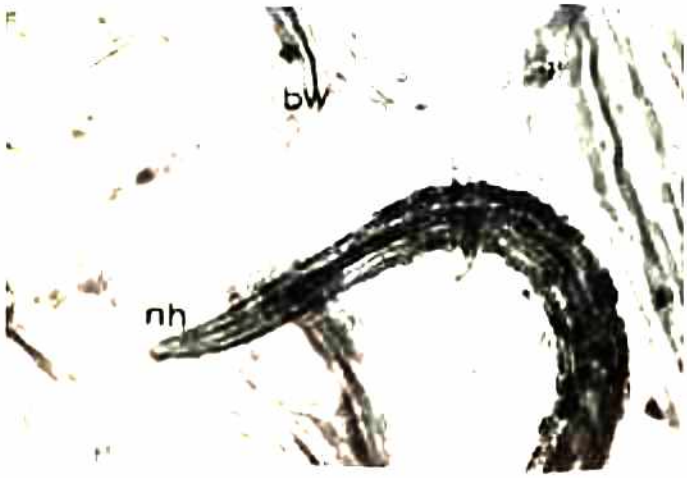
25

26

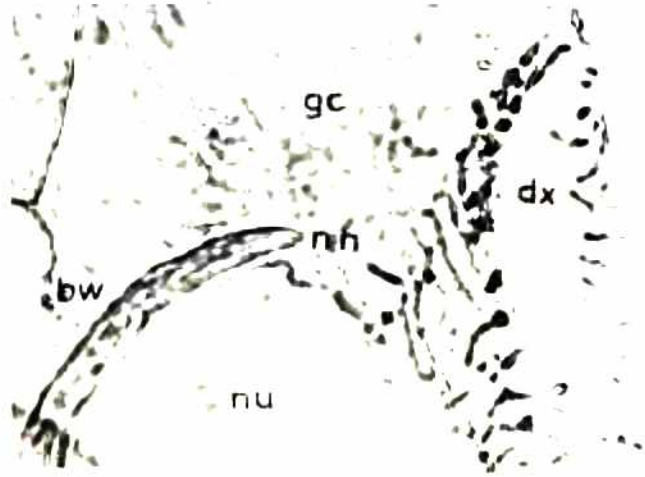
27



28



29



30

Fig. 31 A portion of a S. melongena root in transection showing nuclear hypertrophy in cells adjacent to nematode head (×400).

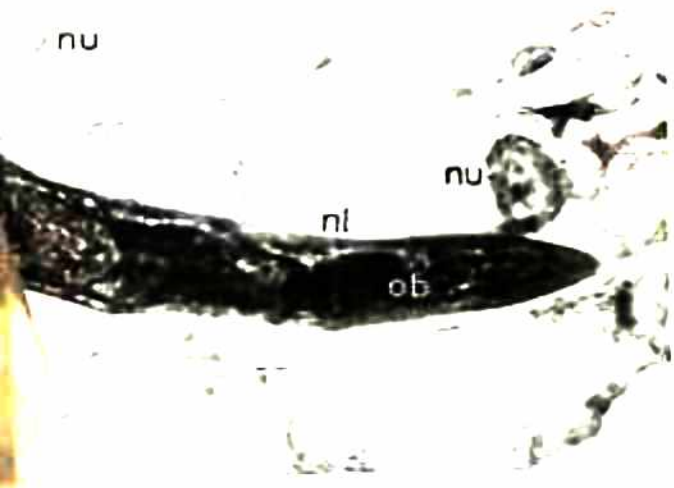
Fig. 32 A longisection of A. esculentus root showing the track along the path of nematode migration (×252).

Fig.32A A giant cell complex produced by a male nematode in S. melongena . The walls remain thin and the cytoplasm is poorly developed. Several hypertrophied nuclei are seen (×230).

Fig. 33 A transection of L. acutangula root showing histological changes induced by a male nematode. Note the necrotic effects, hypertrophy and hyperplasia in nearby cells (×115).

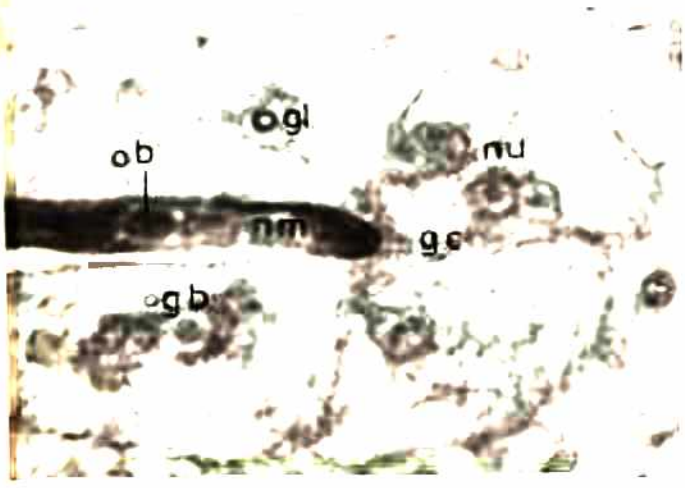
Fig. 34 Giant cell formation in A. esculentus. A longisection showing the orientation of nematode head, formation of giant cells from several types of cells and discontinuously developing xylem at the infected zone (×520).

Fig. 35 Development of hyperplastic tissue around the giant cells in C. sativum. Note the compact appearance of hyperplastic cells and the induction of abnormal xylem in them (×350).

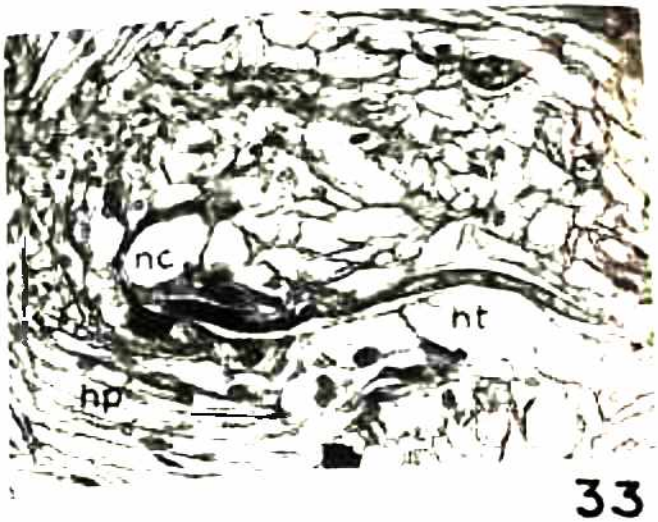


31

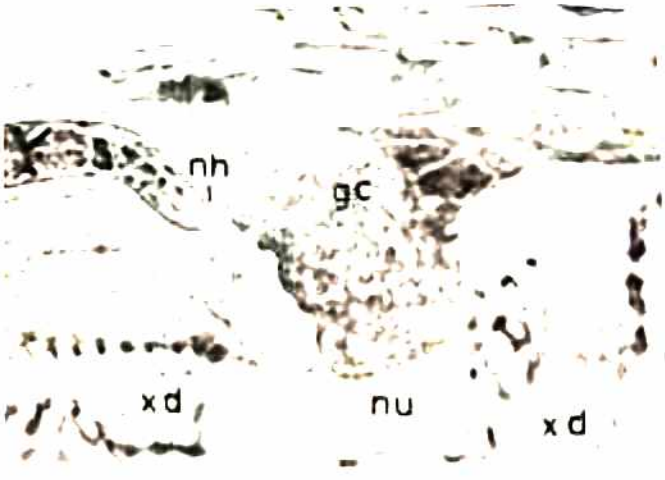
32



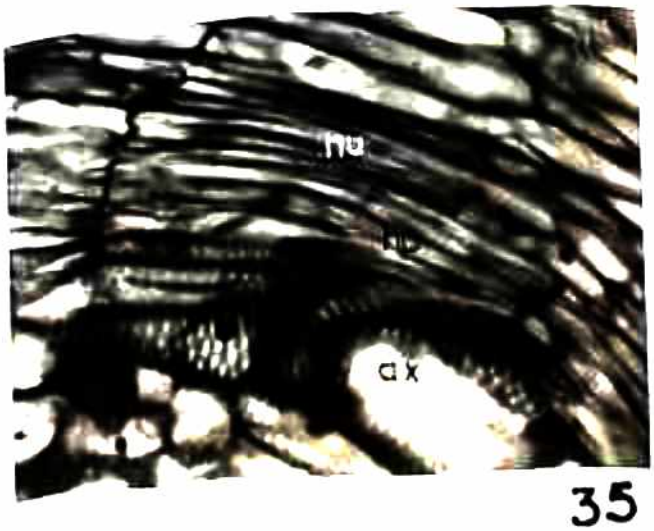
32A



33



34



35

Fig. 36 A longisection of A. esculentus showing giant cell formation along the stele. Note the hypertrophy in giant cell nuclei and nucleoli and the damage to the xylem (×275).

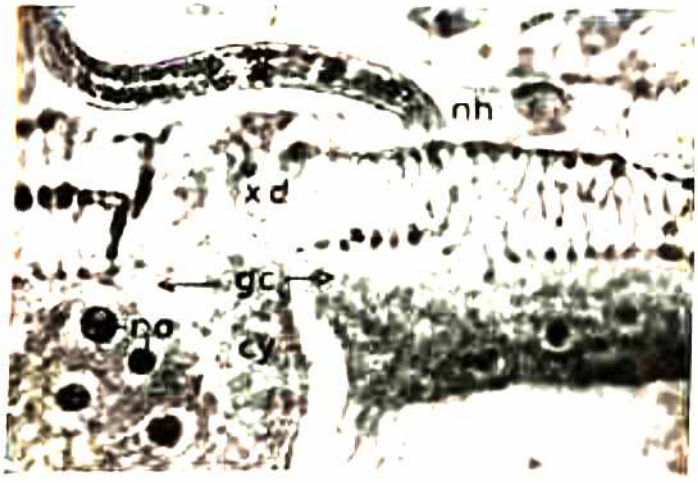
Fig. 37 A longisection of A. esculentus showing the disposition of 2 larvae in stelar region where several giant cells have developed. Note the dense cytoplasm (×240).

Fig. 38 A transection of S. melongena root. Note the formation of a giant cell complex with 6 units. A lateral root originates at this point and abnormal xylem is produced in transverse plane (×360).

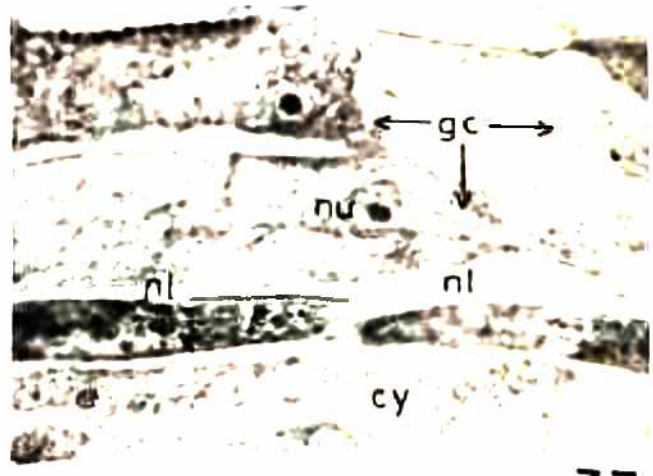
Fig. 39 Transverse section of a giant cell complex in A. esculentus. Several vacuoles and poly-nucleolar nuclei are evident in giant cells (×115).

Fig. 40 A heavily damaged vascular region in A. esculentus. Extensive coalescence of cells by cell wall dissolution has taken place. Xylem is seen in isolated patches (×330).

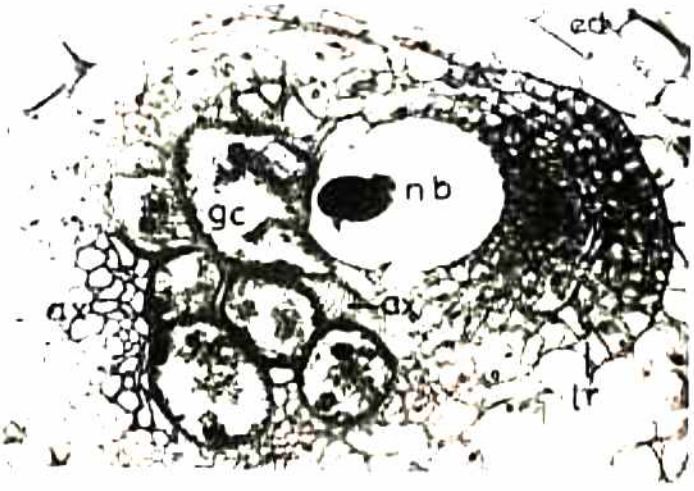
Fig. 41 A full-fledged giant cell complex in C. sativum. Dense cytoplasm with many nuclei accumulate in the centre of giant cells. A lining of cyto-plasm is seen around the giant cell wall (×350).



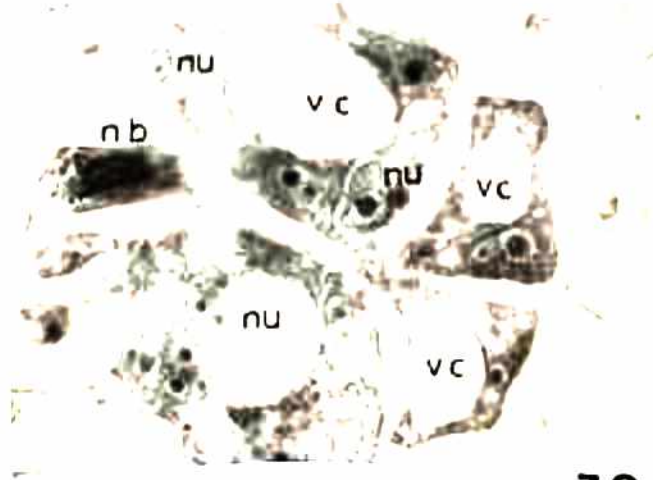
36



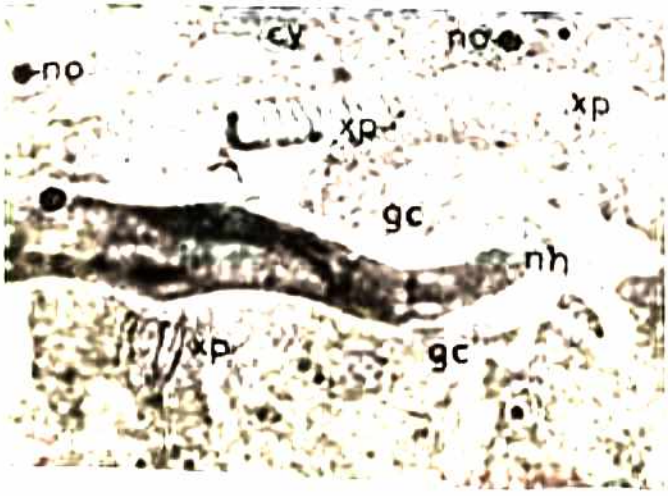
37



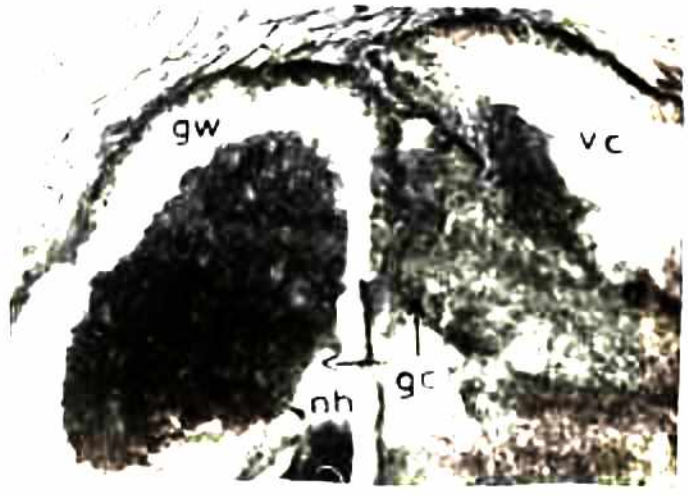
38



39



40



41

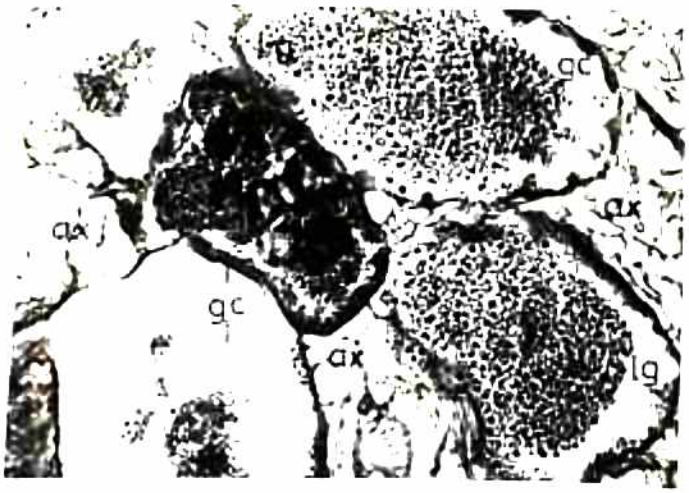
Fig. 42 A transection of L. acutangula gall showing a giant cell complex with several units possessing different organelles. Note the accumulation of lipid globules in 2 units. Several nuclei are seen in another unit (×245).

Fig. 43 A longitudinal section of a giant cell area in S. melongena gall. Note the association of xylem elements with giant cells, and occurrence of cell wall dissolution (×175).

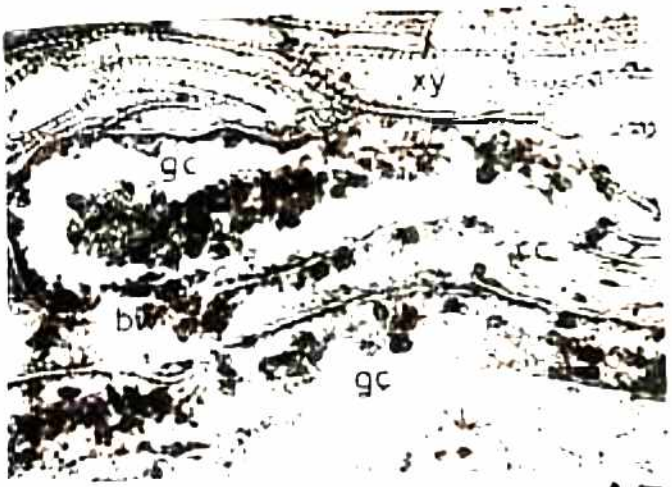
Fig. 44 A longisection of A. esculentus gall showing giant cells induced in primary stelar tissues. Much of the vascular tissues suffered damage although isolated patches of xylem survived (×225).

Fig. 45 A transection of A. esculentus showing a giant cell included in the secondary xylem tissue (×425).

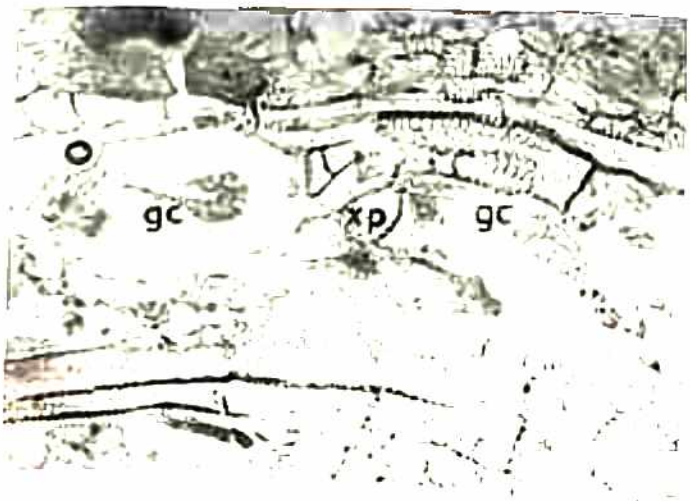
Fig. 46 and Fig. 47 Mature giant cells of L. acutangula associated with adult females. Round, amoeboid and bean-shaped nuclei are noticed in cytoplasm filled with lipid globules (fig. 46 × 225; fig. 47 × 260).



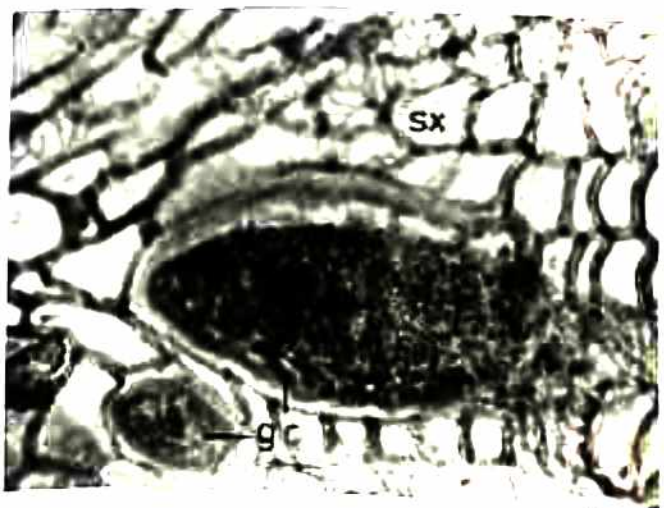
42



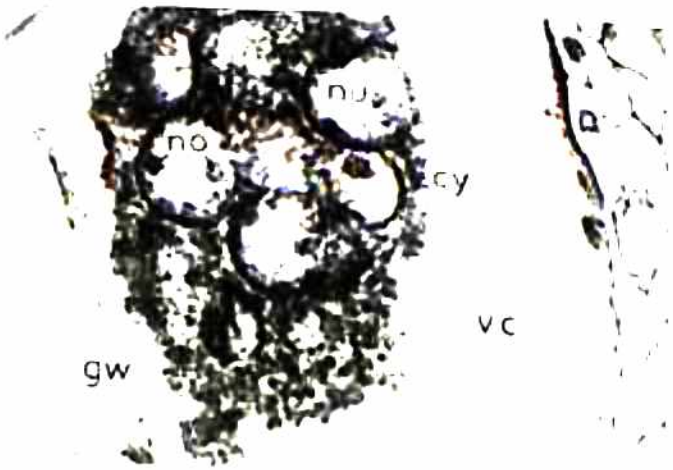
43



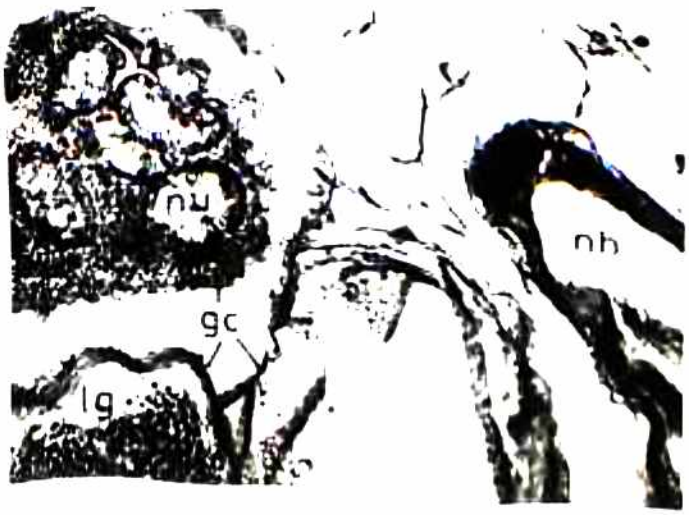
44



45



46



47

Fig. 48 A large nucleus in a L. scitangula giant cell. The chromophilic bodies accumulate around the nuclear membrane which show fissures. Nucleolus includes a nucleolar vacuole (×620).

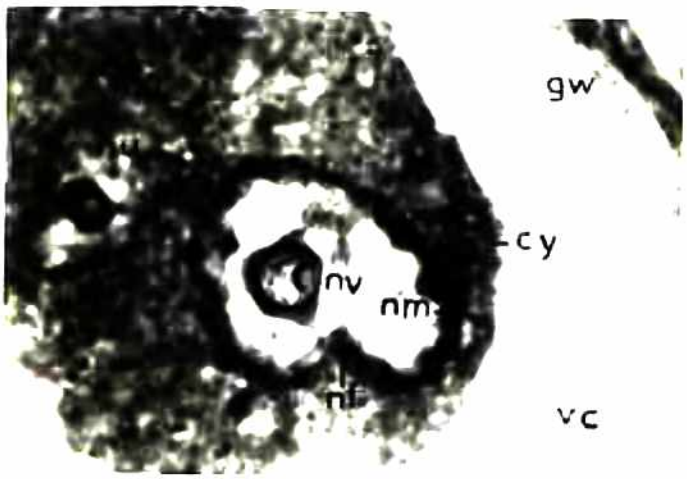
Fig. 49 A transection of a gall on C. frutescens. A cluster of giant cells with varying shapes and sizes of nuclei are seen. Note the lysigenous cavity in the centre of giant cell units, and the irregular patches of xylem. (×115)

Fig. 50 Transection of a primary root of A. esculentus. Nematode infection has converted much stelar tissue into giant cells (×145).

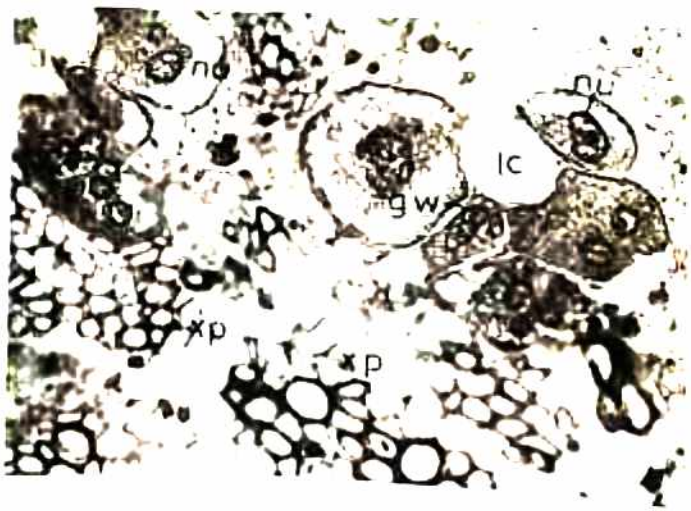
Fig. 51 Transection of a secondary root of A. esculentus, infected with M. incognita acrita. Note the damage to stelar tissues. Cross section of nematodes are also seen (×160).

Fig. 52 Transection of a nematode infected root of C. frutescens. Note the dissected appearance of the stele due to a large female embedded in the root tissues (×96).

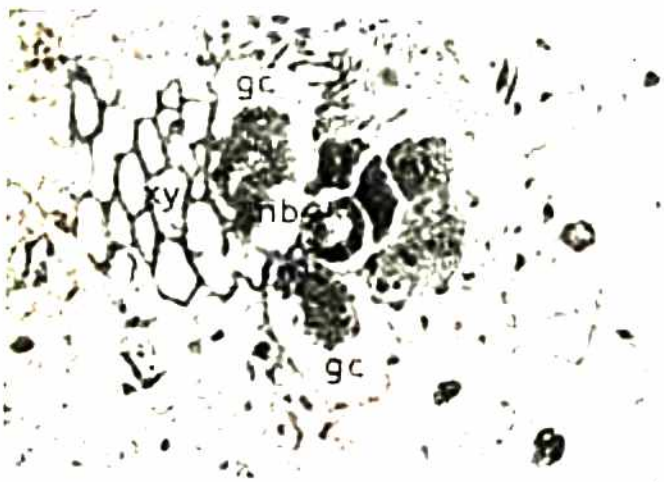
Fig. 53 A transection of an old root of S. melongena. Note the damage and derangement of xylem, and the occurrence of hypertrophied cells in stele (×115).



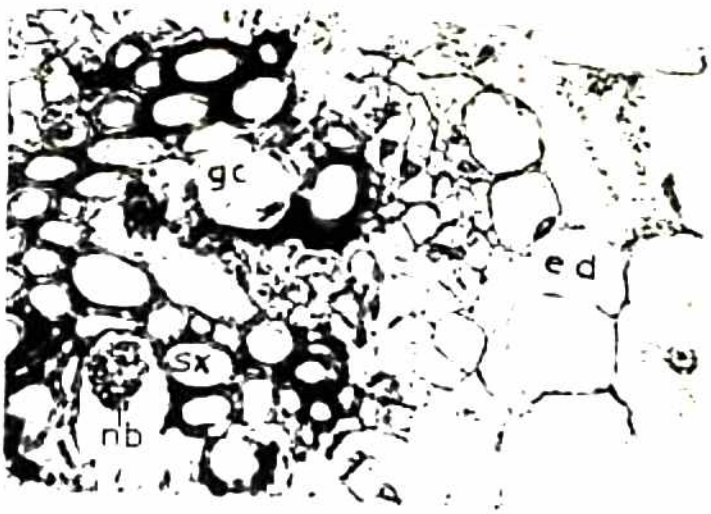
48



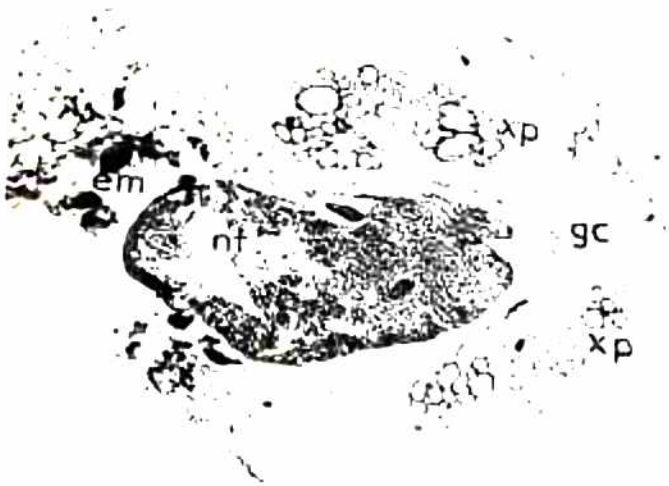
49



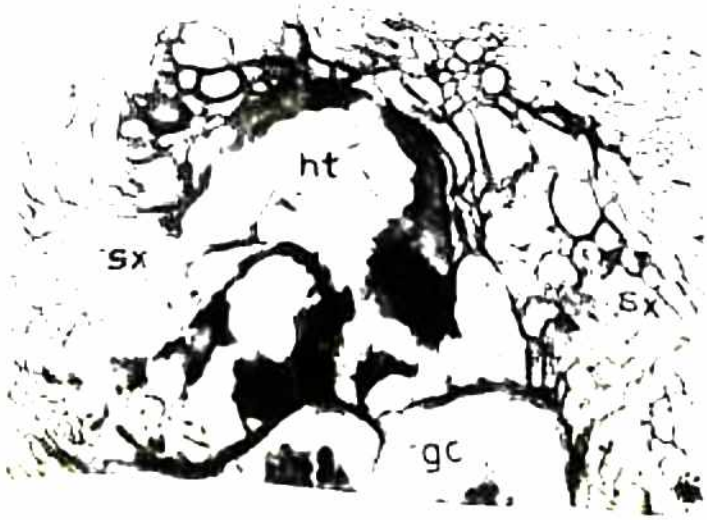
50



51



52

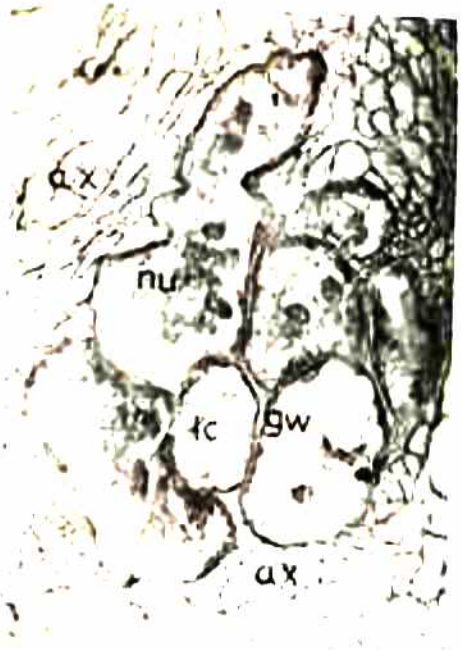


53

Fig. 54 Contour of an old giant cell complex in transverse plane. The abnormal xylem jackets the giant cell area. Note the lysigenous cavity and unequally thickened giant cell walls (×230).

Fig. 55 A portion of a longitudinal section of C. sativum root gall. Hyperplastic cells and abnormal xylem originate in tissues surrounding giant cells (×400).

Fig. 56 A longitudinal section of gall on C. sativum. Note the formation of vascular strands having connections with the main stele (×35).



54

55

56

Fig. 57 A club-shaped root of S. melongena. Nematode infection caused the swelling of the region behind the root meristem (×140).

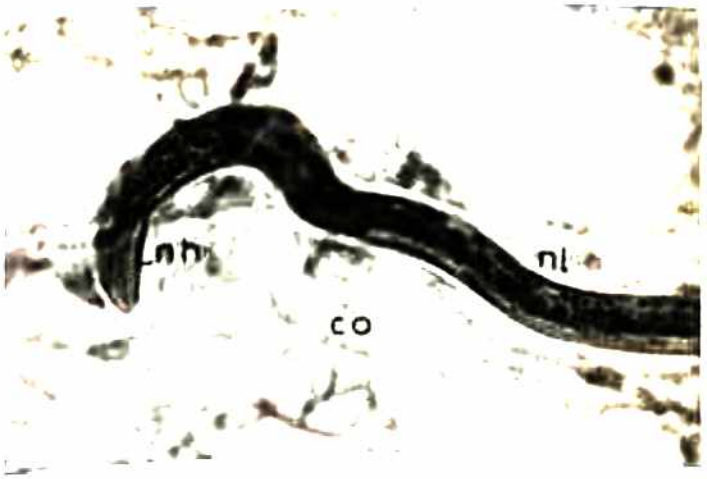
Fig. 58 and Fig. 59 Infective nematodes within the root tissues of S. melongena (fig. 58, ×475 and fig. 59, ×350).

Fig. 60 A nematode head located intracellularly. Broken cell wall and hypertrophied nucleus are seen around its head (×1125).

Fig. 61 A portion of S. melongena root showing the lysigenous intercellular cavity produced during nematode movement. Note the hypertrophy felt in certain nuclei (×1000).

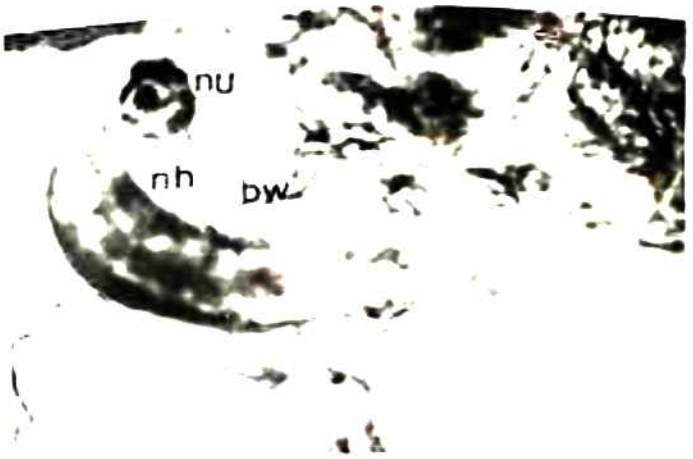
Fig. 62 A young giant cell area in longitudinal section. Note the cytological alterations produced within 5 days after inoculation. Amitotic duplication of nuclei and nuclear aggregates are evident (×190).

57

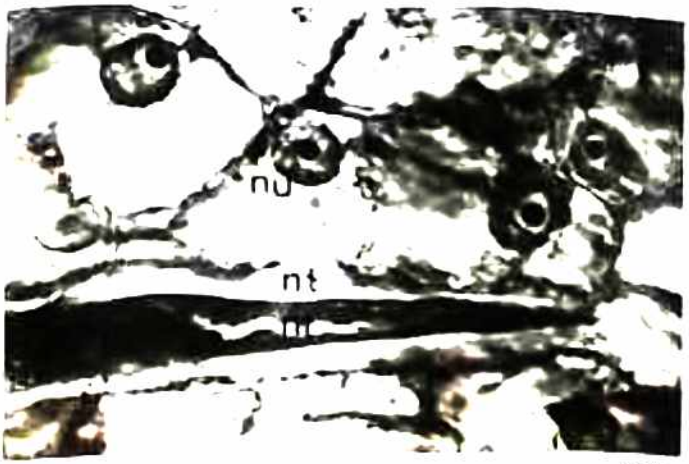


58

59



60



61

62

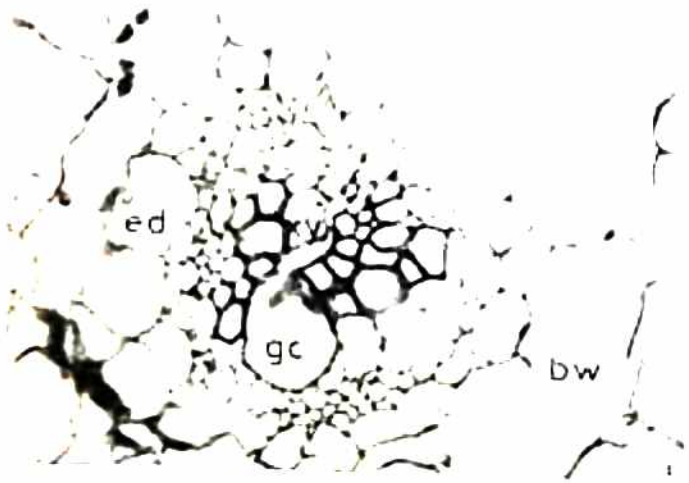
[illegible] early stages of giant cell formation.
[illegible] and two initiation of giant cell on
one side of xylem and in fig. 64 coalescence
of xylem elements with giant cell is seen
(both ×215).

[illegible] Expansion of giant cell complex by coalescence
[illegible] Note the breaks and compression
[illegible] outer cell layers due to giant

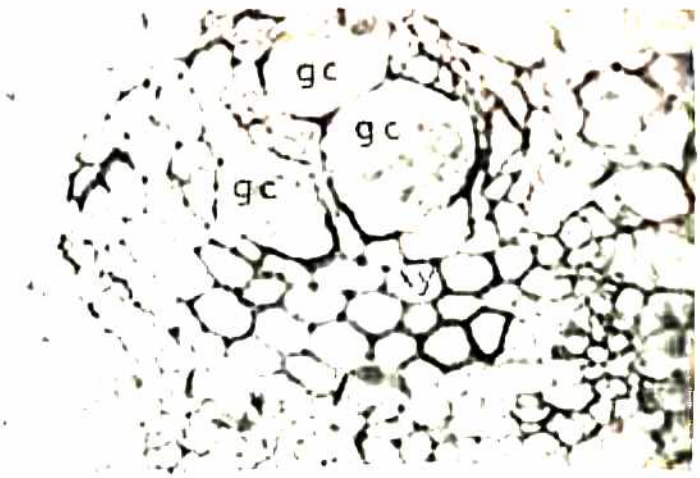
Fig. 66 Cross section of a 10 day old gall. Further expansion of giant cell is evident. Note the nematode head located in the centre of giant cells (×215).

Fig. 67 A transection of the nongalled portion 150 μ away from that in fig. 66. Note the apparently normal appearance of the stele (×215).

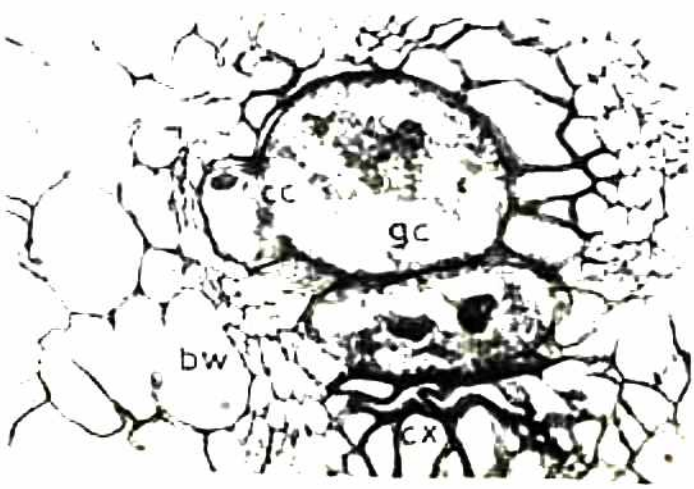
Fig. 68 A longitudinal section showing thick walled giant cells in stele. Note the enormous number of nuclei in one of the giant cell units. Plasmalemma is seen projecting into the vacuole in another unit. Hyperplasia and formation of a lateral root are seen around the giant cells (×480).



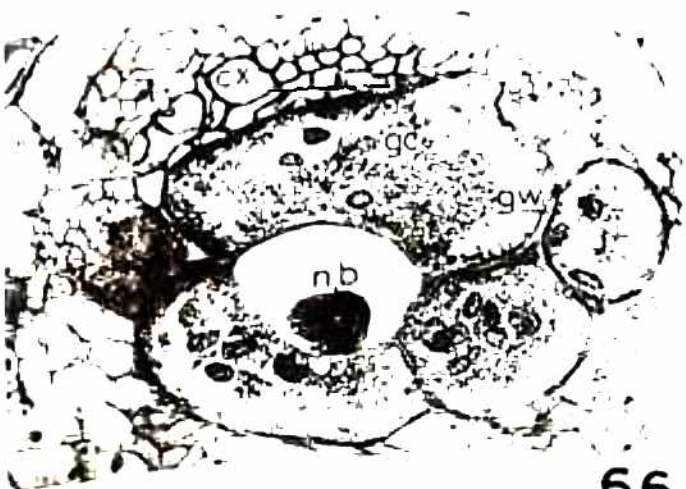
63



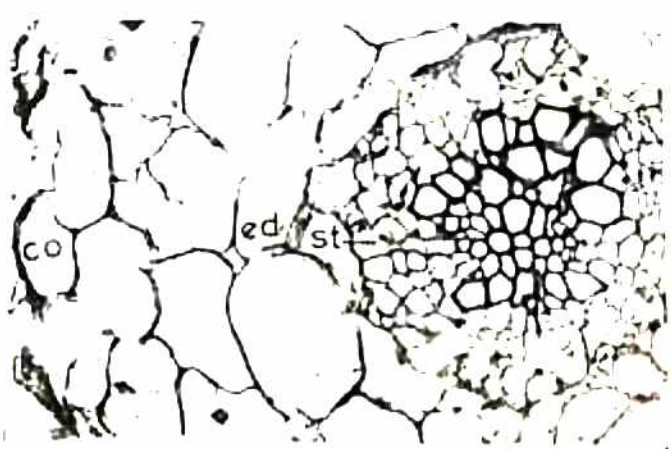
64



65



66



67

68

Fig. 69 A longitudinal section of a large giant cell. The giant cell encroaches into the surrounding tissue causing acquisition of nuclei. Hyperplasia is well marked. Giant cell formation and hyperplasia increased the diameter of infected portion (×90).

Fig. 70 Development of a cluster of giant cell units in the steler tissues. Giant cell units have varying amounts of cell contents (×55).

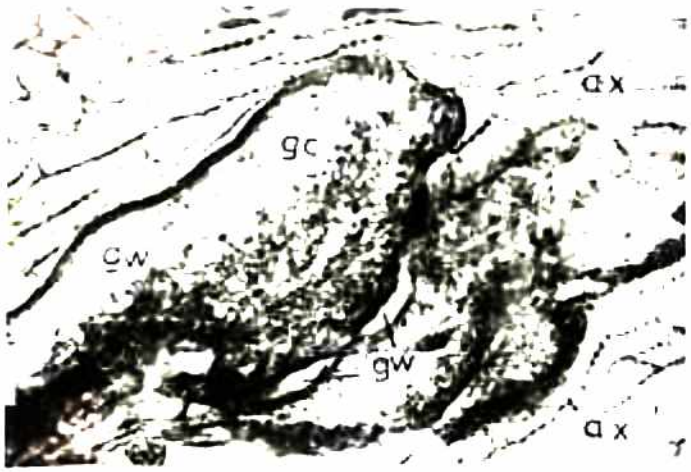
Fig. 71 and Fig. 72 Sections showing the pattern of cell wall thickening. Note the thick and thin areas. Thin areas produced breaks and help in exchange of materials and giant cell expansion (both, ×360).

Fig. 73 A transverse section of a 15 day old gall of S. melongena. Note the round and hypertrophied nuclei in giant cells and neighbouring tissues (×660).

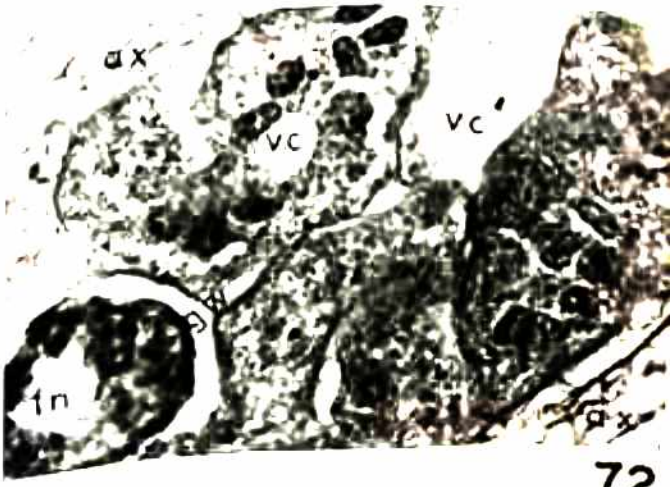
Fig. 74 A longitudinal section of a 15 day old gall. Note the striking difference between the nuclei in giant cells and adjacent tissues. The giant cell nuclei are of varying shapes (×320).

69

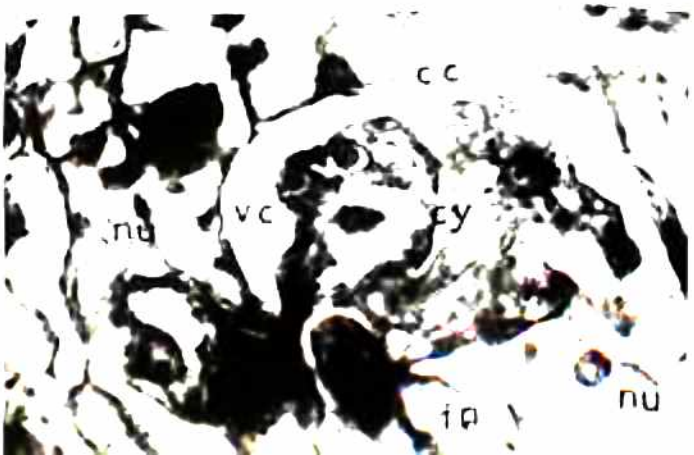
70

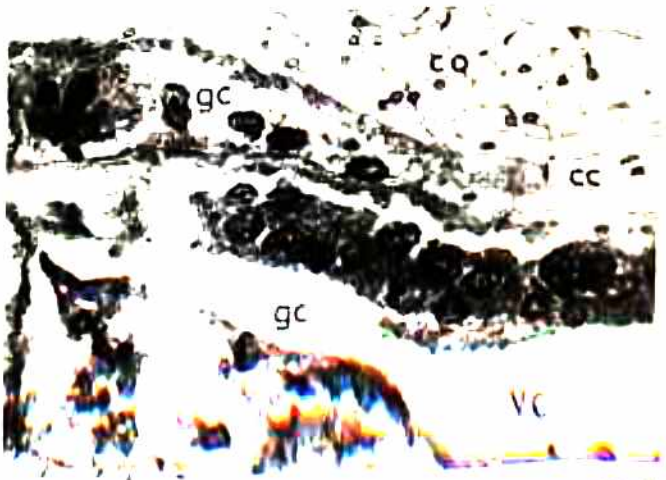


71



72





74

Fig. 75 A full-fledged giant cell 15 days after inoculation. Note the dense contents, thick cell walls and the formation of abnormal xylem around giant cells (×575).

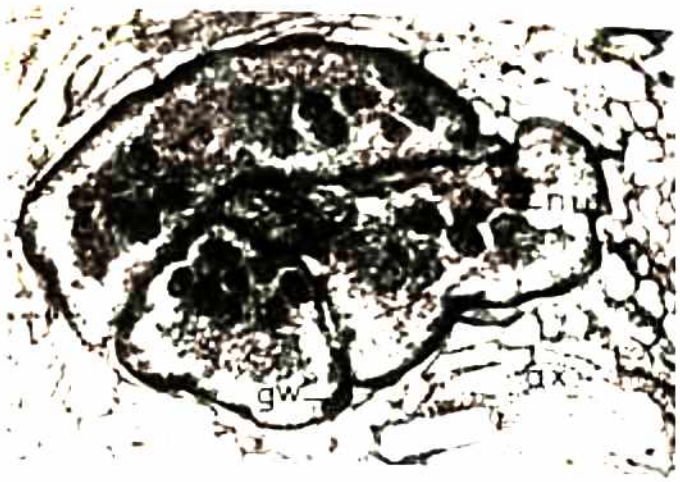
Fig. 76 A longitudinal section of a 15 day old gall. Note the longitudinal extension of giant cells with dense cytoplasm and small vacuoles. Discontinuous xylem and broken endodermal layer surround the giant cells (×320).

Fig. 77 A transverse section of a gall. The giant cell complex is produced by a larva lodged in the stele. Note the cluster of giant cell units radiating around a lysigenous cavity (×355).

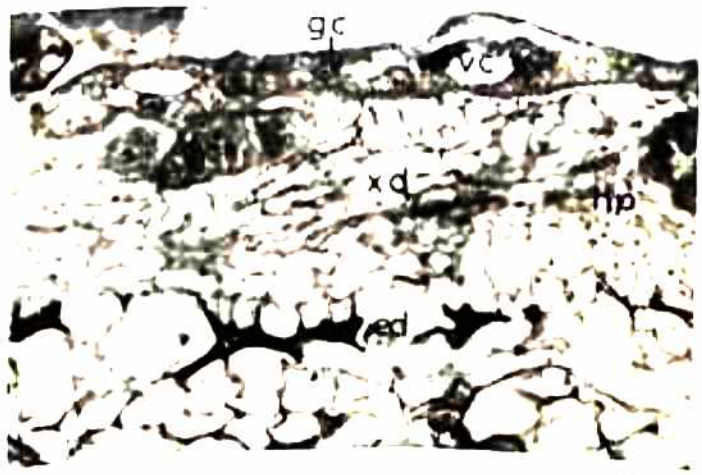
Fig. 78 A longitudinal section of giant cells formed along the stele. Note the breaks in the unequally thickened giant cell walls. Giant cell walls remain thin near the coalescing cells (×360).

Fig. 79 A section of S. melongena gall showing the longitudinal growth of giant cell. Coalescence of cells is quite evident (×90).

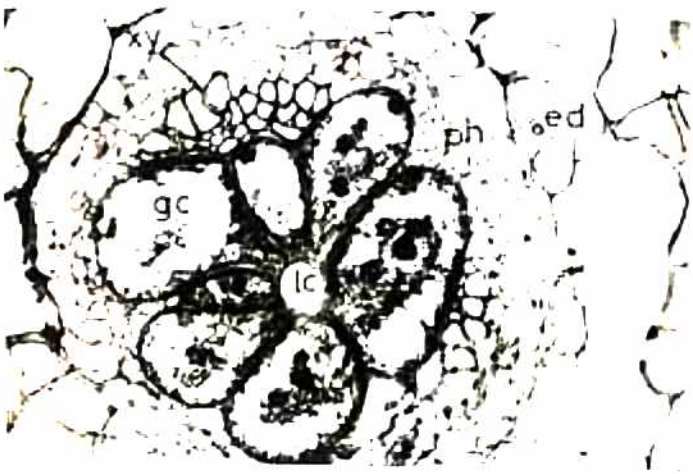
Fig. 80 A portion of giant cell wall showing breaks. Note the round edge of broken cell wall and the continuity of cytoplasm between the two cells (×650).



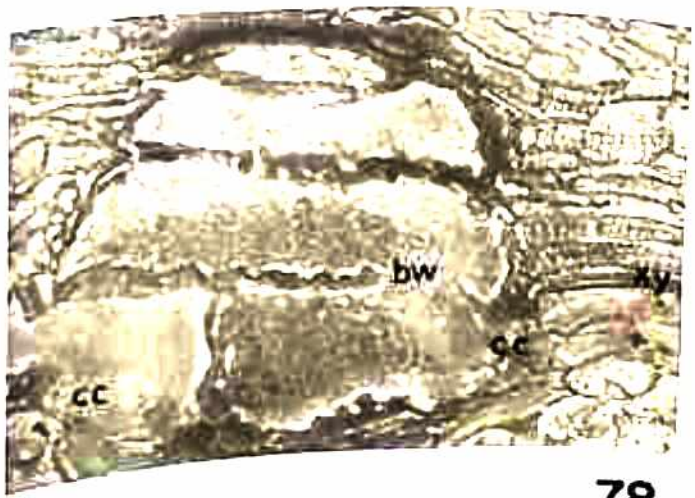
75



76

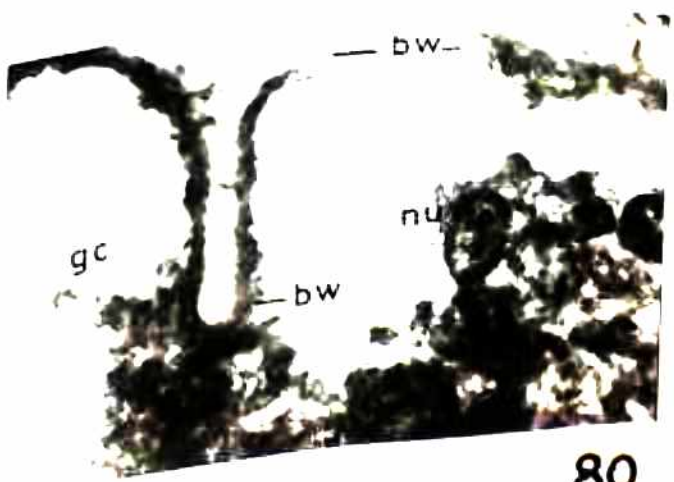


77



78

79



80

Fig. 81 A transverse section of a 20 day old *S. melongena* gall. Cell wall dissolution and nuclear clumping are clear (×400).

Fig. 82 Enlargement of several nuclei from a 20 day old gall. Note the shape of the nuclei and their wavy margins (×880).

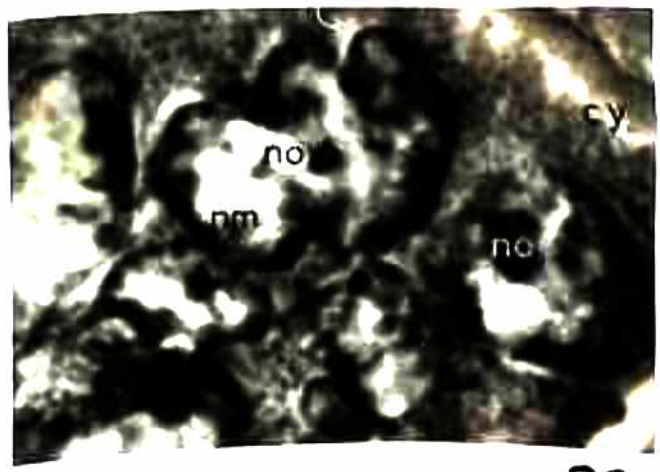
Fig. 83 A longitudinal section of a root-knot, produced as a result of giant cell expansion, production of hyperplastic cells and swelling of the female nematode (×55).

Fig. 84 An enlarged view of hyperplastic cells produced around giant cells (×560).

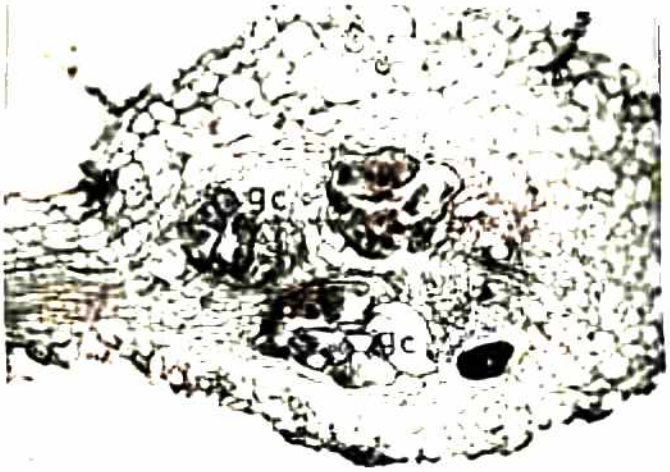
Fig. 85 A longitudinal section showing the merging of cortical cells with the giant cell induced in the stele (×190).

Fig. 86 An egg-laying female enclosed in the root-tissue. Note the large cavity occupied by the female, and the changes in the secondary xylem tissues near the nematode (×35).

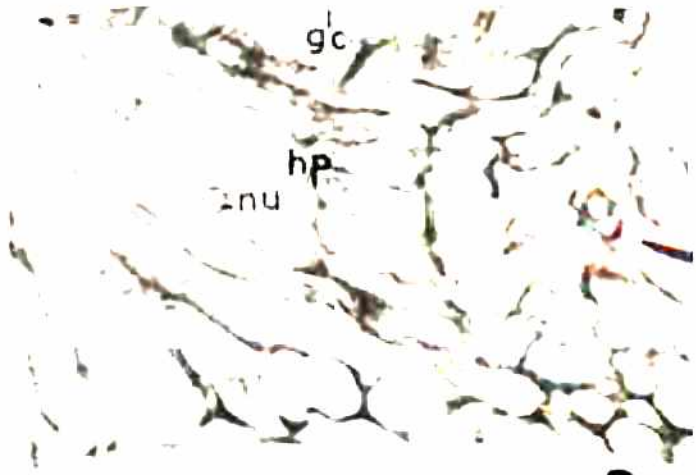
81



82

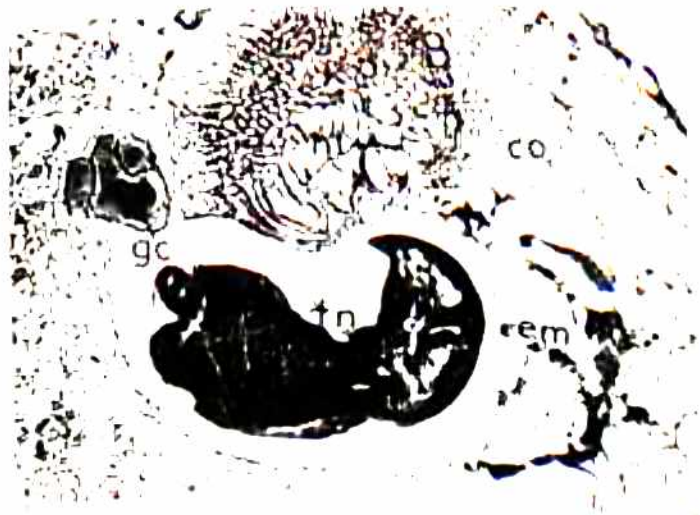


83



84

85



86

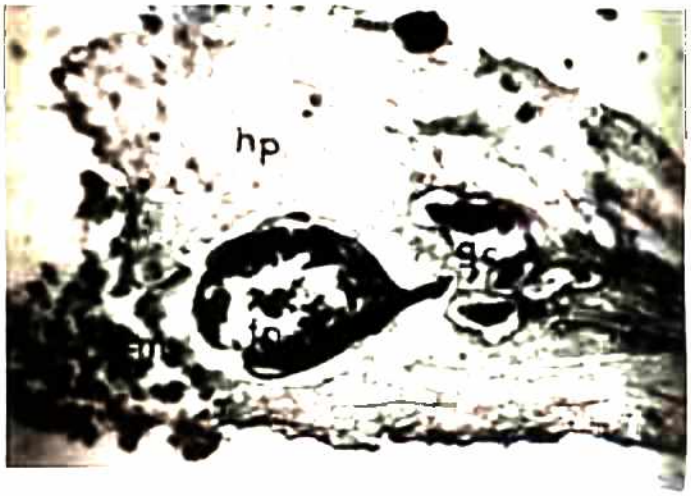
Fig. 87 A longitudinal section of a mature gall on the root tip of S. melongena, showing the position of the giant cells, the nematode and its eggmass (×35).

Fig. 88 A longitudinal section showing 2 adult females located closely in a root. Note the giant cells around their heads, the wedge of parenchyma separating the 2 nematodes. Giant cells show signs of deterioration (×55).

Fig. 89 A giant cell complex 30-40 days after inoculation. Cytoplasm is vanishing from the giant cells; though it shows accumulation towards the leading edge of giant cell units. Several breaks are seen on the walls (×180).

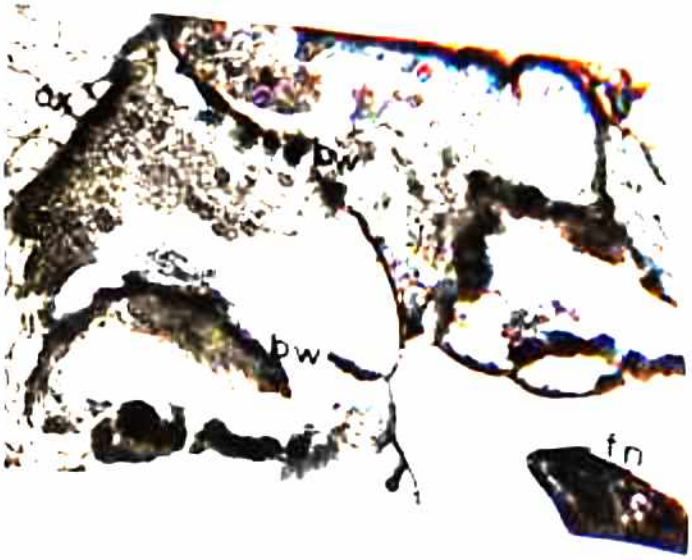
Fig. 90 and Fig. 91 Transverse sections showing the root-knot nematode damage to xylem tissues. Note the abnormal formation of secondary xylem in transverse plane (fig. 91) (both, ×100).

Fig. 92 A transection showing the cavities in cortex occupied by female nematodes. Swellings of the female body and growth of gall parenchyma increase the gall size. Note the giant cells produced in secondary xylem tissues (×100).

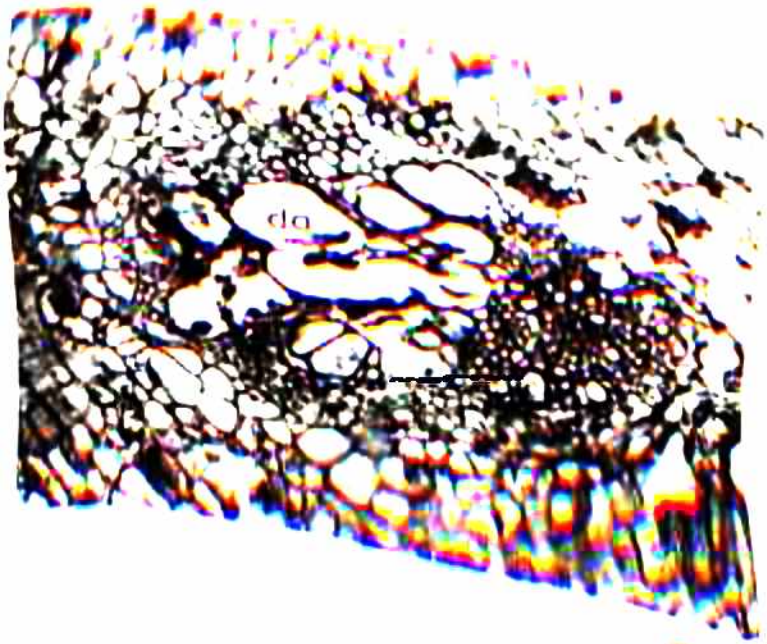


87

88



89



90

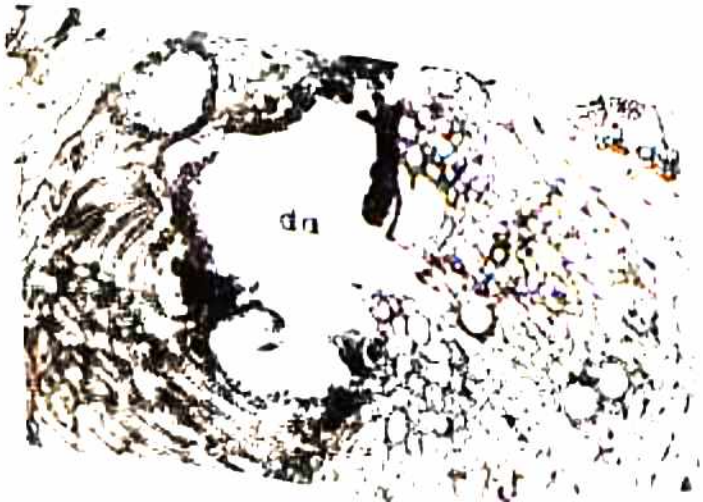


Fig. 93 Longitudinal section of a heavily infected root (×25).

Fig. 94 A transverse section showing a dead nematode and associated pathological tissues in root cortex (×260).

Fig. 95 A transection of a heavily damaged root. Note the disruption to the conducting elements (×55).

93

94

95

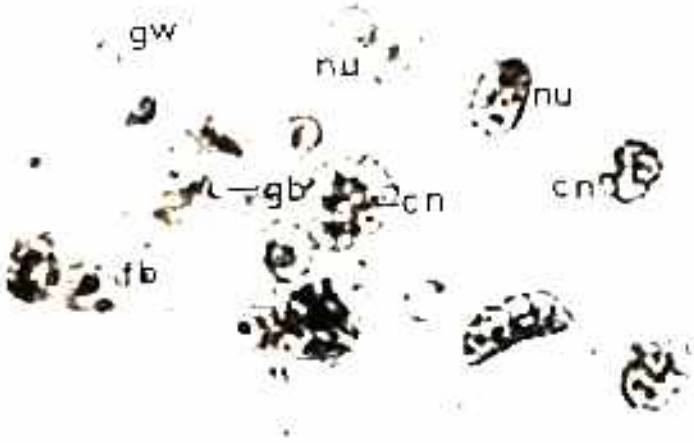
Fig. 96 and Fig. 97 Nuclei in giant cell complexes stained with Schiff's reagent. Nuclei are rich in DNA. Fuelgen bodies are distributed in the nuclear interior and around the nuclear membrane. Note the different sizes and peculiar shapes of the nuclei. Nuclear clumping and amitotic duplications are evident in fig. 96 (fig. 96, ×610 and fig. 97, ×306).

Fig. 98 A giant cell area stained with methyl green-pyronin. Nucleoli and cytoplasm show high concentrations of RNA. Nuclei are rich in DNA. Glistening bodies are also seen (×306).

Fig. 99 A phase contrast photograph of giant cell nuclei and nucleoli stained with Azure B. Nuclei show high concentration of DNA and nucleoli of RNA (×720).

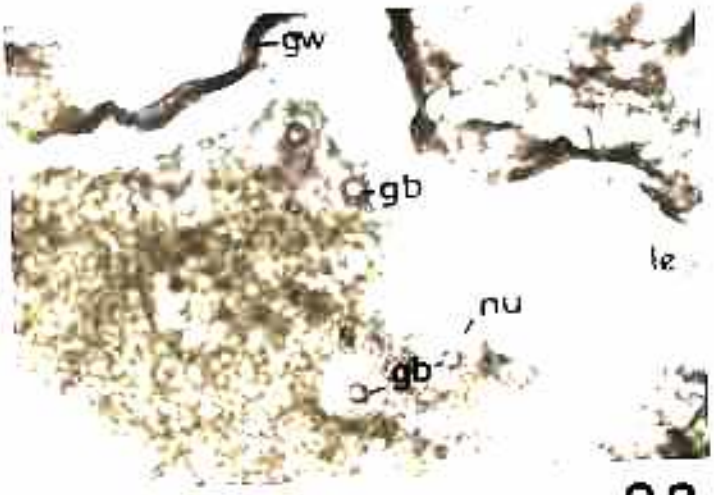
Fig. 100 Wall incrustations stained with Azure B (×1600).

Fig. 101 A transection showing giant cell wall positive in PAS technique (×275).

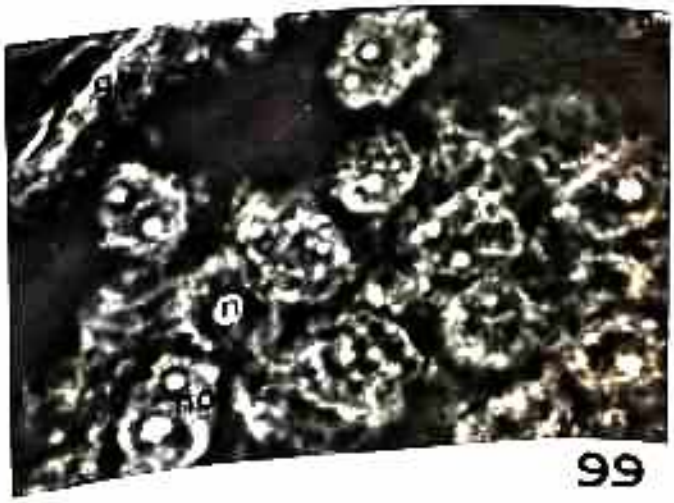


96

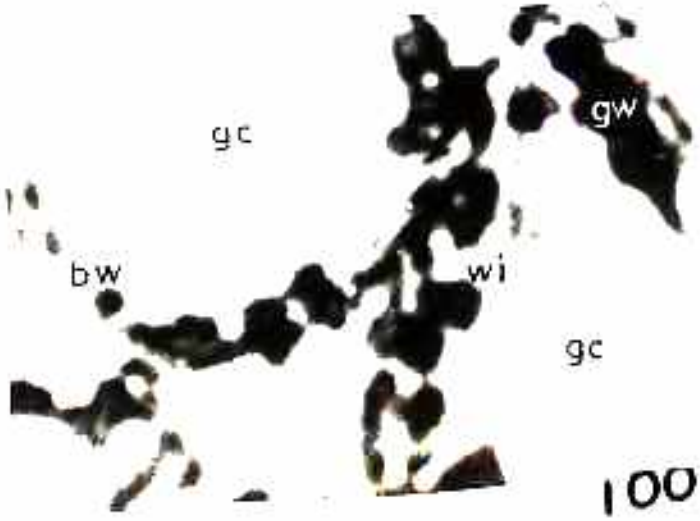
97



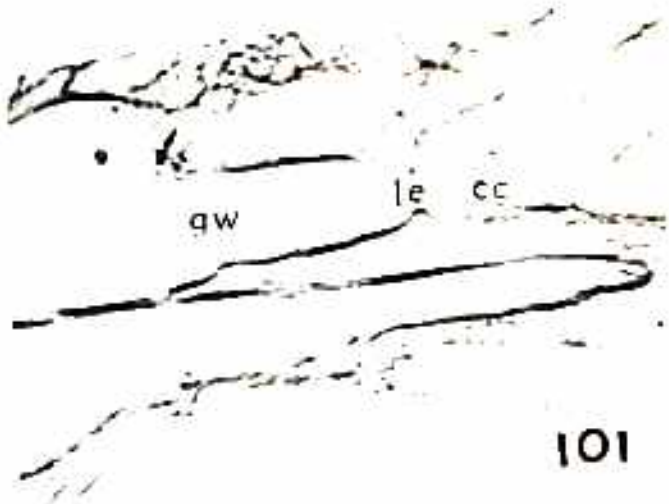
98



99



100



101

Fig. 102 M. incognita acrita eggs cleared in lacto-phenol. Note the detachment of cell contents from egg cuticle in certain cases (×370).

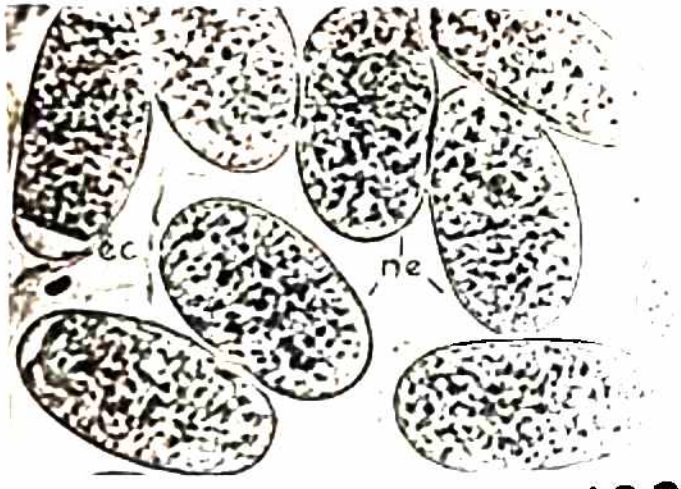
Fig. 103 Infective second stage larvae migrating inside the root of S. melongena. The root was cleared in lactophenol-cotton blue (×90).

Fig. 104 A second stage larva seen embedded in cortical tissues of a cleared root (×100).

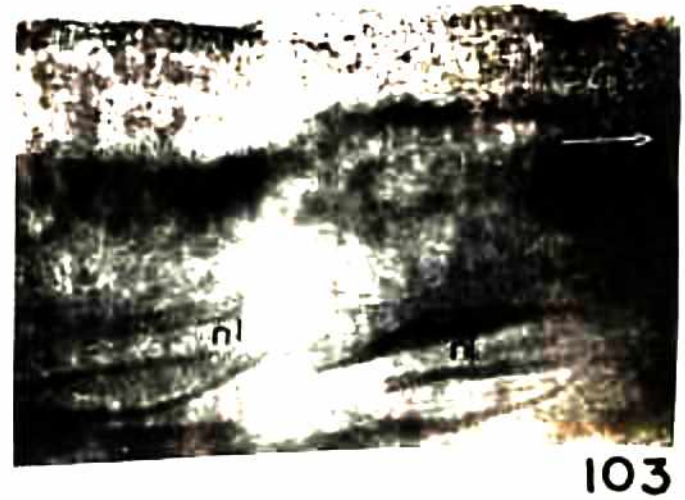
Fig. 105 A root clearing showing several larvae developing around the stele of S. melongena root (×240).

Fig. 106 A sausage-shaped larva embedded in root tissue. Note the head region introduced into the vascular tissues (×100).

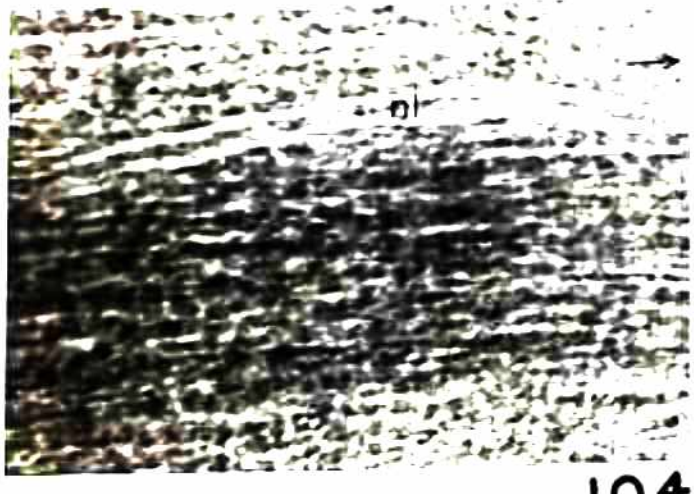
Fig. 107 A female nematode embedded in root tissues. The growth of the nematode has caused swellings of the root (×125).



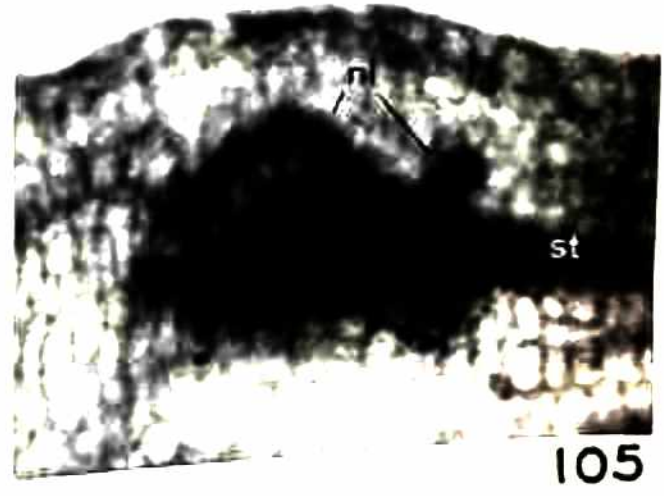
102



103

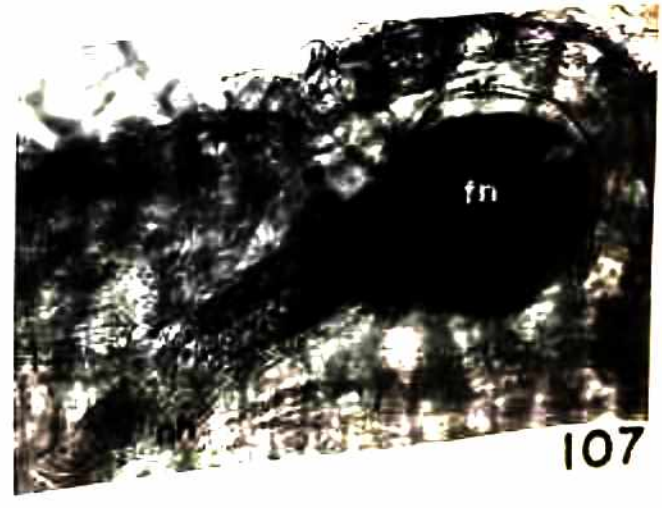


104



105

106



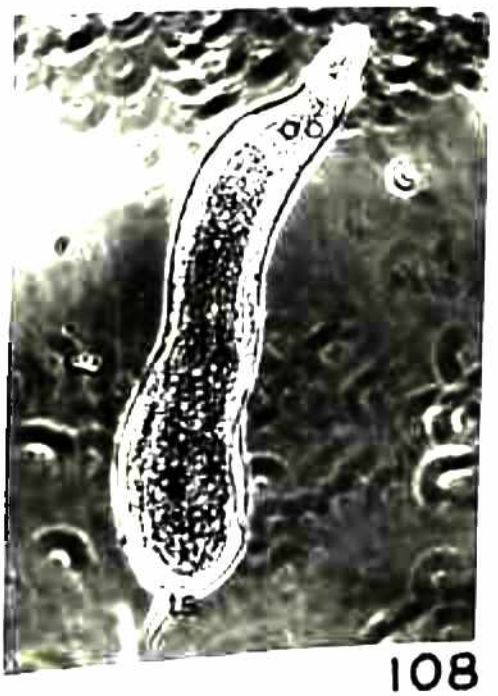
107

Fig. 108 A larva teased out of root 5 days after inoculation. Note the increase in the body size due to feeding. The larva retains a tail-spike. Phase contrast; (×190).

Fig. 109 A sausage-shaped larva teased out of root 7 days after inoculation. Note the body bends caused by the orientation of host cells. Phase contrast; (×150).

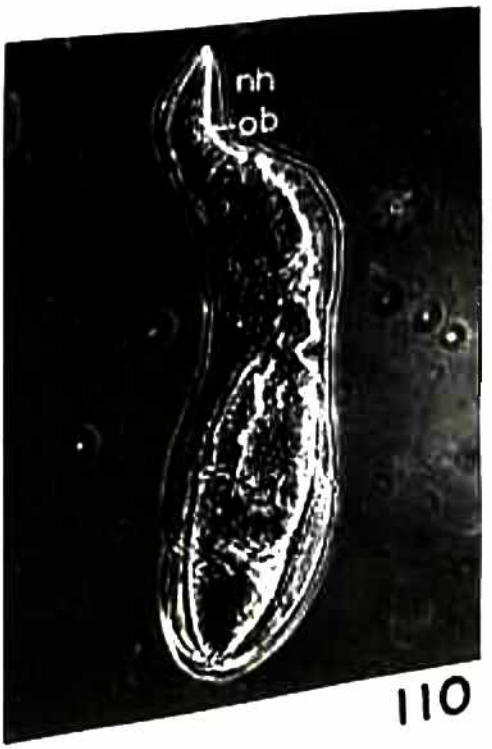
Fig. 110 A third-stage larva developing inside the second stage cuticle. The body contents have withdrawn from the two ends of the cuticle. Phase contrast; (×150).

Fig. 111 A female nematode. Note the paired ovaries and the characteristic perineal cuticular annulations. Phase contrast; (×100).

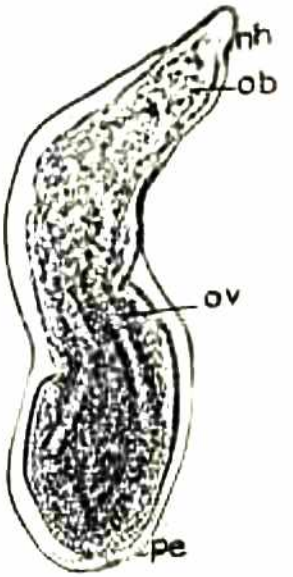


108

109



110



111

Fig. 112 A mature female nematode. Phase contrast; (×120).

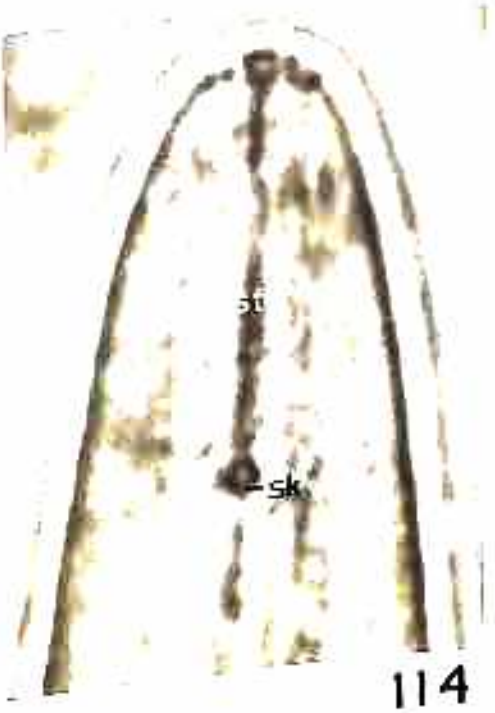
Fig. 113 A male nematode coiled inside the cuticles of the previous instars. The second stage cuticle encloses the successive cuticular layers. Phase contrast; (×150).

Fig. 114 Enlarged view of the head region of a second stage larva (×2240).

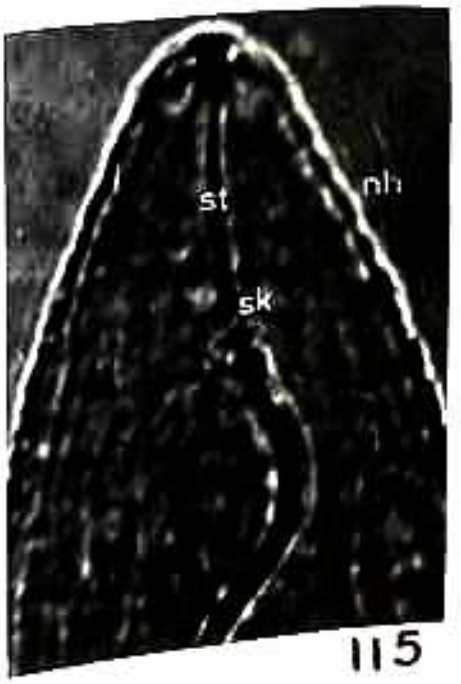
Fig. 115 Head region of a female nematode. Note the buccal spear, the oesophagus and cuticular annulations on the body (×1850).

112

113



114

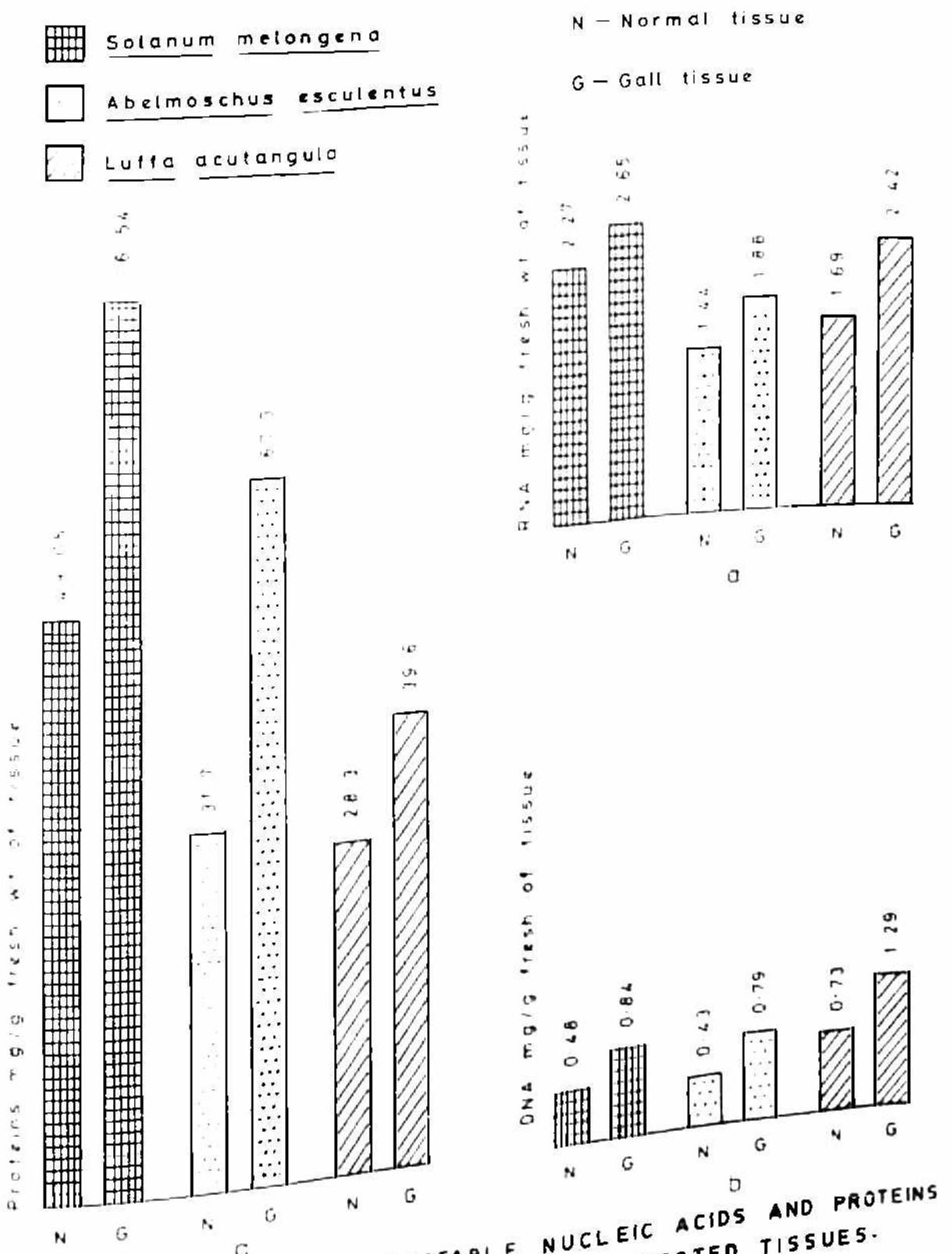


115

Fig. 116A Histograms showing the concentrations of RNA in normal and M. incognita acrita infected root tissues of S. melongena, A. esculentus and L. acutangula.

Fig. 116B Histograms showing the concentrations of DNA in normal and root-knot infected root tissues of the 3 hosts.

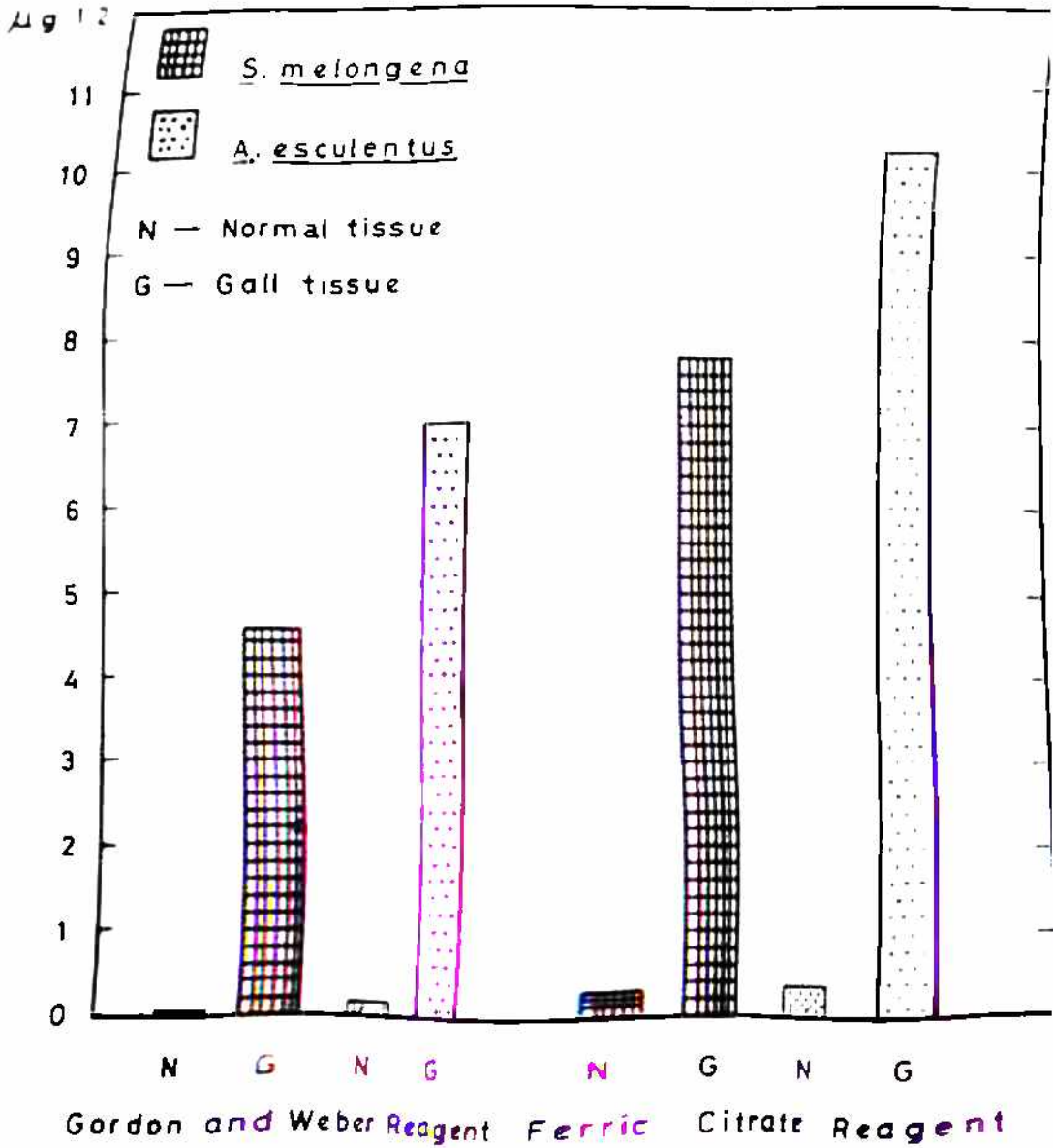
Fig. 116C Histograms showing the concentrations of total proteins in normal and root-knot infected root tissues of the 3 hosts.



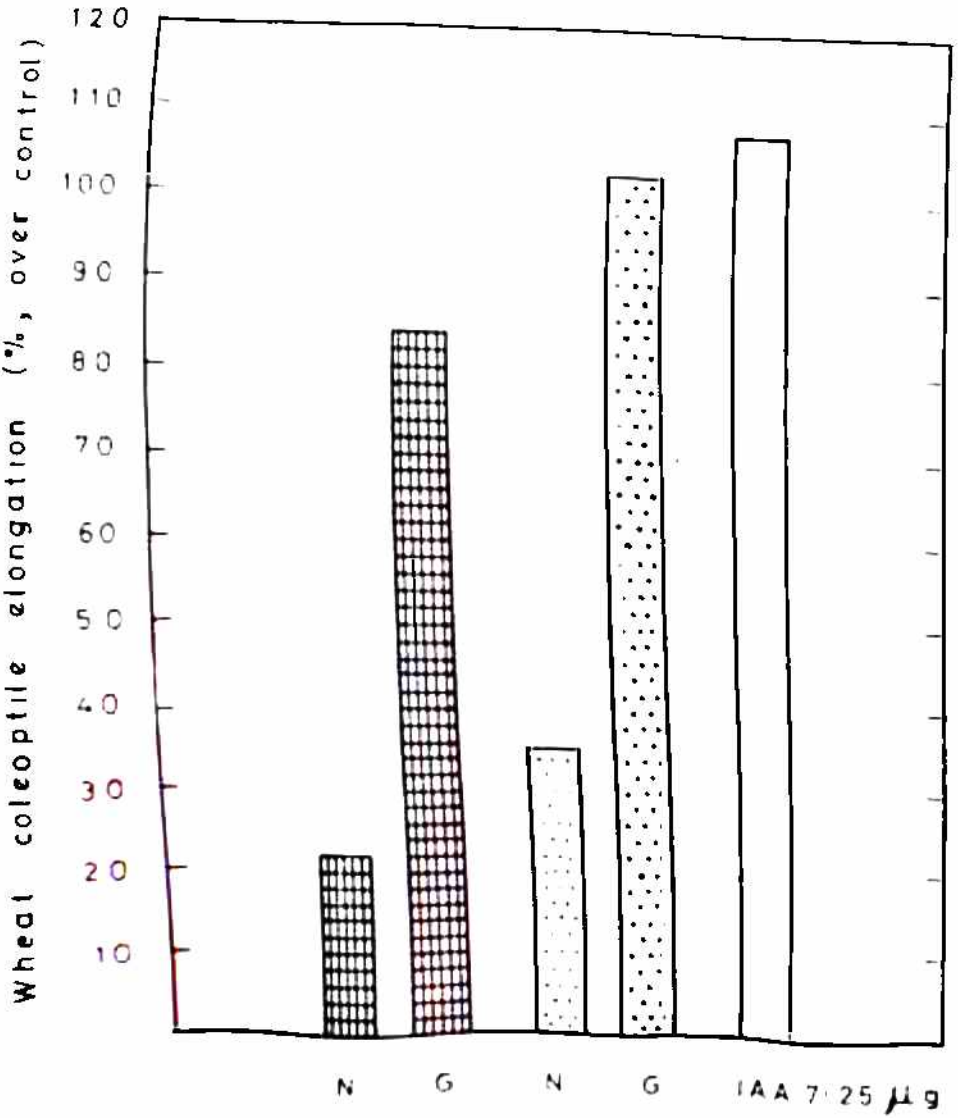
CONCENTRATIONS OF EXTRACTABLE NUCLEIC ACIDS AND PROTEINS IN NORMAL AND M. INCOGNITA ACRITA INFECTED TISSUES.

Fig. 117A Histograms representing the concentrations of indolic growth substances from normal and gall tissues of S. melongena and A. esculentus. Quantitation was made by spectrophotometry after chromogenic reaction with Gordon and Weber and the new ferric citrate reagents.

Fig. 117B Histograms showing the results of the biological activity (wheat coleoptile elongation) of indolic growth substances in normal tissues and M. incognita acrita galls. Growth activity of authentic IAA is also represented.



COLORIMETRIC DETERMINATION OF GROWTH SUBSTANCES FROM NORMAL AND GALL TISSES



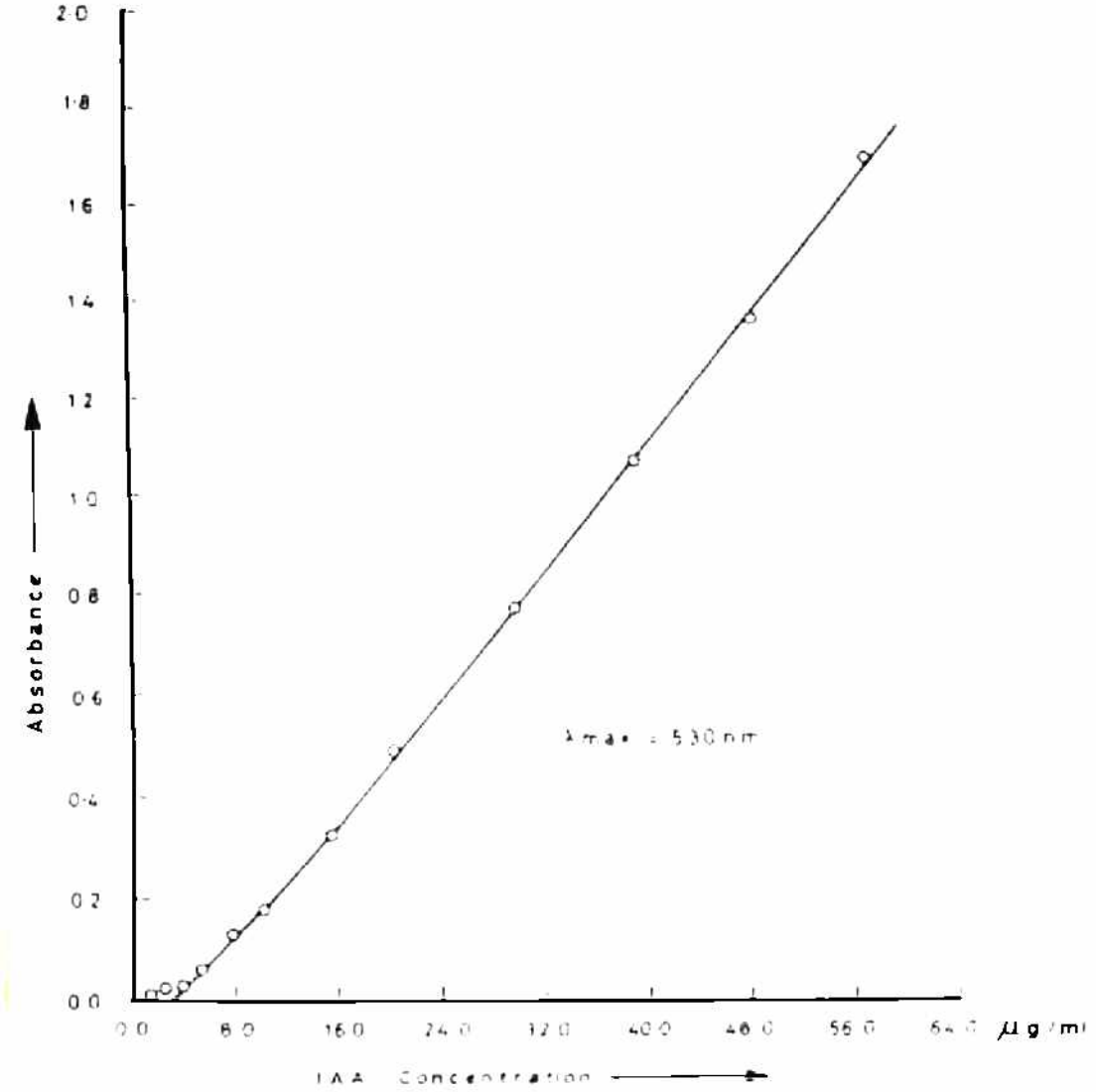
BIOASSAY OF GROWTH SUBSTANCES IN NORMAL AND M. INCOGNITA ACRITA GALLS

117 b

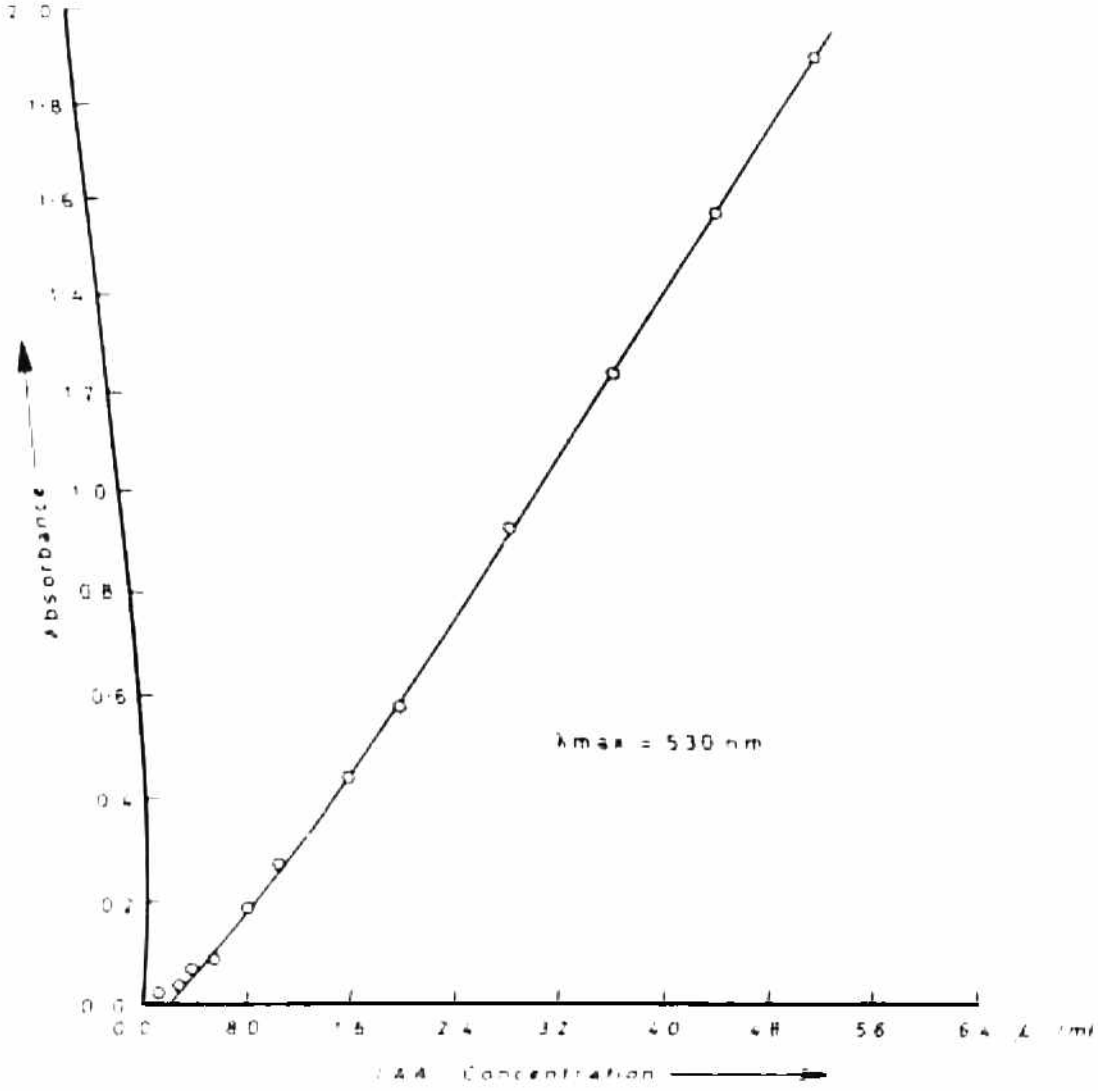
Fig. 118A Standard curve for synthetic indole acetic acid with the Gordon and Weber reagent.

Fig. 118B Standard curve for synthetic indole acetic acid with the new ferric citrate reagent.

Note: IAA produced stronger colour with the new ferric citrate reagent, and the absorbance therefore was higher.



STANDARD CURVE FOR IAA WITH GORDON AND WEBER REAGENT



STANDARD CURVE FOR IAA WITH FERRIC CITRATE

LITERATURE CITED

LITERATURE CITED

Allen, P.J. (1942). Changes in metabolism of wheat leaves induced by infection with powdery mildew. Amer. J. Bot., 29 : 425-435.

Allen, P.J. (1954). Physiological aspects of fungus diseases in plants. Annu. Rev. Plant Physiol., 5 : 559-574.

Arya, H.C., Hildebandt, A.C., and Riker, A.J. (1962). Nucleic acids in callus tissues from grape stem and Phylloxera gall. Phyton (B. Aires), 19 : 27-29.

*Atkinson, G.F. (1890). Nematode root galls. A preliminary report on the life history and metamorphosis of a root-gall nematode, Heterodera radicicola (Greef) Müll., and the injuries produced by it upon the roots of various plants. J. Elisha Mitchell Sci. Soc., 6 : 81-130.

Balasubramanian, M., and Rangaswami, G. (1962). Presence of indole compounds in nematode galls. Nature, 194 : 774-775.

Balasubramanian, M., and Rangaswami, G. (1964). Studies on host range and histopathology of root-knot infections caused by Meloidogyne javanica. Indian Phytopath., 17 : 126-132.

Baldwin, J.G., and Barker, K.R. (1970). Histopathology of corn hybrids infected with root-knot nematode, Meloidogyne incognita. Phytopathology, 60 : 1195-1198.

Barrons, K.C. (1939). Studies on the nature of root-knot resistance. J. Agric. Res., 58 : 263-271.

*Beille, L. (1898). Sur les alterations produites par l' Heterodera radicicola sur les racines du Papaya gracilis. Compt. Rend. Assoc. Franc. Avanc. Sci., 27 : 413-416.

*Bergman, H., and van Duuren, A.J. (1959). Mededel. Inst. Rationale Suikerprod., 29, 3-23. Cited by: Seinhorst, J.W. 1961.

Bernier, G. (1971). Structural and metabolic changes in the shoot apex in transition to flowering. Can. J. Bot., 49 : 803-819.

Bhaskaran, S., and Swaminathan, M.S. (1958). Polyploidy and the genesis of the leguminous root nodules. Nucleus, 1 : 75-88.

Birchfield, W. (1957). The burrowing nema situation in Florida. J. Econ. Entomol., 50 : 562-564.

Birchfield, W. (1964). Histopathology of nematode induced gells of Echinochloa colonum. Phytopathology, 54 : 888 (Abstr.).

Birchfield, W. (1973). Pathogenesis and host-parasite relations of cyst nematode, Heterodera graminophila on grasses. Phytopathology, 63 : 38-40.

Bird, A.F. (1959a). Development of the root-knot nematodes Meloidogyne javanica (Treub) and Meloidogyne hapla Chitwood in the tomato. Nematologica, 4: 31-42.

Bird, A.F. (1959b). The attractiveness of roots to the plant parasitic nematodes Meloidogyne javanica and M. hapla. Nematologica, 4 : 322-335.

Bird, A.F. (1960). Additional notes on the attractiveness of roots to plant parasitic nematodes. Nematologica, 5 : 217.

Bird, A.F. (1961). The ultrastructure and histochemistry of a nematode-induced giant cell. J. Cell Biol. (J. Biophys. Biochem. Cytol.), 11 : 701-715.

Bird, A.F. (1962). The inducement of giant cells by Meloidogyne javanica. Nematologica, 8 : 1-10.

Bird, A.F. (1966a). Esterases in the genus Meloidogyne. Nematologica, 12 : 359-361.

Bird, A.F. (1966b). Some observations on exudates from Meloidogyne larvae. Nematologica, 12 : 471-482.

Bird, A.F. (1967). Changes associated with parasitism in nematodes. I. Morphology and physiology of preparasitic and parasitic larvae of M. javanica. J. Parasitol., 53 : 768-776.

Bird, A.F. (1969). Changes associated with parasitism in nematodes. V. Ultrastructure of the stylet exudation and dorsal oesophageal gland contents of female Meloidogyne javanica. J. Parasitol., 55 : 337-345.

Bird, A.F. (1972). Cell wall breakdown during the formation of syncytia induced in plants by root-knot nematodes. Int. J. Parasitol., 2 : 431-432.

*Bopp, M. (1964). Hemmung der Crown-Gall-Entstehung durch Fluordesoxyuridin. Z. Naturforsch., 196 : 64-71.

Brachet, J. (1953). The use of basic dyes and ribonuclease for the cytochemical detection of ribonucleic acid. Quart. J. Microscop. Sci., 94 : 1-10.

Breton, E.L. and Moule, Y. (1961). Biochemistry and physiology of the cancer cell. In "The Cell" (J. Brachet and A.E. Mirsky, eds.). Vol. V, pp. 497-544. Academic Press, New York.

Chibnall, A.C. (1939). "Protein Metabolism in the Plant", 306 pp. Yale Univ. Press.

Chitwood, B.G. (1949). Root-knot nematodes. I. A revision of the genus Meloidogyne Goeldi, 1887. Proc. Helminthol. Soc. Wash., 16 : 90-104.

Chitwood, B.G. and Oteifa, B.A. (1952). Nematodes parasitic on plants. Annu. Rev. Microbiol., 6 : 151-184.

Chitwood, B.G., Specht, A.W., and Haris, L. (1952). Root-knot nematodes. III. Effects of Meloidogyne incognita and M. javanica on some peach root stocks. Plant and Soil, 4 : 77-95.

Christie, J.R. (1936). The development of root-knot nematode galls. Phytopathology, 26 : 1-22.

Christie, J.R. (1946). Host-parasite relationships of the root-knot nematode, Heterodera marioni. II. Some effects of the host on the parasite. Phytopathology, 36 : 340-352.

Christie, J.R., and Cobb, G.S. (1941). Notes on the life history of the root-knot nematode Heterodera marioni. Proc. Helminthol. Soc. Wash., 8 : 23-26.

Cohn, E. (1965). On the feeding and histopathology of the citrus nematode. Nematologica, 11 : 47-54.

Cook, M.T. (1923). The origin and structure of plant galls. Science, 26 : 6.

Crittenden, H.W. (1958). Histology and cytology of susceptible and resistant soybeans infected with Meloidogyne incognita acrita. Phytopathology, 48 : 461 (Abstr.).

Crittenden, H.W. (1962). Effect of Meloidogyne incognita acrita in nodulating and non-nodulating strains of soybean. Phytopathology, 52 : 163 (Abstr.).

Cutler, H.G., and Krusberg, L.R. (1968). Plant growth regulators in Ditylenchus dipsaci, Ditylenchus triformis and host tissues. Plant Cell Physiol., 9 : 479-497.

Dalessandro, G. (1973). Hormonal control of xylogenesis with pith parenchyma explants of Lactuca. Ann. Bot., 37 : 375-382.

Davis, R.A., and Jenkins, W.R. (1960). Histopathology of gardenia (Gardenia jasminoides Veitchi), infected with three species of Meloidogyne. Nematologica, 5 : 228-230.

Davies, D.D., Patterson, B.D., and Trewvas, A.J. (1968). Studies on the mechanism of action of IAA. In "Plant Growth Regulators", Society for Chemical Industry Monographs, 31 : 208-223.

Demetriades, S.D. (1956). Chromatographic detection of free amino acids in normal and iron-deficient plants of Hibiscus esculentus Linn. Nature, 177 : 95.

Dixon, G.R., and Pegg, G.F. (1972). Changes in amino acid content of tomato xylem sap following infection with strains of Verticillium albo-atrum. Ann. Bot. 36 147-154.

Doney, D.L., Fife, J.M., and Whitney, E.D. (1970). The effect of the sugar beet nematode Heterodera schachtii on the free amino acids in resistant and susceptible Beta species. Phytopathology, 60 : 1727-1729.

Dropkin, V.H. (1959). Varietal response of soybeans to Meloidogyne - a bioassay system for separating races of root-knot nematodes. Phytopathology, 49 : 18-23.

Dropkin, V.H. (1965). Polyploidy in syncytia of hairy-vetch induced by a Meloidogyne species. Nematologica, 11 : 36 (Abstr.)

Dropkin, V.H. (1966). Physiology of nematodes of the soil. Ann. New York Acad. Sci., 139 : 39-52.

Dropkin, V.H. (1969). Cellular responses of plants to nematode infections. Annu. Rev. Phytopathol., 7 : 101-122.

Dropkin, V.H., and Boone, W.R. (1966). Analysis of host-parasite relationships of root-knot nematodes by single larval inoculations of excised roots of tomato. Nematologica, 12 : 225-236.

Dropkin, V.H., Davis, D.W., and Webb, R.E. (1967). Resistance of tomato to Meloidogyne incognita acrita and to M. hapla (root-knot nematodes) as determined by a new technique. J. Amer. Soc. Hort. Sci., 90 : 316-323.

Dropkin, V.H., Martin, G.C., and Johnson, R.W. (1958). Effect of osmotic concentration on hatching of some plant parasitic nematodes. Nematologica, 3 : 115-126.

Dropkin, V.H., and Nelson, P.E. (1960). The histopathology of root-knot nematode infections in soybeans. Phytopathology, 50 : 442-447.

Du Charme, E.P. (1959). Morphogenesis and histopathology of lesions induced on citrus roots by Radopholus similis. Phytopathology, 49 : 388-395.

Dundon, T.R. (1962). Multinucleate giant cell formation in a Pachypsalla gall on Celtis. Amer. J. Bot., 49 : 800-805.

Endo, B.Y. (1964). Penetration and development of Heterodera glycines in soybean roots and related anatomical changes. Phytopathology, 54 : 79-88.

Endo, B.Y. (1971). Synthesis of nucleic acids at infection sites of soybean roots parasitized by Heterodera glycines. Phytopathology, 61 : 395-399.

Endo, B.Y. and Schaeffer, G.W. (1967). Response of Heterodera trifolii in red clover roots to azauracil and other inhibitors. Phytopathology, 57 : 576-579.

Endo, B.Y., and Veech, J.A. (1969). The histochemical localisation of oxidoreductive enzymes of soybeans infected with the root-khot nematode Meloidogyne incognita acrita. Phytopathology, 59 : 418-425.

Endo, B.Y., and Veech, J.A. (1970). Morphology and histochemistry of soybean roots infected with Heterodera glycines. Phytopathology, 60 : 1493-1498.

Epstein, E., and Cohn, E. (1971). Biochemical changes in terminal root galls caused by an ectoparasitic nematode, Longidorus africanus: Amino acids. J. Nematol., 3 : 334-340.

Eversmeyer, H.E. and Dickerson, O.J. (1966). Histopathology of root-knot nematode infected peony (*Paeonia albiflora*) roots. Phytopathology, 56 : 816-820.

Fan, D.F., and Maclachlan, G.A. (1967). Massive synthesis of ribonucleic acid and cellulose in the pea epicotyl in response to indoleacetic acid, with and without concurrent cell division. Plant Physiol., 42 : 1114-1122.

Fielding, M.J. (1959). Nematodes in plant disease. Annu. Rev. Microbiol., 13 : 239-254.

Fife, J.M. (1961). Changes in the concentration of amino acids in sugar beet plants induced by virus yellows. J. Amer. Soc. Sugar Beet Technol., 14 : 133-137.

Flax, M.H., and Himes, M.H. (1950). A differential stain for ribonucleic and desoxyribonucleic acid. Anat. Record, 108 : 529.

Frank, A.B. (1885). Ueber das Wurzelälchen und die durch dasselbe verursachten Beschädigungen der Pflanzen., Landw. Jahrb., 14 : 149-176.

Franklin, M.T. (1971). Taxonomy of Heteroderidae. In "Plant Parasitic Nematodes" Vol. I (B.M. Zuckerman, W.F. Mai, and R.A. Rohde, eds.) pp. 139-162. Academic Press, New York.

Gipson, I., Kim, K.S., and Riggs, R.D. (1971). An ultrastructural study of syncytium development in soybean roots infected with *Heterodera glycines*. Phytopathology, 61 : 347-353.

Godfrey, G.H., and Oliveira, J.M. (1932). The development of root-knot nematode in relation to root tissues of pineapple and cowpea. Phytopathology, 22 : 325-348.

Gomori, G. (1952). "Microscopic Histochemistry, Principles and Practice", 273 pp. Univ. Chicago Press, Chicago.

Goodey, J.B. (1948). The galls caused by Anguillulina balsamophila (Thorne) Goodey on the leaves of Wyethia amplexicaulis Nutt and Balsamorhiza saggitata Nutt. J. Helminthol., 22 : 109-116.

Goodey, J.B. (1963). "Laboratory Methods for Work with Plant and Soil Nematodes", Minist. Agric., Fish and Food Tech. Bull. No. 2, 72 pp. Her Majesty's Stationary Office, London.

Gordon, S.A., and Weber, R.P. (1951). Colorimetric estimation of indole acetic acid. Plant Physiol., 26 : 192-195.

Griffin, A.C., and Rhein, A. (1951). Acta Unio Intern. contracancrom., 7 . 763-767. cited by Breton, C.L. and Moule, Y. (1961). Biochemistry and physiology of the cancer cell. In "The Cell"., (J. Brachet and A.E. Mirsky, eds.). Vol. V, pp. 497-544.

Griffin, G.D. (1969). Attractiveness of resistant and susceptible alfalfa to stem and root-knot nematodes. J. Nematol., 1 : 9 (Abstr.).

Hale, M.G., Lindsey, D.L., and Hameed, K.M. (1973). Gnotobiotic culture of plants and related research. Bot. Rev., 39 : 261-273.

Hamilton, T.H., Moore, R.J., Rumsey, A.F., Means, A.R., and Schrank, A.R. (1965). Stimulation of synthesis of ribonucleic acid in subapical sections of Avena coleoptile by indolyl-3-acetic acid. Nature, 208 : 1180-1183.

Hanks, R.W., and Feldman, A.W. (1966). Quantitative changes in free and protein amino acids in leaves of healthy, Radopholus similis infected and "recovered" grape-fruit seedlings. Phytopathology, 56 : 261-264.

Hawk, P.B., Oser, B.L., and Summerson, W.H. (1954). "Practical Physiological Chemistry", 1439 pp. McGraw-Hill Book Co., Inc., New York.

Heald, C.M. (1969). Histopathology of 'Tifdwarf' bermudagrass infected with Meloidogyne graminis (Sledge and Gordon) Whitehead. J. Nematol., 1 : 9-10 (Abstr.).

*Hotchkiss, R.D. (1948). A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem., 16 : 131-141.

Huang, C.S. (1966). Host-parasite relationships of the root-knot nematode in edible ginger. Phytopathology, 56 : 755-759.

Huang, C.S. (1968). "Mechanism of Giant Cell Initiation and the Subsequent Intracellular Changes Caused by Root-Knot Nematode". Doctoral dissertation. Univ. Calif. 61 pp. Univ. Microfilms Inc. Ann. Arbor, Michigan.

Huang, C.S., and Maggenti, A.R. (1969a). Mitotic aberrations and nuclear changes of developing giant cells in Vicia faba caused by the root-knot nematode Meloidogyne javanica. Phytopathology, 59 : 447-452.

Huang, C.S., and Maggenti, A.R. (1969b). Wall modifications in developing giant cells of Vicia faba and Cucumis sativus induced by root-knot nematode, Meloidogyne javanica. Phytopathology, 59 : 931-937.

Jacobs, W.P. (1952). The role of auxin in differentiation of xylem around a wound. Amer. J. Bot., 39 : 301-309

Jensen, W.A. (1962). "Botanical Histochemistry", 408 pp. W.H. Freeman and Co., San Francisco.

Jimbo, T. (1927). On the budding of nucleoli in the root nodule of Wisteria. Bot. Mag., 41 : 551-553.

Johansen, D.A. (1940). "Plant Microtechnique", 523 pp. McGraw-Hill, New York.

Johnson, R.N. and Viglierchio, D.R. (1969). A growth promoting substance occurring in an extract prepared from Heterodera schachtii larvae. Nematologica, 15 : 159-160.

Jones, M.G.K., and Northcote, D.H. (1972). Multinucleate transfer cells induced in coleus roots by the root-knot nematode, Meloidogyne arenaria. Protoplasma, 75 : 381-395.

Kannan, S. (1967). Activity of dehydrogenases in tomato root-knots infected with the root-knot nematode. Ind. J. Exp. Biol., 5 : 266.

Kannan, S. (1968). Studies in nematode infected root-knots of the tomato plant. Ind. J. Exp. Biol., 6 : 153-154.

Keen, N.T., and Williams, P.H. (1969). Translocation of sugars into infected cabbage tissues during club-root development. Plant Physiol., 44 : 748-759.

Key, J.L., and Hansen, J.B. (1961). Some effects of 2,4-D on soluble nucleotides and nucleic acids of soybean seedlings. Plant Physiol., 36 : 145-152.

Klämbt, D. (1972). Effect of growth substances on rapidly synthesized RNA in sterile tobacco tissue. In "Plant Growth Substances 1970", (D.J. Carr, ed.) pp. 190-195. Springer-Verlag, Heidelberg.

Kingler, J. (1959). Anziehung von collembolen und Nematoden durch Kohlendioxyd-quellen. Mitt. Schweiz. ent. Ges., 32 : 311-316.

Klinkenberg, C.H. (1963). Observations on the feeding habits of Rotylenchus uniformis, Paratylenchus crenatus, P. penetrans, Tylenchorhynchus dubius and Hemicycliophora similis. Nematologica, 9 : 502-506.

*Kostoff, D., and Kendall, J. (1930). Cytology of nematode galls on Nicotiana roots. Centralbl. Bakt., II 81 : 86-91.

Krupsagar, V., and Barker, K.R. (1966). Increased cytokinin concentration in tomato infected with root-knot nematode, Meloidogyne incognita. Phytopathology, 56 : 885.

Krusberg, L.R. (1961). Studies on the culturing and parasitism of plant parasitic nematodes, in particular Ditylenchus dipsaci and Aphelenchoides ritzemabosi on alfaalfa tissues. Nematologica, 6 : 181-200.

Krusberg, L.R. (1963). Host response to nematode infection. Annu. Rev. Phytopathol., 1 : 219-240.

Krusberg, L.R. (1971). Chemical composition of nematodes. In "Plant Parasitic Nematodes", (B.M. Zuckerman, W.F. Mai and R.A. Rohde, eds.) Vol. II, pp. 213-234. Academic Press, New York.

Krusberg, L.R., and Nielsen, L.W. (1958). Pathogenesis of root-knot nematodes to the Porto Rico variety of sweet potato. Phytopathology, 48 : 30-39.

*Kuhn, H. (1959). Zum problem der Wirtsfindung phytopathogener Nematoden. Nematologica, 4 : 165-171.

Kupila-Ahvenniemi, S., and Therman, E. (1968). Morphogenesis of crown gall. Adv. Morphogen., 8 : 45-78.

Larsen, P. (1955). Growth substances in higher plants. In "Modern Methods of Plant Analysis", Vol. III (K. Peach, and M.V. Tracey, eds.) pp. 565-625. Springer-Verlag, Berlin.

Lefkowitz, S.S. (1961). "Plant Growth Substances in Root Galls Induced by Meloidogyne incognita acrita Chit." Doctoral Dissertation. Univ. of Maryland. 32 pp. University Microfilms Inc., Ann Arbor, Michigan.

Leggett Bailey, J. (1967). "Techniques in Protein Chemistry", Elsevier Publishing Co., Amsterdam.

Leopold, A.C. (1955). "Auxins and Plant Growth", 354 pp. Univ. Calif. Press, Berkeley and Los Angeles.

Lewis, S.A. (1973). Identification and metabolism of indoles in Meloidogyne incognita and in cotton resistant and susceptible to Meloidogyne incognita. Diss. Ab., 34 : 484B.

Libbenga, K.R., and Torrey, J.G. (1973). Hormone-induced endore-duplication prior to mitosis in cultured pea root cortex cells. Amer. J. Bot., 60 : 293-299.

Linford, M.B. (1937). Feeding of the root-knot nematode in root tissue and nutrient solution. Phytopathology, 27 : 824-835.

Linford, M.B. (1939). Attractiveness of roots and excised shoot tissues to certain nematodes. Proc. Helminthol. Soc. Wash., 6 : 11-18.

Linford, M.B. (1941). Parasitism of the root-knot nematode in leaves and stems. Phytopathology, 31 . 634-648.

Linford, M.B. (1942). Transient feeding of root-knot nematode larvae. Phytopathology, 32 : 580-589.

Littau, V.C., and Black, L.M. (1949). Cytoplasmic inclusions associated with wound-tumour in Rumex acetosa. Phytopathylogy, 39 : 13 (Abstr.).

Littrell, R.H., (1966). Cellular responses of Hibiscus esculentus to Meloidogyne incognita acrita. Phytopathology, 56 : 540-549.

Loewenberg, J.R., Sullivan, T., and Schuster, M.L. (1960). Gall induction by Meloidogyne incognita incognita by surface feeding and factors affecting the behaviour pattern of the second stage larvae. Phytopathology, 50 . 322-323.

Lownsbery, B.F., and Viglierchio, B.R. (1960). Mechanism of accumulation of Meloidogyne incognita acrita around tomato seedlings. Phytopathology, 50 : 178-179.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin-phenol reagent. J. Biol. Chem., 19 : 265-275.

Mani, M.S. (1964). "Ecology of Plant Galls", Monogr. Biol. XII, (Weisbach, W.W., and Van Oye, P., eds.) 434 pp. Dr. W. Junk, the Hague.

Mankau, R., and Linford, M.B. (1960). Host-parasite relationships of the clover-cyst nematode, Heterodera trifolii Goffart. Univ. Ill. Agric. Expt. Stn. Bull., 667 : 50 pp.

Matthysse, A.G., and Philips, C. (1969). A protein intermediary in the interaction of a hormone with the genome. Proc. Nat. Acad. Sci. (U.S.), 63 : 897-903.

McManus, J.F.A. (1948). Histological and histochemical uses of periodic acid. Stain Technol., 23 : 99-108.

Mollenhauer, H.H. (1967). A comparison of root cap cells of epiphytic, terrestrial and aquatic plants. Amer. J. Bot., 59 : 1249-1259.

*Molliard, M. (1900). Sur quelques caractères histologiques des cecidies produites par l' Heterodera radicicola. Greet. Rev. Gen. Bot., 12 : 156-165.

Morré, D.J., Jones, D.D., and Mollenhauer, H.H. (1967). Golgi apparatus mediated polysaccharide secretion by outer root cap cells of Zea mays. I. Kinetics and secretory pathway. Planta, 74 : 286-301.

Mottram, J.C. (1944). Production of endomitosis in bean roots and its bearing on the genesis of tumours. Nature, 154 : 828.

Mountain, W.B. (1960). Mechanisms involved in plant nematode relations. In: "Nematology" (J.N. Sasser and W.R. Jenkins, eds.). pp. 426-431. The Univ. North Carolina Press, Chapel Hill.

Mountain, W.B. (1965). Pathogenesis by soil nematodes. In "Ecology of Soil-Borne Plant Pathogens", (K.F. Baker and W.C. Snyder, eds.). pp. 285-301. Univ. Calif., Press, Berkeley.

*Müller, C. (1884). Mittheilungen über die unseren Kulturpflanzen schädlichen, das Geschlecht Heterodera bildenden Wurmer. Landw. Jahrb., 13:1-42.

Murray, M.A., and Whiting, A.G. (1946). A comparison of histological responses of bean plants to tryptophane and to low concentrations of indole acetic acid. Bot. Gaz., 108 : 74-100.

*Myuge, S.G. (1956). About the physiology of nutrition of the gall nematode. Doklady Akad. Nauk. SSSR., 108 : 164-165.

*Myuge, S.G. (1957). On the interrelationship of auxins and root-nutrients of tomatoes during meloidogyneosis. Pr Proc. 40th Ann. All-Union Soc. Helminthol. Sci. Conf., Part I, 218.

*Myuge, S.G. (1958). Determination of the buffer-capacity of the galls produced by nematodes. Acad. Nauk. SSSR, Moscow, 30 : 91-92.

*Myuge, S.G. (1960). On the interrelations of some phyto-nematodes with the plant host. pp. 333-338. Conf. Sci. Problems Plant Protection, Budapest.

*Nemec, B. (1910). Das problem der Befruchtungsvorgange und andere zytologische Fragen. Gebruder Borntraeger, Berlin. Part IV. Vielkernige Riesenzellen in Heterodera. Gallen, 151-173.

Niu, M.C., Cordova, C.C., Niu, L.C., and Radbill, C.L. (1962). RNA induced biosynthesis of specific enzymes. Proc. Nat. Acad. Sci. (U.S.), 48 : 1964-1969.

*Nolte, H.W., and Kohler, H. (1952). Pflanzenschädigungen bei Nematodenbefall und ihre Kausalen Ursachen, Nachrbl. Deut. Pflanzenschutzdienst., 32 : 24-28.

*Nooden, L.D., and Thimann, K.V. (1963). Evidence for a requirement for protein synthesis for auxin-induced cell enlargement. Proc. Nat. Acad. Sci. (U.S.), 50 : 194-200.

*Nusbaum, C.J. (1958). The response of root-knot infected tobacco plants to folias applications of maleic hydrazide. Proc. Assoc. South Agr. Workers, 55 : 220.

Oberling, C., and Bernhard, W. (1961). The morphology of the cancer cells. In "The Cell" Vol. V (J. Brachet and A.F. Mirsky, eds.). pp. 405-496. Academic Press, New York.

Orion, D., and Minz, G. (1968). The influence of systemic plant growth substances in the galls induced by the root-knot nematode, Meloidogyne javanica. Phytopathology, 58 : 384-390.

Owens, R.G., and Bottino, R.F. (1966). Changes in host cell wall composition induced by root-knot nematodes. Contrib. Boyce Thompson Inst., 23 : 171-180.

Owens, R.G., and Novotny, H.M. (1960). Physiological and biochemical studies on nematode galls. Phytopathology, 50 : 650 (Abstr.).

Owens, R.G., and Rubinstein, J.H. (1966). Metabolic changes induced by root-knot nematodes in host tissue. Contrib. Boyce Thompson Inst., 23 : 199-213.

Owens, R.G., and Specht, H.N. (1964). Root-knot histogenesis. Contrib. Boyce Thompson Inst., 22 : 471-489.

Owens, R.G., and Specht, H.N. (1966). Biochemical alterations induced in host tissues by root-knot nematodes. Contrib. Boyce Thompson Inst., 23 : 181-198.

Paulson, R.E., and Webster, J.M. (1970). Giant cell formation in tomato roots caused by Meloidogyne incognita and M. hapla (Nematode) infection: A light and electron microscope study. Can. J. Bot., 48 : 271-276.

Paulson, R.E., and Webster, J.M. (1972). Ultrastructure of the hypersensitive reaction in roots of tomato, Lycopersicon esculentum to infection by the root-knot nematode, Meloidogyne incognita. Physiol. Plant Pathol., 2 : 227-234.

Peacock, F.C. (1959). The development of a technique for studying the host-parasite relationship of the root-knot nematode Meloidogyne incognita under controlled conditions. Nematologica, 4 : 43-55.

Piegat, M., and Wilski, A. (1965). Cytological differences in root cells of susceptible and resistant potato varieties invaded by potato root eelworm. Nematologica, 11 : 109-115.

*Pilet, P.E., and Meylan, S. (1953). Polarite electrique, auxines et physiologie des racines des Lens culinaris Medikus. Ber. Schweiz. bot. Ges., 63 : 430-466.

Polli, E.E., and Shooter, K.V. (1958). The sedimentation characteristics of deoxyribonucleic acid from normal and diseased human tissues. Biochem. J., 69 : 398-403.

*Queve, C. (1834). Modifications anatomiques provoquees par l' "Heterodera radicicola" Müll., dans les tubercules dune Dioscoree. Compt. Rend. Assoc. Franc. Avanc. Sci., 23 : 629-633.

Rangaswami, G., and Balasubramanian, M. (1964). Presence of amino acids and indole compounds in the root-knot affected plants. Indian Phytopath., 16 : 234-237.

Rebois, R.V., Epps, J.M., and Hartwig, E.E. (1970). Correlation of resistance in soybeans to Heterodera glycines and Rotylenchus reniformis. Phytopathology, 60 : 695-700.

Riffle, J.W. (1973). Histopathology of Pinus ponderosa ectomycorrhizae infected with a Meloidogyne species. Phytopathology, 63 : 1034-1040.

Riggs, R.D., Kim, K.S., and Gipson, I. (1973). Ultrastructural changes in Peking soybeans infected with Heterodera glycines. Phytopathology, 63 : 76-84.

Roberts, L.W. (1969). The initiation of xylem differentiation. Bot. Rev., 35 : 201-250.

Roychoudhury, R., Datta, A., and Sen, S.P. (1965). The mechanism of action of plant growth substances: The role of nuclear RNA in growth substance action. Biochem. Biophys. Acta, 107 : 346-351.

Rubinstein, J.H., and Owens, R.G. (1964). Thymidine and uridine incorporation in relation to the ontogeny of root-knot syncytia. Contrib. Boyce Thompson Inst., 22 : 491-502.

Russell, C.C., and Perry, V.G. (1966). Parasitic habit of Trichodorus christiei in wheat. Phytopathology, 56 : 357-358.

Sandstedt, R., and Estes, I.W. (1965). Host-parasite relations of Nacobbus batatiformis and the sugar beet and other hosts. J. Amer. Soc. Sugar Beet Technol., 13 : 523-537.

Sandstedt, R., and Schuster, M.L. (1966). The role of auxins in root-knot nematode induced growth on excised tobacco stem segments. Physiol. Plant., 19 : 960-967.

Saran, A.B. (1934). On some anatomical deformities in the root tissue of Hibiscus esculentus L. brought about by the infection of eelworms and their bearing on the growth of the plant. J. Indian Bot. Soc., 13 : 197-199.

Saxena, A.P. (1972). Studies on free amino acid content in Meloidogyne javanica Treub. and in root-knots of jute plant. Z. Parasitenkd., 40 : 101-105.

Seinhorst, J.W. (1961). Plant nematode interrelationships. Annu. Rev. Microbiol., 15 : 177-196.

*Sembdner, G. (1963). Anatomie der Heterodera rostochiensis gallen an tomatenwurzlen. Nematologica, 9 : 55-64.

Setty, K.G.H., and Wheeler, A.W. (1968). Growth substances in roots of tomato (Lycopersicon esculentum Mill.) infected with root-knot nematodes (Meloidogyne spp.). Ann. Appl. Biol., 61 : 495-501.

Sheldrake, A.R. (1973). The production of hormones in higher plants. Biol. Rev., 48 : 509-559.

Shibko, S., Koivistoinen, P., Tratyek, C.A., Newhall, A.R., and Friedman, L. (1967). A method for sequential quantitative separation and determination of protein, RNA, DNA, lipid and glycogen from a single rat liver homogenate or from a subcellular fraction. Anal. Biochem., 19 : 514-528.

Siddiqui, I.A. (1971). Histopathogenesis of galls induced by Meloidogyne naasi in oat roots. Nematologica, 17 : 239-242.

Siddiqui, I.A., and Taylor, D.P. (1971). Histopathogenesis of galls induced by Meloidogyne naasi in wheat roots. J. Nematol., 2 : 237-247.

Smith, J.J., and Mai, W.F. (1965). Host-parasite relationships of Allium cepa and Meloidogyne hapla. Phytopathology, 55 : 693-697.

Stahl, E. (1965). "Thin-Layer Chromatography", 553 pp., Springer-Verlag, Berlin.

Steiner, G., Buhrer, E.M., and Rhodes, A.S. (1934). Giant galls caused by the root-knot nematode. Phytopathology, 24 : 161-163.

Steward, F.C. (1968). "Growth and Organisation in Plants", 564 pp. Addison-Wesley Pub. Co., Massachusetts.

Swamy, B.G.L., and Krishnamurthy, K.V. (1971). Ontogenetic studies on plant gall. II. The histopathology of the roots of Basella alba infected with Meloidogyne javanica. Phytomorphology, 21 : 36-46.

Taha, A.H.Y. (1968). "Interrelationships Between Root-Nodule Bacteria, Plant Parasitic Nematodes and their Leguminous Host". Doctoral dissertation. Univ. Calif. 45 pp. Univ. Microfilms, Inc., Ann Arbor, Michigan.

Thiel, A.F. (1931). Anatomy of the primary axis of Solanum melongena. Bot. Gaz., 92 : 407-419.

Thorne, G. (1961). "Principles of Nematology". 553 pp. McGraw-Hill Book Co., Inc., New York.

*Toennies, G. (1952). Cancer as a problem of protein research: Recent trends. Texas Rept. Biol. Med., 10 : 254-264.

Touzé, P. (1964). Formation of free amino acids and amides during infection of melon by Colletotrichum lagenarium (Pass.). Phytochemistry, 3 : 143-147.

*Treub, M. (1887). Quelques mots sur les effets du parasitisme de l' Heterodera javanica dans les racines de la canne a sucre. Ann. Jard. Bot. Buitenzorg., 6 : 93-96.

*Triantaphyllou, A.C. and Hirschmann, H. (1960). Post-infection development of Meloidogyne incognita Chit. 1949. Ann. Inst. Phytopath. Benaki N.S., 3 : 1-11.

*Ustinov, A.A. (1951). New observations on the study of the gall nematode, H. marioni (Cornu, 1879) Goodey. Trans. Inst. Zool. Acad. Sci. U.R.S.S., G., 405-459.

Vancura, V. (1964). Root exudates of plants. I. Analysis of root exudates of barley and wheat in their initial phases of growth. Plant and Soil, 21 : 231-248.

Van Der Linde, W.J. (1956). The Meloidogyne problem in South Africa. Nematologica, 1 : 177-183.

Varghese, T.M., and Kamalesh Kumari, (1970). Meristem induction in S. melongena roots by Meloidogyne incognita var. acrita. Nematologica, 16 : 457.

Veech, J.A., and Endo, B.Y. (1969). The histochemical localization of several enzymes in soybean infected with the root-knot nematode Meloidogyne incognita acrita. J. Nematol., 1 . 265-276.

Veech, J.A., and Endo, B.Y. (1970). Comparative morphology and enzyme histochemistry in root-knot resistant and susceptible soybeans. Phytopathology, 60 : 896-902.

Viglierchio, D.R. (1971). Nematodes and other pathogens in auxin related plant growth disorders. Bot. Rev., 37 : 1-21.

Viglierchio, D.R., and Lownsbery, B.F. (1960). The hatching response of Meloidogyne species to the emanations from the roots of germinating tomatoes. Nematologica, 5 : 153-157.

Viglierchio, D.R., and Yu, P.K. (1968). Plant growth substances and parasitic nematodes. II. Host influence on auxin content. Expl. Parasit., 23 : 88-95.

Wallace, H.R. (1958). Observations on the emergence from cysts and the orientation of larvae of three species of the genus Heterodera in the presence of host plant roots. Nematologica, 3 : 236-243.

Wallace, H.R. (1960). Movement of eelworms. VI. The influence of soil type, moisture gradients and host plant roots on the migration of the potato root eelworms, Heterodera rostochiensis Wollenweber. Ann. Appl. Biol., 48 : 107-120.

Wallace, H.R. (1961). The nature of resistance in chrysanthemums leaves infested with Aphelenchoides ritzemabosi. Nematologica, 6 : 7-16.

Wallace, H.R. (1963). "The Biology of Plant Parasitic Nematodes", 280 pp. Edward Arnold, London.

Wallace, H.R. (1966). Factors influencing the infectivity of plant parasitic nematodes. Proc. Roy. Soc. B., 164 : 592-614.

Webster, J.M. (1969). The host-parasite relationships of plant parasitic nematodes. Adv. Parasitol., 7 : 1-40.

Webster, J.M. (1972). "Economic Nematology", 563 pp. Academic Press, London.

Weischer, B. (1959). Experimentelle Untersuchungen über Wanderung von Nematoden. Nematologica, 4 : 172-186.

Weischer, B. and Steudel, W. (1972). Nematode diseases of sugar beet. In "Economic Nematology" (J.M. Webster, ed.) pp. 49-65. Academic Press, London.

Wetmore, R.H., and Rier, J.P. (1963). Experimental induction of vascular callus of angiosperms. Amer. J. Bot., 50 : 418-430.

Widdowson, E., Doncaster, C.C., and Fenwick, D.W. (1958). Observations on the development of Heterodera rostochiensis Woll. in sterile root cultures. Nematologica, 3 :308-314.

Wieser, W. (1955). The attractiveness of plants to larvae of root-knot nematodes. I. The effect of tomato seedlings and excised roots on Meloidogyne hapla Chitwood. Proc. Helminthol. Soc. Wash., 22 : 106-112.

Wieser, W. (1956). The attractiveness of plants to larvae of root-knot nematodes. II. The effect of excised bean, eggplant and soybean roots on Meloidogyne hapla Chitwood. Proc. Helminthol. Soc. Wash., 23 : 59-64.

Winter, A. (1966). A hypothetical route for the biogenesis of IAA. Planta, 71 : 229-239.

Wipf, L., and Cooper, D.C. (1940). Somatic doubling of chromosomes and nodular infection in certain leguminosae. Amer. J. Bot., 27 : 821-829.

Yu, P.K., and Viglierchio, D.R. (1964). Plant growth substances and parasitic nematodes. I. Root-knot nematodes and tomato. Expl. Parasit., 15 : 242-248.

*Zinov'ev, V.G. (1964). Some biochemical transformations in the roots of plants susceptible and resistance to root-knot nematode infection. pp. 34-37. Massachusetts Agr. Exp. Sta. Bull. 546.

Zuckerman, B.M. (1960). Parasitism of cranberry roots by Tetylenchus loctus Thorne. Nematologica, 5 : 253-254.

Zuckerman, B.M., Mai, W.F., and Rohde, R.A. (1971). (Eds.) "Plant Parasitic Nematodes", Vol. I and II, 345+347 pp. Academic Press, New York.

---

* Not seen in original