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STUDIES ON THE PLANT PARASITIC NEMATODE *TYLENCHORHYNCHUS DUBIUS*

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1. GENERAL INTRODUCTION

Tylenchorhynchus dubius (Bütschli, 1873) Filipjev, 1936 is probably the most abundant phytophagous nematode in lighter soils in the temperate zones of Europe. Its wide spread and the high density of its population in arable land and meadows is probably related to the polyphagy of the species and to the widespread occurrence of cereals, grasses and other suitable hosts. The nematode has occasionally been recorded as a noxious plant parasite from countries in temperate as well as subtropical zones. It is, however, possible that not all records concern the same species. The *T. dubius* used in this study was derived from a natural population occurring in a field at Wageningen which was identical with the population from which ALLEN selected his neotype in 1955. Little has been reported on the biology, population dynamics and host-parasite relationships of *T. dubius*. This fact, and the widespread occurrence of dense populations, were the reasons to choose this nematode as the subject of our study.

1.1 IDENTITY OF THE SPECIES

The genus *Tylenchorhynchus* was established by COBB in 1913 when he described the species *T. cylindricus*. In 1934 FILIPJEV erected the genus *Bitylenchus* for didelphic species previously placed in the genus *Tylenchus* Bastian, 1865. In 1936 FILIPJEV made *Bitylenchus* a synonym of *Tylenchorhynchus*. ALLEN (1955) studied *Tylenchorhynchus* specimens from various localities in the Netherlands, specimens from the United States Department of Agriculture Collection at Salt Lake City and from the University of California Collection at Berkeley. He made a careful review of the genus, added 22 new species to the 12 already described, and made an identification key based on females of all 34 species. LOOF collected further data on the genus and extended the key to 44 species in 1959. The nematode check-list published by BAKER in 1962 lists 55 species. The key recorded in a doctor's thesis by BAQRI at the Aligarh University in 1969 comprised 83 species.

According to the species card file available at the Landbouwhogeschool, Wageningen, the genus *Tylenchorhynchus* comprised at least 103 described species by the end of 1969, and *T. dubius* was the one described first.

Tylenchus dubius was described by BÜTSCHLI (1873) from a male found around the roots of *Centaurea cyanus* in Germany. His description and figures did not include sufficient information to allow later workers to identify the species with certainty. T. GOODEY (1932) described and illustrated males and females that he believed to be *dubius* from Winches, St. Albans, England. The description and figures were clear, but not detailed enough to identify his *dubius*, which he indicated as *Anguillulina dubia*. When FILIPJEV (1936) made his former genus *Bitylenchus* (1934) a synonym of *Tylenchorhynchus*, he placed the

name *T. cylindricus* Cobb, 1913 in synonymy with *T. dubius* (Bütschli, 1873), which would make the latter species the type of the genus. This, however, was corrected by ALLEN (1955) in his generic monograph. He compared specimens from different countries. The most frequently encountered species of *Tylenchorhynchus* in The Netherlands proved to be one whose males closely resembled BÜTSCHLI's orginal description and illustrations of *dubius*. This species was compared with specimens collected from Winches Farm and was found to be the same species. Since T. GOODEY (1932) had obtained the specimens he described as *dubius* from this place, ALLEN assumed that GOODEY correctly identified his species as *dubius*. This species was not present in material collected in the United States. The specimens described by THORNE (1949) as *dubius* proved to be similar in all respects to *cylindricus*. Since ALLEN's study *T. cylindricus* is therefore again the type species of the genus and the identity of *T. dubius* is clear. ALLEN selected a female as neotype of *T. dubius* from a Dutch population. This specimen is deposited in the University of California Nematode Collection and topotypes of the same populations are available in the Nematode Collection of the Landbouwhogeschool, Wageningen, which were used for comparison with our experimental population.

A full list of the key characters necessary for identification of *T. dubius* females, reads as follows:

Nematode (class Nematoda), stylet tylenchid (order Tylenchida), dorsal oesophageal gland opening close behind stylet knobs (suborder Tylenchina), precorpus and median bulb of oesophagus well separated (superfamily Tylenchoidea), females vermiform with oesophageal glands in terminal bulb not overlapping intestine (Tylenchidae), stylet not very long with three basal knobs, amphid apertures small, ovaries two and female tail rounded (*Tylenchorhynchus*), cuticle with transverse striae only, lateral lines 4, tail with 46–53 striae and striated terminus, lip region offset with 7 annules (*dubius*). Cf. Fig. 5A.

1.2. BIOLOGICAL DATA

T. dubius is a plant parasite which lives as an ectoparasite on the roots of higher plants. Figures 1 and 2 give a picture of the way it feeds and thrives. The nematode has not been found inside roots or other plant tissues. No development has been seen unless growing plant roots were available, and the population density drops to a very low level in fallow soil without higher plants in 1–2 years. No records are available of successful culture of *T. dubius* on other substrates than growing plant roots and it is probably an obligatory parasite of higher plants.

The nematode appears to be very polyphagous, although the few data available indicate the host efficiency and susceptibility to damage vary considerably between plants.

T. GOODEY (1932) recorded that the nematode often occurred in England around the roots of grasses when these are in an unhealthy condition. *T. dubius*

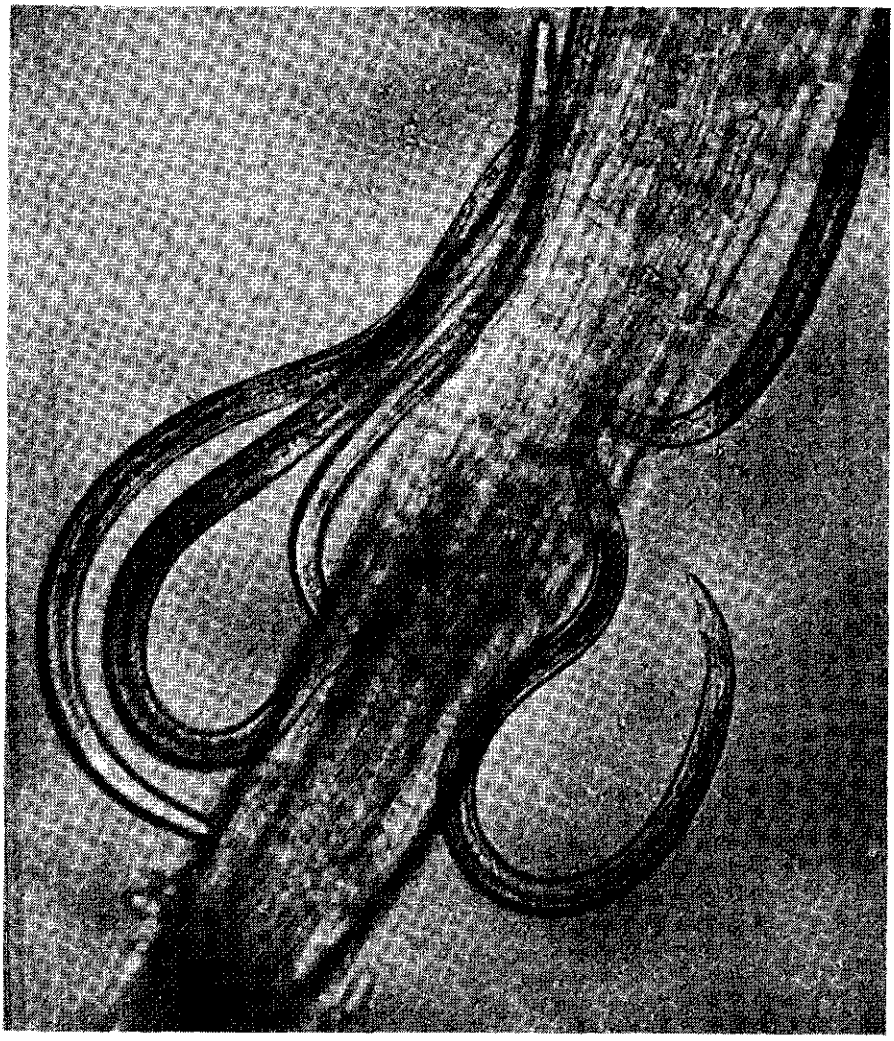


FIG. 1. *T. dubius* crowded around and feeding on elongation zone of a root of ryegrass.

was shown to cause considerable damage to English rye-grass (*Lolium perenne*), the main meadow grass in the Netherlands, in inoculation experiments with normally occurring densities (OOSTENBRINK *et al*, 1963). Ryegrass, oats, rye, barley, corn, swede, red clover and pea were found to be efficient hosts in different field experiments (OOSTENBRINK 1959, 1961). KLEIJBURG and OOSTENBRINK (1959) found marked quantitative differences in nematode distribution between various farms and nurseries in the Netherlands. Sandy soils were characterized by the occurrence of, amongst others, *Tylenchorhynchus dubius*, whereas clay soils contained other species of the genus. OOSTENBRINK *et al.*

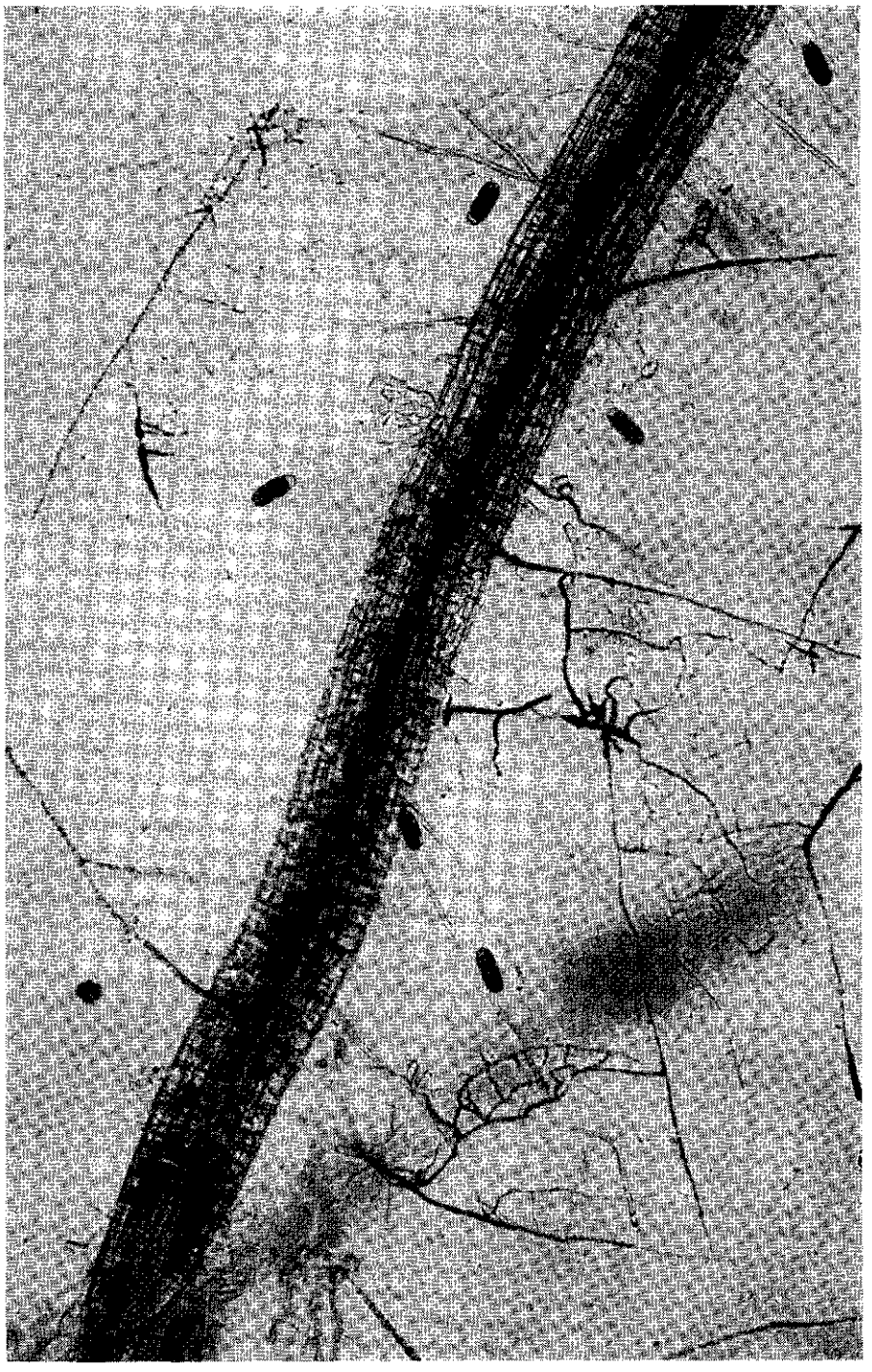


FIG. 2. Eggs of *T. dubius* laid between the root hairs at regular distances from a root of ryegrass.

(1957) showed that the nematicidal plant *Tagetes patula* reduced *Pratylenchus* populations in the soil by 90 per cent, whereas *T. dubius* was reduced to a lesser extent.

T. dubius was recorded several times from crops and soils in the USSR, Poland and Eastern Germany, amongst others from soil and roots of alfalfa crops by TULAGANOV (1949), from soil by BELAEVA (1951), from the rhizosphere of plants by PAESLER (1956), from soil by DEUBERT (1958), from cultivated soils by WITKOWSKA (1958) and WITKOWSKI (1958, 1962), from rye soil (DOMURAT & SANDNER 1960). SKARBILOVIČ (1962 a, b) considered *T. dubius* parasitic with respect to several cultivated plants in the USSR. Repeated cultivation of corn and *Vicia faba* led to population increases of 2–5 times the original density (SKARBILOVIČ, 1967). *T. dubius* has been recorded as the cause of growth reduction of *Gossypium hirsutum* and *Phaseolus acutifolius* under field conditions and in laboratory experiments in the USA by REYNOLDS & EVANS (1953). The species was found associated with roses in India by PRASAD & DASGUPTA (1964). TOBAR JIMENÉZ (1966) found *T. dubius* numerous around the roots of declining carnations in Spain and suspected the nematode to be the cause of the decline. KYROU (1969) found *T. dubius* injurious to potato in pot experiments in England. It is, however, not sure that all these records refer to the same nematode species. KLINKENBERG (1963) studied feeding habits under laboratory conditions of *T. dubius* on root tips of *Trifolium pratense*, *Trifolium repens*, *Poa annua* and *Lolium perenne*. She recorded that *T. dubius* fed on epidermal cells and root hairs by injecting saliva into the cell and soon afterwards sucking in the visibly altered contents. The feeding of this species had no visible effect on attacked cells.

SEINHORST (1959–1962) denied the significance of *T. dubius* as a parasite of English ryegrass and white clover on the basis of laboratory experiments.

Seasonal fluctuations in a population of *T. dubius* and *Rotylenchus robustus* in a sandy soil with an annual crop of peas was reported by STEMERDING (1961) and OOSTENBRINK & STEMERDING (1964), and analysed by OOSTENBRINK (1966). The *T. dubius* population were markedly affected by the pea crop grown, and fluctuated from about 500 to 3000 nematodes per 100 ml of soil. A survey of cabbage fields in Poland and corresponding pot experiments showed that the frequency of occurrence and the reproduction of *T. dubius* increases as soil pH decreases (BRZESKI & DOWE 1969).

1.3. SCOPE OF THE INVESTIGATIONS

The purpose of this study was to contribute to the knowledge of *T. dubius* by studying various aspects concerning:

a. its general biology, notably the life cycle and the morphology and behaviour of different developmental stages;
b. its population dynamics, notably with respect to some unexplained phenomena noticed by others in previous studies:
c. its host/parasite relationships.

2. MATERIALS AND METHODS

Most of the work was done in the Diagnostics Department of the Plantenziektenkundige Dienst (PD) and the Nematology Department of the Landbouwhogeschool (LH). The general working facilities available in these laboratories are not specified, nor is this the case with the optical, photographical and cinematographical instruments used. Special materials or methods used incidentally are described with the corresponding experiments. In this chapter specifications are given of the organisms studied (2.1.), of the fields, soils and plants used (2.2.), of the equipment for control of experimental conditions (2.3.), of the methods for enumeration of nematodes, micro-arthropods and other organisms (2.4.) and of the evaluation of experimental plants (2.5.).

2.1. Nematodes, micro-arthropods and microorganisms

2.1.1. *Nematodes*

The nematodes studied or used for the experiments were natural populations in their original soil, mixed populations extracted from soil, monospecific populations extracted from soil, or monospecific populations which were selected from such mixtures and cultured on selected host plants under laboratory conditions.

The main species in this study was *T. dubius*, but associated species in the main experimental site were taken into account in census work and were sometimes included in the experiments. These field populations comprised measurable populations of *T. dubius*, *Rotylenchus robustus* (De Man, 1876) Filipjev, 1936, *Pratylenchus crenatus* Loof, 1960, *Tylenchus davainei* (Bastian, 1865) Filipjev, 1934, *Aphelenchus avenae* Bastian, 1865, *Trichodorus pachydermus* Seinhorst, 1954. *Mononchus papillatus* Bastian, 1865, and a group of saprozoic nematodes.

T. dubius for experiments were normally taken from a monoculture pea plot, or from a monospecific stock of this nematode maintained in pots with soil on *Lolium perenne* or on *Pisum sativum* at 20°–25°C in a greenhouse. The nematodes for inoculation experiments were extracted from the soil by elutriation, cleaned through cotton-wool filters in water, if necessary stored in water at 5°C, and at any rate inoculated within 48 hours.

Adults of all nematode species used in the experiments, and developmental stages of *T. dubius* used for morphometric studies, were fixed and processed into permanent slides according to the methods described in s'Jacob & Van Bezooijen's manual for practical work in nematology (latest revision 1966). The slides were deposited in the Landbouwhogeschool Nematode Collection, Wageningen.

2.1.2. *Microarthropods*

Microarthropods associated with the nematodes were also extracted and

enumerated from the trial plots, and some of them were cultivated and used for experiments. They were apterygote insects of the order Collembola, notably the species *Tullbergia krausbaueri* (Börner, 1901); *Onychiurus armatus* (Tulberg, 1869), sensu Gisin, 1952 and *Isotomodes productus* (Axelson, 1907), and mites of the suborder Mesostigmata, notably *Rhodacareus roseus* Oudemans s.l. 1902; *Hypoaspis aculeifer* (Canestrini, 1883); *Lasioseius penicilliger* Berlese, 1916; *Histiostoma* sp., *Alliphis siculus* (Oudemans, 1905); *Platyseius borealis* (Berlese, 1904) and *Rhodacarellus sileciacus* Willmann, 1936.

The species used in most experiments are *Tullbergia krausbaueri*, *Onychiurus armatus*; *Rhodacarus roseus*, *Hypoaspis aculeifer*, *Pergamasus runcatellus* Berlese, 1903 and *Lasioseius penicilliger*, cultured on artificial media directly extracted from the soil. Permanent slides of all the species mentioned above are available at the Plantenziektenkundige Dienst.

2.1.3. *Microorganisms*

In some experiments also fungi associated with plant infesting nematodes were determined as a possible cause of plant disease. Some species were isolated and cultured on cherry agar and used for inoculation experiments in combination with *T. dubius*, e.g. *Phoma medicaginis*, var. *pinodella*. Incidentally other small organisms were included in the observations and experiments.

2.2. Fields, soils and plants

2.2.1. *Fields*

The main experimental site was a monoculture pea plot on the permanent crop rotation trial in the garden of the Plantenziektenkundige Dienst (PD), Wageningen. The soil is a sandy loam. The Bedrijfslaboratorium voor Grond- en Gewasonderzoek, Mariendaal, Oosterbeek, the Netherlands, made an analysis of this and of some other soils used in the different experiments. A crop rotation experiment was started in 1958 and continued until now according to a standardized scheme in which 8 annual crops are grown on narrow strips in one direction every two years, with the same crop strips in the same order grown at right angles in the other years (Oostenbrink 1959). In this way a tangential line of monoculture plots is formed. The crops are french marigold (*Tagetes patula* L.), grass/clover (a standardized mixture known as BG 5), oats (*Avena sativa* L.), potato (*Solanum tuberosum* L.) carrot (*Daucus carota* L.), pea (*Pisum sativum* L.), beet (*Beta vulgaris* L.) and fallow (no crop). The monoculture pea plot was used for regular census and as a source of nematodes. Here *Pisum sativum* var. *Rovar* was sown every year at row distances varying from 15–25 cm.

2.2.2. *Soils*

For the laboratory experiments soil from the afore-mentioned pea plot or from other fields was used, depending on the experiment. Specific data will be given in describing the experiments. All soils were prepared by sieving through a

5 mm screen. If necessary they were steamsterilized for 1–2 hours at 121 °C and stored in polythene bags to avoid reinfestation. To maintain fertility of the soils and good growth of the test plants a complete nutrient solution was usually added, namely STEINERS nutrient solution 188 mg/liter, which comprised N, P, S, K, Ca, Mg, Mn, B, Zn, H, Mo, Fe and other microelements (STEINER 1961, 1966).

2.2.3. *Plants*

The main plants used for nematode multiplication as well as for experiments were English rye-grass (*Lolium perenne* L. '*Perma*' and pea (*Pisum sativum* L. '*Rovar*'). Without further specification the indication rye grass or pea always refers to these species and cultivars.

The other crop plants used for host-parasite and nematode population studies were potato (*Solanum tuberosum* L. '*Lekkerlander*'), african marigold (*Tagetes erecta* L. '*Tall Double*'), summer wheat (*Triticum aestivum*), paddy (*Oryza sativa* L. '*Caloro*'), cotton (*Gossypium barbadense* L.), garden bean (*Phaseolus vulgaris* L.), green gram (*Phaseolus radiatus* L.), sorghum (*Sorghum vulgare* Pers. '*Dochna*'), pearl millet (*Pennisetum typhoideum* Rich. *Millet zango*'), (*Brassica campestris* L. var. *rapa*). Common plant names used always refer to the species, varieties or cultivars here, unless indicated otherwise. A number of other plant species used in a host study is listed under 4.4.1.b. The seeds of the plants used were usually obtained from the Afdeling Tropische Plantenteelt, Landbouwhogeschool, Wageningen.

2.3. EQUIPMENT FOR CONTROLLING EXPERIMENTAL CONDITIONS

The equipment for controlling experimental conditions comprised special containers for maintaining the experimental organisms under adjustable moisture conditions and various chambers or apparatuses with either fixed or adjustable temperature and light, as well as facilities and techniques for sterilization of containers, soils, plant seeds and nematodes.

2.3.1. *Containers*

Various containers were chosen or made for the cultivation or observation of the organisms and plants studied, depending on the experiments. For experiments with soil plastic pots of 250 ml, or rectangular tubes of transparent perspex with a diameter of 4 × 4 cm and a height of 20, 16, 8, 4 or 2 cm were often used. Also bottles of 100 ml, tubes of 35 ml and glass tubes 2.5 cm in diameter and 16.5 cm deep were used, as well as bags of 0.25 mm thick polythene of different size. Thin aluminium foils shaped into rectangular containers were used for nematode locomotion studies. Plastic pipes of 3 cm diameter cut into 5 cm sections were joined with tape to form tube-like structures for studying movement of nematodes. Small penicilline bottles were used for survival studies. Petri plates of different sizes, perspex blocks with a depression in the centre, and

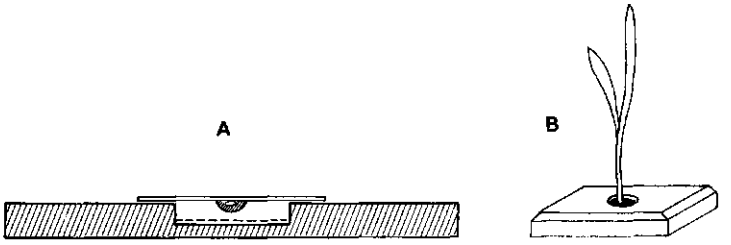


FIG. 3. A. Diagram to illustrate one of the techniques used in studying embryonic development of the egg. Size 7.5 × 2.6 cm (slide), 2.5 cm^2 (cover slip) (as seen in the longitudinal section). B. Diagramatic sketch of the 'micropot' used for studying life cycle of *T. dubius* through larval stages.

normal slides with water, agar or other transparent media were used for observation studies. Very small containers were used for observation of single or very few nematodes in water or on plantlets (Fig. 3). Culturing of mites took place in glass vials with a bottom layer of soft charcoal and gypsum. The tubes, trays and slides used in killing, fixing and mounting were the same as described in S'JACOB & BEZOOIJEN's practical guide (1966).

2.3.2. *Growth chambers and incubators*

Experiments were conducted in rooms with different climatic control facilities, including laboratory rooms, glass-houses, Wisconsin tanks, climate chambers and series of thermostats.

a. Laboratory rooms. The laboratory rooms had a fluctuating air temperature. In winter the temperature varied between 20–22 °C and in summer there was a fluctuation from 20–30 °C.

b. Glass-houses. The glass-houses used were heated in winter and shaded in summer. They had partially controlled temperatures which were, however, not constant because they fluctuated with the environmental temperatures. The temperatures were registered. Artificial light was provided according to the apparent need of the plants.

c. Wisconsin tanks. A range of Wisconsin tanks with constant soil temperatures of 13°, 16°, 19°, 22°, 25°, and 30 °C was available in one of the glass-house compartments. The tanks were filled with tap water, and metal boxes with moist sterilized soil were sunk in this water which was heated up to the controlled temperatures indicated above. The soil was used directly or as a support for plastic or glass containers buried in it. The air temperature varied between 20–25 °C in the compartment. Each temperature tank was provided with artificial light for 16 hours per day.

d. Climate chamber. The climate chamber (113 × 75 × 48 cm) was placed in the laboratory. This climate chamber maintains a constant temperature and artificial tube lighting for 12 to 16 hours per day as required for the experimental plants. Temperature, air moisture and light were automatically controlled.

e. Series thermostat. In a series of small compartments with a cooling

system on one side and a heating system on the other temperature gradients could be obtained which ranged from 0° to 60°C. The temperatures showed a variation of approx. 1 °C due to temperature changes in the environment; the temperature steps between successive compartments varied but they were registered.

2.3.3. *Facilities and methods for sterilization*

Containers, soils and culture media. These could be sterilized by means of high pressure steam autoclaves 121 °C at 15 lb/in^2 for 1–2 hours or for a longer period and lower temperature or a shorter period and higher temperature. A germ free room with ultraviolet light was available for filling sterile dishes and tubes with different culture media.

Seeds and nematodes. In several experiments seeds and nematodes had to be surface sterilized before placing them in culture plates or tubes.

Plant seeds were usually surface sterilized by immersion in 1:1,000 solution of mercuric chloride for 2–3 minutes, then washing in several changes of sterile water. Stainless steel instruments such as needles, scalpels, forceps, scissors, etc., were dipped in alcohol and flamed before and during use.

Surface sterilization of the nematodes was usually practised by submersion in a solution of 0.1% malachite green or 1% streptomycin or 1:1,000 mercuric chloride ($HgCl_2$) for one minute and then washing in several changes of sterile water in watch glasses. Then the nematodes were transferred directly with sterile needles into the culture medium used in the experiment.

2.4. Enumeration of nematodes, microarthropods and microorganisms

The census work in the experimental field and the evaluation of many experiments required sampling, extraction and enumeration of nematodes and of possibly predatory microarthropods, and in some cases of other organisms. The generally applied techniques are described.

2.4.1. *Nematodes*

Periodic sampling of the experimental plot was done by taking cores with a 2.5 cm wide, semicircular auger to a depth of 25 cm. Five cores were taken per sampling date. The core of the soil sample was stripped off by a knife upto the semicircular edges, each core was cut in five sections of 5 cm length, about 30 grams of soil each, which were kept seperate. The plot was yearly grown with rows of peas, 15–25 cm apart, during part of the year and then five cores were taken in the rows as well as between the rows. Core holes were filled with neighbouring soil and marked by sticks; used spots were avoided for subsequent samplings. All sections of all cores were extracted separately.

The soil from pots, tubes or other containers was often extracted whole. Subsamples of 100 ml were taken and extracted when the total amount of soil was too much.

The nematodes were extracted shortly after the samples were taken. The extraction was done by elutriation or, for small samples by the cotton wool filter method; these methods are about 80% efficient OOSTENBRINK (1960, 1969). Both methods are based on mechanical separation of nematodes from soil by means of plenty of water allowing the nematodes by their own activity to pass loose filters in water so that finally only active nematodes are collected (Fig. 4A). They become available in a clean suspension of 100 ml about 24 hours after processing of the sample. Replicated aliquots of 1, 5 or 10 ml or whole suspensions were analysed depending on the densities of nematodes.

The nematodes studied were ectoparasitic or free living species, and extraction of active nematodes from soil according to the methods described was usually sufficient. In some experiments other methods had to be employed to collect nematode eggs, nematode cysts, or in the active stages of normally active species from soil, or to check the presence of endoparasites in plant roots.

2.4.2. *Microarthropods*

Samples for microarthropods, mites and collemboles suspected to act as nematode predators, were taken very close to the nematode samples in the same plot. Comparative studies could thus be made of prey nematodes and predatory mites and collemboles. Microarthropod samples were obtained in the same way as for nematodes (2.4.1.), by using the same augur up to 25 cm depth. Five cores were taken per sampling date. The core of the soil sample, stripped off by knife up to the semicircular edges was cut with a special nail in five sections of 5 cm each, giving about 30 g of soil per section or unit. It was collected in separate sample containers made from iron rings (6.2 cm in diameter and 2.5 cm high fitted with a large sieve 0.2 cm pore) to one side of the ring. Each container with a sample was transferred without further disturbance of the soil into a plastic pot (6.8 × 6.6 × 4.5 cm) containing 20 ml of tap water, with the sieve plate downwards, in such a way that it did not touch the water. The mounted samples were then placed under two tube lights of 40 Watt each for a week, to drive the microarthropods down into the water away from the light, forced by the slow desiccation of the soil which proceeded from the top downwards. This technique is based on modification of the TULLGREN funnel and is expected to reach 80% efficiency for the active microarthropods considered here. The animals reach the water undamaged and float. They were picked up from the water by means of a bamboo needle and preserved in 60% alcohol for indentification; the catch of each sample was counted completely. The method is illustrated in Figure 4B and C.

2.4.3. *Microorganisms*

Of the other small organisms the fungi had to be isolated, cultured, identified, and used for diagnostic work in some experiments. Infected material was surface-sterilized and incubated on selective culture media, such as cherry agar, in petri dishes or culture tubes, with repeated inoculation on fresh plates (cf. AINSWORTH 1961:245).

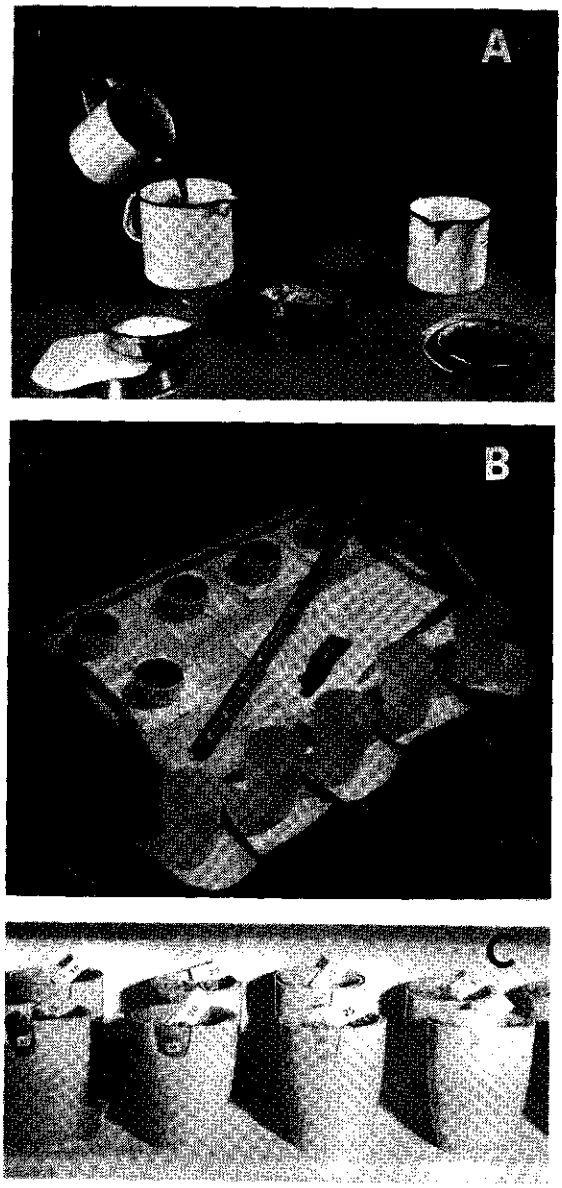


FIG. 4. Essential parts of apparatus used in extraction of nematodes and micro-arthropods from soil. A. Mounted cottonwool filter for the extraction of active nematodes, from a dirty suspension, when most of the soil has been removed by decantation or elutriation (cf. OOSTENBRINK, 1954, 1960). B. A tray with auger, nail, knife, empty iron rings with sieves at the bottom for soil mites extraction and plastic cups for soil samples for nematode extraction. C. Modified TULLGREN funnels for the extraction of micro-arthropods from soil.

2.5. Evaluation of plant growth

Plant growth was evaluated by the common techniques of measuring, weighing and counting. The quantities used for the evaluation of plant growth in relation to nematode attack were usually units or root weight, shoot weight, number of tillers, height of the plants and colour of roots and shoots. In studying relations between nematode density and plant growth, fresh weights of the roots and shoots were usually taken after drying them in cotton cloth or between the fold of filter papers to remove superficial water from the plant surface. Dry weights were taken after keeping the plant material at 90 °C for 2–3 days.

3. REPRODUCTION, DEVELOPMENT AND GROWTH

Few plant parasitic or free-living nematodes have been studied to such an extent that their life cycle, behaviour and significance are fully known. The best studies made up to now are those on *Radopholus similis* (VAN WEERDT 1960), *Ditylenchus dipsaci* (YÜKSEL 1960), *Plectus parietinus* a free-living nematode (MAGGENTI 1961), *Hemicriconemoides chitwoodi* (FASSULIOTIS 1962), *Criconemoides xenoplax* (SESHADRI 1964), *Nacobbus serendipiticus* (CLARK 1967), *Rotylenchulus parvus* (DAS GUPTA and RASKI 1968), *Heterodera betulae* (RIGGS, HIRSCHMANN & HAMBLEN 1969) and *Meloidogyne naasi* (SIDDIQUI & TAYLOR 1970).

The study on reproduction, development and growth of *T. dubius* includes observations and experiments on mating, the egg stage, development of the embryo, the first larval molt inside the egg, hatching of the second stage larva, development and shape of the larval stages and the adults, and morphological variability of the adults. It includes many continuous or intermittant microscopical observations on developing or actively moving specimens in water. Short observations on developing specimens were made by placing single eggs in a hanging drop of water on cover slip inverted over the cavity slide. The water was changed daily and the slides were kept in a dark, moisture chamber placed in a climate chamber at 20 °C (Fig. 3).

A 8 mm colour movie film was made to register several phases. The film is stored in the Plantenziektenkundige Dienst (PD) files and serves as a reference item. A number of drawings and photographs were derived from these observations and this film.

3.1. MATING

3.1.1. *Morphological structures*

T. dubius is a bisexual nematode species with females and males both numerous in normal populations. Apart from the differences in reproductive structures, which include the shape of the tail, there is no extreme sexual dimorphism as in some other plant nematodes. Both types of adults are slender. Females tend to be slightly larger than males and there may be other minor differences in shape (cf. Fig. 5). Intersexes, which occur numerously in some and incidentally in populations of other species of nematodes were not found in my studies of *T. dubius*.

T. dubius is a didelphic amphidelphic species, therefore with two ovaria stretched into opposed directions. The female reproductive tract opens through a vulva, which appears as a transverse slit on the ventral surface of the nematode at about 54% distance from the head end. Its position, however, varied in our stock population from 51% to 57%. Perpendicularly to the body wall leads the vagina, a muscular tube, from the vulva to the middle of the body, after

which it splits up and bends to both sides, head and tail end, towards the uterus of each of the two ovaria. The part of the uterus away from the vagina is modified to a spermatheca, in which several sperms may be visible. Fertilization takes place when the egg-cells pass through the spermatheca. Between the uterus and the spermatheca is the quadricolumella where the egg shell is formed. The spermatheca is followed by the oviduct, then by a single row of oocytes and by the terminal cell (Fig. 5A).

The male has only one testis which is stretched anteriorly towards the head end. The reproductive tract opens into the end of the digestive tract, which is therefore a cloaca, on the ventral body surface near the tail end. Opening into the cloaca is the vas deferens which is divided into tubular and glandular regions. Beyond the seminal vesicle is the single testis which like the ovary comprises several cells side by side. These cells are the sperms in different stages of maturation. In the cloaca is a pair of sclerotised spicules between which the sperm cells are guided when they are inserted into the female vulva in the mating process. Just below the spicules is a sclerotised, curved gubernaculum, which serves to support and guide the spicules. (Fig. 5B).

The presence of numerous males and females with fully equipped copulation organs and the common occurrence of sperms in the female spermatheca indicates that mating normally takes place. Mating was, however, not seen or recorded before in *T. dubius* or other *Tylenchorhynchus* species. Microscopical observations and cinematographic registration were made to study the process.

3.1.2. *The mating process*

Young root tips of grass inoculated with a monospecific population of *T. dubius* were cut loose, lifted from the soil into a tray with water and screened under the dissecting microscope. A male and female of *T. dubius* in mating position were transferred with a bamboo needle into a drop of tap water on a glass slide. A cover slip supported by glass supports was placed over the drop and the slide was left unsealed for aeration; additional water was added if necessary to avoid desiccation. The nematodes were examined under the compound microscope at different magnifications.

The animals had already made contact when the observation started. The mating continued for 70 minutes under the microscope. The spicules and accessory structures were inserted with their tips only into the vulva keeping it open for sperm transfer. During mating the males and females had their anterior ends in opposite directions and made continuous body movements. Their union was firm as if an adhesive material kept the bodies together at the point of contact. Numerous sperms were injected into the vagina and moved up to the reproductive tracts and the spermathecae where they were stored. After 70 minutes the animals separated themselves suddenly and without difficulty.

The photomicrograph motion film indicated above shows the position of male and female in copulation and their body movements, along with the reproductive organs in the act of sperm transfer. The figures 7A, B and C are taken from this film.

3.2. THE EGG

3.2.1 *The egg cell*

A single row of oocytes in different stages of development are visible along the entire length of the ovary, as indicated in (Fig. 5A.). Meiosis, fertilisation, and the extrusion of the polar body, apparently take place when the oocyte passes the spermatheca filled with sperm. As soon as the egg cell has passed through the spermatheca and the adjoining quadricolumella into the uterus,

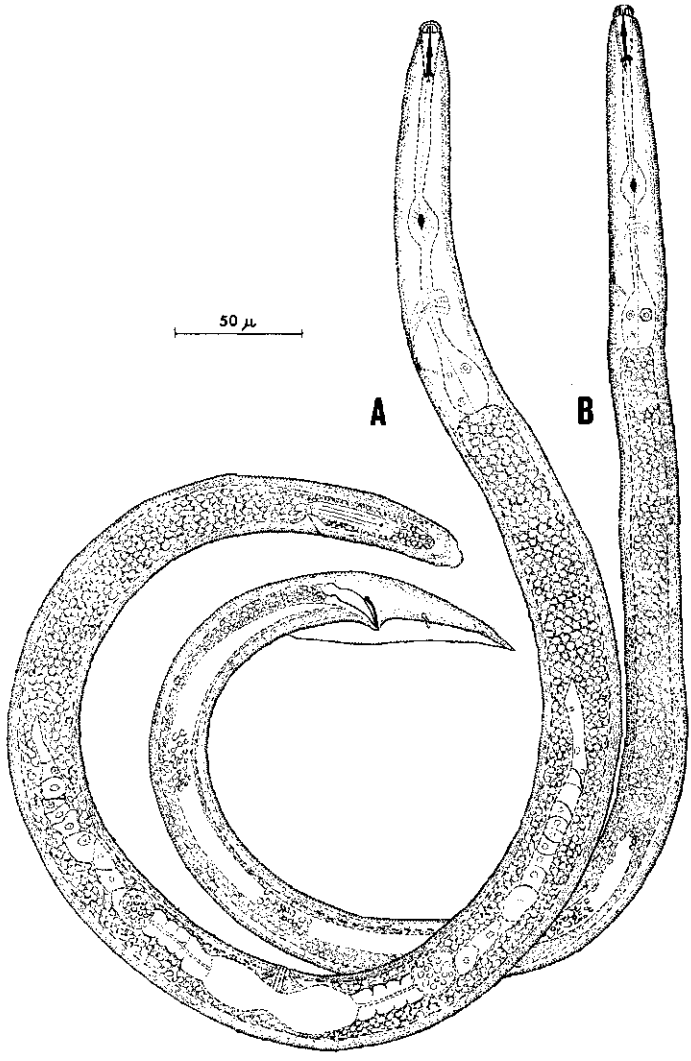


Fig. 5. *Tylenchorhynchus dubius*. A. Female, lateral view. B. Male, lateral view.

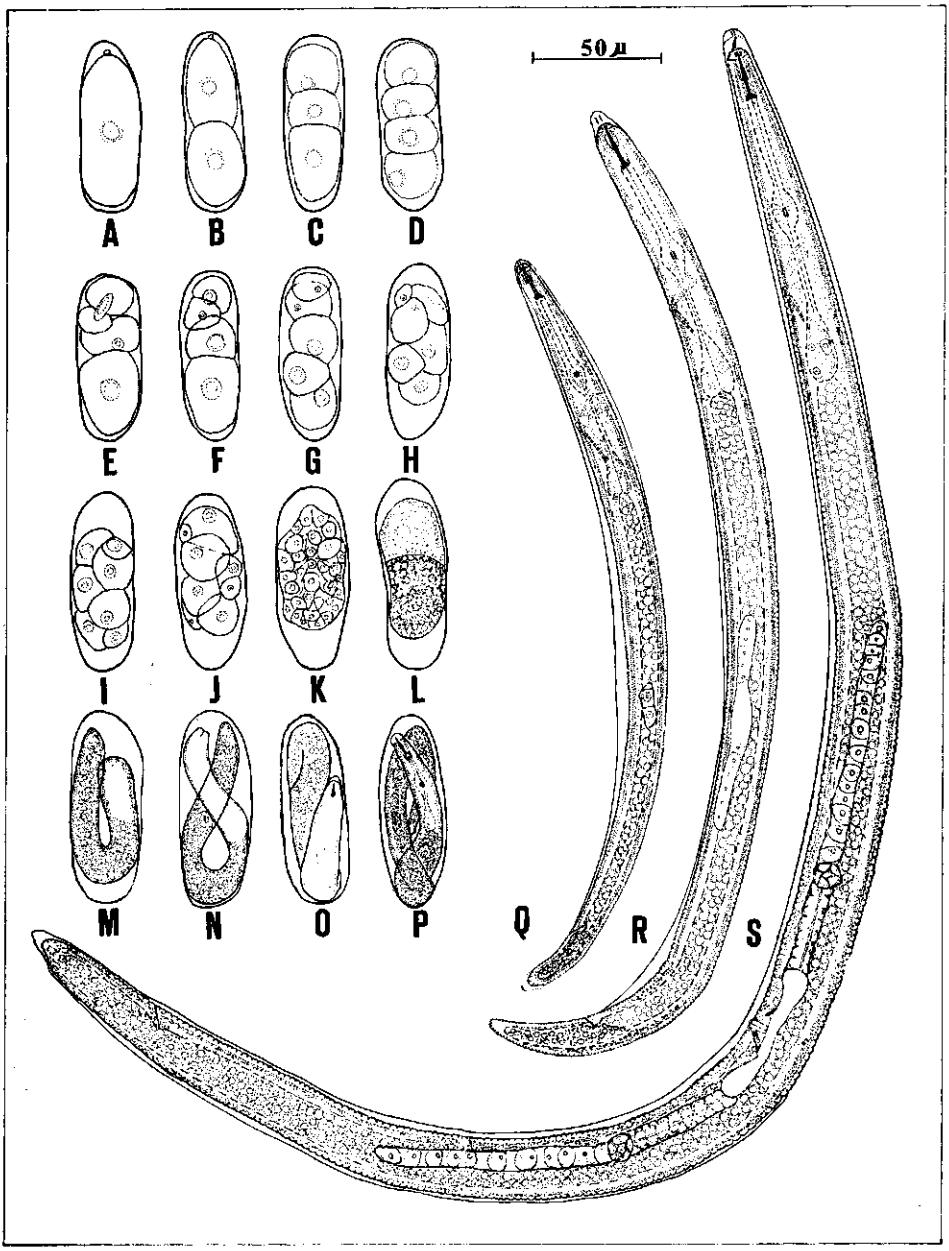


FIG. 6. Embryonic and larval stages of *T. dubius*. A. Single celled stage; B. 2-celled stage; C. 3-celled stage; D–F. 4-celled stage; G. 5-celled stage; H. 6-celled stage; I. 7-celled stage; J. 8-celled stage; K. Many-celled stage; L. Gastrula stage; M–N. First stage larva; O. First molt within egg; P. Second stage larva within egg; Q. Second stage female larva molting; R. Third stage female larva molting; S. Fourth stage female larva molting.

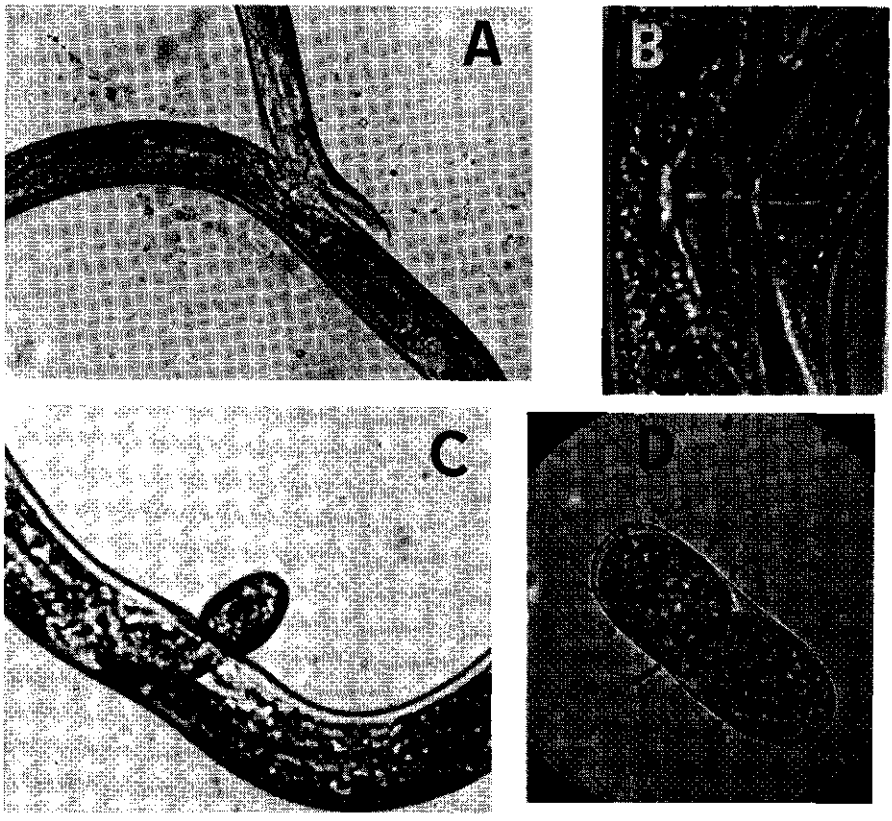


Fig. 7. A. Micro-photograph of *T. dubius* in the act of copulation showing the tip of the spicules in the vagina. B. Position of the male and female bodies during copulation. C. Pouring out of egg from the female body. D. Two-cell stage of the egg.

it has obtained a thin shell which also surrounds the small polar body, situated at one of the narrow sides of the cell. Fig. 6A illustrates the size, shape and position of the egg cell and the polar body within the egg shell. Passage of the oocyte through the sperm stock leading to the fusion of sperm and oocyte nuclei, extrusion of the polar body, the appearance of the egg shell, and the onset of cleavage divisions of an egg cell often immediately after it reaches the uterus, were actually seen during continuous microscopical observations and prove that fertilization takes place. The presence of both types of gametes, does not exclude pseudogamy in which the sperm has only stimulated the egg to further development, or parthenogenesis in which development takes place without sperm cells playing an essential role (NIGON et al, 1960). The fusion of sperm and oocyte nuclei, however, ascertains that the egg cells of *T. dubius* are in fact being fertilized as soon as they reach the uterus.

3.2.2. *Oviposition*

When the egg cell has passed the spermatheca and quadricolumella and has reached the uterus, it may be laid within 4 hours. Sometimes, however, the eggs are held for a long time, up to 2 days, in the uterus before they are laid. Newly deposited eggs are often still in the one-celled stage, but cleavage may take place in the uterus and they may have developed into the two-celled or four-celled stage or, sometimes, even harbour a fully developed larva.

Oviposition is a simple process in *T. dubius*. Females sometimes do not even stop feeding when laying eggs. Usually the activity of the female is increased Generally there is a period of 4 or more hours between successive deposits. A full grown egg nearly fills the whole body width. As soon as the egg in its elastic shell is being pushed out of the vagina through the vulval opening, it forms an elastic dumble when it is partly outside the body (Fig. 7C). The outside portion slowly increases in size until the whole egg suddenly jumps out. Contraction and expansion of uterus and vagina is rapid. Eggs may be retained in the uterus for a long time, but laying proceeds quickly as soon as they reach the vagina. It can readily be observed by placing females with well-developed ovaries in cavity slides in a shallow layer of tap water and observing them under the dissecting or compound microscope. Females usually have 2–3 mature eggs at a time. They usually start egg laying a few hours after they are placed in water. Generally the first egg originates from the anterior ovary and the second from the posterior one. Only 2–3 eggs are deposited per individual female per day and this continued in cavity slides for 3–4 days. The first two eggs from both the ovaries are bigger than the following ones.

When egg laying was studied by placing a single fertilized female on the roots of four days old grass seedlings grown in Petri dishes in water agar under aseptic conditions, it proceeded for about a week. A single female could lay 1–12 eggs which were deposited close to the grass root in a regular pattern: they were deposited among the root hairs in a single row on both sides of the root up to the elongation zone (Fig. 2).

On some occasions females failed to lay the eggs, e.g. when eggs moved simultaneously from both ovaries and got stuck in front of the vagina. This leads to death of the female. In the latter case further development of the egg inside the uterus results in a free second stage larva which, after hatching, makes quick movement and perforates the uterus by its stylet and may break into the pseudocoel. It rarely escapes; usually it dies inside the already dead female. One larvae developing in the anterior ovary moved as far as the excretory pore. Another developing in the posterior ovary moved up to the anal region without being able to escape from the female body.

3.2.3. *Development of the egg*

The mature, and probably fertilized, egg cell is oblong with rounded ends and ranges from 61–70 μ (average 67 μ) in length to 20–25 μ (average 22 μ) in width. It is surrounded by a firm, transparent, somewhat flexible shell or chorion with a smooth surface. Underneath the chorion, in the beginning almost in

contact with it, is the vitelline membrane which encloses the ooplasm. In later stages of cell division it may recede away from the chorion, but it is always clearly visible. Development of the egg may start either when it is still in the uterus or after it has been laid (cf. Fig. 6).

In one-celled eggs cleavage started 15–24 hours after laying. The first division is usually transverse giving rise to an anterior cell and a posterior cell. The polar body is usually visible between the vitelline membrane and the egg shell at the anterior pole of the egg, as was the case in *Radopholus similis* according to VAN WEERDT (1960).

The two blastomeres resulting from the first cleavage are of approximately the same size. The second cleavage is parallel to the first one, dividing the anterior blastomere into two, and usually takes place 24–36 hours after oviposition. The third cleavage, usually within 48 hours after laying, divides the posterior cell in the same way. The four cells are normally arranged in a row, but it is not uncommon that some of them, especially the central cells shift aside and cause a zigzag arrangement. From now on the cells resulting from further divisions shift their position considerably. Observations on several 48–72 hours old blastulas indicate that the fourth and fifth cleavage occur parrallel or longitudinal and at about the same moment in the anterior cells, giving the blastula a five- and six-celled appearance in which no fixed regular pattern was noticeable. At 72–96 hours the posterior cells also divide, usually longitudinally, resulting in a seven- and eight-celled structure. At this stage the cell contents, which previously was in continuous streaming motion became more quiet. 6 days after oviposition a many-celled stage, considerably shrunken and separated from the egg shell, by an empty space apparently comparable to the gastrulation phase, is formed. The structure, which so far was uniformly filled with cells with dense, opaque globules, begins to show an orderly arrangement of small cells with transparent, light areas. By the end of the 6th day the gastrula appears to develop into an oblong structure with a hyaline part, developing later into the oesophageal region, and a dark section, indicating the future intestine of the embryo. The embryo elongates in a few hours to a body with a single flexure and two blunt ends. At this stage the first movements of the organism inside the egg are noted. Further elongation and reduction of the body width in the next few hours leads to a distinct larva with 2–3 body flexures, making to and fro movements, as well as lateral rolls and twists. 7 days after the one-celled egg has been laid the larva is so active, that it hardly remains in one position for any length of time. The body wall and internal organs are not yet clearly marked, and a mouth stylet has not yet been formed (Fig. 6M), but it has to be considered as the fullgrown first-stage larva as will be shown under 3.2.4.

3.2.4. *First larval molt inside the egg*

During the 8th day the larva develops further and appears to molt inside the egg. The oesophageal region becomes well separated from the dark intestine, an initially dimly visible stylet with three basal knobs is being formed and a mol-

ting skin appears. When the larva was taken out of the egg shell by gently puncturing it, the moulting skin was clearly visible. It was very thin and tender and disappeared after some time as little pieces in the surrounding water. At the end of the 8th day fully developed, second-stage larvae with strong stylets and clearly visible other organs were present in the eggs. A well-developed second-stage larva occupies the entire space inside the egg shell, with its body in 2 to 3 folds (Fig. 6P). They became very active and started piercing the egg shell as an introduction to hatching.

3.3. HATCHING

The hatch of nematode larvae from their egg shells has been studied for some plant parasitic nematodes only. It appears to be an important aspect of the plant/nematode relationship, with much variation. DONCASTER & SHEPHERD (1967), showed, that the hatch of certain *Heterodera rostochiensis* required strong, systematic efforts of the larvae and that it was an active process. Environmental conditions, however, are of influence. These include moisture, temperature and oxygen; the hatch of nematode eggs usually demonstrates optimum curves in relation to each of these factors (WALLACE 1959, OOSTENBRINK 1967, DAO 1970). Apart from general environmental factors, plant root exudates may influence or determine the hatch of plant nematodes. It is known since long that the hatch of several *Heterodera* species is strikingly influenced by root exudates of their specific host plants, whereas other species of the genus do not show this relationship. BAUNACKE (1922), SHEPHERD (1962), and OOSTENBRINK (1967) demonstrated that in the case of *H. rostochiensis* diapause may prevent or delay hatching, despite the presence of host exudates and favourable environmental conditions. There are, on the other hand, several records of certain plant nematodes hatching in water without plant root exudates. Hatching, therefore, shows much variation depending on the host/nematode association, and the knowledge is far from complete. In any case, it has to be kept in mind that the possible influence of host exudates in hatch has to be considered separate from the question as to how far such exudates form a gradient in the soil which attracts hatched larvae to the roots.

Nothing is known about the hatch of *T. dubius*. For this reason observations were made about the process of hatching and experiments were carried out to determine the influence of host exudates.

3.3.1. *Process of hatching*

The development from egg laying to hatch seems to be a continuous process in *T. dubius* kept in water. The second-stage larva in the egg is nearly always moving, the activity increasing before hatching. The larva moves its head in various directions and tries to perforate the egg shell by making 20 to 52 stylet punctures per minute at one spot, especially at the narrow side of the egg. Sometimes this process may go on for three hours. The larva may become inac-

tive for a short time, and then start to puncture again at the same spot. Just before hatching the larva exerts a pressure in the elastic egg shell by expanding its body so that only two halves are seen. When the shell membrane breaks, the larva is out of the egg in a few seconds. After hatching, the liberated larva remains active.

It appears from these observations that hatch of *T. dubius* takes place readily without delay, and that the larva plays an important role in actively liberating itself from the egg shell by means of its stylet and body power.

3.3.2. *Influence of host diffusates*

Fullgrown *T. dubius* females were taken from the monospecific stock culture grown on peas. Three experiments were carried out to test the influence of two suitable host plants, pea and rye-grass with a water control.

a. Pea-root diffusate

Root-diffusate from pea was collected by growing two-day old seedlings in 10 ml tap water for 48 hours, and filtering it through a no. 3 sintered glass filter. This filtrate was used as the 'normal' solution, and at dilutions of 10, 100, 1000 and 10,000 times. Tap water, filtered like the diffusate, was used as control. All liquids were stored at 5 °C. The experiments were started on 26th June, 1968.

For each of the six test liquids, five cavity slides were each filled with 4 drops, making a total of 30 slides. Two gravid females of *T. dubius* were added to each slide. The five replicate slides of each liquid were placed together in a closed Petri dish with moist filter paper; all dishes were kept at room temperature of about 22 °C.

After 24 hours each slide had 5–7 eggs. Only 5 eggs, in the one- or two-celled stage, were left in each slide by picking out the excess eggs and the mother nematodes. The test liquids were renewed daily from the stock solutions. Also daily all slides were observed under the dissecting microscope.

The first hatch was observed and recorded 7 days after the eggs had been laid; hatched larvae were removed. One day later, after 8 days, all eggs hatched in all treatments. There were no significant differences (cf. Table 1 A).

b. Grass-root diffusate

Root-diffusate from rye-grass was collected by growing 100 four day old seedlings in 10 ml tap water for 48 hours and filtering it as indicated under a. Tap water again served as a control. The liquids were stored at 5 °C.

Four replicates of one egg were treated with the liquids, as indicated above. This material was placed in a climatic cell at 19 °C.

After 10 days the four larvae had hatched in both liquids. Also in this experiment no significant differences occurred. The slower hatch (viz. those taking more time), may be due to the lower temperature used in this experiment (cf. Table 1 B).

c. Water

Five replicates with 10 eggs each were treated with tap water only, at 20°C–22°C. After 7 days 34%, after 8 days 76% and after 196 hours 100% of the larvae had hatched (cf. Table 1 C).

It appears from the experiments that *T. dubius* larvae hatch readily and quantitatively in tap water and that the host-root exudates did not influence the process.

TABLE 1. Percentage of hatched *T. dubius* larvae in different root-diffusates and water:
A. Root-diffusate from pea at different dilutions. Percentages based on 5 replicate with 5 eggs each. Experiment at ± 22°C.
B. Root-diffusate from ryegrass. Percentages based on 4 replicate slides with 1 egg each. Experiment at 19°C.
C. Water. Percentage based on 5 replicate slides with 10 eggs each. Experiment at 20°–22°C.
(n.s.) = differences not statistically significant at 5% point.

	After 7 days	After 8 days	After 9 days	After 10 days
A. Pea-root diffusate				
Undiluted diffusate	32	100		
1:10	52	100		
1:100	64	100		
1:1000	60	100		
1:10000	40	100		
Water (control)	52	100		
	(n.s.)	(n.s.)		
B. Grass-root diffusate				
Undiluted diffusate		0	75	100
Water (control)		25	100	100
		(n.s.)	(n.s.)	(n.s.)
C. Water	34	76	100[1]	

[1] This percentage was in fact reached after 196 hours.

3.4. THE LARVAL STAGES AND THE ADULTS

Nematodes in their development pass through a number of stages marked by molts, during which a new cuticle is formed beneath the old one, which is shed at each molt in most species, although there are exceptions. CHITWOOD & CHITWOOD (1950) stated that 'there are usually four molts in the development of a nematode, the stage following the fourth molt being the fifth or adult stage'. Four molts have been observed in several important plant nematodes, such as *Meloidogyne* (NAGAKURA 1930), CHRISTIE & COBB 1941) and *Heterodera* species (RASKI 1950, CHITWOOD & BUHRER 1946, HAGEMEYER 1951). On the other hand LINFORD and OLIVEIRA (1940) failed to detect the pre-hatch molt in *Rotylenchulus reniformis*. The first molt was recorded to occur inside the egg in *Heterodera rostochiensis* (HAGEMEYER 1951). *Heterodera trifolii* (MULVEY 1959), *Tylen-*

chulus semipenetrans, (VAN GUNDY 1958), *Radopholus similis* (VAN WEERDT 1960) and *Criconemoides xenoplax* (SESHADRI 1964).

According to our results described under 3.2.4. there is a first molt inside the egg of *T. dubius*. Information about the number of molts, and therefore of stages, in the development of this nematode, was collected by examining and measuring specimens of cultures and by analysing a natural population.

3.4.1. *Identification of the different stages*

The life cycle of *T. dubius* from L_2 to adult, and the different stages separated by molts, were studied by examination at short intervals of a population grown from known inoculum on rye-grass under controlled conditions. Perspex blocks of 3 × 2 × 0.5 cm with a central pit of 0.8 mm diameter and 0.4 mm depth as shown in Figure 3 were used as micropots. In each of a few micro-pots a three-days old grass seedling in sterilized sand was inoculated with five freshly hatched second-stage larvae on 5 November 1968. The nematodes were picked by hand and transferred in two drops of distilled water per pot. The pots were placed in Petri dishes with a moist filter paper at the bottom in a climate chamber at 20 °C and 12 hours artificial light per day. STEINER's nutrient solution was added every third day and distilled water according to the apparent need of the plant.

Every 4th day one micropot was emptied into a 50 ml beaker with water, and the nematodes were collected. After complete washing of the roots and adhering soil, the suspension was stirred vigourously and decanted after a few seconds when the sand had settled down. Fresh water was added, stirred and decanted, and this process was repeated 3–4 times. The decanted supernatants were poured together in counting dishes and examined under a binocular microscope. The supernatant was clear, without any debris, and nearly all nematodes originally inoculated could be recovered. The duration of each larval stage and the approximate time of its molt was determined by this method, and the collected nematodes were preserved for the morphometric studies recorded under 3.4.2.

The interval of 4 days was chosen because preliminary observations had shown that each larval stage, including its molting period during which the old skin was visible, lasted more than 4 days. There was variation in the individual speed of development and the specimens extracted at one date were not always of the same stage. The series of extractions, ten in succession covering a period of 40 days, furnished specimens of all stages of the life cycle, and they could be recognized without difficulty from their size and shape.

The inoculated, freshly hatched L_2 were still present as L_2 after 4 days. They had started to molt after 8 days and had reached the L_3 stage after 12 days. After 16 days the L_4 stage had been reached by most specimens. This stage lasted about 12 days: 28 days after the inoculation of the L_2 fullgrown females and males were available. Egg laying had started 4 days later, therefore after 32 days, but this was 40 days in other specimens. Because maturing of the egg up to hatch of the L_2 takes about 8 days (cf. 3.3.2.), the whole life cycle of *T. dubius* under our experimental conditions takes 40–48 days.

The series of observations, therefore, established that four molts, including

the one inside the egg, occur, The molting specimens which are easily recognizable because the newly-formed stage shrinks markedly away from the old skin in all three stages, L_2, L_3, and L_4, offered the key to determine the number of stages. Figures 5 A, B and 6 P, Q, R, and S illustrate all the key stages, viz. the newly molted L_1–L_4, and also fully developed males and females.

3.4.2. *Morphology of the different stages*

a. Population from ryegrass

Representative non-molting specimens of the different stages observed in the experiment under 3.4.1. have been collected and processed into permanent slides. Morphometric studies were made on the limited number of identified specimens of a certain stage available from this study. The range of measurements indicated may be larger when more specimens are taken into consideration. In the descriptions sizes are given in microns and indicated by letters, namely L = body length (not to be confused with the larval stage indications L_1, L_2, L_3, and L_4); W = greatest body width, S = length of stylet, E = length of esophagous, T = length of tail. Also De Man's ratios are used, namely α = body length divided by greatest body width, β = body length divided by oesophagous length, and γ = body length divided by tail length.

Second-stage larva (L_2). cf. Figure 6.
$L = 211$–290, $W = 13$–18, $S = 11$–13, $E = 53$–110, $T = 23$–33, $\alpha = 15$–18, $\beta = 2.9$–4.1, $\gamma = 9$–10.

Shortest and thinnest free stage, head blunt and slightly set-off, with lightly sclerotized cephalic frame, spear clearly visible with strong basal knobs, oesophagus clearly structurated with large median bulb and conspicuous valves and with a well-marked terminal bulb not overlapping the intestine. Intestine darkly coloured by globules, anus clearly visible. The genital primordium often visible as a 1–3 celled structure of a few micron lengths at about 60–70% of the body length.

Molting starts in the head end where a new, constricted head becomes visible and a new strong stylet is formed as soon as the skin withdraws from the top, leaving the top part from the old stylet connected with the old skin (Fig. 6Q). At the molting stage the oesophagous is still clearly visible in detail, the intestine is still darkly coloured, and the genital primordium is still small, although it may have grown somewhat.

Third-stage larva (L_3).
$L = 353$–491, $W = 18$–24, $S = 15$–16, $E = 111$–124, $T = 29$–45, $\alpha = 20$–21, $\beta = 2.8$–4.2, $\gamma = 11$–12.

The L_3, after it has escaped from the L_2 skin, is markedly longer and thicker than the L_2 and has a longer stylet, oesophagous and tail: the difference in size between L_2 and L_3 is so great that there is probably little overlap. The general body shape is the same as L_2. The blunt head is somewhat more set-off, the intestine is less dark although with much coarser granules than the intestine of L_2.

From the genital primordium, two oval, lightly coloured enlargements of some tens of microns initiate the development of the ovaries. A vulva is not formed. A hemizonid of about 3 μ is visible.

At molting the following larval stage starts to from a constricted head end which withdraws from the skin and leaves the top part of the L_3 stylet attached to the old skin whereas it forms a stout, new stylet. At the molting stage the oesophagus is still clearly structured and the gonads have reached considerable length (Fig. 6R).

Fourth-stage larva (L_4).
$L = 444–726$, $W = 21–30$, $S = 15–19$, $E = 109–138$, $T = 43–75$, $\alpha = 20–27$, $\beta = 4.0–5.5$, $\gamma = 10–13$.

The L_4, escaped from the L_3 skin, resembles the L_3. It is longer and thicker and has a larger tail and stylet. although the sizes of the two stages show considerable overlapping. The head is clearly set-off, the ovaries are larger and there is a beginning of a vulva. The colour of the intestine is again well-stocked with dark globules.

At molting the newly-formed new head is narrow and markedly constricted. Molting specimens continue to move, although in a clumsy way. Inside the L_4 skin, the adult males and females develop and their ovaries and other sex organs develop in a clearly visible way. (Fig. 6S).

Adult female (♀), Fig. 5A.
$L = 725–1009$, $W = 29–35$, $S = 18–20$, $E = 123–179$, $T = 60–83$, $\alpha = 25–32$, $\beta = 5.3–6.8$, $\gamma = 11–15$.

The adult female after molting from the last larval skin resembles the earlier stages; its sizes overlap those of the L_4. It was never found with sperms at molting.

Adult male (♂), Fig. 5B.
$L = 743–801$, $W = 25–29$, $S = 18–21$, $E = 120–164$, $T = 41–58$, $\alpha = 27–32$ $\beta = 4.7–6.6$, $\gamma = 14–19$.

The adult male is somewhat smaller than the female, but it has the same morphology apart from the sex organs. The numerical data are collected in Table 2.

The morphometric differences between the successive stages are great, but the size of the characters usually overlap each other. For this reason qualitative characters, specially concerning the development of the gonads and sex organs, are useful for relating specimens of a certain size to their proper stage.

TABLE 2. Measurements of the non-molting specimens in microns from *T. dubius* grown on rye-grass in the experiment described under 3.4.1 which could be placed with certainty in the stage categories indicated.
n = number of specimens. Other symbols explained in text.

	L	*W*	*S*	*E*	*T*	α	β	γ
L_2 (n = 6)	211–290	13–18	11–13	53–110	23–33	15–18	2.9–4.1	9–10
L_3 (n = 3)	353–491	18–24	15–16	111–124	29–45	20–21	2.8–4.2	11–12
L_4 (n = 13)	444–726	21–30	15–19	109–138	43–75	20–27	4.0–5.5	10–13
♀ (n = 11)	725–1009	29–35	18–20	123–179	60–83	25–32	5.3–6.8	11–15
♂ (n = 5)	743–801	25–29	18–21	120–164	41–58	27–32	4.7–6.6	14–19

b. Population from pea

Seventy molting specimens and adult males and females of various sizes were picked at random from a population extracted from around pea roots in the experimental field plot in July, 1967. Temporary mounts were prepared of all the specimens in tap water and the slides were sealed with paraffin and stored at 5 °C till their body length was measured. Temporary mounts were preferred to avoid the danger of shrinkage during preparation. The length of 10 eggs was also measured. The data are summarized in Fig. 8.

It appears from Fig. 8, that the length of the various molting stages does not show much overlapping.

It should, however, be kept in mind that they are selected specimens which do not represent a whole population, and therefore that the ranges may be wider.

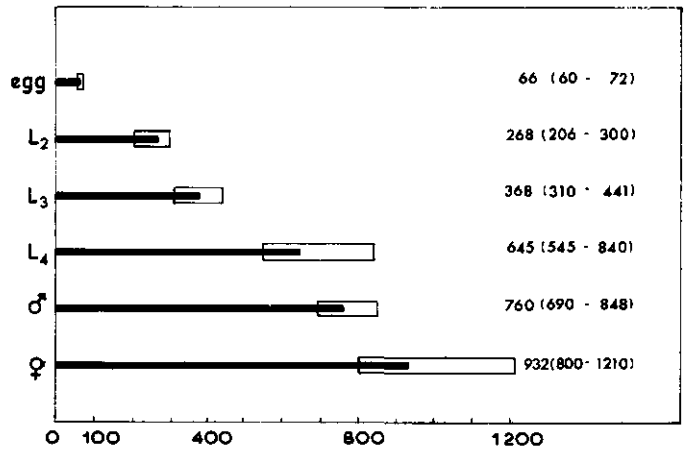


FIG. 8. Length in microns of eggs (10 specimens), molting second-stage larvae L_2 (13 specimens), molting third-stage larvae L_3 (20 specimens), molting fourth-stage larvae L_4 (13 specimens), adult males ♂ (12 specimens), adult females (12 specimens), collected from around pea roots in the monoculture pea plot in July, 1967. Only molting specimens of the larval stages are measured. Abscissa: length of specimens in microns. Ordinate at left: indication of stages. Ordinate at right: average length of stages (graphically represented by solid bars); range of measurements between brackets (graphically represented by open bars).

3.4.3. *Morphometric analysis of a developing population*

Morphological studies of a developing population have proved that four molts, including the one in the egg, occur (cf. 3.2.3).

Measuring non-molting specimens selected from a population in grass (Table 2) as well as molting specimens selected from a natural population on pea (Fig. 8) suggests, that the free stages in soil (L_2, L_3, L_4, adults) differ considerably in size, although there is an overlap. In the afore- mentioned studies measured specimens were selected in one way or another. Therefore morphometric data on all specimens of a natural, developing population on rye grass are added. The stages from L_2 to adults were quantitatively extracted from a soil grown with grass on 5.5.1970. The population was extracted from the soil by decantation and sieving, without using a filter to avoid every possible loss of inactive specimens. The length of 264 non-molting specimens, taken without any selection, was determined. The results are summarized in Fig. 10. In addition 23 molting specimens were collected. They were measured and the results were summarized in Fig. 9.

Fig. 10, shows that the frequency of different size categories of non-molting specimens does not clearly indicate the L_2, L_3, L_4 and adult stages. There are minor peaks at 175–275, 325–375 and 425–525, and a great peak at 625–825 μ. The peaks at 175–275 and 325–375 may indicate high frequencies of L_2 and L_3 respectively, but the L_4 is not characterized by the peak of 425–525 because many L_4 specimens were also observed in the length categories from 625 μ on. There is apparently too much overlapping between the lengths of the different stages to separate them clearly by means of the non-molting specimens.

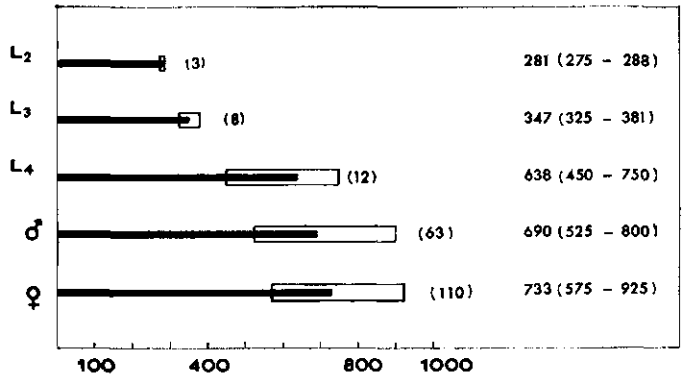


FIG. 9. Length in microns of molting second-stage larvae L_2 (3 specimens), molting third-stage larvae L_3 (8 specimens), molting fourth-stage larvae L_4 (12 specimens), adult males ♂ (63 specimens) and adult females ♀ (110 specimens). All molting L_2, L_3, and L_4 specimens occurring together with the 264 non-molting larvae and adults collected at random from a natural population grown on ryegrass (cf. Figure 10) were measured. Abscissa: length of specimens in microns. Ordinate at left: indication of stages. Ordinate at right: average length of stages (graphically represented by solid bars); range of measurements between parenthesis (graphically represented by open bars).

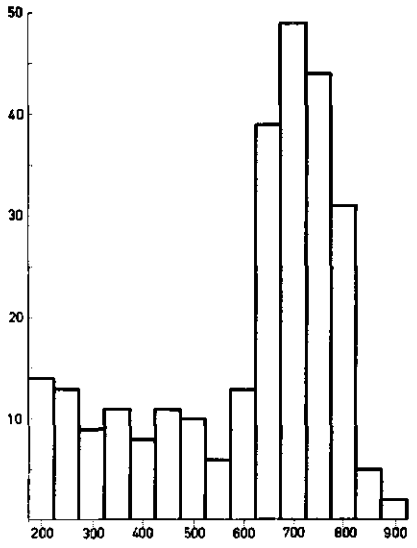


FIG. 10. Frequency of specimens in size classes when 264 non-molting specimens, randomly chosen from a natural population extracted from soil grown with ryegrass, were measured. Abscissa: Size classes, indicating length in μ = microns 200 = 175–225 μ, 250 = 225–275 μ etc. Ordinate: number of nematodes.

The size of the molting specimens, however, gave separation between L_2, L_3, and L_4 (cf. Fig. 9), whereas the adults could be recognized from the sexual organs. These results are in accord with the data obtained with pea (cf. Fig. 8).

3.5. INFLUENCE OF TEMPERATURE ON MORPHOLOGY OF THE ADULTS

Adult females and males of the stock population of *T. dubius* were reared on rye-grass in sterilized PD soil at 13°, 16°, 19°, 22° and 25°C respectively, and one extra batch was selected from the natural population in the monoculture pea plot from which the stock population was also drawn some years earlier (cf. 2.2.1). These six populations were used for morphological studies.

Fourteen characters known in nematode taxonomy, of which 10 concerned females as well as males, were measured or calculated from measurements on 25 females and 25 males of each population in water slides. The characters are indicated by the symbols *L; α; β; γ; T/ABW; BW/LiW; OW/LiW; E; S;* g_1; g_2; *V; T; Spi; Gu*, which are explained in Fig. 11.

Table 3 gives the results. All data indicated are averages of measurements on 25 specimens; the significance of differences between populations, reared at the different temperatures, is also given.

In both sexes the characters T/ABW, α and γ did not show any distinct differences. In addition, in females the averages of the characters Gl and E were constant, in males Spi, Gu, OW/LiW and BW/LiW.

Significant differences were found in both sexes for L, β and S; in females furthermore for BW/LiW and V, in males for E and Te.

The absence of significant differences for α and γ does not make these charac-

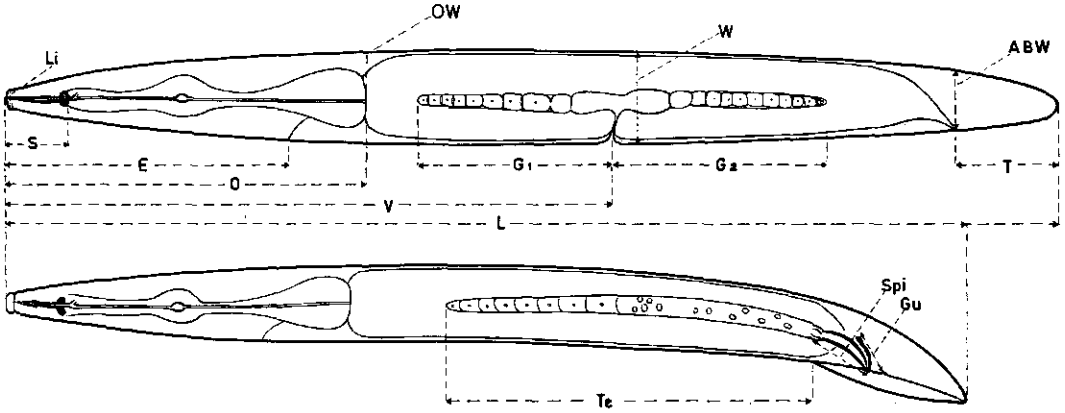


Fig. 11. Definition and illustration of morphometric characters used in the studies on *Tylenchorhynchus dubius* (Table 3).

L	= body length in microns
W	= maximum body width in microns
O	= distance from head end to base of oesophagus in microns
α	= body length divided by greatest body width (= L/W)
β	= body length divided by distance from head end to junction of oesophagus and intestine (= L/O)
γ	= body length divided by tail length (= L/T)
T/ABW	= tail length divided by anal body width
BW/LiW	= body width divided by width of lip region
OW/LiW	= body width at base of oesophagus divided by width of lip region
E	= distance from head end to excretory pore as percentage of body length (= E/L × 100)
S	= stylet length in microns
G_1 and G_2	= length of anterior resp. posterior female gonad as percentage of body length (= $G_1/L \times 100$ and $G_2/L \times 100$)
V	= distance from head end to vulva as percentage of body length (V/L × 100)
Te	= length of male gonad as percentage of body length (= Te/L × 100)
Spi	= spicule length in microns
Gu	= gubernaculum length in microns

ters of special value for the description of *Tylenchorhynchus* species because this absence was due rather to great variation than to stability of the characters. The results as whole convey a warning against describing new *Tylenchorhynchus* species on the basis of the characters mentioned without determining the influence of temperature.

A short comment with respect to the individual characters follows.

L, body length, reached a greater length for both sexes at 25 °C than at lower temperatures. Females are longer than males. Temperature caused differences of about 9 % in the females and 4 % in the males, and it is remarkable that in both sexes the smallest specimens were formed at the intermediate temperatures.

It is also remarkable that the natural field population comprised females and males which are significantly larger than the reared specimens at any of the temperatures used.

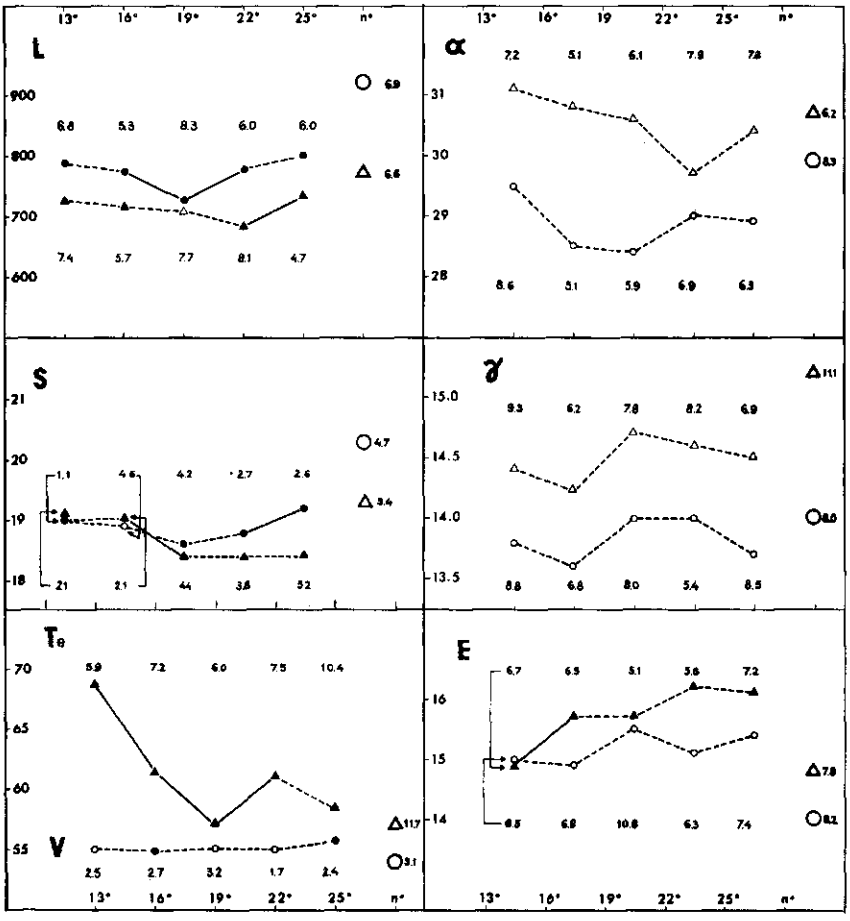


FIG. 12. Influence of rearing temperatures on six morphological characteristics of *T. dubius* females (o, o) and males (△, △). Filled points (●, ▲) are significantly different at 5% level from at least one other point in the temperature range, open points (o, △) indicate no significance. Solid line (—) between two neighbouring points means that the difference is statistically significant at 5% level, whereas broken line (- - -) indicates no significance. The larger dots and triangles (○, △) indicate the results for the population taken from the field which was not included in the temperature experiment; significance of differences of these data from the others are recorded in the text but are not indicated in the graphs. Abscissa: temperatures at which the nematode populations were reared, °C. Ordinate: *L* and *S* are expressed as microns. *V*, *T*, *E* as percentages, and α, *B*, δ and *BW*/*Liw* are ratios; cf. Figure 11 for explanation of the symbols and Table 3 for 25 specimens from which the average values indicated are determined. The figures indicate PEARSON's variation coefficient.

α, body length divided by greatest body width, was maximal at the lowest temperature for both sexes, 13 °C. The differences between populations were, however, not significant, similarly when the natural population was taken into account. Females are apparently not only longer, but also thicker than males.

TABLE 3. Morphometric characters of adult females and adult males of *T. dubius* populations cultivated at different temperatures on ryegrass for 15 months, or at naturally fluctuating temperature = t° on peas in the field; all figures are averages of 25 specimens measured. Cf. Fig. 11 for definition and illustration of the characters, and Fig. 12 for significance of the differences.

Sex and rearing temperature in C°	*L*	*α*	*β*	*γ*	*T/ABW*	*BW/LiW*	*OW/LiW*	*E*	*S*	g_2	g_2	*Vresp. Te*	*Spi*	*Gu*
(♀) 13°	787	29.5	5.8	13.8	2.9	3.2	3.0	15.0	19.0	24.6	23.2	55.1	–	–
(♀) 16°	775	28.5	5.4	13.6	3.0	3.4	2.9	14.9	18.9	25.3	26.1	54.8	–	–
(♀) 19°	727	28.4	5.3	14.0	2.9	3.0	2.9	15.5	18.6	23.5	23.9	55.1	–	–
(♀) 22°	778	29.0	5.5	14.0	2.9	3.1	3.0	15.1	18.8	23.6	23.8	54.9	–	–
(♀) 25°	800	28.9	5.6	13.7	2.9	3.3	3.1	15.4	19.2	24.3	25.0	55.7	–	–
(♀) t°	921	29.9	6.3	14.0	2.9	3.6	3.1	14.0	20.3	25.9	25.5	53.9	–	–
(♂) 13°	725	31.1	5.4	14.4	3.0	3.0	2.9	14.9	19.0	–	–	68.7	24.8	13.0
(♂) 16°	715	30.8	5.1	14.2	2.9	3.0	2.9	15.7	19.0	–	–	61.4	24.9	13.0
(♂) 19°	706	30.6	5.1	14.7	2.8	3.0	3.0	15.7	18.4	–	–	57.0	24.8	12.7
(♂) 22°	684	29.7	5.0	14.6	2.8	2.9	2.8	16.2	18.4	–	–	61.0	24.8	12.8
(♂) 25°	733	30.4	5.1	14.5	2.8	3.1	3.0	16.1	18.4	–	–	58.4	24.9	13.0
(♂) t°	774	30.7	6.0	15.2	3.0	3.2	3.0	14.8	19.3	–	–	57.0	24.8	13.3

β, body length divided by length of oesophageal region, was 8–9 % greater at 13 °C than at higher temperatures, for both sexes. β of both sexes in the natural population, however, was again significantly greater than in any of the experimental populations, as for *L.*

γ, was greater for males than for females, indicating the longer tail of the males, but there were no significant differences between the populations, including the natural population.

BW/LiW, maximum body width divided by width of lip region, was not influenced by temperature in males, but some significant, although irregular differences occurred in females. This is not surprising, because body width in females is dependent on the developmental stage of the gonads. The specimens from the natural populations again showed the highest values for both sexes.

OW/LiW, body width at the base of oesophagus divided by width of lip region, was not influenced by temperature in males; in females some slight and irregular differences were found; the explanation may be the same as in BW/LiW.

E, the position of the excretory pore was more forward on the body of both sexes at low than at high temperatures, and the differences in males were significant. In specimens of the natural population the position was far forward again for both sexes.

S, stylet length, was influenced somewhat by temperature in both sexes, with the lowest values at intermediate temperatures. Specimens of the natural field population showed again the highest values for both sexes.

V, the vulva position was hardly influenced, although some significant differences occurred.

Te, the relative testis length, was considerably greater at low temperatures than at 19 °C or higher. This is a remarkable result.

A significant influence of temperature is therefore measurable for most morphological characters of both sexes, but the influence is generally small and does not follow a regular pattern.

3.6. Discussion

The experiments, observations and cinematographic registrations have revealed the hitherto unknown course of the reproduction, development and growth of *T. dubius*. A number of general concepts about the life cycle of nematodes are confirmed and others are added or modified.

Males and females are both numerous in *T. dubius* and Figure 5 shows that the sex organs are normally developed, but typical for the genus and the species. Mating takes place: it was observed and registered for the first time in a member of the genus, and it is probably that the procedure is typical for many nematode species with both sexes slender and active throughout their lives. The reproduction is amphimictic, leading to a zygote. No special chromosome studies were made, but fusion of the nuclei of sperm and oocyte followed by cell division and extrusion of a polar body was observed. Usually the fertilised egg in the one-cel-

led stage is laid within a few hours, although further development may take place inside the uterus, and sometimes even aberrations may occur. Egg laying is an uncomplicate process, due to the flexibility of the egg (Fig. 7C). The eggs are deposited singly in a more or less regular pattern between the root hairs around the elongation zone of the roots, namely at a favourable site (Fig. 2). This is related to the fact that the eggs are laid by feeding females, which do not even stop feeding when the egg parts from the body. The number of eggs laid per female did not exceed 12 in our experiments. This number seems low for a small animal, but it is well in line with the great persistance and correspondingly high chance of survival on the one side, and with the slow reproduction rate of the nematode on the other side. Slow reproduction is a characteristic of most plant nematodes, and this may be due more often the low number of eggs per female than to excessive waste of young animals as in the case of *Heterodera, Meloidogyne* and some other sessile species, which are known to deposit packets of a large numbers of eggs.

When a one-celled egg is deposited it developes into an infective L_2, still inside the egg shell, in 8 days, at a temperature of about 20°C. Cleavage, embryonisation and the first molt inside the egg could clearly be seen and registered (Figures 6A-P). The continuous turbulent moving and streaming of the cell contents up to a seven- or eight-celled structure, reached after about 4 days, is fascinating to watch on time-lapse film. After 6 days the many-celled blastula, a more quiet stage, is formed and shrinks away from the cell wall. Further development into once more an again activily moving embryo, and then into a distinct larva with 2–3 body flexures, does not take more than a day. Again one day later again, therefore 8 days after a one-celled egg has been deposited, the first molt takes place (Figure 6O) and the now well-structured L_2 with a strong stylet is moving vigorously and starts to pierce the egg shell as an introduction to hatching, which normally follows after a few hours. The whole process, from fertilization to hatching is apparently continuous without noticeable rest periods in *T. dubius*.

Hatching is also an active process: the L_2 liberates itself by egg shell perforation and using body pressure. Root exudates of host plants do not influence hatching as in some other nematodes, which again indicates continuity of the development of this species.

The life cycle of *T. dubius*, from egg to egg, took about 40–48 days at 20°C and could be analysed due to experiments and morphometrics, in which the characters of molting specimens appeared to be most useful. The cycle comprised five stages apart from the egg, therefore four molts of which the first molt took place inside the egg. The fifth stage are mature adults. This is the usual pattern which was known from some other nematodes.

Taxonomic nematode studies are usually based on the morphology of adult specimens. As temperature has a significant influence, morphometric characters of new *Tylenchorhynchus* species should not be given without taking the temperature effect into account. DAO (1970) reached the same results with *Aphelenchus avenae* and *Ditylenchus dipsaci*, and it probably holds for other nematodes as well.

4. ECOLOGICAL RELATIONS

The studies comprise observations and experiments concerning movement, feeding, host plants, survival with and without hosts, influence of the physical environment, influence of the biotic environment, and a discussion.

ERRATA

Contents 2.4: . . . other organisms.
should be read as: . . . microorganisms.

2.4.3: Other organisms.
should be read as: Microorganisms.

Page 35: . . . and 5 (4.7 – 5.3) per hour . . .
should be read as: . . . and 5 (4.7 – 5.3) cm per hour . . .

Page 48: . . . (winter)temperures should be read as:
. . . (winter)temperatures

Page 90: under 3 and 4
should be read: under 4 and 5

Page 109: figures 33 should be read as: 39

Page 121: . . . densities and climatic figures for . . .
should be read as: . . . densities for . . .

dulated trails printed on moist agar surfaces by moving L_2 and by adult females and males are visible in Fig. 13, which prints are based on photographs.

The real undulating distances covered on moist cherry agar measured from such ichnograms, was 1(0.7–1.2), 4(3.8–4.2), and 5(4.7–5.3) per hour for L_2, female and male specimens respectively. In a thin sand layer the movements are principally the same, although distorted because the body has to follow the space between the heavy sand particles which are not shifted aside by the nematodes. The concept that nematodes in soil move through water films around soil pores without changing the position of soil particles is confirmed

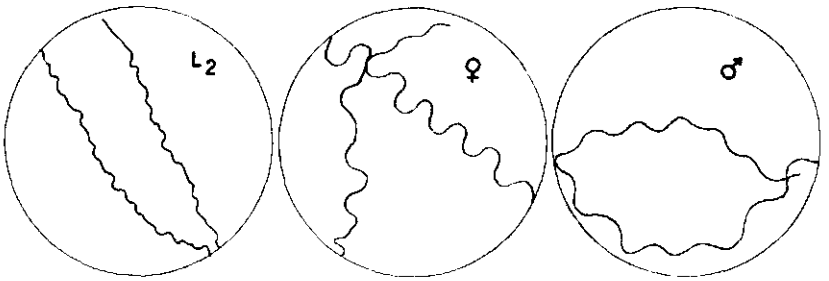


FIG. 13. Trails of *T. dubius* on moist agar surfaces, by second-stage larva (L_2), by adult female (♀) and by adult male (♂).

by these observations. A very thin water film is apparently sufficient to allow a nematode to move, because it collects an amount of water around its body and the water is following the creeping nematode.

It can be seen from Fig. 1, that *T. dubius* may reach great densities around and on root tips, where they apparently feed peacefully and undisturbed, although they are lying over and against each other, and despite the fact that they are constantly moving and touching each other. The movement of a nematode is not noticeably influenced by the presence of other specimens of the same species.

From the great density which they can reach in soil (several tens per ml, cf. 4.4.1a) and the distance which they travel ($\pm$ 1 cm per hour, cf. 4.1.2) it can be derived, that the tolerance between individuals of the species is considerable. In a densely populated soil each nematode of about 1 mm length has the space of no more than 25 mm^3 of soil, i.e. less than three times its body length in each direction, whereas they travel the diameter of this space in a few minutes. Each nematode must meet and touch other specimens of the populations many times per hour, and this does not apparently impede the activity or prevent a high density of the population.

When the density in a suspension in water is great, *T. dubius* sometimes forms unusual clusters of 15–30 constantly moving nematodes, in which adults and the larger larval stages may be included. This phenemenon, which was known from some other species, may be due to the uniform and constantly undulating movement of the specimens or to some other, unexplained mechanism (DONCASTER & WEBSTER. 1968).

b. Soil

One experiment was made to study the horizontal, and another to study the vertical movement of *T. dubius* in soil.

Horizontal movement

Rectangular trays of 10 × 1 × 1 cm were made from thin aluminium sheets and filled with a 0.2 mm thick layer of nematode-free, coarse sand which was known to be a suitable substrate for *T. dubius*. At one side of the tray a 1 cm long section was separated from the rest by 45 μ copper gauze, and herein a plant of rye grass in the two-leaf stage with well-developed roots was planted.

The gauze kept the roots away from the rest of the container, but root exudates and nematodes could easily pass. After 2 days 25 specimens of *T. dubius* (5 specimens of each stage: L2, L3, L4, males and females) were inoculated in the soil of each container in the 5th cm section away from the gauze. The soil was kept moderately moist and the containers were placed in Petri dishes with moist filter paper. There were in total 5 containers and 1 of them was evaluated 4, 8, 48, 72 and 144 hours after the inoculation. The movement of the nematodes was determined by cutting the containers in ten 1 cm sections. The contents of each sections was washed into a counting dish and the nematodes were counted and recorded. The results are summarized in Table 4. The numbers of L2, L3 and L4 are given as a sum, although they were noted separately.

It appears that nearly all nematodes inoculated could be recovered alive, also after 144 hours, and that all nematode stages could cover a distance of 5 cm in the soil within 144 hours, except the males of which none reached more than 3 cm. There were no consistant differences between the other stages in distance covered or in direction of movement. The few nematodes which had already travelled 4 cm after 4 hours were one female and one L_4, which suggests that the larger nematodes can move somewhat quicker.

The table shows that most nematodes were still at their site of inoculation after 4 and after 8 hours. Movement in this initial period was rather away from than towards the host plant. After 48 hours nearly all nematodes had left the site of inoculation, but again specimens of all stages moved at the same rate in both directions. It is clear that the distribution of the nematodes was not much influenced by the host plant. The nematode spreading seems to have occurred at random, with a slight concentration at both ends of the tray.

The experiment shows that all stages of *T. dubius* can travel horizontally at least 5 cm through the soil, except the males which are apparently less motile or less persistent in their motility.

Vertical movement

5 cm pieces of 3-cm-wide plastic pipes were joined together to make a column calibrated in 0–5, 5–10, 10–15, 15–20 and 20–25 cm. In the laboratory 18 columns, filled with steam-sterilized sandy soil, were placed upright in Petri dishes with water, at 22 °C and 12 hours of artificial light per day. In 9 tubes a germinated pea seed was sown. All plants grew well and two weeks after sowing the roots grew down to the bottom of the column. Then 150 *T. dubius*, a natural mixture of larvae, males and females in 1 ml water were pipetted at 2.5 cm depth in each of the sown and unsown tubes.

5 ml of water was added on top of each tube to avoid quick drying of the top layer. Three replicate tubes with peas and three without peas were evaluated 10, 20 and 30 days after the inoculation. The soil columns were cut into 5 cm sections and all nematodes were extracted from each section separately. The results are summarized in Fig. 14.

No significant differences were noticed between the planted and the unplanted soil. The observation period of 30 days was apparently short enough to avoid

TABLE 4. Horizontal movement in soil of males (♂), females (♀) and larvae (L, i.e. $L_2 + L_3 + L_4$) of *T. dubius* at different moments after the inoculation of 5 specimens of each stage at a distance of 5 cm from a host plant, which was ryegrass.

Movement towards host plant in cm \ Hours after inoculation	4			8			48			72			144		
	(♂)	(♀)	(L)	(♂)	(♀)	(L)	(♂)	(♀)	(L)	(♂)	(♀)	(L)	(♂)	(♀)	(L)
+5 (= plant site)	0	0	0	0	0	0	0	1	2	0	1	3	0	1	4
+4	0	0	0	0	0	0	0	0	0	0	0	3	0	0	2
+3	0	0	0	0	0	0	2	0	0	2	2	0	2	2	1
+2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
+1	0	1	0	0	0	2	0	0	5	2	0	1	2	0	0
0	4	2	10	2	1	10	1	0	1	0	0	1	0	0	1
−1	1	0	2	1	1	0	0	1	0	0	1	0	0	1	0
−2	0	1	1	1	3	1	1	1	3	0	0	2	0	0	0
−3	0	0	0	1	0	1	1	1	2	1	1	3	1	1	2
−4	0	1	1	0	0	0	0	1	1	0	0	2	0	0	3
Total nematodes	5	5	14	5	5	14	5	5	14	5	5	15	5	5	13

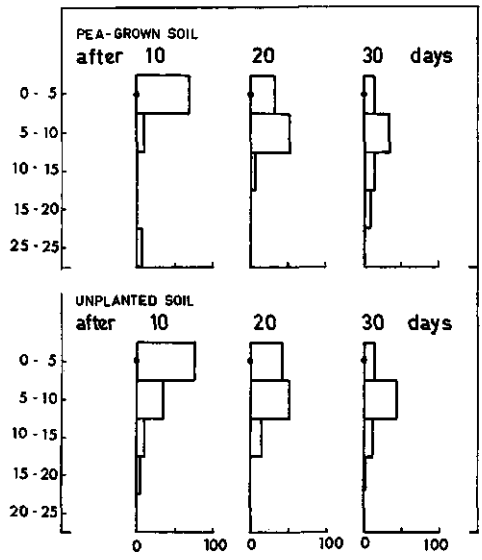


FIG. 14. Vertical movement of *T. dubius* in 25 cm high columns of sandy soil in 3 cm wide tubes which were placed upright in petri dishes with water. Inoculation of a natural mixture of 150 nematodes at 2.5 cm depth, as indicated by a dot in each diagram. Tubes were kept unplanted, or grown with one pea plant per tube; evaluations were made after 10, 20 and 30 days, as indicated in the graphs. Abscissa for each individual diagram: number of nematodes extracted from the sections of each column; average of 3 replicates. Ordinate for each individual diagram: depth of column sections in cm.

the complicating effect of a new nematode generation, and the pea plants did not influence the distribution pattern of the nematodes specifically.

In both soils the larger part of the population was still in the upper 5 cm after 10 days, viz. 82% in the planted and 60% in the unplanted soil. At this moment 7% and 1% respectively had reached a depth of 20–25 cm, which means that they must have moved actively downwards for more than 17.5 cm in 10 days. These nematodes were of different stages viz. L_2, L_3, L_4 and adult males and females.

After 20 days both the planted and unplanted soil harboured most of the nematodes in the 5–10 cm layer (56% and 46% respectively). Appreciable numbers were still present in the top 5 cm (36% and 38% respectively) and low numbers occurred in layers deeper than 10 cm (about 7% and 13% respectively).

This situation was essentially the same after 30 days, except that the numbers in the top 5 cm decreased appreciably in both the planted and unplanted soil. This may be due to lethal influences of drought in this layer.

The conclusion is that most of the nematodes moved only about 5 cm down into the 5–10 cm deep layer, although some of the nematodes, especially L_3, L_4 and males, had moved down for more than 17.5 cm within 10 days. This was not noticeably influenced by the presence of the pea plant, which is known to be a good host.

4.1.2. *Feeding*

T. dubius is a polyphagous ectoparasite of roots of higher plants. The permanent motility, the presence of fairly strong mouth stylets and the presence of much stored food in the intestine, as indicated before (Chapter 1), suggest that all stages from L_2 to adults are active feeders. None of the stages of *T. dubius* has been observed feeding on other organs of higher plants than roots, nor on lower plants or other organisms or dead substrates.

The feeding procedure was studied by placing surface-sterilized nematodes close to 2–5 cm long roots of rye-grass growing in Petri dishes with a thin layer of sterile water agar. The dishes were kept in plastic bags at 20 °C under artificial light and the nematodes could be examined, photographed and filmed easily under the dissecting or the compound microscope. Their occurrence in an agar medium and the microscope light apparently did not prevent them to start or continue feeding.

Figures 1 and 15 indicate the general position and body attitude of feeding *T. dubius*. All active stages including the males, are indeed feeding and no difference in behaviour was noticed. Most nematodes feed in the root elongation zone just behind the root tip, where several nematodes may be found together apparently without disturbing each other. They may also be feeding on the root tip further back along the root between the root hairs, but none was observed feeding on a root hair itself. The nematode in search of a feeding site

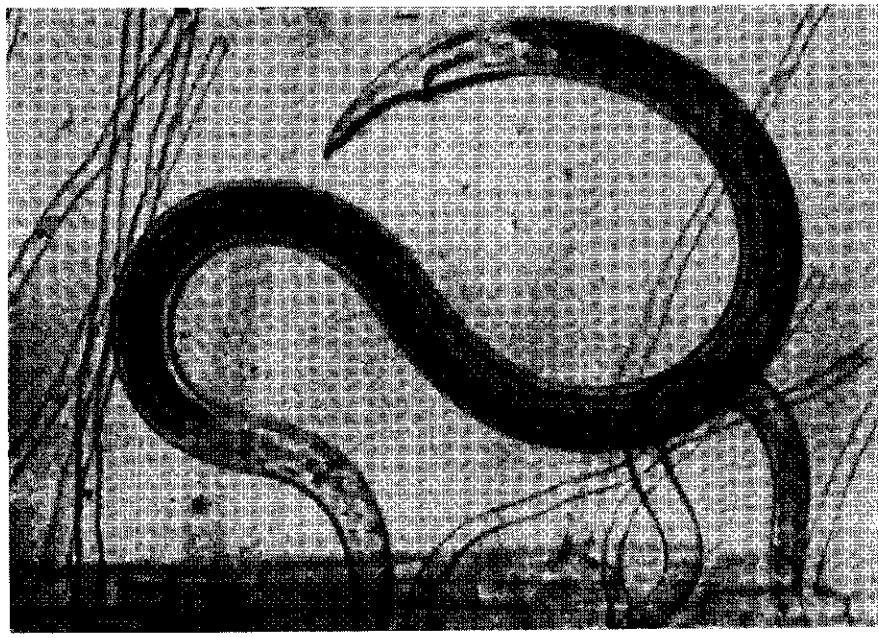


FIG. 15. A male and a second-stage larva of *T. dubius* feeding on epidermal cells of ryegrass root growing in agar medium. Cf. also Fig. 1.

glides over the root surface with the head and stylet approximately at right angles to it when exploring the root epidermis. As the nematode moves to and fro tentative jabs are made by the stylet at various epidermal cells. After finding a suitable feeding site a series of rapid jabs, about 20–25 per minute, is often made with the stylet until the epidermal cell is perforated or punctured. The stylet is never inserted further than its tip. Sometimes the nematode fixes its mouth against an epidermal cell for 15–20 minutes without movement of the sylet. During feeding the nematode body generally lies in one or two loose curves close to the root, with the anterior somewhat raised above the root to place the lip region and stylet towards the epidermal cell. The general body attitude is changed regularly by quiet movements. Lips are pressed against the epidermal cell, but the head does not penetrate the root. *T. dubius* specimens were never found inside the root. The pulsation of the median esophageal bulb may reach a very large frequency when the stylet is used for cell penetration or actual feeding. Feeding sometimes continued for about half an hour at the same spot, but it may be much shorter. *T. dubius* is apparently a feeder on superficial cortical cells only. Cells used for feeding generally show a yellow-brown discolouration visible only with the aid of the compound microscope. Similar light to dark browning was observed when *T. dubius* was cultured under sterile conditions on *Phaseolus radiatus*, *Brassica campestris* and on pea.

4.2. Survival without hosts

The only literature on the survival of *T. dubius* without hosts concerned two natural populations in soil (Oostenbrink 1966). A population in sandy soil following pea, appeared to maintain its density at a high level throughout the autumn and winter. Another population in a sandy pea plot which was kept fallow for several years dropped to about 45% after one year and to about 5% after two years of fallow. In my field experiments *T. dubius* survived from July to the following spring, despite a cold spell from December to February during which the temperature in the soil went down to +2°C. It also showed a high degree of resistance to adverse conditions in my laboratory experiments.

Special experiments were conducted to study the survival of *T. dubius* without host plant under different environmental conditions.

4.2.1. *Starvation*

T. dubius, as an obligatory feeder on higher plants, is exposed to starvation in fallow soil. Experiments were made to study the fate of a natural population inoculated in soil with and without host (a), and of single nematode stages in soil without host (b).

a. Population in soil with and without host

60 plastic pots were filled with 200 g sterilized PD sandy soil and each inoculated with 100 *T. dubius* randomly selected from the stock culture on ryegrass and

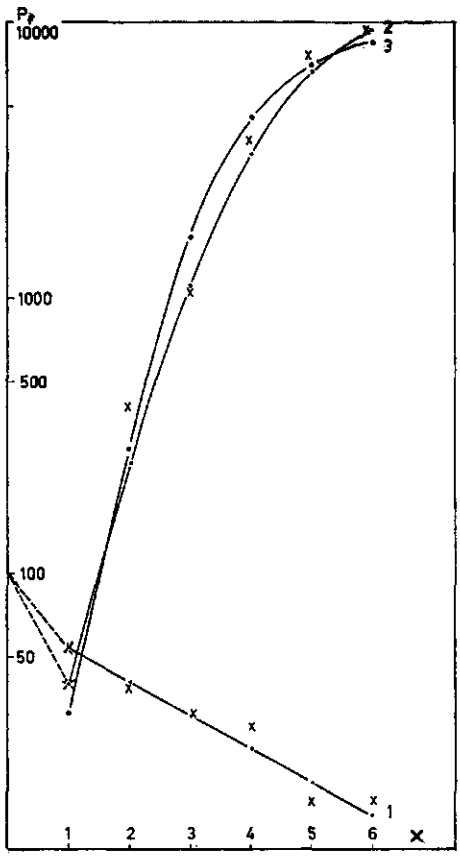


FIG. 16. Starvation of *T. dubius* with time in moist fallow soil, compared to soil grown with ryegrass, when a mixed population of 100 nematodes was inoculated into pots with 200 g sterilized sandy PD soil. Observed nematode densities indicated by x are averages of 5 pots. The values indicated by dots are calculated according to three formulae recorded on the corresponding graphs, viz. $\log P = 1.853 - 0.121\ X$ (1), $\log P = 0.625 + 1.059\ X - 0.084\ X^2$ (2) and

$$\log P = \frac{3.986}{1 + e^{1.474 - 0.967\ x}}$$ in which e is the base of natural logarithms (3). Abscissa: time in months (= X) after which nematode densities were determined. Ordinate: final nematode densities per pot (P_f) on a logarithmic scale.

transferred by means of a micropipette. 30 pots were kept fallow and 30 were sown with ryegrass, leaving 8 seedlings per pot. All pots were placed in random order on the bench of a glasshouse with a temperature fluctuating from 10° to 20°C. Sufficient water was provided to keep the soil moist; the planted pots were also provided with nutrient solution to maintain good growth of the ryegrass. Nematode numbers were determined in 5 pots of each of the two series after 1, 2, 3, 4, 5 and 6 months. The results are described below and summarized in Fig. 16.

After 1 month 53 and 39 of the 100 inoculated nematodes were recovered on an average from the fallow and grass-grown pots respectively. The difference was not significant. The figures, however, may include loss of nematodes due to the inoculation and extraction procedures, in addition to decline of the populations with time. From this moment on great differences between the two series were found.

The population density in the fallow soil pots decreased further to 28 in the next 3 months or to 15 in the next 5 months, and males, females as well as larvae

were involved in this decrease to about the same extent. The decrease of the total population in the period between 1 and 6 months can well be represented by a rectilinear regression of the logarithmically transformed nematode density on time as indicated in the legenda of Fig. 16. The regression formula indicates that the population lost about 6 % of its log. density each month.

In the pots with ryegrass the population density rose steeply up to 9688 nematodes per pot, i.e. 48 nematodes per g of soil, in the period between 1 and 6 months, and then it had probably even not reached its saturation density yet. Females were more numerous than males throughout the experiment and larvae were far more numerous than adults from the 2nd month on. The population increase with time can be represented by the well-known logistic curve which is exponential (cf. OOSTENBRINK 1966) or by a second-degree curve calculated to fit the experimentals results as closely as possible; the formulae and corresponding graphs of both curves are indicated in Fig. 16.

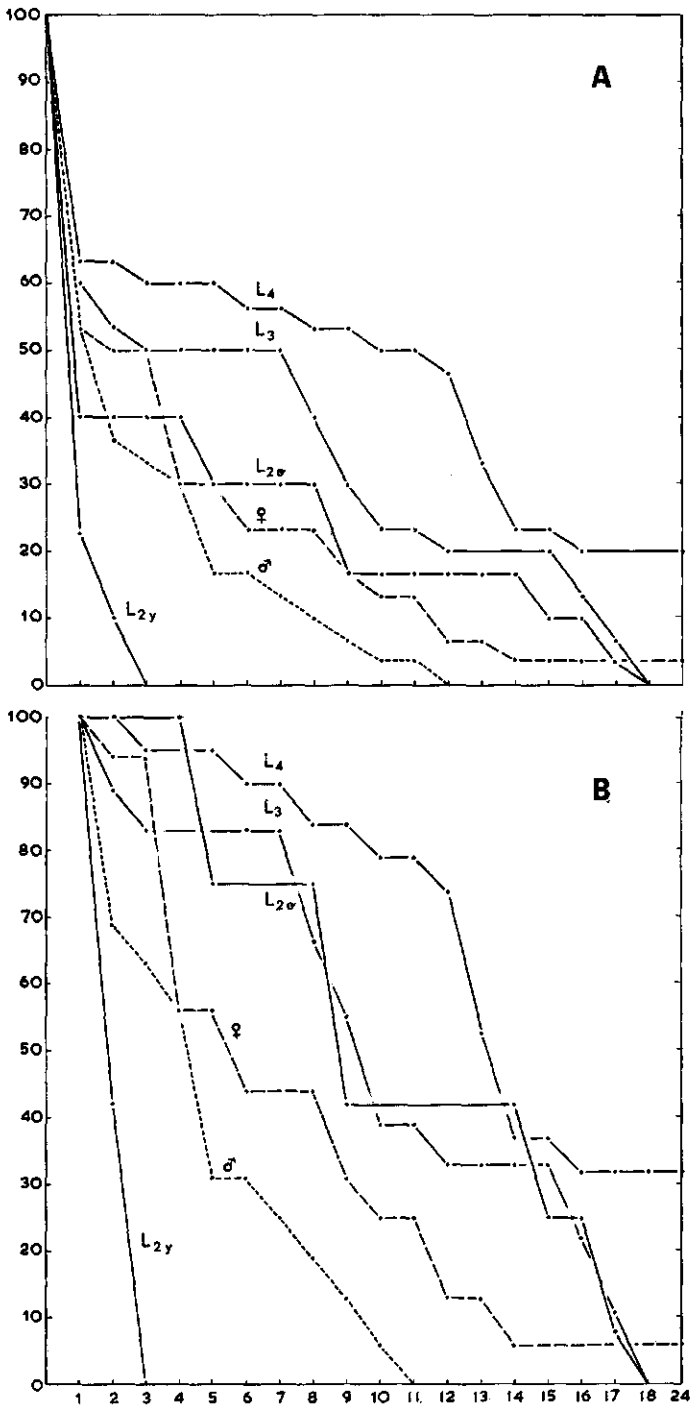
The result indicates that the host plant is necessary for reproduction of *T. dubius* and that there is a gradual decline of the population without host plant. This is probably due to starvation. It was noticed, however, that the food reserve in the intestinal cells of specimens recovered after 6 months starvation had apparently not yet been exhausted and that the population still comprised males, females and larvae.

b. Single stages in soil without host

342 small glass bottles were each filled with 10 g of nematode-free sandy soil. Ten specimens of a certain stage were inoculated per bottle. There were six nematode stages, viz females (♀), males (♂), second-stage larvae immediately after hatching which were called second stage 'young' (L_{2y}), older second-stage larvae (L_{2o}), third-stage larvae (L_3) and fourth-stage larvae (L_4). For each stage 57 bottles were inoculated. The 10 nematodes for each of the bottles were collected in a drop of water by picking them by hand from the monospecific stock population cultured in ryegrass. Then the drop of water with the nematodes was deposited with a micropipette into a central hole up to half the depth of the soil in the bottle. The holes were covered with the surrounding soil. 1 ml of water was added to bring the soil in a moist condition and a thin cover of coarse sand was added to each bottle. The soil in the bottle was kept moderately moist throughout the experimental period by adding distilled water when necessary. All bottles were kept in a dark cupboard in the laboratory, in which the air temperature was about 22 °C in winter and varied from 20° to 30 °C in summer.

The inoculation was performed on 13 May 1966. From then on each month the contents of 3 bottles of each series were extracted by means of the decantation-cottonwool filter method. This was done for 18 months in succession, followed by a final count after 24 months. The results are summarized in Figures 17A and B.

It appears from Fig. 17A that a marked decline of the populations occurred after the first months, followed by a more gradual decline, with interesting


A
B
L4
L3
L2σ
♀
♂
L2y
100
90
80
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 24

significant differences between the various stages. The percentages recovered after one month in decreasing order were 63% for L_4, 60% for L_3, 53% for both the adult stages, ♀ and ♂, 40% for L_{2o} and 23% for L_{2y}. However, it is possible that the decline in the first month was partly due to an artefact, namely the fact that a certain percentage of the inoculated nematodes is lost in the extraction process or because they suffered from the inoculation procedure. This loss may be highest for the smallest stages. Extrapolation of the graphs suggests that this loss could have been 30–40% for the larger stages (adults, L_4 and L_3) and 50–70% for the smaller stages (L_{2y} and L_{2o}). Anyway this possibility cannot be excluded. This possible artefact is excluded in the comparison of the results of the successive extractions in Fig. 17B. Fig. 17B shows, that the L_{2y} is least persistent: live specimens were recovered after 2 months, but none any after 3 months. The population apparently declined rapidly from the beginning on. L_{2o} was about equally persistent as L_3 and L_4: a large percentage of these three stages were still alive after 14 months. From the 14th or 15th month on L_{2o} and L_3 declined more rapidly and they were apparently dead or at any rate inactive after 18 months. L_4, the pre-adult larval stage, appeared to be more persistent: there was hardly any further decline from th 14th to the 24th month. After 24 months 20% of the original inoculum, 41% of the nematodes found after 1 month, were still alive and active.

The ♀ density dropped somewhat quicker than the L_4, but also here no further decline occurred between the 14th and the 24th month, and 5% of the inoculated number, 10% of the number extracted after 1 month, were alive after 24 months. It is not impossible that these were females which had just molted from the L_4 stage or perhaps had not yet molted at all, and therefore were L_4. The males are more persistent than L_{2y}, but less than the other stages. Their population was fully extinct after 12 months.

4.2.2. *Temperature stress*

a. Monospecific culture at different temperatures in soil

50 gram portions of a well-mixed sandy polder soil with a monospecific culture of *T. dubius*, freed from roots and debris, were filled in 39 thin polythene bags of 0.25 mm thickness. The bags were closed by making twisted knots at both sides. Three bags were processed immediately after filling, on 5th May, 1969 to determine the initial nematode density. The other bags were placed at constant temperatures of −11 °C, 5 °C and 30 °C, 12 bags per temperature. 3 replicate bags for each treatment were evaluated after 2, 7, 14 and 42 days. The data are summarized in Table 5 and the statistical significance of nematode

FIG. 17. Percentage survival in vials with moist sandy soil without host plant, of different stages of *T. dubius*, viz. females (♀), males (♂), young second-stage larvae (L_{2y}), old second-stage larvae (L_{2o}), third stage larvae (L_3) and fourth-stage larvae (L_4). These were 10 specimens per vial, 3 replicate vials for each separate stage, and for each extraction date. Abscissa: extraction dates as months after inoculation (which took place on 13.5.1966). Ordinate: percentage nematodes extracted, with inoculated number = 100% (A), and with the number present at the first evaluation date after 1 month = 100% (B).

TABLE 5. Survival and development of a monospecific culture of *T. dubius* in moist soil kept at −11 °C, 5 °C and 30 °C for various lengths of time without host.
Figures are averages of 3 replicate bags with 50 g of soil each for second-stage larvae (L_2), third-stage larvae (L_3), fourth-stage larvae (L_4), males (♂), females (♀) and total populations. The maximum figure of each column is printed bold.
The decrease of nematode densities with time is checked by Kendall's test and its significance is indicated as follows: − = non-significant, + = significant at 90%, ++ = at 95%; ± means an increase which is significant at 90%. Cf. also figure 18.

Temperature in °C	Time of	L_2	L_3	L_4	♂	♀	Total (%)
− 11 °C	0	*103*	*1426*	*596*	*140*	*146*	2411 (100)
	2	3	480	166	46	23	718 (30)
	7	0	283	140	26	10	459 (19)
	14	0	113	55	5	5	178 (7)
	42	0	5	4	0	1	10 (0.4)
		++	++	++	++	++	++
+ 5 °C	0	103	*1426*	*596*	140	146	2411 (100)
	21	96	1173	513	176	143	2101 (87)
	42	123	1313	376	256	163	2231 (93)
	63	*138*	1056	573	*270*	223	2260 (94)
	84	90	1100	383	203	*253*	2029 (84)
		−	−	−	±	±	−
+ 30 °C	0	*103*	*1426*	*596*	*140*	*146*	2411 (100)
	21	38	838	505	108	120	1609 (67)
	42	76	1090	201	138	101	1606 (67)
	63	6	716	413	86	10	1231 (51)
	84	10	730	290	123	83	1236 (51)
		+	+	+	−	+	+

densities with time is indicated. It appears that decreases occurred at − 11 °C and at 30 °C and slight increases at 5 °C.

At − 11 °C all stages, and therefore the total population, declined to barely measurable densities. For L_2 this was already so after 2 days. All the other stages had also lost a large part of their populations after 2 days, but a decrease of their densities to very low levels occurred between 7 and 14 days for the adults ♂ and ♀, and between 14 and 42 days for the L_3 and L_4. Long spells of − 11 °C are therefore fatal for the whole population, with L_2 as the most susceptible and L_3 and L_4 as he most resistant stages. The few surviving specimens of L_3, L_4 and the one surviving female after 42 days had abnormal, large vacuoles in their intestine and were obviously in a poor condition. This indicates that the nematodes lost must have been dead and not temporarily paralyzed by the treatment. Because there was only decrease in numbers, and no increase at all, at − 11 °C the decrease of each nematode stage with time is calculated and drawn as rectilinear regressions for this temperature; correlation coefficients between nematode densities and exposure time are also indicated in Fig. 18. All correlations and regression coefficients are negative and highly significant and the graphs confirm the weak persistence of L_2 and the great persistence of L_3, L_4, with the adults intermediate.

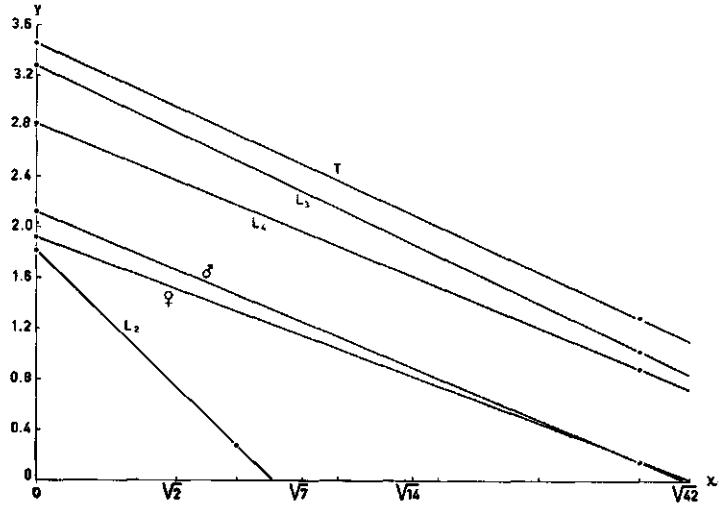


FIG. 18. Regression lines in time for the different nematode stages, viz. second-stage larvae (L_2), females (♀), males (♂), fourth-stage larvae (L_4), third-stage larvae (L_3) and all nematode stages together (T), when they were kept at –11 °C for 2, 7, 14 and 42 days (cf. Table 5).
Y = logarithm of number of nematodes plus one.
X = square root of exposure time in days.
r = correlation coefficient.
The formulae of the regression lines, together with between brackets the correlation coefficients, which are statistically very significant, are as follows for the different stage categories:
Total: $Y = -0.36\,X + 3.46$ ($r = -0.98$)
L_3: $Y = -0.37\,X + 3.28$ ($r = -0.97$)
L_4: $Y = -0.32\,X + 2.82$ ($r = -0.97$)
♂: $Y = -0.33\,X + 2.12$ ($r = -0.96$)
♀: $Y = -0.29\,X + 1.92$ ($r = -0.92$)
L_2: $Y = -0.76\,X + 1.82$ ($r = -0.90$)

None of the stages declined significantly in 84 days when the population was kept at 5 °C; there were on the contrary significant increases for the adults.

This temperature seems to conserve the population as a whole; the final density of the total population was 84 %. Scrutiny of the results reveals a special difficulty for the interpretation which is due to the use of a natural population in soil with all stages mixed. It appears that after 21 days some L_4 larvae were molting whereas all specimens found after 42 days were molting. So even at 5 °C some development takes place. It is therefore possible that hatching of the eggs (which could not be counted) has caused the slight increase of L_2 after 42 and 63 days, and that further development of L_2, L_3 and L_4 has caused some increase of L_3, L_4 and adults respectively in the course of the experiment. The numbers of L_2 are relatively low and it is improbable that the development has caused a great shift in the figures. Nevertheless the phenomenon may explain why the adults increased slightly, but significantly during the experiment. This result indicates, that the temperatures chosen for storage of soil samples by OOSTEN-

BRINK (1960a) should rather be somewhat lower than 4°–5°C, as indicated by him.

At 30°C the density of all stages declined gradually during the exposure period of 84 days, except L_2 which had already declined noticeably after 21 days and of which less than 10% was found active and alive after 63 days. The density of the total population dropped to 51%. The temperature of 30°C was apparently too high for *T. dubius*. The rapidly decreasing L_2 density indicates, that hatching of eggs has not been important at this temperature. Further development and molting of the L_3 and L_4 may have taken place, and this may explain the relatively high numbers of males throughout the experiment. The unfavourable high temperature may have increased the male/female ratio, as was shown to occur in other nematodes by DAO (1970). The results obtained at 30°C agree well generally with the results obtained at –11°C.

b. Polyspecific mixture in soil at alternating temperatures

The effect of high (summer) and of low (winter) temperaures, and of sudden temperature changes, was studied with respect to *T. dubius* and associated nematodes in the soil taken from the monoculture pea plot used in several of our experiments, as indicated under 2.2.1. The sandy soil was taken from the field on 20 January 1969 while it was freezing weather. It was brought into the laboratory, freed from root-remains and debris, thoroughly mixed and put into polythene bags in 50 g portions, as described under b. Four bags were processed immediately after filling, to determine the initial density of *T. dubius* and of the other predominant species in this soil.

Half of the other bags were placed at 22°C and half at –6°C; four bags of each series were evaluated after 1, 2, 3 and 4 weeks. After 4 weeks the bags were interchanged, i.e. the bags of –6°C were transferred to 22°C and the bags of 22°C were transferred to –6°C (with some of the bags left at 22°C). 1 and 2 weeks later, i.e. 5 and 6 weeks after the onset of the experiment, further evaluations were made. In all cases the nematode communities were fully analyzed, for *T. dubius* even up to the different developmental stages. The results are summarized in Fig. 19; for *T. dubius* they are recorded in greater detail in Table 6.

The soil was brought from the freezing temperature outdoors into the laboratory. In some hours it obtained the laboratory temperature of 22°C during mixing and filling the bags. Then the bags were placed at their experimental temperatures. The temperature treatments caused similar differences in population densities of *T. dubius* and the other species or groups studied (cf. Fig. 19).

After one week at 22°C the densities of all populations rose somewhat, which may be due to the hatching of eggs existing in the soil. In the following 5 weeks they gradually declined to a level which was finally 74% 55%, 89% and 74% of the initial density of *T. dubius* (T), *Rotylenchus robustus* (R), other possible plant parasitic species mainly *Meloidogyne hapla*, *Tylenchus davainei*, *Aphelenchus avenae* and *Trichodorus Pachydermus* (*O*) and saprozoic nematodes (*S*), respectively. When the populations were placed at –6°C for the last two weeks the densities dropped suddenly further, namely to 61%, 34%, 28%, 51% respectively.

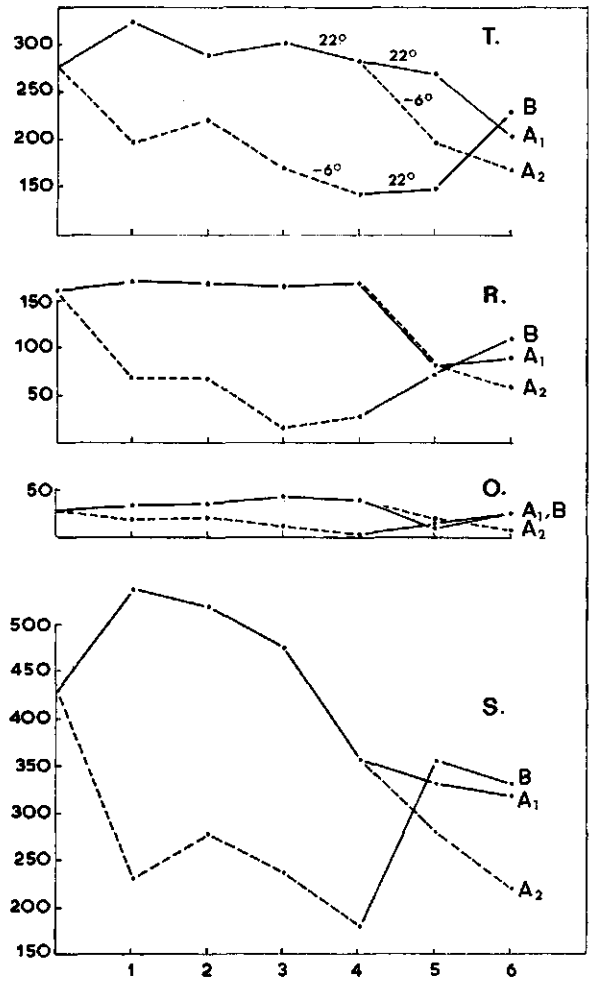


FIG. 19. Survival of *T. dubius* (T), *Rotylenchus robustus* (R), other plants parasitic nematodes (O) and saprozoic nematodes (S) as a polyspecific community in their original soil, kept at 22°C for 6 weeks (Graph A_1), at 22°C for 4 weeks + at – 6°C for 2 weeks (Graph A_2) and at – 6°C for 4 weeks + at 22°C for 2 weeks (Graph B), as indicated in the graphs. Figures are averages per 50 g of soil. Experiment started on 20.1.1969. Abscissa: exposure time in weeks. Ordinate: nematode numbers per 50 g of soil.

At –6°C all the populations declined markedly within 1 week, which decline continued gradually until after 4 weeks they were transferred to 22°C. Then all populations expanded within the next 2 weeks, and their final densities at the end of the experiment were as high, or even somewhat higher, than of the populations continuously kept at 22°C. They were 83%, 67%, 100% and 77% of the initial densities, respectively.

It is therefore, clear that the low temperature of –6°C does initially kill some

TABLE 6. Survival of *T. dubius* in moist soil with a polyspecific natural nematode community, when given different treatments with alternating temperatures, A and B.
Figures are averages per 50 g of soil for males (♂), females (♀), second-stage larvae (L_2), third-stage larvae (L_3), fourth-stage larvae (L_4) and total populations.
Starting date: 20 January 1969.

Exposure time in weeks	A. 22 °C	Between brackets: 22 °C for 4 weeks, followed by −6 °C for 2 weeks				
	L_2	L_3	L_4	(♂)	(♀)	Total
0	78	98	43	25	33	277
1	48	165	18	40	53	324
2	41	130	28	35	55	289
3	68	133	28	45	28	302
4	60	145	33	18	28	284
5	40 (13)	170 (113)	10 (20)	40 (30)	10 (20)	270 (196)
6	40 (15)	120 (108)	15 (20)	5 (8)	25 (18)	205 (169)

Exposure time in weeks	B. −6 °C	Between brackets: −6 °C for 4 weeks, followed by 22 °C for 2 weeks				
	L_2	L_3	L_4	(♂)	(♀)	Total
0	78	98	43	25	33	277
1	20	115	13	15	33	196
2	25	120	25	23	28	221
3	16	93	15	25	20	169
4	23	78	28	0	13	142
5	? (20)	? (87)	? (16)	? (13)	? (13)	? (149)
6	? (53)	? (105)	? (17)	? (30)	? (25)	? (230)

of the nematodes, but nevertheless conserves the populations to such an extent, that after two weeks of favourable temperature the populations are higher than those kept permanently, in this case for 6 weeks, at the favourable temperature of 22 °C. The populations in soil can apparently over winter in the Dutch climate without damage. For the soil temperature seldom reaches such a low for such a long period. This holds for populations in soil and not for populations in water. When different stages of *T. dubius* were kept in tap water at −6 °C for an hour, they did not revive at all.

Table 6 allows a more detailed analysis with respect to the population fluctuations of *T. dubius*, because nematode stages are recorded separately (apart from eggs which are lost in the extraction procedure). The initial population increase at 22 °C is apparently due to maturation of the population (shift from L_4 to adults and from L_2 to L_3 after 1 week, whereas the L_2 density must have been complemented by new larvae hatched from eggs); the gradual decline at 22 °C, which is most obvious in the 5th and the 6th week, is mainly due to the decline of the L_2 and ♂ densities; the stronger decline during the last two weeks when the population was transferred to −6 °C accentuated the lower persistence of the L_2 and the ♂. The population placed immediately at −6 °C had lost most of its

L_2 and all its ♂ after 4 weeks. Transfer to 22 °C increased the densities of all stages in 2 weeks, however especially those of L_2, L_3 and ♂.

The details of Table 6 confirm the conclusions that L_2 and ♂ of *T. dubius* are more susceptible to adverse conditions than the other stages, and that L_3, L_4 and ♀ densities, and probably the eggs, are preserved by low temperatures in such numbers that they can restore the population to its original density and composition in two weeks as soon as the temperature becomes favourable.

The similarity of the graphs for different nematodes suggests that their reactions to the temperatures and temperature changes are similar, although there may be differences with respect to the stages in their respective populations.

4.2.3. *Moisture stress*

a. Drought

Soil infested with a monospecific culture of *T. dubius* on ryegrass was freed from roots, mixed, and put into 21 small plastic bags in 50 g portions on 15 April 1970. The soil was placed in shallow layers and the bags were left open at 20–26 °C in the laboratory room without adding water. The soil from three randomly chosen bags was elutriated after 0, 1, 2, 3, 4, 5 and 6 weeks to determine the density and composition of the *T. dubius* populations. The results are summarized in Fig. 20 and described below.

The weight reduction of the soil was recorded each week to determine the loss of water. After one week the weight remained almost constant at 47 g, obviously because the soil was air-dry. At that moment the nematode population was reduced on an average from about 135 to 9.3 per unit of soil, whereas further reduction to 5.6, 3.6, 1.3, 0.3 and 0.0 had occurred after 2, 3, 4, 5 and 6 weeks respectively. The graphs in Fig. 20 indicate the percentage reduction of

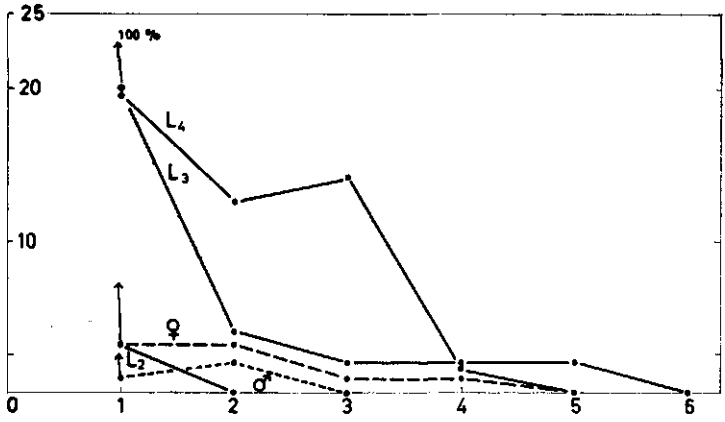


FIG. 20. Influence of soil drying on the survival of different stages of *T. dubius* of a natural population in soil, viz. males (♂), females (♀), second-stage larvae (L_2), third-stage larvae (L_3) and fourth-stage larvae (L_4), when samples of 50 g of soil were dried as thin layers at 20–26 °C in a laboratory room. Abscissa: drying period in weeks. Ordinate: percentage survivors.

the different nematode stages. L_2 is apparently very susceptible; it was reduced to 3% after one week and to zero after two weeks. The adults were reduced to about the same extent after one week, but the zero level was reached for males after 3 and for females after 5 weeks. The L_3 and L_4 stages were more resistant than the others. 20% of each stage was alive after one week, and full extinction was not reached for L_3 until after 5 weeks and for L_4 until after 6 weeks. The whole active population of *T. dubius* thus appears to be susceptible to drought, but it is also clear that part of the population, especially consisting of L_3 and L_4, can survive air-dry conditions for 4 to 5 weeks.

b. Excess of moisture

Another lot of soil with a monospecific culture of *T. dubius* as indicated under a, was filled in 50 g samples, into 21 glass bottles, after which 10 ml of tap water was added per bottle to flood the soil. Each bottle was closed in a plastic bag to prevent evaporation and the soil remained soaking wet throughout the experiment. All bottles were kept in a laboratory room at 20–26°C. The soil from three randomly chosen bottles was elutriated after 0, 1, 2, 3, 4, 5 and 6 weeks to determine the nematode populations. The results are described below and illustrated in Fig. 21.

The total nematode population of an average 188 specimens per botlle was reduced to 23.0 after one week, and to 15.6, 11.8, 10.0, 10.3 and 8.6 after 2, 3, 4, 5 and 6 weeks respectively. Fig. 21 indicates, that L_2 was again the most susceptible stage: after one week only 3.6% survived and this reduced to zero after

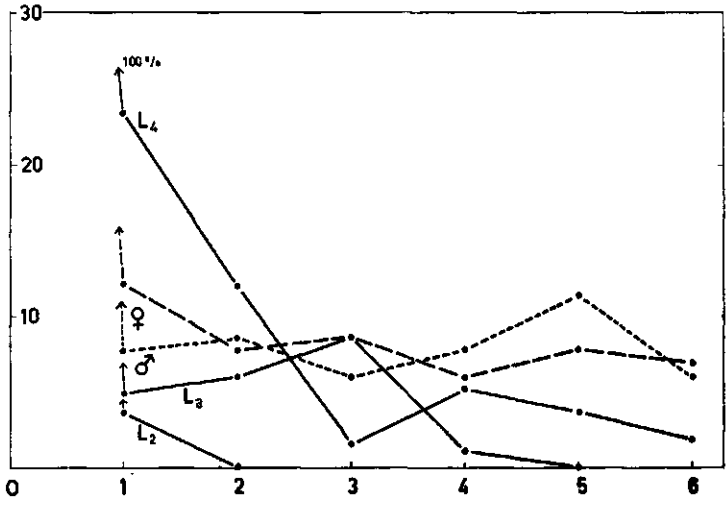


FIG. 21. Influence of flooding on the survival of different stages of *T. dubius* of a natural population in soil, viz. males (♂), females (♀), second-stage larvae (L_2), third-stage larvae (L_3) and fourth-stage larvae (L_4), when samples of 50 g of soil in glass bottles were flooded and placed at 20–26°C in a laboratory room. Abscissa: flooding period in weeks. Ordinate: percentage survivors.

two weeks. L_3 was reduced to about 5% after one week, and became extinct after 5 weeks. Males and females were both reduced to about 10% after one week, but this percentage survivors was, surprisingly enough, still present after 6 weeks. L_4 was again the least susceptible stage, with about 23% survivors after 1 week. After 6 weeks the percentage of survivors, however, was lower than that of the adults, viz. 2. Flooding was therefore effective in reducing the populaton, but there were surviving adults and L_4 even after 6 weeks.

4.3. Influence of the physical environment on development

The influence of the environment on a developing nematode population is very complex, as was illustrated by Dao in a detailed scheme (1970).

The fact that we study an obligatory plant parasite limits our possibilities to control experimental conditions, the more so for studies in the complex biotope soil. A living host plant or living host tissue is always involved in the experiments, and there are mutual relations between the host and the nematode. All abiotic and biotic soil factors which influence the nematode directly may also influence the host or be influenced by it; this holds for temperature, moisture, soil structure, chemistry of the soil solution and soil air, as well as other organisms. In our study on the influences of certain environmental factors, we kept other factors as constant as possible. Differences in plant weight or root weight which nevertheless occur, are usually taken into account by expressing nematode densities not only per container but also per unit of plant weight and root weight.

The main single abiotic factors are no doubt soil temperatures, soil moisture and soil type. The chemistry of the soil solution and soil air may also play a role but it is usually not determinant for the fate of a nematode population within the range of concentrations which are normally met under natural conditions, (Upadhyay 1969) and is therefore not especially studied.

Temperature influences all biological activities and processes. It is shown for different activities of a number of nematode species, that the well known optimum curve reflects the activity with successive zones of kill – cold rigor – increasing activity – optimum – decreasing activity – heat rigor and kill, in a gradient from low to high temperatures. The temperature requirements differ with nematode species and within the species with biotypes (Dao 1970). No experimental and hardly any other data are available about *T. dubius*.

Soil moisture-content is also known to be a determinant factor in nematode biology. Activities of certain nematodes are shown to be closely correlated with the soil moisture characteristic, and the moisture conditions which nematodes prefer or can stand varies widely with species (Wallace 1960). Again no experimental data are available with respect to *T. dubius*.

Soil type is shown to comprise factors which determine or regulate the establishment of many nematodes, including plant parasitic species. The most recent study was by Upadhyay (1969) who also did some experiments with Dutch populations of *T. dubius*.

The influence of temperature, moisture and soil type on a developing population of *T. dubius* was studied in the experiments recorded under 4.3.1., 4.3.2. and 4.3.3. respectively.

4.3.1. *Soil temperature*

Experiment 1.

The reproduction rate of *T. dubius* was determined in Wisconsin tanks kept at 20°, 25° and 30°C. Twenty adult nematodes, 10 females and 10 males, were added to glass tubes of 4 cm wide and 19 cm long with 150 g of nematode-free sandy soil, and 3 twelve-days old grass seedlings were planted in each tube on 26.5.1967. There were 5 replicate inoculated tubes for each temperature, and equal numbers of tubes were kept without inoculation as controls, making a total of 30 tubes. STEINER's nutrient solution and water were given at intervals and artificial light was provided 12 hours per day. The plants grew well. After 60 days the tubes were evaluated by determining the fresh root and shoot weights of the plants and the numbers of various stages of the nematode. The results are summarized in Fig. 22 and Table 7.

The thermogram of Fig. 22A indicates reproduction, which was strong at 25°C (85 fold), moderate at 20°C (34 fold) and low at 30°C (5 fold). The differences in temperatures for total populations and larvae were highly significant. The optimum for reproduction is 25°C, or possibly somewhat lower. At 25°C the numbers of males, females and larvae were all higher than at the other temperatures; the very high percentage of larvae accentuated the thrift of population at this temperature. Males were somewhat less numerous than females at all temperatures. But the male to female ratio was rather lower at the unfavourable temperature of 30°C than at 25°C or at 20° in this experiment. The male to female ratios of individual replicates varied strongly and differences between

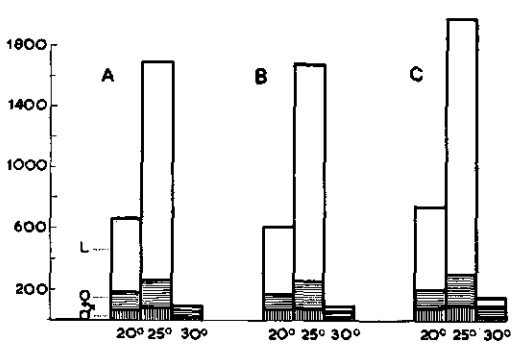


FIG. 22. Histograms of final numbers of *T. dubius*, when 20 adult nematodes, 10 females and 10 males, were inoculated on 26.5.1967 into glass tubes with 150 g of soil, in which grew 3 ryegrass seedlings each and kept in Wisconsin tanks for 60 days at 20°, 25° and 30°C. Abscissa: temperatures applied in °C. Ordinate: nematode numbers per tube as average of 5 replicates, calculated per tube (A), per 2 g of plant weight (B) or per g of root weight (C). Males (♂), females (♀) and larvae (L) are recorded separately as indicated.

TABLE 7. Inoculation of 20 *T. dubius* (10 females + 10 males) per tube of 150 g soil with three seedlings of ryegrass, at three different temperatures, 20°C, 25°C and 30°C. Final nematode densities (P_f) are numbers per tube, final plant weights and root weights are given as g; figures are averages of 5 replicates, start of the experiment on 26.5.1967, evaluation after 60 days.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, xx = highly significant ($P \leqslant 1\%$), x = significant ($1\% < P \leqslant 5\%$), – = non-significant ($P > 5\%$).

	Nematodes:		
	Not inoculated	Inoculated	Averages
P_f			
20°C	0	685 (log. = 2.79)	
25°C	0	1703 (log. = 3.15)	
30°C	0	95 (log. = 1.90)	
		(L.S.D. = 0.42)	
Plant weight			(L.S.D. = 0.25)
20°C	2.31	2.24	2.27
25°C	3.26	2.03	2.65
30°C	3.42	2.05	2.74
Averages			
(L.S.D. = 0.21)	3.00	2.11	
Root weight			(L.S.D. = 0.17)
20°C	0.99	0.92	0.95
25°C	1.37	0.86	1.11
30°C	1.44	0.65	1.04
Averages			
(L.S.D. = 0.14)	1.26	0.81	

Analysis of variance:

Plant weight		P values	Root weight		P values
F temperature	= 8.03	xx	F temperature	= 1.91	–
F nematodes	= 78.93	xx	F nematodes	= 46.56	xx
Interaction	= 17.07	xx	Interaction	= 9.48	xx

temperatures were not significant. Total weight of plants or weight of roots differed with temperature, but the foregoing conclusions were the same when final nematode populations were calculated per unit of plant weight or per g of roots (Figs. 22 B and C).

Comparison of the inoculated with the un-inoculated plants (cf. Table 7) shows that the inoculation has caused significant damage to the plants at 25°C and at 30°C, but not at 20°C. This result will be discussed further in Chapter 6.

Experiment 2

140 plastic tubes of 4 × 4 × 20 cm were each filled with 340 g of sterilized sandy PD soil and 10 g of soil with on an average 100 *T. dubius* (13 males + 45 females + 42 larvae) from a monospecific stock culture on rye grass Then, on 20.4.1966, four one-week old ryegrass seedlings were planted in each tube.

Five batches of 25 tubes were placed in Wisconsin tank departments at 13°, 16°, 19°, 22° and 25°C respectively. The nematode populations in five tubes of each series were extracted and analysed after 3, 6, 8, 12 and 15 months respectively. The nematode populations after 15 months were not only evaluated for densities, but also for morphological characters of the adults (cf. 3.5.).

Three batches of 5 tubes received treatments at alternating temperatures before they were evaluated, namely 6 months at 13°C + 6 months at 25°C, 6 months at 25°C + 6 months at 13°C and 9 months at 19°C + 3 months at 30°C, respectively.

All tubes were placed in a glasshouse with normal daylight in Wisconsin-tank departments at the temperatures indicated, and in addition 12 hours of artificial light per day to complement the daylight. STEINER's nutrient solution was added once per month and water at regular intervals according to the apparent need of the plants. All plants grew well throughout the experiment. The grass was cut every three months, and at these moments evaluations took place. Root weights were also determined.

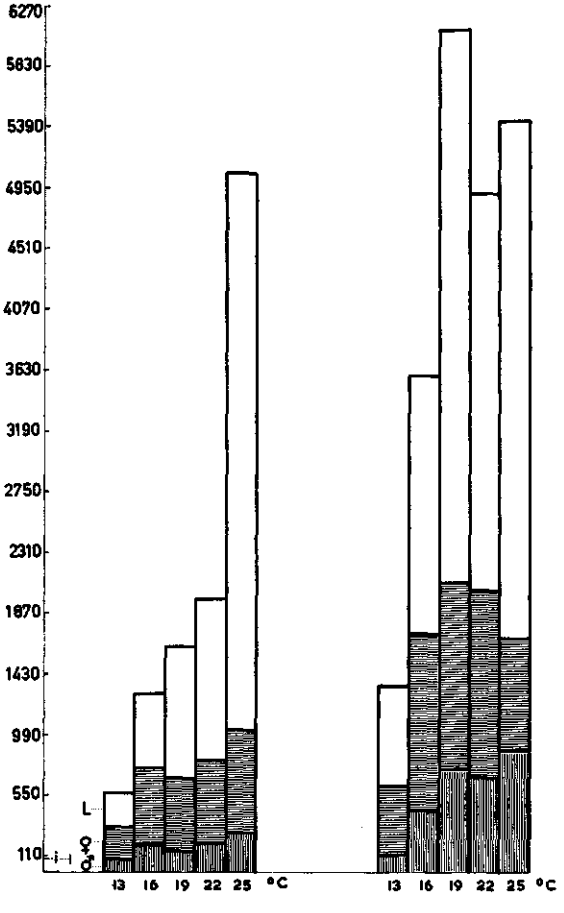


FIG. 23. Histograms of final numbers of a *T. dubius* population on ryegrass seedlings grown in tubes of 340 g of sandy PD soil, 3 months after inoculation with about 100 specimens (13 males + 45 females + 42 larvae) from a monospecific stock culture (left hand graphs) and 15 months after inoculation (right hand graphs), as a function of temperature. Abscissa: constant temperatures in the Wisconsin tanks. Ordinate: final nematode density as an average of five replicate tubes. Males, females and larvae are recorded separately as indicated in the graphs. The total inoculated density is indicated by i.

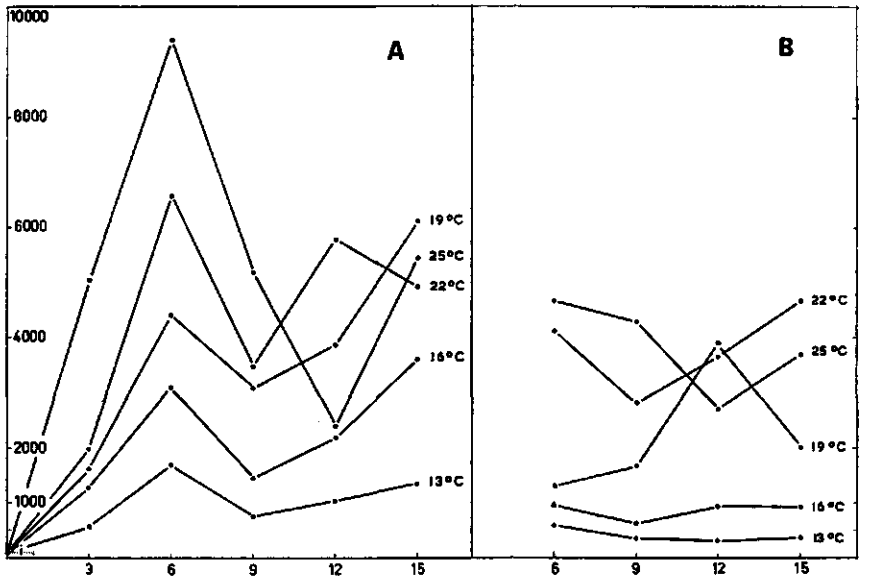


FIG. 24. Densities of *T. dubius* with time after inoculation of grass seedlings in tubes with 340 g of sandy PD soil with about 100 specimens (13 males + 45 females + 42 larvae) from a monospecific stock culture, at five different temperatures as indicated in the graphs. Abscissa: breeding time in months at the various Wisconsin tanks temperatures from 13° to 25°C. Ordinate: total number of nematodes per tube (A), or per g of fresh root weight (B); average of five replicates. The initial density is indicated by i.

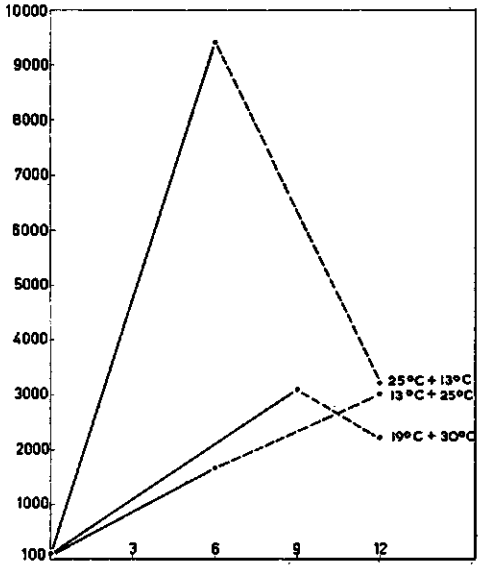


FIG. 25. Densities of *T. dubius* with time, after inoculation of ryegrass seedlings in tubes with 340 g of sandy PD soil with about 100 specimens (13 males + 45 females + 42 larvae) from a monospecific stock culture, at three different temperature regimes, viz 13°C for 6 months + 25°C for 6 months, 25°C for 6 months + 13°C for 6 months, and 19°C for 9 months + 30°C for 3 months. Abscissa: breeding time in months at the various temperature regimes indicated in the graphs. Ordinate: total number of nematodes per tube; average of five replicates.

The population densities are summarized in Fig. 23 and 24 for the tubes kept at constant temperatures and for the tubes at alternating temperatures in Fig. 25.

The thermograms of Fig. 23 show that after 3 months some reproduction had taken place at all temperatures, with 25° far superior to 22° and the lower temperatures. After 15 months the population density at 19° and 22° were about as high as at 25 °C, but 16° had a lower and 13 °C a much lower score. The numerical differences were again mainly due to the numbers of larvae. Fig. 24A illustrates the populations with time at the different temperatures calculated per tube. It appears that at all temperatures there was an increase at 3 months and a further increase towards an optimum at 6 months after the onset of the experiment. The population had dropped at all temperatures after 9 months (in the month of January), but started to rise again in the next spring and summer (with 25 °C after 12 months and 22 °C after 15 months as aberrant points). The populations, therefore, showed a seasonal decrease between October and April, synchronical for the different temperatures, when the numbers were calculated per tube.

Fig. 24B, however, shows that the seasonal population changes and the incidental aberrant figures disappear, when the numbers of nematodes are calculated per g of root. The decline of the populations per tube was therefore due to the fact that there were fewer roots. This may be due to the fact that the plants showed lack of light during the winter season (despite 12 hours of artificial light) and indicates at any rate that there was no seasonal population fluctuation due to intrinsic characters of the nematode population itself.

It is obvious from these experiments, that 25 °C originally favoured the population increase most, but that 19° and 22° were as favourable in the long run, whereas the lower and higher temperatures were unfavourable.

Fig. 25 confirms these results: the population rose steeply at 25 °C and then dropped heavily at 13 °C; at 19 °C there was a moderate increase, followed by a decrease when the temperature went up to 30 °C; at 13° the population rose moderately and continued to rise at 25 °C. In the same period the other two populations dropped at 13° and at 30°.

4.3.2. *Soil moisture*

The effect of high moisture content of the soil on maintenance and reproduction of *T. dubius* was studied by growing rice (*Oryza sativa* 'Caloro') as 'upland rice' in relatively dry soil water being given once a week and as flooded 'paddy rice' under aquatic conditions with water always covering the soil. 24 glass tubes were filled with 175 g of nematode-free sandy soil and all were planted with a two-days rice seedling. 12 tubes were inoculated with 3750 *T. dubius* specimens as a mixed population from the monospecific stock culture on ryegrass; the other 12 tubes were left uninoculated. Half of the inoculated and of the un-inoculated tubes were maintained in 'dry' condition and the rest was kept 'flooded'. All tubes were maintained in a climate chamber at 25 °C with 16 hours of artificial light. Water and STEINER's nutrient solution were added

TABLE 8. Inoculation of 3750 *T. dubius* specimens of a mixed population per tube of 150 g soil with a seedling of *Oryza sativa*, under 'dry' and 'flooded' conditions. Final nematode densities (P_f) are numbers per tube, final plant weights and root weights are given as g; figures averages of 6 replicates; start of the experiment on 12.3.1968, evaluation after 102 days.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, xx = highly significant ($P \leqslant 1\%$), x = significant ($1\% < P \leqslant 5\%$), – = non-significant ($P > 5\%$).

	Nematodes:		
	Not inoculated	Inoculated	Averages
P_f			
Dry	0	211 (log. = 2.298)	
Flooded	0	6 (log. = 0.650)	
		(L.S.D. = 0.386)	
Plant weight			(L.S.D. = 0.31)
Dry	2.65	1.55	2.10
Flooded	1.95	0.85	1.40
Averages			
(L.S.D. = 0.31)	2.30	1.20	
Root weight			(L.S.D. = 0.14)
Dry	0.57	0.38	0.47
Flooded	0.41	0.21	0.31
Averages			
(L.S.D. = 0.14)	0.49	0.29	

Analysis of variance:

Plant weight		P values	Root weight		P values
F flooding	: 21.72	xx	F flooding	: 6.06	x
F nematodes	: 53.15	xx	F nematodes	: 9.37	xx
Interaction	: 0.00	–	Interaction	: 0.01	–

according to the apparent need of the plants, without disturbing the 'dry' and 'flooded' conditions. The plants grew well, although differences were noted in connection with the treatments. 102 days after the start of the experiment on 12.3.1968, the plant shoot and root weights and the nematode numbers of each tube were evaluated. The results are summarized in Table 8.

It appeared that the final nematode numbers were lower than the inoculated ones in both, dry and flooded soils. It is possible that the large number of L_2 in the inoculum, which were found to be susceptible to adverse conditions (cf. 4.2), did not survive the inoculation. Taking this into account it is still remarkable that the nematode populations did not thrive well in this experiment. There were highly significant differences between treatments. The nematode density was 211 (100–300) per tube with 'dry' soil and 6 (2–10) per tube in the 'flooded' soil. Flooding appears to be very unfavourable to *T.dubius*, although some specimens in each tube survived the flooded condition for 102 days.

Comparison of the inoculated with the un-inoculated plants showed, that the inoculation had caused damage to the rice, both in the 'dry' and the 'flooded' soil. The colour of the roots of the un-inoculated plants in dry as well as flooded soil were white, whereas those of the inoculated plants were light brown in the dry soil and dark brown in the flooded soil. The inoculated plants in the dry soil also had lighter green shoots than the un-inoculated plants. Total plants and roots were both significantly heavier in the un-inoculated soils, dry as well as flooded, as can be seen from Table 8. The aspect of damage will be discussed further in Chapter 6.

4.3.3. *Soil type*

Five soils of a different type were used, the characteristics of which are given in Table 9.

a = polder soil (sandy soil from Oostelijk Flevoland),
b = sand/clay (a 50% mixture of soils d and e);
c = potting soil (a mixture of sand and peat);
d = sand soil (sandy soil from the crop rotation trial field at the PD, Wageningen),
e = clay soil (from the Binnenhaven, Wageningen).

Each of these soils in itself were mixed, freed from stones and debris, and steam-sterilized. One month later a sample of each was analysed for its physical and chemical composition. Glass tubes were each filled with 75 g of this soil on a bottom layer of perlite, whereas plastic straws were placed in the tubes to further aeration and drainage of excess of water if necessary; there were five replicate tubes for each soil. All tubes were sown with ryegrass; after germination three plants per tube were left. One fortnight later each tube was inoculated with a mixture of 10 females and 10 males of *T. dubius*, added with a little water into a hole made in the centre of each tube and covered with surrounding soil. All tubes were placed in a climate chamber at 18 °C with 12 hours of artificial light per day. Water and STEINER's nutrient solution were added at intervals to maintain good growth of the plants. All plants had a healthy dark-green colour in all soils throughout the experiment, although growth differences occurred. 60 days after inoculation all tubes were evaluated by determining root and shoot weights and numbers of males, females and larvae of the nematode. The data on nematode numbers are summarized in Table 9 and Fig. 26.

Fig. 26 indicates that the final nematode populations differ markedly with soil type as well as with development of the host plant. This was confirmed by statistical treatment of the data. The total number of nematodes per tube for the polder soil a, is significantly greater than for the soils c, d and e; the total number for the sand/clay mixture b is significantly greater than for the clay soil e. When the final population is calculated per 2 g of plant tissue (diagram B) or per g of root tissue (diagram C), the sandy soil d appears to be as favourable as the polder soil a. The number of nematodes per g of root tissue is probably the best statistic to measure the influence of the soil itself on the nematode's reproduction. According to diagram C the sandy soil d and the polder soil a have

TABLE 9. Numbers of *T. dubius* in five different soils, when 20 nematodes (10 females + 10 males) were inoculated to tubes with 75 g of soil each grown with three seedlings of ryegrass. Inoculation on 22.9.1966, evaluation after 60 days, five replicate tubes per soil. L.S.D. = least significant difference at 5% level.

1	2		3	4	5	6	7	8	9	10	11	12
	% Sand									Nematodes:		
	16 μ	105 μ	% Clay	% Humus	% $CaCO_3$	pH-KCl	N-total	Total plant weight g	Root weight g	per tube	per 2 g of plant	per g of root
a. Polder soil	79	26	14	2.2	5.0	7.0	0.09	1.329	0.547	263	396	480
b. Sand/clay	54	43	44	2.2	0.1	4.9	0.12	1.899	0.885	204	215	231
c. Potting soil	73	64	10	17.2	0.1	5.7	0.33	2.149	0.736	148	138	201
d. Sandy soil	89	72	9	1.9	0.1	6.0	0.09	0.837	0.405	140	334	346
e. Clay soil	17	11	80	2.5	0.1	4.0	0.16	1.786	0.874	133	148	152
L.S.D.										46		

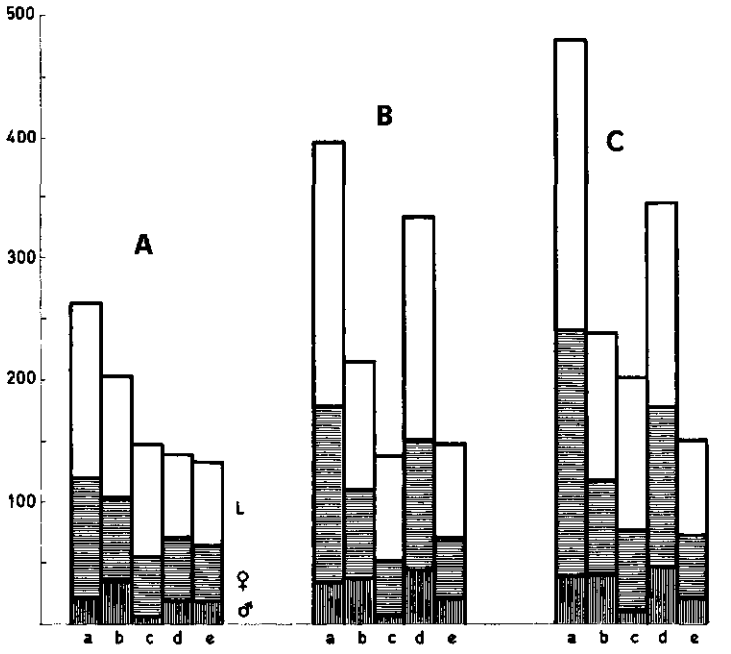


FIG. 26. Final numbers of *T. dubius* in five different soils, when 10 females + 10 males were inoculated to ryegrass seedlings, grown in tubes with 75 g of soil. Evaluation 60 days after inoculation, which took place on 22.9.1966. Each figure is the average of 5 replicate tubes. Abscissa for each diagram: indication of soil types: a = polder soil, b = sand/clay mixture, c = potting soil, d = sandy soil, e = clay soil (cf. text). Ordinate: nematode numbers, cumulative for males (♂), females (♀) and larvae (L) as indicated, calculated per tube (diagram A) or per 2 g of plant weight (diagram B) or per g of root weight (diagram C).

been more favourable than the sand/clay mixture b, the potting soil c and the clay soil e. Fig. 26 shows also, that the final populations in all soils comprise numerous males and females, in addition to larvae. The male/female ratios calculated from the average figures are 0.23–0.53–0.14–0.36–0.42 for the soils a, b, c, d, e respectively There is much variation between the replicates within soils. The notably low ratio for the potting soil (cf. Fig. 26) appears to be the only ratio which differs significantly from the others.

Table 9 furnishes 12 columns, with data about the soils, plant weights and final nematode densities, for comparisons. The total plant-and root-weights (columns 8 and 9) differ with soils and are apparently correlated with soil fertility, notably with the total nitrogen contents (col. 7) which may have influenced plant growth despite regular addition of STEINER's nutrient solution. Phosphorus, potassium and magnesium contents were also determined, but are omitted in the table because no relation whatsoever could be found with plant weights or nematode densities.

The total number of nematodes per tube (col. 10) could not be related to any

of the soil- or plant-statistics. However, when the differences in plant growth were taken into account, highly significant relations appeared. The final nematode numbers per 2 g of plant tissue, or better still: per g of roots, appeared to show a positive correlation with the sand contents (particles $> 16\ \mu$) and correspondingly a negative correlation with the clay contents (particles $< 16\ \mu$) of the soils, with the peaty potting soil (17% humus) as a marked exception. These results agree in all details with UPADHYAY's results (1969). It is clear that the development of *T. dubius* is favourably influenced by a high sand contents of the soil, but also that a high peat contents, as in our potting soil, and in the peaty sand soils of the Veenkoloniën, may suppress or prevent the nematode's reproduction. Some further conclusions can be drawn from table 9. It appears that coarse sand of $> 105\ \mu$ is no more favourable than fine sand of 16–105 μ, which is predominant in the polder soil a. It is also clear that at high $CaCO_3$ content, as in soil a, is not unfavourable to the nematode. It further appears that the nematode's reproduction is not directly favoured by low pH; as was suggested by BRZESKI and DOWE (1969): lowest reproduction occurred in the clay soil e with pH 4.0. The polder soil a with a pH of 7 was one of the two most favourable soils for reproduction.

T. dubius, therefore, is markedly influenced by soil type. It reproduces best in sandy soils, except those with a high humus (peat) content.

4.4. INFLUENCE OF THE BIOTIC ENVIRONMENT ON DEVELOPMENT

Under controlled temperature, moisture and other abiotic conditions, a plant nematode population may still be strongly influenced by the type, age and amount of plant tissue (host plant, quality and quantity of food), the density of the nematode population (space available per specimen, intraspecific competition) and by the presence of other organisms (interspecific competition, predation and parasitism, and other phenomena). Food, space and other organisms were studied in a number of experiments.

T. dubius was recorded to be a polyphagous, and probably obligatory parasite of the roots of higher plants by OOSTENBRINK (1959a), KLINKENBERG (1963) and others (cf. 1.2.). The nematode apparently finds the host roots and reproduces on them (cf. 4.2.). We concluded from earlier experiments (cf. 3.3.), that *T. dubius* hatches readily in water and that this process is not influenced by host root exudates prevailing in water. It is nevertheless possible, that host roots attract such polyphagous root nematodes in soil by a CO_2 gradient or another unspecific mechanism which is not transferable to vitro-tests in water. There is no doubt that the activity of certain plant nematodes is stimulated by exudates of host roots. This has been studied extensively for *Heterodera* species, especially *H. rostochiensis*. But even in these cases, literature is inconclusive on the question whether direct attraction plays a role. KÜHN (1959) and others suppose that active nematodes arrive at the roots due to random movements and not due to a gradient of attractants, because such a gradient is not likely to be established

in soil. Concentrations around, on, or in plant roots, or special sections of plant roots, are recorded for several plant nematodes, e.g. *Heterodera* species (BAUNACKE 1922, GADD & LOOS 1941, SHEPHERD 1959, WALLACE 1958), different styletbearing nematodes (STEWART 1921), *Helicotylenchus multicintus* (LINFORD 1939), *Meloidogyne* species (LINFORD 1939, WEISER 1955, BIRD 1959, 1960) and others. WINOTO (1969) supposed that high density of *Pratylenchus* species in and around host roots was due to strong reproduction of specimens which happened to meet and penetrate the roots. The literature is inconclusive even in respect to the question whether active concentrations of plant nematodes around host roots takes place. Three experiments were designed to study the problems of nematode attraction by its host and of host suitability (cf. 4.4.1.).

The influence of age and amount of host tissue on nematode population increase and equilibrium density has not been extensively studied. Some studies on *Pratylenchus penetrans* indicate, that the population increased more when the host plants were pruned or kept under suboptimal nutrient, light and temperature conditions (DOLLIVER 1961, MACDONALD & MAI 1963, MACDONALD 1966, WILLIS & THOMPSON 1969). Three experiments were carried out with *T. dubius* to study the influence of host density, host age and host pruning (cf. 4.4.4)

Apart from the host plant, other organisms in the soil, including other nematodes, do not seem to have a determinant influence on *T. dubius* or other plant nematode populations. This appears from the general occurrence of polyspecific nematode communities and from the great persistance of specific populations. Interspecific competition was not purposely studied. On the other hand there was an unexplained population decrease in *T. dubius* at the end of each growing season of the host crop in our experimental plot, which could be due partly or wholly to predation or parasitism, as mentioned in the introduction under 1.2. Several types of organisms have been recorded as parasites and predators of nematodes, such as fungi, protozoa, predatory nematodes, tardigrada, mites and collembola (WALLACE 1963:80–84). We concentrated our experimental- and fieldstuides on micro-arthropoda, especially mites and springtails, for several reasons, Mites and collembola are usually present in soil in great density, second only to the density of nematodes. The few data published, suggest that many species are predatory. Their capacity to destroy nematodes in vitro is impressive, and preliminary experiments suggested that they may be of some significance in the regulation of nematode populations (SHARMA & WINDRICH 1966, V. D BUND 1970). KARG (1961) showed for different Gamasidae, inhabiting arable soil, that they actively prey on other small species of the soil fauna. Predation on nematodes was observed for *Hypoaspis aculeifer*, *Alliphis siculus*, *Platyseius montanus*, *Dendrolaelaps hirschmanni*, *D. rectus*, *Rhodacarus roseus* and *Rhodacarellus silesiacus*. He also found a considerable increase of nematode predatory Gamasidae (*Rh. silesiacus*, *Rh. roseus* and *H.aculeifer*) in potato fields heavily infested with *Heterodera rostochiensis*. *Tyrophagus sp.* and *Tullbergia krausbaueri* also increased in numbers. It is therefore possible that these Gamasidae also reacted on a number of other mites and collembola. A number of

experiments were therefore carried out to study the role of micro-arthropods as an element in the environmental resistance in soil as explained previously (cf. 4.4.4). Some observations were made on other predators, parasites and diseases of *T.dubius* (cf. 4.4.5).

4.4.1. *Host plants*

In the following pot experiments we studied concentration of *T. dubius* around host roots. We also determined the host suitability of a large number of plants from temperate as well as tropical climates and of two common weeds in our main experimental field plot.

a. Concentration around host roots

One hundred g of naturally infested, moist sandy PD soil was filled into 30 plastic pots. 15 were planted with a pea seedling in the two-leaf stage; and the other 15 were left unplanted. The pots were placed in random position on a tray in a laboratory room with temperatures fluctuating from 10 to 21 °C and 12 hours of artificial light per day. The tray was covered by plastic sheets to avoid loss of moisture; no water was given during the experiment which ran for 20 days. The plants grew well. After 4, 8, 12, 16 and 20 days 3 replicate pots of both series were evaluated. The 100 g of soil from the unplanted pots was extracted by elutriation. The contents of the planted pots were treated as follows: the root bunch with soil was removed from the pot and pressed gently by hand to loose soil, which was elutriated. This was 100 g minus the amount of soil adhering to the plant roots. The latter was weighed with the plant and washed onto a stack of fine sieves. After this, the small amount of soil which had adhered to the roots was determined by again weighing the plant and by subtraction, also the amount of loose soil per pot. The soil adhering to the roots was extracted by decantation, as it was only a small amount. The nematode populations collected from the unplanted control soil, the loose pot soil and the soil adhering to the roots were quantitatively analyzed and calculated per g of soil for comparison. Fig. 27 summarizes the results.

The data show, that already after 4 days, *T. dubius* density in the soil adhering to the pea roots was about 4 times the density in the loose pot-soil or in the unplanted control soil. This cannot be due to the extraction techniques: the elutriation technique for 100 g of soil is known to extract about 80% of the nematodes, and decantation of the small amount of soil which had adhered to the roots is about equally effective. It also confirms the fact, that not all nematode species yield higher densities around the roots. The differences are real and they must be due to concentration and not to breeding. The density in the soil around the roots, and therefore the difference with the loose soil and the unplanted soil, increased somewhat further in the next 4 days, but then remained stable up to the 20th day. The data prove that *T. dubius* is concentrated around pea roots, but also that the larger part of the population was not mobilized and stayed away from the roots in the surrounding loose soil even after 20 days. The number of nematodes collected after 20 days from the adhering soil, 3.5 g from 3 plants,

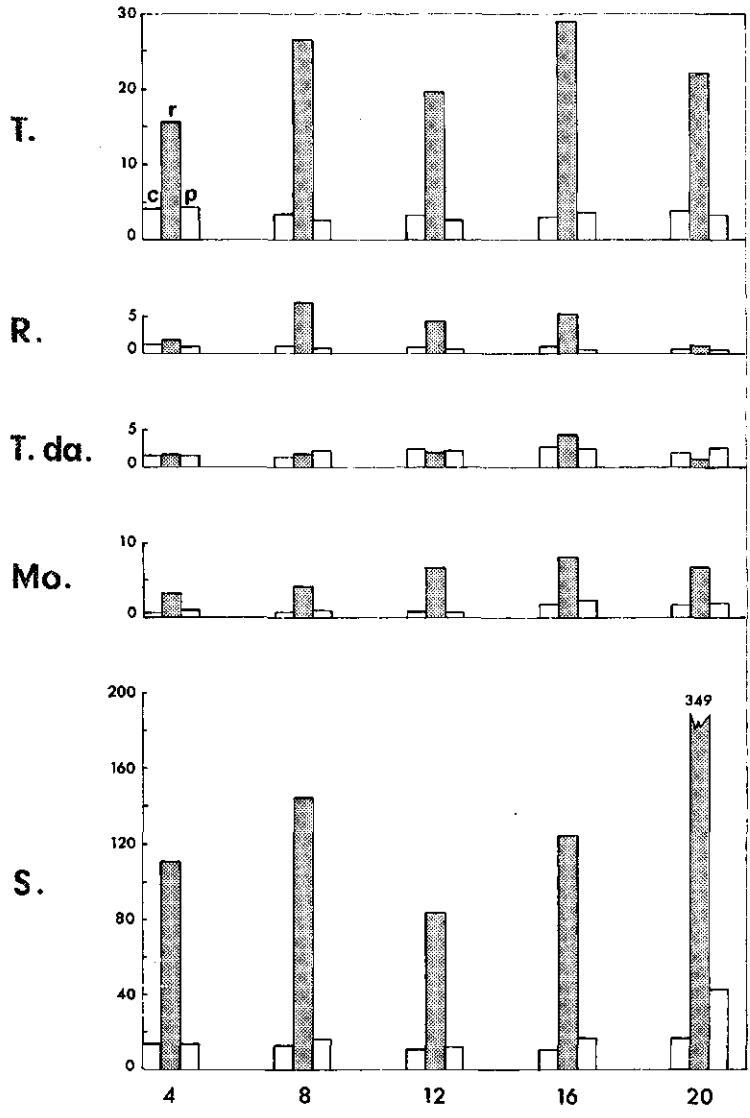


FIG. 27. Density of nematodes in soil adhering to the roots of pea seedlings grown in pots with 100 g naturally infested soil for 4, 8, 12, 16 or 20 days (central columns, r), compared to the rest of the pot soil (right columns, p) and to the unplanted control soil (left columns, c).
T. = *T. dubius*, R. = *R. robustus*, T.da. = *Tylenchus davainei*, Mo = *Mononchus papillatus*, S. = saprozoic nematodes.
Abscissa for all nematode categories: time in days after planting Ordinate for all nematode categories: number of nematodes per g of soil, as average of 3 replicates pots.

was 77 and the number of nematodes from the loose pot soil, 296,5 g from 3 pots, was 1010. Of all the nematodes available, 93% were therefore not participating in the attack on the root systems.

R. robustus increased its density around the roots to about the same degree as *T. dubius* after 4–16 days, but after 20 days this concentration was lost again. This may be linked to the fact, that pea is not a suitable host for this nematode (cf. 5.4).

The *Tylenchus davainei* density was not significantly higher around the roots than in the loose pot soil or in the unplanted soil.

The saprozoic nematodes reached high densities around the roots from the 4th day on, and these densities tended to increase with time. This last phenomenon may be a breeding effect because saprozoic nematodes usually have a short generation time. The fact that the density in the loose soil also increased after 20 days supports this explanation.

The significant concentration of *Mononchus papillatus* around the roots may be related to the density of the other nematodes, which serve as prey animals.

The conclusion is, that part of the populations of *T. dubius*, of saprozoic nematodes and of the predator *Mononchus papillatus* are concentrated around pea roots and stays there. *R. robustus* was concentrated there temporarily. *Tylenchus davainei* was not affected. Increase in density which could also be due to reproduction of the nematodes was found for saprozoic nematodes and perhaps for *Mononchus*, but not for the other species. Probably because the period of 20 days was only long enough for the first mentioned groups to demonstrate measurable reproduction.

b. Host suitability of 41 crop plants

Plastic pots, filled with 150 g of nematode-free sandy soil were inoculated with 10 adult nematodes, five males and five females. 41 plant species were grown in these pots, from 25 February to 3 June, 1967, four replicate pots per plant. The experiment is published in detail (SHARMA 1968). The results are summarized here as three groups to illustrate the polyphagous nature of the nematode.

a. Plants allowing more than a 10-fold multiplication, in the order of decreasing host suitability, are: rice (*Oryza sativa* 'Caloro'), cauliflower (*Brassica oleracea* var. *botrytis*), turnip (*Brassica campestris* var. *rapa*), black gram (*Phaseolus mungo*), sorgum (*Sorghum vulgare* 'Dochna'), perennial rye grass (*Lolium perenne*), rape (*Brassica campestris* var. *rapa*), green gram (*Phaseolus radiatus*), oats (*Avena sativa* 'Marne'), pearl millet (*Pennisetum typhoideum* 'Millet zango'), summer rye (*Secale cereale*), and pearl millet (*Pennisetum typhoideum*, 'zaria local').

b. Plants allowing less then a 10-fold multiplication, in the order of decreasing host suitability, are: sorghum (*Sorghum vulgare* 'Selor kuning'), pearl millet (*Pennisetum typhoideum* 'Cumbu'), sorghum (*Sorghum vulgare* 'Feterita'), rice (*Oryza sativa* 'Ketan Gadijk'), italian millet (*Setaria italica*), swede (*Brassica campestris* var. *napobrassica*), corn (*Zea mays* 'Goudstein'), tomato (*Lycopersicum esculentum* 'Moneymaker'), barley (*Hordeum vulgare*), pearl millet (*Pennise-*

tum typhoideum 'Bultul), pea (*Pisum sativum* 'Rovar'), radish (*Raphanus sativus* 'non plus ultra'), cotton (*Gossypium barbadense*), bitter gourd (*Momordica charantia*), chick pea (*Cicer arietinum* 'Brown'), spinach (*Spinacia oleracea*).

c. Plants not allowing noticeable multiplication are: roselle (*Hibiscus sabdariffa*), carrot (*Daucus carota*), cotton (*Gossypium hirsutum*), globe amaranth (*Gomphrena globosa*), chenopodium (*Chenopodium amaranticolor*), white gram (*Cicer arietinum* 'White'), sugar beet (*Beta vulgaris*), cucumber (*Cucumis sativus* 'Long yellow cross'), petunia (*Petunia alba*) chilli (*Capsicum annuum* 'Friesdorp'), flax (*Linum usitatissimum*), datura (*Datura stramonium*), tobacco (*Nicotiana tabacum* 'White Burley').

c. Host suitability of two weeds

Two weed plants found on the monoculture pea plot throughout the year 1965/66. *Agropyron repens* and *Poa annua*, were studied in addition to the plants recorded under b.

Ten glass jars of about 100 ml were provided with 50 g of sterilized sandy PD soil. 5 jars were planted with one nematode-free cutting of *Agropyron repens*, and in 5 other jars *Poa annua* was sown and after germination thinned down to four plants per jar. Ten days later, on 22.12.67, each jar was inoculated with 10 adult *T. dubius*, 5 females and 5 males. The females contained no visible eggs, but may or may not have been fertilized. The nematodes were collected from a stock culture grown on grass and were inoculated together in one ml of water and covered with some soil, after which more water was added. The jars we randomly arranged on a glasshouse bench. Water and nutrient were provided and the plants grew well in all jars. 60 days after the inoculation the nematode numbers in all jars were determined. The results are summarized in Table 10.

TABLE 10. Multiplication of *T. dubius* on the weeds *Agropyron repens* and *Poa annua*, 10 adult nematodes, 5 female + 5 males, were inoculated to jars with 50 g of soil grown with these two plants. Final nematode numbers were determined after 60 days, figures are averages of 5 replicates jars. Between brackets the range of the totals of individual replicates is given.

Plant species	Final nematode numbers:			
	Males	Females	Larvae	Totals
Agropyron repens	27	52	84	163 (36–354)
Poa annua	5	10	12	27 (2–65)

The inoculated density increased 16-fold on an average on *Agropyron repens* and 3-fold on *Poa annua*. Both weeds may therefore serve as an alternate host in the monoculture pea plot. Especially *A. repens*, of which rhizomes are continuously present, may obtain a certain population density in the absence of a main crop.

4.4.2. *Quantity and quality of food*

Three experiments were carried out to study the influence of plant density, the effect of plant pruning and the effect of plant age on nematode reproduction. In all cases the plant and root weights were determined and used for the calculation of relative nematode numbers in addition to total numbers.

a. Plant density

Four series of 5 six-centimeter wide plastic pots were filled with 96 g of nematode-free sandy soil, and planted with 1, 2, 4 and 8 ten-day old seedlings of ryegrass. Then 12 females and 12 males of *T. dubius* of the stock culture reared on grass were inoculated per pot. The pots were placed in a random position in a climate chamber at 22 °C and 12 hours of artificial light per day. Each week 20 ml of STEINER's nutrient solution was provided per pot and some distilled water was added daily to water the plants. All plants grew well throughout the experiment. Planting and inoculation of the pots took place on 15.10.1968, and 78 days later all pots were evaluated. Plants weights, root weights and nematode populations were determined. The results are summarized in Table 11.

Table 11 shows that the final nematode population increases significantly with the plant density. The final number of males, females, larvae and total nematodes all show significant rectilinear regressions on the number of plants per pot, after logarithmic transformation of the figures. The number of nematodes per g of roots does not differ much between objects, and this indicates that the final nematode density was determined mainly by the amount of food plant roots.

Final nematode densities, however, are not exactly proportionate to root weight, and other elements in the 'environmental resistance' may not be neglected. The relations between final nematode numbers and plant weight, y_1, or root weight, y_2, cannot be represented by rectilinear regressions, for the corresponding regression coefficients r_{y1} and r_{y2} are not significant. They are rather top curves, which can better be represented by the modified logistic formulas already mentioned in the introduction of this section. The final nematode numbers per g of plant or per g of root recorded in table 11 also indicate an optimum at medium plant density or medium root weight per pot.

It appears from these results that the 'environmental resistance' was mainly determined by the amount of roots, but that other elements which increased in weight at higher densities were also significant.

It is noteworthy that the male to female ratio was about 1 in all environments of this experiment, and therefore that food scarcity or increasing population density did not influence the ratio despite the fact that they reduced population growth.

b. Plant pruning

One hundred forty four glass bottles were filled each with 100 g of nematode-free sandy soil and planted with two seedlings of ryegrass. Half of the bottles were inoculated with 100 specimens of a monospecific. *T dubius* culture grown

TABLE 11. Influence of plant density on the reproduction of *T. dubius*. 12 females and 12 males, therefore, 24 nematodes (= P_i), were inoculated into pots with 96 g of soil, planted with 1, 2, 4 or 8 seedlings of ryegrass. Final plant weights (= y_1), final root weights (= y_2) and final nematode numbers (= P_f) are recorded per pot, as average of 5 replications. Planting and inoculation date 15 October 1968, evaluation after 78 days.

Number of plants per pot = n	Log. n (coded) = n_1	Total plant weight in g = y_1	Root weight in g = y_2	Nematode numbers					
				Males	Females	Larvae	Total per pot = P_f	Total per g of plant	Total per g of root
1	0	0.854	0.292	106	110	1174	1390	1628	4760
2	1	0.780	0.220	152	84	1626	1862	2387	8464
4	2	1.188	0.540	130	138	1862	2130	1793	3944
8	3	1.474	0.684	208	204	2828	3240	2198	4737

Regression formula of the logarithm of nematode numbers (log. P_f) on the logarithm of the number of plants (log n, coded = n_1) for different nematode categories; r_{n1}, r_{y1} and r_{y2} are regression coefficients of Log. P_f on n_1, y_1 and y_2; – = not significant, x = significant at 5% level, xx = significant at 2% level.

Males : $\log P_f = 2.00 + 0.090\ n_1$ $r_{n1} = 0.49^*$ $r_{y1} = 0.32 -$ $r_{y2} = 0.27 -$
Females: $\log P_f = 1.89 + 0.110\ n_1$ $r_{n1} = 0.49^*$ $r_{y1} = 0.38 -$ $r_{y2} = 0.44 -$
Larvae : $\log P_f = 3.02 + 0.120\ n_1$ $r_{n1} = 0.53^{**}$ $r_{y1} = 0.24 -$ $r_{y2} = 0.27 -$
Total : $\log P_f = 3.10 + 0.115\ n_1$ $r_{n1} = 0.55^{**}$ $r_{y1} = 0.27 -$ $r_{y2} = 0.29 -$

on grass, the other half were left uninoculted. In half the bottles of each series the grass was cut four times a month (the yield being determined). The rest remained untouched. All bottles were placed in randomized position in a climate chamber at 20 °C and 12 hours of artificial light. STEINER's nutrient solution was provided to all bottles once every three weeks, and water according to the apparent need of the plants. Each month, starting from the inoculation date 3.6. 1969, a number of bottles were evaluated for root and shoot weight and for nematode population (L_2, L_3, L_4 and adults). For each of the first five months 6 replicates of each treatment were evaluated. For the 6th and 7th month this number was 3. The results are summarized in Table 12, and in the Figs. 28, 29 and 30.

Table 12 shows that the plant pruning (cutting of the ryegrass at short intervals) has considerably reduced plant growth, especially root growth, as well as the growth of the nematode population. Fig. 28 illustrates the population densities with time in both environments for the total populations per bottle, and also for the numbers per g of plant tissue and per g of root tissue. It is again clear that the nematode densities were as in experiment a, mainly determined by the amount of food (plant roots): the number of nematodes per g of plant or per g of roots was as high or higher on the poorly developed, cut plants as compared to the uncut plants.

TABLE 12. Influence of cutting of ryegrass on the reproduction of *T. dubius* on the roots. Bottles with 100 g of soil were planted with two ryegrass seedlings, inoculated with 100 nematodes each on 3.6.1969. The ryegrass was either cut 4 times a month or left uncut. Each month, for 7 months in succession, 6 replicate bottles of each series (3 for the 6th and 7th month) were evaluated, and averages were determined for nematode numbers, fresh root weight and fresh total plant weight per bottle.

Evaluation date: number of months after inoculation	Total nematodes per bottle	Plant weight in g	Nematodes per g of plant weight	Root weight in g	Nematodes per g of root
Uncut					
1	292	1.42	206	0.37	789
2	3052	3.29	928	1.73	1764
3	24905	5.65	4408	2.79	8927
4	26961	6.54	4123	3.16	8532
5	30765	8.22	3743	4.23	7273
6	23150	7.28	3180	4.07	5688
7	11634	10.55	1103	5.20	2237
Cut					
1	106	0.40	265	0.10	1060
2	2718	1.27	2140	0.34	7994
3	7010	1.46	4801	0.35	20029
4	9184	1.72	5340	0.37	24822
5	5998	1.86	3225	0.27	22215
6	3150	1.49	2114	0.14	22500
7	1560	1.63	957	0.13	12000

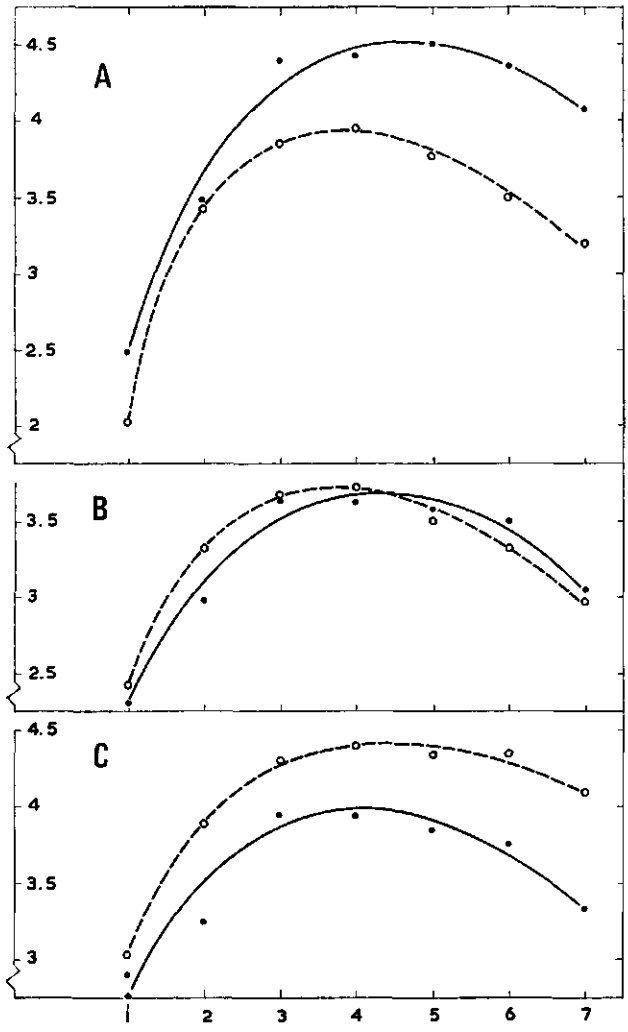


FIG. 28. Density of *T. dubius* with time on cut (o) and uncut (•) ryegrass, when 100 nematodes, a natural mixture of adults and larvae, were inoculated into glass bottles with 100 g of soil grown with 2 grass seedlings each on 3.6.1969 and kept in a climate chamber at 20°C and 12 hours of artificial light per day. The graphs are drawn to approximate the points after the inoculation.
Abscissa: time in months after the inoculation.
Ordinate: logarithm of nematode numbers as an average of 6 replicates (3 replicates for the 6th and 7th month).
A = for populations per bottle;
B = for populations per g of total plant weight;
C = for populations per g of root weight.

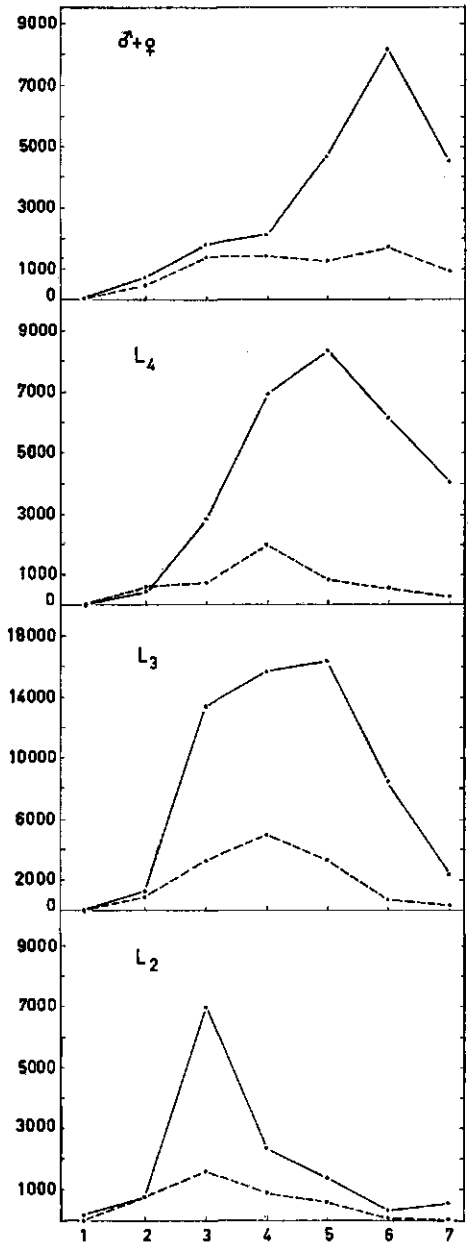


FIG. 29. Aging of the *T. dubius* population with time on cut (o—o) and uncut (● - - ●); cf. legend of Fig. 28. Figures are untransformed numbers per bottle of 100 ml soil as average of 6 replicates, separately for second-stage larvae (L_2), third-stage larvae (L_3), fourth-stage larvae (L_4) and adults (♂ + ♀).
Abscissa: time in months after the inoculation.
Ordinate: nematode number per bottle as an average of 6 replicates (3 replicates for the 6th and 7th month), illustrated separately for the different nematode stages as indicated in the graphs.
(The scale size for L_3 is half the size for the other stages).

In both environments the total population reached a maximum density, after which it declined considerably.

The decline started in November and continued in December and January. It occurs independent of root weights and is not due to changing climatic condi-

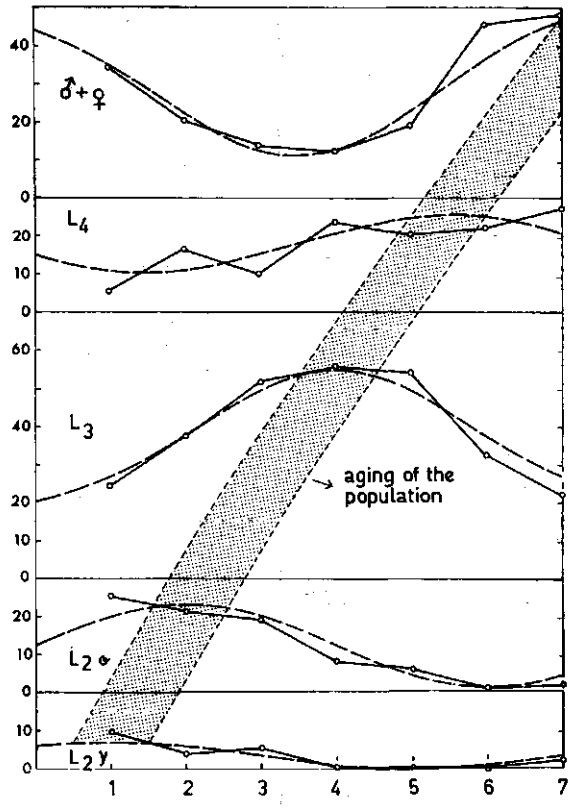


FIG. 30. Aging of the *T. dubius* population with time on ryegrass, when the results of cut and uncut bottles are joined; cf. legenda of Fig. 28 and of Fig. 29. Figures for young second-stage larvae (L_2y), old second-stage larvae (L_2O), third-stage larvae (L_3), fourth-stage larvae (L_4) and adults (♂ + ♀), as monthly averages of 12 replicates, are expressed in percentages of the total populations. Solid lines (——) are real, broken lines – – –) are approximations. The connection of the curve peaks by means of the sloping bar illustrates the process of aging of the population. Abscissa: time in months after the inoculation. Ordinate: percentage of the population, represented by the different stages as indicated in the graphs, calculated from the monthly average numbers.

tions, for temperature, light as well as moisture and additions of fertilizer were maintained. It must be related to aging of the population or of the host plant, or to another factor increasing the environmental resistance. Aging of the population with time takes place, as illustrated in Figs. 29 and 30.

Fig. 29 shows, that the uncut grass caused temporary high densities for all nematode stages, but the peaks were reached for L_2 after 3 months, for L_3 and L_4 after 4–5 months, and for the adults after 6 months. The same was true for the cut grass, although the peaks were much lower and less marked. The peaks for the successive stages, therefore, shift with time, which illustrates gradual aging, and finally a decrease in the populations as a whole.

Fig. 30 illustrates the result, when cut and uncut replicates are joined and when the average numbers are calculated as percentages of the total population. The aging process of the population is clearly indicated by the sloping bar which connects the curve peaks. It appears that the aging continues throughout the experiment, and that the decrease in the total population density in the 6th and 7th month is due particularly to the decrease in L_3. However, Fig. 29 has shown that the numbers of L_4 and adults also decreased during these months. Apparently an adverse factor is becoming important as from the 5th month. This may be related to aging of the host plant. This problem is studied further on.

c. Plant age

Plastic pots were filled with 200 g of a nematode-free sandy soil and potting soil. Half of the pots were planted with seedlings of ryegrass in sandy soil and the other half with seedlings of pea in potting soil, thus making two series. From each series sets of 6 pots were inoculated at different instants after planting, namely after 1, 4, 7, 10, 13 and 16 weeks. The inoculum per pot consisted of 15 nematodes, viz. 10 apparently fertilized females + 5 males, individually selected in all cases from the stock population. All pots were kept in randomized position in a climate room at 20–26 °C and 12 hours of light per day; water was given according to the apparent need of the plants. STEINER's nutrient solution was given at intervals of 3 weeks. Two months after each inoculation the corresponding pots of both series were evaluated by weighing fresh plant weights and root weights and by separately determining the nematode densities, for males, females, L_2, L_3 and L_4. The results are summarized in the graphs of Figs. 31 A, B, C.

The ryegrass developed well and all weights of shoots and roots were increasingly higher: the later the inoculation had taken place, the longer had been the growing period of growth, viz. the period between planting and inoculation, plus two months. The increasing weights were not strictly rectilinear (Fig. 31 A), but they could be calculated as significant rectilinear regressions by means of the formulae $y = 0.67x + 1.25$ and $y' = 0.86x - 0.69$ for shoots and roots respectively. The nematode populations reached higher densities in the two months reproduction period on the 4- and -7weeks old grass plants than on the 1-week old plants, which were obviously still too weak. Cf. Fig. 31 B. The number of nematodes per g of roots was highest on the 1 week old plants, therefore the quality of these roots was best. The 10-, 13- and 16-week old plants apparently did not furnish the right quality of roots, for the total population was much less, despite the fact that the quantity of roots was greater and still growing.

Fig. 31 C shows, all stages being always present at all inoculation dates. There were marked differences, however, in the percentages of different stages. The percentages males and the percentages L_4 were not greatly influenced by the age of the plant. The percentages of females were significantly higher and the percentages of second- and third-stage larvae were correspondingly lower, for inoculations of 1-week old plants as well as of 10 weeks old plants, probably for different reasons. This is evident when the populations resulting from all inocu-

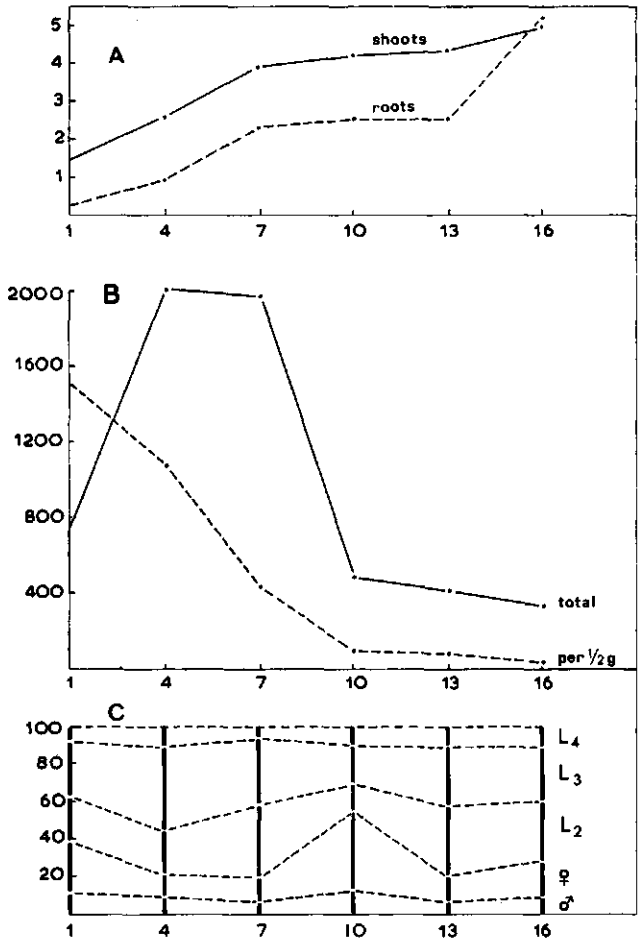


FIG. 31. The influence of the age of ryegrass seedlings on the multiplication of *T. dubius*. Fifteen nematodes (10 females + 5 males) were inoculated to 200 g pots at 1, 4, 7, 10, 13 and 16 weeks after planting, 6 replicates. Two months after each inoculation nematodes, plant weights and root weights were determined; the figures recorded are averages of the 6 replicates. Abscissa: age of plants in months at the moment of inoculation. Ordinate: for A – fresh weight of shoots and of roots, in g per pot; for B – number of *T. dubius* as totals per pot and per ½ g of roots, for C – percentages for the populations of males (♂), females (♀), second-stage larvae (L_2), third-stage larvae (L_3), and fourth-stage larvae (L_4) drawn over each other extracted from the plants of various ages.

lations are considered. The population development on 1-week old plants was obviously delayed as compared to that on 4- and -7week old plants due to lack of roots. The percentage of females must be relatively high because the offspring, L_2 and L_3, had not yet been formed due to lack of time, not lack of fertility or unfavourable conditions.

It is different for the populations inoculated to 10-week old plants. The 10-,

13- and -16week old plants were too old to allow good reproduction, and it is probable that in this case the 10-week old plants were marginal. They apparently allowed abundant development of females in the population, but these were not fertile and could not cause abundant reproduction as in the younger grass plants.

The pea series was involved in complications. The peas grew well during the first two months, but later yellowing and drying of the lower leaves due to infection caused by *Phoma medicaginis* var. *pinodella* occurred. It gradually proceeded to the higher leaves of all plants. In the series with inoculation applied last, i.e. 13 weeks after planting, the plants had dark green tops whereas the other leaves were dried up and the stems had become light green to yellow and finally brown. Older plants, therefore, had lower weights of fresh shoots than younger ones. It was also true for the fresh root weight, which indicated, that root systems were gradually disintegrating. The decreased weight of shoots and roots could be indicated as significant rectilinear regressions, by means of the formulae $y = -0.17x + 1.83$ and $y' = -0.18x + 0.98$. The sick condition of the plants must have influenced the results. It explains that the nematode density rose very high for the inoculation to 1-week old plants, less high on 4-week old plants, still less on 7-weeks old plants and neglibible on the 10- and 13-weeks old plants. This holds too when the numbers are expressed per g of roots, which indicates that the quantity as well as the quality of the roots decreased with increasing age of the plants (cf. Fig. 32 A, B, C). Graph C shows, as in Fig. 31 C, that the percentages males and the percentages L_4 were greatly not influenced by the age of the plant. It also shows that the percentage females became significantly higher again, and the percentage second- and third-stage larvae significantly lower, the moment the root systems became unsuitable as a source for food and reproduction at the moment of inoculation i.e. for plants of 10 weeks. It can again be explained as being an accumulation of specimens which had reached adult female stage but could cause abundant reproduction no more.

Thus in both cases, plants with young roots were required to enable the nematode to multiply strongly. It is obvious that in ryegrass the oldest plants with maximum root production were correlated with lowest nematode density, which phenomenon may influence the role of *T. dubius* as a parasite in permanent grassland. It is not likely that intrinsic periodicity of the nematode was involved in these differences, because peak populations of *T. dubius* were collected from young plants of the same hosts species at the moment when the populations in the experiments collapsed (cf. Fig. 31B, 32B). The maximum multiplication rate in two months, starting from 10 females, was about 200 on grass and about 130 on pea. Because the difference was significant, grass, under experimental conditions was therefore a more suitable host than pea. Further it is noteworthy that the onset of unfavourable conditions, i.e. on 10-weeks old plants, caused an accumulation of females and not of males. Normally unfavourable circumstances cause an increase of the male to female ratio in other nematodes.

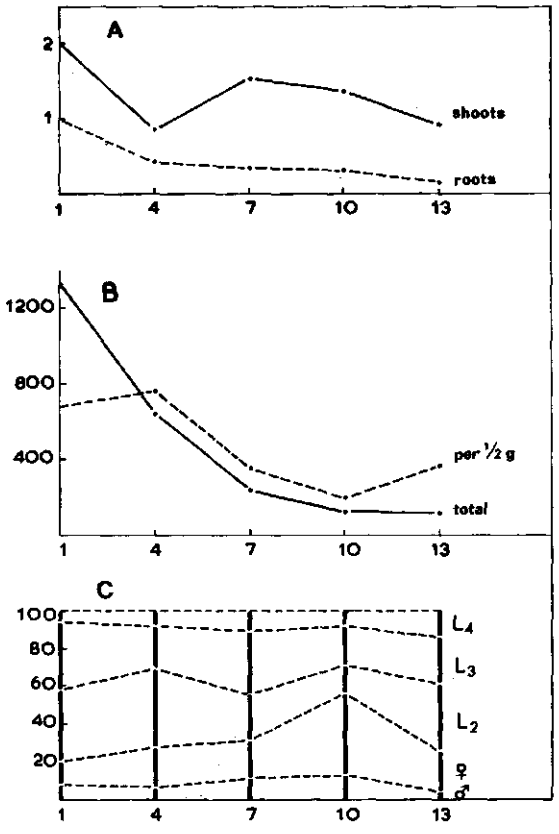


FIG. 32. The influence of the age of pea seedlings on the multiplication of *T. dubius*. Fifteen nematodes (10 females + 5 males) were inoculated to 200 g pots at 1, 4, 7, 10 and 13 weeks after planting, 6 replicates. Two months after each inoculation nematodes, plant weights and root weights were determined; the figures recorded are averages of the 6 replicates. Abscissa: age of plants in months at the moment of inoculation. Ordinate: for A – fresh weight of shoots and of roots, in g per pot; for B – number of *T. dubius* as totals per pot and per ½ g of roots, as indicated; for C – percentages for the populations of males (♂), females (♀), second-stage larvae (L_2), third-stage larvae (L_3), and fourth-stage larvae (L_4) drawn over each other extracted from the plants of various ages, as indicated.

4.4.3. *Population density*

a. Varying space with one host plant

Four series of 5 six-centimeter wide plastic pots were filled with 24, 48, 96 and 192 g of nematode-free sandy soil respectively. Each pot was planted with 1 ten-days old seedling of ryegrass and inoculated with 12 females and 12 males of *T. dubius* of the stock culture reared on grass. The general set-up and treatment of the pots was the same as in the experiment described under 4.4.1.a. Growth of the plants was fair throughout the experiment, but in the pots with 96 g of soil 2 of the 5 replicate plants grew poor for unknown reasons. 78 days after the onset

of the experiment, which took place on 15.10.1968, all pots were evaluated for plant weights, root weights and nematode populations. The results are summarized in Table 13.

Table 13 shows that the final nematode density P_f increases significantly with the amount of soil used, but also that this increase is correlated with development of the test plants. In general this development was only fair and great differences occurred between replicates, especially in the series with 96 g of soil which comprised some plants with very poor growth. Although the correlations between final nematode density and plant growth or root growth were marked, for total nematodes as well as for the different stages separately, it is clear from the table that straight correlation lines did not represent the relation very closely and that top curves according to a modified logistic curve formula would probably give a closer approximation of the observed densities. The results indicate that the highest nematode density per ml of space or per g of plant tissue was reached in '48 g of soil', the highest density per g of roots in '48 g of soil' and the highest total density in '192 g of soil'. The complex situation which arises when 1 plant is grown in varying amounts of soil only allows for the general conclusions, that the population is strongly correlated with the amount of roots, but has also been influenced otherwise, directly or indirectly, by the size of the environment.

On an average the male to female ratio in this experiment was also high, although with considerable variation. It was > 1 in the lowest as well as the highest amounts of soil and no consistent correlation with any environmental factor could be found.

b. Varying space with homogeneous host density

Rectangular open trays of 4 cm height, and with a bottom surface of 4×2, 4×4, 4×8, and 4×16 centimeters, were provided with 32, 64, 128 and 256 grams of sandy soil in an even layer of about 2.5 cm and grown with 1, 2, 4 and 8 fourdays old ryegrass seedlings at equal distances (Fig. 33). 16 females and 16 males of *T. dubius* were inoculated per tray, evenly distributed amongst the plants. In this way the 32 nematodes were brought into environments which were uniform in structure and food density, but the initial space available per nematode was 1, 2, 4 and 8 ml in the four series respectively. The trays were placed in a growth chamber at 18 °C and 12 hours of artificial light. Optimum moisture conditions were maintained throughout the experiment and STEINER's nutrient solution was added at regular intervals. All plants grew well throughout the experiment. At intervals of 16 days 5 trays of each series were evaluated by extracting all nematodes and analysing the populations for the first 4 observations; thereafter intervals of 32 days were maintained. After 176 days only one tray of each series was evaluated for each interval and from then on the figures became more variable. The results are summarized in Tables 14 and 15 and in Fig. 34A, B, C, D.

Table 14 summarizes the average final nematode densities, the totals per container (P_f) as well as per 32 g of soil for all 19 examination dates. It contains

TABLE 13. Multiplication of *T. dubius* in varying amounts of soil grown with ryegrass. 24 nematodes (p_i), 12 females and 12 males, were inoculated into pots with 24, 48, 96 and 192 g of soil, all planted with one seedling of ryegrass.
Plant and root weights and nematode numbers are recorded per pot as average of 5 replicates.
Planting date 15.10.1968, evaluation after 78 days.

Soil weight in g = n	Log n (coded) = n_1	Total plant weight in g = y_1	Root weight in g = y_2	Nematode numbers					
				Males	Females	Larvae	Total per pot = P_f	Total per g of plant	Total per g of root
24	0	0.190	0.072	46	38	210	294	1547	4083
48	1	0.640	0.170	166	174	1560	1900	2969	11176
96	2	0.678	0.136	58	110	1136	1304	1923	9588
192	3	1.192	0.332	266	168	2120	2554	2143	7693

Regression formula of the logarithm of nematode numbers (log P_f) on the logarithm of the amount of soil (log n coded = n_1) on the plant weight (y_1) and on the root weight (y_2), for different nematode categories; between brackets the corresponding correlation coefficients; − = not significant, * = significant at the 5% level, ** = significant at the 1% level.

Males	$\log P_f = 1.64 + 0.20\ n_1$ (0.49 *)	$\log P_f = 1.43 + 0.76\ y_1$ (0.61 **)	$\log P_f = 1.51 + 2.47\ y_2$ (0.62 **)
Females:	(0.33 −)	$\log P_f = 1.36 + 0.80\ y_1$ (0.53 *)	$\log P_f = 1.43 + 2.65\ y_2$ (0.55 *)
Larvae	$\log P_f = 2.43 + 0.31\ n_1$ (0.68 **)	$\log P_f = 2.13 + 1.13\ y_1$ (0.83 **)	$\log P_f = 2.32 + 3.20\ y_2$ (0.74 **)
Total	$\log P_f = 2.58 + 0.28\ n_1$ (0.68 **)	$\log P_f = 2.31 + 1.03\ y_1$ (0.84 **)	$\log P_f = 2.48 + 2.94\ y_2$ (0.75 **)

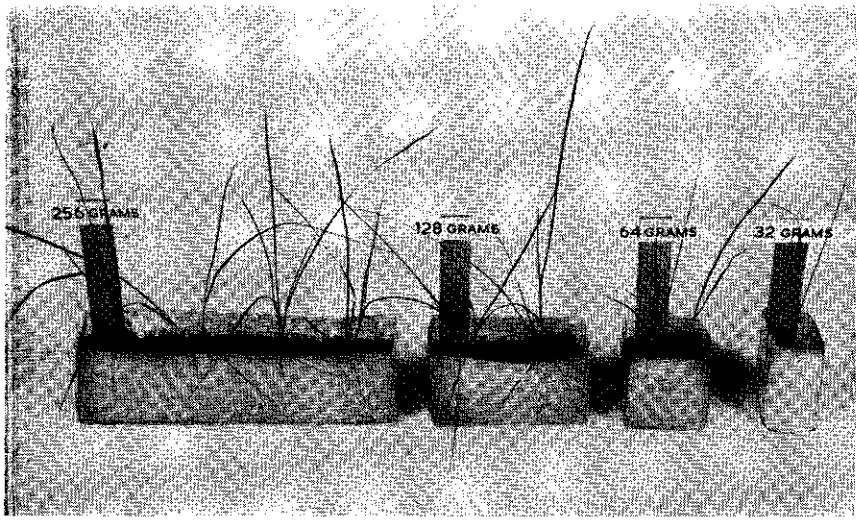


Fig. 33. Trays with 32, 64, 128 and 256 grams of soil, grown with 1, 2, 4 and 8 ryegrass seedlings to obtain environments which were different in space, but uniform in structure and root density. All trays were inoculated with 32 *T. dubius*, 16 males and 16 females, which were evenly distributed.

much information. The differences between P_f were originally small and the significance of these differences is added for the intervals up to 144 days. It is obvious that none of the differences was significant after 16 and 32 days, that some were significant after 48 and 64 days, and that all were significant at later dates, as well as for the intervals after 144 days, which are omitted. After 16 days 23 to 26 nematodes on an average were recovered, against 32 initially inoculated. After 32 days 3- to 6 fold reproduction had taken place, without differences induced by space. This indicates that 32 nematodes per 256 g of soil was a density high enough to avoid underpopulation. Also that the inoculated density was still only determining the reproduction rate and not yet competition for space. After 48 days, P_f at 32 g started to lag behind the populations with more space and later this did not change. The P_f at 64 g and 128 g started to lag behind P_f at 256 g after 64 days, and at later dates the influence of space was always marked. All this refers to P_f, total populations per container. The density per unit of soil (32 g) was originally lower in the larger containers due to the dosage inoculated, but after 112 days this difference had gone. The final densities per g of soil reached at 144 days on, was about 50–80 in the different containers (with 32 g lower at 144 days and higher (more than 100) at 176 days, but in line at and after 208 days).

Table 14 indicates, that the populations in all containers increased according to top curves (cf. also Figs. 28 and 31), although at the end of the observation period (after 240–304 days) some of the curves seemed to rise again. The curves are probably of the logistic type, but the observed P_f could be approximated by

TABLE 14. Multiplication of *T. dubius* with time on ryegrass in environments which were different in space but uniform in structure and root density. 32 nematodes, 16 males and 16 females, were inoculated on 26.11.1965 to trays with 32, 64, 128 and 256 g of soil in a 2.5 cm thick layer, grown with 1, 2, 4 and 8 ryegrass seedlings respectively. Final nematode numbers (P_f) were determined after 16, 32, 48 etc. days (intervals of 16 days numbered). Figures are averages of 5 replicate trays except for intervals from 11 on when 1 replicate was taken. Between brackets are the figures calculated per 32 g of soil.

Space (g of soil per container) \ Time: Interval nr =	1	2	3	4	7	9	11	13	15	17	19
Days =	16	32	48	64	112	144	176	208	240	272	308
32 g	24	93	113	178	282	834	4100	2340	500	800	3320
64 g	26	81	230	429	1520	3644	5540	4480	3400	900	4320
	(13)	(41)	(115)	(215)	(760)	(1822)	(2770)	(2240)	(1700)	(450)	(2160)
128 g	26	92	326	500	4012	7668	7960	8320	8560	11800	4590
	(7)	(23)	(82)	(125)	(1003)	(1917)	(1990)	(2080)	(2140)	(2950)	(1148)
256 g	23	137	190	313	6066	12144	19020	14960	14760	10080	15870
	(3)	(17)	(24)	(39)	(758)	(1518)	(2378)	(1870)	(1845)	(1260)	(1984)

The significance of differences between P_f totals in the different amounts of soil after 16, 32, 48, 64, 112 and 144 days (– = not significant, x = significant at the 2% level):

	256 g						128 g						64 g					
	16	32	48	64	112	144	16	32	48	64	112	144	16	32	48	64	112	144
32 g	–	–	–	–	x	x	–	–	x	x	x	x	–	–	–	x	x	x
64 g	–	–	–	x	x	x	–	–	–	–	x	x						
128 g	–	–	–	x		x												

TABLE 15. Number of days after which the maximum density was reached for larvae, females and males in the containers with 32 g and with 256 g of soil. Cf. Figure 34. The differences were significant according to FRIEDMAN's test.

Space (g of soil per container)	Larvae	Females	Males
32 g	176	187	202
256 g	197	216	232

separately calculating the corresponding second-degree formulae (cf. Fig. 16), for each environment. Within each environment formulae were calculated for males (♂), females (♀), larvae (L) and totals (T), after logarithmic transformation of the numbers. Fig. 34 A, B, C, D indicate the formulae and the corresponding graphs for the environments 32 g, 64 g, 128 g, and 256 g respectively. Comparison of the graphs reveals the following points.

All curves have about the same shape and in all populations larvae are more numerous than females and females more numerous than males throughout the observation period (with one aberrant point for males in the 64 g trays after 272 days). The number of days after which the maximum is reached, however, was somewhat different for larvae, females and males, viz. 189, 198 and 229 on an average in all containers. Increased space caused a higher P_f for totals, larvae, females as well as males. The maximum is also reached at a later date when more space was available. This holds for the total population, but also for all separate nematode categories. The differences were shown to be significant by comparing the results of 32 g and 256 g in Table 15. It also appeared that the 'aberrant' population increase at the end of the observation period occurred at a later moment and to a lesser degree in the larger containers. The phenomenon suggests periodicity, i.e. in the nematode's reproduction or the growth of the host plant, but it cannot be explained, for the circumstances in the growth chamber and in the trays were kept as constant as possible.

The experiment therefore indicates, that 16 females + 16 males per 256 g was enough to avoid underpopulation, that the population needed more than 64 but less than 112 days to compensate for the 8 time lower inoculum density per g of soil in the largest containers, and that the P_f was nearly proportionate to the space available. It is further obvious that the population in all its stages passes a maximum, that more space causes a higher population density, and that the maximum is reached later when more space is available.

c. Varying inoculum levels on different hosts

Experiment 1

The relation between P_i initially inoculated population and P_f final population extracted after a fixed period, was studied under controlled conditions on different hosts.

Four series of glass tubes, 4 cm in diameter and 19 cm in length, were filled with 150 g sterilized PD soil, and planted with a week-old grass seedling, a one

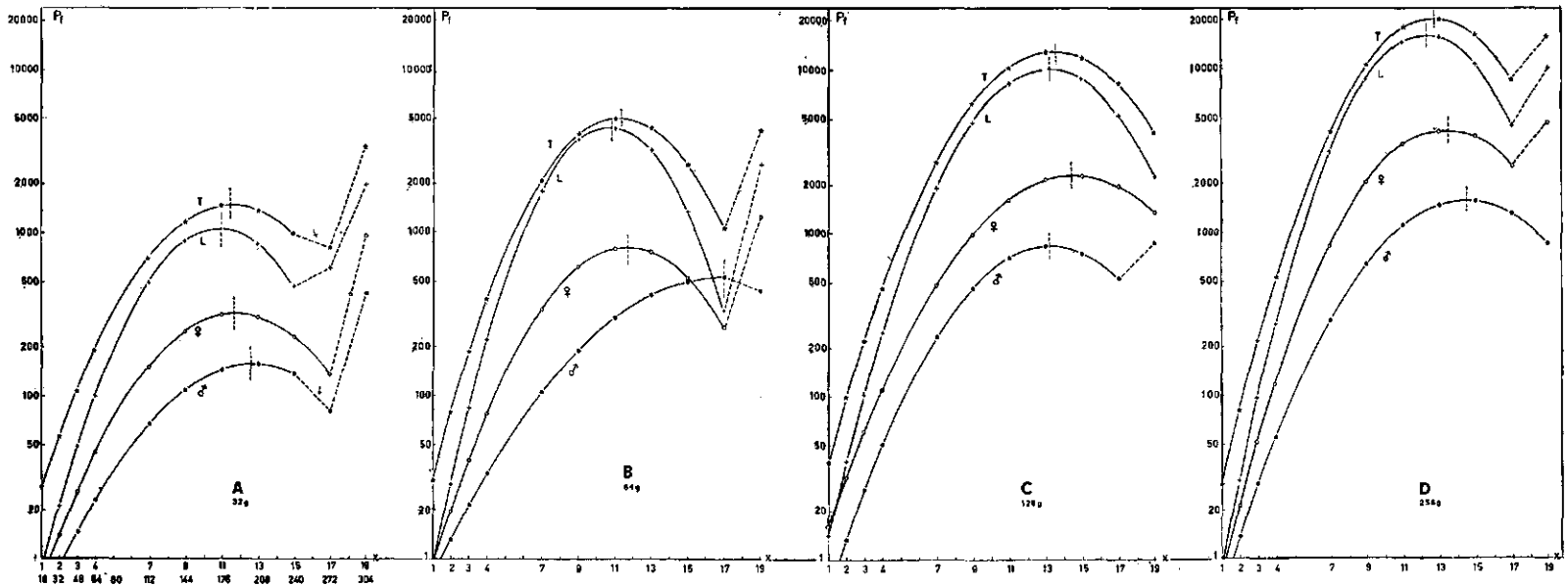


FIG. 34. Multiplication of *T. dubius* with time on ryegrass in environments which differed in space but were uniform in structure and root density (Cf. Fig. 33 and Table 14), calculated and drawn separately for males (♂), females (♀), larvae (L) and totals (T). Densities at the end of the observation period are omitted in the calculations and the points are connected with the calculated curves by broken lines. Vertical broken lines indicate the curve maxima.
Abscissa: X = time, indicated by interval numbers (each interval is 16 days). Also days are recorded under the interval numbers.
Ordinate: P_f = logarithm of the average nematode numbers (average of 5 repilcates; 1 replicate from interval 11 on).

Formulae: *A*.
P_f (T) = 1.103 + 0.357 X — 0.015 X^2
P_f (L) = 0.487 + 0.463 X — 0.021 X^2
P_f (♀) = 0.550 + 0.331 X — 0.014 X^2
P_f (♂) = 0.450 + 0.272 X — 0.011 X^2

B.
P_f (T) = 1.033 + 0.472 X — 0.021 X^2
P_f (L) = 0.333 + 0.617 X — 0.029 X^2
P_f (♀) = 0.547 + 0.405 X — 0.017 X^2
P_f (♂) = 0.657 + 0.247 X — 0.007 X^2

C.
P_f (T) = 1.185 + 0.434 X — 0.016 X^2
P_f (L) = 0.666 + 0.508 X — 0.019 X^2
P_f (♀) = 0.869 + 0.341 X — 0.012 X^2
P_f (♂) = 0.429 + 0.376 X — 0.014 X^2

D.
P_f (T) = 0.936 + 0.530 X — 0.021 X^2
P_f (L) = 0.316 + 0.634 X — 0.026 X^2
P_f (♀) = 0.446 + 0.478 X — 0.018 X^2
P_f (♂) = 0.424 + 0.385 X — 0.013 X^2

week old *Tagetes erecta* seedling, a rooted potato eye taken from the tuber one-week before, or left unplanted (fallow), respectively. At the time of planting 5 tubes of each series were inoculated in the centre with a suspension comprising 0, 3, 30, 300, 3000 and 30000 *T. dubius* from the stock culture reared on grass. After inoculation and planting all tubes were saturated with water and covered with the surrounding soil. All tubes were randomized in the climate chamber running at 25 °C and 12 hours of artificial light. STEINER's nutrient solution and water was given regularly, avoiding excess of moisture, to maintain good growth. After 85 days the tubes were evaluated by determining nematode densities and root and shoot weights.

The results are summarized in Table 16 and in Fig. 35.

Table 16 lists the results for each of the 'crops'. For ryegrass the populations multiplied 19-, 58-, and 9-fold at inoculum densities, P_i, of 3, 30 and 300 per tube respectively in 85 days, whereas the final populations, P_f, were less than the inoculated densities when 3000 and 30000 nematodes were added. For potato the general pattern was the same. *Tagetes erecta* caused a P_f which was lower than P_i for all inoculated densities. The same was true for 'fallow' conditions. The suppressing effect of *T. erecta* was considerably higher than for 'fallow' at all densities, which illustrates the nematicidal effect of this plant for *T. dubius*

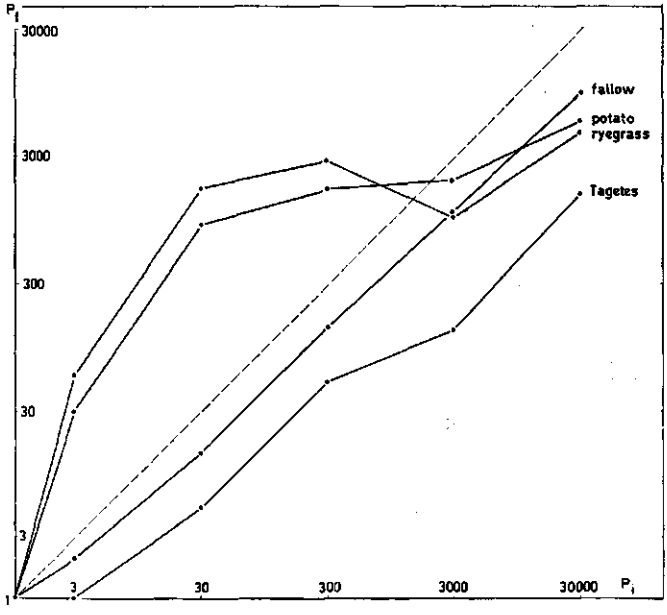


FIG. 35. Final populations of *T. dubius* per tube (P_f) at increasing inoculum levels (P_i), for ryegrass, potato, *Tagetes* and fallow. The straight line is the maintenance line for P_i. Inoculation on 12.12.1966; evaluation 85 days later; all points indicate average results of 5 replicate tubes with 150 g soil each. Abscissa: log inoculated number = P_i. Ordinate: log final number = P_f.

TABLE 16. Final numbers of *T. dubius*, when geometrically increasing numbers, P_i were inoculated per tube of 150 g of soil grown with ryegrass, potato, *Tagetes erecta* or left fallow, respectively. Also root weights in g, y_1, and total plant weights in g, y_2 are recorded, as well as the final populations per g of roots or per g of plant weight. Planting and inoculation date 21.12.1966, evaluation 85 days later. All figures are averages of 5 replications.

	ryegrass					potato					*Tagetes*					fallow
P_i	y_1	y_2	P_f	P_f/y_1	P_f/y_2	y_1	y_2	P_f	P_f/y_1	P_f/y_2	y_1	y_2	P_f	P_f/y_1	P_f/y_2	P_f
0	0.252	1.710	0	0	0	1.376	6.712	0	0	0	0.236	2.358	0	0	0	0
3	0.292	1.700	57	195	34	1.320	6.341	30	23	5	0.360	2.348	0	0	0	2
30	0.064	1.099	1733	27078	1577	1.143	6.939	892	780	129	0.172	2.520	5	29	2	14
300	0.078	0.958	2802	35923	2925	0.892	6.966	1696	1901	243	0.164	1.910	50	304	26	140
3000	0.060	1.045	1034	17233	989	0.891	6.514	1972	2213	303	0.150	1.754	133	887	76	1094
30000	0.066	0.856	4720	71515	5514	0.523	6.916	5888	11258	851	0.182	2.542	1558	8560	613	10000

(cf. WINOTO 1970). Comparison of the three plants on the basis of nematode numbers per g of root weight reveals maximum figures for grass, potato and *Tagetes* of about 71515, 11258 and 8560. The density per g of grass roots, therefore, is extremely high, whereas the density for potato roots was not much higher than for *Tagetes* roots. Density figures per g of total plant weight were low and they differed little between plants.

Fig. 35 illustrates that the courses of P_f for grass and for potato are top curves (sensu OOSTENBRINK, 1966). This is most obvious when we subtract the inoculated levels and consider the maintenance line as the basis. It also reveals that P_f at the highest inoculum levels is suppressed less in fallow than by ryegrass and potato, which indicates that mobilization of a population by suitable hosts may be fatal to the nematodes when they are too numerous.

The general course of the graphs is the same when the densities per g of root weight or per g of plant weight are plotted against P_i. The root weights for all plants and the total plant weight for ryegrass decreased with increasing P_i indicating damage. This is considered in Chapter 6 in more detail. It is obvious from these data that high nematode densities cause damage and that the amount of damage should be taken into account in population studies.

Experiment 2.

A second experiment, similar to Experiment 1 was conducted with rice, wheat, pearl millet and fallow, inoculated with a series of 0, 10, 100, 1000, 10000 *T. dubius* in 150 g soil per tube. All the treatments were replicated five times. The experiment was started on 8.3.1968 and evaluated after six months in the glasshouse with an average temperature of 25 °C, ranging from 23 °C to 27 °C. The results are summarized in Table 17 and Fig. 36.

Table 17 and Fig. 36 show that the inoculated population decreased strongly in fallow soil, whereas reproduction according to top curves occurred on wheat, rice and pearl millet. This was the case for numbers per tube as well as for numbers per g of roots, also when the inoculated densities were not subtracted.

Host suitability decreased in the order wheat-rice-pearl millet. Wheat was apparently susceptible to damage. This was apparently true for pearl millet as well, although it did not maintain high nematode densities. Rice did not show damage and correspondingly maintained higher populations than the other plants when the inoculum dosage was high (cf. Chapter 6).

d. Underpopulation

Experiment 1.

Twenty plastic pots were each filled with 200 g of sterilized sandy PD soil and divided into two series of 10 pots each.

The first series was sown with peas, one seed per pot, and inoculated one week later around the roots by loosening the soil on 1.9.1964 and adding 50 fertilized females and 50 males per pot from the monoculture pea plot population multiplied on peas. The pots were maintained per room temperature, about 20 °C, in

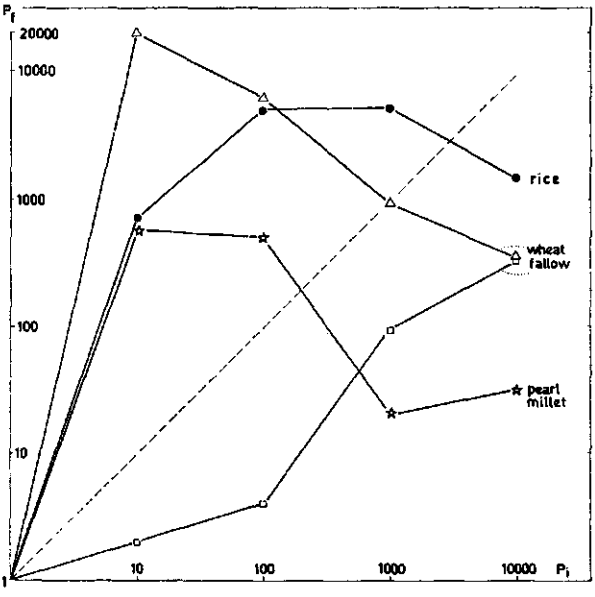


FIG. 36. Final populations of *T. dubius* per tube (P_f) at increasing inoculum levels (P_i) for wheat, rice, pearl millet and fallow. The straight line is the maintenance line for P_i. Inoculation on 8.3.1968, evaluation 6 months later; all points indicate average results of 5 replicate tubes with 150 g soil each. Abscissa: log inoculated numbers = P_i. Ordinate: log final numbers = P_f.

the laboratory; water and nutrient solution were provided regularly. The pots were evaluated for nematode populations 87 days after the inoculation.

The second series was set up and maintained as a parallel experiment, with the following differences: it was started 11 days later and evaluated after 84 days, and the inoculum per pot consisted of 50 obviously fertilized females from the stock culture as in the first series but no males.

The results of both experiments are summarized in Table 18. The numbers of males, females, larvae and totals were all similar; none of the differences was statistically significant. It is obvious that females which are fertilized initially do not need adult males to establish a population. In both these experiments the male to female ratio was about 4 to 10 and differences from the 1 to 1 ratio were highly significant in both cases; in fact males were less numerous than females in all ten replicates of both experiments. This result accentuates the aberrant male to female ratios obtained in the following experiment.

Experiment 2.

Thirty eight plastic pots, 7 cm wide were each filled with 200 g of sterilized sandy PD soil and sown with one pea seed. One week after germination of the seed, on 15.8.1964, each plant was inoculated with one young female and one male of *T. dubius* collected from the monoculture pea plot. The two nematodes

TABLE 17. Final populations of *T. dubius*, when geometrically increasing numbers, P_i were inoculated per tube of 150 g of soil, grown with rice, wheat, pearl millet or left fallow, respectively. Also root weights in grams, y_1, and total plant weights in grams, y_2, are recorded, as well as the final populations per g of root or per g of plant weight. Planting and inoculation date 8.3.1968, evaluation 6 months later. All figures are averages of five replications.

	rice					wheat					Pearl millet					fallow
P_i	y_1	y_2	P_f	P_f/y_1	P_f/y_2	y_1	y_2	P_f	P_f/y_1	P_f/y_2	y_1	y_2	P_f	P_f/y_1	P_f/y_2	
0	3.41	13.97	0	0	0	7.70	18.38	0	0	0	2.88	21.10	0	0	0	0
10	3.58	13.58	697	195	51	4.52	11.80	19406	4293	1645	2.68	16.24	570	213	35	2
100	3.14	13.58	5144	1638	379	5.36	12.52	6324	1180	505	2.12	13.88	504	238	36	4
1000	2.50	12.78	5292	2117	414	4.30	11.02	930	216	84	1.56	10.78	20	13	2	95
10000	3.12	13.26	1520	487	115	3.66	9.68	350	96	36	3.20	14.54	32	10	1	328

TABLE 18. Number of *T. dubius* recovered when 50 young females and 50 males (A) or when 50 obviously fertilized females (B) were inoculated onto a one-week old pea plant grown in 200 g of sandy soil in a plastic pot. Figures are averages of 10 replicate pots, determined 87 (for A) or 84 (for B) days after inoculation.
Significance of differences: – = not significant.

Inoculum per pot	Period of reproduction	Final nematode populations:			
		Males	Females	Larvae	Total
A. 50 females + 50 males	1.9.64–27.11.64	158	395	777	1330
B. 50 fertilized females	12.9.64– 5.12.64	153	387	821	1361
Significance of differences:		–	–	–	–

were inoculated simultaneously at the same spot. All pots were kept at room temperature in the laboratory and regularly provided with water and nutrient solution. 23 pots were randomly examined for nematode reproduction after 87 days and the other 15 pots after 94 days. The results of the two series were not significantly different, and therefore all 38 pots were considered as one series.

From 13 of the 38 pots no *T. dubius* was recovered; 6 yielded 1 adult male only, and the remaining 19 pots yielded more and other specimens of *T. dubius* than were inoculated. The catch from these 19 pots is recorded in Table 19.

Table 19 indicates that the number of nematodes per pot varied from 0 to 47, with an average of 20 for the pots with reproduction and an overall average of 10 for all pots. Therefore, reproduction takes place in most cases when one pair of nematodes is inoculated together. The great variability in the figures may be due to missing or retarded contacts between the single female and the single male inoculated. In addition it may be related to the degree of fertility and the state of fertilization of the young female at the moment of inoculation immediately after being collected from the natural field population.

The most remarkable result is the fact that the male/female ratio was extremely high (3 on an average) and fluctuated strongly between pots. Usually males are less or about as numerous as females. This was the case in only 7 pots. In most of the pots, however, males were far in excess, and there were several pots, including pots with a high number of offspring in which many males were accompanied by only 1 or 2 females only (numbers 1, 2, 16, 17, 34) or by no females or larvae at all (numbers 4, 27, 36). This result is significant in this experiment, but it cannot be explained. It may stimulate further research concerning environmental influence on sex determination (cf. DAO 1970). It is probably not due to the fact that only one male per female was present, for such combinations yielded significantly more females than males in the experiments recorded under 3 and 4.

TABLE 19. Number of *T. dubius* recovered after 87–94 days when one young female and one male were inoculated onto the same root of a one-week old pea plant grown in 200 g of sandy soil in a plastic pot. Thirty eight plants were inoculated; 19 plant yielded newly-formed specimens. The populations are recorded under the plant number given when evaluation took place.

Plant numbers	Nematode specimens recovered:			
	Males	Females	Larvae	Total
1	25	2	2	29
2	33	1	0	34
3	9	4	8	21
4	2	0	0	2
5	0	1	1	2
16	5	2	0	7
17	8	1	5	14
21	28	6	4	38
22	0	2	0	2
23	0	0	2	2
25	1	1	2	4
27	10	0	0	10
30	0	2	0	2
31	10	17	8	35
32	13	13	10	36
33	29	8	10	47
34	28	1	12	41
35	18	6	13	37
36	8	0	0	8
Average	12	4	4	20
19, 20, 24, 26, 29, 37	1	0	0	1
6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 18, 28, 38	0	0	0	0
Overall average	6	2	2	10

Experiment 3.

Twenty plastic pots were filled with 100 g sterilized sandy PD soil. Half of the pots were sown with pea and the other half with ryegrass. One week after germination of the plants, each pot was inoculated with a single fertilized female taken from the stock culture on grass. The pots were randomly arranged in a climate chamber at 20 °C and 12 hours of artificial light per day. STEINER's nutrient solution was given at 2 weeks intervals. The plants grew well in both the series without significant differences in growth. The inoculations were made on 15.6. 1969; 50 days later the final nematode populations were determined.

The results are summarized in Table 20. Four of the pea pots comprised males and females, but one of the pots comprised 47 males against 1 female, and another 4 males against 1 female with a number of larvae showing male development. Four other pots comprised only females and female larvae. The rest two

TABLE 20. Number of *T. dubius* recovered after 50 days when one fertilized female was inoculated onto the roots of one week old peas and ryegrass plants, in 100 g of sandy soil grown in a plastic pot. 10 pots with pea were inoculated; 8 plants yielded newly formed specimens. 10 pots with grass were inoculated; all pots yielded newly formed specimens. The populations are recorded as the average of 10 replications. Significance of differences: – = not significant.

Plants	Nematode specimens recovered:			
	Males	Females	Larvae	Total
Peas	10	8	5	23
	(0–48)	(0–23)	(0–18)	(0–66)
Ryegrass	16	16	6	38
	(0–58)	(1–45)	(1–14)	(7–71)
Significance of difference	–	–	–	–

pots had no reproduction. Of the total population males, females and larvae covered 45.2%, 34.3% and 20.5% respectively.

In the grass pots eight out of ten had reproduction with males, females and larvae. In two pots only females and female larvae were present. The composition of the total population was 43% males, 41.5% females and 15.5% larvae.

The average figures, therefore, mask the fact that individual females often produce only females or males, in large numbers. The hypothesis that more males are formed when food is scarce or adverse environmental conditions exist cannot explain these results, which require further examination.

Experiment 4.

Thirty six plastic pots were filled with 128 g of sterilized sandy PD soil and planted with 4 one-week old grass seedlings each. Then 6 series of 6 replicate pots were inoculated with a geometrically increasing number of *T. dubius* around the host roots, namely 1 single female, 1 female + 1 male, 2 females + 2 males, 4 females + 4 males, 8 females + 8 males and 16 females + 16 males. The nematodes were extracted from a stock culture on pea and were picked from the suspension after extraction without selection with respect to their state of fertilization. Females and males of each inoculum were together inoculated at one spot onto the roots. The spot were randomly arranged on a glass house bench at about 25 °C temperature and 12 hours of artificial light per day. Water and nutrient solution were added at intervals. The plants grew well at all inoculum densities without significant growth differences between the series. The inoculations were made on 4.12.1965; 100 days later the final nematode populations were extracted from all pots and analysed. The results are summarized in Table 21 and in Fig. 37.

The nematode numbers recorded in Table 21 indicate that reproduction occurred at all inoculum densities, but also that there was underpopulation at low densities.

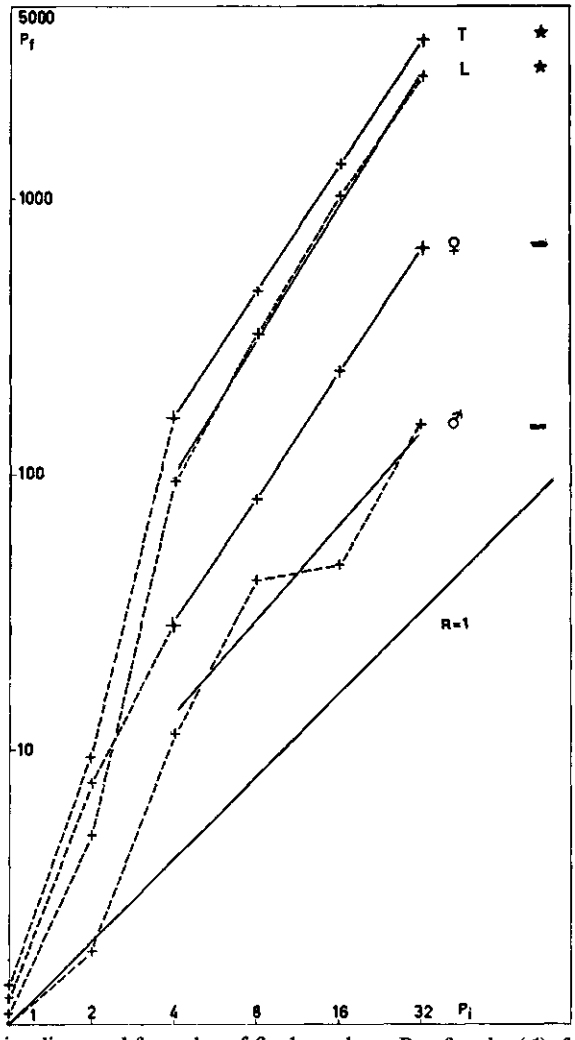


FIG. 37. Regression lines and formulae of final numbers, P_f, of males (♂), females (♀), larvae (L) and totals (T) of *T. dubius* on inoculated densities, (P_i), from 4 to 32 nematodes per pot. The observed values are indicated from 1 to 32 nematodes per pot, averages of 6 replicates, and connected by broken lines. The calculated regression lines are drawn solid; the maintenance line for which the reproduction rate R = 1 is also added; the regression coefficients of the lines for T and L differ significantly from the maintenance line at the 5% point (*), and are therefore steeper, whereas the lines for ♀ and ♂ are not. Abscissa: P_i = log number of nematodes inoculated per pot of 128 g soil. Ordinate: P_f = log final number of nematodes per pot after 100 days. Regression formulae of the logarithm of the final nematode numbers (log P_f) on the logarithm of the inoculated numbers (log P_i, however coded so that x = 1, 2, 3, 4 for P_i = 4, 8, 16, 32) for the different nematode categories:

log P_f (T) = 1.755 + 0.460X
log P_f (L) = 1.505 + 0.496X
log P_f (♀) = 1.008 + 0.456X
Log P_f (♂) = 0.784 + 0.344X

TABLE 21. Number of *T. dubius* recovered after 100 days when 6 geometrically increasing densities were inoculated to pots of 128 g soil grown with four ryegrass seedlings. Figures are per 6 replicate pots, separately for males (♂), females (♀), larvae (L) and totals (T). The average reproduction rates for males, females and total populations are recorded between brackets (R = P_f/P_i, i.e. final numbers divided by initially inoculated numbers).

Inoculated nematodes	Final nematode populations:			
	♂	♀	L	T
1 (1♀) × 6	0 (0.0)	2 (0.3)	2	4 (0.7)
2 (1♀ + 1♂) × 6	7 (0.6)	41 (3.4)	45	93 (7.8)
4 (2♀ + 2♂) × 6	129 (5.4)	295 (12.3)	986	1410 (58.7)
8 (4♀ + 4♂) × 6	315 (6.6)	651 (13.6)	2690	3656 (76.2)
16 (8♀ + 8♂) × 6	450 (4.7)	2020 (21.0)	8980	11450 (119.3)
32 (16♀ + 16♂) × 6	1090 (5.7)	5570 (29.0)	20430	27090 (141.1)

Reproduction from the single females was practically nil; in two of the six replicate pots the inoculated female was recovered, and no offspring was found except in one pot where 2 larvae were present. The female in this pot had probably mated before it was collected and inoculated. Mating apparently is necessary for reproduction of *T. dubius*, for reproduction occurred in five out of six replicate pots when the single female was provided with a single male.

The reproduction rates at low densities were significantly lower than at high densities, which indicates an effect of underpopulation, despite the fact that a single fertilized female can already establish a colony. This was certainly the case for densities up to 4 nematodes per pot of 128 g of soil, but it may hold for higher densities. In Fig. 37 final densities (P_f) are plotted against initial densities (P_i) on a double logarithmic scale and the resulting line is similar to the sinusoid part of the reproduction curve for which KLOMP *et al.* (1964) and OOSTENBRINK (1966) published a formula. The course of $R = P_f/P_i$ in this figure appears to be nearly rectilinear for the inocula from 4 nematodes onwards per pot. This holds for males, females, larvae as well as total numbers. The final densities of all four categories are therefore calculated and drawn as rectilinear regressions on the inoculated numbers from 4 to 32 nematodes per pot. The regression coefficients of these lines are compared to the regression coefficient of the maintenance line ($R = 1$); the difference is significant for total nematodes and larvae and this means that the reproduction rate increases with increasing P_i also in the area of P_i between 4 and 32 nematodes per pot. This suggests that underpopulation was still effective at these inoculated densities, although to a lesser extent than at densities from 1 to 4 nematodes per pot. The lines also indicate that larvae were more numerous than females and that females were more numerous than males at all inoculated densities from 4 to 32 per pot.

Fig. 38 illustrates the male to female ratios for all inoculum categories, from 2 to 32 nematodes per pot, calculated from individual replicates. The results in-

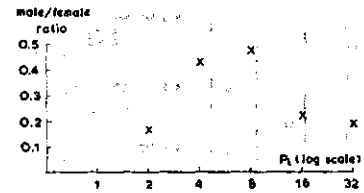


FIG. 38. Male/Female ratio of *T. dubius* with 2 to 32 nematodes (1 to 16 pairs of adults) per pot.

dicate that males were less numerous than females at all inoculum densities, also when one female and one male were combined. The dominance of males in the offspring found in some earlier experiments, can therefore not be due to the presence of one male per female.

Experiment 5.

One hundred eight plastic tubes of 4 × 4 × 20 cm were filled with 350 g of sterilized sandy PD soil and planted with 2 one-week old ryegrass seedlings. Then 6 series of 18 tubes were inoculated, as in Experiment 4, with 1 single female, 1 female + 1 male, 2 females + 2 males, 4 females + 4 males, 8 females + 8 males, and 16 females + 16 males. The nematodes were extracted from a stock culture on grass and were handpicked from the suspension; only recently molted, obviously unfertilized females were selected in this experiment. All individuals i.e. males and females were inoculated at regular distances from each other at 2.5 cm depth in the soil. These horizontal distances varied between males and females from about 0.7 to about 2.8 cm, depending on inoculated numbers. The tubes were randomly arranged in trays in the climate chamber at 18°C and 12 hours of artificial light per day. Water and STEINER's nutrient solution were added at intervals. All plants grew well without significant differences between the different inoculation series. The inoculation were made on 19.4.1966.

The final nematode populations were evaluated from three replicate tubes of each series after 1, 2, 3, 4, 5 and 6 months (= at an interval of 30 days). The results are summarized and illustrated in Table 22 and Figs. 39 and 40.

Table 22 shows, that the single females did not show reproduction in any of the tubes. Reproduction occurred when 1 female + 1 male were introduced, but it was slight and erratic and did not lead to well-established populations through out the experimental period. The same held true, when 2 females + 2 males were inoculated, although populations became distinctly higher after 4 months. Higher inocula reached well-established populations after 2 months, although the differences between 4, 8 and 16 pairs of nematodes were still measurable after 6 months. Here also underpopulation obviously influenced the results. The male to female ratio was, as in Experiment 4, lower than 1, also when 1 female + 1 male were inoculated.

Table 22 shows that the reproduction rate for the total populations after 4, 5 and 6 months, P_f, increases with inoculum density, P_i, as in experiment 4, and this suggests the influence of underpopulation for densities of 1 up to 16, and possibly 32 nematodes per tube of 350 g of soil. When log P_f is plotted against

TABLE 22. Final *T. dubius* numbers for males (♂), females (♀), larvae (L) and totals (T), recovered after 1, 2, 3, 4, 5 and 6 months when 6 geometrically increasing densities were inoculated to tubes of 350 g soil grown with two ryegrass seedlings. Inoculating date: 19.4.1966. Figures are averages per 3 replicate tubes.

Time in months	1				2				3				4				5				6			
Inoculated nematodes	♂	♀	L	T	♂	♀	L	T	♂	♀	L	T	♂	♀	L	T	♂	♀	L	T	♂	♀	L	T
1(1♀)	0	1	0	*1*	0	1	0	*1*	0	1	0	*1*	0	1	0	*1*	0	1	0	*1*	0	1	0	*1*
2(1♀ + 1♂)	1	1	3	*5*	1	1	7	*9*	5	8	6	*19*	1	12	15	*28*	1	0	0	*1*	0	6	0	*6*
4(2♀ + 2♂)	1	2	3	*6*	1	1	5	*7*	1	3	2	*6*	12	14	45	*71*	9	3	7	*19*	26	71	129	*226*
8(4♀ + 4♂)	3	3	1	*7*	10	5	32	*47*	55	42	74	*171*	7	82	102	*191*	43	180	445	*668*	80	366	420	*866*
16(8♀ + 8♂)	4	4	1	*9*	7	13	130	*150*	55	66	145	*266*	64	92	139	*295*	123	500	2260	*2883*	580	620	1760	*2960*
32 (16♀ + 16♂)	9	5	6	*20*	11	11	128	*150*	94	124	268	*486*	183	817	2527	*3527*	480	1056	6396	*7932*	413	1346	3030	*4789*

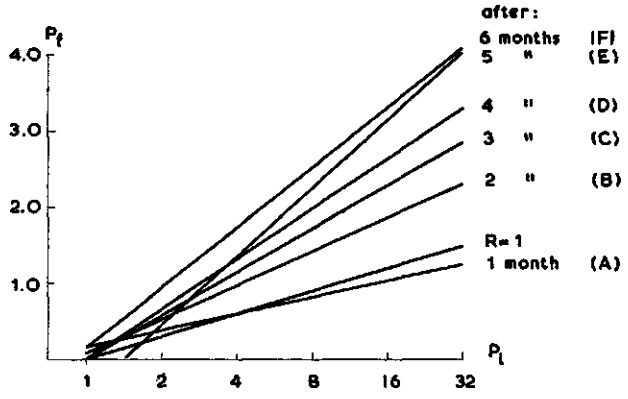


FIG. 39. Regression lines of final *T. dubius* densities on inoculated densities per pot for the different observation or reproduction periods as indicated in the graphs, calculated from the averages of 3 replicates. The regression line R = the maintenance line for all inoculated densities. Abscissa: P_i = number of inoculated nematodes per tube of 350 g of soil (log scale). Ordinate: P_f = final number of nematodes per tube after 1, 2, 3, 4, 5 and 6 months (log scale). The regression formulas are not recorded, but the significance of the differences between the regression coefficients, i.e. the reproduction rates illustrated by the slope of the lines, are calculated and recorded below:

B	C	D	E	F	R	
**	***	***	***	***	*	A
	–	*	***	***	*	B
		–	**	**	***	C
			–	–	***	D
				–	***	E
					***	F

– = difference not significant
* = significant at 0.05
** = significant at 0.01
*** = significant at 0.001

log P_i we obtain reproduction curves of which the major part does not deviate much from rectilinear regressions. Therefore the regression lines were calculated and drawn separately for the different inoculum dosages (Fig. 39) and for the different observation periods (Fig. 40), and statistical significance of differences was calculated and added below the figures.

Fig. 39 shows that the reproduction rates change from lower to higher inoculum levels, and that many of the differences are significant. A, after 1 month, differs significantly from all other observation periods and the slope indicates that in this case the corresponding reproduction rate *decreases* significantly from lower to higher inoculum level. This must be due to higher extraction loss or less survival when more nematodes are inoculated; reproduction had apparently not yet taken place after 1 month. The reproduction rates at all other observation dates, 2 to 5 month, increase with increasing inoculum level. The slope of B is significantly different from D, E, F, but not from C; the slope of C differs significantly from E and F, but not from D, the slopes of D, E and F do not differ significantly from each other. This means that after 2 and 3 month the highest inoculum densities had still not yet reached optimum reproduction rate, due to the effect of underpopulation.

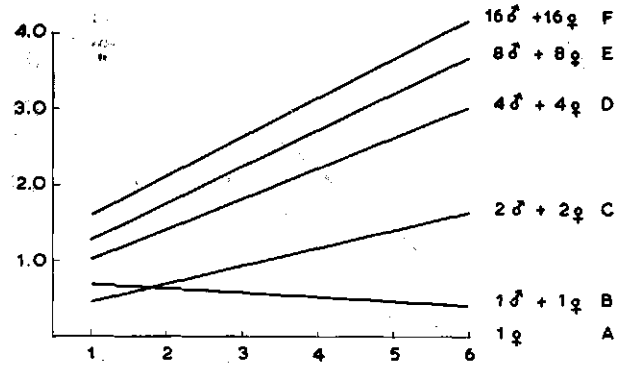


FIG. 40. Regression lines of final *T. dubius* numbers on observation period in months, separately for the different inoculation levels as indicated in the graphs, calculated from the averages of 3 replicates. Abscissa: observation or reproduction period in months. Ordinate: P_f = final number of nematodes per tube of 350 g of soil (Log scale). The regression formulas are not recorded, but the significance of the differences between the regression coefficients, i.e. the reproduction rates illustrated by the slope of the lines, are calculated and recorded below:

B	C	D	E	F	
–	*	***	***	***	A
	*	***	***	***	B
		–	*	*	C
			–	–	D
				–	E

– = difference not significant
* = significant at 0.05
** = significant at 0.01
*** = significant at 0.001

Fig. 40 shows, that the reproduction rate, i.e. the slope of the lines, changes with the duration of the observation period, except for A and B, the inoculum dosages of 1 ♀ or 1 ♀ + 1 ♂ per tube, which show no or no persistent reproduction. All the other inoculum dosages show not only an increasing reproduction rate with time, but also that the rate of reproduction increases less steep for the lower values of P_i, which again characterizes underpopulation. It appears that the reproduction rate of C, 2 ♀ + 2 ♂ per tube, is significantly less than E and F, but not than D; D, E and F are not significantly different. This indicates that the inoculum dosage C, 2 ♀ + 2 ♂ per tube, was too low to reach the same rate of reproduction as the higher inoculum levels in the period of 6 months, due to the effect of underpopulation. With 1 ♀ + 1 ♂ per tube reproduction is erratic and on an average not yet measurable, and with 2 ♀ and 2 ♂ it is still suboptimal after 6 months.

4.4.4. *Predation by micro-arthropods* (Fig. 41 A).

Experiments were made under controlled conditions to study predation of *T. dubius* by the mite *Lasioceius penicilliger* in vitro (a) and in soil (b), by the mite *Rhodacarus roseus* in soil (c), and by several predatory mites and springtails, alone or in combinations, in soil (d).

a. *Lasioceius penicilliger* in vitro

The predatory mite *L. penicilliger* was inoculated into vials with a pure population of *T. dubius* to study the predation capacity of the mite in vitro. Ten glass vials, 3 cm in diameter and 1.5 cm deep, were filled with gypseum and soft coke powder up to 1 cm deep after which water was added. This provided a hard, porous substratum which was continuously moist, but which could not be penetrated by the nematode or the mites. The behaviour of the predator and prey animals could easily be observed against the black background under the dissecting microscope.

One hundred adult *T. dubius* specimens, 50 males and 50 females, were handpicked from the stock culture on grass and transferred into each vial in a drop of water by means of a micropipette. In five vials 10 adult specimens of *L. penicilliger* were transferred by means of a bamboo pick and the five remaining vials were kept as controls. Care was taken that the substratum was saturated with water, without however a water layer above the surface. All vials were covered with a glass slide and sealed with vaseline to avoid escape of the mites from the vials. They were placed in the moist atmosphere of a large petridish with some water on the bottom and kept at room temperature in the laboratory.

Examination of the vials under the dissecting microscope showed, that the mites could easily catch the nematodes and started eating them immediately after inoculation. They often swallow a whole nematode from the tail or from the middle of the body in about 5 minutes, while the prey still moved. Large and thick nematodes may be cut into pieces, which are swallowed or chewed and sucked empty; in the latter case the empty skin often left behind. During feeding the mites are not easily disturbed, but the approach of other mites makes them agressive. Shortly after feeding they may not take notice of nematodes moving or lying before them. The mites evidently felt comfortable in the containers; even copulations took place.

After 48 hours all vials were evaluated by washing the nematodes and the mites into a dish and by counting them. The results are summarized in Table 23. All nematodes from the uninoculated and all mites and nematodes from the inoculated vials were recovered. It appears from the table, that the mites consumed 90% of the nematodes, therefore 9 nematodes per mite, within 48 hours.

TABLE 23. Predation of *T. dubius* by the mite *Lasioceius penicilliger* in vitro, when 10 adult mites were inoculated to vials with 100 adult nematode specimens. Nematode number and mite numbers determined after 48 hours; averages of 10 replicates. The percentages predation was 89.8 ± 1.04, and was statistically significant at the 5% confidence level.

Treatments	Nematodes alone	Nematodes + mites	Difference (= predation)	Mites recovered
Animals recovered	100	10 (9–11)	90 (89–91)	10

The experiment illustrates the predacious nature and the great predation capacity of the mite.

b. *Lasioceius penicilliger* in soil

This mite was also inoculated in soil with a population of *T. dubius* artificially established on pea. Fifteen plastic pots of six centimeter diameter were partially filled with 100 g sterilized sandy soil and one pea seed was sown per pot. One week later each pot was provided with 100 adult specimens, 50 males and 50 females, of *T. dubius* from the stock culture on grass. Ten of the pots were further inoculated with 5 adult specimens of *L. penicilliger* and the other 5 were kept as controls. The mites were picked from a culture raised on nematodes in glass vials, but originally collected from the soil of the crop rotation trial field at the PD, Wageningen. The edges of all pots were smeared with vaseline against escape or penetration of mites. All pots were placed in trays with water and mineral oil to avoid contamination, and randomized on a glasshouse bench. Distilled water and nutrient solution were provided at intervals. All plants grew well and the pots were evaluated after 5 weeks. The nematodes were extracted by elutriation from the 5 un-inoculated pots and from 5 of the inoculated pots; the other 5 inoculated pots were used for extraction of the mites in modified TULLGREN funnels. The results are summarized in Table 24:

It appears from the results in Table 24 that the nematodes as well as the mites reproduced during the experiment, but also that the inoculation of the mites caused a significant reduction of the nematode numbers with 44%.

c. *Rhodacarus roseus* in soil

The mite *R. roseus* was inoculated in soil with a population of *T. dubius* artificially established on ryegrass.

Twelve glass tubes were filled with 30 g sterilized sandy soil and a one-week old seedling of ryegrass was planted per tube. Each tube was provided with 35 adult specimens, 25 mature females and 10 males, of *T. dubius* selected from the stock culture on grass. Eight of the tubes were further inoculated with 5 adult specimens of *R. roseus* and the other 4 were kept as controls. The mites were picked from a natural population extracted from the grass/clover plot in the

TABLE 24. Predation of *T. dubius* by *Lasioceius penicilliger* in soil, when 5 mites were inoculated to plastic pots with a pea plant growing in 100 g sterilized PD sandy soil provided with 100 adult nematodes each. Nematode numbers and mite numbers determined after five weeks; averages of 5 replicates. The percentage predation was 44.4 ± 6.6 and was statistically significant at the 5% confidence level.

Treatments	Nematode alone	Nematodes + mites	Difference (= predation)	Mites recovered
Animals recovered	191 (145–229)	106 (73–138)	85 (33–154)	6 (4–7)

crop rotation trial field at the PD, Wageningen. The edges of all tubes were smeared with vaseline and kept randomized but apart from each other in a rack placed in the laboratory under artificial light for 16 hours per day. The temperature of the tubes was 22° to 24°C, but rose to 25°C when the lights were on. Distilled water and nutrient solution were provided at 3 days intervals. All plants grew well and the tubes were evaluated after one month. Extraction was done as in the previous experiment, now with 4 replicates for 'nematodes alone', 'nematodes + mites' and 'mites'. The nematode populations are analysed in detail and the results are summarized in Table 25.

TABLE 25. Predation of *T. dubius* by the mite *Rhodacarus roseus* in soil, when 5 mites were inoculated to glass tubes with a ryegrass seedling growing in 30 g sterilized sandy soil provided with 25 mature female and 10 males of the nematode each. Nematode numbers and mite numbers determined after one month, are averages of four replicate tubes. For the nematode populations males (♂), females (♀), larvae (L) and totals were recorded.
Significance of differences: − = not significant, x = significant at 5% level, xx = significant at 1% level.

Treatments	♂	♀	L	Total
Nematodes alone	7.5	20.3	120	147.8
Nematodes + mites	5.3	13.8	3.5	22.5
Difference (= predation)	2.2^{-}	6.5^{-}	116.5^{xx}	$125.3^{xx} = 85\%^{xx}$
Mites recovered				2

Table 25 shows that the nematode numbers had increased more than fourfold in the tubes without mites. In the tubes with mites there must also have been some reproduction, for all populations comprised larvae in addition to adults, but the total numbers were lower than the inoculated numbers. Predation significantly reduced the total numbers to about 85% of the initial value. The detailed figures show that the number of larvae was much more reduced than the number of males or the number of females, and therefore that *R. roseus* probably catches larvae much more efficiently than adults. Despite strong predation on the nematodes the number of mites recovered was lower than the inoculated number. This may be due to incomplete extraction of the final mite population or to cannibalism, which is known to occur within the populations of *R. roseus* and other predatory mites.

d. Several mites and springtails in soil

The micro-arthropod population in the PD trial field comprised several species which were known to prey upon nematodes in vitro. The most numerous predators, extracted from this soil, were the mites *Rhodacarus roseus*, *Pergamasus runcatellus*, and *Hypoaspis aculeifer*, and the springtail *Tullbergia krausbaueri*. These species, and also the springtail *Onychiurus armatus*, of which a culture was available in the laboratory, were studied separately and in combination for their predatory effect on *T. dubius* in soil.

One hundred twenty glass jars of 100 ml capacity were filled with 18 g sterilized fine gravel and, on top of it, 75 g sterilized sandy soil from the PD trial field; two straws were inserted for better aeration of the bottom layer and for draining away excessive moisture if necessary. An aseptically-raised six-day-old seedling of grass was planted in each jar. Each of 114 jars was provided with 25 adult specimens, 20 females and 5 males, of *T. dubius* from the stock culture on grass; 6 jars were left nematode-free (Control I). Throughout the experiment STEINER's nutrient solution was supplied fortnightly (and distilled water was added to maintain favourable moisture conditions). All jars were placed in metallic trays filled with water and mineral oil; the trays were placed in a climate chamber at 22 °C and 12 hours artificial light. All plants grew well and 51 days after the inoculation of the nematodes their population was determined by extraction and analysis of 6 inoculated jars (Control II). This population density served as the initial population for the second phase of the experiment which started with the inoculation of the predators and lasted 77 days.

The three mite species and two springtail species were inoculated in such a way that apart from the above mentioned controls I and II nine further treatments, number 1–9, were formed, namely; (1) nematodes alone (no predators), (2) all three mites + two springtails, (3) all three mites, (4) *Rhodacarus roseus*, (5) *Pergamasus runcatellus*, (6) *Hypoaspis aculeifer*, (7) the two springtails, (8) *Tullbergia krausbaueri* (9), *Onychiurus armatus*. There were 12 nematode-infested replicates for each treatment. In treatment (2) 2 adult specimens of each of the five mite and springtail species were inoculated per pot. In treatment (3) 2 adults of each of the three mite species and in treatment (4) 3 adults of each of the two springtail species were inoculated per pot. In the other treatments with single predator species the inoculum was always 6 adult specimens per jar.

The plants were treated as before and maintained good growth, although growth difference appeared between as well as within treatments during the second phase of the experiment. Seventy seven days after inoculation of the possible predators all jars were evaluated; nematodes were extracted by elutration from six replicates and predators by funnel extraction from the remaining six replicates of each treatment. The results are summarized in Table 26.

The results were analysed statistically, and can be described as follows: The un-inoculated pots (Control I) were still nematode-free at the end of the experiment. The pots inoculated with 25 nematodes had built up a total density of 508 nematodes per pot after 51 days (Control II). This density (Treatment (1) = no predators) increased further in the following 77 days for all nematode categories, and the total final density was 5567 per jar with 75 g soil. All the other treatments (2) to (9), had lower final densities than (1), although (4) and (5) were only slightly lower, whereas (3), (6), (2) and (7) were considerably lower. Analysis of variance, followed by comparison of logarithmic nematode densities of different treatments indicated that (1), (4) and (5) differed from (3), (6), (2) and (7). Despite great and significant differences between the 9 treatments, (1) did not differ significantly from the rest, when all treatments (2) to (9) were taken together. This difference, however, was very significant when young second

TABLE 26. Predation of *T. dubius* by 3 mite species, 2 springtail species, or combinations, in soil. The predators were inoculated to glass jars, 51 days after a ryegrass seedling was planted in 75 g sterilized sandy soil to which 20 females and 5 males of *T. dubius* were added. Nematode numbers and predators number, determined 77 days after the predators were inoculated, are averages of six replicate jars. For the nematode populations the young second-stage larvae (L_{2y}), old second-larvae (L_{2o}), third- and fourth-stage larvae ($L_3 + L_4$), adult (♂ + ♀) and totals were recorded.

Treatments	Total number of predator specimens:		Nematodes:				
	Inoculated	Recovered	L_{2y}	L_{2o}	$L_3 + L_4$	♂+♀	Total
Control I = no nematodes	0	0	0	0	0	0	0
Control II = nematodes at the time of inoculation of predators	0	0	15	82	277	134	508
(1) No predators = nematodes alone	0	0	788	1277	2562	940	5567
(2) All mites + all springtails	10	0	53	390	1327	445	2215
(3) All mites	6	0	50	285	996	350	1681
(4) *Rhodacarus roseus*	6	5	133	710	3320	952	5115
(5) *Permagasus runcatellus*	6	2	180	972	2841	781	4774
(6) *Hypoaspis aculeifer*	6	1	52	198	995	540	1785
(7) Two springtails	6	323	172	592	1355	583	2702
(8) *Tullbergia krausbaueri*	6	47	195	560	1975	903	3633
(9) *Onychiurus armatus*	6	305	43	323	1530	693	2589

stage larvae were considered, which category is apparently most susceptible to predation. This appears from the numbers in Table 26, but also when the frequency of different nematode categories is calculated as a percentage. Table 27 shows that the second stage larvae, especially the young specimens (L_{2y}) are relatively less frequent when predators are added, compared to 'no predators'.

It is clear that several treatments have suppressed the nematode population, whereas others have not. All mites (3), *Hypoaspis aculeifer* (6), all mites + springtails (2) and all springtails (7), have been effective, decreasingly in this

TABLE 27. (cf. Table 26). Frequency of the different nematode stages, expressed as percentages of the total populations, for (1) = no predators, compared with all treatments with predators (2) to (9).

Treatments Nematode stages	(1) = no predators	(2) to (9) = predators
L_{2y}	*14*	*4*
L_{2o}	*23*	*16*
L_3	40	51
L_4	6	8
♂	9	11
♀	8	11

order according to Table 26. Suppression of the nematode density, however, was not closely related to the final predator densities. The springtail densities were high except for treatment (2) in which they were inoculated together with the mites. There were also differences between treatments for plant and root weights which may have been caused by the nematodes, or by springtails which are known to feed also on living roots) or by other causes, but which at any rate may have influenced the final nematode densities. In fact the results in Table 26 may comprise all interrelations between nematode, micro-arthropod, and plant. It is probable that the differences between the treatments recorded in Table 26 are the complex result of several processes, as under natural conditions. The fact that the inoculation of micro-arthropods in soil resulted in reduction of the nematode population, as in earlier experiments, stands. The significance, however, of this phenomenon for the control of nematodes in practice cannot be estimated from these data.

4.4.5. *Other predators, parasites and diseases*

Three organisms were noticed on or in *T. dubius* which are probably noxious to the nematode. None of them could be identified and they may be new. They were not extensively studied, but they are briefly described under a, b and c.

a. Large, one-celled blister-like organisms filled with protoplasm were found attached to the body of incidental specimens of *T. dubius* without causing visible damage to the cuticle of the nematode (Fig. 41B). They may be Protozoa related to *Duboscqia penetrans* (Thorne 1940, Kuiper 1958), but their shape is different and they are much larger.

b. Incidental nematodes were found with their body completely filled up with large spore-like structures in which dense protoplasm and a nucleus was visible (Fig. 41 C). Their origin is unknown. The shape of the nematode body suggests that it was still turgescent when it was filled up with the spore like-structures, and therefore that they may have killed the nematode.

c. Eggs were sometimes covered by numerous rod-shaped bacteria, which were all orientated in concentric rows perpendicular to the egg surface (Fig. 41 D). Neither the role of these bacteria nor their identity is known.

4.5. Discussion

The data about movement and feeding, host plants, survival and influence of the physical and biotic environment have given a general insight into the ecological relations of the nematode.

All larval and adult stages of the nematode, including the molting specimens, are active crawlers. This is in accordance with body length and frequency of undulation in water, i.e. with Wallace's 'Length Frequency product', which is about 2 for the L_2 and 8 for the adults. Their locomotion, an undulation of the whole body in the horizontal plane, causes regular ichnograms on a smooth agar surface (Fig. 13) but adapts itself to the structure of the soil under natural con-

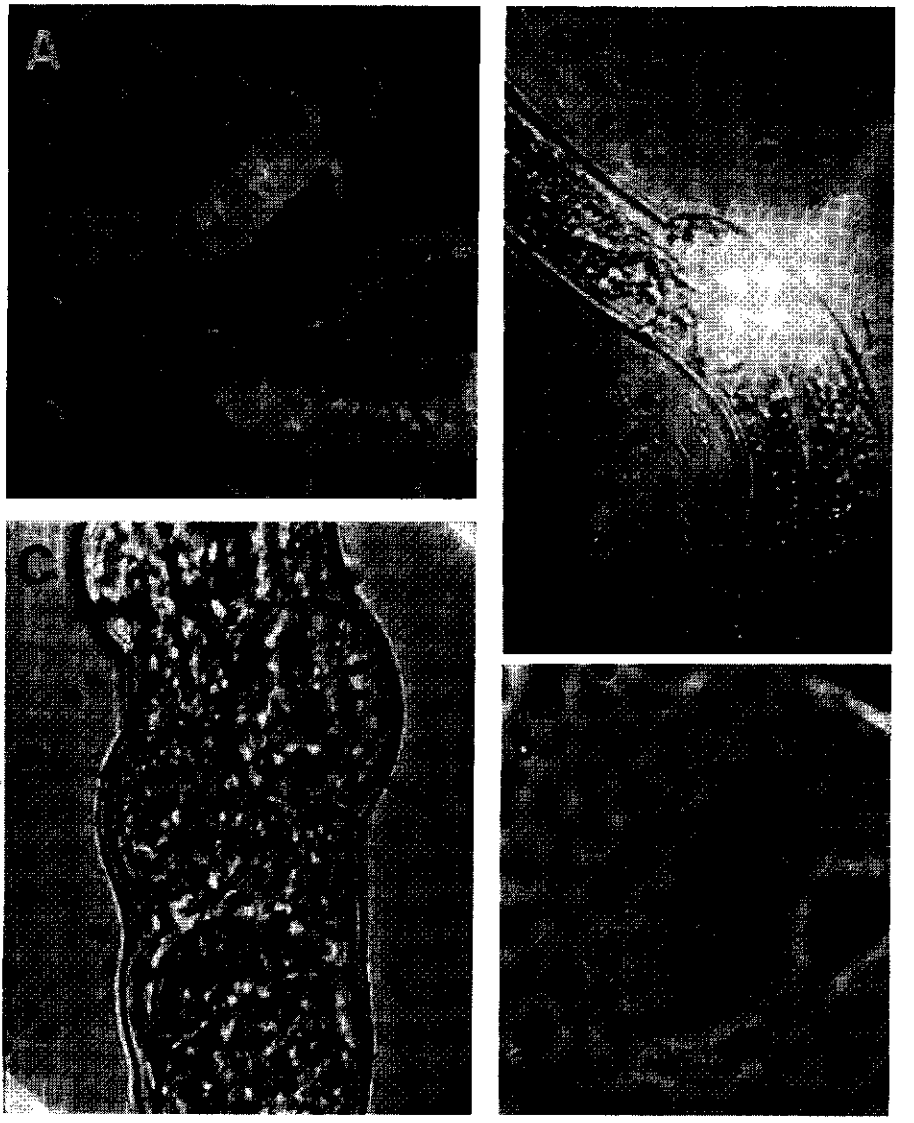


FIG. 41. Predation and parasitism of *T. dubius*
A. The springtails *Onychiurus bicampatus* eating a nematode.
B. One-celled blister-like organisms on the body of *T. dubius*.
C. *T. dubius* body filled with spore-like structures.
D. Rod-shaped bacteria in concentric rows perpendicular to egg surface.

ditions, as known for some other species. Observations suggest that *T. dubius* specimens move continuously, or are ready to move without delay as soon as conditions allow this. They are not even at rest during copulation or feeding.

Constant motility of nematodes, as long as environmental conditions permit this, may be general for many ectoparasitic and free-living species.

Another phenomenon, which may hold for many nematode species, is the great tolerance of specimens of each other. The great densities they reach in soil and on roots (Fig. 1) and the fact that specimens frequently touch each other obviously does not disturb them. The formation of clusters of moving specimens in water in *T. dubius* as in a number of other species, is unexplained, but it is unusual and need not be considered here further. Experiments showed that all stages of *T. dubius* can travel horizontally for at least a few centimeters through the soil within a few days. All stages could travel vertically for more than 17,5 cm within 10 days, although most specimens did not reach more than 5 cm. *T. dubius* specimens, therefore, will normally be able to reach rootlets of a growing crop. It is remarkable that the host itself does not attract the nematodes or influence the direction of the movement in this species.

T. dubius is an ectoparasite of roots of higher plants. All active stages, from L_2 to adult females and males, were seen feeding on the cortex cells of host plants in agar cultures illuminated by the microscope lamp (Figs. 1 and 15).

They were apparently not annoyed by the artificial conditions under which the observations took place. None of the stages was ever seen feeding on other organs of higher plants or on lower plants or other organisms or dead substrates, and they were never found inside the roots. The nematode can thrive on many plant species. The facts that populations are reduced to very low densities in fallow soil after 1–2 years, and that no measurable densities are ever found in unplanted soil, indicate that algae and fungi are not suitable as a food source. Also the possibility cannot be excluded that the nematodes feed and maintain a very low density on algae or fungi. Most nematodes feed in the root elongation zone or on the tip of host rootlets of 0.1–1 mm thickness; feeding on the root hairs was never observed. They apparently require roots of a certain thickness. Cells used for feeding generally show a yellow-brown discoloration as was observed for a number of plants. These details complement the description of the feeding process by KLINKENBERG (1963).

T. dubius populations have been recorded by OOSTENBRINK (1966) to be resistent in the field. Our laboratory experiments confirmed this, for a mixed population lost only 6% of its log density per month in pots with fallow soil at temperatures between 10°–20°C, whereas the population increased according to an exponential curve or a second-degree curve when ryegrass was grown (Fig. 16). The result illustrated in Fig. 17 that the persistance against starvation is very low for young second-stage larvae (L_{2y}) in comparison to the other stages is considered of much interest. The L_2 was also more susceptible than the other larval stages and the adults to low soil temperatures of −11°C (Fig. 18) and of −6°C (Table 6) and to a high soil temperature of 30°C, whereas 5°C conserved the populations and even allowed some development (Table 5). Long spells of low temperature, −11°C, were fatal to the whole population, but *T. dubius* populations survived alternating temperatures which simulated the Dutch winters without much damage. The same was true for *R. robustus*, several other plant

parasitic nematodes and the group of saprozoic nematode species occurring in the same soil (Fig. 19). Moisture stress, drought, as well as excess of moisture also affected the population of *T. dubius* in soil, and these effects can be used for nematode control in practice. The population was extinct after 6 weeks when thin layers of soil were gradually dried in the air at 20°–26°C in the laboratory. There were still 5% survivors, belonging to the L_4 and adult stages, when infested soil was kept flooded with water under similar laboratory conditions. L_2 was again more susceptible than the other stages to both these adverse conditions, drought and flooding. The low persistence of the young L_2 specimens against starvation and unfavourable conditions of temperature and moisture is a striking phenomenon, which helps to explain the marked seasonal population fluctuation of *T. dubius*.

The influence of the environment on a developing population of a plant nematode in soil is very complex as was illustrated in detail by DAO (1970). The abiotic factors studied in our experiments are soil temperatures, soil moisture and soil type. The chemistry of the soil solution and soil air were not included: other workers found that they were usually not determinant for the fate of an established nematode population (UPADHYAYA 1969). The graphs in Figs 22–25 indicate that the influence of temperature on the reproduction of *T. dubius* can be represented by the common type of thermogram with an optimum. It appears that a soil temperature of 25°C initially favours population increase most, but that 19° and 22°C are as favourable in the long run, whereas 16°C or lower and 30°C or higher are unfavourable. The favourable range from 19°–25° is considerably higher than was expected from the results published by DAO (1970) and from the main distribution area of the nematode. It may well be that especially the potency to start activity at 13°C (or perhaps even at 5°C – cf. Table 5) determines the fact that this nematode is particularly widespread in temperate climates with a cool spring. Excess of moisture was unfavourable to the nematode, even when the suitable host rice was grown (Table 8). Inoculation of the nematode in different soils extended the insight already obtained from field observations. The nematode reproduces best in sandy soils, except when a high humus (peat) content is present as in the 'Veenkoloniën' and in some pot soils, and pH is not determinant. The results stress the necessity to choose the proper soil type for experiments with a plant nematode, especially when the experiment are meant to simulate field conditions. Therefore the study on abiotic factors, confirmed that temperature, moisture and type of the soil influence the development of *T. dubius* markedly, but it did not add essential new information.

It is obvious from Fig. 27 that *T. dubius* and some other nematodes accumulate rapidly around the roots of a host and stay there, whereas other species do not. However, the fact that 93% of the *T. dubius* population did not come close to the roots confirms the conclusion that attraction by the roots is weak or absent and supports the hypothesis that often only a fraction of the population of polyphagous plant nematodes participates in the infestation and reproduction process (WINOTO 1970). The polyphagy of *T. dubius* appears from the fact that more than 30% of the 41 crop plants tested caused more than 10-fold reproduc-

tion in pot experiments, and it is remarkable that many tropical or subtropical plants are suitable hosts. Polyphagy may be rather the rule for plant nematodes, and not the exception as for parasitic fungi. The fact that *Agropyron repens* and *Poa annua*, originally common as weeds in our monoculture pea plot, cause nematode reproduction, may help to explain the aberrant, broad population peak in the first year of our field study (cf. Figs 46 and 48).

The amount of food (host roots) as well as the quality of the food (age) are apparently both important for the reproduction of *T. dubius*. This appears from Tables 11 and 12 and Figs 28, 31 and 32. It is obvious that the final nematode density (P_f) per pot is lower when fewer plants are grown or when the plants are weakened by pruning, but P_f per g of roots is often higher in the same pots. It is also clear that populations decline, even per g of roots, when plants are getting old; this holds also for a perennial such as ryegrass (Fig. 28), on which the population as a whole aged with time (Figs 29, 30). This must be due to the fact that older plants are less suitable for reproduction of *T. dubius* than young plants. It is probable that the quality of the roots is determinant here, but other factors correlated with aging of the plants in soil may have played a role. The fact that aging of ryegrass was correlated with decrease of *T. dubius* density may be of importance although this does not exclude periodic fluctuation of the population density under permanent grass (cf. Fig. 34). It is noteworthy that the onset of unfavourable conditions, i.e. on 10-week old plants, caused an accumulation of females and not of males (Figs. 31 and 32).

The study about the influence of initial nematode density, or space per animal, on a developing population of *T. dubius* is complicated by the fact that a living host plant is always involved. Final density (P_f) per container, per g of soil and per g of roots have to be considered separately (Table 13), and time always influences the results. When a certain low number of nematodes was inoculated to ryegrass in trays of different size with uniform root density (Tables 14 and 15, Fig. 34), the total P_f was proportional to the size of the tray. but its maximum was reached later in larger trays. The maximum density per g of soil was about the same for all trays, because the original differences were liquidated with time. This density was unnaturally high, owing to the high root density in our trays. Maximum densities in the field are lower, but the results and the mechanisms are not principally different. Also here each plant causes a host-specific maximum density per g of soil which is always reached when the growing period of the host crop or of the series of host crops is long enough, or when the initial density is high enough. It is further obvious (Fig. 34) that the population in all trays fluctuated with times, i.e. they decreased after passing the maximum and then started to rise again. Population fluctuation under permanent grass may be due to periodicity in the growth of the grass roots, or to the biology of the nematode, namely to the fact that the reproduction rate $R = P_f/P_i$ falls when P_i is too high. Tables 14 and 15, and Figs. 35 and 36 show that the reproduction curve of *T. dubius* for several host plants studied was a top curve, and this must lead to fluctuation of the population density in the course of time (OOSTENBRINK 1966). At very low initial density underpopulation was observed by us in

several cases. This was suggested to hold for nematodes by KORT (1962), SEINHORST (1968) and MERNY (1970). It was not noticeable at a P_i of 32 nematodes per 256 g of soil (Table 14, Fig. 33), but it was marked at 4 and even 16 per 128 g (Table 21, Fig. 37) and at 32 per 350 g (Table 22, Figs. 33 and 40). A critical density for underpopulation may be somewhere near 12 specimens per 100 g of soil.

Micro-arthropods, mites as well as springtails, are the most numerous Metazoa next to nematodes in the soil, and they comprise several species which prey on nematodes. Their predation potency in vitro is impressive, according to direct observation (Fig. 41 A) and to the results of experiments (Table 23). Inoculation of certain predator species to soil with nematodes also leads to significant results, and to the indication that second-stage larvae are suppressed far more than the older and larger stages (Tables 24–26). When one or more predators are brought together with nematodes around a growing host plant, a complex situation exists in which not only direct predation, but several interactions between predators, nematodes and the plant play a role, as must be the case under natural conditions (Table 26). It is difficult to give a satisfactory interpretation of all the results and, therefore, to estimate the significance of these predators for nematode control in practice. However, the facts, that the presence of predatory mites and springtails in the soil decreases the density of *T. dubius*, and that the young second-stage larvae are selectively suppressed, are supported by all experiments, and this makes it probable that micro-arthropods play a role in the regulation of the nematode population.

Several organisms, apart from micro-arthropods, are able to prey on or cause damage to nematodes. They were not included in our studies, but three organisms found on or in *T. dubius* are nevertheless recorded because they may be noxious and are probably not recorded earlier (Figs. 41 B, C, D).

5. THE FIELD POPULATION

5.1. Occurrence and density

The widespread occurrence of dense populations of *T. dubius* in lighter soils in The Netherlands and other countries in Western Europe, mentioned in the general introduction, has been amply documented by several workers (c.f. 1.2).

The field studies in this work were limited to the population in one field, the crop rotation trial field in the garden of the PD at Wageningen, and were concentrated particularly on the monoculture pea plot in this trial.

The trial was started on a homogeneously infested field in 1958, according to a socalled 'cross trial' or 'chess board' scheme, recommended for the study of crop rotation effects in relation to nematodes and other causes (Oostenbrink 1959, Hijink & Oostenbrink 1968). In this trial 8 crops, including fallow, were grown in a fixed order alternatively in the north-south and the east-west direction. The scheme of the trial and the crops is recorded in Fig. 42.

The composition of the nematode communities of each of the 64 plots was quantitatively determined every spring. The original nematode community, analyzed in the spring of 1958, consisted of 550 *T. dubius* + 274 *Rotylenchus ro-*

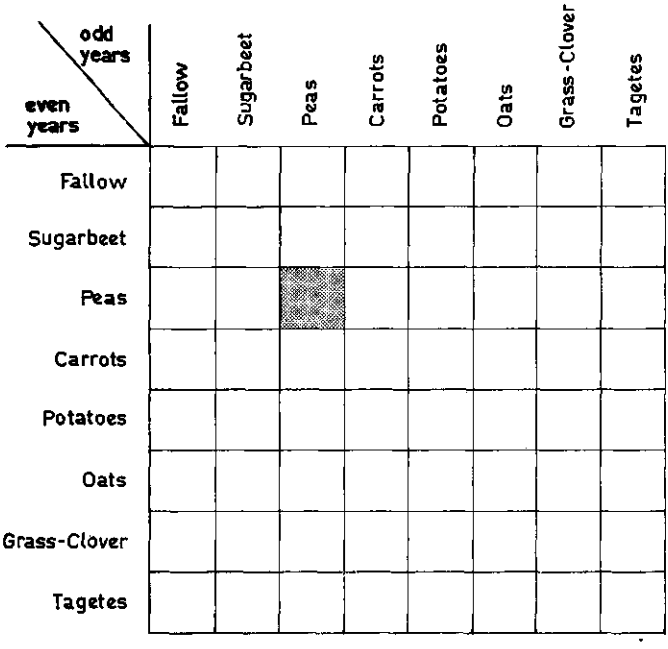


Fig. 42. Crop rotation trial field according to the so called 'cross trial' of 'chess board' scheme, running from 1958–1970. The monoculture pea plot, which was the main experimental site for this study, is shaded.

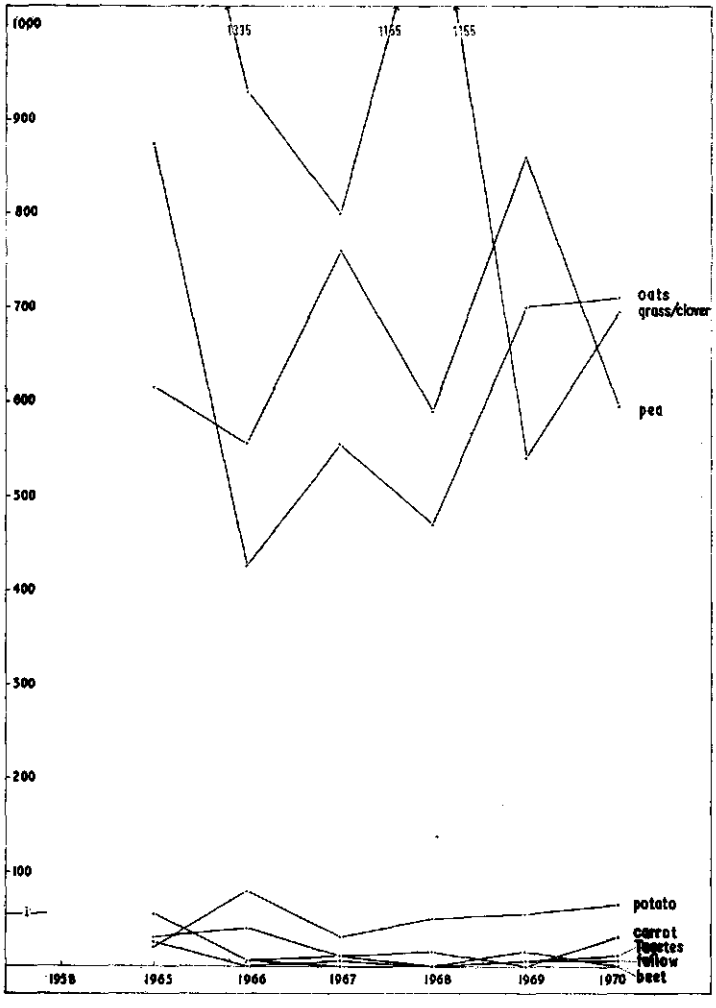


FIG. 43. Population density per 100 ml of soil of *T. dubius* in the 8 monoculture plots of the crop rotation experiment at the PD, Wageningen in the spring of each year of the observation period 1965–1970. The overall density at the start of the experiment is indicated by i.

bustus + 472 *Pratylenchus crenatus* + 385 other Tylenchida + 2434 saprozoic nematodes, per 100 ml of soil. The densities of *T. dubius* in the monoculture plots of all 8 crops in the spring of the last six years are illustrated in Fig. 43, and the densities in two-years' rotations of these crops with the main experimental plant, pea, are illustrated in Fig. 44. It is clear from Fig. 43 that only grass/clover, oats and pea maintain a high density of *Tylenchorhynchus*, usually between 500 and 1000 per 100 ml of soil, and that the population is maintained at a low or very low level under potato, carrot, beet, *Tagetes* and fallow. These data are in accordance with the results of the experimental host plant studies recorded under

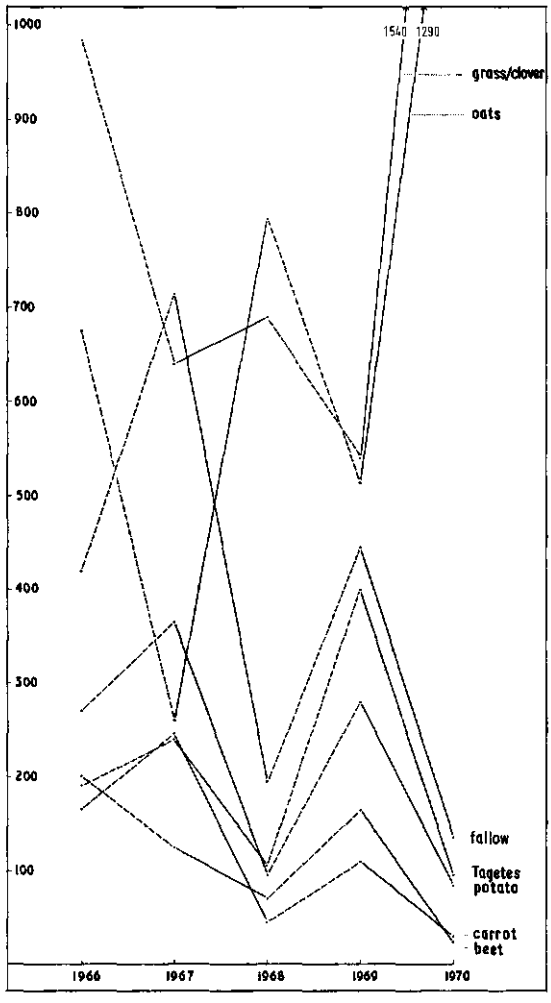


FIG. 44. Population level of *T. dubius* in the observation period 1965–1970 when 7 different crops were alternated with pea on the crop rotation experiment at the PD, Wageningen. The course of the population during the years that pea is grown, is indicated by broken lines.

4.4.1.b, although potato was found a more suitable host in the experiments than according to the field study. The alternate growth of pea with the other crops, as illustrated in Fig. 44, indicates that the populations of grass/clover and oats are somewhat suppressed by pea, and that all the other populations are increased by pea. This seems to put the host suitability of pea somewhat below that of grass/clover and oats, but definitely above that of the other crops. The fact that the population does not reach complete extinction even in the fallow plots, may be due to infections from neighbouring plots, or to the fact that germinating seeds or stolons of weeds may have maintained a low density. The population of *Tylenchorhynchus* in the monoculture pea plot is *T. dubius*, whereas some of the other plots comprise a number of *T. nanus* Allen, 1955, in addition to *T. dubius*. It is therefore not quite sure that grass/clover and oats are more suitable than

TABLE 28. Nematode densities per 100 ml of soil of the monoculture pea plot in the spring of the years 1966, 1967, 1968, 1969 and 1970. The plot had yearly been grown with pea, since 1958.

Nematodes \ Years	1966	1967	1968	1969	1970
Tylenchorhynchus dubius	555	760	590	860	595
Rotylenchus robustus	375	1445	375	430	290
Pratylenchus crenatus	0	0	5	20	10
Other Tylenchida and saprozoic nematodes	420	1100	1125	1355	1205
Total	1350	3305	2095	2665	2100

pea as hosts for *T. dubius*.

Most observations were done in the monoculture pea plot. The nematode populations, determined in the spring of the years 1966, 1967, 1968, 1969 and 1970 are recorded in Table 28. The average populations densities of the years 1965, 1966, 1967, 1968 and 1969 are summarized in Table 29. The densities of the plant parasites recorded must be mainly due to the pea crop, which was in all years *Pisum sativum* 'Rovar'. Some influence may have been exerted by the weeds present in the plot. In the year that observations started, 1965–1966, the pea plot was not free from weeds in the autumn and included one or more of the following plant species: *Agropyron repens* (L.) P. B., *Taraxacum officinale* L., *Lamium amplexicaule* L., *Senecio vulgaris* L., *Viola arvensis* Murr., *Capsella bursa-pastoris* (L.) Med., *Galinsoga parviflora* Cav. and *Poa annua* L. Since then the plot was kept free from weeds and volunteer pea plants after the growing of the pea crop from April to July, and weeds were also removed carefully as soon as they became visible in the crop. In this pea plot horizontal and vertical distribution of *T. dubius* was studied (cf. 5.2), whereas seasonal fluctuation of the

TABLE 29. Average population densities per 150 g of soil for each of the years 1965–1969, calculated from the monthly averages upon which the graphs of Fig. 48 are based.

Subjects \ Years	1965	1966	1967	1968	1969	Overall average
Tylenchorhynchus dubius						
Total	2232	1476	1791	1700	1597	1759
Larvae	1656	979	1367	1440	1282	1345
Females	405	375	302	135	182	280
Males	171	122	122	125	133	135
Rotylenchus robustus	574	648	734	589	418	593
Other Tylenchida	369	128	70	101	79	149
Saprozoic nematodes	1576	1139	1061	1758	1757	1458
Micro-arthropods	37	23	24	14	7	21

population of *T. dubius*, associated nematodes and possible predators was studied by monthly sampling of the soil up to 25 cm depth (cf. 5.3 and 5.4).

5.2. Horizontal and vertical distribution

5.2.1. *Horizontal distribution*

Ploughing, digging and harrowing the soil before planting mixes the nematodes with the soil, but aggregation of the parasitic species and of the saprozoites takes place, according to the experiments described under 4.4.1.a.

Experiment 1.

Samples were taken from the monoculture pea plot to compare the nematode densities in the pea rows and between the pea rows on 20.5.1966 and again on 20.6.1966. The soil had been pulverized in March and peas had been sown on 17.4.1966; the rows were 25 cm apart and the distance between plants was about 3 cm. On each of the afore-mentioned sampling dates 5 soil cores were taken by means of an auger to the depth of 25 cm in the rows as well as between the rows. Each core consisted of about 150 g of soil and was examined separately for nematodes. The results are summarized in Table 30.

Table 30. Density of nematodes per core of 150 g of soil in the pea rows and between the pea rows in the monoculture pea plot, determined 33 and 64 days after sowing of the peas on 17.4.1966. Figures are average of five replicate cores. Significance of differences indicated by P values: – = not significant, ** = $P \leq 0{,}01$.

Nematode species	Density in the rows	Density between the rows	P values for place
Tylenchorhynchus dubius			
33 days after sowing	942	1104	–
64 days after sowing	4848	2080	**
P values for time	**	**	
Rotylenchus robustus			
33 days after sowing	432	484	–
64 days after sowing	400	658	–
P values for time	–	–	
Other Tylenchida			
33 days after sowing	92	128	–
64 days after sowing	144	128	–
P values for time	–	–	
Saprozoic nematodes			
33 days after sowing	854	1272	–
64 days after sowing	1806	2022	–
P values for time	**	**	

The data of Table 30 indicate that *T. dubius* was equally numerous between the rows as in the rows at the first sampling date. In the period between the first and the second sampling date, the density increased about 2-fold between the rows and about 5-fold in the rows. Thus agglomeration in the rows took place due to strong reproduction. *R. robustus* did not show significant reproduction nor concentration in the rows. There was, on the contrary, a slight concentration between the rows at the second sampling date, which may indicate some deterrent effect of the pea roots. The density of the other Tylenchida was hardly influenced, neither in nor between the rows. The saprozoic nematodes reproduced rather strongly in as well as between the rows, but there was no noticeable agglomeration effect in the rows.

It is obvious that *T. dubius* and the saprozoic nematodes multiplied on or around the pea roots, whereas the others did not. *T. dubius* agglomerated markedly in the rows, where the root density was high.

Experiment 2.

Further observations were made on 18.7.1969 on the densities of *T. dubius*, *R. robustus*, the other Tylenchida and the saprozoic nematodes during growth of the pea crop in the same plot in rows 15 cm apart, by taking four samples, namely in row A, one perpendicular to row A at 5 cm distance, one at 10 cm distance and one at 15 cm distance i.e. in row B. The densities per 150 ml of soil were respectively:

		Mean	S.E.
for *T. dubius*	: 2540–1260– 840–2790	1858	477
for *R. robustus*	: 150– 180– 290– 250	218	32
for other Tylenchida	: 130– 120– 180– 90	130	19
for saprozoic nematodes	: 3610–2620–1930–4460	3155	555

These figures indicate aggregation of *T. dubius* and of the saprozoic nematodes near to the pea roots, but no influence on the distribution of *R. robustus* and other Tylenchida. They are therefore in accordance with the experimental data obtained earlier and recorded under 4.4.1.a.

5.2.2. *Vertical distribution*

Experiment 1.

The vertical distribution of the nematodes, and of possibly associated predatory arthropods, was studied by extracting samples from the monoculture pea plot at monthly intervals from March 1965 to February 1966. The soil was ploughed and pulverized in March 1965 (just after the first sampling). Peas were sown on 5 april and harvested at the end of July. The field was left undisturbed until the middle of November, when stable manure was spaded in, and the soil was pulverized in December. Samples were taken with a 2.5-cm wide auger which could be opened, so that the core sections from 0–5 cm, 5–10 cm, 10–15 cm, 15–20 cm and 20–25 cm could be taken out and extracted separately. Each month, during the period from March 1965 through February 1966, 5 samples (cores) were taken from random places in the plot for nematode extraction, and 5 replicate samples (cores) were taken side by side with the ne-

matode samples for arthropod extraction. In both cases the cores were extracted in sections as indicated before, according to the methods described under 2.4.1. Each core section consisted of 30 g of soil. The results are summarized in the diagram of Fig. 45 and the graphs of Fig. 46.

The diagrams of Fig. 45 shows that all animal species or groups were present at measurable densities throughout the year at all strata from 0–25 cm. The overall average per core of about 150 g, i.e. when the averages over the whole period are summed for all strata, was: 2117 for T., 597 for R., 356 for O., 1536 for S. and 35 for Ar. The overall density in the tilth, therefore, decreased in the order T, S, R, O. and Ar. The total number of nematodes per average core was therefore 4606 against 35 for the micro-arthropods.

The average core figures allow the following conclusions:

a. T. was about equally numerous in all strata from 0–25 cm, with slightly higher figures for the strata 10–15, and 15–20 cm;
b. R. was most numerous in the strata 5–10 cm and 10–15 cm;
c. O. was most numerous in the strata deeper than 10–15 cm;
d. S. was most numerous in the top strata from 0–5, 5–10 and 10–15 cm;
e. the micro-arthropods were more numerous in the top 15 cm than below.

These are the average figures over the whole period, and they have to be analyzed in relation to time, and therefore to plant growth, for the plot was grown with peas in the months April through July.

The graphs of Fig. 46 indicate the following fluctuations with time for the total numbers per core, representing the whole tilth:

a. T. appears to show a marked peak during the growth of the pea crop. This will be discussed further under 5.3.
b. R. shows some minor density fluctuations not related to the crop.
c. O. does not show a distinct fluctuation.
d. S. shows a marked peak in April and this may be correlated with the first growth of the crop or be related in an other way to the spring season, e.g. to accelerated decay of organic material due to a rising temperature. Less marked peaks occurred in September and in December and they may reflect decay of the crop roots and of the stable manuring respectively.
e. The micro-arthropods do not show a distinct density fluctuation, apart from a density fall from March to April.

It is possible, that the rise of S. and T. at the same moment are causally related to this decrease of the arthropods. It is probably rather a consequence of the ploughing of the soil which occurred on 20.3.1965. Comparison of the diagram of Fig. 45 and the graphs of Fig. 46 suggest the following points with respect to the vertical distribution of the animals:

a. T. was apparently numerous throughout the tilth in March 1965 and further throughout the whole season. Rototilling the soil in this month did not change the position much. In May and June, and also in September 1965 the highest density occurred at 10–20 cm, apparently due to multiplication on pea roots. It is aberrant, and probably due to sampling errors, that the higher density in this layer was not found in July and August. The decrease of the popula-

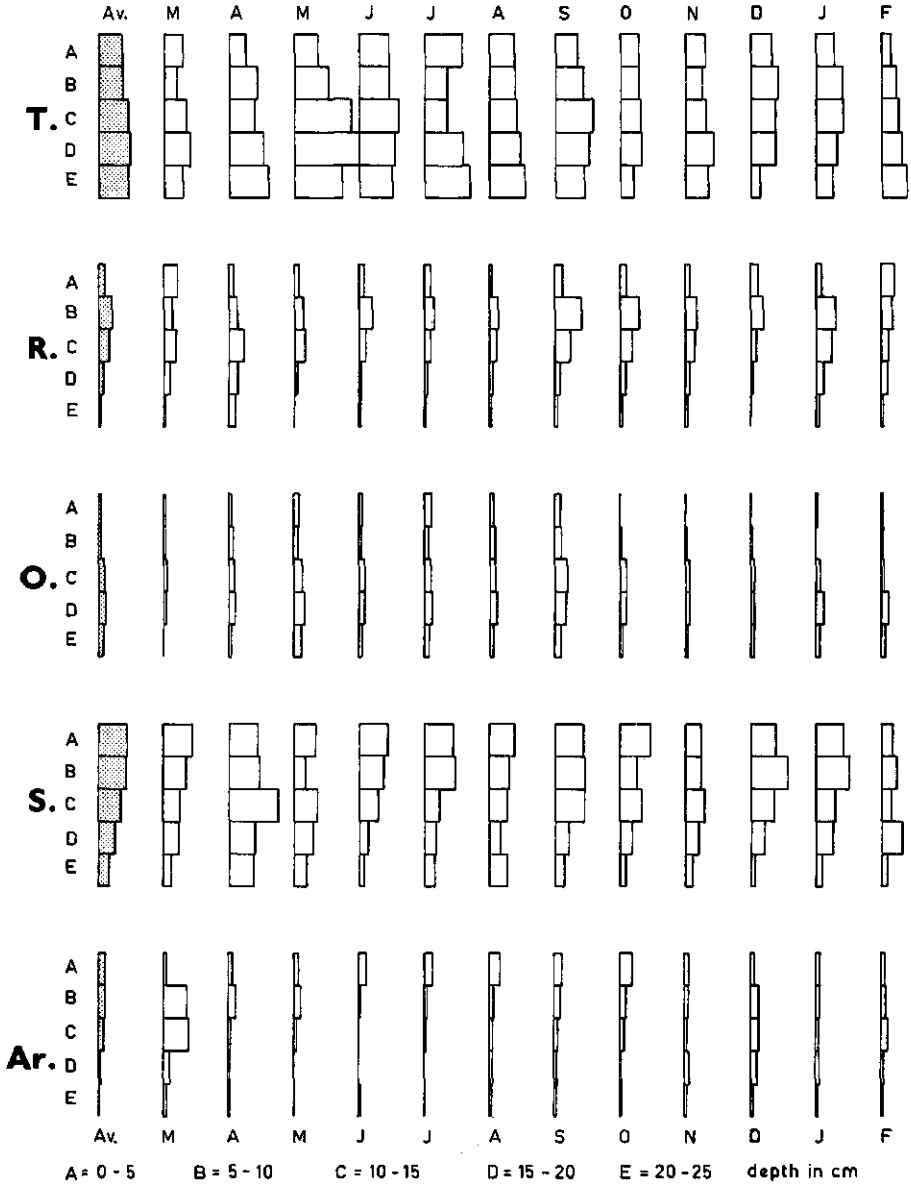


FIG. 45. Vertical distribution of *T. dubius* (T.), *Rotylenchus robustus* (R.), other tylenchid nematodes (O.), saprozoic nematodes (S) and micro-arthropods, mainly mites and collembola (Ar.), in the monoculture pea plot at each month of the period March 1965 through February 1966, as indicated. Twelve-month average for each animal is also recorded as Av.
Abscissa of each diagram: number of animals per soil core section of 5 cm length, i.e. about 30 g of soil (1 mm = 125 animals).
Ordinate of each diagram: depth in cm of the corresponding sections.

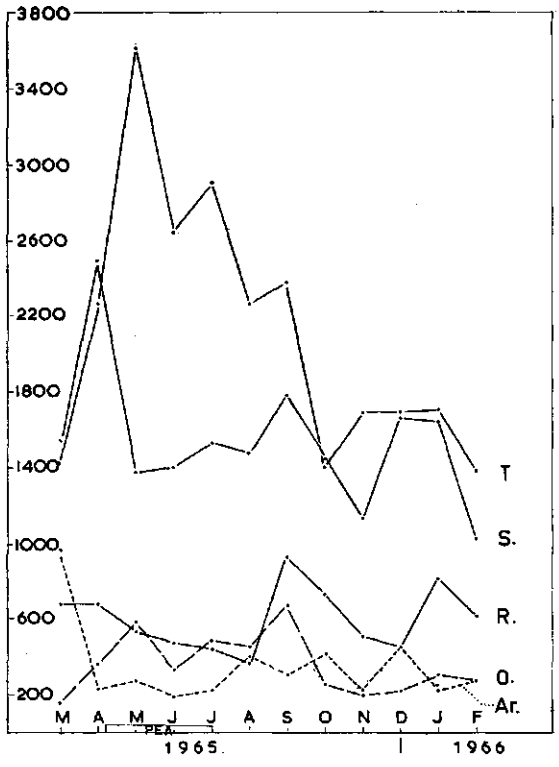


FIG. 46. Fluctuation with time of the total numbers per core of 150 g, taken up to 25 cm depth, for all animal groups recorded in Fig. 45.
Abscissa: month of sampling.
Ordinate: number of animals per core.

tion density in the higher layers in February may be due to the extreme cold conditions in this month.

b. R. was less numerous than T., but there was apparently some preference for the layers 5–15 cm. Some fluctuations in certain layers occurred in certain months, but they did not appear to correlate with plant growth or another known factor.

c. O. which comprised *Tylenchus davainei, Aphelenchus avenae* and others, apparently showed some preference for the deeper layers of 10–25 cm. They were significantly more numerous throughout the whole tilth in September 1965 than in any other month. This may be due to the facts that at least some species of this group are fungus feeders, and that the fungus flora was probably dense on decaying organic material in this month.

d. S., the saprozoic nematodes which are mostly bacterial feeders, were most numerous in the upper soil layers, down to 15 cm. They were numerous in general throughout the year. Their density shows some peaks in April and perhaps in September and December, which may in all cases be due to increased decay processes, and therefore increased bacterial densities in the soil.

e. Ar, the micro-arthropod fauna, consisting mainly of mites and collembola, was most numerous in the layers 5–15 cm before tillage, i.e. in March, and

after the summer season, i.e. after October when stable manure was spaded into the soil. Throughout the summer and autumn season they were most numerous in the upper soil layers, in April and May up to 10 cm, but in the period June, July, August and October markedly in the upper 5 cm of the soil. It is therefore clear that their density was related to season, probably to crop growth or amount of organic material or nematode density. It is not possible to indicate further the extent to which density was directly related to the nematodes.

Experiment 2.

An additional observation was made on the same plot on 29.8.1966, one month after the peas had been harvested. The soil had been left undisturbed. Three twin cores were taken with the afore-mentioned auger, now to the depth of 40 cm, and the cores were again subdivided into 5 cm sections for nematode and micro-arthropod extraction. The results are summarized in the diagrams of Fig. 47.

T. dubius was again found to be numerous throughout the whole tilth from 0–25 cm. Very few occurred in the layer from 25–30 cm and none was found in deeper layers. The numbers of males, females and larvae had been counted separately. It appeared that the male to female ratio was about 1:5 throughout the tilth, and that more than half of the population consisted of larvae, except in the layers 5–10 cm and 10–15 cm where the percentages were 35 and 47 respectively.

R. robustus was again most numerous in the top 15 cm, with a peak in the layer of 5–10 cm.

The other Tylenchida were present in relatively low numbers, but they occur-

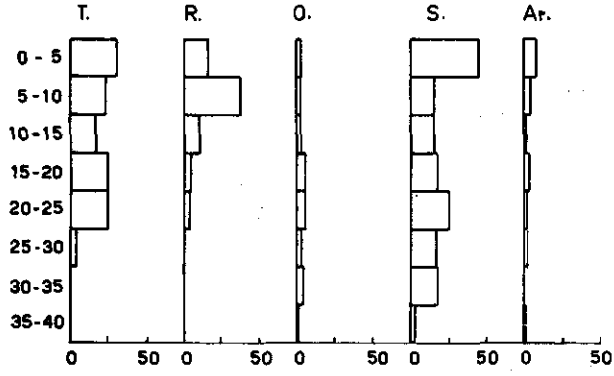


FIG. 47. Vertical distribution of *T. dubius* (T.), *R. robustus* (R.), other tylenchid nematodes (O.), saprozoic nematodes (S.) and microarthropods (Ar.), in the monoculture pea plot on 29.8.1966, one month after harvest of the pea crop. Averages of 3 replicate cores. Abscissa of each diagram: number of animals per soil core section of 5 mm length, i.e. about 3 g of soil. Ordinate of each diagram: depth in cm of the corresponding sections.

red up to 40 cm depth and they were somewhat more numerous in the layers of 15–35 cm than in higher or deeper layers. The non-tylenchid stylet-bearing nematode *Trichodorus pachydermus*, present in low numbers, was noticed in the layers from 20–40 cm and not above.

The saprozoic nematodes were again numerous throughout the tilth up to 35 cm depth, with a significant peak in the top 5 cm. There was also a measureble density in the layer from 35–40 cm.

The micro-arthropods were also most numerous in the upper 5 cm and slightly less numerous in the next 5 cm. Their numbers in deeper layers were low, but they were present up to 40 cm. *Rhodacarus roseus* was mainly found at 15–25 cm depth. *Tullbergia krausbaueri* was found down to 40 cm depth. Significant correlations between the micro-arthropods and the nematode densities can not be traced in these data.

5.3. Seasonal fluctuation curves of *T. dubius*

The fluctuation with time of the populations of *T. dubius* and associated nematodes in the natural community of the afore-mentioned monoculture pea plot was studied for a period of 58 months, from March 1965 to December 1969. Each month five soil cores were taken to the depth of 25 cm with a semi-cylindrical auger of 2.5 cm width. The cores were taken at random spots in the plot, independent of plant rows if a pea crop was present. Each core, after levelling it up to the edges, delivered about 125 ml, i.e. 150 g of soil, which was examined as a whole for nematodes by elutriation of the soil and detailed analysis of the nematode catch. The average numbers of the five replicate cores are recorded in the graphs of Fig. 48 for *T. dubius* males, females, larvae and totals, and for the totals of *R. robustus*, other Tylenchida as a group and saprozoic nematodes as a group. The numbers of *T.dubius* for the last two years, 1968 and 1969, are also presented in an other way and in more detail, namely for males, females, fourth-stage larvae, third-stage larvae and second-stage larvae separately.

Side by side with the cores taken for nematode extraction, replicate cores were always taken for enumeration of the micro-arthropods densities (mites and springtails), to study their density in relation to the density of the nematodes, which are prey animals for several of the micro-arthropods species. The cores taken for micro-arthropod enumeration were separated into 5 cm sections, each about 30 g of soil, which were put separately in the extractors. The total number of micro-arthropods per full core, as an average of five cores per sampling date, are also represented in Fig. 48.

In addition for a number of climatic factors, the daily averages of each month are graphically recorded in Fig. 48 namely:

soil temperature in °C in the layer from −10 cm to −20 cm (maximum and minimum at −10 cm were averaged, the same for −20 cm, then the mean was taken of the average temperatures of both depths; all figures are based on daily records);

relative humidity of the air as a percentage, based on daily average of three readings;

rainfall in mm, based on daily sums;

air temperature at +10 cm height (maximum and minimum were averaged; all figures are based on daily records);

dew point in °C, based on daily records.

The data on which the average figures are based were collected and furnished by the Physics and Meteorology Department of the Landbouwhogeschool, Wageningen. The distance between the site where the meteorological instruments are placed and the experimental plot is less than 2 km, and we have therefore considered these data representative for the climatic condition in the experimental plot.

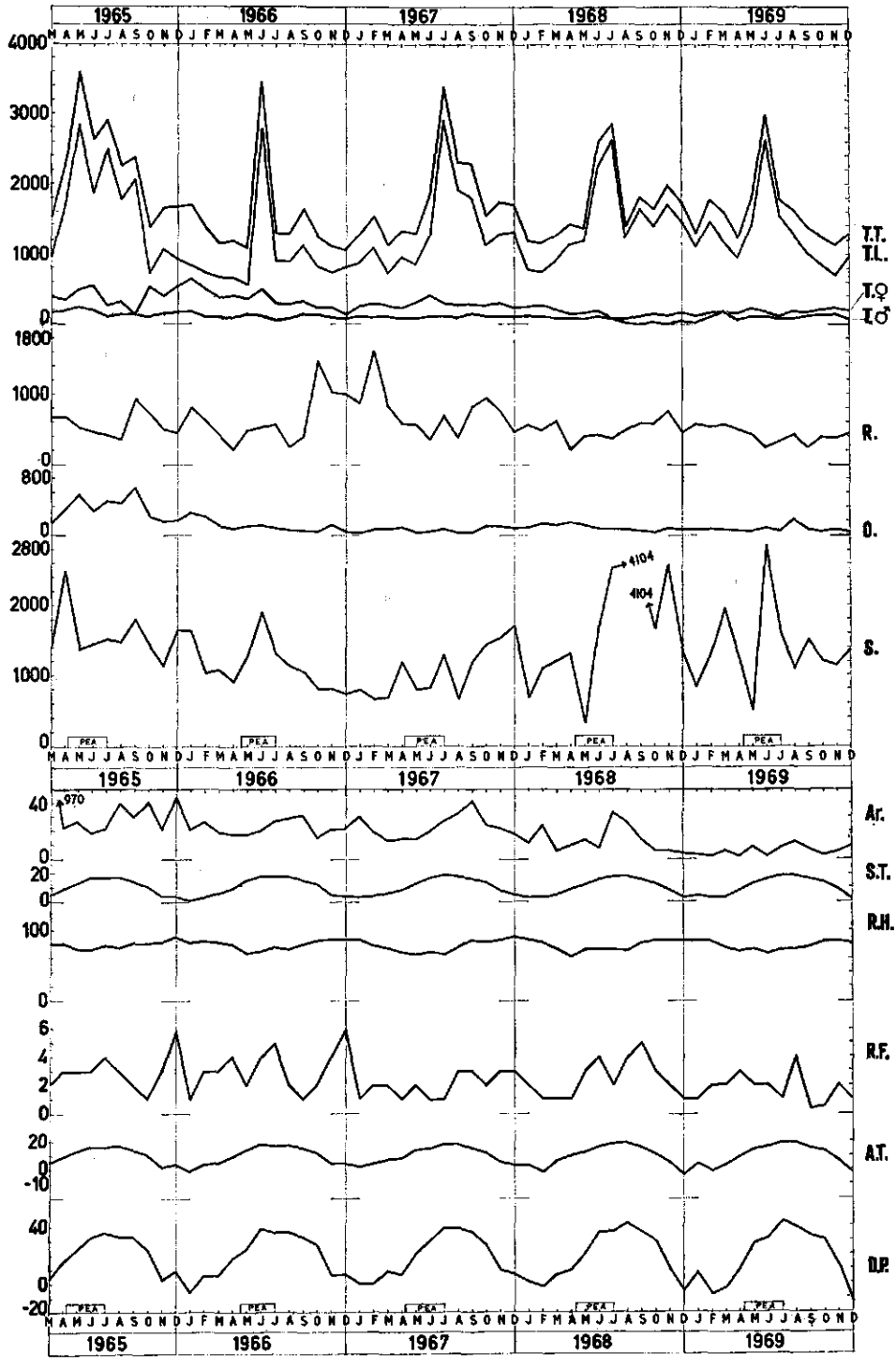
The average numbers for each species or group of species and the average values of climatic factors for each month of the observation period, are graphically represented in the Fig. 48.

The average figures from which the graphs are drawn, as well as the results of the replicate cores from which the averages are composed, need not be added in general, but they will be cited below if they are useful to substantiate certain points or to indicate statistical significance of certain differences in describing the results. The average population densities and climatic figures for each of the years 1965–1969 are already recorded in Table 29.

T. dubius, the main experimental animal, appears to maintain a high density, between 1000 and 2000 (per core of about 150 g of soil), throughout the years, with every year a sharp peak up to about double this density during the growth of the pea crop. This is a remarkably regular pattern, which illustrates the great persistance of the population as well as the affinity of the nematode to its host plant. It is also obvious from the graphs, that the peaks in the density of the total population (T.T.) are only due to peaks in the density of the larvae (T.L.), whereas the density of the males (T.♂) and the females (T.♀) do not show noticeable fluctuations in relation to the crop. The population peak in the year 1965 is considerably broader than in the other years, because high densities for total numbers and larvae were present until October, and not August as in the later years. In this year the density rose from 1520 in March to 3598 in May. It was 2638 in June, 2906 in July, 2264 in August and 2380 in September, and dropped in October to 1392. The high density in the period between July and October must be due to the fact that the plot harboured *Poa annua* and *Agropyron repens*, both suitable hosts of *T. dubius*, as common weeds after the harvest of the pea crop in this particular year. In the following years the plot was kept weed-free.

A closer examination of the *T. dubius* fluctuations by means of Fig. 49, which illustrates the densities for all larval and adult stages during the period April 1968 to December 1969 in a series of graphs, reveals the following points.

The preplant population density was 1430 in April 1968 and 1228 (per 150 g of soil) in April 1969, thus illustrating the fact that the population hibernates in great density, and must therefore be very persistent against adverse conditions


1965
1966
1967
1968
1969
T.T.
T.L.
T.♀
T.♂
R.
O.
S.
4104
4104
Ar.
970
S.T.
R.H.
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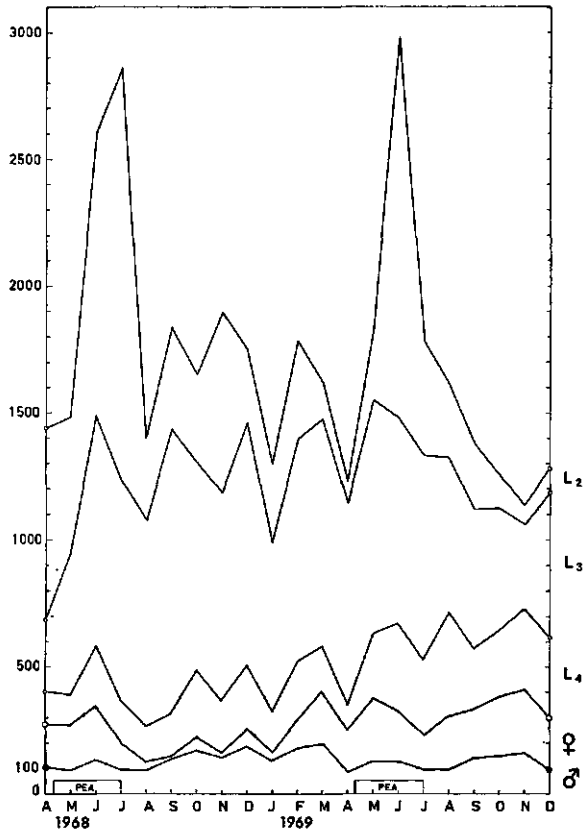


FIG. 49. Fluctuation of *T. dubius* populations in detail for the period April 1968 to December 1969 (cf. Fig. 48). Males (♂), females (♀), fourth-stage larvae (L_4), third-stage larvae (L_3) and second-stage larvae (L_2) are recorded separately. Abscissa: time in months. Ordinate: numbers per core of 150 g soil for each of the nematode categories indicated in the graphs.

including starvation and low temperatures.

Both adult stages and all free larval stages, L2, L3 and L4, were present in the spring. The strong population increase occurred from May on in both years,

FIG. 48. Seasonal fluctuation of *T. dubius*, indicated separately for males (T♂), females (T♀), larvae (TL) and totals (TT), *R. robustus* (R.), other tylenchid nematodes (O.), saprozoic nematodes (S.) and micro arthropods, mainly mites and springtails (Ar.), in the monoculture pea plot at each month of the period from March 1965 through December 1969 as indicated. Average daily climatic figures are also recorded. Soil temperature in °C (ST), relative humidity in % (RH), rainfall in mm (RF), air temperature in °C (AT) and dew point in °C (DP). Abscissa of each diagram: time in months. Ordinate for all nematode categories: number of animals per core (i.e. 150 g of soil), soil temperature, relative humidity, rainfall, air temperature and dew point, as average figures (cf. text).

i.e. about one month after sowing the crop, and the peaks, which went up to nearly 3000, were over in both years in August. The peak coincides with the growing period of the crop, from the middle of April to the end of July, after which the populations remain stable up to the following season with some minor fluctuations. It is clear from the numbers of the different stages, i.e. from the width of the zones between the graphs of Fig. 49, that the heavy increase and sudden fall are mainly due to the density of L2. The peak formation must be due to strong multiplication on the host crop pea, causing an excess of birth rate over mortality rate, and the fall to the rapid disappearance of the numerous L2 at a moment that no new eggs were laid and hatch.

The number of females was relatively low in the period from August 1968 to January 1969, but for the rest fluctuations in the numbers of adults and L_4 and L3 were not marked, as can be read from the width of the corresponding zones in Fig. 49, and they certainly did not contribute much to the general fluctuation of the total density with time. This agrees with the earlier experimental results (cf. 5.2.2). The general course of the population density graph of *T. dubius* appears to be mainly determined by food supply and poor survival of the L2 in comparison to the other stages.

The small irregular fluctuations occurring outside the plant growing season may be due to sampling errors, or they may be due to processes which influence birth or mortality. Increased hatch of eggs, either newly laid or with retarded development, cannot be excluded; the data available do not give information about eggs in the soil, because these are not caught in the extraction procedure. Also adverse factors, such as very low winter temperatures and tilling the soil, may be involved and there are more possible factors. These influences, however, are relatively slight and they do not obscure the general phenomenon of stability and persistence of the whole population outside the plant growing season, which is a major characteristic of it.

R. robustus also maintained a fairly high density of, on an average, 593 specimens during the whole period 1965–1969, thus showing great persistance as well. Its population showed unpredictable fluctuations, but the density was always below the average during the growing season of the crop. There were strong population rises in October 1965 (from 348 to 928 specimens), January 1966 (from 450 to 804), October 1966 (from 394 to 1468), February 1967 (from 860 to 1612), September 1967 (from 370 to 822), March 1968 (from 478 to 614) and November 1968 (from 572 to 760 nematodes). The maintenance of a rather high general density under pea despite the absence of affinity for the plant and the capriceous changes of the population of this plant-parasitic nematodes are both puzzling phenomena, which remain to be explained.

The other Tylenchida, as a group, are continuously present at a rather low density, on an average 149 per 150 g of soil over the whole period, but they do not show population changes which could be related to the crop or to other known factors. From April through September 1965 the density was high, and temporary increases were noticed at other times. It should be kept in mind that

the graph represents the density of a group, and that the graphical presentation obscures relatively great fluctuations of low densities. The possibility that certain species in the mixture with a low population density are specifically influenced by the crop, or another systematic factor, is real. The detailed analysis of our samples indicates that this actually holds for several plant parasitic species, e.g. *Meloidogyne hapla* and *Trichodorus pachydermus*.

The saprozoic nematodes, again a group, were numerous at all sampling dates (av. 1458 nematodes per 150 g of soil), but their density varied strongly with time and showed temporary high peaks, which cannot easily be related with crop growth or another known factor, as in the case of *T. dubius*. The density rose in April 1965 (from 1422 to 2500 per 150 g of soil), was maintained at a fairly high level from May to August, rose again in September 1965 (from 1472 to 1800), December 1965 (from 1132 to 1656), June 1966 (from 1272 to 1910), April 1967 (from 680 to 1184), July 1967 (from 818 to 1282), September 1967 (from 660 to 1182, with a continuous increase up to 1704 in December 1967), June 1968 (from 324 to 1694, with a continuous increase up to 4104 in September 1968), November 1968 (from 1672 to 2576), March 1969 (from 1282 to 1954) and May 1969 (from 1254 to 5000, with the fairly high although decreasing numbers of 2866 and 1586 left in June and July 1969 respectively). An increase apparently takes place in most years early in the spring, during the growing period of the crop, shortly after the crop has been harvested, and sometimes late in the autumn. There were no significant general correlations with soil temperature or rainfall, as can already be seen from the graphs. It is, however, probable that the increase of the saprozoic nematodes as a group, which are mainly bacterial feeders, is closely correlated with the intensity of decay of organic material in the soil.

The micro-arthropods, mites and springtails, are a complicated group of several species, including predators, facultative predators, saprozoic species and possibly some primary plant parasites. As a group they were found each month, in average numbers varying from 3 to 97 per core. The springtail *Tullbergia krausbaueri* and the predatory mite *Rhodacarus roseus* were regularly found, the afore-mentioned species mainly in the upper ten cm of the soil and the latter between 15 and 25 cm depth. Other predatory species, which occurred not sporadically, were the springtail *Isotomodes productus* and the mites *Hypoaspis aculeifer* and *Pergamasus* sp. The numbers of individual species per sample were too low to be considered separately in relation to the nematodes, particularly to the marked seasonal fluctuation of the *T.dubius* density. In the corresponding graph of Fig. 48 the total numbers of micro-arthropods per average soil core are recorded. They indicate significant fluctuations of the group, but the cause of these fluctuations and their influence on the nematodes is difficult to interpret. It is obvious that the micro-arthropods are present in measurable densities throughout the year. Close examination of the graph reveals that their densities showed a peak in the autumn of every year, August or September, and usually also early in the spring. The autumn peak, therefore, followed the decline of the *T. dubius* peak and occurred at the moment that the

crop roots decayed in the soil. It may well be that the micro-arthropods community, being a heterogeneous group of species which also interact among themselves, is directly associated with the nematode density. Literature records and the experimental results obtained earlier (cf. 4.4.4) and under 5.4. indicate the existence of both relations. The data obtained in the present study support the insight obtained until now, but they are not detailed enough to reveal the significance of specific relations.

The climatic factors include rainfall and four factors which are all closely related with temperature. There was rainfall in every month of the five years' period, varying from 0.3 to 6 mm as daily average of the different months, therefore with several peaks, but a significant direct correlation with the nematode or micro-arthropod densities was not noticeable. This was different for the other factors. Soil temperature, air temperature and dew point followed a regular yearly course, with which the relative humidity was correlated. Each of these factors showed a seasonal curve with which the growth of the pea crop, and therefore the corresponding fluctuation of the *T. dubius* and other animal populations were correlated. This secondary relation of monthly averaged temperature and other factors with the nematode population, however, has not been directly determinant, as appears from the fact that the nematode population peak collapses far before the temperature and other curves decline. Temperature and moisture are known to influence nematode population fluctuations (cf. DAO 1970, WILLIS & THOMPSON 1969), and they may even have decisive trigger effects on movement, development and reproduction (WALLACE 1969a, OOSTENBRINK 1967). Monthly averages, however, are too coarse to measure such direct effect, and can in this case only illustrate the annual course of the climate and demonstrate that general climatic factors are not influencing the population density of *T. dubius* and other nematodes to a noticeable degree. When the steep density peak to plant growth is removed, one must in fact conclude that the *T. dubius* population is very resistant against climatic influences.

Subsequent to this field study soil from the same trial field was placed in pots in a glasshouse which was permanently grown with pea by sowing a new crop every six weeks while temperatures were maintained comparable to those of a normal growing season of pea. In this way the influence of a pea crop and of the summer season was simulated throughout the year.

5.4. POPULATION FLUCTUATION UNDER PARTIALLY CONTROLLED CONDITIONS

Soil with its natural biocenose was taken from the peripheral region of the monoculture pea plot (cf. 5.3.), and was sieved and mixed. On 13th October 1967, 230 plastic pots were each filled with 150 g of soil and randomly arranged on the bench of a glasshouse. Half of the pots were kept unplanted and in the rest of the pots peas were sown, of which two plants were maintained for about six weeks. On 27th November the pea shoots were cut and removed,

and two new germinating pea seeds were placed in the soil of the pots without removing the old roots or disturbing the soil otherwise. This procedure, removal of shoots and replanting of new seedlings, was repeated on 10th January 1968, 24th February, 7th March, 21st April, 4th June, 18th July and 1st September 1968. Fairly good plant growth could be maintained throughout the experimental period due to normal care and monthly additions of 25 ml of STEINER'S nutrient solution per pot, except for the seedlings planted on 24th February which were therefore replaced on 7th March. Unplanted pots were kept moderately moist, and received nutrient solution as the ones containing pea plants.

The pots were placed under 16 hours of artificial light per day until the end of April; daylight was complemented in the period that days were short. Air temperature was maintained at 18 °C for the first three months, but on 27th December all pots were transported into a glasshouse with limited heating, so that temperatures were 19–20 °C on an average, but fluctuations on warm days and cool nights occurred, which simulated outdoor conditions during a normal growing season from April to August. These conditions were maintained until the end of the experiment. Temperatures from January to September 1968 were continuously recorded by means of a thermograph. Mean daily averages, based on the temperatures at 0, 8 and 16 o'clock, are recorded for each month in Table 31. It appears that for the month of February, and to a lesser extent for March, the average temperatures have been lower and for June and July they have been higher than was intended.

Five unplanted pots and five pea-grown pots were randomly selected on the 13th of every month from October 1967 to September 1968 for enumeration of the nematodes; similar sets of fallow and pea-grown pots were then used for microarthropod extraction. The nematode catch was analyzed directly after extraction and the micro-arthopods were collected in 60% alcohol and identified later. The results are summarized in Table 31 and Figs. 50, 51, 52 and described below.

Table 31 shows that the *T. dubius* population in the pea-grown pots rose to considerably higher level than was found in the field (cf. 5.3.), probably because of the great root density in the pots. Multiplication occurred at all seasons. There were population fluctuations, which will be discussed later. The population in the fallow pots declined gradually to about 20% of its original density in the course of 12 months. This must be natural decline of the population. It is possible, but not probable that the monthly additions of nutrient solution influenced it, because the monthly amount of fertilizer salts added was only about 1/3 of a regular dosage in practice, namely less than 200 mg per litre of soil. The fact that saprozoic nematodes and saprozoic mites increased their densities in the course of the experiments supports the conclusion that fertilization has not caused unfavourable conditions in the unplanted pots.

The density of the saprozoic nematodes also rose sharply, with some marked fluctuations, in the grown pots. In the unplented pots the density dropped somewhat during the first three months, but then it rose again to densities between one and two times the initial density.

TABLE 31. Population densities of nematodes and of micro-arthropods with time in pots with 150 g natural soil from the monoculture pea plot, permanently grown with peas (A) or kept unplanted (B). Figures are numbers per pot, as the average of five replicate pots which were monthly taken from a uniform stock of pots.

Nematode communities analyzed as follows: T. = *T. dubius;* R. = *R. robustus;* O. = other stylet-bearing nematodes, mainly *Tylenchus davainei; Aphelenchus avenae* and *Trichodorus pachydermus;* S. = saprozoic nematodes.

Micro-arthropod populations analyzed as followes: Spr. = springtails; mainly *Tullbergia krausbaueri;* Pred. M. = predatory mites, mainly *Pergamasus runcatellus, Rhodacarus roseus, Hypoaspis aculeifer* and *Lasioseius penicilliger;* Sapr. M. = saprozoic mites, mainly *Tectocepheus velatus, Tyrophagus putrescentia, Eupodes sp,. Histiostoma sp., Prostigmata* Gen. spp.

Sampling months; between brackets average temperature for the month in °C		Nematodes:				Micro-arthropods		
		T.	R.	O.	S.	Spr.	Pred. M.	Sapr. M.
A = with pea								
October 1967	(18)	1170	620	60	1000	1	0.4	0.0
November	(18)	1030	400	100	6800	13	0.4	0.6
December	(18)	3340	460	70	5540	28	42.0	1.4
January 1968	(19)	2000	300	90	4780	157	0.2	308.8
February	(14)	1780	170	30	7620	113	0.0	17.0
March	(17)	2050	90	0	5580	181	8.6	21.8
April	(19)	5330	100	190	5390	482	0.0	134.4
May	(20)	3180	20	10	5070	326	10.4	168.8
June	(23)	6880	20	80	4780	531	18.2	239.2
July	(24)	3950	0	50	11430	112	0.0	434.3
August	(20)	3530	0	130	8530	75	4.0	288.8
September	(20)	1390	0	90	8280	125	16.2	296.4
B = unplanted								
October 1967	(18)	1170	620	60	1000	1	0.4	0.0
November	(18)	1060	480	60	950	5	0.6	0.6
December	(18)	760	330	110	600	30	2.4	0.2
January 1968	(19)	680	210	80	670	40	1.2	0.4
February	(14)	740	220	60	2390	71	0.2	7.6
March	(17)	550	160	90	1230	39	0.2	32.0
April	(19)	400	90	40	1790	42	0.0	52.8
May	(20)	350	60	30	1110	56	0.0	17.6
June	(23)	250	30	40	1730	34	0.6	88.0
July	(24)	270	10	90	2040	0	0.0	124.2
August	(20)	180	10	30	1820	46	0.4	412.6
September	(20)	200	10	10	1700	3	0.2	263.4

The *R. robustus* population declined gradually from 620 to a barely measurable density, under peas as well as in the unplanted pots.

The other stylet-bearing nematodes maintained their initial low density under pea and in the unplanted pots.

The micro-arthropods were determined as to species, and were combined later into three groups: springtails, predatory mites and saprozoic mites. All

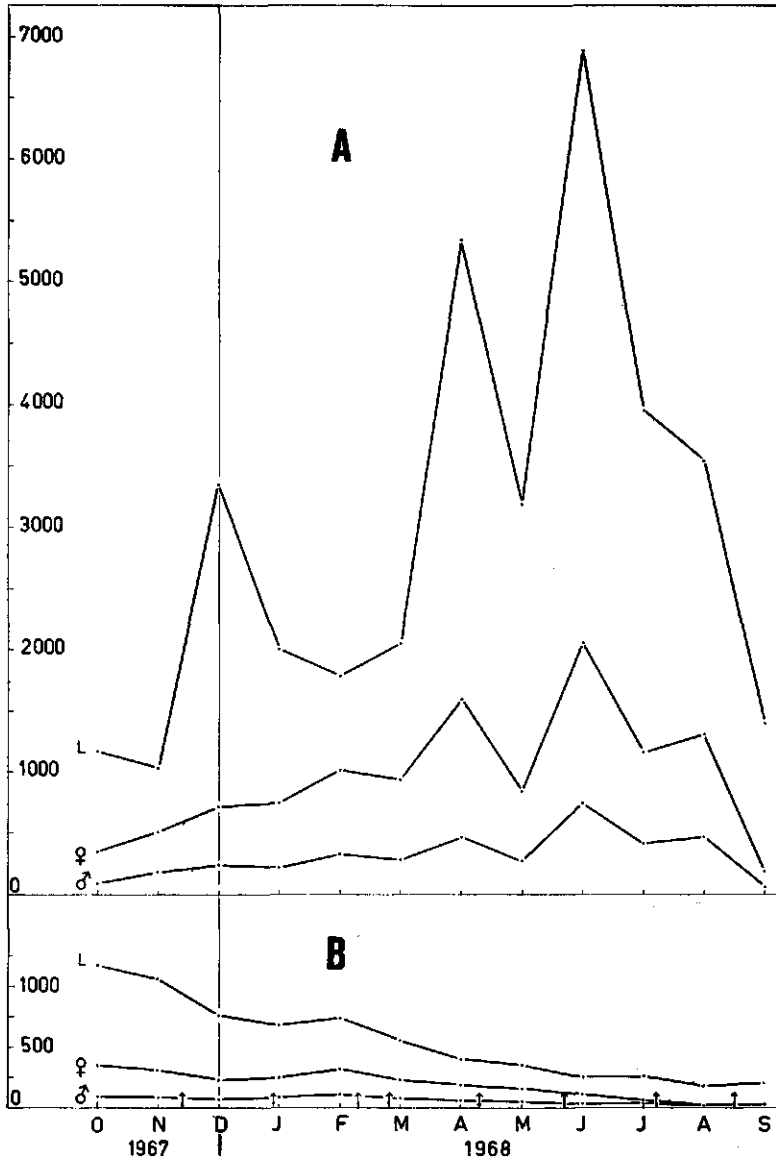


FIG. 50. Development of the different stages, males (♂), females (♀) and larvae (L) of *T. dubius* with time in the experiment recorded in Table 31, when 150 g pots with naturally infested soil from the monoculture pea plot were permanently grown with pea, by removing and resowing the crop about every six weeks, at the moments indicated by arrows (A), compared to unplanted (B). Abscissa: time, with months indicated. Ordinate: numbers of nematodes of the stages indicated.

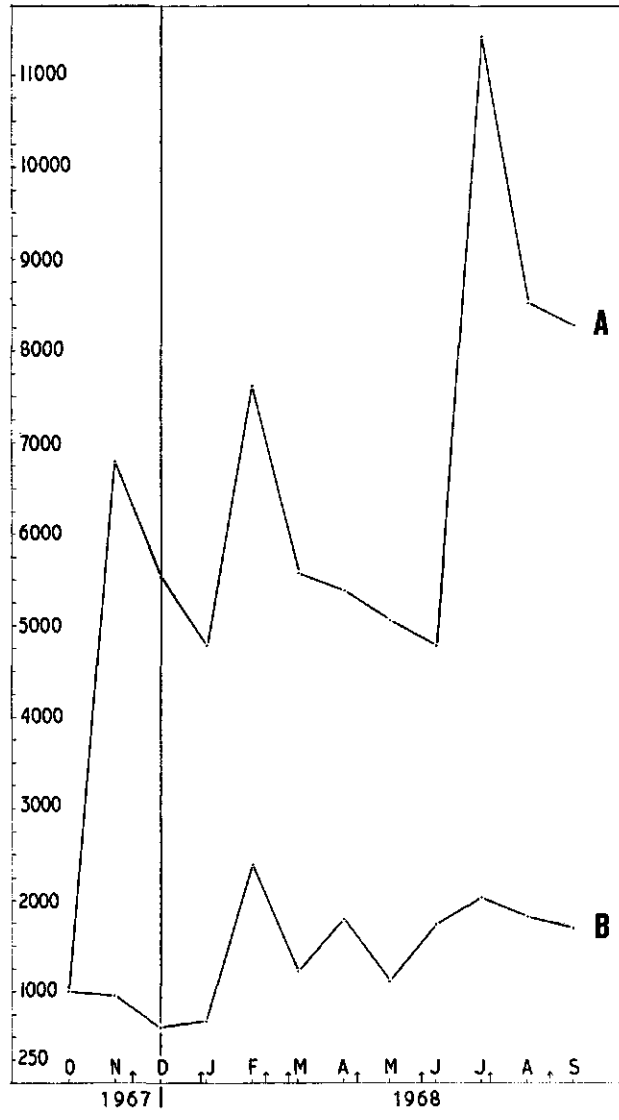


Fig. 51. Legend as Fig. 50, now for the Saprozoic nematodes.

three groups showed significant population fluctuations. The springtails, among which *Tullbergia krausbaueri* was the dominant species, increased gradually during the first three months and reached a great density which fluctuated in close correlation with the *T. dubius* density. In the unplanted pots their density increased somewhat, but then remained stable at about one tenth of the density in the pea-grown pots.

The predatory mites showed some significant density peaks, but their numbers

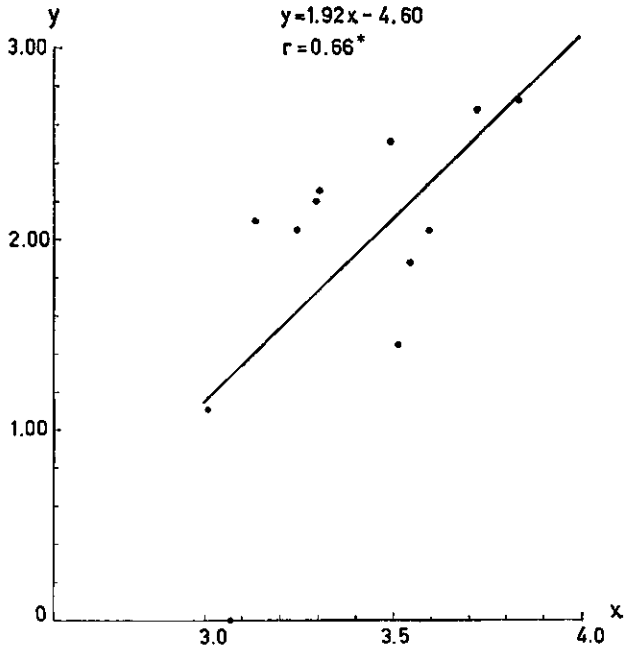


FIG. 52. Calculated from Table 31. Correlation between the number of *T. dubius* and the number of springtails, mainly *Tullbergia krausbaueri*, in pots with soil grown permanently with peas. Abscissa: nematodes per pot as average of 5 replicates, log scale. Ordinate: springtails per pot as average of 5 replicates, log scale. * = $P<0.05$

never became very high and were not closely correlated with the other organisms. In the unplanted pots their numbers remained always at a barely measurable density.

The density of the saprozoic mites in the pea-grown as well as in the unplanted pots rose gradually from October to March, and reached a high density beginning in April. There was one steep density peak in January, which was due mainly to the sudden reproduction of the *Histiostoma* sp. It is probable that the increase of the saprozoic mites was mainly regulated by temperature: the average temperatures, but also the minimum temperatures rose somewhat from 15 April on. The sudden increase of *Histiostoma* sp. in January, which occurred only in the pea-grown, in fact in 2 out of the 5 replicate pots, however, cannot be fully explained by temperature only, because it did not occur in the unplanted pots.

Fig. 50 demonstrates some important population fluctuations of *T. dubius* in the pea-grown pots, which are again mainly due to fluctuations in larval densities as in the earlier experiments. The fresh crops started every six weeks developed reasonably well, and feeding roots were available throughout the year, Regular oscillation of the population density in successive nematode generations or after successive crops, may perhaps be expected from the fact that the multiplication curve of *T. dubius* on pea is a top curve, which involves a factor

less than 1 at very high densities (cf. Figs. 35, 36, 49, and also Figs.2 and 3 in OOSTENBRINK 1966). Three important fluctuations, however, are irregular and remain to be explained, viz. the very low density found in February, the fact that the peak in July was higher than any other, and the continuous decrease of the population, down to a low level, in the period from June to September.

5.5. DISCUSSION

Results of the crop rotation trial field confirm the polyphagy of *T. dubius*, but also that host suitability varies much between plants: only grass/clover, oats and pea maintain a high density through the years, whereas the population was low under the other plant species. The characteristic nematode community in the monoculture pea plot comprised *R. robustus*, some other tylenchids and a high density of saprozoic nematodes, in addition to *T. dubius*, which was the dominant species.

Stratified and periodic samplings reveal, that distribution of *T. dubius* in the tilth up to 25 cm is more uniform than in the case of other nematodes. A high *T. dubius* density, between 1000 and 2000 per core of 150 g, occurs throughout the years, with every year a sharp peak during the growth of the pea crop. This regular pattern illustrates the dominant influence of the host plant on density, as well as the great persistence of the population when no host plants are present. The peak illustrates a rising density, due to strong multiplication of nematodes on young pea roots, and a sudden fall of populations, which is particularly due to rapid disappearance of the numerous L_2 at the moment that egg laying and harching stagnate. Data recorded in this chapter and in chapter 4 suggest that the great susceptibility of the second-stage larvae to starvation and the preferential predation of micro-arthropods on the second-stage larvae each play a role. There is no doubt that second-stage larvae disappear rapidly when no growing food plant is available, and this must be an important part of the mechanism. Predators, however, are also active, and inoculation experiments (cf. 4.4.4.) as well as the correlations found in this study suggest that they are effective especially in the suppression of the density of young second-stage larvae. Natural starvation of second-stage larvae is therefore complemented by preferential predation of second-stage larvae by micro-arthropods, especially *Tullbergia krausbaueri*. It is clear that more detailed information about the complex association of nematodes and other organisms is needed for a full evaluation of the significance of predators, parasites and diseases (cf. also Fig. 41) in the control of plant nematodes.

6. HOST RESPONSE

It appears from the foregoing chapters that *T. dubius* is a parasite of higher plants, and that plant species differ in host suitability and susceptibility to damage. The literature comprises some contradictory records about its pathogenic potentials, as indicated in the general introduction.

Following sections include data about damage recorded in experiments described in earlier chapters (6.1), results of inoculations with a single dosage or with increasing dosages of nematodes on a number of plants (6.2) and results of a study on a complex infestation of pea by *T. dubius* and *Phoma medicaginis* (6.3), followed by a discussion (6.4).

6.1. Damage recorded in earlier experiments

In a number of experiments recorded before, noticeable damage was caused to test plants when inoculated with *T. dubius*. Such results were observed in pots in which the stock population of the nematode was reared when sorghum was planted in this soil (cf. 2.1.1), in Table 7 for ryegrass, in Table 8 for rice, and in Tables 16 and 17 for several plants.

6.1.1. *Sorghum*

Plastic buckets, filled with 3000 g of sandy soil, were inoculated with 1500 specimens of *T. dubius* (males, females and larvae) collected from the PD crop rotation trial field, whereas other buckets were left uninoculated. Inoculation took place on 6.7.1966, and ryegrass was grown to obtain large numbers of nematodes as a stock. On 6.1.1967 an inoculated and an uninoculated bucket were freed from ryegrass and sown with equal numbers of seeds of *Sorghum vulgare* 'Dochna'. On 19.7.1967, root and shoot weights of the plants were determined, as well as nematode densities. Plants had developed well, but growth of uninoculated control plants had been much better than growth of inoculated plants (cf. Fig. 55A). Root weights per bucket were 56 g and 29 g and shoot weights were 59 and 30 g for control and inoculated respectively. The number of *T. dubius* had increased to 4500 per 100 g in inoculated soil, against nil in control soil.

6.1.2. *Ryegrass*

Results of Table 7 show, that inoculation of 20 *T. dubius*, 10 females + 10 males, per tube of 150 g soil caused significant damage to ryegrass after 60 days at 25 °C and at 30 °C, but it is noteworthy that the final nematode density had increased about 85-fold at 25 °C, 34-fold at 20 °C and only 5-fold at 30 °C. At 30 °C, therefore, damage occurred despite the fact that the nematode did not multiply strongly.

6.1.3. *Rice*

Inoculation of 3750 *T. dubius* per tube of 150 g of soil had caused significant damage to rice after 102 days for root weight as well as for total plant weight in dry soil and in flooded soil, although final nematode populations declined, according to the data in Table 8.

6.1.4. *Ryegrass, potato, and Tagetes*

Data in Table 16 show that increasing densities of *T. dubius* caused decreasing weight for ryegrass roots and total plants, for potato roots (not for total plants) and for Tagetes roots (not for total plants). These significant relations are illustrated by the graphs in Fig. 53A, and for ryegrass also in Fig. 55B. It is remarkable that root systems of potato and *Tagetes*, which are both poor host plants, may be reduced without measurable reduction of total plant weight. This reduction is already measurable for all plants at inoculation densities of 30 nematodes per pot with 150 g of soil. For potato roots, rectilinear regression of root weight on log nematode density is drawn in Fig. 54 and the corresponding formula is calculated.

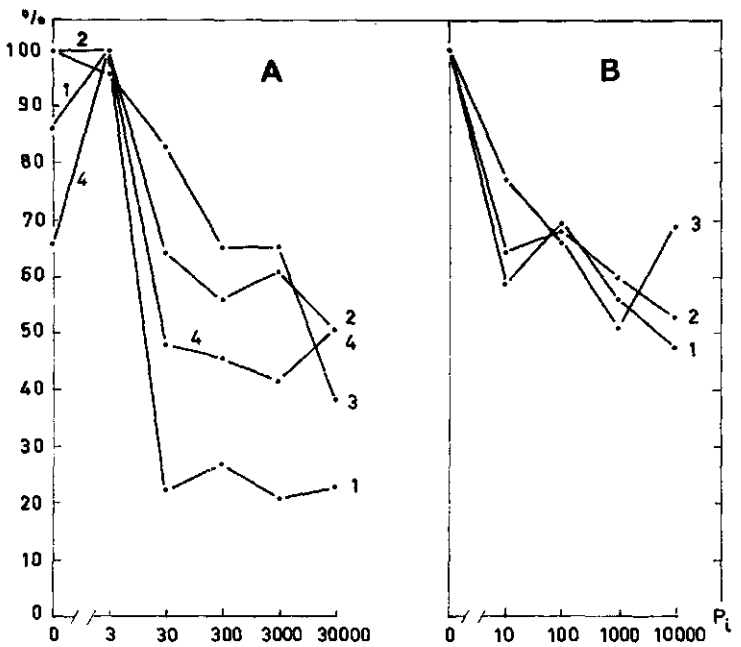


Fig. 53. Regression of root weight or plant weight of different plants on inoculated densities of *T. dubius*, calculated from the data in Tables 16 (A) and 17 (B).

A – 1 = ryegrass roots
2 = ryegrass total plants
3 = potato roots
4 = *Tagetes* roots

B – 1 = wheat roots
2 = wheat total plants
3 = pearl millet total plants

Abscissa: inoculated nematode densities, log scale.
Ordinate: root or plant weights, as percentages of highest value.

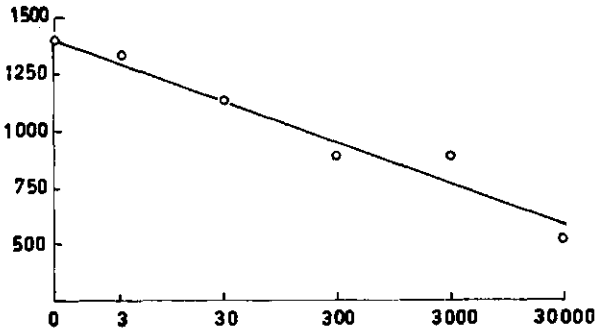


FIG. 54. Regression of root weight of potato on density of *T. dubius* inoculated to tube on 21.12.1966 (cf. Table 16). Abscissa: X = number of *T. dubius* inoculated per 150 g of soil, log scale. Ordinate: Y = root weight of potato in mg per tube, average per 5 replicates. Formula: $Y = -0.18X + 1.40$; $r = -0.976$.

6.1.5. *Wheat, pearl millet and rice*

Results of a similar inoculation experiment, summarized in Table 17, show that increasing *T. dubius* densities caused decreasing weight for wheat roots and total plants and for pearl millet total plants (not for roots), whereas the weight of rice roots and total plants were not influenced in this expriment. The significant relations are illustrated by graphs in Fig. 53B. Results in Table 17 demonstrate that susceptibility to damage is not necessarily correlated with host suitability of a plant, for pearl millet was susceptible to damage although it suppressed nematode population, whereas rice did not show any damage in this experiment despite the fact that nematodes at moderate densities multiplied strongly.

6.2. ADDITIONAL INOCULATION EXPERIMENTS

6.2.1. *Single nematode dosage*

Four experiments were made in which a single dosage of *T. dubius* was inoculated to tubes or pots with soil, compared to un-inoculated. The plants were cotton, sorghum green gram and pearl millet and the corresponding experiments are described below under a–d.

a. Cotton

Ten plastic tubes 4 × 4 × 20 cm were filled with 300 g of sandy soil, a month after the soil was mixed and steam sterilized. The tubes were each sown with one cotton seed, (*Gossypium barbadense*) and ten days later, on 25.1. 1967, five tubes were each inoculated with 10000 *T. dubius* freshly extracted from the stock culture on ryegrass. The tubes were sunk in steam sterilized sand in a Wisconsin tank at 25 °C constant temperature and 12 hours of artificial light was provided per day. The plants were watered regularly and STEINER'S nutrient solution was added once per three weeks. The plants grew well, although less in

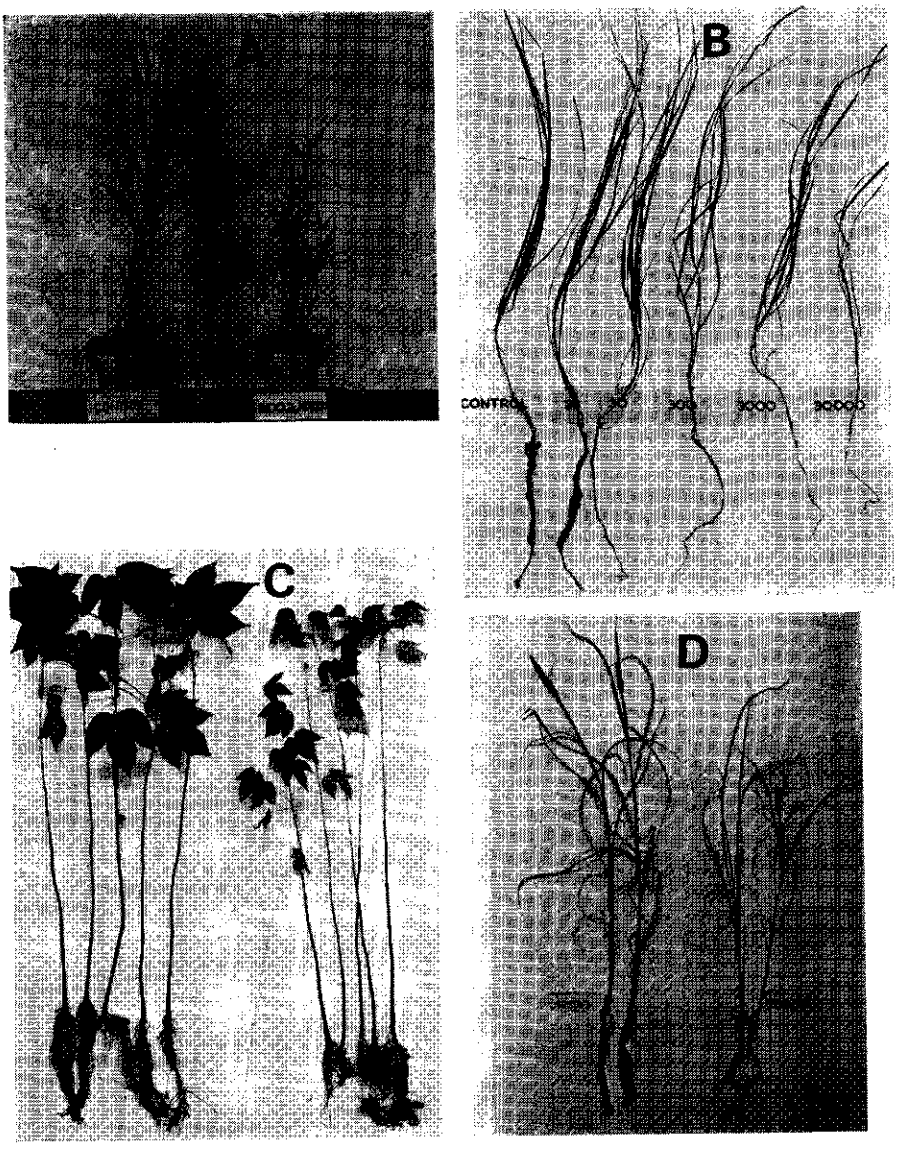


Fig. 55. Results of inoculation experiments. A. *Sorghum*. At left: control; at right inoculated with 1500 *T. dubius* per pot of 3000 g soil on 6.7.1966, after which ryegrass was grown and then *Sorghum* was sown on 6.1.1967, one replication; photograph taken 6 months later. B. Ryegrass. From left to right inoculated with 0, 3, 30, 300, 3000 and 30000 *T. dubius* per tube of 150 g soil on 12.12.1966; photograph taken after 85 days. Cf. Table 16. C. Cotton. At left: control, five replicates; at right: inoculated with 10000 *T. dubius* per tube of 300 g soil on 25.1. 1967, five replicates; photograph taken 135 days later. Cf. Table 32. D. Pearl millet. At left: control, two replicates; at right: inoculated with 5800 *T. dubius* per tube of 225 g soil on 28.9.1967, two replicates; photograph taken 102 days later. Cf. Table 32.

the inoculated tubes than in the controls. The experiment was evaluated 135 days after inoculation. Data on shoot and root weight and on nematode density are summarized in Table 32, and in Fig. 55C.

The inoculation of *T. dubius* caused a reduction of the root system with browning, a reduction of the leaf size and stunted growth (cf. Fig. 1A). The fresh weights of shoots as well as roots for the inoculated plants were significantly lower than for the un-inoculated controls. The nematode density on the inoculated plants, however, did not increase but dropped considerably as compared to the initial population. Cotton must, therefore, be considered as very susceptible to damage, but it is not a suitable host for *T. dubius*.

b. Sorghum

Simultaneously with the experiment as described under a and according to the same scheme an experiment was made with sorghum (*Sorghum vulgare* 'Dochna'). The only difference was that now two plants per tube were maintained and not one. The results are summarized in Table 1.

The inoculated *Sorghum* plants were slightly stunted. Shoot and root weights were on an average lower than for the control plants, but the differences were not statistically significant.

c. Green gram

One week old plants of green gram (*Phaseolus radiatus*), grown singly in pots with 200 g of sterilized sandy soil, were each inoculated with 2900 freshly extracted *T. dubius*, on 13.3.1968. Five pots were inoculated and five were kept as un-inoculated controls. The plants were randomly arranged in a climate chamber with 25°C constant temperature and 16 hours artificial light per day. STEINER's nutrient solution was provided once in three weeks to all the pots. The experiment was terminated 80 days after inoculation and nematode densities as well as shoot and root weights of plants were determined. The results are summarized in Table 32 and Fig. 56A.

The inoculated plants grew irregularly, their roots were small, brittle and very dark, and the leaves were small and lightly coloured compared to the control plants. Shoot weight and root weight were both less than half the weight of the control plants and these differences were significant. The nematodes multiplied strongly, to 11.4 times the inoculated numbers, and reached densities of more than 160 per g of soil on an average and up to 230 per g in an incidental replicate (in pots with 200 g of soil). Green gram, therefore, is a very suitable host for *T. dubius* and is susceptible to damage.

d. Pearl millet

One week old seedlings of pearl millet (*Pennisetum typhoideum* 'Millet zango') grown singly in glass tubes with 225 g of sterilized sandy soil, were each inoculated with 5800 freshly extracted *T. dubius* on 28.9.1967.

Five tubes were inoculated and five were kept as un-inoculated controls. The tubes were randomly arranged in the climate chamber with 25°C temperature

TABLE 32. Inoculation of *T. dubius* to cotton (sown) in plastic tubes with 300 g of soil, *Sorghum* (grown in plastic tubes with 300 g of soil), green gram (grown in pots with 200 g of soil) and pearl millet (grown in glass tubes with 225 g of soil). Figures are nematode numbers, fresh shoot weights and fresh root weights per tube or pot, as average of 5 replicates. Significance of differences between un-inoculated and inoculated are indicated by – = not significant, * = significant at 5%, ** = at 1%, *** = at 0.5%.

Experiment and crop	Nematode numbers: Inoculated	Final (range between brackets)	Shoot weight in g		Root weight in g	
			Inoculated	Uninoculated	Inoculated	Uninoculated
a. Cotton	10000	910 (560–1520)	3.66* (3.00–5.00)	4.86 (4.00–7.00)	1.50* (0.50–2.50)	3.30 (2.00–5.00)
b. Sorghum	10000	11472 (8050–17550)	3.64– (2.70–4.50)	4.40 (3.00–6.50)	0.78– (0.3–1.4)	0.96 (0.5–1.5)
c. Green gram	2900	32660 (3600–46000)	2.00* (1.00–3.00)	4.00 (3.75–4.25)	1.34** (0.20–2.25)	3.05 (2.75–3.25)
d. Pearl millet	5800	1238 (850–1500)	2.00*** (1.15–2.57)	4.28 (4.11–4.65)	0.27*** (0.06–0.41)	0.93 (0.71–1.35)

and 16 hours of artificial light per day. STEINER's nutrient solution was given once in three weeks. The experiment was evaluated 102 days after inoculation. Data on plant weight and nematode population are summarized in Table 32 and Fig. 55 D.

The inoculated plants grew poorly, whereas root systems were reduced with discoloured roots, leaves had a lightgreen colour and ear formation was suppressed or much retarded. The un-inoculated plants grew much better, roots were better developed and ears were formed. The shoot weight of the inoculated plants was reduced to about 47% and the root weight to about 30% of the controls, and these differences were statistically very significant. The final nematode density, however, was considerably lower than the inoculated density. Pearl millet, therefore, is very susceptible to damage, but it is apparently not a suitable host plant for *T. dubius*, as was the case with cotton.

6.2.2. *Increasing nematode dosages*

Thirty plastic pots were filled with 200 g of sterilized sandy polder soil. Each was sown with one seed of bean, *Phaseolus vulgaris* 'brown'. A week later, on 21.6.1968, 5 groups of 6 pots were inoculated with 0, 2500, 5000, 7500 and 10000 *T. dubius* respectively. The plants grew well for about a month; then *Tetranychus* mites attacked the plants and killed them even after spraying with an acaricide. The aerial parts of the plants were cut and removed and *Phaseolus radiatus* was grown in each pot. These plants grew well and they were cut at the surface level after about 50 days. Then, on 11.9.1968, seeds of turnip (*Brassica campestris* var. *rapa*), were sown in each pot. One plant was maintained after germination as a test plant. The pots were randomly arranged in the glasshouse with fluctuating temperature. STEINER's nutrient solution was given once per three weeks. The plants grew well, although better in the control pots than in the inoculated pots. Ten weeks after sowing of the turnips the experiment was evaluated. Nematode densities and weights of plant roots and shoots were determined. The results are summarized in Table 33, and in Fig. 56B.

The inoculated plants grew much more poorly than the controls. Growth reduction increased with inoculum dosages, although this increase was barely significant. This may be due to the fact that the difference between the initally inoculated populations were levelled off by the two plants which preceded turnips. Inoculated turnip plants had reduced root systems with fewer root hairs and smaller leaves with a lighter colour than the control plants. Root, shoot and total plant weights of the inoculated plants were only a fraction of the weights of control plants. The final *T. dubius* densities were much higher than the inoculated numbers in all series and passed average densities of 175 per g of soil (in pots of 200 g) and of 600000 per g of plant tissue or 900000 per g of root tissue. The final densities cannot be compared directly to the inoculated densities, due to the fact that turnip was not the first crop following inoculation. But it may safely be concluded that turnip is susceptible to damage and that it is a suitable host plant for *T. dubius*. It is noteworthy that the densities of saprozoic nematodes also rose to high densities in all inoculated series, whereas their number in the un-inoculated controls was still negligible.

TABLE 33. Inoculation of increasing densities of *T. dubius* to turnip grown in plastic pots with 200 g of soil. Figures are nematode numbers, fresh shoot weights or fresh root weights per pot, as averages of 6 replicates. L.S.D. = least significant difference.

Inoculated nematode numbers	Final nematode numbers (range between brackets)	Shoot weight in g	Root weight in g
0	0	2.64 (1.30–5.70)	0.52 (0.20–1.30)
2500	35350 (20000–51600)	0.88 (0.53–1.50)	0.06 (0.02–0.13)
5000	32516 (7600–46000)	0.57 (0.08–0.98)	0.12 (0.06–0.22)
7500	25767 (17800–38600)	0.57 (0.08–1.31)	0.11 (0.01–0.30)
10000	30833 (16000–57800)	0.50 (0.09–0.98)	0.03 (0.01–0.09)
L.S.D. at 5%	0.21	0.92	0.22

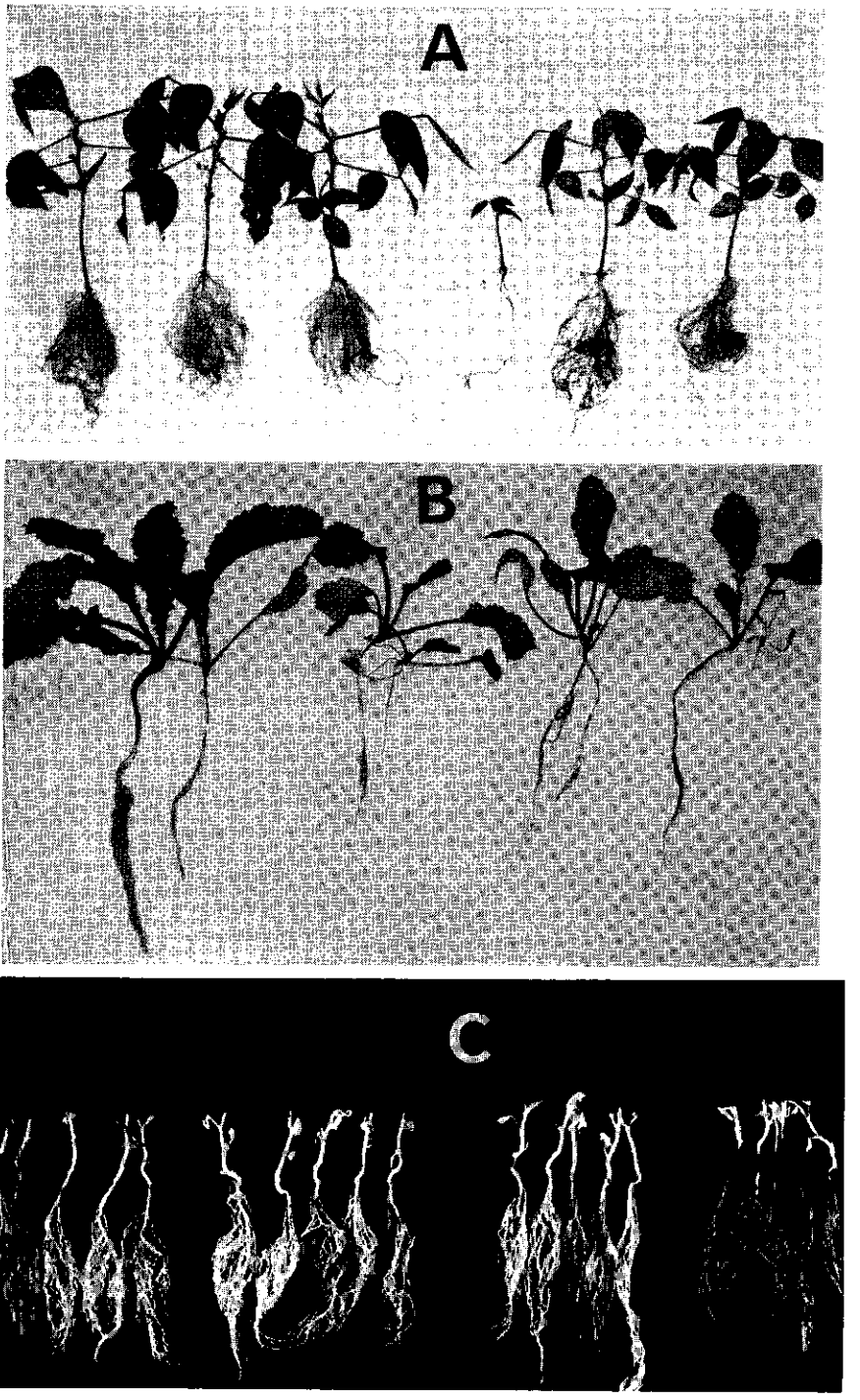
Analysis of variance:
F for root weight = 6.53
F for shoot weight = 8.25

6.3. COMPLEX INFESTATION

The pea crop of 1969 on the monoculture pea plot showed very poor growth compared to the neighbouring plots where peas were grown in rotation with other plants and this difference could not be due to nematode infestation alone. The plants started drying up very early accompanied by leaf discolouration and limited pod formation. The root system was reduced and darkly coloured and the foot of the plant stems became brown to black. From the black stem foots the fungus *Phoma medicaginis* var. *pinodella* (Jones) Boerema comb. nov. was isolated and identified in the Mycology Section of the Diagnostics Department of the PD. Every year this fungus is found in many samples of pea and other leguminosae with such black stem symptoms sent from all over the country to this Department. Experimental work with the fungus by BOEREMA *et al* (1965) and other literature data indicate that it must be considered a weak parasite, which may harm plants if they are weakened by other unfavourable factors or conditions.

The preplant nematode community comprised 860 *T. dubius*, 430 *R. robustus*,

FIG. 56. Results of inoculation experiments. A. Green gram. At left: control, three replicates; at right: inoculated with 2900 *T. dubius* per pot of 200 g soil on 13.3.1968, three replicates; photograph taken 90 days later. Cf. Table 32. B. Turnip. From left to right inoculated with 0, 2500, 5000 and 7500 *T. dubius* per pot of 200 g soil on 21.6.1968, after which first *Phaseolus radiatus* was grown, and then turnip was sown on 11.9.68; two replicates; photograph taken ten weeks later. Cf. Table 33. C. Pea (root system with black stem symptoms only to indicate black stem foot). From left to right control, nematodes only (2560 *T. dubius*), fungus only (suspension of *Phoma medicaginis*), and nematodes + fungus (2560 *T. dubius* + suspension of *P. medicaginis*), five replicates. Inoculations on 12.8.69, photographs taken after 9 weeks. Cf. Table 34.


A
B
C

1375 other stylet-bearing nematodes and 2665 saprozoic nematodes (cf. Table 28). *T. dubius* was the only numerous species which appeared to be closely related to pea and was shown to decrease growth of the plant in earlier experiments (cf. 6.2.1.a-d). Therefore this effect of *T. dubius* was studied as a possible partner in a complex infestation with the afore-mentioned fungus. Synergistic relations between *T. dubius* and micro-organisms in the causation of plant disease are unknown. Several other nematode-fungus combinations. however, are recorded as the cause of complex diseases in various crops (NEWHALL 1958, MORGAN 1957, THOMASON *et al*, 1959, LABRUYÈRE *et al* 1959, HOLDEMAN 1956, POWEL 1963).

In order to study the pathogenicity of the *T. dubius – Phoma medicaginis* association two inoculation experiments were made to pea, with a nine weeks interval between them, one with a low inoculum density of the nematode, the fungus and the combination, and the other with a high inoculum density of the organisms. *T. dubius* was extracted from a monospecific culture grown on ryegrass at 20 °C in initially sterilized sandy soil; the population was in water but was not made complete aseptic by further sterilization. *Phoma medicaginis* var. *pinodella* was isolated from blackened stem foots of pea from the monoculture pea plot cultured on cherry agar in tubes with 7 ml cherry agar each at room temperature.

6.3.1. *Nematode-fungus interaction at low inoculum densities*

Twenty plastic pots were each filled with 200 g of sandy soil (of the crop rotation trial field) which was sterilized one month earlier and stored at 5 °C. An one week old pea seedling, germinated in sterilized peat soil, was transplanted in each pot on 12.8.1969 and the same day batches of five pots were inoculated as follows:

a. *Control* (10 ml of distilled water, per pot)
b. *Nematodes only* (2560 specimens of a *T. dubius* population with all developmental stages in 10 ml of water, per pot)
c. *Fungus only* (10 ml of a suspension of mycelium and pycnidia of *P. medicaginis* in the 14 ml cherry agar of two culture tubes mixed with 40 ml of distilled water, per pot)
d. *Nematodes + fungus together* (dosages mentioned under b and under c, per pot).

All pots were randomly arranged in a tray with distilled water to avoid penetration of micro-arthropods; the tray was placed in a climate chamber at 20 °C and 12 hours of artificial light per day. Twenty ml of STEINER's nutrient solution was added per pot every week, as well as distilled water according to the apparent need of the plants. The plants developed well, although growth differences occurred which were recorded. Nine weeks after inoculation the pots were evaluated. Nematode numbers and weight of roots, shoots and total plants were determined and symptoms of shoots and roots were recorded and photographed. The results are summarized in Table 34 and Fig. 56C and described below.

Table 34 indicates, that the plants inoculated with nematodes + fungus had

TABLE 34. Separate and combined effects of *Tylenchorhynchus dubius* (2560 specimens per pot) and *Phoma medicaginis* var. *pinodella* (10 ml suspension of mycelium and spores: cf. text) on peas grown in pots with 200 g of sterilized sandy soil. Evaluation 9 weeks after the inoculation on 12.8.1969. Final nematode numbers and 'fresh' plant weights in g are per pot, as averages of five replicates; between brackets ranges are recorded. L.S.D. = least significant difference at 5%; N.S. = no significant difference.

Inoculations	Root weight	Shoot weight	Total plant weight	Final nematode numbers
Control	1.16 (0.75–1.58)	2.84 (1.71–4.11)	4.00 (2.84–5.11)	–
Nematodes only	0.91 (0.68–1.35)	3.06 (2.16–5.12)	3.96 (2.86–6.47)	7700 (1000–11850)
Fungus only	0.94 (0.77–1.18)	2.39 (1.78–3.21)	3.33 (2.55–4.26)	–
Nematodes + fungus	0.52 (0.43–0.73)	1.98 (1.58–2.36)	2.50 (2.02–3.09)	16270 (5600–22950)
L.S.D. 5%	0.33	N.S.	N.S.	N.S.

significantly less root weight than the other series. These plants ripened much earlier than the plants in the other series, and they were the only plants with a black foot and with a dark-coloured root system. These differences are conspicuous as can be seen from Fig. 56C. The blackening of the stem foot appeared as small stripes four weeks after inoculation and finally covered the whole stem basis up to a height of 2.5 cm. Shoot weight and total plant weight was also lower for the nematode + fungus series than for the others, but these differences were statistically not significant. Nematodes only have not shown significant damage to the growth of the peas, although root weight and total plant weight were somewhat lower than for the controls. This was also true for shoot weight of fungus only, but not for shoot weight of nematode only. The plants with nematodes only had green tops, and were therefore not ripened as far as the others, at the moment of evaluation. It is at any rate clear that the influence of nematodes only and of fungus only was neglibible or light in this experiment, whereas nematodes + fungus caused marked effects: darkened and reduced roots, blackened stem foots and early ripening. It is is further noticed that the inoculated *T. dubius* increased from 2560 per pot to 7700 on the plants with nematodes only, and to 16270 on the plants with nematodes + fungus. Strong nultiplication occurred without a significant difference between the two series, but it is worth to note the tendency that nematodes + fungus caused higher final densities than nematodes only.

6.3.2. *Nematode-fungus interaction at high inoculum densities*

The experiment described under 6.3.1. was repeated on 11.11.1969 according to the same scheme, but with inoculum levels which were about six times higher. The number of *T. dubius* per pot was now 16000 and the amount of fungus per pot was the whole contents of a petriplate with 13 ml agar grown with *P. medicaginis* for two weeks. The final evaluation was again made 9 weeks after inoculation. The results are summarized in Table 35 and described below.

The fresh weights of shoots and total plants, as well as the dry weights of the total plants, showed significant differences; they decreased in the order Control – fungus only – nematodes only – nematodes + fungus. The figures for nematodes + fungus were less than 2/3 for the control plants, but the differences between nematodes only and 'control' were also significant, as well as between nematodes + fungus and fungus only.

Fresh root weight decreased in the order control – fungus only – nematodes + fungus – nematodes only, but the figures were more variable and their differences were only significant at the 10% level. There were, however, distinct qualitative differences. Black stem foots were present in all plants inoculated with nematodes + fungus, in 3 out of the 5 replicate plants inoculated with fungus alone and in none of the plants of the other two series. Root browning and early yellowing was obviously correlated with black stem foot. This experiment indicates that a heavy dosage of the fungus alone can cause black stem foot of pea, and consequently root browning and early yellowing, but again that the nematode promotes it, and therefore that there is nematode-fungus interaction with

TABLE 35. Separate and combined effects of *T. dubius* (16000 specimens per pot) and *Phoma medicaginis* var. *pinodella* (mycelium and spores of a whole petriplate per pot: cf. text) on peas grown in pots with 200 g of sterilized sandy soil. Evaluation 9 weeks after the inoculation on 11.11.1969. Final nematode numbers and plant weights in g are per pot, as averages of five replicates; between brackets ranges are recorded L.S.D. = least significant differences at 5%; N.S. = no significant difference.

Treatments	Fresh root weight	Fresh shoot weight	Fresh plant weight	Dry plant weight	Final nematode numbers
Control	1.99 (1.30–2.59)	4.81 (3.80–5.45)	6.80 (5.10–7.95)	1.23 (0.90–1.50)	–
Nematodes only	1.24 (1.03–1.40)	3.50 (2.92–4.02)	4.73 (4.32–5.32)	0.98 (0.70–1.19)	2580 (1050–3900)
Fungus only	1.84 (1.21–2.20)	3.94 (2.65–4.80)	5.78 (3.86–6.79)	1.14 (0.94–1.30)	–
Nematodes + fungus	1.56 (1.09–2.34)	2.71 (1.73–3.58)	4.27 (2.82–5.26)	0.78 (0.50–1.04)	4390 (2100–5250)
L.S.D. 5%	N.S.	0.90	1.37	0.27	N.S.
10%	0.48	–	–	–	0.23

respect to pathogenicity. It is also clear that the heavy dosage of the nematode alone caused significant growth reduction without causing black stem foot.

The high density of *T. dubius* dropped on an average in the inoculated pots. Numbers were higher on the plants when also fungus was added; the difference between the two series was significant at the 10% level.

6.4. Discussion

T. dubius is an obligatory root parasite of higher plants which reproduces strongly on certain species. This does not necessarily imply that the plants suffer from the attack. Our experiments, however, prove that several plants are seriously damaged by the nematode at densities which also occur in the field. Each nematode infestation is a specific relation between a certain nematode species (or rather strain or trophotype) and a plant species (or rather variety or cultivar) The amount of damage is largely determined by the preplant nematode density, but it is usually influenced by the environmental conditions (cf. Oostenbrink, 1966). It is in this context important to keep in mind that our experiments have largely been made in pots, the results of which are supplemented with field observations.

The experiments prove that sorghum, ryegrass, wheat, green gram, turnip and pea were all susceptible to damage and were suitable hosts, because the inoculated nematode density increased. Tropical plants may obviously be damaged by *T. dubius* in addition to its common hosts. The data about rice are not consistent. Pearl millet was also very susceptible to damage, although it did not increase nematode populations. This stresses the necessity to consider host suitability and susceptibility to damage as separate phenomena in the nematode/plant relationship which are not necessarily correlated. It is further remarkable that under our experimental conditions root systems of potato and *Tagetes*, which are both poor hosts, were reduced without significant reduction of the total plant weight. The final nematode densities reached per g of soil or per g of plant roots when inoculated plants are grown in pots are unnaturally high due to strong concentration of the roots, and it is probable that the initial inoculum density in pot experiments has to be high as well to simulate infestation in the field where usually far more nematodes are available per plant than are inoculated into small containers. It is noteworthy in this context, that damage of, for example, ryegrass occurred when 20 nematodes were inoculated per 150 g of soil (Table 7) or at densities from 30 nematodes per 150 g of soil (Table 16, Figs 53A and 55B). Results of the PD crop rotation field trial, which are not recorded in detail in this study, also revealed a significant regression of the growth of grass on the preplant nematode density. The data, therefore, indicated that *T. dubius* causes damage at densities which normally occur in the field, and therefore it must be considered a noxious parasite in agriculture.

Effect of combined inoculations of *T. dubius* and the fungus *Phoma medicaginis* to pea proves for the first time, that *T. dubius* is a partner in complex infestations.

SUMMARY

By means of investigations in the laboratory, in greenhouses and in the field the author collected data on the biology, population dynamics and economic importance of *Tylenchorhynchus dubius*, the most common of the nematode species that live ectoparasitically on the roots of higher plants in sandy soils in the Netherlands and neighbouring countries.

After reviewing the literature and the methods used the author devoted some chapters to multiplication, development and growth, ecology, the field population and the damage to plants.

The study of the life cycle comprised observations and experiments on copulation, egg-stage, first larval moult in the egg, the emergence of the L_2, the development and morphology of the larval and adult stages and the morphological variation of the latter. The life cycle could be followed fairly completely; it was found to take 40–48 days. The data are fixed in Tables 1–3, photographs and drawings (Figs. 1, 2, 5–12), and in a documentary film.

As to ecology and behaviour, the following aspects were studied: movement, feeding, host plants, survival with and without host plants, influence of the physical environment and of the biotic environment in the soil.

The observations and experiments are represented in Table 4–27 and Fig.13–41. They give a picture of the biology of the nematode in relation to its environment, which is now more fully documented than that of the other ectoparasitic nematode species so far.

The field research was done chiefly on a population in the garden of the Plant Protection Service, and supplemented by pot experiments. During five years the author studied effects on host plants, density and distribution in the soil, and seasonal population fluctuations in relation with physical and biotic factors. The results are summarized in Tables 28–31 and Figs. 42–52.

Inoculation experiments and observations on damage showed, that, apart from discoloration of cortical cells, *Tylenchorhynchus dubius* does not produce conspicuous symptoms of the roots. The infestation results in growth decrease, e.g. in English rye-grass, wheat and turnip, but also in cotton, millet and other tropical crops. In combination with *Phoma medicaginis*, *T. dubius* induces black stem foot disease in peas which is not produced by either of these organisms separately (Table 32–35, Fig. 53–56). The fact, that this nematode is widely found in densities judged harmful, indicates that it is economically important and that attention should be given to its control.

SAMENVATTING

Door onderzoek in laboratorium, kas en veld werden gegevens verzameld over biologie, populatiegedrag en economische betekenis van *T. dubius*, de meest voorkomende ectoparasitair op de wortels van hogere planten levende aaltjessoort op zandgrond in Nederland en omgevende landen.

Na een overzicht van de literatuur en de gebruikte methoden werden hoofdstukken gewijd aan vermeerdering, ontwikkeling en groei, de ecologie, de veldpopulatie en de schadelijkheid.

De studie van de levenscyclus omvatte waarnemingen en proeven over paring, ei-stadium, embryo-ontwikkeling, eerste larvale vervelling in het ei, het uitkomen van de L_2, de ontwikkeling en vorm van de larvale stadia en de volwassen dieren en de morfologische variatie van de volwassenen. In 40–48 dagen is de levenscyclus, die vrij volledig kon worden gevolgd, voltooid. De gegevens zijn vastgelegd in tabellen, foto's en tekeningen (Tab. 1-3, Fig. 1, 2, 5-12) en in een documentatie-film.

Het onderzoek over ecologie en gedrag had betrekking op beweging en voeding, waardplanten, overleving met en zonder waardplanten, invloed van het fysische milieu en van de levende componenten in de grond. De waarnemingen en proeven zijn weergegeven in Tab.4–27 en Fig.13–41. Zij geven een beeld van de biologie van het aaltje in verband met zijn milieu dat uitvoeriger gedocumenteerd is dan van de andere tot nu toe onderzochte ectoparasitaire soorten.

Het veldonderzoek werd vooral gericht op een populatie in de PD-tuin, aangevuld met proeven in potten. Invloed van waardplanten, dichtheid en verdeling in de grond, en seizoens-populatie schommelingen in verband met fysische en biotische factoren werden gedurende 5 jaren bestudeerd. De resultaten zijn samengevat in Tab. 28-31 en Fig. 42-52.

Uit inoculatieproeven en waarnemingen over schade bleek, dat *T. dubius*, behalve verkleuring van schorscellen, geen opvallende symptomen aan de wortels veroorzaakt. De aantasting veroorzaakt wel groeivermindering, o.a. bij Engels raaigras, tarwe en koolraap, maar ook bij katoen, gierst en andere tropische gewassen, terwijl *T. dubius* samen met de schimmel *Phoma medicaginis* bij erwt een voetziekte verwekt die niet optreedt als de organismen afzonderlijk voorkomen (Tab. 32–35, Fig. 53–56). Het wijd verspreid voorkomen van schadelijk geachte dichtheden van het aaltje wijst er op, dat het economisch van belang is en dat de bestrijding aandacht verdient.

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* Original not seen.