

The Biology of  
Plant Parasitic Nematodes

by  
H. R. Wallace

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*Rothamsted Experimental Station*



LONDON

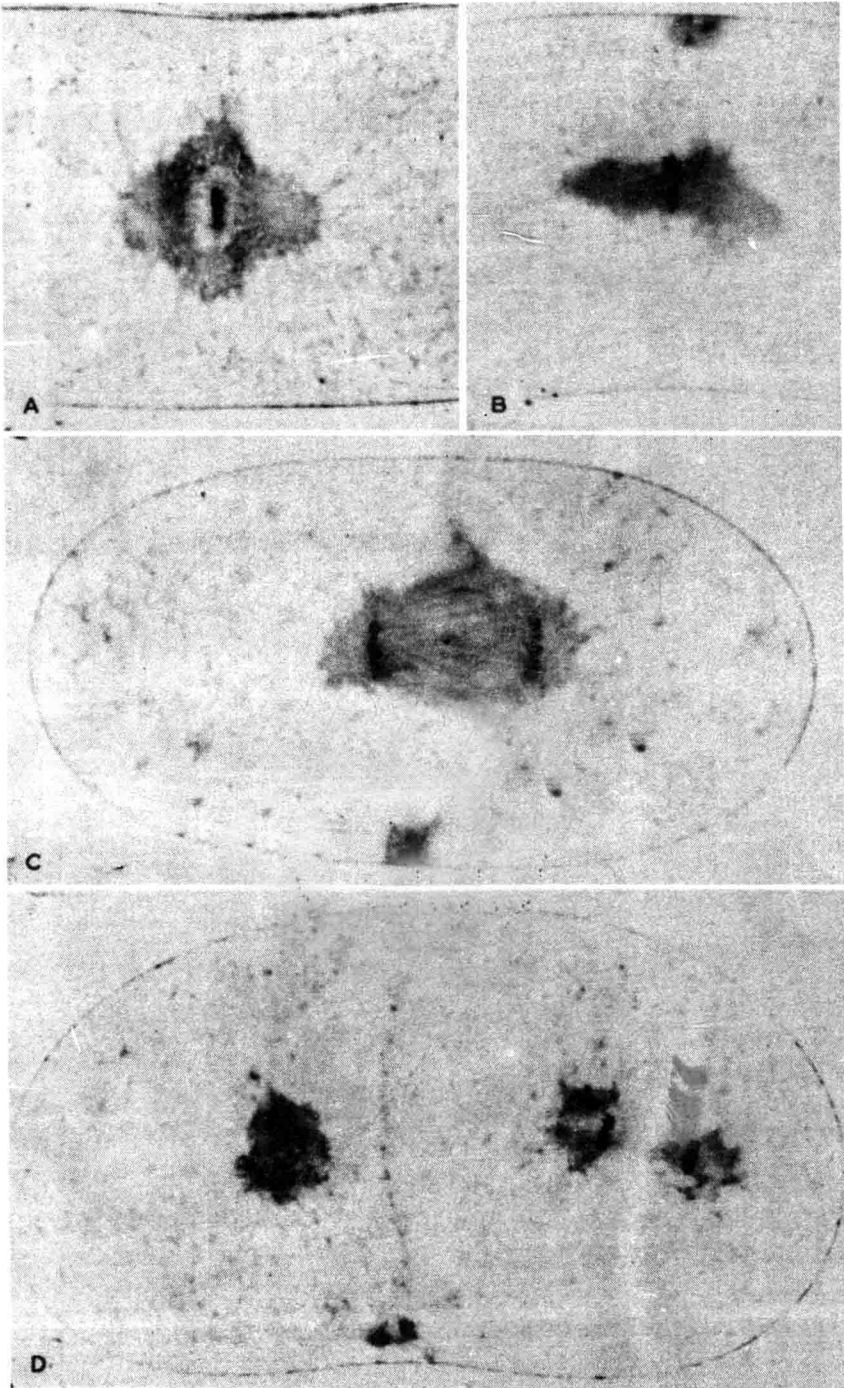
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FRONTISPIECE. Cleavage divisions in *Heterodera glycines*. (A) Metaphase of first cleavage: the chromosomes form a single metaphase plate; (B) Metaphase of first cleavage: sperm and egg chromosomes form separate metaphase plates on a common plane; (C) Anaphase of first cleavage (D) Second cleavage division: the two polar nuclei are still visible ( $\times 1200$ ). See page 12. Triantaphyllou and Hirschmann, *Nematologica*, 1962. E. J. Brill. Reproduced by permission.

## PREFACE

Plant nematology is a young subject, for it is only during the last ten to fifteen years that it has begun to emerge from the early descriptive stage into an experimental one. Previous books dealing with plant nematodes have been concerned mainly with the taxonomy or with the disease nematodes cause and this is the first attempt to draw together what is known of their biology. In addition to summarising current knowledge of their behaviour and of the ways that multiplication, movement and survival of nematodes are affected by changes in the environment, the book indicates some of the major gaps in knowledge and shows that some hypotheses, which have been widely accepted as fact, are of doubtful validity. It is now possible to indicate some general principles underlying nematode behaviour without too much speculation unsupported by experimental evidence.

I thank those who kindly gave me photographs for use in the text; acknowledgments for other illustrations are made where the figures occur. I am grateful for the discussion, criticism and advice given by my colleagues in the Nematology Department at Rothamsted, particularly Mr. F. G. W. Jones and Dr. Audrey Shepherd and to Mrs. Janet Cowland who drew the figures and helped a great deal with the typing and checking of the text.

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## CHAPTER 1

# Introduction

### ECONOMIC IMPORTANCE

The impetus for the rapidly and steadily increasing amount of work on the biology of plant nematodes has largely come from the increasing awareness of the diseases nematodes cause to agricultural and horticultural crops. It is impossible to assess their economic importance even roughly, for in many parts of the world they have still to be sought, but it is probably safe to say that every country and every crop will be troubled by nematodes. Their importance is often evident enough without needing any precise tests to measure percentage losses of yield; a potato crop planted on land heavily infested with *Heterodera rostochiensis* or tobacco on land with root knot can be rendered worthless and in bush or tree crops such as tea and citrus, yields can be halved by the depredations of plant nematodes.

Some workers have attempted to express these losses in terms of money. Thus, *Pratylenchus vulnus* causes a loss of half a million dollars in walnuts in California alone (Lownsbery and Thomason, 1959), and the total annual losses from plant nematode attacks in that State are put at more than 90 million dollars (Allen and Maggenti, 1959). Over the U.S.A. as a whole, estimates of the annual loss in cultivated crops due to plant nematodes range from 250 million dollars (Hutchinson *et al.*, 1961) to 500 million dollars (Cairns, 1955). Comparable losses probably occur elsewhere in the world. An assessment in England and Wales in 1949, for example, showed that the annual loss of potatoes caused by *Heterodera rostochiensis* was of the order of 200,000 tons, representing £2 million (Southey and Samuel, 1954). Such figures must, however, be treated with caution because present methods of estimating crop losses are open to question. There are dangers in deriving figures from nematicide-treated plots, as Seinhorst (1960) has pointed out, and extrapolation from small experimental fields to areas as big as the U.S.A. is obviously liable to give results which may be well wide of the true figures. This does not necessarily mean that the figures quoted over-estimate the position, on the contrary, they may under-estimate the economic importance of plant nematodes. For example, losses in yield can occur without obvious symptoms of nematode attack and so the effects of the nematodes may easily be overlooked.

Economic importance is not the only stimulus for interest in plant nematodes, however. From the biological aspect they present many fundamental problems in the fields of animal behaviour, soil biology, plant pathology and pest control.

## GENERAL CHARACTERISTICS

Plant nematodes are colourless and most have an elongated cylindrical shape (Fig. 1). In genera like *Heterodera* and *Meloidogyne*, however, the adult female is more or less spherical or lemon-shaped. In length, most lie in the range of 0.5 to 5 mm and in width from 20 to 100  $\mu$ .

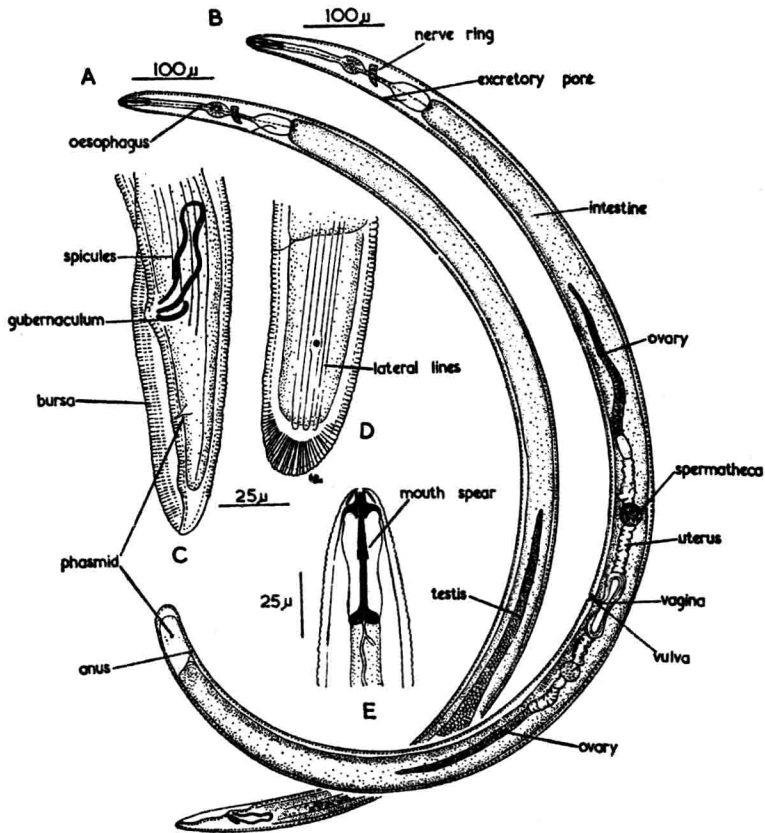


FIG. 1. General morphology of a plant parasitic nematode, *Tylenchorhynchus icarus*. (A) male, (B) female; (C) male tail; (D) female tail; (E) head. (Wallace and Greet, 1963. Reproduced by permission of Rothamsted Experimental Station).

Plant nematodes occur chiefly in the soil, where they attack plant roots, but the genera *Aphelenchoides*, *Ditylenchus* and *Anguina* contain species that parasitize plants above soil level, in leaves, stems or flowers. The nematodes can be classified according to their parasitic habits. Some are ectoparasitic and feed by thrusting the mouth-spear into plant tissues without actually entering the plant. Others are endoparasitic and spend

all or part of their life-cycle within the plant tissues. Of the endoparasites, some, like *Pratylenchus* spp., move freely within the tissues as larvae and adults and often emerge from the root into the soil. Species, which develop spherical or ovoid females, lose the power of locomotion and so remain immobile within the tissues. The distinction between ecto- and endoparasitism is not clear-cut, however; some species like *Aphelenchoides ritzemabosi* may be endoparasitic within the mature leaves of chrysanthemums but ectoparasitic on the young leaves. Some ectoparasites have also been observed with about half their body embedded in the plant tissues, whereas usually only the mouth spear penetrates the plant; *Tylenchorhynchus macrurus* on grass roots is an example.

The life-cycle of plant nematodes consists of the egg, four larval stages and the adult. There are, therefore, four moults, the first of which usually occurs within the egg. The larvae usually resemble the adults except in gonadal development. By definition the term larva is, therefore, incorrect, 'juvenile' would be more appropriate, but usage has now established the term 'larva' in plant nematology and there is no point in changing it now.

## GENERAL MORPHOLOGY

The nematode body wall has an outer non-cellular cuticle which may have transverse, lateral or longitudinal thickenings giving characteristic striations. The cuticle is composed of three main layers, the cortex, matrix and fibre layers. Bird (1958) identified fifteen amino acids in the cuticle of *Meloidogyne hapla* and *M. javanica* and he suggested that the chemical reactions of the cuticle indicated the presence of collagen as well as phenolic compounds and polyphenol oxidase. Within the cuticle is a cellular syncytial layer, the hypodermis. The ventral, dorsal and lateral chords are merely thickenings of the hypodermis. The body muscles, which are responsible for the undulatory type of movement in nematodes, consist of a single layer of elongated cells attached to the hypodermis. The muscles are distributed in four longitudinal bands down the length of the body between the chords and so lie dorso- and ventro-laterally. During movement, contractions of these muscle cells bend the body in the dorso-ventral plane. There are no circular muscles. Between the body wall and the inner digestive tube is a fluid-filled cavity containing the reproductive system and some of the gland cells.

The digestive system consists of an anterior terminal mouth usually surrounded by six lips in which lie structures resembling sense-organs, but whose function has not yet been demonstrated. The most anterior part of the digestive tube is the cuticle-lined stoma which, in the plant nematodes, is in the form of a mouth spear which can be protruded from the mouth to penetrate plant tissues during feeding and invasion. The stoma leads into the oesophagus, which is muscular and has a triradiate lumen. Ducts of the oesophageal glands join the lumen of the oesophagus.

There then follows the intestine, a straight tube, one cell thick, ending, in females, in the rectum with a posterior, sub-terminal opening, the anus. In males, the vas deferens of the reproductive system joins the rectum to form a cloaca.

The female reproductive system consists of either one or two ovaries which connect with the ventral vaginal opening, the vulva, by tubular uteri. In species with two ovaries the branches are usually directed anteriorly and posteriorly and the vulva is more or less median except in *Heterodera* and *Meloidogyne* which have a terminal vulva and ovaries directed anteriorly. Where there is only one ovary the arrangement varies in different genera. Spermathecae are sometimes present in the uteri. The male reproductive system has one or two testes entering into the vasa deferentia which join with the rectum to form a cloaca. The male usually has a pair of cuticularised spicules dorsal to the cloaca and a single additional structure, the gubernaculum. These organs appear to function as a holding mechanism during copulation. Most plant nematodes are bisexual, but parthenogenesis and hermaphroditism also occur in some species.

The structure of the excretory system is very variable but often consists of a transverse canal leading from one or two excretory cells to the ventral excretory pore which is usually in the region of the oesophagus. Flame cells are absent.

Little is known of the nervous system in nematodes, but it is usually assumed that they possess the same organisation as the larger animal parasitic nematodes, which have been more closely studied. In many plant nematode species it is possible to see only the nerve-ring, which encircles the oesophagus. It seems likely that nerves from the nerve-ring extend anteriorly to innervate the amphids and other structures in the head region. The cephalids, deirids, phasmids and hemizonion are possibly sensory, but their relationships to the nervous system are not known. A clear area in the hypodermis on the ventral side of the nematode in the region of the nerve-ring has been described by J. B. Goodey (1959). This is the hemizonid and appears to be a ventro-lateral commisure of the nervous system; its function is unknown.

One of the striking features of nematodes is the lack of variation in form and organisation between different species with quite different habits and habitats. This uniformity has been attributed to the need to satisfy the rigorous requirements of mechanical strength and efficiency (Harris and Crofton, 1957). Thus, in animals that move by undulatory propulsion, there is little scope for modification of the characteristic elongated cylindrical shape. The organisation of the internal anatomy may also be correlated with the mechanical principles involved in locomotion. Harris and Crofton (1957) show that the cuticle, with its spiral basketwork of inextensible fibres and elastic layer, is an efficient system which gives at any given internal volume, a constant length against which the pull of the dorsal or ventral muscles can give a powerful bending

movement of the body. The system functions, not only at a high internal turgor pressure, but over a wide range of pressures. They suggest that the triradiate pharynx provides a highly efficient pumping mechanism for keeping the alimentary canal open when localised increases in turgor pressure during movement tend to collapse it. Harris and Crofton take their arguments even further and suggest that the structural organisation of the excretory system and reproductive system is influenced by the mechanical features of nematodes.

Uniformity is also apparent in some aspects of behaviour. The pattern of locomotion in nematodes, for example, is very similar. In fact, in studying nematode movement I have found it useful to consider these animals in terms of simple mechanical models. Such an approach can be taken too far, of course, for there are many obvious differences between the behaviour of different species. However, at the functional and mechanical levels it appears to be a valid working hypothesis.

## TAXONOMY

Nematodes include species that are saprophytic and those that are parasitic on many hosts of plants and animals. They occupy almost every conceivable habitat—freshwater, salt water and soil, in fact all situations where there is sufficient moisture and a source of organic food supply, and this includes such unlikely places as vinegar vats and beer mats.

There are at present conflicting views on the classification of the higher groups of nematodes. Thorne (1961) considers the group as a Phylum, Nemata, divided into two classes, the Secernentea and Adenophorea. J. B. Goodey (1963) considers the group to have the status of Class, the Nematodea, and he rejects the initial binary grouping. There are also differences at the Order level between these two classifications. Such differences are mostly a question of subjective opinion, however, and need not concern the experimentalist. Neither do they affect the general statements that the majority of plant-parasitic nematodes occur in the Order Tylenchida, with a few in the Dorylaimida, and that all of them are distinguished by the possession of a mouth spear. In the Tylenchida the spear is a hollow cuticular structure, usually with basal knobs. In the Dorylaimida the spear has a different origin, developing sub-ventrally from a tooth or teeth and is often asymmetrical; basal knobs are usually absent.

The primary concern of the experimentalist is to know the identity of the animal he is working with. Correct identification as distinct from taxonomy is therefore an essential prerequisite to research and, for this, accurate detailed descriptions of species are required. Such descriptions often give a range of dimensions of males and females, together with diagnostic morphological characters. In other words, the description is based on a sample from a population and the reliability of any measurements depends on the size of the sample. The number of individuals

which have to be measured to give a reliable indication of the degree of variability can be decided by a simple statistical procedure and I see no reason why morphometric descriptions of species should not be accompanied by a mean and standard error so that comparisons can be made between different populations. Furthermore, some indication of variation in morphological characters could be indicated by a series of drawings to show the range of variation. Thus, de Coninck (1962) has stressed the importance of intra-specific variability and Taylor and Jenkins (1957) and Coomans (1962) give statistical data in their descriptions of *Pratylenchus* spp. and *Rotylenchus goodeyi* respectively. By this approach descriptions of species become more objective and less the product of subjective opinion.

## RELATIONSHIPS TO OTHER ORGANISMS

It is often useful in research to use techniques developed in other branches of biology and this has been done in plant nematology. Methods of culturing nematodes on plant tissues under aseptic conditions, the Baermann funnel technique for extracting nematodes from soil, histological studies of nematodes and infested plants are a few examples of techniques which originated in other biological fields. Furthermore, observations from experiments with other kinds of animals may suggest a useful approach to a problem in plant nematology, although it is dangerous to take such analogies too far. Nematodes occupy a distinct ecological niche in the soil, so much so that Haarløv (1960) considers that there is little direct competition between nematodes and micro-arthropods. Nematodes, unlike micro-arthropods and earthworms, are continually surrounded by water on which they are entirely dependent for their activities, i.e. they are hydrophilous as opposed to aerophilous. Nematodes, like protozoa and rotifers, move in the water films in the soil pore spaces without disturbing the soil particles, unlike the larger members of the soil fauna. These nematode characteristics present to the biologist problems which are in many ways distinct from those in other animal groups, hence it is often necessary to use new techniques and new concepts in studies on nematode biology.

The animal parasitic nematodes, as might be expected, have features in common with the plant parasitic species. There are close similarities between the two groups in their temperature, oxygen and osmotic requirements and in locomotion and orientation responses (Wallace, 1961). Although the biochemical aspects of the physiology of plant parasitic nematodes are little understood, Krusberg (1960) has shown that *Ditylenchus dipsaci* has a metabolism which follows the general pattern established for the animal parasitic nematodes. Rogers (1960) discusses the mechanisms controlling the hatching of eggs and moulting of larvae of nematodes. He suggests that, in parasitic species, part of the internal mechanism determining these processes has been lost, so that the parasite

is dependent on the host to replace it. In animal nematodes the host appears to provide a stimulus which causes the infective stage to produce the internal secretions necessary for hatching and moulting. Rogers suggests that in plant nematodes the host may provide the missing internal secretions which initiate these processes. The mechanisms involved in hatching and moulting of plant nematodes are unknown, but there is no doubt that chemical exudates from plant roots do stimulate them. Here then is at least one example where both animal and plant nematodes should be considered as a whole.

Such a common approach would also be useful in the ecological field. Plant parasitic nematodes which leave the soil and ascend plants to invade leaves, stems or flowers are more active than the root parasites that stay in the soil. This increased activity is associated with the ability to swim upwards in water films, and I suggest, therefore, that plant nematodes can be divided into two groups; those which, by their inherent high activity, can escape from the soil and invade new habitats above soil level, and those which are confined to the soil because they are too inactive to swim upwards in the water films on the outside of plants. There is a similar division in the saprophytic nematodes between soil forms and those which occur in moss cushions, tree boles, etc. The fact that many species of animal parasitic nematodes ascend herbage which is then eaten by the host suggests that this group may have this ecological division as well.

The lack of interchange of experience which has existed up to the present between the animal and plant nematologists is certainly unfortunate for I think that each could learn from the other techniques and methods of approach to different problems. Seinhorst's ingenious sedimentation and elutriation techniques for the recovery of nematodes from soil could be used equally well for the soil-inhabiting stages of animal nematodes, and his mistifier method for extracting nematodes from plant tissues might be adapted for recovering animal nematodes from herbage or animal tissues. The influence of oxygen and carbon dioxide on the hatching of eggs in the centre of a *Heterodera* cyst and on the hatching of trichostrongyle eggs in the centre of faecal pellets seem to me to be similar problems; in fact one could compile many such examples.

The absence in this book of a chapter on physiology reflects the large gaps in our knowledge of the plant nematodes, but at least it is useful to know where the gaps exist. Work on the physiology of animal nematodes as described by von Brand (1952) and Rogers (1962) provides valuable information on which the plant nematologist can base his research.

#### REFERENCES

- ALLEN, M. W. and MAGGENTI, A. R. (1959). Plant nematology in California. *Calif. Agric.* **13** (9), 2-3.
- BIRD, A. F. (1958). The adult female cuticle and egg sac of the genus *Meloidogyne* Goeldi, 1887. *Nematologica*, **3** (3), 205-212.

- VON BRAND, T. (1952). *Chemical physiology of ectoparasitic animals*. New York: Academic Press Inc.
- CAIRNS, E. J. (1955). Nematodes—tiny but mighty. Research under way points to development of better and cheaper controls. *Highlights of Agricultural Research*, 2 (1).
- DE CONINCK, L. (1962). Problems of systematics and taxonomy in nematology today. *Nematologica*, 7, 1-7.
- COOMANS, A. (1962). Morphological observations on *Rotylenchus goodeyi* Loof and Oostenbrink 1958. 1. Redescription and variability. *Nematologica*, 7, 203-215.
- GOODEY, J. B. (1959). The excretory system of *Paraphelenchus* and the identity of the hemizonid. *Nematologica*, 4, 157-159.
- GOODEY, T. (1963). *Soil and freshwater nematodes*. Revised edition, edited by J. B. Goodey. London: Methuen & Co. Ltd.
- HAARLØV, N. (1960). Microarthropods from Danish soils. *Oikos*, suppl. 3, 9-176.
- HARRIS, J. E. and CROFTON, H. D. (1957). Structure and function in the nematodes: internal pressure and cuticular structure in *Ascaris*. *J. exp. Biol.* 34 (1), 116-130.
- HUTCHINSON, M. T., REED, J. P., STREU, H. T., EDUARDO, A. A. and SCHROEDER, P. H. (1961). Plant parasitic nematodes of New Jersey. *New Jersey agric. exp. Sta. Rutgers*, Bull. 796.
- KRUSBERG, L. R. (1960). Hydrolytic and respiratory enzymes of species of *Ditylenchus* and *Pratylenchus*. *Phytopathology*, 50 (1), 9-22.
- LOWNSBERY, B. F. and THOMASON, I. J. (1959). Progress in nematology related to horticulture. *Proc. Amer. Soc. hort. Sci.* 74, 730-746.
- ROGERS, W. P. (1960). The physiology of infective processes of nematode parasites; the stimulus from the animal host. *Proc. roy. Soc. B.* 152, 367-386.
- (1962). *The nature of parasitism*. New York and London: Academic Press.
- SEINHORST, J. W. (1960). Over het bepalen van door aaltjes veroorzaakte opbrengstvermindering bij cultuurgewassen. *Meded. LandbHoogesch. Gent.* 25 (3/4), 1026-1039.
- SOUTHEY, J. F. and SAMUEL, G. G. (1954). Potato root eelworm. 1. A review of the situation. 2. Research in progress. *Min. Agric. & Fish. N.A.A.S.* 12 pp.
- SUIT, R. F. and DUCHARME, E. P. (1957). Spreading decline of citrus. *State Plant Board Florida* 2, Bull. 2.
- TAYLOR, D. P. and JENKINS, W. R. (1957). Variation within the nematode genus *Pratylenchus*, with the descriptions of *P. hexincisus* n.sp. and *P. sub-penetrans* n.sp. *Nematologica*, 2, 159-174.
- THORNE, G. (1961). *Principles of nematology*. New York: McGraw-Hill Book Company Inc.
- WALLACE, H. R. (1961). The bionomics of the free-living stages of zooparasitic and phytoparasitic nematodes—a critical survey. *Helm. Abs.* 30 (1), 1-22.
- WALLACE, H. R. and GREET, D. N. (1963). Observations on the taxonomy and biology of *Tylenchorhynchus macrurus* (Goodey, 1932) Filipjev, 1936 and *Tylenchorhynchus icarus* n.sp. *Parasitology* (in press).



## CHAPTER 2

# Reproduction, Development and Growth

### REPRODUCTION

Reproduction in plant nematodes is mainly bisexual with no scarcity of males, although the sex ratio may vary considerably under different environmental conditions. Tyler (1933a), studying the reproduction of root knot nematodes (*Meloidogyne* sp.), found that although males were rare they were more frequent in old, unhealthy or heavily parasitised roots; 16.4 per cent of the population were males in primary infections but 56.5 per cent in secondary infections. Tyler suggested that the proportion of males increased under adverse conditions. Sex determination in *Heterodera rostochiensis* may depend on abundance of food (Den Ouden, 1960) for with high larval infestations competition for food might result in more males. This would account for the decrease in the rate of multiplication of this species with increasing initial infestation (Fenwick and Reid, 1953). Bird (1960) found that in nitrogen deficient plants, where there might be a scarcity of food, only males of *Meloidogyne javanica* were present. Similarly, a preponderance of males may occur in large populations of mermithids parasitising grasshoppers (Cobb *et al.*, 1927).

Changes in sex ratio may be caused by differential mortality rates of males and females but there is no evidence for this. Ellenby (1954) suggests that sex in *Heterodera rostochiensis* is determined by the environment and he discounts a differential death rate between the sexes (Fig. 2). Triantaphyllou (1960) studied sex determination in *Meloidogyne incognita* and found that when few larvae entered tomato roots, most or all of them developed into females. When large numbers entered the apical portions of the roots, however, galls were formed, rootlet growth ceased and the nematodes, which were crowded together, developed mostly into males. Triantaphyllou, like Tyler, concluded that unfavourable conditions for development induce maleness. Triantaphyllou, however, took the problem a stage further. He showed that, in incubated, galled tomato roots, all second stage male larvae developed into adult males with one testis. Furthermore, many second stage female larvae underwent sex reversal and developed into adult males with two testes. Anatomical examination of second stage larvae substantiated the hypothesis of sex reversal which, Triantaphyllou suggests, is caused chiefly by nutritional deficiencies.

Sex ratio is therefore density dependent and possibly controlled by nutrition but further research is needed before the specific factors are