CLIMATIC INFLUENCE ON THE DISTRIBUTION PATTERN OF PLANT PARASITIC AND SOIL INHABITING NEMATODES

FEDERICO DAO D.

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(With English, Spanish and Dutch summaries)

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWWETENSCHAPPEN
OP GEZAG VAN DE RECTOR MAGNIFICUS, DR. IR. F. HELLINGA,
HOOGLERAAR IN DE CULTUURTECHNIEK,
TE VERDEDIGEN TEGEN DE BEDENKINGEN VAN EEN
COMMISSIE UIT DE SENAAT
VAN DE LANDBOUWHOGESCHOOL TE WAGENINGEN
OP VRIJDAG 13 FEBRUARI 1970, TE 16.00 UUR

DOOR

FEDERICO DAO D.

H. VEENMAN & ZONEN N.V. - WAGENINGEN - 1970

THEOREMS

I

Black kernels in wheat samples from ancient graves in the Agricultural Museum in Dokki, Cairo, are probably galls of *Anguina tritici*.

II

Local inspections are of great and border inspections of small value in quarantine and other regulatory control of plant nematodes.

III

Certain fungi inhibit nematodes by the formation of nematicidal substances. This has to be considered of importance to the pathogenic activity of these nematodes.

IV

Cultivation of the African oil palm in Venezuela should be extended despite unusual risks due to the 'red ring disease'.

G. Malagutti (1953) 'Pudricion del cogollo de la palmera de aceite africana (Elaeis guineensis Jacq) en Venezuela'.

V

Nematodes are involved as vectors of viruses, and other soil born pathogens to a much larger extent than is reported up to now.

VI

The best incentive for the development of tropical countries is international levelling of market prices for important agricultural products.

VII

Modernization of agriculture in developing countries should start from pilot areas, under the condition that products can be sold at reasonable prices.

VIII

The family structure of part of the Venezuelan population, as well as in other Latin American countries is a major social problem, which demands special education measures together with efforts to improve the level of living.

IX

The procedure for gaining a doctorate in the Netherlands could be improved by accepting some principles from the American system.

X

The title 'professor' to address certain university teachers should be abolished.

F. DAO D.
Wageningen, 13 February 1970

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1. GENERAL INTRODUCTION

Nematodes are among the most abundant and widespread of all organisms (COBB 1915). They are numerous in nearly every moist habitat in which organic material is present, such as soil, fresh and salt water, plants, animals and a wide variety of organic substrates. Zoological analyses indicate that nematodes, being the most ubiquitous group of the animal kingdom, constitute more than 80% of all multicellular animals (STÖCKLI 1946). This is mainly due to their great density in soil. The dominant soil animals, apart from Protozoa, are either nematodes, the most numerous in terms of individuals, or arthropods, the most numerous in terms of species (GHILAROV 1967).

In order to give an impression of the numbers of animals of different groups that may be present in the soil, the results of some of the most thorough analyses of soil microfauna are summarized in table 1. It should be noted, that sampling and extraction techniques are diverse and that their efficiency differs for the various animal groups and for individual workers on a particular group. This is borne out by the reports of two colloquia on such techniques organized at Rothamsted in 1958 by the Soil Zoology Committee of the International Society of Soil Science (MURPHY 1962), and at Paris in 1967 by the Unesco International Biological Program (PHILLIPSON, in print). It is however not likely that other animal groups are lost to a much larger extent than nematodes, so that there is no reason to doubt the numerical dominance of the nematodes as illustrated in table 1.

TABLE 1. Number of nematodes and other numerous multicellular animals in millions beneath one square meter of soil calculated from the data published by different authors

Records / Animal groups	After GISIN (1947) Meadow soil	After STÖCKLI (1946), modified by MACFADYEN (1957) Meadow soil	After FRANZ (1950)			
			Meadow peat	Meadow sand	Arable peat	Arable sand
Nematoda	6	1.8 – 120	18.2	5.8	4.7	4.5
Acari	0.4	0.02 – 0.12	0.024	0.015	0.009	0.014
Collembola	0.2	0.01 – 0.04	0.013	0.007	0.018	0.025
Other Arthropoda	0.02		0.003	0.002	0.001	0.003
Rotatoria and Tardigrada	0.1		0.27	0	0	0
Enchytraeidae	0.01	0.002 – 0.02	0.003	0.028	0.003	0.033
Lumbricidae	0.0004	0.00003 – 0.002	0.00007	0.00035	0	0.00014

Early records of nematode densities in the soil are often lower. Summaries are made by HYMAN (1951), FILIPJEV & STEKHOVEN (1941), PETERS (1955), STÖCKLI (1943) and OOSTENBRINK (1956). The last mentioned two authors indicate, that the nematode densities found by different authors increased more than thousand fold in the course of some decades owing to the use of improved techniques and that the actual numbers are as a rule several millions per square metre of soil.

The experimental part of this study is restricted to soil inhabiting nematodes and to plant parasites, which all have a soil phase as well, with emphasis on the nematofaunas in Venezuela and in the Netherlands. OOSTENBRINK (1966) recorded average nematode densities per 100 ml of soil of 3004 and 5620 in arable land and meadows in the Netherlands, and of 794 in various arable grassland and bush plots in Venezuela; on an average 30%, 41% and 67% of these nematode communities respectively were known and suspected plant parasites.

Nematodes are widely spread as a group. They are known to occur in nearly every soil, but pertinent knowledge about the geographic distribution of individual species is scarce. This is due to incomplete coverage of areas and of the habitats within a given area, and to erratic collection and poor identification of the nematodes.

Most of the earth's surface has never been included in regional inventories despite the fact that numerous incidental surveys have been made for over a century. These inventories were normally local, they included only a small fraction of the possible nematode habitats of the area and only a part of the fauna at each sampling site was normally caught.

Up to some years ago inventories were also very erratic and selective due to lack of quantitative methods for the extraction of collected samples. Recent surveys with so called quantitative extraction techniques, however, also overlook the larger part of the species established in a field, unless many large samples are taken and several thousands of nematodes are identified. Usually 10 to 20 species occurring in high densities are recorded from one sample after identification of a few hundred specimens, which means that species occurring irregularly distributed or at densities below 1 per 10 ml of soil often go undetected. Scrutiny of one old meadow in Belgium by taking eight samples at different places and times and identification of about 5000 specimens revealed the presence, however, of at least 69 species, which was then the greatest number of species recorded from one single field (COOMANS 1962). Other habitats may even be richer in species: Filipjev found up to 140 species of nematodes in a single sample of sea sand at the Murman coast (FILIPJEV & STEKHOVEN 1941).

Identification of the nematodes raises special problems (STEINER 1956, ALLEN 1960, LOOF 1960, DE CONINCK 1968). Few specialists are able to identify all nematodes; and plant parasitic and free-living species belong to widely different taxonomic groups. Old species descriptions are often based on a group of related species which may cause uncertainty about the identity of a

record. The occurrence of several species of a particular genus in the same nematode community is by no means uncommon (OOSTENBRINK 1957, COOMANS 1962); this is not realized in many survey reports. Up to January 1969, the number of nematodes species in plants, soil and fresh water was about 2500, according to the card index at the Landbouwhogeschool, Wageningen. A large part of the existing nematode species, however, is still undescribed. Estimates differ widely but may exceed 90% and go as high as 98% or 99% when all nematode categories are considered (HYMAN 1951, THORNE 1961). FILIPJEV & STEKHOVEN estimated in 1941 that at that moment about 2000 of more than 30000 existing species of plant, soil, fresh water and marine nematodes were described; i.e. about 7%. The actual percentage of undescribed nematode species is difficult to estimate because it is also dependent on the results of further experimental work concerning the species concept of nematodes, i.e. concerning the variability of morphological characters and the physiological specificity or polyphagy. Many species are undescribed anyway, and in many survey reports new species are denoted by the name of the closest related species described.

Finally, biological differentiation within species appears to be common at least within plant parasitic species, and an inventory would only be complete as a basis for experimental work and for agricultural evaluation if strains which differ in host affinity or in another important aspect are recorded separately. This has hardly been done in any inventory up to now. It is clear therefore that factual knowledge about nematode distributions is of a local nature and that it mainly concerns widespread species with dense populations or some plant parasites which receive special attention from quarantine agencies.

Data to explain the situation are incidental and hardly sufficient to formulate hypotheses of general validity, whereas experimental data have so far been too scarce to provide a basis for generalization.

1.2. PUBLISHED FAUNISTIC INVENTORIES

The total number of publications on plant, soil and fresh water nematodes was 4 in the 17th, 25 in the 18th and about 1.000 in the 19th century, after which it increased rapidly to some 20.000 items now, according to the literature card index available at the Landbouwhogeschool at Wageningen. Faunistic studies and taxonomy developed together, side by side, as could be expected, and the corresponding literature amounts to about 20% of the total literature. Nematode taxonomy has been comprehended in some textbooks; but a recent review of faunistic data which is needed as a basis for our study is not available and has to be made. It is not possible to keep marine nematodes from a literature review on plant, soil and freshwater forms, because the data are often interwoven. In the review we distinguish an initial preliminary period, which is considered to end with DE MAN's classic faunistic studies, then followed by a period of general zoological inventories based on random samplings and general expeditions which declined during the second world war period, although some

workers are still engaged on this type of work, and finally the post-war period, which is characterized by a boost of phytonematology and hence by many reviews or surveys of plant and soil nematodes for agricultural purposes. In our attempt to give a near comprehensive review of faunistic nematode studies made over the years it appeared to be necessary to use different patterns in summarizing or compilating the available information about the successive periods, and also to refer often to the bibliography on plant, soil and freshwater nematodes by BAKER, OOSTENBRINK & VAN BERKUM (1967) and some other published lists to complete the literature references.

1.2.1. *Initial surveys*

BORELLUS (1656) discovered the first free-living nematode, the vinegar eelworm *Turbatrix aceti;* several pioneers of microscopical research, e.g. POWER, HOOKE, VAN LEEUWENHOEK, BAKER, SPALLANZANI, showed interest in this nematode. In 1743 NEEDHAM recorded the first plant parasitic nematode, the wheat gall nematode *Anguina tritici.* MÜLLER (1786) described several species of free-living marine and freshwater nematodes. His work was followed by studies by DUJARDIN (1845), LEIDY (1852), CARTER (1859), CLAUS (1862), EBERTH (1863) and others. Their efforts resulted in the discovery of about eighty species of free-living nematodes found in various parts of the world. Then followed the outstanding studies by BASTIAN (1865), A. SCHNEIDER (1866), BÜTSCHLI (1873) and DE MAN (1876, 1880, 1884) which are among the most famous faunistic records.

DUJARDIN (1845) was evidently the first who tried to collect notes on the extent and probable diffusion of free nematodes and their pluriformity. His general work on helminths, which is primarily an extensive compilation, comprised a long chapter on nematodes. These were mainly zooparasites, but the now well known genera of soil inhabiting nematodes *Dorylaimus* and *Rhabditis* were also described. He considered *Rhabditis* species, among which he counted the vinegar eelworm and the wheat gall nematode, as the group which delivered the most precious deductions for biology and he speculated on such phenomena as drought resistance, spontaneous generation and transmigration of free-living soil and water rhabditids into animal parasites.

LEIDY described nematodes from stagnant water near Philadelphia in 1851.

Later CARTER (1859) published a local faunistic survey on 'Dracunculus and microscopic Filaridae in the island of Bombay', in which he described ten new species from water in the tanks and marshes of the peninsula. He stated that the free-living nematodes 'abound in species, and are spread in myriads probably all over the world where there is vegetable matter for them to feed upon, in salt as well as in freshwater, in the sea and on the land' while the zooparasitic nematodes 'inhabit all animals, perhaps more or less down to the lowest worms', and also that 'these worms at first apparently insignificant from their simple thread-like form and scarcity are seen to assume an importance in organic creation which calls for a much more extended study of them than they have as yet received'.

Some years later CLAUS (1862) reported on some nematodes in humus and EBERTH (1863) gave a general review and described 23 new nematode species from the Mediterranean.

In 1865 BASTIAN made the first extensive faunistic survey in Great Britain. Up to then an estimated 550 distinct species of zooparasitic nematodes had been described. Of the other nematodes only the vinegar eelworm, the wheat gall nematode and one or two other species were reported as existing in Great Britain. Within fifteen months he obtained from a few limited regions no less than 20 new genera, 100 new species of soil, freshwater and marine nematodes. The nematodes from soil and freshwater represented 13 genera, 9 of which were new, viz. *Aphelenchus*, *Cephalobus*, *Ironus*, *Monohystera*, *Mononchus*, *Plectus*, *Trilobus*, *Tripyla* and *Tylenchus*. By his own account he found nematodes 'in all the specimens of soil examined, in moss, various species of lichen, about the roots of fungi, also the roots of grasses, and between the sheaths of their leaves, amongst the mud of ponds and rivers, on the freshwater algae, amidst decaying liverworts and mosses, and on submerged aquatic plants. The marine species exist in great abundance in the surface-mud of rivers and estuaries, in the sand and amongst the small stony debris under the shelter of rocks as well as in the tide pools, where they swarm about the roots of the corallines and on some of the smaller and finer sea-weeds'. Further studies followed in 1866.

In the same year A. SCHNEIDER (1866) recorded and described in his monography on the Berlin zoological museum collection a number of free-living and plant parasitic nematodes from Germany, including *Anguillula tritici* (= *Anguina tritici)*. He described 180 species, of which only 30 free-living, but he had not seen BASTIAN's work before the taxonomic part of his monography was finished.

The knowledge about the free-living nematodes in Germany was greatly extended by BÜTSCHLI's 'Beiträge zur Kenntnis der freilebenden Nematoden' in 1873. He was stimulated by BASTIAN's results in England and found 61 soil and water species in Germany more than half of which were new to science. 14 of his species were considered to be identical with U.K. species described by BASTIAN. One year later Bütschli (1874) continued his observations by a study on free-living marine nematodes in the port of Kiel, now stimulated as he was by MARION's (1870 a, b, 1872) publications on marine nematodes in the Gulf of Marseilles. GREEFF (1870) and VON LINSTOW (1876, 1877) added their observations on soil and marine nematodes in Germany while describing several new species. JOSEPH (1879) reported on endemic nematodes in the caves of Krain.

The Hungarian nematofauna from soil and water was first described by OERLEY in 1880, who added descriptions of five new species and also reported on nematodes in the possession of the British Museum (1882).

A milestone were the faunistic studies by DE MAN (1876, 1880, 1884) on the soil and water nematodes in the Netherlands. They were the first faunistic studies in this country. In his 'Onderzoekingen over vrij in de aarde levende nematoden' he found more than 50 species, largely new, and he described 5 new genera: *Tylopharynx*, *Tylencholaimus*, *Teratocephalus*, *Leptolaimus* and *Bastia-*

nia. In 1880 in a preliminary descriptive article he published all the genera and species observed in the Netherlands up to then; in total 141 species of 38 genera, 15 of which were new, viz. *Alaimus*, *Deontolaimus*, *Aphanolaimus*, *Desmolaimus*, *Microlaimus*, *Ethmolaimus*, *Choanolaimus*, *Aulolaimus*, *Prismatolaimus*, *Cylindrolaimus*, *Macroposthonia*, *Rhabdolaimus*, *Odontolaimus*, *Diphtherophora* and *Tylolaimophorus*. His exhaustive monography of 1884 on 'Die frei in der reinen Erde und im süssen Wasser lebenden Nematoden der niederländischen Fauna' dealt with 143 species of 38 genera, but he added records of species observed by others and also faunistic lists of nematode species which he extracted himself from a single grass sod sample from each of 6 other countries, viz. 33 species from Erlangen in Bavaria, 24 from Laibach in Krain (Yugoslavia), 13 from Luzern, Altdorf and Schönegg in Switzerland, 10 from Montpellier in France, 17 from Sydenham in England and 20 from Christiania (Oslo) in Norway. He filed his soil and freshwater nematodes of the Netherlands into five ecological groups: omniphagous nematodes, 'meadow' nematodes, sand nematodes, brackish water nematodes and freshwater nematodes, and he stated that the freeliving soil and freshwater nematodes are largely associated with certain soil types and with freshwater, although some species occur almost everywhere. A large proportion of the nematodes observed in the Netherlands was also observed in Germany, Hungary, France, England and Norway, i.e. probably in whole Europe, though others were not widespread. He knew (1876, 1884) that the nematodes in the Mediterranean differed from those in the North Sea, and that nematodes in the Indian Ocean differed from those in the Gulf of Mexico. Confident about the value of his work he stated: 'Durch meine Untersuchungen ist jetzt die niederländische Nematodenfauna am vollständigsten bekannt geworden'.

Consequently at that time some substantial data were available on the occurrence of soil and plant nematodes in Great Britain, Germany, Hungary and the Netherlands, with stray bits of information concerning other areas, suggested that interesting, and perhaps important research could be done on this group all over the world. This evidently stimulated the activity of many zoologists.

1.2.2. *Zoological surveys and expeditions*

From 1884 on, faunistic work, side by side with taxonomy and accompanied by ecological observations, developed enormously. It included nematodes from soil, freshwater, and the sea as well as plant, moss and special habitats, obtained in many parts of the world from chance samplings and expeditions. Some workers, in Europe as well as in America, switched from one category of nematodes to the other and worked on a truly international basis. The period up to the second world war is dominated by a number of specialized zoologists, including DE MAN, COBB, DADAY, MICOLETZKY, STEFANSKI, HOFMÄNNER, MENZEL, STEINER, FILIPJEV, KREIS, STEKHOVEN, DE CONINCK, SCHNEIDER, ALLGEN and PARAMONOV, with some 40 others contributing notably. After the war period CHITWOOD, GERLACH, ALTHERR, PAESLER, MEYL, ANDRÁSSY, GADEA, HOPPER,

LOOF and others continued this type of general inventories although most nematologists turned to surveys of plant parasitic nematodes (cf. 1. 2. 3). Our review of general faunistic work is essentially a comprehensive description of the activity of the different workers, whose results are only discussed where they show general aspects or are for other reasons essential.

DE MAN published further faunistic studies on nematodes in the Netherlands, Germany and Russia (1885), on nematodes in the North Sea and the Channel (1886, 1888, 1889, 1890, 1893), from the Arctic region (1904), from Walcheren and the coasts of Zealand (1906, 1907a), from the Seine (1907b), from a lake in Tibet (1908a), from soil in Norway (1917), soil and water in the Netherlands (1919, 1921a, 1923a), from the sea near Walcheren (1922a) and the Zuiderzee (1920, 1922b, 1923b), from a channel near Caen (1928) and from the Batu Caves at Selangor (1929). The soil nematodes observed in several Dutch areas over a period of 5 years were listed in 1922. He also made a number of records from plant material, such as algae, orchids, moss, oak slime flux and paper (1892, 1895, 1908b, 1910, 1913). His total work comprised 168 articles including more than 40 on nematodes, and was ably summarized by VAN BENTHEM JUTTING (1951), in whose biography all of DE MAN's articles are listed.

COBB's numerous publications dealt with nematodes in various habitats from all continents. His work comprises studies on soil nematodes from Jena (1889), on Arabian nematodes (1890), on marine nematodes from Australia (1898), on many new species from Australia, Fiji Islands and Europe (1893a), on freshwater nematodes from New Zealand (1904), on nematodes from sugar cane roots *inter alia* from Hawaii (1893b, 1906), on new genera found in freshwater and soil (1913). In his work in the period from 1914 on, largely compilated in his 'Contributions to a Science of Nematology', he described *inter alia* the Antarctic marine nematodes of the Shackleton Expedition (1914a, 1915a), the North American free-living freshwater nematodes (1914b), nematodes in Lake Michigan (1915b), mononchs from the USA and other countries (1917), nematodes of filter-beds in American cities (1916, 1918), type species from a hundred new genera from marine and some other habitats from all over the world (1920), from hot springs (1921a) nematodes collected by the Canadian Arctic Expedition (1921b), nematodes inhabiting trees (1923), nematodes on commercial seed (1924), nematode pests of fruits and vegetables (1926a) and of narcissus (1926b), a stratigraphic survey of nematodes in marine beach sand (1919, 1929), marine free-living nematodes of Australian expeditions (1930), whereas after his death a key to the genera of free-living nematodes appeared (1935).

COBB's contribution to what he indicated as 'a science of nematology' is tremendous. Apart from his faunistic and taxonomic work he published numerous incidental records, biological studies and observations, developed many extraction and other techniques and terms, and is often called 'the father of phytonematology', because he understood and described the widespread occurrence of dense populations of parasitic nematodes and their significance as parasites of plants, as well as of man and animals, in a clear and thought provo-

king way. His comprehensive sketch of the nematode situation (1915, 1927), which has been cited by nematologists again and again, comprises *inter alia* the following elements:

'Nematodes are amongst the most abundant and widespread of all organisms...
If all the matter in the universe except the nematodes were swept away, our world would still be dimly recognizable...
We could recognize the soil and tell where had been one kind of soil and where another...
Trees would still stand in ghostly rows...
The location of various plants and animals would still be decipherable...'

He also stated that the value of manure and of crop rotation should be explained not simply on the basis of the exhaustion of certain elements in the soil, but also as an accumulation of certain organisms. Contrary to the retrospective, analytic DE MAN, COBB made visionary generalizations which were daring in his time but which have been found correct and of great value in recent years.

Further contributions were made in Europe by DADAY. He studied nematodes in alkaline waters (1893a, b), the Balaton Lake (1894, 1903a), the Tatra lakes (1896, 1897a), Hungarian waters (1897b, c), New Guinea (1899, 1901a), the Quarnero (1901b), Patagonia (1902), Asia Minor (1903b), Turkestan (1903c, 1904), Paraguay (1905a, b), Mongolia (1906a, 1913), Swiss waters (1906b, 1911, 1914), the Nile (1910a) and East Africa (1908, 1910b). Other early records on freshwater or marine nematodes were made by CHATIN (1885), DU PLESSIS-GOURET (1885), PLOTNIKOV (1899, 1901, 1906), VON RATZ (1900), DE ROUVILLE (1903a, b, 1905, 1907), TÜRK (1903), ZSCHOKKE (1900), KLAUSENER (1908), JÄGERSKIÖLD (1894, 1901, 1909, 1913), FRIEND (1911), also by JOHNSON (1912), VON LINSTOW (1907a, b, c, 1909) SAVELJEV (1912), STEWART (1914), LEIPER & ATKINSON (1914), DE CILLIS (1917), DITLEVSEN (1919, 1921, 1922, 1923, 1926, 1928 a, b, 1930). DITLEVSEN also published (1911, 1928 a, b) on free-living soil nematodes in Denmark, BRAKENHOFF (1914) and SKWARRA (1922, 1924) recorded nematodes from soil and water from N.W. Germany, MARCINOWSKI 1909) gave an extensive review on plant parasitic nematodes, LUDWIG (1910) reported on nematodes of trees and other plants, DIXON (1905), RICHTERS (1908), HORN (1909) and HEINIS (1908, 1914, 1929) reported on moss inhabiting nematodes, FUCHS (1915) on fungiphagous nematodes, MAUPAS (1900) on nematodes in decaying material, SEURAT (1913) on nematodes from South Tunisia. HOFMÄNNER (1913a, b), HOFMÄNNER & MENZEL (1914, 1915), MENZEL (1912, 1914), STEINER (1913, 1913/1914, 1914a, b) and STEFANSKI (1913, 1914a) all worked on the nematodes in lakes and in soils or plant habitats in Switzerland and surroundings. MENZEL also made a record on nematodes from the Arctic (1920), from the Malay Archipelago (1925a) and the Dutch East Indies (1922, 1925b, 1929, 1930), notably also the Sunda Islands (1943). After initial studies in Switzerland STEFANSKI made several studies on the nematofauna in the river Inn (1915a, 1916a) and in all kind of habitats in Poland (1914b, 1915b, 1916b, c,) including brackish water (1922) and several lakes, especially in the Tatra

mountains (1923, 1924a, b, 1933, 1937, 1938). He also published on nematodes from streaming water in Rumania (1926, 1927).

One of the prominent workers on soil and freshwater nematodes in Europe since DE MAN was no doubt MICOLETZKY, who worked mainly on nematodes in different habitats of the Eastern Alps and Bukowina in Austria (1912, 1913a, b, 1914, 1915, 1917, 1922a, 1923a), but he also studied nematodes from Africa (1916), from the Bodensee (1922c), and from lakes in Northern Germany (1922b), from the Volga (1923b, 1927), from Suez (1922d, 1924) freshwater and peat nematodes from Denmark (1925a), nematodes from the Alai-Pamir Expedition (1929) and marine nematodes from Surinam, Trinidad and East Afrika (1925b) and from the Sunda Islands (1930). His 1922 book is a comprehensive study of great value, and he himself was convinced that it put Austria on the same high level as the Netherlands and Switzerland. COBB was blamed by MICOLETZKY for neglecting the results of European work or treating it superficially. MICOLETZKY in his turn synonymized or neglected many of COBB's species. It is clear now that MICOLETZKY was wrong in doing so and that the taxonomy in his otherwise valuable work caused confusion due to his approach. He divided the nematodes in six ecological groups, viz. ubiquitous forms, nematodes in bogs and swamps, in meadow soils, in humus of forests, in moss, and in sandy soils.

MICOLETZKY found no consistent differences between habitats in low lands and mountains.

After his studies of nematodes in Switzerland, STEINER published on marine, freshwater, soil and plant nematodes in general (1916a, 1918a, 1921a) and reported on them from habitats all over the world, including Sumatra (1915), S.W. and W. Africa (1916b, c, d, 1918b) the Barentssee and Nowaja Zemlja (1916 e, f), the German deep-sea expedition (1918c), the Elbe (1918d), the Neuenburgersee (1919), high-mountain lakes in Perú (1920), different seas including East-Asian (1921 b.c), peanuts in Africa (1925, 1926), the Volga river (1929), the slime flux of poplars (1930), the German South-pole expedition (1931 a, b). Until 1960 he added numerous records and observations on nematodes on plants in the USA, Puerto Rico and other areas.

FILIPJEV worked on free-living nematodes in the collection of the Zoological Museum at Leningrad (1916) and studied the nematodes of the Caspian Sea, the Black Sea and the Sea of Azow (1917, 1918a, 1922a, b), the Polar seas near Sebastopol (1918b, 1921, 1924, 1926) and other seas (1927), the Oka river (1928), the Neva Bay (1929, 1930), Abyssinian freshwaters (1931) and the Teleckoje sea (1933), before he made his famous classification of the free-living nematodes (1934a) and the Tylenchinae (1936a, b) and before he wrote his well-known textbook (1934b) which is largely incorporated in FILIPJEV & STEKHOVEN's Manual of Agricultural Helminthology (1941). His contribution on the free-living nematodes from the Northern Arctic Ocean (1946) was published posthumously.

KREIS worked in the same period on marine nematodes from different origin, *inter alia* from Surinam, France, Sebastopol, Spitzbergen and the Sunda Islands

(1924, 1926, 1928a, b, 1929a, 1932a, 1933), also on freshwater nematodes from Paraguay (1932b) and Madras (1936), soil nematodes from areas in China (1929b, 1930), plantparasitic nematodes (1932c) and new nematodes from the Southern Pacific (1938). RAHM worked on moss nematodes (1925), on nematodes from Brasil (1928, 1929), Chile (1932), North China (1937a, b) and the Island Hainan (1938). CASSIDY published on nematodes from Hawaii (1926) and Java (1930). THORNE (1929) reported on nematodes from a big mountain peak in Colorado and found most of them identical with European species, but different from the desert fauna. HOEPPLI (1925, 1926, 1932a, b, c) reported on free-living nematodes in Yellowstone Park and in the thermal waters of this park, and on nematodes found on the island of Amoy (1932b), HOEPPLI & CHU reported on nematodes from hot springs in China and Formosa (1932), WU & HOEPPLI (1929) on free-living nematodes from Fookien and Chekiang.

Other records in the same period were by KISTLER (1922) on nematodes in the Chiemsee peat area, by TANI (1925) on free-living nematodes in faeces of man, by PETERS (1930) on nematodes in sewage, by GOFFART on nematodes in 'Westfälischer Hochmoore' (1925, 1928), by OKHOTINA on nematodes in lake Valda (1926), by LIEBERMANN on free-living nematodes in sugar factory ponds (1927), in the river Moldau (1928 a, b,) and in some Tatra lakes (1931), by BURKHALTER on soil nematodes in the high rocky massive of Naye (1928), by BROWN on nematodes in China (1928), by HNATEWYTSCH on nematodes in oar mines in the 'Erzgebirge' (1929), by ROBERTSON on freeliving nematodes in North-Scottish arable soil (1929), by OMER-COOPER on nematodes in Abyssinia (1930), and by DE CONINCK on free-living nematodes in Belgium (1930a, b, 1932). LEVASHOV recorded nematodes of the periphyton of Volga ships (1928), of Volga plankton (1929) and of plants in Abkhasia (Asia Minor) and the Black Sea coast (1935); ALYAVDINA (1929) recorded them as food of Volga fish. MARCHANT (1934) studied nematodes in the Canadian province of Manitoba and indicated that soils in cold areas develop rich nematode faunas and that different soil types bring about nemic faunas of varying composition. SINITZKII (1932) recorded nematodes from decaying beet roots; MESCHKAT (1934) from reed in the Balaton lake; FRENZEL (1936) recorded nematodes from meadows; VALKANOV recorded nematodes from Bulgarian waters and the Black Sea (1934, 1936, 1937/1938, 1957); Soós worked on moss nematodes in Hungary (1940, 1941a, b, c, d) and on freshwater nematodes from Hungary (1943). STANTSCHEFF on nematodes from lakes in Holstein (1944), SCHULZ (1932, 1934, 1935a, b) and REMANE & SCHULZ (1935) reported on marine nematodes at Kiel. The first mentioned author also worked on marine nematodes from Sicily and Gran Canaria (1935). In the same year TAYLOR (1935) published a review of the few fossil nematodes known.

W. SCHNEIDER studied the nematodes from freshwater and salty springs (1923a, b, 1924, 1925a, b) and from lakes and other biotopes in Nort-West Germany and published a general treatise on free-living and plant parasitic nematodes (1930). Other publications dealt with nematodes from West Africa (1935), from caves and springs in Yugoslavia (1940) and from the Okrida lake (1943).

In 1937 he thoroughly treated the free-living nematodes of the German Limnological Sunda Expedition to Sumatra, Java and Bali and concluded that the prevailing species were circumtropic endemic forms, inhabiting a limited geographical area, although a small number of forms known from Europe were found. He considered the pH of the habitat important (a concept which was not supported by later workers) and distinguished between acidobiontic, acidophilic, eurytopic and acidophobic nematodes.

Several studies were made on marine nematodes of the Belgian coast (STEKHOVEN & ADAM 1931a, STEKHOVEN & DE CONINCK 1933, STEKHOVEN 1935a), on marine nematodes in the Zuiderzee and in the 'Nord & Ostsee' and of the Snellius Expedition (WÜLKER & STEKHOVEN 1933), STEKHOVEN 1929/1930, 1931b, 1935b, STEKHOVEN & PUNT 1935c, 1944a, BRESSLAU & STEKHOVEN 1940). DE CONINCK studied nematodes from marshes and warm springs in the Congo (1935), STEKHOVEN plant parasitic nematodes in the Congo (1936), STEKHOVEN & TEUNISSEN nematodes from the national 'Park Albert' (1938).

STEKHOVEN's other faunistic studies deal with nematodes in the port of Plymouth (1935d), Ostende's 'bassin de chasse' (1942a), the Mediterranean coast of the Camargue (1942b), the Balearic Islands (1942c), the fishing grounds of Alexandria (1943a, b), caves and springs in Belgium (1943c), freshwater habitats (1944b, together with TEUNISSEN), the Skagerrak and the surroundings of Stockholm (1946), caves and subterranean water in Rumania (1950a,) the Mediterranean Bay of Villefranche (1950b), free-living nematodes from the Belgian Congo (1951) and from other areas, mostly in Asia (1954) and free-living marine nematodes from the Kerguelen Island (1955). Much of his work and of others was summarized in his chapter in 'Bronn's Klassen und Ordnungen des Tierreichs' (1937) and in the 'Manual of Agricultural Helminthology' by FILIPJEV & STEKHOVEN (1941).

DE CONINCK continued his studies with publications on free-living nematodes from the caves of Han (1939) and from warm springs, water and brackish soil on Iceland (1940, 1943, 1944), on nematodes from Lake Tanganyika (1964, together with HASPESLAGH) and on nematodes, including root knot nematodes, in the Congo (1962a, b). His experience was recently brought together in a manual, GRASSÉ's 'Traité de zoologie' (1965).

PARAMONOV made faunistic studies of free-living nematodes in salt lakes (1927, 1929) and in the Klyazina river (1938), reviewed saprozoic nematodes in the USSR (1951) as well as the parasitic forms (1952, 1956) and treated the ecological relationships of nematodes in general in his recent textbook (1962, 1964, 1968).

ALLGEN wrote more than 170 articles on nematodes in the period 1921/1960. They are largely short faunistic records on marine, brackish water and freshwater nematodes in and around the Scandinavian countries. Other articles, in chronological order of appearing, concern marine nematodes from Tasmania, the Campbell and Staten Islands, Antarctics, the Macquarie Island, Siberia, the Lofoten Islands, the Mediterranean (with a summary of all nematodes known from the Mediterranean in 1942), the Sea of Marmora, East Greenland and

Jan Mayen, Greenland and other Arctic regions (with reviews and analyses in 1957 of all Arctic nematode faunas known at the time), the East coast of South America and Grahamland in Antarctica. His work includes studies on marine nematodes from collections of the Hamburg zoological museum and from four expeditions, viz. the Swedish South Pole Expedition 1901/1903, Mortensen's Pacific Expedition 1914/1916, the Swedish Deep-Sea Expedition 1947/1948 and the Swedish Albatross Expedition 1948. Detailed reference to all these studies on marine nematodes is not given here, but can be found in the forementioned bibliography which does comprise a full list of ALLGEN's publications. ALLGEN compared the marine nematodes of Norway with the marine nematofauna of the Arctic (1955a), America (1954a, 1955b), the Atlantic coast of Europe (1956a), the Antarctic-Sub-Antarctic area (1956b), the Mediterranean and surrounding areas (1956c), the tropics (1957a, b), the subtropical and temperate areas in the Southern hemisphere (1958), the Far East (1959). In some general articles he discussed the marine tylenchids (1934a), distribution and frequency of marine nematodes (1933), their 'bipolarity' (1934b, 1954b), and the biology and ecology of free-living marine nematodes in general (1955c). Moreover, he published incidental articles on nematodes from other habitats or from special areas, viz. on some free-living nematodes in moss (1929), nematodes from the Congo (1933b), some saprozoic and terrestrial nematodes from Sweden (1950, 1951a, b), freshwater and terrestrial nematodes in Southern waters (1951c), some freshwater nematodes from Mount Kenya (1952), terrestrial nematodes from Jan Mayen (1953) and arctic moss nematodes collected by the Swedish Greenland Expedition 1899 (1954c).

CHITWOOD, too, reported on the occurrence of marine nematodes collected during a deep sea expedition near Puerto Rico (1934), on marine nematodes of North Carolina (1936, 1937), the Gulf of Mexico (1954, together with TIMM), Northern California (1960) and North America (1951), as well as on nematodes from the caves of Yucatan (1938).

A number of workers in Europe spent most of their time on general faunistic studies of nematodes also after the second world war; most of them are still active in this field.

ALTHERR reported on nematodes in and near Switzerland, namely from the mines of Bex (1938), from Valais (1950a), from the 'Parc National Suisse' (1950b, 1952, 1955), from soils and lakes in the Jura (1953, 1954a, b), and from forest soils in alpine valleys (1963a). He also studied nematodes from habitats abroad, namely from the Weser basin and the dunes of Helgoland (1958), and freshwater nematodes from French Cameroon (1960), South America (1963b), Lorraine (1963c) and from the rivers Rhine (1965) and Saale (1968).

PAESLER studied nematodes in East Germany from general and soil biotopes (1939, 1941), from dung (1936, 1946) and also from mountainous areas (1959) and from the rhizosphere of lucerne plants (1956), from mushroom cultures (1957, a, b) and from forest litter (1962).

The studies by MEYL dealt with nematodes from volcanic soils (1953a), from

soil and water on the island of Ischia (1953 b, 1954a) and from Italy in general (1954b), from saline soils (1954c, 1955a) and from higher fungi in woods (1954d). He also studied free-living nematodes from Lake Tanganyika (1955b, 1957a), Brasil (1957b) and Perú (1957c) as well as soil and freshwater nematodes in general (1961a, b).

ANDRÁSSY published on nematodes from the Bükk-mountains (1952), from peat (1953a), from cotton roots (1954), from barley (1956a) and from several habitats in Hungary (1953b, 1962d, e, 1964a), Bulgaria (1958a), Rumania (1959a) and Yugoslavia (1959b). He paid special attention to nematodes in the Adige (1959c, 1962a) and the Danube river (1960d, 1962b, 1962c, 1966a), as well as to nematodes in caves in Hungary (1959d, e, with a review of the nematodes known from caverns). Several studies concerned nematodes from countries in other continents, namely from French West Africa (1956b), from Egyptian waters and the Red Sea (1958b, 1959f), from China (1960a), Afghanistan (1960b), Belgian Congo (1960c), East Africa (1961, 1964b), Angola (1963a), Ghana (1965, 1966b), Mongolia (1964c, 1967b), Argentina, Brasil and other South-American countries (1963b, 1967a, 1968a) and from Swedish lakes (1967c) and groundwater in Congo-Brazzaville (1968b).

GADEA published about free-living soil and freshwater nematodes from different areas of Spain (1952a, b, c, 1953a, b, c, d, 1954a, 1955a, 1956a, b, 1957, 1964f), about moss and marine nematodes from Cerdeña (1968), San Marino (1954b), Sanabria (1954c), Sierra de la Demanda (1955b), Cabo Verde and Madeira Islands (1958), the Spanish Mediterranean coast (1960a), the Medas and Pitiusas Islands (1964d, e), high mountain biotopes (1964b) and the Andes in Perú (1965). He also reported on marine nematodes in the Gulf of Guinea (1960b), soil nematodes of the island of Annobón in this Gulf (1960c), brackish water nematodes from Nepal (1961) and from Chile (1963b), moss, lichen and freshwater nematodes from the island of Menorca, the Mediterranean and elsewhere (1962a, b, 1963a, 1964c), nematodes from paddy fields in Spain (1962c), nematodes from lichens on the small Spanish Mediterranean islands (1964a).

During the period 1948–1964, GERLACH, sometimes in conjunction with others, published at least 34 faunistic studies, 5 general comunications and several taxonomic studies, all on marine nematodes, which are completely listed in the forementioned Bibliography, to which we refer. They deal with nematodes from sea, beach or groundwater of the coasts of Germany, the Bulgarian Black Sea coast, the Italian coast and the Thyrrenic Sea, the coasts of Finland, Portugal, Tunisia and Algeria, France, Madagascar, Brasil, San Salvador, Nicaragua, Congo, the Red Sea, the Gulf of Aden, the Maledives and Spitzbergen.

HOPPER studied marine nematodes from the Gulf of México (1961a, b, 1963), Rhode Island waters (1962) and Florida (1967a, b, also WIESER & HOPPER 1967). CHANDLER (1954) had published earlier on nematodes from the Gulf of Mexico. There are also studies on marine nematodes from Australia and from Antarctic and Sub-Antarctic Stations (MAWSON, 1953, 1956, 1957a, b, 1958a, b), from Arcachon (RENAUD-DEBYSER 1959), and the Roscoff region in France (LUC & DE CONINCK 1959), from the Lodz Island and the Puck

Gulf (JANIK 1962, 1963), from Chile (MURPHY 1965, 1966), from Durban (INGLIS 1966), from the West coast of Schleswig-Holstein (LORENZEN 1966), and from the European Arctics (PLATONOVA 1967) and from coastal ground waters of the island Hiddensee (PETZOLD 1956). COLES (1958) published on nematodes parasitic on sea weeds; WIESER *et al* on marine nematodes (1956, 1959a), on subterranean nematodes from Chesapeake Bay (1959b) and on nematodes from algae (1960). TIMM surveyed marine nematodes from Maryland, St. Martin's Island, the Bay of Bengal, East Pakistan and the Arabian Sea (1952, 1954, 1956, 1957a, 1958, 1959, 1962) and reported also on soil nematodes from East Pakistan (1957b, 1964), Thailand and the Philippines (1965).

Other recent faunistic surveys deal with nematodes from very diverse biotopes, such as still and running waters in India (KHERA 1965, 1966, 1967), the shore of waters in Germany (HIRSCHMANN 1952, ALTHAUS 1954/1955) and Italy (AMICI 1966), Lake Champlain in Australia (FISHER 1968), small forest pools in Poland (CHODOROWSKA 1961), lake shores in Poland (STRADOWSKI 1964), subterraneal waters in Germany (GOFFART, 1949, 1950), caves in South France (CAYROL 1967a) and in the Pyrenees (CHODOROWSKA 1963), the river Blyth estuary (CAPSTICK 1959), irrigation canals in Washington (FAULKNER & BOLANDER 1967), waste water (CALAWAY 1963) and sewage filter beds (MURAD 1965). Other biotopes were the periphyton in Lake Tajty (PIECZYNSKA 1959), mosses in the Georgian SSR (ELIAVA 1966), boggs in the upper Harz (KISCHKE 1956), Mount Ontaki in Japan (YAMAGUTI 1954), the slime flux of living trees (HEINDL–MENGERT 1956), decaying wood (KÖRNER 1954), wood poles along the North-Eastsea Channel (SCHÜTZ & KINNE 1955), bark beetle holes (RÜHM 1956), sawdust compost (KUX & REMPE 1954), other compost (GUNDHOLD 1952,WEINGÄRTNER 1953), cattle dung (SACHS 1950), tung soils (WHITLOCK *et al* 1963), different biotopes in the Congo (COOMANS 1966), peat, heather, forests and other biotopes in Poland (BRZESKI 1961a, b, 1962, 1963a, b and in 1964 the faunistic and taxonomic inventory on freeliving nematodes in Poland), woods and humus soils (MIHELCIC 1953a, b, PEARSE 1946, VOLZ 1949, 1951), meadows in West-Böhmen (LELLÁKKOVÁ–DUŠKOVÁ 1964), alkaline spots in Germany (PAETZOLD 1955, 1958), plants, soil and freshwater in Canada and Alaska and biotopes in the Canadian high Arctics (MULVEY 1963, 1969), native prairie soils and plants in Kansas (ORR *et al* 1965, 1967, dune sands (YEATES 1967a, b, c, d, e, f, g, h,), soil and water in Venezuela (RAPOPORT & TSCHAPEK 1967), soil in Madrás city (KANNAN 1960, 1961), soil and wood fungi in the Gorki district (KRUGLOVA 1960), soils from the Macquarie Island (BUNT 1954), from the Kerguelen Island (PLATONOVA 1958), from Switzerland (STÖCKLI 1952, 1957a, b), from Uzbekistan (GUSHANSKAYA 1951), from the Chuvash SSR (SUDAKOVA 1958), from Kara-Kalpakia and the Kashka-Dar basin (BELYAEVA 1959a, b, 1961), from Central Asia (TULAGANOV 1947), from the Netherlands (LOOF & OOSTENBRINK 1962a), from Sweden and Lapland (VAN ROSSEN & LOOF 1962), from Germany (WEISCHER 1962, 1963), from the Orkney Islands (WILLIAMS 1964), from Yugoslavia (KRNJAIČ 1968), from Puerto Rico (THORNE *et al* 1964, 1968).

Many economic surveys of agricultural soils and plants comprise faunistic data as well. They are summarized in Section 1.2.3.

In addition to the data of general zoological surveys are the monographic treatises, usually taxonomic revisions, of nematode groups. They furnish integrated, selected information on the geographic distribution of the nematode groups studied and are therefore extremely valuable. Books containing such information about a number of nematode groups were published by: (FILIPJEV & STEKHOVEN (1941), T. GOODEY (1933), J. B. GOODEY (1963) and THORNE (1961, whereas data on plant parasitic groups are also found in GOODEY (1933), CHRISTIE (1959) and SOUTHEY (1965). Important monographic treatises on higher nematode categories in which valuable fannistic data are collected, deal with Dorylaimoidea (THORNE 1939), Tylenchidae (FILIPJEV 1936a, THORNE 1949), Rhabditata (PARAMONOV 1956), Mononchidae (CLARK 1960/1 1963, MULVEY 1961/67), Cephalobidae (THORNE 1937, BUANGSUWON & JENSEN 1966), Aporcelaimidae, Nygolaimidae and Nygolaimellidae (HEYNS 1965, 1968), Hoplolaiminae (SHER 1961–1966) and Criconematidae (DE GRISSE 1968). The same holds for the genus monographies and revisions, concerning: *Meloidogyne* (CHITWOOD 1949, WHITEHEAD 1968), *Heterodera* (FRANKLIN 1951), *Tylenchorhynchus*, *Pratylenchus*, *Rotylenchulus*, *Leptonchus*, *Tylencholaimus* (LOOF 1959, 1960a, 1964, LOOF & OOSTENBRINK 1962b, LOOF & JAIRAJPURI 1968), *Rotylenchulus* (DASGUPTA *et al.* 1968), *Hemicycliophora* (TARJAN 1952, THORNE 1955, LOOF 1968), *Hemicriconemoides* (CHITWOOD & BIRCHFIELD 1957), *Criconemoides* (TAYLOR 1936, RASKI 1958, RASKI & GOLDEN 1966, TARJAN 1966), *Paratylenchus* (GERAERT 1965), *Hirschmanniella* and *Radopholus* (SHER 1968a, 1968b), *Anguillulina* and *Aphelenchus* (GOODEY 1928, 1932), *Paraphelenchus* (BARANOWSKAYA 1958), *Anaplectus* (ALLEN & NOFFSINGER 1968), *Mylonchulus* (COETZEE 1967), *Dorylaimus*, *Aporcelaimus* and *Pungentus* (THORNE & SWANGER 1936), *Dorylaimoides* (THORNE & SWANGER 1936, SIDDIQI 1964), *Xiphinema* (SIDDIQI 1959, LUC 1961, HEYNS 1966), and *Trichodorus* (ALLEN 1957, HOOPER 1962, SIDDIQI 1963).

It appears from the results of this great number of diverse faunistic surveys, that nematodes occur almost everywhere in measurable densities, as was already pointed out under 1.1. The habitats recorded include fresh and salt water and soils all over the world, from the tropics to the artics, from mountain tops to caves and mines and subterranean springs. They include all kinds of organic substrates from very diverse origins, including roots and other subterranean parts, stems, leaves, flowers, fruits and seeds from higher plants, trees and tree slime fluxes, paper, sawdust, decaying wood, peat, compost, dung, sewage and filter beds, also algae, liverworts, mosses and fungi. Nematodes are also widespread as associates and parasites of animals and animal derivates.

Many authors noticed the density and pluriformity of the populations and some commented on the relation between density and composition of nematode populations and environmental factors, of which food, moisture, soil type, geographic altitude and climatic condition were mentioned. Most of the authori-

tative faunistic workers agreed that the nematode species generally differed from one climatic area or sea to another, but that a number of them were ubiquitous and occurred everywhere. This view was expressed *inter alia* by DE MAN, COBB, FILIPJEV, STEKHOVEN, MARCHANT, ALLGEN and SCHNEIDER. MICOLETZKY on the other hand, found no consistent differences between habitats in low lands and mountains; he also denied any difference between many described species from widely varying geographic zones, and synonymized them.

1.2.3. *Inventories for agricultural purposes*

During the second world war random exploration and faunistic studies declined, whereas food shortage and the development of methods for population evalution, diagnostics and control of nematodes in the post-war period stimulated the study of nematology, especially plant nematology. There was, however, considerable overlapping both ways. A number of zoologists continued general faunistic studies, and phytonematologists made incidental contributions in this type. On the other hand many early observations on plant nematology were made by COBB, STEINER, THORNE, CHRISTIE, GODFREY and others in the USA. and by MARCINOWSKY, GOFFART, GOODEY and others in Europe. Also, early handbooks in the field of nematology dealt primarily with plant nematology: T.GOODEY (1933), FILIPJEV(1934)and FILIPJEV & STEKHOVEN(1941). These manuals paved the way for the rapid development of phytonematology in the post-war period and were followed by several others, as well as by numerous reports of colloquia, symposia and congresses and by some journals on plant nematology.

In the last 20 years the number of nematologists increased from some tens to more than a thousand, and is still growing rapidly. Some 1200 workers published more than once on plant, soil and water nematodes in the last decade (VAN BERKUM 1970), and the two main international societies, the European Society of Nematologists and the Society of Nematologists in America, have more than 700 members. The phytonematologists now dominate the field and most of the faunistic, taxonomic and ecological work is connected with agriculture. This determines the amount as well as the type of information. The period of faunistic studies by a few outstanding specialists has given way to a period of countless short records on various aspects of plant nematology by a thousand workers from all over the world, with at the moment few authoritative reviews and few well-planned surveys for agricultural purposes. Most records are concerned with new or interesting plant/nematode relationships, descriptions of new species and revisions of groups, biological observations and control experiences. Compilation of these data, and of many chance observations during routine, quarantine, diagnostic or advisory work, gives some insight into the geographic distribution of certain nematodes or nematode problems. Planned economics surveys may aim at providing an insight into the occurrence of all nematodes in a certain area, or of nematodes associated with certain plants, or of one particular nematode or nematode group; such surveys may be qualitative, or quantitative if the population densities are also determined; they may

further serve to establish possible relations with crop damage by systematic evaluation of crop growth at the sampling sites. In addition to morphological identification, specification of nematode species into strains with difference in host affinity may lead to surveys supported by plant tests.

Although we summarize the nematode surveys made for agricultural purposes, tabulation of the records of plant/nematode associations would be beyond the scope of this work. The more comprehensive information, survey reports and inventories based on compilation already comprises about 200 publications up to mid 1969. They are listed separately in a publication by DAO *et al* (1970), to which I shall refer later. The information obtained from these surveys is presented below under the following:

a. Overall nematode surveys per area, without limitation to crop;
b. Overall nematode surveys per plant or plant group;
c. Surveys for a particular species or group of plant nematodes.

It should be noted that nematodes as parasites in animals are not mentioned in these surveys, although they are of agricultural and medical importance. They are seldom encountered in work with plant and soil nematodes and fall outside the scope of our study.

a. Overall nematode surveys per area, without limitation to crop

The published surveys for all nematodes, or all plant parasitic nematodes, vary widely in set-up and value. They comprise planned surveys, both quantitative and qualitative, with or without registration of crop damage, and also records from literature or from slide collections per area. Specially designed economic surveys require a plan, an organisation and a budget; the number of such surveys is relatively small and most of them are of recent date. They resemble general faunistic surveys as described under 1.2.2, but may differ in that the samples are deliberately chosen from cultivated land and that the non-plant parasitic nematodes are often not registered in detail. An example of the different types of overall survey is given below.

KLEYBURG *et al* (1959) extracted and analysed the nematode community of all the fields of ten typical farms and nurseries. The significance of the nematodes was determined in field trials and inoculation experiments (HIJINK *et al* 1968). Such data are the basis for advisory work in routine laboratories where several hundred farms in the Netherlands have so far been examined for all plant nematodes present. Such specially designed, quantitative surveys, including economic evaluation of the nematode populations found, require a standard sampling procedure and central identification to obtain comparable results. Their realization is normally limited to individual farms or special areas for which somebody feels responsible and pays the cost. The number of such surveys is still small.

Overall surveys designed to cover large areas are sometimes quantitative with respect to population densities, but most of them are qualitative in that the frequency is indicated with which certain species are encountered, e.g. MAI *et al*, (1961), on nematodes in the Northeastern states of the USA and

JIMÉNEZ-MILLÁN *et al*, (1965), on nematodes in Spain. For such surveys a series of samples is selected at random or according to a particular plant to represent the area or country, and the techniques for evaluation are standardized. Growth deficiencies in crops may be correlated with the nematodes encountered as was done in the surveys in Venezuela by MCBETH (1956) and in Central Africa and Congo-Brazzaville by LUC *et al* (1963, 1964).

Reviews of the overall plant nematode situation in a certain country or area may be purely a bibliography, e.g. STEINER & RAMIREZ (1964) on agro and plant nematodes of the American Tropics, or include the author's experience, e.g. LUC 1968, on nematode problems in Africa.

A survey of nematodes in the Netherlands based on slides in a collection was published by LOOF & OOSTENBRINK (1962), already cited in Chapter 3.

Many surveys comprise results of actual surveying along with information from other sources.

In the Survey List by DAO *et al.* more than 260 overall nematode surveys of all types by about 200 workers are collected. They deal with nematodes from many areas of the world. For the sake of brevity we refer to this List and cite only the areas which are covered, either by one or by several different workers:

The USA and several of its states and regions in addition to the already mentioned Northeastern states and New York, namely Alabama, California, Colorado, Florida, Georgia, Kansas, Kentucky, Louisiana, Maryland, Maine, Massachusetts, Michigan, Minnesota, Mississippi, New Jersey, North Dakota, Oregon, Rhode Island, South Carolina, Texas, Utah, Virginia, West Tennessee, including surveys in the South-eastern states, the Southern states and also Puerto Rico and Hawai; Canada, inclusive Ontario, Quebec, Alaska;

Latin America and several regions and countries, namely Central America, Mexico, Panama, Cuba, Venezuela, Surinam, the Windward Islands, the Lesser Antilles, Peru, Brazil inclusive Sao Paulo, Uruguay, Costa Rica;

Europe and several countries and areas, namely the Netherlands, including the Ysselmeer polders, Belgium, Britain, including N. Scotland, Denmark, Sweden, Finland, Poland, France, inclusive W. France and S. France, Spain including Granada, Sevilla, and the Canarian Islands, Portugal, Italy including Sicily, W. Germany, including Westphalia-Lippe, Bavaria and Hessen-Nassau, DDR, Czechoslovakia, Yugoslavia, Creece, Cyprus, Turkey;

The USSR and several of its republics and other regions, namely the western districts, Moldavian SSR, Lithuanian SSR, Estonia, Eastern Georgia SSR, Ferghana SSR, Amu-Darya SSR, Turkmen SSR, Tashkent, Azerbaidzhan, Tadjikistan, Dagestan, Uzbekistan, Bydgoszez, Tbilisi, Kara-Kalpakia;

Africa and different countries and regions, namely West Africa, the former French territories and Madagascar, Gold Coast, Algeria, Tunesia, Egypt, the UAR., Sudan, Kenia, Malawi, Rhodesia, Nyasaland, Central-African Republic, Ghana, Congo-Brazzaville, South-Africa;

Asian countries and areas, namely Israel, Palestine, Ceylon, India, including

different states and regions, namely Hyderabad, Mysore, Rajasthan, N. India and New Delhi, Japan, Thailand, Cambodia, Indonesia;

Australia and different regions, namely Western Australia and Queensland.

The general information from the above overall surveys reveals that plant and other nematodes are omnipresent and numerous as a group in cultivated soils, that several species and genera normally occur in the same field, that these species differ from area to area, that also among the plant parasites many species are still undescribed, that the density and composition of the species present in a community within a climatic area are strongly influenced by the plants grown as well as by soil type and/or moisture conditions. Most plant parasites appear to be extremely widespread if circumstances are favourable, whereas some have apparently not yet reached their potential distribution range. It also appears that damage by root nematodes is seldom specific enough to be used as indication for the presence of a species.

b. Overall nematode surveys per plant or plant group

Overall surveys of all nematodes or all parasitic species per plant or plant group are normally made for economic purposes, but produce general faunistic information as well. They are based on standard sampling procedures and central identification, or they are compilations from literature, slide collections or inquiries. Also in this case planned surveys may be quantitative or qualitative and the associated crop damage may be registered or not. Planned technical surveys are normally restricted to a certain country or area as in the category recorded under a, although they are limited to certain plants. The surveys which are wholly or partly based on compilation include many advisory leaflets and textbook chapters on diseases of certain crops. One or more examples of the different types of surveys per plant are given.

HOESTRA (1968) published a systematic survey for all nematodes in apple orchards in the Netherlands and correlated them with poor growth of the trees. Other systematic surveys for all nematodes in a special plant or plant group are concerned for example with roses in the USA (DAVIES 1959, 1960), rice in Thailand (TAYLOR, 1968), banana in the western Canary Islands (BELLO-PEREZ *et al* 1965). Dozens of such surveys have been made of various crops all over the world under the auspices of local, national or international organisations.

The number and diversity of summarizing publications based on literature and files is considerable. Authoritative reviews of recent data are available for few crops only. Good examples are reports by WEHUNT & EDWARDS (1968), in which 56 known and possible parasitic nematodes of banana are listed complete with their published distribution all over the world and by RÜHLE (1967) on the distribution of plant-parasitic nematodes associated with forest trees of the world. GOFFART (1951) in his 'Nematoden der Kultuurpflanzen Europas' summarized known records of all nematode infestation on cultivated plants in Europe. Anonymous advisory communications often list the

nematode problems of certain crops. Other are based on specially designed inquiries.

The number of surveys, inventories and compilations concerning nematodes per plant or per plant group listed by DAO *et al* (1970) is more than 660. Suffice it to mention here the plant groups and the more than 100 different particular plants involved in such surveys.

General groups include plant categories indicated as economic, commercial, agricultural, horticultural, field, forest, garden, greenhouse, ornamental, flower, vegetable, woodland, forage, grassland, pastures, meadow, golf courses and turf; also permanent crops in general, woody crops in general, hardwood, trees, shrubs, vines, fruit crops (tropical, deciduous, stone fruit, small fruit, oyster nut and others); further, nurseries of seedlings in general, of trees, forest trees, stone fruit rootstocks, coniferous seedlings, turf. Special crops of topical interest in many nematode surveys are banana and other *Musa* species, beet and beet seedlings, cereals and grasses (especially rice but also wheat, oats, rye, small grains, corn and millet, and of the grasses especially blue grass, Pangola grass, pastures, fodder grasses and native grasses), citrus, coffee, cotton, mushrooms, strawberry, sugar cane and tobacco.

Other surveys deal with abacá, *Anemone*, *Anthurium*, *Azalea*, apple, avocado, bean, boxwoods, cactuses, *Camelia*, also *C. sinensis*, carnation, carrot, celery, also celery seedlings, cherry, clovers, also red clover, white clover and alfalfa, cocoa, *Convallaria*, cucurbitaceous crops, also cucumber, gherkin, melon and cantaloup, dahlia, daffodil, fig, gladiolus, grape vineyards and nurseries, herbs, also medical herbs, jute, kenaf, lettuce, lilies, also red spiderlilies and Croft Easter lilies, mango, manioc, maple, okra, olive, onion and other *Allium* species, palms, also coconut, date and sabal palmetto palms, papaya, parsley, pea and other legume crops, peanut, pear, *Pelargonium roseum*, peppermint, phlox, pineapple, pines, also white pine, red pine, jack pine and blue spruce, potato and potato tubers, *Polianthes tuberosa*, *Pyrethrum*, raspberry, rose and other Rosaceae, sirih, soybean, *Stephanotis*, sweet potato, tau-saghyz, tea, also young tea, tomato, violets, also African violets, and wild and cultivated yams.

Some special plants included in such surveys were blueberry, cranberry, rhizomes of ginger, marigolds, mosses, orchids, seaweeds, weeds and herbs.

Several rare and most of the important cultivated plants have been the subject of an overall nematode survey at some place; many plants were studied several times at several places. The findings make it clear that nearly every crop can be parasitized by a number of plant nematodes and that the parasitic fauna differs strongly between plants. This is probably a common feature of plants. Plants which were not made the subject of special agricultural surveys are probably infested by or associated with as many nematodes as the studied plants, as can be concluded from T. Goodey's 'The nematode parasites of plants catalogued under their hosts' (J. B. GOODEY, FRANKLIN & HOOPER 1965). The same plant grown in similar climatic zones, however, reveals a series of parasites which largely, although not in details, is the same in all these zones.

c. Surveys for a particular species or group of plant nematodes

Here again, planned surveys and compilations of locality records obtained from various sources are included.

The planned surveys are usually directed against well defined noxious or suspected species in limited areas, and normally give a reliable insight into the local distribution of the species. Such surveys concern:

Heterodera species in general, e.g. in the Netherlands, Sweden, Spain and some other countries in Europe;

H. rostochiensis, e.g. in most european countries, the USA, Canada, N. India;

H. avenae, e.g. in Great Britain, the Netherlands, USSR;

H. schachti, in Great Britain, Sweden, Austria, USSR;

H. glycines, in several states of the USA;

H. cruciferae, in Great Britain, the Netherlands, and Germany;

H. carotae, in Germany;

Ditylenchus destructor, in the USA, Canada, Austria, USSR;

D. dipsaci, in the Netherlands, Great Britain, S. Germany, Switzerland:

D. angustus, e.g. in Madagascar;

D. convallariae, e.g. in German Democratic Republic;

D. radicicola, in meadows in the Netherlands;

Meloidogyne species in many countries all over the world;

Tylenchulus semipenetrans, in the USA, India, UAR, USSR, and many other citrus growing countries;

Nacobbus batatiformis, in the USA;

Radopholus similis, e.g. in Florida and California;

Several *Pratylenchus* species, e.g. in the Netherlands, Great Britain, USA, Cuba, UAR;

Some *Tylenchorhynchus* species, e.g. in the USA, India;

Several *Xiphinema* and *Longidorus* species, e.g. in the USA, Great Britain, Germany, Israel;

Several *Trichodorus* species, e.g. in the USA, Great Britain, the Netherlands, Japan;

Rhadinaphelenchus cocophilus, e.g. in the Caribbean area.

Distribution ranges derived by compilation of stray records are based on various sources and they are numerous. The ranges recorded in chapters on plant nematodes in handbooks, in monographies, and in advisory leaflets concerning particular plant parasitic nematodes are compilations of literature records, sometimes supplemented by unpublished information of the authors. The method is simple and covers published information from all over the world, but the result may be biassed by incomplete coverage of the potential distribution areas and by misidentification.

Many distribution ranges recorded in species descriptions or revisions of genera are based on collections of permanent slides. Here identification is relatively safe but the specimens used are not normally representative to indicate the potential geographic range.

A third source for compilating international and national distribution ranges

of particular nematodes, are the lists derived from routine observations at quarantine, diagnostics, advisory and control centres. Examples are the annual USDA lists of intercepted plant pests and diseases (e.g. ANONYMUS 1969), which reflects the international distribution of certain nematodes, and the annual reports of national plant protection or advisory laboratories which often reflect the occurrence and distribution of certain species in a country. Also here identification is generally safe, but the results may be biassed if chance samplings are involved.

Finally specific inquiries may yield unpublished information on certain species in a country or on an international basis. Well-known are the annual reports of the European and Mediterranean Plan Protection Organization (EPPO) on the occurrence of *Heterodera rostochiensis* in member countries (ANONYMUS 1966). *H. rostochiensis* is probably the nematode whose national and global distribution pattern has been studied more thoroughly than that of any other species. The international distribution is known owing to organized inquiries, reporting activity by EPPO and by the USDA Pest Control Organisation, and is illustrated in Fig. 1. The distribution range of this nematode was mapped by OOSTENBRINK in 1950, who reported the presence of the nematode in 14 countries or special areas. In 1961 a symposium review by him listed 30 countries. In his Golden nematode handbook SPEARS (1968) records 39 countries or areas with recognized infections. They are, in chronological order of the records: Germany, East and West (1913), Scotland (1913), England and Wales (1917), Ireland (1922), Sweden (1922), Denmark (1928), Northern Ireland (1933), Jersey (1938), the Netherlands (1941), USA (1941), Finland (1946), Poland (1946), France (1947), USSR (1948), Creece (1951), Faeroe Islands (1951), Austria (1952), Belgium (1952) Guernsey (1952), Saarland (1952), Peru (1952), Bolivia (1952/1954), Iceland (1953), Algeria (1953), Spain (1953), Czechoslovakia (1954), Luxembourg (1955), Argentina (1955), Norway (1956), Portugal (1956), Israel (1957), Switzerland (1958), Canary Islands (1960), India (1961), Italy (1962), Canada (1962), Yugoslavia (1964), Panamá (1967), Chile (1967). Additional but doubtful records exist on Mexico, Malta and UAR and on damage observed along the Trans-Siberian and South-Manchurian Railway. These data indicate that the nematode is widespread in the potato culture all over the world and that it is spreading further. Detailed information of local surveys performed by precise techniques reveals, however, that its occurrence may be incidental or widespread in particular countries or areas and that the host range differs essentially between areas. In tropical countries it occurs only at high altitudes. This case thus clearly demonstrates the incompleteness and relative value of compilated faunistic records and the need for careful formulation of the results, even in the case of a well-studied nematode.

The inventories for agricultural surveys reveal the wide spread of dense populations of plant nematodes and other species as a group. The nematodes occur in practically every cultivated soil in all areas surveyed and plant parasitic species were found in all crops surveyed. The survey results suggest that plant and soil factors and an area's geographic position have a strong if not decisive

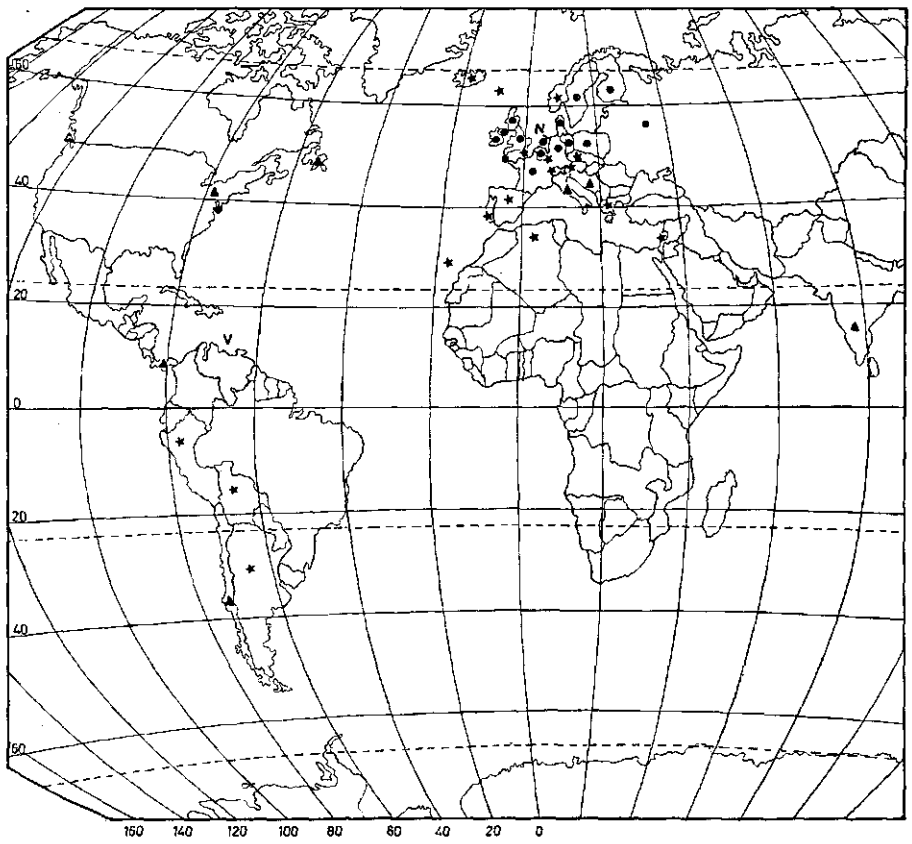

FIG. 1. Distribution range of *Heterodera rostochiensis:* records known in 1950 (●), records added in 1961 (✱), and further records added in 1968 (▲). Venezuela (V) and Netherlands (N) are specially indicated on the map, which is made according to equivalent Mollweide projection (Nieuwe Winkler Prins Atlas 1963).

influence on the composition and density of nematode populations. The data of all these surveys, however, are insufficient to give a near complete picture of the nematode fauna in any specific area and so indicate the actual and potential distribution range of any species with confidence; this even applies to the best-studied plant parasite *Heterodera rostochiensis*.

1.3. THE NEMATODE'S ENVIRONMENT

Early works on animal ecology contain few references to soil and plant nematodes, but the faunistic studies summarized under 1.2 comprise many incidental observations and remarks. FILIPJEV & STEKHOVEN (1941) gave a short account on the ecology of plant parasitic nematodes. HYMAN (1951) dealt with it more extensively. NIELSEN (1949) and ANDRÁSSY (1956) assessed the importance of the nematofauna as soil biomass; the first mentioned author also

furnished experimental data on nematode food preferences and oxygen consumption. DROPKIN (1955), SEINHORST (1961) and OOSTENBRINK (1966a) reviewed the relationship between nematodes and plants, JONES (1959) discussed the influence of plants and soil. Valuable information on the ecology of plant nematodes can also be found in the works of T. GOODEY (1933), GOFFART (1951), CHRISTIE (1959), DECKER (1963), JENKINS & TAYLOR (1967), J. B. GOODEY (1963), PARAMONOV (1968), STEMERDING & KUIPER (1968) and KIRYANOVA & KRALL (1969). WINSLOW (1960), in a review on nematode ecology, described the nematodes in relation to their habitats and geographic distribution and indicated influences on their distributions and abundance. WALLACE's book (1963) summarizes the knowledge of environmental influences on nematode biology. NIELSEN (1967) added some new data, stressed that nematodes do not significantly affect the mechanical or physical properties of the soil and that their ecological significance is mainly related to their feeding on plants and algae (primary production), on fungi and bacteria (primary decomposition) or as predators on other animals (higher consumer levels). Despite the long list of publications on the topic the pertinent knowledge about the relation between nematodes and their environment is scarce and is often not conclusive or fit for generalization.

1.3.1. *A nematode population in relation to its environment*

The environmental influences which are recorded as instrumental in the biology of soil and plant nematodes are of a physical, chemical and biological nature and can be listed as follows:

climatic influences, related to temperature, moisture, light and other radiation, periodicity;

soil influences, related to composition and structure, moisture, chemistry of the soil solution and of soil air;

food influences, related to host characters, quantity and quality of organic material;

influence of other organisms, due to interspecific competition, predation, parasitism and diseases, other complex relations;

intraspecific influences, due to competition, cooperation.

These influences are generally interdependent and comprise interactions and variations. Strong temperature and moisture fluctuations, disturbance of the soil by cultural methods, alternation of host plants, fertilization of soils, disinfection of soils, and similar changes fall within the scope outlined above. They are aberrant elements in the study of nematode biology, but all of them are incorporated in agricultural practices and this makes them important as topics for phytonematological research.

The above mentioned relations between nematodes and their environment can also be summarized in a functional scheme (Figure. 2). It is adapted for nematodes from a scheme for animals in general by BAKKER (1964), with the essential modifications that migration is omitted and that a direct influence of climate, soil and other physico-chemical components on nematode populations

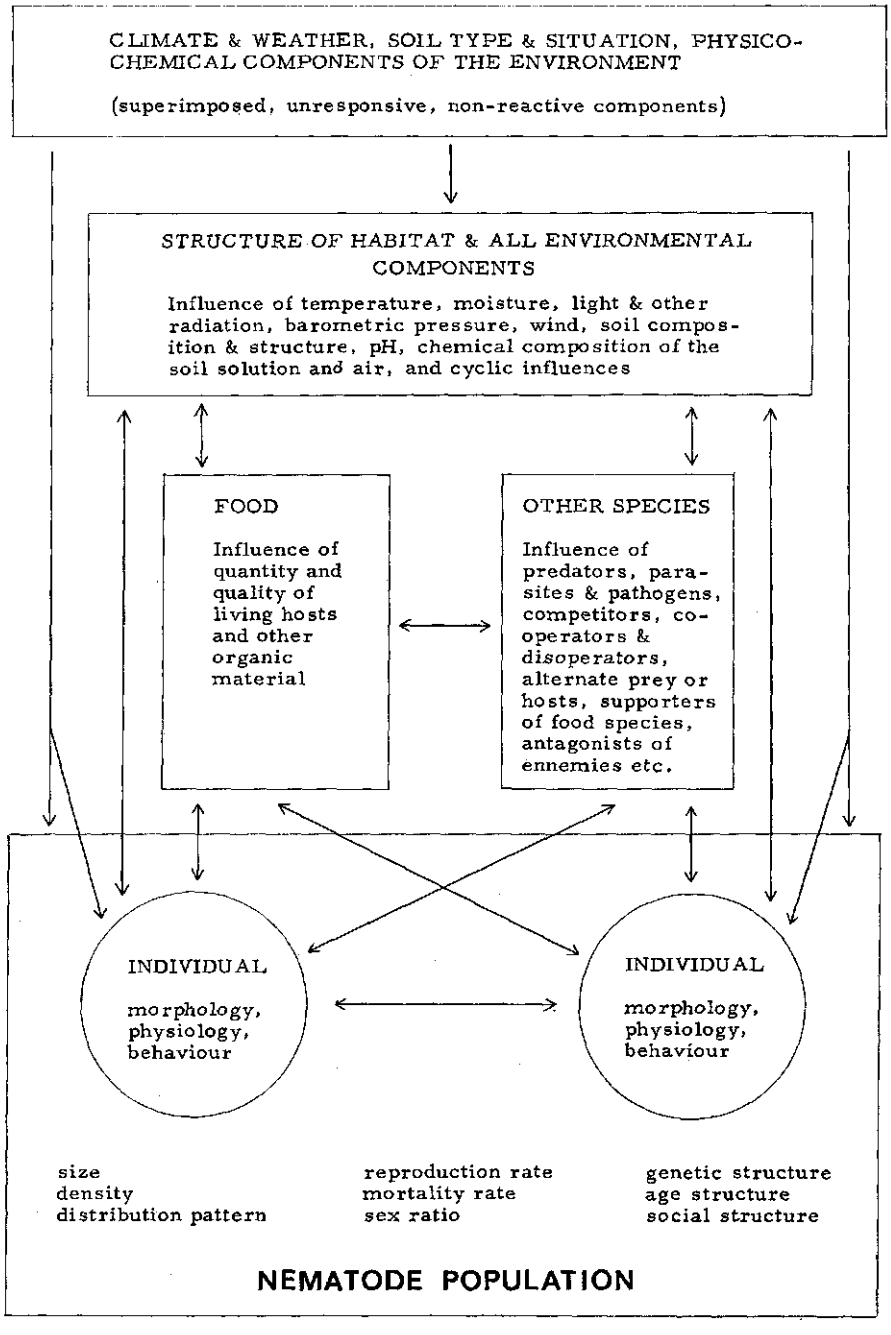

FIG. 2. Scheme of a nematode population in relation to its environment, adapted for nematodes from BAKKER (1964).

is introduced. The reasons for these modifications will be given later.

In this scheme the population of a species is taken as the central unit, although allowance is made for environmental influences on the single nematode, on the monospecific population and on the community of all species in the environment. The population and its properties are a composite from its constituent individuals and their porperties. The web-like relations between the population (sum of individuals) and four main groups of environmental components are sketched; an exponentially rising number of (inter)relations has to be indica – ted if the invironment is split up further. Climate etc., the group of components which influence habitat and nematodes directly but which are not influenced reversely, is superimposed on the others. The other groups – habitat, food and other species – comprise responsive or reactive (in the sense of NICHOLSON 1954) components.

The scheme is descriptive for a certain population in a certain environment at a certain moment. It is functional and causal in so far that the properties and/or influences of the environmental components are added to the environmental labels. The scheme lacks a time scale, and therefore does not describe the results of the (inter) actions, e.g. the fate or development or genetic adaptation of the population. It is therefore not evolutionary nor is it quantitative. But despite its limitations it is a useful review of the position of nematodes in their environment and as such it will be used for reference purposes.

A study of the background of the geographic distribution of nematodes is a population study with special aspects concerning the distribution and abundance of animals and the changes which ultimately take place in their number. Soil is a safe kind of environment, in which the requisites are relatively constant and in which also climatic and weather fluctuations are damped. Nematodes differ strongly in their morphology, physiology and behaviour from insects, birds and other groups of animals which were generally used for population studies. In our case the type of organism and habitat influences views on the relative importance of the different processes which appear on populations, although the processes may be fundamentally the same. OOSTENBRINK (1966a) concluded on the basis of empiricism and experiments, that climate, soil and plant factors are the main determinants for the development of plant nematode populations, and that the widespread occurrence of polyspecific nematode mixtures in grown soil could be explained by a combination of four characters, viz. great persistance, polyphagy, little interspecific competition and efficient spread. The last mentioned point requires special attention in this case. The active locomotion of nematodes is negligeable, and this accounts for the fact that emigration from and immigration into a special environment is practically zero and was therefore omitted from the scheme in Figure 2. The passive spread of nematodes, however, is extremely efficient owing to their small size (genetally less than 1 mm), great density (in general several tens per ml of soil, several thousands per plant) and often great persistence against drought and starvation. Every cubic centimetre of moist soil, every plant or bit of plant tissue and even dried soil and plant material on vehicles, man, birds, insects or

other carriers, will introduce one or more nematodes, often a community of several species, into the new environment. As a result soil and plant nematodes often seem to be omnipresent, the environment determining the establishment of a measurable population, as in the case of non-specific microorganisms. Nematodes, therefore, are normally carried passively but alive into a habitat after which the environment becomes operative. This passive spread is only partly related to nematode biology in the strict sense and is more a matter of artificial inoculation. It normally results in infections of single or several specimens. These have to survive and reproduce before the population or community can be measured and studied in relation to the geographic environment. At that stage it is already certain that the habitat affords suitable conditions for the existence and propagation of the species under consideration.

In the course of our study it will appear that soil, plant and other local components of the environment cannot account for the geographic variation of the nematofauna and that we have to focus our attention on climate, notably on temperature. For this reason only climate and weather will be discussed more fully in this introduction, with special emphasis on temperature.

1.3.2 *Climate and weather*

Climate is the sum or the average of weather conditions over many years. Weather is a composite of atmospheric conditions, temperature, precipitation and humidity, air pressure, wind velocity and direction, sunlight and other radiation at a particular time or over a short period. The influence of climate and weather, long term and short term effects, are not always clearly separated.

Climate apparently has a determining influence on the position of the border of the range of any species, animal or plant. This influence may be direct, but also indirect, for example via food (living host plants or animals, dead organic material or nutritional elements) and via the soil. The literature on the topic in general is vast. Main climatic factors for the flora and fauna are temperature and moisture (KÖPPEN 1923), light and perhaps cyclic influences. HOPKINS (1938) attempted to relate plant response to geographic location and elevation and worked out a 'bioclimatic law' that covered a response to climate, mainly temperature. He suggested values for latitude, longitude and elevation in determinig the time at which plants of the same species will flower. General discussions on the influence of climate and weather on the occurrence of plant diseases, insect pests, worm parasites of livestock and human diseases are given by HUMPHREY, HYSLOP, LUCKER and HIRSCH respectively in 'Climate and man, Yearbook of agriculture of the U.S.D.A.' (1941).

In his survey on the influence of climate and weather on animal populations, KLOMP (1962) distinguished clearly between *the influence of climate on the mean density level of the population* and *the influence of weather on density fluctuations*. He summarizes several studies, field work and experiments, which demonstrate the influence of climate or climatic components on the mean density of insects and mites. The same has been done earlier by BIRCH (1957) and others. By deduction KLOMP denies, that climate or weather can regulate popu-

lation density directly, e.g. bring about permanent fluctuation around a mean density level, because his calculations show that all populations will soon become extinct unless density-related proccesses become operative. This, however, is exactly what happens with most nematode infections carried into unsuitable climate or soil environments – they become extinct because reproduction is nil or does not compensate for mortality. It is obvious that most studies on populations dynamics concern existing populations, i.e. the populations that have been established and for this very reason are the selected and successful combinations of animal species and environment. This fact makes the processes studied unsuitable for evaluating the influence of environmental components on geographic distribution. Each nematode inoculum which reaches a measurable density must have acquired reproduction and is therefore more or less adjusted to its environment. A countless number of inoculates however must become extinct, owing to the direct influence of climatic, soil or other abiotic environmental conditions, immediately or after a number of generations. This direct influence is shown in Figure 2. It is more a matter of determination or destruction than of regulation, but it certainly must be one of the main governing processes in determining the borders of the range of nematodes species. Climate, and the same holds for soil, seems to have components which are determinant for the establishment or extinction of nematode populations. On the other hand established populations show very regular fluctuations and are surprisingly little influenced by weather and other abiotic factors (OOSTENBRINK 1966a). A full analysis of the influence of weather on the dynamics of nematode populations has never been made as in the case of insects and other animals, but is less relevant because established nematode populations are persistently present in a high density. When climate and soil are favourable enough to allow a certain population level to exist, food and other environmental components may become operative *in situ*. For plant nematodes apparently the host plant is a dominant factor. An established plant nematode population in a soil periodically grown with host plants bears resemblance to a population of animals in a cage under adequate, controlled conditions. Nematodes, therefore, are special animals in a special environment; they show high population levels with modest, regular fluctuations.

As indicated before, temperature will appear as the dominent component of climate, and the known data on temperature effects on nematodes are cited below.

1.3.3. *Temperature*

Temperature influences all biological processes. Its effect is mostly considered to be the resultant of many chemical and physical reactions in an organism and can be represented by mathematical equations although much of this effect is unexplained (VON BRAND 1960, OOSTENBRINK 1967). Nematodes are poikilothermic animals, and their rate of metabolism, activity and behaviour follows the temperature in the environment without much delay. Environmental temperature is therefore one of the most important factors in nematode biology.

WALLACE (1963) and OOSTENBRINK (1967) indicate that the nematological thermograms known are the normal type of biological optimum curve, demonstrating successive areas of *kill*, cold stupor, limited activity, optimum, limited activity, heat stupor and kill in a gradient from low to high temperature. This should hold for each nematode, but complete data on any one species are not available despite the fact that one or more temperature requirements of some 47 different nematodes species were traced. They are summarized in Table 2, which lists more than 140 different cases of a nematode's activity related to temperature; only in few cases, however, were minimum, optimum and maximum temperatures indicated all three and in only 12 cases were the figures precisely indicated.

It appears that soil and plant nematodes are normally active and thriving at temperatures between 15° and 30°C and become motionless in the ranges from 5°–15° and 30°–40°C. Close observation of the figures, however, reveals interesting differences between the species studied for minimum and optimum temperatures of some activities.

The minimum temperature for some essential activities was found to be low for *Heterodera avenae* (emergence at 2°C), *H. trifolii* (emergence at 4°C), *Ditylenchus dipsaci* (invasion at 5°C), *Meloidogyne hapla* (invasion at 5°C), *H. rostochiensis* (activity at 5°–10°C), *H. schachti* (activity at 5°–10°C). They are known as plant parasites in temperate climates. High minimum temperatures for hatch or reproduction are recorded for *Hemicycliophora arenaria* (hatch at 21°C), *Tylenchulus semipenetrans* (hatch at 15°C, reproduction at 21°–22°C), *Trichodorus christiei* (reproduction at > 16°C), *Scutellonema brachyurum* (reproduction at about 18°C), *H. glycines* (emergence at 16°C) and *Meloidogyne* spec. (reproduction at 15°–16°C). They are all known as nematodes from warmer regions. Exceptional was a high minimum temperature recorded for reproduction of *M. hapla* on alfalfa and the low minimum temperature of 5°C recorded for egg production of *Hemicycliophora arenaria*, which appears to be a thermophil nematode in all other respects.

The optimum temperatures for several activities were also generally low for above-mentioned plant parasites in temperate climates, viz. below 20°C for *Heterodera avenae*, *H. trifolii*, *D. dipsaci*, and below 25°C for *H. rostochiensis* and *H. schachti*. They were generally 30°C or higher for *Acrobeles complexus*, *Aphelenchus avenae*, *Belonolaimus longicaudatus*, certain *Ditylenchus* species, *Fictor anchicoprophaga*, *Hemicycliophora arenaria*, *Hoplolaimus coronatus*, *Meloidogyne incognita*, *Paraphelenchus acontioides*, *Paroigolaimella bernensis*, *Tylenchulus semipenetrans* and certain *Trichodorus* species from the USA. None of these species occurs in the Netherlands, as will appear from Chapter 3.

The maximum temperatures for activity are generally between 30°C and 35°C; they do not show much correlation with the minimum and optimum temperatures and with the distribution range of nematodes.

Apart from biological response to normal temperature, tolerance against extreme temperature may be determinant for the geographic range of a species. Nematodes try to escape from unsuitable temperatures (CLAPHAM 1931), but

TABLE 2. Minimum, optimum and maximum temperatures recorded for activities of a number of soil and plant nematode species

Nematode species	Activity or process	Temperature in °C			
		min.	opt.	max.	Record
Acrobeles complexus	Reproduction	10–15	34	–	Thomas 1965
A. buetschlii	Reproduction	–	27–32	–	Nicholas 1962
Aphelenchoides composticola	Reproduction	–	25	–	Arrold & Blake 1967
A. composticola	Hatch	13	18–23	–	Cayrol 1967b
A. composticola	Maturation of larvae	13	23	–	Cayrol 1967b
A. composticola	Growth	–	23	–	Cayrol 1967b
A. limberi	Growth	–	18	–	Cayrol 1967b
A. ritzemabosi	Reproduction	8	–	–	Dolliver et al. 1962
Aphelenchus avenae	Egg production	–	25	35	Fisher 1969
A. avenae	Hatch	10	35	–	Pillai & Taylor 1968
A. avenae	Development	15	35	–	Pillai & Taylor 1968
A. avenae	Hatch	10		38	Taylor 1962
Belonolaimus longicaudatus	Reproduction	–	32	–	Rivera Camarena 1964
Criconemoides curvatum	Reproduction on vetch	–	25	–	Malek et al 1965
C. curvatum	Reproduction on carnation	–	20	–	Malek et al 1965
C. curvatum	Reproduction	±15	25	–	Malek & Jenkins 1964
C. xenoplax	Reproduction	–	22–26	–	Seshadri 1964
Ditylenchus dipsaci	Invasion	5	20	30	Griffin 1968
D. dipsaci	Activity	15	–	38	Courtney & Latta 1934
D. dipsaci	Reproduction	–	18	–	Barker 1959
D. dipsaci	Activity	–	10–20	–	Seinhorst 1950
D. dipsaci	Activity	–	21	–	Sayre & Mountain 1962
D. dipsaci	Mobility	–	15–20	–	Wallace 1958,
D. dipsaci	Mobility	–	15–20	–	Blake 1962
D. dipsaci kept at 10°	Activity	–	15	–	Croll 1967
D. dipsaci kept at 20°	Activity	–	20	–	Croll 1967
D. dipsaci kept at 30°	Activity	–	25	–	Croll 1967
D. myceliophagus	Growth	–	18	–	Cayrol 1967c
D. myceliophagus	Reproduction	–	18	–	Cairns 1954
D. spec. (parthenogenetic)	Hatch	10	35	–	Pillai & Taylor 1967c
D. spec. (parthenogenetic)	Development	15	30	–	Pillai & Taylor 1967c
D. spec. (bisexual)	Hatch	10	35	–	Pillai & Taylor 1967c
D. spec. (bisuexal)	Development	15	30	–	Pillai & Taylor 1967c
D. spec.	Infestation	7–13	–	–	Cairns 1954
Fictor anchicoprophaga	Hatch	10	32–35	–	Pillai & Taylor 1968 a
F. anchicoprophaga	Development	15	32–35	–	Pillai & Taylor 1968a
Hemicycliophora arenaria	Egg production	5	24	36	McElroy & Van Gundy 1966
H. arenaria	Mobility	–	30–36	–	McElroy & Van Gundy 1966
H. arenaria	Hatch	21	±33	36	McElroy & Van Gundy 1966
Heterodera avenae	Emergence	2	10–15	25–30	Fushtey & Johnson 1966
H. avenae	Emergence	–	20	–	Winslow 1955
H. glycines	Emergence	16	24	36	Slack & Hamblen 1959
H. glycines	Development	10	23	–	Ichinohe 1955
H. glycines	Development	10	–	–	Ross 1960
H. rostochiensis	Invasion and development	11	15–16	–	Chitwood & Buhrer 1945
H. rostochiensis	Activity	5–10	25	30–35	Kämpfe 1955
H. rostochiensis	Activity	16	–	–	Mai & Harrison 1959
H. rostochiensis	Activity	9	–	–	Chitwood & Buhrer 1945
H. rostochiensis	Emergence	–	21	–	Lownsbery 1950
H. rostochiensis	Emergence	–	25	30	Fenwick 1951
H. rostochiensis	Development	–	18–24	29	Ferris 1957
H. rostochiensis	Development	–	–	32	Fenwick 1951
H. rostochiensis	Emergence	–	–	37	Mai 1952
H. rostochiensis	Development	–	–	29	Mai & Harrison 1959
H. rostochiensis	Emergence	12	22	31	Oostenbrink 1967
H. rostochiensis	Emergence	10	20	31	Oostenbrink 1967
H. schachti	Activity	5–10	25	30–35	Kämpfe 1955
H. schachti	Emergence	10	25	35	Wallace 1955
H. schachti	Reproduction	–	21–27	–	Raski & Johnson 1959
H. schachti	Reproduction and development	–	27,5	32.5	Thomason & Fife 1962
H. schachti	Mobility	–	15	–	Wallace 1958
H. schachti	Emergence	10	20	31	Oostenbrink 1967
H. schachti	Penetration	15	20–25	±35	Johnson & Viglierchio 1969
H. schachti	Development	15	20–25	30	Johnson & Viglierchio 1969
H. trifolii	Emergence	4	17	31	Oostenbrink 1967
Hoplolaimus coronatus	Reproduction	–	32	–	Rivera Camarena 1964
Meloidogyne hapla	Hatch	–	25	–	Bird & Wallace 1966
M. hapla	Mobility	–	20	–	Bird & Wallace 1966
M. hapla	Invasion	–	15–20	–	Bird & Wallace 1966
M. hapla	Growth	–	20–25	–	Bird & Wallace 1966
M. hapla	Hatch after 30 days incubation	–	21	–	Wuest & Bloom 1966
M. hapla	Hatch after 3 days incubation	–	27	–	Wuest & Bloom 1966
M. hapla	Hatch after 30 days incubation	–	12	–	Wuest & Bloom 1966
M. hapla	Invasion	5	25	–	Griffin 1969
M. hapla	Larval development	15	25	30	Griffin 1969

Tabel 2. (Continued)

Nematode species	Activity or process	Temperature in °C			Record
		min.	opt.	max.	
Meloidogyne hapla	Reproduction on susceptible alfalfa	±20	25	–	Griffin 1969
M. hapla	Resistant alfalfa	–	25	–	Griffin 1969
M. hapla	Sexratio on susceptible alfalfa	–	15–20	–	Griffin 1969
M. hapla	Resistent alfalfa	–	25	–	Griffin 1969
M. hapla	Pathogenicity in susceptible alfalfa	–	25	–	Griffin 1969
M. hapla	Resistant alfalfa	–	30–35	–	Griffin 1969
M. hapla	Pathogenicity	±15	20–30	±35	Griffin & Jorgenson 1969
M. hapla	Reproduction	–	25	–	Griffin & Jorgenson 1969
M. incognita	Development	15	30–35	–	Davide & Triantaphyllou 1967
M. incognita	Sex ratio	–	30–35	–	Davide & Triantaphyllou 1967
M. incognita	Development in sweet potato: susceptible var. Allgold	–	24 day/ 20 night	–	Jatala & Morrison 1967
M. incognita	resistant var. Tinian	–	24 day/ 20 night	–	Jatala & Morrison 1967
M. incognita	resistant var. Heartogold	–	32 day/ 28 night	–	Jatala & Morrison 1967
M. incognita	resistant var. Nemagold	–	32 day/ 28 night	–	Jatala & Morrison 1967
M. incognita acrita	Survival of eggs and larvae	–	9.5	–	Bergeson 1960
M. incognita acrita	Hatch	–	27	–	Bergeson 1960
M. incognita acrita	Development	–	30 day/ 20 night	–	Bryant & Wyllie 1968
M. javanica	Reproduction	–	24–28	–	Wallace 1969
M. javanica	Invasion	–	24–28	–	Wallace 1969
M. javanica	Hatching	–	30	–	Bird & Wallace 1965
M. javanica	Mobility	–	25	–	Bird & Wallace 1965
M. javanica	Growth	–	25–30	–	Bird & Wallace 1965
M. javanica	Infectivity	–	15	–	Thomason et al 1964
M. javanica	Mobility	–	15	–	Thomason et al 1964
M. javanica	Hatch	10	30	35	Wallace 1966
M. javanica	Movement	–	25	–	Wallace 1966
M. javanica	Invasion	–	20–30	–	Wallace 1966
M. spp.	Reproduction	15–16	25–32	–	Thomason & Lear 1961
M. sppp.	Reprocution	9	27	–	Tyler 1933
[illegible]	Infestation	10–12	20–25	[illegible]	Godfrey 1926
Nacobbus serendipiticus	Development	–	25	–	Prasad & Webster 1967
N. serendipiticus	Sex ratio	–	20	–	Prasad & Webster 1967
Neotylenchus linfordi	Hatching	10	25	30	Pillai & Taylor 1968
N. linfordi.	Development	15	25	30	Pillai& Taylor 1968
Panagrellus silusiae	Growth in length	8	10	35	Gisels 1964
Paraphelenchus acontioides	Hatch	10	35	–	Pillai & Taylor 1968
P. acontioides	Development	15	35	–	Pillai & Taylor 1968
Paratylenchus amblycephalus	Infestation	–	16–21	–	Reuver 1959
P. nanus	Moulting	–	20	±30	Fisher 1966
P. neoamblycephalus	Reproduction	–	20	30	Fisher 1967
P. projectus	Moulting	10	–	35	Rhoades & Linford 1959
Paroigolaimella bernensis	Hatch	10	30–32	–	Pillai & Taylor 1968
P. bernensis	Development	15	30	–	Pillai & Taylor 1968
Pratylenchus minyus	Reproduction	–	30	–	Faulkner & Bolander 1969
P. penetrans	Reproduction	15	22.5	30	Patterson & Bergeson 1967
Pratylenchus vulnus	Reproduction	–	25	30	Lownsbery et al 1967
P. vulnus	Survival	–	15	–	Lownsbery et al 1967
Rhabditis filiformis	Movement	14–16	26–27	–	Tanaguchi 1935
Rotylenchus parvus	Reproduction	–	30	–	Dasgupta & Raski 1968
Scutellonema brachyurum	Reproduction	±18	28	–	Malek & Jenkins 1964
Trichodorus christiei	Reproduction	–	25	–	Malek et al 1965
T. christiei	Reproduction	–	25	–	Malek & Jenkins 1964
T. christiei	Reproduction	–	25–30	–	Bird & Mai 1967
T. christiei	Reproduction	16	27	–	Young & Ben Struble 1966
T. spec.	Development	–	35	–	Rhode & Jenkins 1957
Tylenchorhynchus claytoni	Reproduction on tobacco	–	29–35	–	Krusberg 1959
T. claytoni	Reproduction on wheat	–	21–27	–	Krusberg 1959
Tylenchulus semipenetrans	Development	–	25–31	–	Baines 1950
T. semipenetrans	Hatch	15	30	35	O'Bannon 1968
T. semipenetrans	Penetration	–	20	–	O'Bannon et al 1967
T. semipenetrans	Development	–	25	–	O'Bannon et al 1967
T. semipenetrans	Reproduction	–	25	–	O'Bannon et al 1967
T. semipenetrans	Infection and development	–	25–30	–	Baines 1950
T. semipenetrans	Reproduction	21–22	28–31	31	Kirkpatrick et al 1965
T. semipenetrans	Survival of freeliving stage	0	10	30	Cohn 1966
Xiphinema americanum	Reproduction on tomato	–	20	–	Griffin & Barker 1966
X. americanum	Reproduction on strawberry	–	24	–	Griffin & Barker 1966
X. diversicaudatum	Embryogeny	10	25	30	Flegg 1969
X. diversicaudatum	Laying – Gastrulation	10	25	–	Flegg 1969
X. diversicaudatum	Gastrula-larva	–	10	25	Flegg 1969
X. diversicaudatum	Larva – hatch	10	25	–	Flegg 1969

their mobility is very limited and it is clear that lethal temperatures as well as drought, often determine the fate of an inoculated nematode dosage under natural conditions. All temperatures below the minimum and above the maximum for activity must ultimately be lethal. Such temperatures, however, do not normally occur permanently in nature. Most nematode species can stand temperatures below 5 °C for a long time. They survive the temperature range chosen to preserve populations in stored soil samples, which is 0–5 °C, for several months (OOSTENBRINK 1960), as well as normal winter freezing temperatures, which do not noticeably influence the densities of natural populations. Many nematodes can endure extremely low temperatures in an anabiotic state or when they are encysted. Some moss-inhabiting species withstand even a temperature of –270 °C for $7\frac{3}{4}$ hours (RAHM 1928). Quiescent larvae of *D. dipsaci* survive –80 °C for 20 minutes (BOSHER & MCKEEN 1954). Eggs in cysts of *H. glycines* survive –40 °C for 7 months and –24 °C for 18 months (ICHINOHE 1955, SLACK & HAMLEN 1961). COBB (1921) thawed living nematodes out of blocks of polar ice.

High temperatures, above 40 °C, are normally lethal after a short time. Species which are active in a limited, normal temperature range, however, may comprise stages which under certain conditions can survive temperatures above 40 °C for a long period. There is such evidence with respect to quiescent *D. dipsaci* (COURTNEY & LATTA 1943) and wheat galls with *Anguina tritici* (BYARS 1920). Some nematodes survive in natural habitats with a high temperature, such as hot springs (VON LINSTOV (1901).

Further, some special temperature aspects should be mentioned in addition to activity thermograms and lethal temperatures. The data about minimum, optimum and maximum for a number of nematodes are in Table 2, related to studies at temperatures with little fluctuation. Temperatures in natural habitats, however, show diurnal, seasonal and other vairable fluctuations depending on climatic zone and depth in the soil. Such fluctuations may increase or decrease certain nematode activities (cf. WALLACE 1963).

TYLER (1933) and ICHINOHE (1955) correlated certain nematode activities with a 'heat sum', but temperature may also act as a trigger for nematode activity, or its influence may be obscured or distorted by diapause effects (OOSTENBRINK 1967). And it is obvious that the influence of temperature may be modified by other environmental factors, as already indicated in the scheme of Figure 2. It should further be noted, that there may be physiological differences, genetically determined or not, within a species and that the influence of tempeture on different processes or activities of different stages–eggs, larval stages, adults, or cysts–may be different.

The data from Table 2 and the considerations about lethal temperature suggest a strong influence of temperature on the distribution pattern of nematodes.

1.4. Scope of the investigations

The purpose of the following study is to ascertain:

a. whether great geographic variation in the nematofauna exists;
b. which influences are instrumental in causing such differences;
c. to what extent climatic influences are involved;
d. which climatic factor is determinant;
e. how this determinant factor influences nematodes.

It has been assumed in the foregoing sections that the nematofauna differs between areas, that climate influences nematodes and that temperature is an important factor in this context. Needed next are a thorough comparison of populations from different climate areas, an analysis of the different factors involved and experimental determination of effects and confirmation of the propounded theories. They are pursued here by comparing the situation in a tropical country, Venezuela and a country with a temperate climate, the Netherlands, and by conducting experiments with selected nematodes from both countries.

CHAPTER 2

MATERIALS AND METHODS

In this chapter regularly employed materials and methods are discussed. In appropriate cases, details are given of other techniques used in conducting the experiments.

2.1. SAMPLING TECHNIQUES

The author was involved in the nematode surveys in Venezuela published by MCBETH (1956) and LOOF (1964), and took additional soil and plant samples from the field to supplement the faunistic data (Chapter 3) and to find suitable populations for his experimental work (Chapters 4, 5, 6). A special shovel was used for taking samples from growing crops or natural vegetation including one or more plants complete with roots and about 1 litre of surrounding soil. When poor growth was noticed in a crop a separate sample from the poor patch and its surroundings was taken. Samples from trees were composited by bulking small quantities of soil and roots obtained from at least four different spots around the tree. All samples were packed in plastic bags to avoid drying-up and transported to the laboratory in Venezuela or occasionally by air mail to the Netherlands, for nematode extraction. They were stored at 5 °C in case they could not be processed immediately. In the laboratories subsamples were taken, after thorough mixing, of 100 ml of soil for the extraction of active nematodes, 100 ml of soil for the extraction of cysts and 10 g of roots or other plant tissue for the extraction of endophytic populations. The extraced populations were completely examined and subsequently preserved without further subsampling of the suspensions.

The experimental work described in Chapters 4, 5 and 6 was normally accompanied by quantitative evaluation of population densities in the soil or other culture media as pots, tubes, culture dishes and similar small containers, or in the corresponding plants or plant tissues. The sampling of these soils and materials was effected according to different techniques, depending on the material, it being understood that soil samples were normally not smaller than 100 ml and plant samples not smaller than 10 g, and that the samples were as representative as possible for the container. In this work aliquots of the extracted suspension were often taken for quantitative microscopical analyses. The variability of the experimental work including the sampling procedures was always suppressed and controlled by taking replicates and making statistical checks (cf. 2.6).

2.2. NEMATODES

The nematodes used for supplementing faunistic lists and for taxonomic studies were specimens available or brought into permanent slides, which are all deposited in the collection at the Landbouwhogeschool at Wageningen (referred to as LH Collection). They are isolated, fixed, and embedded in glycerine on aluminium microscopical slides, according to the methods described in s'JACOB & VAN BEZOOIJEN's manual for practical work in nematology (latest revision 1967).

The nematode populations for experiments were some natural, mixed populations in their original soil and a number of selected species from Venezuela and the Netherlands obtained after extraction, inoculation and propagation as monospecific populations under controlled conditions in greenhouse compartments or in the laboratory of the Nematology Department of the Landbouwhogeschool, Wageningen. The following populations were maintained as monospecific cultures:

a. Meloidogyne incognita (Kofoid & White, 1919) Chitwood, 1949. A population was collected from tomato at Maracaibo in the tropical lowland of Venezuela and indicated as (V); another population was collected from tomato in a greenhouse at Wageningen, the Netherlands and indicated as (N). Both populations were reared and kept as a stock on tomato in standard potting soil.

b. Meloidogyne hapla (Chitwood, 1949). A (V) population was collected from potato at Sanare in the mountains of Venezuela, and an (N) population originally from *Berberis buxophilus* L. at Wageningen was subsequently kept as a stock on tomato in Wageningen. These populations were also reared on tomato.

c. Ditylenchus dipsaci (Kühn, 1858) Filipjev, 1936. A standard (V) population was collected from garlic at Burbusay, 2000 m above sea level in the mountains of Venezuela; a standard (N) population was originally collected from onions in the Netherlands but stored as a dry stock (population U-6 from Dr. J. W. Seinhorst, Wageningen). Other (N) populations were strains from tulip and from narcissus which were furnished by the Plantenziektenkundige Dienst. All populations could be stored as dry, fourth-stage larvae or kept breeding in onion bulbs.

d. Aphelenchus avenae Bastian, 1865. A (V) population was collected from soil around tomato roots at Cagua in the tropical lowland of Venezuela, and an (N) population from soil around carrot roots at Wageningen. Both populations were propagated and maintained on oat meal agar cultures of the fungus *Alternaria solani* at 25 °C in a laboratory incubator.

e. Helicotylenchus dihystera (Cobb, 1893) Sher, 1961. A (V) population was collected from soil around roots of maize at Cagua in the tropical lowland of Venezuela and propagated as a stock culture at Wageningen on maize in pots with standard potting soil.

Special, monoxenic cultures of *A. avenae* were maintained on sterile oat meal agar with *Alternaria solani*. To avoid other organisms, apart from the nematode

and the fungus, sterilization of the nematode in solution of streptomycin 1% and Aretan 0.2% was practised before each inoculation. Such monoxenic cultures were maintained as a stock in an incubator at 25 °C for two years by regular inoculation onto new culture plates; subcultures were used for experiments.

2.3. Plants, soils and containers

The main materials used for nematode propagation and experiments were plants of tomato (*Lycopersicum esculentum* L. var. Moneymaker), maize (*Zea mays* L.) and garden phlox (*phlox paniculata* L.) and bulbs of onion (*Allium cepa* L.), tulip (*Tulipa* spp.) and daffodil (*Narcissus* spp.). *Aphelenchus avenae* was always reared on the fungus *Alternaria solani*.

The soil used as a rule was steam-sterilized standard potting soil, consisting of 75 parts by volume of peat, 10 parts of sand, 3 parts of clay and 12 parts of leaf mold, which mixture was found suitable for the propagation of many nematode species by Winoto (1969). In some experiments natural soils collected in Venezuela and the Netherlands were used.

The containers normally used were common clay pots, rectangular plastic tubes of 4 × 4 × 20 cm and petri dishes of 9 cm diameter.

2.4. Temperature equipment

Four types of facilities or apparatus were used to obtain controlled temperatures in the experiments, namely greenhouse compartments and climatic cells, Wisconsin tanks, a series thermostat and special incubators.

a. Greenhouse compartments and climatic cells. In a greenhouse of the Landbouwhogeschool at Wageningen, cabinets were available in which the temperature was automatically kept at 10°, 15°, 20°, 25° and 30 °C (28°–30 °C). Higher temperatures could not be attained in this installation. The plants were exposed to artificial light if necessary to obtain continuous assimilation periods of 18 hours per day. In another part of the greenhouse climatic cells with a more rigid control of all climatic factors were available, and the same range of temperature were used.

b. Wisconsin tanks. A greenhouse of the Plantenziektenkundige Dienst at Wageningen accommodated a series of sand tanks immersed in water which was automatically kept at temperatures of 15°, 20°, 25°, 30° and 35 °C. Lower temperatures could not be attained here. The air temperature in the greenhouse was between 20°–25 °C varying with the temperature outdoors. The plants were exposed to artificial light for 18 hours per day.

c. Series thermostat. In a series of small compartmens 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. Up to 16 compartments were available, each having a thermometer, and permitting selection of appropriate temperatures. The temperatures showed a variation of ± 1 °C owing to temperature changes in the environment; the temperature steps between successive compartments

varied. There was no artificial light in the thermostat. The apparatus was available in the building of the Plantenziektenkundige Dienst.

d. Special incubators. Several incubators with precise automatic control based on heating and in some cases on cooling, were used for the aseptic cultures of *Aphelenchus avenae* and in a number of other experiments.

2.5. Estimation of nematode densities and infestation levels

The materials to be examined in the laboratory were soil and plant samples taken from the field, soil and plant materials in experimental containers, inoculated bulbs, or agar plates with fungivorous nematodes. Most of the methods are described by Oostenbrink (1960b) or in s'Jacobs & van Bezooijen's syllabus (1967); modifications had to be applied with some materials. Evaluation methods for soils differ widely from those for other substrates.

2.5.1. *Soils*

Samples taken from the field were passed trough a coarse sieve to remove plant materials and stones and were thoroughly mixed, whereupon 100 ml of soil was used for the extraction of active nematodes and, if desired, another 100 ml for the extraction of nematode cysts. Active nematodes were extracted either according to the decantation cotton-wool method (in Venezuela) or by means of Oostenbrink's elutriator (in Wageningen), after which the nematode suspensions obtained were microscopically analyzed according to standard procedures. Both methods are considered to give comparative results. Extraction of *Heterodera* cysts was pursued by floating small samples in a bowl of water, followed by hand-picking (in Venezuela) or by using a modified Fenwick apparatus (in Wageningen).

In most of our experiments the extraction of nematodes from plastic tubes containing test plants was essentially the same, although some modifications were necessary. Soil and roots were normally found to adhere tightly to the tube wall and had to be loosened with water. The roots were then washed free, weighed and kept separate for extraction (cf. 2.5.2). The tube's soil contents already dispersed in water, were then extracted as a whole in the elutriator referred to above. The amount of soil per tube is 300 ml and the nematode figures of our experiments are therefore often expressed per 300 ml of soil.

2.5.2. *Plant tissues and agar cultures*

Nematodes from roots, phlox plants or bulbs were normally extracted by placing the material for 7 days in the funnel-spray apparatus (Seinhorst 1950, Oostenbrink 1960); 10 g of material or the whole root system, plant or bulb was used after weighing the material and cutting it to pieces. Counting of the catch was done quantitatively, after which the number of nematodes was expressed per whole plant or bulb or per gram of material. This method was efficient for the extraction of endoparasitic root nematodes, e.g. *Pratylenchus* spp. and of *Ditylenchus dipsaci.* It was however, not suitable for the evaluation

of *Meloidogyne* infestations, because these nematodes become immobile in the roots.

The degree of penetration and development and of infestation by *Meloidogyne* spp. was estimated by staining the root systems with cotton blue lactophenol and examination of the nematodes inside the roots and of the galls on the roots. The degree of galling, as a measure of penetration, development and infestation, was expressed according to a gall index, by enumerating the number of galls comprising nematodes or correlated with the percentage of the root surface estimated to be transformed by the nematodes into gall tissue. The index ran from 0–4 as follows: 0 = no galls present, 1 = 25% of the root infested, 2 = 25%–50% infested, 3 = 50%–75% infested, 4 = 75%–100% infested.

Aphelenchus avenae in the fungus grown agar cultures was counted by adding water to the oat meal agar cultures, which lifted the agar block into a floating position in the petri dish. After 24 hours the nematodes were extracted and counted in the water. When the numbers of nematodes were difficult to count in the petri dish by the high numbers present, the suspension was completed up to 100 ml and an aliquot was counted.

2.6. Statistical treatment of the data

Most experiments were set up with sufficient replicates to be able to calculate the statistical significance of the results. The figures concerning nematode densities, infestation levels or morphological characters were often converted into percentages or other ratios or into logarithms before treatment. The transformations were usually to natural logarithms. Some results are transformed to the normal 10-logarithm; this is consequently indicated with the corresponding experiments. Different statistical treatments had to be applied, including analysis of variance, covariance and Student's test, and least significant differences were often calculated (Snedecor 1962, Cochran & Cox 1964, Salmon and Hanson 1964). Most of the calculations were made on the computer of the Mathematics Department of the Landbouwhogeschool at Wageningen.

CHAPTER 3

ANALYSIS OF THE NEMATOFAUNAS IN VENEZUELA AND IN THE NETHERLANDS

3.1. INTRODUCTION

Venezuela and the Netherlands are chosen as suitable climatic test regions for determining and comparing their nematofaunas and for selecting suitable nematode species for experiments.

Special reasons for the choice of these two countries are the following:

a. The countries represent two widely separated, completely different climatic, floristic and faunistic zones (cf. Figure 1), but each comprises local conditions which are typical of the other country, namely temperate conditions in the high mountain areas of Venezuela and tropical conditions in the greenhouses of the Netherlands.

Venezuela is a country of 912,000 km² in tropical South America situated between 1° and 11°N. of the equator. It is mountainous, with altitudes varying from 0 to 5000 m. It can roughly be divided in the North-Western plain lowlands, the South-Eastern highlands and the high Andes mountain range which cuts the plain lowlands into two parts. The climate is tropical and fairly humid with an annual drought period in the plains, it is tropical and dry in the highlands, and temperate and humid at high altitudes in the Andes mountains.

The Netherlands, 36,000 km², is situated in the West-European plain between 51° and 54°N. of the equator. It is flat, with altitudes from 6 m below to about 300 m, above sea level. The climate is a temperate sea climate with rain all over the year and with little geographical variation. Artificial, warm climates are established on a commercial scale for horticultural crops in greenhouses.

b. The nematofauna of the Netherlands is probably better known than that of any other country. This was so at the time of DE MAN's faunistic studies (1884), it was still the case according to MICOLETZKY (1922) and may be true up to the present time. In comparison with other tropical countries Venezuela's nematofauna is also fairly well known because of some special surveys which will be recorded later.

c. The nematofaunas of both countries have to a large extent been identified or checked by the same workers, at the Landbouwhogeschool Wageningen, so that the risk of misidentification is reduced.

d. The author himself has been involved in nematode studies in Venezuela and in the Netherlands and could readily obtain populations for observations and experimentation from these two countries.

3.2. Nematodes in Venezuela

Three extensive faunistic studies of the nematofauna in plants, soil and freshwater in Venezuela, were made at the request of Compañía Shell de Venezuela or the Fundación Shell de Venezuela. They are supplemented by unpublished data of the author.

McBeth, in co-operation with the author, collected nematodes from 75 separate samples from 19 crops in 1955. The nematodes were studied at the University of California. At least 50 nematode species, of which 20 were known or suspected plant parasites, were recorded by him in a technical report for limited distribution published by Shell Development Company (McBeth 1956).

Oostenbrink, in co-operation with Gonzalez and the author, collected 155 soil and root samples from 81 fields or localities involving some 25 crops in 1962. The nematodes were extracted in Venezuela and studied by Loof at the Landbouwhogeschool Wageningen. Additional material was furnished by the author in the course of this study. 219 species were studied and recorded complete with the collection localities and particulars about substratum and geographic altitude were indicated (Loof 1964).

The third survey was made by Torrealba (1967). He recorded 76 genera of nematodes obtained from soil and root samples of various plants from different regions in Venezuela. 9 of these genera were found for the first time in Venezuela, 43 genera reported from Venezuela by McBeth or Loof were not reported in this survey.

All nematodes reported from Venezuela in the aforementioned inventories are listed and marked with V in Table 3 and to these are added the unpublished records of species observed by the author in samples or slide collections.

Table 3. List of nematode species recorded from Venezuela (V) and from the Netherlands (N) by different authors or in unpublished records

BUNONEMATIDAE			*M. spiculigera* (Steiner, 1936) Dougherty, 1953	N	
Bunonema ditlevseni Micoletzky, 1925	N		*M. ultima* (Körner, 1952) Dougherty, 1955		V
B. penardi Stefanski, 1914	N		*Pelodera chitwoodi* (Bassen, 1940) Dougherty, 1955	N	
B. poligraphi (Fuchs, 1930) Sachs, 1949	N		*P. icosiensis* (Maupas, 1916) Dougherty, 1955	N	
B. reticulatum Richters, 1905	N		*P. strongyloides* (A. Schneider, 1860) A. Schneider, 1866	N	
B. richtersi Jägerskiöld, 1905	N		*P. teres* A. Schneider, 1866	N	
Craspedonema styriacum Micoletzky, 1922	N		*Protorhabditis filiformis* Bütschli, 1873	N	
RHABDITIDAE			*P. oxyuris* (Claus, 1862) Doutherty, 1955	N	
Cruznema lambdiense (Maupas, 1919) Osche, 1952	N	V	*Rhabditis axei* (Cobbold, 1884) Dougherty. 1955		V
Diploscapter coronatus (Cobb, 1893) Cobb, 1913	N	V	*R. brevispina* (Claus, 1862) Bütschli, 1873	N	
Mesorhabditis capitata Loof, 1964		V	*R. buetschlii* de Man, 1876	N	
M. monohystera (Bütschli, 1873) Dougherty, 1955	N				
M. paucipapillata (Paetzold, 1955) Paetzold, 1958		V			

R. intermedia de Man, 1880 N
R. longicaudata Bastian, 1865 N
R. marina Bastian, 1865 N
R. musicola Rahm, 1928 N
R. oxycerca de Man, 1895 N
R. producta (A. Schneider, 1866) Oerley, 1886 N
R. terricola Dujardin, 1845 N

NEOAPLECTANIDAE
Neoaplectana bibionis Bovien, 1937 N V

PANAGROLAIMIDAE
Panagrolaimus rigidus (A. Schneider, 1866) Thorne, 1937 (N V)

DIPLOGASTERIDAE
Butlerius filicaudatus Adam, 1930 N
B. monohystera Taylor, 1964 N
Demaniella cibourgensis Steiner, 1914 N
Diplenteron colobocercus Andrássy, 1964 N
Diplogaster rivalis (Leydig, 1854) Bütschli, 1873 N
Eudiplogaster armatus (Hofmänner, 1913) Paramonov, 1952 N
Mononchoides elegans (Weingärtner, 1955) Meyl, 1961 (N V)
M. fictor (Bastian, 1865) Goodey, 1963 N
M. striatus (Bütschli, 1876) Paramonov, 1952 N
Odontopharynx longicaudata de Man, 1912 N
Paroigolaimella spirifera (Skwarra, 1921) Andrássy, 1958 N
Pristionchus lheritieri (Maupas, 1919) Paramonov, 1952 N
Tylopharynx striata de Man, 1876 N

CEPHALOBIDAE
Acrobeles ciliatus v. Linstow, 1877 N V
A. complexus Thorne, 1925 N V
A. undulatus Loof, 1964 V
Acrobeloides buetschlii (de Man, 1884) Steiner & Buhrer, 1933 N V
A. emarginatus (de Man, 1880) Thorne, 1937 N V
A. enoplus Steiner, 1938 V
A. tricornis (Thorne, 1925) Thorne 1937 N
Cephalobus nanus de Man, 1880 (N V)
C. persegnis Bastian, 1865 (N V)
Cervidellus serratus (Thorne, 1925) Thorne 1937 N
C. vexilliger (de Man, 1880) Thorne, 1937 N
Chiloplacus bisexualis (Micoletzky, 1916) Thorne, 1937 N
C. demani (Thorne, 1925) Thorne 1937 N
C. obtusicaudatus (Kreis, 1930) Thorne, 1937 V
C. propinquus (de Man, 1921) Thorne, 1937 N
C. quadricarinatus (Thorne, 1925) Thorne, 1937 V
C. symmetricus (Thorne, 1925) Thorne, 1937 N
C. trifurcatus (Thorne, 1925) Thorne, 1937 N
C. trilineatus Steiner, 1940 N
Eucephalobus oxyuroides (de Man, 1876) Steiner, 1936 N V
E. striatus (Bastian, 1865) Thorne, 1937 N
Heterocephalobus elongatus (de Man, 1880) Andrássy, 1967 N
H. filiformis (de Man, 1880) Andrássy, 1967 N
H. longicaudatus (Bütschli, 1873) Andrássy, 1967 N V
H. pulcher (Loof, 1964) Andrássy, 1967 V
H. teres (Thorne, 1937) Andrássy, 1967 V
Myolaimus heterurus Cobb, 1920 V
Panagrobelus stammeri Rühm, 1956 N
Plectonchus sp. N
Stegelleta ophioglossa Andrássy, 1967 V
Turbatrix aceti (Müller, 1783) Peters, 1927 N

TERATOCEPHALIDAE
Euteratocephalus crassidens (de Man, 1880) Andrássy, 1958 N V
E. palustris (de Man, 1880) Andrássy, 1958 N
Teratocephalus costatus Andrássy, 1958 N
T. terrestris (Bütschli, 1873) de Man, 1876 N V

TYLENCHIDAE
Anguina agrostis (Steinbuch, 1799) Filipjev, 1936 N
Basiria magnidens (Thorne, 1949) Geraert, 1968 N
Ditylenchus convallariae Sturhan and Friedman, 1965 N
D. destructor Thorne, 1945 (N V)
D. dipsaci (Kühn, 1858), Filipjev, 1936 N V
D. intermedius (de Man, 1880) Filipjev, 1936 (N V)

D. myceliophagus Goodey, 1958	N
D. radicicola (Greeff, 1872) Filipjev, 1936	N
Macrotrophurus arbusticola Loof, 1958	N
Pseudhalenchus sp.	N
Psilenchus hilarulus de Man, 1921	(N V)
Telotylenchus ventralis Loof, 1963	N
Tetylenchus joctus Thorne, 1949	N
Trophurus imperialis Loof, 1957	N
T. sculptus Loof, 1957	N
T. sp.	V
Tylenchorhynchus acutus Allen, 1955	V
T. brevidens Allen, 1955	N
T. bursifer Loof, 1959	N
T. capitatus Allen, 1955	N V
T. clarus Allen, 1955	N
T. claytoni Steiner, 1937	N
T. contractus Loof, 1964	V
T. dubius (Bütschli, 1873) Filipjev, 1936	N
T. icarus Wallace & Greet, 1964	N
T. judithae Andrássy, 1962	N
T. lamelliferus (de Man, 1880) Filipjev, 1936	N
T. lenorus Brown, 1956	N
T. macrurus (Goodey, 1932) Filipjev, 1936	N
T. martini Fielding, 1956	V
T. maximus Allen, 1955	N
T. microdorus Geraert, 1966	N
T. microphasmis Loof, 1959	N
T. nanus Allen, 1955	N
T. nothus Allen, 1955	N
T. obscurus Allen, 1955	N
T. ornatus Allen, 1955	N
T. striatus Allen, 1955	N
T. tessellatus Goodey, 1952	N
Tylenchus agricola de Man, 1884	N V
T. costatus de Man, 1921	N V
T. davainei Bastian, 1865	N V
T. graciloides Micoletzky, 1925	N
T. leptosoma de Man, 1880	N
T. thornei Andrássy, 1954	N
T. vulgaris Brzeski, 1963	N
HOPLOLAIMIDAE	
Helicotylenchus digonicus Perry, 1959	N
H. dihystera (Cobb, 1893), Sher, 1961	V
H. erythrinae (Zimmerman, 1904) Golden, 1956	V
H. multicinctus (Cobb, 1893) Golden, 1956	N
H. pseudorobustus (Steiner, 1914) Golden, 1956	N
H. varicaudatus Yuen, 1964	N
H. vulgaris Yuen, 1964	N
Hirschmanniella gracilis (de Man, 1880) Luc & Goodey, 1963	N
H. loofi Sher, 1968	N
H. oryzae (Soltwedel, 1889) Luc & Goodey, 1963	V
H. spinicaudata (Schuurmans Stekhoven, 1944) Luc & Goodey, 1963	V
Hoplotylus femina s'Jacob, 1959	N
Nacobbus serendipiticus Franklin, 1959	N
Peltamigratus holdemani Sher, 1964	V
P. macbethi Sher, 1964	V
P. pachyurus Loof, 1964	V
Radopholus similis (Cobb, 1893) Thorne, 1949	N V
Rotylenchulus borealis Loof & Oostenbrink, 1962	N
R. reniformis Linford & Oliveira, 1940	V
Rotylenchus fallorobustus Sher, 1965	N
R. buxophilus Golden, 1956	N
R. goodeyi Loof & Oostenbrink, 1958	N
R. robustus (de Man, 1876) Filipjev, 1936	N
Pratylenchoides crenicauda Winslow, 1958	N
P. laticauda Braun & Loof, 1966	N
P. maritimus Bor & s'Jacob, 1966	N
Pratylenchus brachyurus (Godfrey, 1929) Goodey, 1951	V
P. coffeae (Zimmerman, 1898) Filipjev & Schuurmans Stekhoven 1941	V
P. convallariae Seinhorst, 1959	N
P. crenatus Loof, 1960	N V
P. hexincisus Taylor & Jenkins, 1957	V
P. fallax Seinhorst, 1968	N
P. flakkensis Seinhorst, 1968	N
P. neglectus (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941	N
P. penetrans (Cobb, 1917) Filipjev & Schuurmans Stekhoven 1941	N
P. pratensis (de Man, 1880) Filipjev, 1936	N
P. pseudopratensis Seinhorst, 1968	N
P. scribneri Steiner, 1943	N
P. thornei Sher & Allen, 1953	N V
P. vulnus Allen & Jensen, 1951	N
P. zeae Graham, 1951	V
NEOTYLENCHIDAE	
Boleodorus thylactus Thorne, 1941	N

Deladenus durus (Cobb, 1922) Thorne, 1941 (N V)
Ecphyadophora tenuissima de Man, 1921 N
Ecphyadophoroides tenuis Corbett, 1964 V
Hexatylus viviparus Goodey, 1926 N
H. sp. N
Nothotylenchus acris Thorne, 1941 N
N. sp. V
Thada cancellata Thorne, 1941 N

TYLENCHULIDAE
Tylenchulus semipenetrans Cobb, 1913 N V

HETERODERIDAE
Heterodera avenae (Wollenweber, 1924) Filipjev, 1934 N
H. cacti Filipjev & Schuurmans Stekhoven, 1941 N
H. carotae Jones, 1950 N
H. cruciferae Franklin, 1945 N
H. fici Kirjanova, 1954 N
H. galeopsidis (Goffart, 1936) Filipjev & Schuurmans Stekhoven, 1941 N
H. goettingiana Liebscher, 1890 N
H. humuli Filipjev, 1934 N
H. punctata Thorne, 1928 N
H. rostochiensis Wollenweber, 1923 N
H. schachti Schmidt, 1871 N
H. sp. V
H. trifolii (Goffart, 1932) Oostenbrink, 1949 V
Meloidogyne arenaria (Neal, 1889) Chitwood, 1949 N V
M. exigua Goeldi, 1887 V
M. hapla Chitwood, 1949 N V
M. incognita (Kofoid & White, 1919) Chitwood, 1949 N V
M. javanica (Treub, 1885) Chitwood, 1949 N V
M. naasi Franklin, 1965 N

PARATYLENCHIDAE
Paratylenchus amblycephalus Reuver, 1959 N
P. elachistus Steiner, 1949 V
P. goodeyi Oostenbrink, 1953 N
P. hamatus Thorne & Allen, 1950 N
P. microdorus Andrássy, 1959 N
P. straeleni (de Coninck, 1931) Oostenbrink, 1960 N

CRICONEMATIDAE
Criconema boettgeri (Meyl, 1954) de Grisse & Loof, 1965 N
C. celetum Wu, 1960 V
C. menzeli (Stefanski, 1924) Taylor, 1936 N
C. murrayi (Southern, 1914) Taylor, 1936 N
Criconemella parva (Raski, 1952) de Grisse & Loof, 1965 N
Criconemoides amorphus de Grisse, 1967 N
C. informis (Micoletzky, 1922) Taylor, 1936 N
Hemicycliophora conida Thorne, 1955 N
H. epicharoides Loof, 1968 N
H. macristhmus Loof, 1968 N
H. nucleata Loof, 1968 N
H. robusta Loof, 1968 N
H. thienemanni (W. Schneider, 1925) Loos, 1948 N
H. thornei Goodey, 1963 N
H. triangulum Loof, 1968 N
H. typica de Man, 1921 N
Macroposthonia annulata de Man, 1880 N
M. annulatiformis de Grisse & Loof, 1967 N
M. crenata (Loof, 1964) de Grisse & Loof, 1965 N
M. curvata (Raski, 1952) de Grisse & Loof, 1965 N
M. dherdei de Grisse, 1967 N
M. irregularis (de Grisse, 1964) de Grisse & Loof, 1965 N
M. onoensis (Luc, 1959) de Grisse & Loof, 1965 V
M. oostenbrinki (Loof, 1964) de Grisse & Loof 1965 V
M. ornata (Raski, 1958) de Grisse & Loof, 1965 V
M. peruensiformis de Grisse, 1967 V
M. pseudosolivaga (de Grisse, 1964) de Grisse & Loof, 1965 N
M. raskiensis (de Grisse, 1964) de Grisse & Loof, 1965 N
M. rotundicauda (Loof, 1964) de Grisse & Loof, 1965 N
M. rustica (Micoletzky, 1915) de Grisse & Loof, 1965 N
M. solivaga (Andrássy, 1962) de Grisse & Loof, 1965 N
M. sphaerocephala (Taylor, 1936) de Grisse & Loof, 1965 N V
M. vadensis (Loof, 1964) de Grisse & Loof, 1965 N
M. xenoplax (Raski, 1952) de Grisse & Loof, 1965 N
Nothocriconema annuliferum (de Man, 1921) de Grisse & Loof, 1965 N

N. demani (Micoletzky, 1925) de Grisse & Loof, 1965	N
N. loofi de Grisse, 1967	N
N. mutabile (Taylor, 1936) de Grisse & Loof, 1965	N V
N. princeps (Andrássy, 1962) de Grisse & Loof, 1965	N
Xenocriconemella macrodora (Taylor, 1936) de Grisse & Loof, 1965	N
APHELENCHIDAE	
Anomyctus xenurus Allen, 1940	N
Aphelenchoides bicaudatus (Imamura, 1931) Filipjev & Schuurmans Stekhoven, 1941	V
A. blastophthorus Franklin, 1952	N
A. composticola Franklin, 1957	N
A. fragariae (Ritzema Bos, 1891) Chiristie 1932	N
A. helophilus (de Man, 1880) Goodey, 1933	N
A. hunti Steiner, 1935	N
A. limberi Steiner, 1936	N
A. parietinus (Bastian, 1865) Steiner, 1932	N
A. ritzemabosi (Schwartz, 1911) Steiner & Buhrer, 1932	N
A. saprophilus Franklin, 1957	N
A. subtenuis (Cobb, 1926) Steiner & Buhrer, 1932	N
Aphelenchus avenae Bastian, 1865	(N V)
Bursaphelenchus digitulus Loof, 1964	V
B. gonzalezi Loof, 1964	V
Rhadinaphelenchus cocophilus (Cobb, 1919) Goodey, 1960	V
Seinura demani (Goodey 1928) Goodey, 1960	N
S. paratenuicaudata Geraert, 1962	V
S. tenuicaudata (de Man, 1895) Goodey, 1960	N
S. winchesi (T. Goodey, 1927) J. B. Goodey, 1960	N
PARAPHELENCHIDAE	
Paraphelenchus myceliophthorus, Goodey, 1958	N
P. pseudoparietinus (Micoletzky, 1922) Micoletzky, 1925	(N V)
PLECTIDAE	
Anaplectus granulosus (Bastian, 1865) de Coninck & Schuurmans Stekhoven, 1933	N V
A. submersus (Hirschmann, 1952) Maggenti, 1961	N
Chronogaster boettgeri Kischke, 1956	N
C. daoi Loof, 1964	V
C. typica (de Man, 1921) de Coninck, 1935	N
Haliplectus bickneri Chitwood, 1956	N
Paraplectonema pedunculatum (Hofmänner, 1913) Strand, 1934	N
Plectus acuminatus Bastian, 1865	N
P. assimilis Bütschli, 1873	N
P. cirratus Bastian, 1965	N
P. elongatus Maggenti, 1961	N
P. geophilus de Man, 1880	N
P. longicaudatus Bütschli, 1873	N
P. palustris de Man, 1880	N
P. parietinus Bastian, 1865	N V
P. parvus Bastian, 1865	(N V)
P. rhizophilus de Man, 1880	N
P. tenuis Bastian, 1865	N
Rhabdolaimus terrestris de Man, 1880	N V
Tylocephalus auriculatus (Bütschli, 1873) Anderson, 1966	N
Wilsonema otophorum (de Man, 1880) Cobb, 1913	N V
TRIPYLOIDIDAE	
Tripyloides gracilis Ditlevsen, 1919	N
TRIPYLIDAE	
Tobrilus allophysis (Steiner, 1919) Andrássy, 1959	N
T. consimilis (W. Schneider, 1925) Andrássy, 1959	N
T. gracilis (Bastian, 1865) Andrássy, 1959	N
T. grandipapillatus (Brakenhoff, 1914) Andrássy, 1959	N
T. pellucidus (Bastian, 1865) Andrássy, 1959	N
Tripyla affinis de Man, 1880	N
T. filicaudata de Man, 1880	N
T. papillata Bütschli, 1873	N
T. setifera Bütschli, 1873	N
Trischistoma arenicola (de Man, 1880) Schuurmans Stekhoven, 1951	N V
T. monohystera (de Man, 1880) Schuurmans Stekhoven, 1951	N
CAMACOLAIMIDAE	
Aphanolaimus aquaticus Daday, 1894	N
A. attentus de Man, 1880	N
Bastiania gracilis de Man, 1876	N
B. longicaudata de Man, 1880	N
Deontolaimus papillatus de Man, 1880	N
Dintheria tenuissima de Man, 1921	N
Leptolaimus papilliger de Man, 1876	N

AXONOLAIMIDAE

Aulolaimus oxycephalus de Man, 1880	N
Cylindrolaimus communis de Man, 1880	N
C. melancholicus de Man, 1880	N
Rogerus orientalis (Hoeppli & Chu, 1932) Hoeppli & Chu, 1934	V

MONOHYSTERIDAE

Diplolaimelloides altherri Meyl, 1954	N
Monohystera agilis de Man, 1880	N
M. dispar Bastian, 1865	N
M. filiformis Bastian, 1865	N
M. gracilior Johnston, 1938	N
M. longicaudata Bastian, 1865	N
M. macrura de Man, 1880	N
M. microphthalma de Man, 1880	N
M. paludicola de Man, 1881	N
M. similis Bütschli, 1873	N
M. simplex de Man, 1880	N
M. stagnalis Bastian, 1865	N
M. villosa Bütschli, 1873	N
M. vulgaris de Man, 1880	(N V)
M. sp.	V
Prismatolaimus dolichurus de Man, 1880	N
P. intermedius (Bütschli, 1873) de Man, 1880	(N V)
P. stenolaimus de Man, 1921	N

SPHAEROLAIMIDAE

Sphaerolaimus gracilis de Man, 1876	N

LINHOMOEIDAE

Desmolaimus zeelandicus de Man, 1880	N

CHROMADORIDAE

Chromadorina bioculata (Schultze, 1857) Wieser, 1954	N
Chromadorita leuckarti (de Man, 1876) Filipjev, 1930	N
Hypodontolaimus geophilus (de Man, 1876) Wieser, 1954	N
Prochromadora oerleyi (de Man, 1881) Filipjev, 1930	N
Spilophorella paradoxa (de Man, 1880) Filipjev, 1918	N

MICROLAIMIDAE

Microlaimus globiceps de Man, 1880	N

CYTHOLAIMIDAE

Achromadora dubia (Bütschli, 1873) Micoletzky, 1925	N
A. pseudomicoletzkyi van der Linde, 1938	N
A. ruricola (de Man, 1880) Micoletzky, 1925	(N V)
A. terricola (de Man, 1880) Micoletzky, 1925	N
Choanolaimus psammophilus de Man, 1880	N
Ethmolaimus pratensis de Man, 1880	N
Odontolaimus chlorurus de Man, 1880	N
Paracyatholaimus intermedius (de Man, 1880) Filipjev, 1930	N

IRONIDAE

Cryptonchus tristis (Ditlevsen, 1941) Filipjev, 1935	N
Cryptonchus sp.	V
Doticholaimus marioni de Man, 1888	N
Ironus ignavus Bastian, 1865	N
I. longicaudatus de Man, 1884	(N V)
Syringolaimus striatocaudatus de Man, 1888	N

DESMODORIDAE

Metachromadora vivipara (de Man, 1907) Filipjev, 1918	N

ONCHOLAIMIDAE

Adoncholaimus thalassophygas (de Man, 1876) Filipjev, 1918	N

MONONCHIDAE

Anatonchus tridentatus (de Man 1876) de Coninck, 1939	N
Cobbonchus sp.	V
Granonchulus schulzi (Meyl. 1955) Andrássy, 1958	N
Iotonchus sp.	V
Miconchus digiturus (Cobb, 1893) Andrássy, 1958	V
M. studeri (Steiner, 1914) Andrássy, 1958	N
Mononchus papillatus Bastian, 1865	N V
M. parvus de Man, 1880	N V
M. truncatus Bastian, 1865	N V
M. venozolanus Loof, 1964	V
Mylonchulus brachyuris (Bütschli, 1873) Andrássy, 1958	N
M. minor (Cobb, 1917) Andrássy, 1958	V
M. rotundicaudatus (Skwarra, 1921) Andrássy, 1958	N
M. sigmaturus (Cobb, 1917) Andrássy, 1958	N V
M. subtenuis (Cobb, 1917) Andrássy, 1958	N
Prionchulus muscorum (Dujardin, 1845) Wu & Hoeppli, 1929	N

P. punctatus (Cobb, 1917) Andrássy, 1958 — N

ONCHULIDAE

Stenonchulus troglodytes W. Schneider, 1940 — N

BATHYODONTIDAE

Bathyodontus cylindricus Fielding, 1950 — V

B. mirus (Andrássy, 1956) Andrássy in Hopper & Cairns, 1959 — N

Oionchus obtusus Cobb, 1913 — V

ISOLAIMIDAE

Isolaimium sp. — N

DORYLAIMIDAE

Amphidorylaimus infecundus (Thorne & Swanger, 1936) Andrássy, 1960 — V

Aporcelaimellus amylovorus (Thorne & Swanger, 1936) Heyns, 1965 — N V

A. capitatus (Thorne & Swanger, 1936) Heyns, 1965 — V

A. obscurus (Thorne & Swanger, 1936) Heyns, 1965 — N V

A. obtusicaudatus (Bastian, 1865) Altherr, 1968 — N V

Aporcelaimus regius (de Man, 1876) Thorne & Swanger, 1936 — N

A. superbus (de Man, 1880) Goodey, 1951 — N

A. vorax Thorne & Swanger, 1936 — N

Carcharolaimus dentatus Thorne, 1939 — N

C. drepanodon Loof, 1964 — V

C. rotundicauda (de Man, 1880) Thorne, 1939 — N

Discolaimium cylindricum Thorne, 1939 — V

D. gracile Thorne, 1939 — V

Discolaimoides bulbiferus (Cobb, 1906) Heyns, 1963 — V

Discolaimus affinis Loof, 1964 — V

D. major Thorne, 1939 — (N V)

D. mucurubanus Loof, 1964 — V

D. similis Thorne, 1939 — V

D. texanus Cobb, 1913 — N

Dorylaimus annulatus Daday, 1905 — V

D. crassus de Man, 1884 — N

D. stagnalis Dujardin, 1845 — N

Enchodelus macrodorus (de Man, 1880) Thorne, 1939 — N

Eudorylaimus acuticauda (de Man, 1880) Andrássy, 1959 — N

E. agilis (de Man, 1880) Loof, 1969 — N

E. andrassyi (Meyl, 1955) Andrássy, 1959 — N

E. asymmetricus (Thorne & Swanger, 1936) Andrássy, 1959 — N

E. brunettii (Meyl, 1953) Andrássy, 1959 — V

E. bryophilus (de Man, 1880) Andrássy, 1959 — N

E. carteri (Bastian, 1865) Andrássy, 1959 — N

E. diadematus (Thorne & Swanger, 1936) Andrássy, 1959 — V

E. doryuris (Ditlevsen, 1911) Andrássy, 1959 — N

E. ettersbergensis (de Man, 1885) Andrássy, 1959 — V

E. granuliferus (Cobb, 1893) Andrássy, 1959 — V

E. holsaticus (W. Schneider, 1925) Andrássy, 1959 — N

E. incisus (Thorne & Swanger, 1936) Andrássy, 1959 — V

E. iners (Bastian, 1865 Andràssy, 1959 — N

E. labiatus (de Man, 1880) Andrássy, 1959 — N

E. leuckarti (Bütschli, 1873) Andrássy, 1959 — N

E. lugdunensis (de Man, 1880) Andrássy, 1959 — N

E. minutus (Bütschli, 1873) Andràssy, 1959 — V

E. minor (Thorne & Swanger, 1936) Andrássy, 1959 — V

E. miser (Thorne & Swanger, 1936) Andrássy, 1959 — (N V)

E. modestus (Altherr, 1952) Andrássy, 1959 — N

E. monohystera (de Man, 1880) Andrássy, 1959 — N V

E. nitidus (Thorne & Swanger, 1936) Andrássy, 1959 — V

E. parvus (de Man, 1880) Andrássy, 1959 — N

E. pratensis (de Man, 1880) Andrássy, 1959 — N

E. rhopalocercus (de Man, 1876) Andràssy, 1959 — N

E. similis (de Man, 1876) Andrássy, 1959 — N

E. simplex (Thorne & Swanger, 1936) Andrássy, 1959 — N

Labronema czernowitzense (Micoletzky, 1922) Thorne, 1939 — N

L. mauritiense Williams, 1959 — V

Longidorella microdorus (de Man, 1880) Goodey, 1963 — N

L. morbida (Loof, 1964) Jairajpuri & Hooper, 1969 — V

L. parva Thorne, 1939 — V

L. penetrans (Thorne & Swanger, 1936) Goodey, 1963 V
L. xenura E. Khan & M. A. Siddiqi, 1963 V
Longidorus caespiticola Hooper, 1961 N
L. elongatus (de Man, 1876) Thorne & Swanger, 1936 N
L. goodeyi Hooper, 1961 N
L. leptocephalus Hooper, 1961 N
L. macrosoma Hooper, 1961 N
L. vineacola Sturhan & Weischer, 1964 N
Lordellonema porosum (Heyns, 1963) Heyns, 1963 V
Mesodorylaimus aberrans Loof, 1969 N
M. attenuatus (de Man, 1880) Andrássy, 1959 N
M. bastiani (Bütschli, 1873) Andrássy, 1959 N
M. centrocercus (de Man, 1880) Geraert, 1966 N
M. filiformis (Bastian, 1865) Goodey, 1963 N
M. globiceps Loof, 1964 V
M. hofmänneri (Menzel, 1914) Goodey, 1963 N
M. litoralis Loof, 1969 N
M. mesonyctius (Kreis, 1930) Andrássy, 1959 V
M. parasubtilis (Meyl, 1957) Andrássy, 1959 V
M. subtilis (Thorne & Swanger, 1936) Andrássy, 1959 N
M. subulatus (Thorne & Swanger, 1936) Andrássy, 1959 N
Nygolaimus altherri Heyns, 1968 N
N. aquaticus Thorne, 1930 N
N. brachyuris (de Man, 1880) Thorne, 1930 N
N. clavicaudatus Altherr, 1953 N
N. hartingii (de Man, 1880) Thorne, 1930 N
N. intermedius (de Man, 1880) Loof, 1961 N
N. laevis Thorne, 1939 N
N. loofi Heyns, 1968 N
N. macrobrachyuris Heyns, 1968 N
N. trophurus Heyns, 1968 N
N. vulgaris Thorne, 1930 N V
Paractinolaimus macrolaimus (de Man, 1880) Andrássy, 1964 N
Paralongidorus maximus (Bütschli, 1874) Siddiqi, 1964 N
Paraxonchium orthodon (Loof, 1964) Altherr & Loof, 1969 V
P. laetificans (Andrássy 1956) Altherr & Loof, 1969 N
Prodorylaimus brigdammensis (de Man, 1876) Goodey, 1963 N
P. longicaudatus (Bütschli, 1874) Andrássy, 1959 N
Pungentus silvestris (de Man, 1912) Coomans & Geraert, 1962 N
Thornenema calvalcantii (Lordello, 1955) Andrássy, 1959 V
Thornia steatopyga (Thorne & Swanger, 1936) Meyl, 1954 N
Xiphinema diversicaudatum (Micoletzky, 1927) Thorne, 1939 N
X. index Thorne & Allen, 1950 V
X. sp . V

BELONDIRIDAE

Axonchium coronatum (de Man, 1906) Thorne & Swanger, 1936 N
A. propinquum (de Man, 1921) Thorne, 1939 N
Belondira clava Thorne, 1939 V
Dorylaimellus aequalis (Cobb, 1918) Thorne, 1939 N
D. longicollis Loof, 1964 V
D. montenegricus Andrássy, 1959 N
D. nygellurus Loof, 1964 V
D. parvulus Thorne, 1939 V
D. porosus Thorne, 1939 V
D. spicatus Loof, 1964 V
D. vexator Heyns, 1963 V
D. virginianus Cobb, 1913 N
Nygellus sp. V
Oxydirus oxycephaloides (de Man, 1921) Thorne, 1939 N
O. oxycephalus (de Man, 1885) Thorne, 1939 (N V)

LEPTONCHIDAE

Basirotyleptus sp. V
Dorylaimoides elegans (de Man, 1880) Thorne & Swanger, 1936 N
D. limnophilus (de Man, 1880) Loof, 1964 N
D. micoletzkyi (de Man, 1921) Thorne & Swanger, 1936 N
D. parvus Thorne & Swanger, 1936 V
D. riparius Andrássy, 1962 V
D. sp. V
D. teres Thorne & Swanger, 1936 N
Doryllium longicaudatum Loof, 1964 V
D. uniforme Cobb, 1920 V
Leptonchus granulosus Cobb, 1920 V
L. scintillans Loof, 1964 N
Poronemella porifer (Loof, 1964) Siddiqi 1969 V
Tylencholaimellus affinis (Brakenhoff, N

1914) Thorne, 1939		*T. anemones* Loof, 1965	N
T. magnidens Thorne, 1939	V	*T. christiei* Allen, 1957	V
T. sagittifer (de Man, 1921) Thorne, 1939	N	*T. monohystera* Allen, 1957	V
T. striatus Thorne, 1939	N V	*T. nanus* Allen, 1957	N
Tylencholaimus crassus Loof & Jairajpuri, 1968	N	*T. pachydermus* Seinhorst, 1954	N
T. formosus Loof & Jairajpuri, 1968	N	*T. primitivus* (de Man, 1880) Micoletzky, 1922	N
T. leptonchoides Loof, 1964	V	*T. similis* Seinhorst, 1963	N
T. maritus Loof & Jairajpuri, 1968	V	*T. teres* Hooper, 1962	N
T. minimus de Man, 1876	N	*T. viruliferus* Hooper, 1963	N
T. mirabilis (Bütschli, 1873) de Man, 1876	N	*Tylolaimophorus typicus* de Man, 1880	N
T. nanus Thorne, 1939	V	ALAIMIDAE	
T. stecki Steiner, 1914	N	*Alaimus arcuatus* Thorne, 1939	V
T. zeelandicus de Man, 1876	N	*A. elongatus* de Man, 1906	N
Tyleptus gymnochilus Loof, 1964	V	*A. meyli* Andrássy, 1961	N V
		A. minor Cobb, 1893	V
DIPHTEROPHORIDAE		*A. parvus* Thorne, 1939	N V
Diphterophora brevicollis Thorne, 1939	N	*A. primitivus* de Man, 1880	(N V)
D. communis de Man, 1880	N	*A. striatus* Loof, 1964	V
D. obesus Thorne, 1939	N	*Amphidelus dolichurus* (de Man, 1876) Thorne, 1939	(N V)
D. perplexans (Cobb, 1913) de Coninck, 1931	V	*A. elegans* (de Man, 1921) Thorne, 1939	N
Trichodorus aequalis Allen, 1957	N	*A. hortensis* Andrássy, 1961	N V
		A. uniformis Thorne, 1939	N

3.3. Nematodes in the Netherlands

The list of plant, soil and freshwater nematode species observed in the Netherlands was composed with the aid of several sources. They comprise a file of described species in general, the large nematode collection available at the Landbouwhogeschool including De Man's collection, a list by Loof & Oostenbrink (1962) of 258 species from Dutch soils present in these collections in 1960, earlier lists of nematodes published by De Man (1884, 1922), or shorter articles by him and other authors, and unpublished records collected by the author from the above slide collections or from files at the Landbouwhogeschool and the Plantenziektenkundige Dienst, Wageningen.

All nematodes known to be reported from the above-mentioned biotopes in the Netherlands are marked with a N in Table 3.

3.4. Discussion

It is immediately clear from Table 3 that the nematode faunas of the two countries differ markedly. Of the 362 species in the Netherlands and the 113 species in Venezuela few are reported from both countries, and scrutiny of these cases reveals that most of them concern an aberrant climatic biotope in one of the countries or are doubtful for other reasons.

The difference between nematode faunas of both countries does not appear clearly at the family level: all families recorded have representatives in both

countries except for a few families with one species or with species from biotopes which were up to now not yet sampled in Venezuela and except perhaps the Bunonematidae. The species of this family, however, are only found when special techniques are used and may be absent in the Venezuelan list for this reason.

The difference is evident however at the genus level, and certainly in the species range of genera with many species. The genus *Heterodera*, for example, is of widespread occurrence with 12 species in the Netherlands, but was not encountered in Venezuela except in some samples with a few larvae of an unidentified species. The same holds for *Hemicycliophora* (9 species N only), *Longidorus* (6 species N), *Bunonema* (5 species N), *Pelodera* (4 species N) and *Peltamigratus* (3 species V only). Within many other genera marked differences occur in the species range, e.g. in *Mesorhabditis*, *Rhabditis*, *Chiloplacus*, *Helicotylenchus*, *Hirschmanniella*, *Paratylenchus*, *Criconema*, *Macroposthonia* (only one of 18 species in both countries), *Aphelenchoides*, *Monhystera*, *Eudorylaimus* (only one of 28 species in both countries), *Mesodorylaimus*, *Dorylaimellus*, and *Trichodorus*, but also in *Tylenchorynchus* and *Pratylenchus* because the single or few overlapping species were found in Venezuela at high altitudes, and even in *Meloidogyne* because the thermophil species in the Netherlands are found only in greenhouses *(M. arenaria, M. incognita* and *M. javanica)*, whereas one cryophil species in Venezuela was only found at high altitude *(M. hapla)*.

The 63 species reported preliminarily from both countries deserve special scrutiny. A subsequent check of permanent slides available in the LH Collection revealed, that 5 of these species do not occur in both countries, the Venezuela specimens being specifically different from those of the Netherlands, viz. *Ditylenchus destructor*, *Ironus longicaudatus*, *Discolaimus major*, *Eudorylaimus miser*, *Oxydirus oxycephalus*. Two species are probably different, for differences were already indicated by Loof (1964), viz. *Achromadora ruricola* and *Paraphelenchus pseudoparietinus*. Of 13 other species recorded from both countries the identity is not wholly certain, either because of poor state of taxonomy of the genus in question or because of insufficient species diagnosis, viz. *Panagrolaimus rigidus*, *Mononchoides elegans*, *Cephalobus nanus*, *C. persegnis*, *Ditylenchus intermedius*, *Psilenchus hilarulus*, *Deladenus durus*, *Aphelenchus avenae*, *Plectus parvus*, *Monhystera vulgaris*, *Prismatolaimus intermedius*, *Alaimus primitivus* and *Amphidelus dolichurus*. For all these 20 species the indications N and V are placed between brackets. Finally: at least 16 species were found in an aberrant biotope in one of the countries. They are *Acrobeloides buetschlii*, *Euteratocephalus crassidens*, *Ditylenchus dipsaci*, *Tylenchorhynchus capitatus*, *Pratylenchus crenatus* and *P. thornei*, *Meloidogyne hapla*, *Anaplectus granulosus*, *Plectus parietinus*, *Mononchus papillatus* and *Aporcelaimellus amylovorus*, which were found at high altitudes only (1500–3000 m) in Venezuela, and further *Radopholus similis*, *Tylenchulus semipenetrans*, and *Meloidogyne arenaria*, *M. incognita* and *M. javanica*, which occur under greenhouse conditions only in the Netherlands. Six species found in Venezuela at intermediate altitudes of 1200–1500 m only should perhaps be added to this group, viz. *Teratocephalus terrestris*,

Tylenchus agricola, *T. davainei*, *Mylonchulus sigmaturus*, *Aporcelaimellus obtusicaudatus* and *Alaimus meyli*.

This leaves us with 21 species which may live in the temperate as well as the tropical areas studied. They are *Cruznema lambdiense*, *Diploscapter coronatus*, *Neoaplectana bibionis*, *Acrobeles ciliatus*, and *A. complexus*, *Acrobeloides emarginatus*, *Eucephalobus oxyuroides*, *Heterocephalobus longicaudatus*, *Tylenchus costatus* and *T. leptosoma*, *Macroposthonia sphaerocephala*, *Nothocriconema mutabile*, *Rhabdolaimus terrestris*, *Wilsonema otophorum*, *Trischistoma arenicola*, *Mononchus truncatus*, *Aporcelaimellus obscurus*, *Eudorylaimus monohystera*, *Nygolaimus vulgaris*, *Tylencholaimellus striatus* and *Alaimus parvus*.

Most of these 16 species are saprozoic or predatory. It is important for plant quarantine to note that, despite efficient spread and extreme polyphagy of many species, plant parasitic species were not found to thrive in the temperate as well as in the tropical climate, except *Macroposthonia sphaerocephala* and *Nothocriconema mutabile*. These two criconematid species were found in the Netherlands, and also in Venezuela at 400 m altitude or higher; they deserve special attention as possible ubiquitous plant parasites. *Ditylenchus destructor*, which was recorded from the Netherlands and from localities in Venezuela at 400 m altitude or higher, is omitted from the list of species which occur in both climates because the species collected in Venezuela differs significantly from *D. destructor* known as a parasite of potato and other plants. The data of later experiments confirm, that the plant parasites from one climatic region do not normally thrive or even survive at conditions of the other climate (cf. chapters 4 and 6.8).

Taxonomical identity of course does not exclude physiological differences between the populations studied and therefore does not prove ubiquity of the species; this requires transmission and other experiments.

For experimental analyses and determination of climatic influences on nematodes some species were selected from the list and some of their populations were collected and cultured. Apart from some natural, mixed populations the following experimental animals were considered suitable as a basis for such work: (V) and (N) populations of *Meloidogyne incognita*, *M. hapla*, *Ditylenchus dipsaci* and *Aphelenchus avenae* and a (V) population of *Helicotylenchus dihystera*. The origin of the populations was already described in detail under 2.3.

CHAPTER 4

TRANSMISSION AND INOCULATION EXPERIMENTS

4.1. INTRODUCTION

The conclusion from the data in the foregoing chapter was that the nematode faunas of tropical Venezuela and of the Netherlands differ markedly and that very few species have established themselves in the field in both areas. This cannot be attributed to the geographic situation of the two countries per se or to insufficient transportation of initial inoculum into the areas, for many species recorded from the tropics are found in greenhouses in the Netherlands and several endemic species from the temperate regions are found at high altitudes in Venezuela. Table 4 summarizes all records concerning plant parasitic species.

These data show that environmental influences must be instrumental in causing the differences and they suggest that climate is the main determinant factor. The environment however, includes soil, food and other organisms in addition to climate, as indicated under 1.3.1. and in Figure 1.

The type of soil may be determinant for the success with which certain species establish and maintain themselves (SEINHORST 1950, OOSTENBRINK 1954, UPADHYAY *in litt.*). It is unlikely however that this factor determines much of the differences between the two nematofaunas, for soil type varies widely in each of the countries, and in the aberrant climatic niches indicated in Table 4 infections of several species from the other zone took place.

Quality and quantity of food, i.e. host plants for the plant parasites, small animals for the predatory nematodes and bacteria and other micro-organisms for the so called saprozoic nematodes, affect all species, but it is unlikely that food is responsible for the different faunas. Most nematodes are highly polyphagous; this holds for most plant parasites, such as *Pratylenchus*, *Tylenchorhynchus*, *Meloidogyne* and other species. *Pratylenchus penetrans* reproduced on each of 182 plants tested (OOSTENBRINK *et al*, 1957). The host suitability of the plants varies and may determine whether a population will reach a measurable density, but good hosts of nematodes endemic in a temperate zone are not restricted to temperate crops. SHARMA (1968) showed that many tropical plants are excellent hosts for *Tylenchorhynchus dubius*, which is widespread in the Netherlands and is evidently limited to temperate zones.

Nor can the great difference between the faunas be accounted for by other organisms in the soil, since experimental inoculations in sterilized soils as well as natural infections in foreign soils (Table 4) are often successful.

In order to obtain pertinent information about the influence on nematode populations of climatic factors when the influence of soil, host plant and other organisms was excluded, a number of transplantation and inoculation experiments were made.

TABLE 4. Plant parasitic nematodes in aberrant climatic niches in the Netherlands and in Venezuela (cf. Table 3); authoritative record between brackets.

Thermophil plant nematodes, endemic in tropical or subtropical zones, found as natural infections in greenhouse plants in the Netherlands:

Tylenchorhynchus claytoni	(OOSTENBRINK 1959)
Radopholus similis	(KUIPER 1969)
Pratylenchus scribneri	(OOSTENBRINK 1960)
Pratylenchus vulnus	(OOSTENBRINK 1960)
Pratylenchus coffeae	(OOSTENBRINK 1961)
Nacobbus serendipiticus	(DE BRUIJN & STEMERDING 1968)
Tylenchulus semipenetrans	(s'JACOB, *in litt.*)
Heterodera cacti	(FILIPJEV & STEKHOVEN 1941)
Heterodera fici	(OOSTENBRINK 1958)
Meloidogyne arenaria	(OOSTENBRINK 1955)
Meloidogyne incognita	(OOSTENBRINK 1955)
Meloidogyne javanica	(OOSTENBRINK 1955)
Xiphinema diversicaudatum	(OOSTENBRINK 1959)
Helicotylenchus multicinctus	(s'JACOB, *in litt.*)

Cryophil plant nematodes, endemic in temporate zones, found as natural infections at high altitudes (1500–3000 m) in Venezuela:

Ditylenchus dipsaci	(MALAGUTTI 1950)
Tylenchorhynchus capitatus	(LOOF 1964)
Pratylenchus crenatus	(LOOF 1964)
Pratylenchus thornei	(LOOF 1964)
Meloidogyne hapla	(LOOF 1964)

The experiments comprised inoculations of different nematodes at low and high altitudes in Venezuela (4.2), transmission of a naturally infested lowland soil to a high altitude and of a naturally infested mountain population to a low altitude in Venezuela (4.3), and planting of *Meloidogyne* infested material from warm countries outdoors in Wageningen (4.4).

4.2. INOCULATION EXPERIMENTS IN VENEZUELA

Clay pots of 15 cm diameter were filled with about one kg of steam-sterilized sandy-loam soil; in each pot a two-week old, nematode-free tomato seedling was planted. The pots were inoculated with nematodes over the next two months. Eight pots were used for each of the nematodes tested. Four pots of each series were placed at the Estación Experimental de Mucuchies, at 2800 m above sea level, and the other at Facultad de Agronomia, Universidad del Zulia, Maracaibo, at 0 m above sea level.

The five nematode populations used for establishing infections by inoculation were *Meloidogyne incognita* (L) (= from lowland), *Meloidogyne hapla*

(H) (= from high mountains), *Pratylenchus zeae* (L), *Helicotylenchus dihystera* (L) and *Aphelenchus avenae* (H.) I failed to establish infection in any of the two climatic zones with four others species namely *Ditylenchus dipsaci* (H), *Criconemoides* sp. (H), *Tylenchorhynchus claytoni* (L) and *Rotylenchulus reniformis* (L.) This may be due to unsuitability of the soil, of the plant, of the inoculum or of the inoculation procedure. Anyway, these species were lost for further comparison.

The inoculum of each species consisted of 200 hand-picked nematodes per pot, randomly chosen from a natural population, except for the *Meloidogyne* species, of which four egg masses per pot were inoculated. All inoculations took place at the lowland station at Maracaibo. They were performed in succession for the different nematodes. After two months all series have been inoculated and half of the pots were transported to the Estación Experimental de Mucuchies. This was in June 1965.

The inoculated plants were left for 18 months in each of the localities in an open nursery, so that at any rate one full winter and one full summer passed. They were adequately taken care of in both places according to normal practice. The tomato plants were replaced regularly by young nematode-free seedlings during this period as soon as the plants declined on account of age; the old roots were left in the pots. At the end of this period all pots were transported by plane to the Nematology laboratory of the Landbouwhogeschool, Wageningen, the Netherlands, and the population densities in a sample taken from each pot were determined according to the methods described in Chapter 2; of some series two separates samples per pot were taken and analyzed. The results are summarized in Table 5.

TABLE 5. Inoculation results with 5 different nematodes into pots with sterilized sandy loam grown with tomato for 18 months at two different altitudes in Venezuela.
Inoculation April 1965; transportation to final site June 1965.
Figures after 18 months, are average numbers of four replicate pots per 100 ml of soil.

Nematode populations (L) = low land origin (H) = high mountain origin	Inoculated nematode per pot with ± 1000 ml of soil	Final nematodes densities		Smallest valid level of significance of differences, according to one-sided Wilcoxon test (n.s. = non-significant)
		at 0 m	at 2800 m	
Meloidogyne incognita (L)	4 egg masses	340	18	.025
Meloidogyne hapla (H)	4 egg masses	2810	13040	.10
Helicotylenchus dihystera (L)	200	114	41	.025
Pratylenchus zeae (L)	200	119	26	.025
Aphelenchus avenae (H)	200	111	100	n.s.

4.3. Transmission experiments in Venezuela

From the lowland, (L) = 0 m, as well as from the mountains, (H) = 2800 m, a sandy loam with a natural mixed nematode population was selected. An amount of each soil was thoroughly mixed without sterilization and filled into 8 pots of 15 cm diameter which were then planted with a tomato seedling as in the former experiment. About one month later 4 pots of each series were placed in the lowland as well as the high mountain nurseries as indicated before. Also in this experiment the tomato plants were renewed a number of times when they aged during the 18 months that the experiment lasted.

The nematode populations were assessed in Venezuela in 100 ml of each soil before the experiment started. After 18 months 100 ml samples from each individual pot were examined in Wageningen, yielding four replicate counts per series. The results are summarized in Table 6.

4.4. Transmission experiments in the Netherlands

Three experiments were made in the Diagnostics Department of the Plantenziektenkundige Dienst (PD) to study the behaviour of thermophil *Meloidogyne* species outdoors in the Netherlands. The author did not make the observations himself, but obtained the experiment records from Ir. K. Kuiper for study and inclusion in this report.

a. *Meloidogyne javanica* in potato

Potato tubers with a knotty surface which were heavily infested by *M. javanica* were obtained for observation from South Rhodesia in May 1957. Three tubers were planted in a pot with sterilized greenhouse soil in which this nematode species can thrive according to other observations. The tubers were planted on 18th May 1957 and sunk into a PD garden plot outdoors. There were no control plants in the greenhouse during this experiment.

None of the root systems was galled by the end of the year. A laboratory examination on 22nd January 1958 did not reveal any *Meloidogyne* specimens in the soil, in the roots or in the peels of newly formed tubers. Thus the heavily infested tubers did not cause infection outdoors and the population was completely extinct in one season.

b. *Meloidogyne arenaria* in *Polianthus tuberosa*

Tubers of the ornamental plant *Polianthus tuberosa*, heavily infested with *M. arenaria*, were specially imported from Israel to see whether the nematode would maintain itself when the tubers were planted outdoors in the Netherlands.

An infested tuber was planted in each of 6 ten-litre pots with sterilized potting soil in May 1957; 3 pots were placed in the greenhouse and 3 were sunk into a PD garden plot outdoors. All plants developed well. After 18 months, in November 1958, the roots of both the greenhouse and the outdoor plants still contained *Meloidogyne* larvae. There were, however, no larvae in the soil of the outdoor plants, whereas the soil around the greenhouse plants contained 20 to

TABLE 6. Natural nematode populations per 100 ml of soil of lowland (L) and high mountain (H) origin, each placed in pots and grown with tomato for 18 months at two different altitudes in Venezuela. Start of the experiment in June 1965.
Figures after 18 months are average numbers of four replicate pots.

Nematode species	Initial nematode densities	Final nematode densities		Smallest valid level of significance of the differences according to one-sided Wilcoxon-test (n.s. = non-significant)
		at 0 m	at 2800 m	
(L) soil				
Helicotylenchus dihystera	200	1400	30	.025
Paratylenchus sp.	60	300	0	.025
Meloidogyne incognita	50	20	30	n.s.
Aphelenchus avenae	80	30	60	.05
Other tylenchids	95	220	180	n.s.
Saprozoic nematodes	350	400	492	.10
Total		2370	792	
(H) soil				
Helicotylenchus erythrinae	80	30	130	.025
Meloidogyne hapla	1900	2000	17000	.025
Aphelenchus avenae	90	20	120	.025
Other tylenchids	200	390	410	n.s.
Saprozoic nematodes	400	800	550	.10
Total		3240	18210	

200 larvae per 100 ml, indicating that reproduction took place in the greenhouse pots and probably not outdoors. This was confirmed in December 1959: the outdoor plants were completely free from *Meloidogyne*, also in the tubers and the roots. Consequently the *Meloidogyne* population of the infested *Polianthus* tubers was extinct after 31 months.

c. *M. arenaria* and *M. javanica* on white clover and potato

9 pots of 750 ml were inoculated with a mixed *Meloidogyne* population of *M. arenaria* and *M. javanica* extracted from greenhouse-grown *Caladium* plants; 15 egg masses were inoculated per pot and they were sown with white clover in March 1967. 3 pots were placed in a heated greenhouse, 3 in an unheated greenhouse and 3 outdoors under a glass plate.

In December 1967 the plants of all three series appeared to be infested with *Meloidogyne*, although of each series, according to laboratory examination of one pot, damage to the roots and degree of infestation were severest in the greenhouse series and slightest in the series placed outdoors under a glass plate. The other two replicates of each series were resown with white clover. In June 1968 the contents of each pot were mixed as inoculum with sterilized garden soil and placed in a five-litre plastic bucket, and a potato tuber, variety Maritta, was planted in each bucket. The buckets of each series were placed again in the heated greenhouse, the unheated greenhouse and outdoors under cover of a glass plate.

In November 1968 the experiment was evaluated. The results are summarized in Table 7. The plants in the greenhouse were severely infested and in a very poor condition, those in the cold greenhouse were rather heavily infested and intermediate in growth. The results show that the plants placed outdoors had completely got rid of their infestation and that they grew much better.

TABLE 7. Development and infestation of potato in pots infested with *M. arenaria* and M. *javanica* when placed under 3 different climatic conditions (cf. 4.4.c.). Planting date June 1968, evaluation date November 1968.

Place of the pots	Length of stems in cm	Weight of tubers in g	Galling rate of rootball (5 = severe, 0 = nil)	Infection rate of stained roots (5 = severe, 0 = nil)
Heated greenhouse	20	90	5	5
	50	95	5	
Cold greenhouse	165	215	5	4
	112	180	5	
Outdoors, covered	392	380	0	0
with a glass plate	384	310	0	

4.5. DISCUSSION

Table 4 shows, that many nematodes are essentially widespread outside their endemic area, since they occur in aberrant climatic niches of the other country. The experiments confirm that climate is determinant for the establishment and reproduction of many nematodes.

Inoculation in the same soil with the same plants shows (Table 5) that *M. incognita* (L), *P. zeae* (L) and *H. dihystera* (L) thrive better at low altitude than at high altitude. The reverse holds for *M. hapla* (H), whereas no influence of altitude was noticed for *A. avenae* (H). The population differences are great and significant for the four reactive species, but none of them dissapeared completely from the pots in the unsuitable locality over the period of 18 months, which therefore included at least one full season. Essentially the same results were obtained with the transplanted natural populations (Table 6). It appears that *H. erythrinae* and *M. hapla* of the (H) population declined at low altitude, whereas *H. dihystera* and *Paratylenchus* sp. of the (L) population declined at high altitude. This was not so with the saprozoic nematodes and the other tylenchids as a group. *M. incognita* (L) did not reach a high density in the lowlands and therefore showed no significant difference in this experiment, and the result obtained with *A. avenae* (H) suggested that the population tends to prefer high altitudes. Also in the transplantation experiments the recorded species except *Paratylenchus* sp. did not disappear completely from the soil in the 18 months period. However the influence of altitude on the composition of the population, is clearly and generally in line with the inoculation experiment.

These are probably the first experimental data to show that geographic

altitude greatly influences nematode poulations, although several authors have noticed the great and consistant differences between the faunas at low and high altitudes (KRUSBERG & HIRSCHMANN 1958, WHITEHEAD 1960, JONES 1961, WHITEHEAD & DE GRISSE 1962 LOOF 1964, OOSTENBRINK 1966). Our experiments exclude the direct influence of locality, soil, plants and other growth factors such as water and fertilization, and leave us with temperature and possibly air pressure as instrumental influences which have to be studied further.

The transmission experiments with tropical *Meloidogyne* species in the Netherlands show, that these species cannot maintain themselves outdoors, although they are known to be noxious parasites on greenhouse crops. Many other tropical parasites also occur in greenhouse crops in the Netherlands (Table 4). It is clear that the cause lies in climatic influences and that air pressure cannot be instrumental here. The experiments in the Netherlands were restricted, however, to *Meloidogyne* species. For this reason the possible influence of air pressure on some nematodes will be studied (Chapter 5) in addition to temperature (Chapter 6).

CHAPTER 5

AIR PRESSURE EFFECTS

5.1 INTRODUCTION

Air pressure was left as a possible factor, next to temperature, to explain the influence of different geographic altitudes on nematode reproduction. Little is known about the effect of air pressure on nematodes; conclusive experimental data are lacking.

Therefore some experiments were carried out in exsiccators to study reproduction of the fungus feeder *Aphelenchus avenae* (V) and (N) and of the plant parasite *Ditylenchus dipsaci* (V) and (N) at the normal atmospheric air pressure at sea level, about 76 cm Hg (mercury), and at the air pressure prevailing at 3000 m above sea level, which is about 54 cm Hg.

5.2. APHELENCHUS AVENAE (V) AND (N)

Experiment 1. 60 petri dishes of 5 cm diameter with oat-meal agar plus the fungus *Alternaria solani* as culture medium were used. 30 dishes were inoculated with a single larva of population (V), 30 others with a single larva of population (N). Then 20 inoculated dishes of each series, 40 in total, were placed together in an 11 litre exsiccator in which the air pressure was reduced to 54 cm Hg by means of a water vacuum pump. This low pressure was maintained and checked every other day during the period of incubation. The 10 remaining dishes of each series, 20 in total, were placed together in another closed exsiccator under normal atmospheric pressure, about 76 cm Hg. The experiment started on 16th January 1968 and lasted one month. The culture dishes were subsequently filled with water and allowed to stand for 24 hours, whereupon the nematodes were collected by decantation of the suspension. The nematodes were counted as outlined in Chapter 2. The results are summarized in Table 8.

TABLE 8. Populations of *Aphelenchus avenae* (V) and (N) after inoculation of a single larva per culture dish, placed for one month at 25°C at two different air pressures (54 cm Hg and about 76 cm Hg). 40 petri dishes were placed together in the low pressure exsiccator, 20 in the normal pressure exsiccator.

Population	Total number of culture dishes per exsiccator	Average number of nematodes per dish:	
		at 54 cm Hg air pressure	at normal, 76 cm Hg air pressure
A. avenae (V)	40	0	
	20		878
A. avenae (N)	40	0	
	20		295

The results in Table 8 show no reproduction in the exsiccator with 40 culture dishes submitted to 54 cm Hg pressure. A fair reproduction occurred in the cultures of both populations placed in the exsiccator with 20 dishes at an air pressure of 76 cm Hg, although with strong variation between individual cultures. The 'average' offspring of one inoculated larva was 878 in the (V) and 295 in the (N) population; this difference between the two populations was significant in this experiment. The experiment confirms the known ability of *A. avenae* to thrive on a fungus and to reproduce monosexually. The results seem to indicate that low pressure suppresses reproduction of the nematode, but the difference between low and normal pressure cultures appears to be due to another cause, as is shown herein after and in the following experiments. Upon being opened the low pressure exsiccator containing the 40 dishes gave out a strange smell, while in the other exsiccator containing 20 dishes the smell was less strong. The *A. solani* fungus colonies in the low pressure exsiccator appeared unhealthy and had a greyish colour, against a normal dark colour of the fungus culture in the other exsiccator. This suggested the posibility of the cultures having produced substances with a toxic effect on the fungi and perhaps, via the fungus or directly, on the nematodes. The fact that this influence was strongest in the low pressure exsiccator could be due to the low pressure, but also to the greater number of cultures, 40 in the low pressure exsiccator against 20 in the normal pressure one. The toxic influence disappeared rapidly from the cultures in the open air, for when the same 40 cultures of the low pressure exsiccator were reinoculated with *A. avenae* from both populations and placed in an open incubator at 25 °C reproduction was readily obtained in all the dishes.

Experiment 2. The above-mentioned experiment was repeated at low air pressure (54 cm Hg) with different numbers of Petri dishes per exsiccator. The same method and materials were used, except that four larvae were inoculated per dish. The results in Table 9 show that very strong nematode reproduction occurred at low air pressure with 1 dish per exsiccator, as tested for the (N) population. In this case a more than 9000-fold reproduction of the inoculum was obtained in one month. The reproduction rate dropped when the number of

TABLE 9. Populations of *Aphelenchus avenae* (V) and (N) after inoculation of 4 larvae per culture dish, placed for one month at 25°C at a low air pressure of 54 cm Hg when 20, 10, 5 and 1 petri dishes were placed together in one exsiccator.

Population	Total number of culture dishes per exsiccator	Average number of nematodes per dish:		
		Adults	Larvae	Total
A. avenae (V)	20	0.2	0.8	1
	10	58	1260	1318
	5	52	1000	1025
	1			
A. avenae (N)	20	0.1	0.5	0.6
	10	370	1300	1670
	5	67	704	771
	1	8900	29750	38650

dishes per exsiccator was 5 or 10. With 20 dishes per exsiccator some reproduction occurred, although it was very limited. There were no noticeable differences between the two nematode populations.

Experiment 3. The experiment 2 was repeated, but now with 1 larva per petri dish under normal air pressure during $2\frac{1}{2}$ weeks. Table 10 shows no reproduction with 40 dishes per exsiccator, whereas the offspring increased with fewer dishes per exsiccator. This was true, without much difference, for both populations.

TABLE 10. Population of *Aphelenchus avenae* (V) and (N) after inoculation of a single larva per culture dish, placed for $2\frac{1}{2}$ weeks at 25°C at normal air pressure of about 76 cm Hg when 40, 20, 10 and 5 petri dishes were placed together in one exsiccator

Population	Total number of culture dishes per exsiccator	Average number of nematodes per dish:		
		Adults	Larvae	Total
A. avenae (V)	40	0	0	0
	20	8	7	15
	10	17	20	37
	5	85	118	203
A. avenae (N)	40	0	0	0
	20	8	11	19
	10	30	50	80
	5	210	950	1160

5.3. DITYLENCHUS DIPSACI (V) AND (N)

Similar experiments as mentioned in section 5.2. were carried out with *D. dipsaci* (V) and (N) populations in onion bulbs. Onion bulbs were inoculated in a hole 2 cm deep and 0.5 cm wide made with a borer in the centre of the bulb and into this hole a suspension containing 50 hand-picked adult female larvae were pipetted. The hole was then plugged with cotton and sealed with paraffin.

After inoculation the bulbs were placed in an exsiccator to 20°C and at 54 cm Hg pressure for 4 weeks. For each of the populations 20, 10 or 5 bulbs were placed together in three exsiccators at 54 cm Hg pressure, making a total of 40, 20 and 10 bulbs per exsiccator. There were control exsiccators at normal, atmospheric pressure, but with half the number of inoculated bulbs, namely 20, 10 and 5 in total per exsiccator.

The results in Table 11 indicate that with 40 bulbs in one exsiccator no reproduction of the nematodes took place. The onion bulbs were decaying and covered with fungi after three weeks. With 20 bulbs reproduction was already fairly high, although less than with 10 bulbs. This was true for both populations. There was no significant difference between low and normal air pressure; nematode reproduction at low pressure was even somewhat higher than at normal air pressure.

TABLE 11. Population of *Ditylenchus dipsaci* (V) and (N) after inoculation of 50 larvae per onion bulb, placed for 4 weeks at 20°C at two different air pressures (54 cm Hg and about 76 cm Hg), when different numbers of bulbs were placed together in one exsiccator.

Population	Total number of bulbs per exsiccator	Average number of nematodes per bulb:	
		at 54 cm Hg air pressure	at normal, 76 cm Hg air pressure
D. dipsaci (V)	40	0	
	20	194	130
	10	644	213
	5		250
D. dipsaci (N)	40	0	
	20	172	120
	10	478	200
	5		1000

5.4. DISCUSSION

From the experiments with *A. avenae* and *D. dipsaci*, (V) and (N) populations it appears that there was certainly no negative effect of low air pressure as prevailing in high mountain areas on the reproduction of the nematodes when the number of petri dishes or bulbs in the exsiccators was low.

There were odours in the exsiccator associated with changes in the colour of the fungus cultures and with suppression of nematode reproduction, when many fungus cultures or many onion bulbs were placed together in closed containers, but this is apparently not related to air pressure. It is probably due to the fact that living or decaying organic materials release fungicidal and nematicidal agents, a point also noticed by WINOTO (1969). The volatile, smelling vapour which was noticed in the exsiccators, may comprise this agent. It was brought into a gas chromatograph in the Department of Organic Chemistry of the Landbouwhogeschool and probably was a product from the fungus metabolism, a carbohydrate compound, but further indentification failed owing to the small amount of volatile material.

The experiments in this chapter reveal that low air pressure, such as encountered at high geographic altitudes, does not determine the reproduction of the (V) and (N) populations of *A. avenae* and *D. dipsaci*. It is therefore unlikely that it is a determinant factor in the establishment and thrift of nematode populations in different geographic areas. This leaves temperature as the only important factor for further study.

CHAPTER 6

TEMPERATURE EFFECTS

6.1. INTRODUCTION

It appeared from Chapters 3, 4 and 5 that climatic influences on nematode communities are great, and by exclusion of the other influences the conclusion seems justified that the key factor involved must be temperature. The known effects of temperature on different nematodes are summarized in the literature review under 1.3.3. The data suggest that the temperature is a dominant selective factor determining the geographic distribution of nematodes, but more thermograms of species from different climatic zones are needed to support or reject this hypothesis. In our study we determined the influence of temperature ranges on one or more biological aspects (hatching, penetration, reproduction, and influence on the host plant, as well as survival, acclimatization and morphology) of a range of nematode species.

The test species were selected with reference to the faunistic lists of the nematofaunas in Venezuela and the Netherlands, and two natural, mixed nematode communities from Dutch origin were added. Four of the test species were represented by a population from Venezuela (V) as well as the Netherlands (N), namely *Meloidogyne incognita*, *M. hapla*, *Ditylenchus dipsaci*, and *Aphelenchus avenae*. Further a population of *Helicotylenchus dihystera* from Venezuela was added. Thus the test animals included representative populations from both climatic zones. They were brought together and maintained as stocks in greenhouse compartments; the data about origin and history of each population were given before (cf. 2.2).

6.2. MELOIDOGYNE INCOGNITA (V) AND (N)

Comparative experiments were conducted in temperature gradients with the (V) and (N) populations of *M. incognita* to study hatching (section 6.2.1), penetration, reproduction and gall formation (section 6.2.2), and morphology (6.2.3).

6.2.1. *Hatching*

Egg masses of both populations propagated on tomato at a temperature of 25 °C, were collected from the roots by means of a forceps. They were placed on filter paper moistened with distilled water in order to select egg masses of the same size, shape and colour.

Single egg masses were placed on soaked filter paper touching distilled water in cylindrical holes of 1 by 1 cm in small plastic blocks. Each block with a single egg mass was placed in a closed petri dish with a thin layer of water on the

bottom. Four replicate egg masses of each population were placed in compartments of the series thermostat at each of the following temperatures: 5°–10° 15°–20°–25°–30°–35° and 40°C. The real temperatures fluctuated somewhat (± 1°) and were approximately those recorded here. The experiment was started on 8th September 1967. Counts or hatched larvae were made after 3, 6, 9, 12 and 15 days by washing all free larvae of each egg mass from the block holes into the petri dish and re-establishing the same egg mass on new filter paper in a clean block for further hatching. The total emergence of the larvae is recorded in Figure 3, the rate of the emergence with time in Figure 4.

After 15 days all egg masses were subjected to 25°C under the same conditions for another 15 days. The number of hatched larvae from the first period of 15 days at different temperatures and from the additional 15 days with a treatment at 25°C are listed together in Table 12.

It appears from figures 3 and 4 that the egg masses of the populations (V) and (N) were about equally responsive and liberated near 700 larvae per egg mass at their optimum temperatures. The thermograms of Figure 3 are of the same shape, but they show two minor differences, namely that the (V) population had

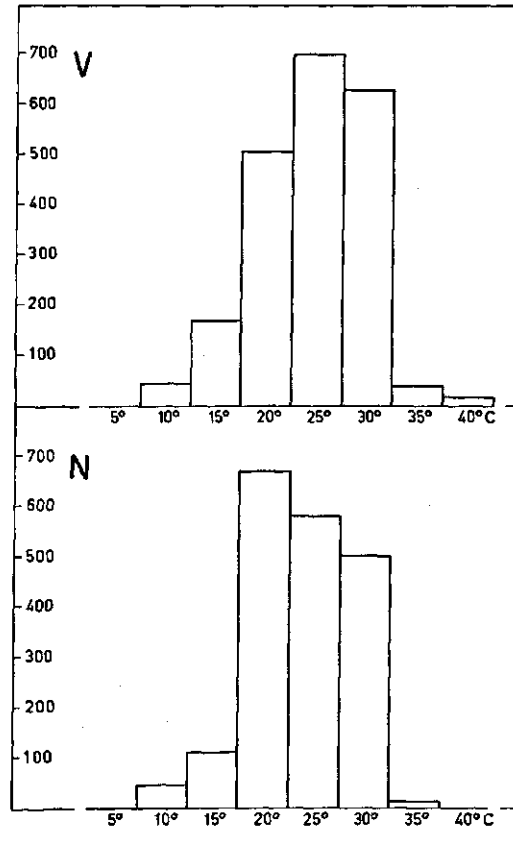

FIG. 3. Hatching thermograms of *Meloidogyne incognita* from Venezuela (V) and from the Netherlands (N) after 15 days.
Ordinate: final number of hatched larvae per egg mass; average of four single egg masses.
Abscissa: temperatures in the different thermostat compartments (5° to 40°C).

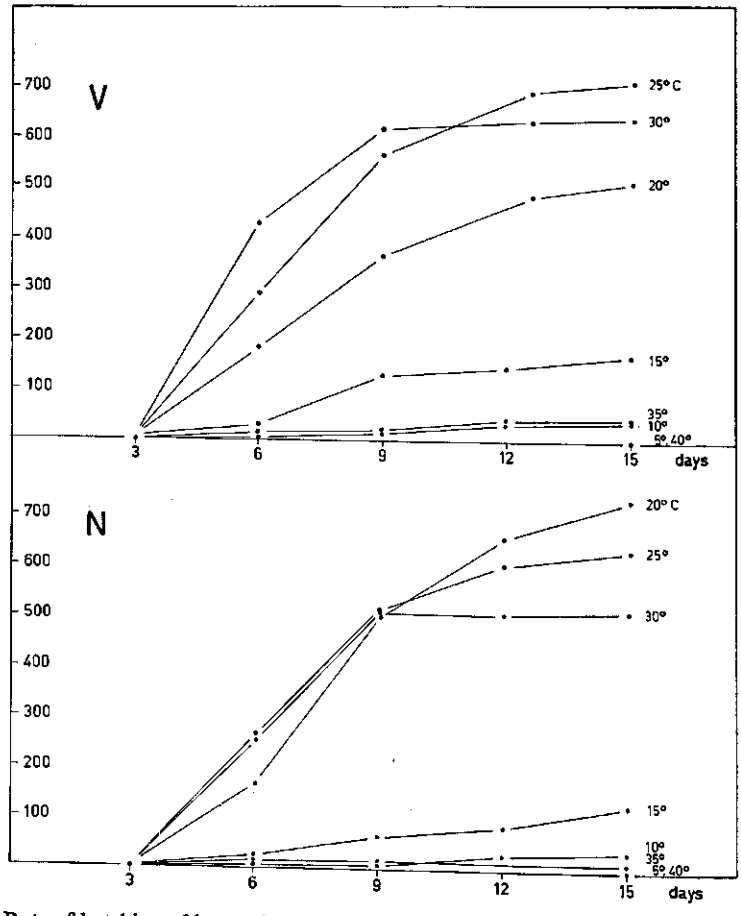

FIG. 4. Rate of hatching of larvae from egg masses of *Meloidogyne incognita* from Venezuela (V) and from the Netherlands (N) at eight different temperatures with time.
Ordinate: number of hatched larvae per egg mass; average of four single egg masses.
Abscissa: duration of exposure to distilled water at different temperatures, varying from 5° to 40 °C, as indicated in the graphs.

its maximum hatch at the higher temperature and showed a better hatch at 35 °C and 40 °C, than with the (N) population. In both populations only a few larvae hatched at 5 °C, viz. 3 per four egg masses in the (V) and 4 per four egg masses in the (N) population. Hatching was measurable but still low in both populations at 10 °C. It was moderate at 15 °C, but a strong hatch in both populations was only obtained at the temperature range from 20 °to 30 °C. At 35 °C hatching was again low in both populations, although lower in the (N) population; at 40 °C the (V) population still released 68 larvae per 4 egg masses against nil in the (N) population. The differences between the two thermograms are therefore that the (V) population had its optimum at 25 °C, against 20 °C in the (N) population, and that the (V) population released 68 larvae per four egg

TABLE 12. Number of hatched larvae per four egg masses of *Meloidogyne incognita* from Venezuela (V) and the Netherlands (N) after 15 days in various temperatures and after 15 days more at constant temperature of 25°C

Temperatures °C	*M. incognita* (V)		*M. incognita* (N)	
	After 15 days at the temperatures indicated	After an additional 15 days at 25°C	After 15 days at the temperatures indicated	After an additional 15 days at 25°C
5	3	400	4	546
10	163	2233	162	825
15	695	860	527	886
20	2016	2162	2781	2859
25	2809	2823	2368	2402
30	2513	2532	2044	2046
35	184	341	53	55
40	68	68	0	25

masses at 40°C, against 0 in the (N) population. Figure 4 indicates that the hatching process started after 3 to 6 days, increased rapidly until 9th day and then slowed down. As can be seen from Figure 4 hatching at the highest temperatures, 40°C for the (V) and 35°C for the (N) population, occurred mainly or extensively during the first 6 days of the 15 days' period; also the cumulative hatch at 20°C was always lower than at 25° and 30°C in the (V) population, whereas in the (N) population it surpassed at 25° and 30°C only after 9 days.

Table 12 shows that neither of the two populations was completely killed during their 15 days' stay at any of the extreme low or high temperatures. A percentage of larvae, however, lost their activity or were killed; this percentage was more than 80% for both populations at 5°C and at 35°C and 40°C. At 10° and 15°C part of the population may also have been killed, although this indication is less pertinent. There is no consistent difference between the two populations of this species with respect to their survival at extreme temperatures; the (V) population survived at temperatures of 10° and 15°C and the (N) population survived at 40°C equally well or better than the other.

6.2.2. *Penetration and development, root galling, reproduction*

a. Experiments in greenhouse compartments

Egg masses of the (V) and (N) populations were collected and selected as indicated under 6.2.1. They were inoculated to young tomato plants sown in wooden boxes and transplanted in square plastic tubes of 4 cm diameter and 20 cm long, filled with 300 ml of soil; one week after transplanting one egg mass was inoculated in each pot. 10 pots were placed in greenhouse compartments with the following general temperatures: 10°–15°–20°–25° and 30°C (in fact 28°–30°C). Two plants were removed from each temperature unit for evaluation after 4, 6, 8, 10 and 12 weeks to make up a series with time. The number of larvae in the roots were quantitatively checked, and the degree of galling and the number of larvae in the soil were determined by the methods

described under 2.5. The results are discussed below and summarized in Tables 13 and 14 and in Figure 5.

TABLE 13. Inoculation of *Meloidogyne incognita* (V) and (N) onto tomato in greenhouse compartments. Root gall index (0 = no galling, 4 = 100% of the root system transformed to galls; cf. 2.5.2) 4, 6, 8, 10 and 12 weeks after inoculation of one egg mass per tube of 300 ml with one plant, at 5 different temperatures. Each figure is the root knot index per plant (x) plus one and transformed to the natural logarithm, according to the formula $^{e}\log(x+1)$; average of two replicates. L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leqslant 0.01$), * = significant ($0.01 < P \leqslant 0.05$), (*) = nearly significant ($0.05 < P \leqslant 0.10$), – = non-significant ($P > 0.10$).

Greenhouse temperatures / Evaluation time	*M. incognita* (V) 10°C	15°C	20°C	25°C	28°–30°C	Time averages (L.S.D. = 0.44)
After 4 weeks	0.00	0.00	0.69	1.24	1.39	0.66
After 6 weeks	0.35	0.35	1.10	1.24	1.24	0.86
After 8 weeks	0.00	1.10	1.35	1.15	1.50	1.02
After 10 weeks	0.00	0.35	1.10	1.39	1.61	0.89
After 12 weeks	0.00	0.35	1.39	1.50	1.61	0.97
Temperature averages (L.S.D. = 0.44)	0.07	0.43	1.13	1.30	1.47	

Greenhouse temperatures / Evaluation time	*M. incognita* (N) 10°C	15°C	20°C	25°C	28°–30°C	Time averages (L.S.D. = 0.47)
After 4 weeks	0.90	0.90	1.24	1.15	1.39	1.11
After 6 weeks	1.24	1.24	1.04	1.50	1.61	1.33
After 8 weeks	1.15	0.69	1.61	1.61	1.61	1.33
After 10 weeks	0.35	1.50	1.39	1.61	1.61	1.29
After 12 weeks	0.69	1.04	1.61	1.61	1.61	1.31
Temperature averages (L.S.D. = 0.47)	0.87	1.07	1.38	1.50	1.56	

Analysis of variance:

		P values
M. incognita (V)		
F temperature	= 53.75	**
F time	= 2.76	*
Interaction	= 1.59	(*)
M. incognita (N)		
F temperature	= 11.60	**
F time	= 1.11	–
Interaction	= 1.90	(*)

TABLE 14. Inoculation of *Meloidogyne incognita* (V) and (N) onto tomato in greenhouse compartments. Free larvae in the soil 4, 6, 8, 10 and 12 weeks after inoculation of one egg mass per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the natural logarithm $^e\log(x+1)$ of the number of larvae per tube (x) plus one; average of two replicates.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leqslant 0.01$), * = significant ($0.01 < P \leqslant 0.05$), (*) = nearly significant ($0.05 < P \leqslant 0.10$), – = non-significant ($P > 0.10$).

Evaluation time \ Greenhouse temperatures	*M. incognita* (V) 10°C	15°C	20°C	25°C	28°–30°C	Time averages (L.S.D. = 3.57)
After 4 weeks	0.00	0.00	0.00	0.00	0.00	0.00
After 6 weeks	0.00	0.00	2.31	3.45	3.11	1.77
After 8 weeks	0.00	1.52	2.06	5.29	7.75	3.22
After 10 weeks	0.00	0.00	0.00	8.16	8.44	3.32
After 12 weeks	0.00	0.00	3.37	8.99	8.14	4.10
Temperature averages (L.S.D. = 3.57)	0.00	0.30	1.55	5.18	5.49	

Evaluation time \ Greenhouse temperatures	*M. incognita* (N) 10°C	15°C	20°C	25°C	28°–30°C	Time averages (L.S.D. = 3.38)
After 4 weeks	0.00	1.86	0.00	1.52	4.78	1.63
After 6 weeks	0.00	0.00	3.74	7.29	7.80	3.77
After 8 weeks	1.52	1.86	2.54	6.94	7.38	4.05
After 10 weeks	0.00	1.52	4.60	9.65	8.39	4.83
After 12 weeks	3.34	3.00	7.95	10.30	10.66	7.05
Temperature averages (L.S.D. = 3.38)	0.97	1.65	3.76	7.14	7.80	

Analysis of variance:

		P values
M. incognita (V)		
F temperature	= 16.06	**
F time	= 6.11	**
Interaction	= 1.92	(*)
M. incognita (N)		
F temperature	= 24.71	**
F time	= 9.77	**
Interaction	= 1.16	–

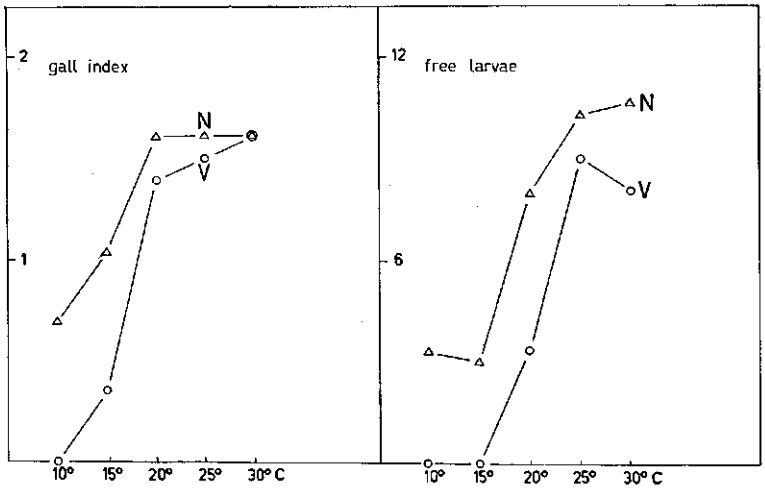

FIG. 5. Comparison of the (V) and (N) populations of *Meloidogyne incognita* with respect to penetration and root gall formation and with respect to free larvae in the soil, 12 weeks after inoculation to tomato growing in 300 ml tubes in the greenhouse experiments (cf. tables 13 and 14).
Ordinate: lefthand graph: natural logarithm $^e\log(x + 1)$ of root gall index per plant (x) plus one; righthand graph: of number of free larvae per tube (x) plus one; in both cases average of two replicates.
Abscissa: temperatures in the various greenhouse compartments.

Microscopic examination revealed that penetration and development of the larvae were strongly correlated with gall formation. Developing larvae were always surrounded by swollen root tissue, and swollen tissues always harboured larvae or further developed stages of the nematode. The root gall index was therefore a useful statistic for the evaluation of penetration and development as well as galling.

Table 13 shows that root galling increased significantly with temperature in both populations according to a linear trend. There was a similar increase with time, statistically significant for the (V) population, but not for the (N) population, which will be explained later.

Hardly any larvae of the (V) population penetrated at 10 °C, and only incidental plants, 1 out of 10 during the experiment, showed the presence of a developing female and a corresponding slight galling. At 15 °C penetration was common; galling became visible on most plants, although with some delay, and never exceeded the gall index 2 for individual plants or 1.1 for an average of two plants. Only one plant yielded free larvae of a new generation in the soil according to Table 14. At 20°, 25° and 30 °C, nematode penetration and development and gall formation were strong on all plants, although slightly less strong and occurring with more delay at 20 °C than at the higher temperatures. Free larvae occurred at all these high temperatures after 6 weeks and more, but not before 4 weeks.

Numerous larvae of the (N) population penetrate in all plants and on the first evaluation date, i.e. after 4 weeks, had already caused considerable gall formation, at all temperatures from 10° up to 30°C. Free larvae of a new generation in the soil were found under some of the plants at 10°C, and they were generally present at 15°C. This is a significant and interesting difference between the two populations. The (N) population also thrived best at the temperature range from 20° to 30°C, with possibly some preference for the higher temperatures, a tendency also shown by the (V) population. From the low temperatures of the range, it is clear, however, that the (N) population is relatively less thermophil or less cryophob than the (V) population.

The data about free larvae in the soil (Table 14) are generally in accordance with the data about infestation and galling (Table13). This is not surprising since both sets of data are derived from plants and soils from the same tubes. The numbers of free larvae in the soil also increase with temperature and time, highly significant for both. Although the interactions between temperature and time are not significant at the 5% level, they are nearly significant for galling in both populations and for free larvae in soil in the (V) population. Interaction, indicating that gall formation and nematode reproduction proceed at a slower rate at the low temperatures, would probably have shown better if the first evaluation had been made on an earlier date and if more replicates could have been evaluated at each date. It should be noted here, that the free larvae of the (N) population present after 4 weeks, and not after 6 weeks, at 15°C, not the lower or higher temperatures, may be attributed to another aspect, namely partial survival of the inoculum. The same question arises and is discussed further in the next experiment.

The difference between the (V) and the (N) population is stressed in Figure 5, which shows the gall index and the free larvae in the soil after 12 weeks, i.e. at the end of the experiment. It appears that the (N) population causes galls at 10°C and produces new generation larvae at 10° and at 15°C, whereas the (V) population does not. The clearcut difference in free larvae in the soil at the end of the experiment ascertains that it is caused by difference in reproduction and not by difference in survival of the inoculum, the more so since the (N) population did not produce free larvae after 4 and 6 weeks.

b. Experiments in Wisconsin tanks

Similar experiments with *M. incognita* (V) and (N) as described under a. were carried out in Wisconsin tanks at soil temperatures of 15°–20°–25°–30° and 35°C. The soil temperatures were controlled as indicated, whereas the air temperature in the room fluctuated between 20° and 25°C. One week after planting tomato plants in the plastic tubes, they were placed in the Wisconsin tanks and left there for three days to attain the desired soil temperatures, which were checked by means of soil thermometers. The nematodes were subsequently inoculated, again one egg mass per tube with one plant. Again there were ten plants for each temperature, two plants being harvested 4, 6, 8, 10 and 12 weeks after inoculation. In this case the temperature range of 15°–35°C was some-

what different from that used in the greenhouse experiments owing to the capacity of the facilities. Nematode penetration, degree of galling and number of free larvae in the soil were evaluated in the same way in the experiment under a. The results are described below and summarized in Tables 15 and 16 and Figure 6.

TABLE 15. Inoculation of *Meloidogyne incognita* (V) and (N) onto tomato in Wisconsin tanks. Root gall index (0 = no galling, 4 = 100% of the root system transformed to galls; cf.2.5.2) 4, 6, 8, 10 and 12 weeks after inoculation of one egg mass per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the root knot index per plant (x) plus one and transformed to the natural logarithm, according to the formula °log (x + 1); average of two replicates.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), * = significant ($0.01 < P \leq 0.05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non significant ($P > 0.0$).

Wisconsin tanks temperatures / Evaluation time	*M. incognita* (V)					
	15 °C	20 °C	25 °C	30 °C	35 °C	Time averages (L.S.D. = 0.36)
After 4 weeks	0.69	1.10	1.24	0.90	1.50	1.09
After 6 weeks	0.90	1.10	1.50	0.69	1.04	1.05
After 8 weeks	1.24	1.10	1.39	1.50	1.61	1.37
After 10 weeks	0.35	1.04	1.61	1.61	1.61	1.24
After 12 weeks	0.00	0.90	1.61	1.61	1.61	1.14
Temperature averages (L.S.D. = 0.36)	0.64	1.05	1.47	1.26	1.47	

Wisconsin tanks temperatures / Evaluation time	*M. incognita* (N)					
	15 °C	20 °C	25 °C	30 °C	35 °C	Time averages (L.S.D. = 0.08)
After 4 weeks	1.10	1.10	1.39	1.61	1.61	1.36
After 6 weeks	1.10	1.24	1.61	1.61	1.10	1.33
After 8 weeks	1.61	1.61	1.61	1.61	1.61	1.61
After 10 weeks	1.50	1.61	1.61	1.61	1.61	1.59
After 12 weeks	1.61	1.61	1.61	1.61	1.61	1.61
Temperature averages (L.S.D. = 0.08)	1.38	1.48	1.56	1.61	1.51	

Analysis of variance:

	P values
M. incognita (V)	
F temperature = 27.24	**
F time = 3.73	*
Interaction = 4.77	**
M. incognita (N)	
F temperature = 32.38	**
F time = 74.81	**
Interaction = 17.49	**

TABLE 16. Inoculation of *Meloidogyne incognita* (V) and (N) onto tomato in Wisconsin tanks. Free larvae in the soil 4, 6, 8, 10 and 12 weeks after inoculation of one eggs mass per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the natural logarithm $^e\log(x + 1)$ of the number of larvae per tube (x) plus one; average of two replicates.
L.S.D. = least significant difference at 5%, F = variance ratio, P= probability, ** = highly significant ($P \leq 0.01$), * = significant ($0.01 < P \leq 0.05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non significant ($P > 0.10$).

Wisconsin tanks temperatures / Evaluation time	*M. incognita* (V)					
	15 °C	20 °C	25 °C	30 °C	35 °C	Time averages (L.S.D. = 3.00)
After 4 weeks	0.00	0.00	0.00	4.07	2.68	1.35
After 6 weeks	0.00	2.65	7.74	6.34	6.48	4.64
After 8 weeks	0.00	3.38	5.77	2.40	7.91	3.89
After 10 weeks	1.52	5.34	10.20	8.89	7.79	6.75
After 12 weeks	1.52	7.47	8.92	9.28	9.09	7.24
Temperature averages (L.S.D. = 3.00)	0.61	3.77	6.53	6.18	6.79	

Wisconsin tanks temperatures / Evaluation time	*M. incognita* (N)					
	15 °C	20 °C	25 °C	30 °C	35 °C	Time averages (L.S.D. = 1.66)
After 4 weeks	1.52	0.00	0.00	7.86	2.39	2.09
After 6 weeks	0.00	2.31	7.44	8.64	7.64	5.20
After 8 weeks	6.80	9.20	9.18	9.00	8.01	8.44
After 10 weeks	7.68	10.60	8.98	10.03	10.42	9.54
After 12 weeks	5.52	9.45	8.92	11.98	9.47	7.09
Temperatures averages (L.S.D. = 1.66)	4.30	6.31	6.90	9.50	7.62	

Analysis of variance:

	P values
M. incognita (V)	
F temperature = 22.07	**
F time = 18.25	**
Interaction = 1.83	(*)
M. incognita (N)	
F temperature = 42.34	**
F time = 105.68	**
Interaction = 7.43	**

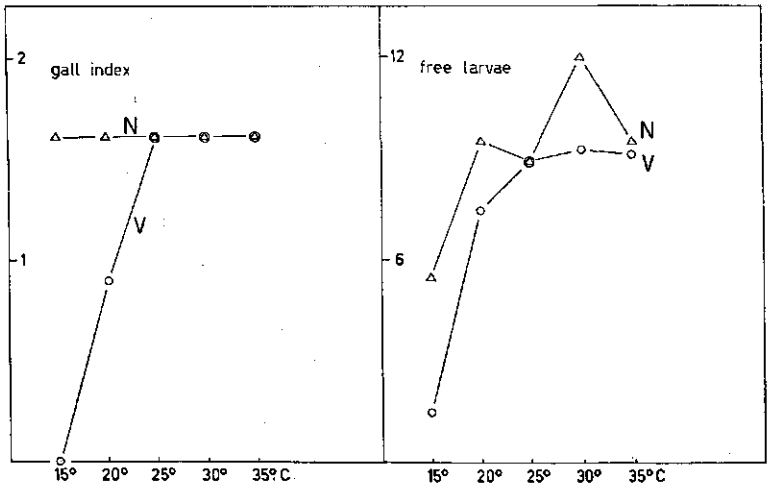

FIG. 6. Comparison of the (V) and (N) populations of *Meloidogyne incognita* with respect to penetration and root gall formation and with respect to free larvae in the soil, 12 weeks after inoculation to tomato growing in 300 ml tubes in Wisconsin tank experiments (cf. Tables 15 and 16).
Ordinate: lefthand graph: natural logarithm $^e\log(x + 1)$ of root gall index per plant (x) plus one; righthand graph: of number of free larvae per tube(x) plus one; in both cases average of two replicates.
Abscissa: temperatures in the various Wisconsin tanks.

The results are generally the same as those obtained in the greenhouse experiments where not only the soil but also the ambient conditions were constantly kept at the temperatures indicated. Here again galling and free larvae in the soil, indicating reproduction, increased with temperature as well as with time, and the interactions were generally significant.

Both populations had already caused gall formation at all temperatures after 4 weeks (Table 15). The nematodes had evidently entered the roots and started developing with the corresponding root tissue swelling, already at 15 °C. The (V) population caused little galling at 15 °C (the zero figure after 12 weeks must be chance escape, for there were newly formed larvae in these tubes according to the figures of Table 16). The galling at 20 °C was moderate and it was strong at 25 °–35 °C. Gall formation by the (N) population was already high at the low temperatures; it rose somewhat with temperature shortly after inoculation but not at later evaluation dates. All temperatures were evidently favourable for penetration and early development with corresponding root tissue swelling, and no differences were visible after 8 or more weeks.

The numbers of free larvae in the soil (Table 16) confirmed and emphasized the above temperature relations. In the tubes inoculated with the (V) population no free larvae were present at 15 °C before the fourth evaluation date, i.e. after 10 weeks, and even then the densities remained low. The (N) population had apparently formed many new larvae at 15 °C already after 8 weeks. At

20 °C and 25 °C both populations had apparently formed new generation larvae after 6 weeks, and at all the higher temperatures after 4 weeks. These results explain the highly significant interactions between the influence of temperature and time found in the statistical analysis of the data.

The incidental and unexpected presence of a few free larvae at 15 °C after 4 weeks probably indicates partial survival of the inoculum, as was already suggested under a. *M. incognita* free larvae in the soil may survive for 4 weeks if temperatures are not extreme (BERGESON 1960). It is, however, unlikely that they would survive 4 weeks at the higher temperatures or 8 weeks at 15 °C.

The difference between the (V) and (N) populations is further illustrated in Figure 6, which reflects the degree of galling and of nematode reproduction (free larvae). At the end of the experiment, after 12 weeks, the degree of galling by the (V) population is still much lower at 15 ° and 20 °C than at higher temperatures; this is not so with the (N) population. The formation of free larvae by the (V) population is also much lower at 15 ° and 20 °C than by the (N) population. Both populations prefer the higher temperature range of 25 °–35 °C. Accordingly, the difference between the populations is that the (N) population can start its activity and reproduction at lower temperatures than the (V) population; free larvae of the first mentioned population may also survive longer in the soil at 15 °C.

c. Experiments in climatic cells

Similar experiments with *M. incognita* (V) and (N) as described under a. and b. were carried out in climatic cells with controlled temperatures, air moisture (75 %) and light (18 hours per day). The temperatures were 10 °–15 °–20 °–25 ° and 30 °C. The tomato plants in the plastic tubes were inoculated with 3 egg masses per tube (=per plant) and before being placed in the climatic cells all plants were kept in a greenhouse at 25 °C for three days in order to obtain a homogeneous penetration of larvae. Four plants at each temperature were evaluated after 2, 4, 5 and 6 weeks. In this experiment infestation and gall formation was not evaluated by a root gall index indicating the percentage of swollen roots, but by the number of galls comprising nematodes according to microscopic scrutiny of stained root systems (Table 17). Free larvae in the soil were determined in the same way as in the earlier experiments described under a. and b. (Table 18). The evaluation data obtained at the end of the experiment are shown in Figure 7.

As was the case in the experiments a. and b. infestation and gall formation increased with temperature and time for both populations; the interaction was also high due to the fact that hardly any penetration and gall formation was noticed throughout the experimental period for any of the two populations in the 10 °C series. On one of the 4 evaluation times only one plant of the four replicates was found infested by two larvae, namely after 5 weeks in the (V) population and after 6 weeks in the (N) population. On the basis of the earlier experiments this was to be expected for the (V) population, but not for the (N) population. Infestation and gall formation by the (N) population at 10 °C in

TABLE 17. Inoculation of *Meloidogyne incognita* (V) and (N) onto tomato in climatic cells. Number of nematode-inhabited root galls 2, 4, 5 and 6 weeks after inoculation with three egg masses per tube of 300 ml with one plant, at 5 different temperatures. Each figure is the total number of the nematode-inhabited root galls in four replicate plants (x) plus one and transformed to the natural logarithm, according to the formula $^e\log(x + 1)$.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), * = significant ($0.01 < P \leq 0.05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non-significant ($P > 0.10$).

Climatic cell temperatures / Evaluation time	*M. incognita* (V) 10°	15°	20°	25°	30 °C	Time averages (L.S.D. = 0.68)
After 2 weeks	0	4.0	4.6	4.5	4.6	3.6
After 4 weeks	0	5.3	4.9	4.9	4.4	3.9
After 5 weeks	1.1	5.0	5.0	5.9	6.3	4.7
After 6 weeks	0	6.9	6.1	6.5	6.5	5.2
Temperatures averages (L.S.D. = 0.74)	0.3	5.3	5.2	5.5	5.4	

Climatic cell temperatures / Evaluation time	*M. incognita* (N) 10°	15°	25°	20°	30 ° C	Time averages (L.S.D. = 0.62)
After 2 weeks	0	5.1	5.6	3.8	3.8	3.7
After 4 weeks	0	5.7	5.5	5.3	4.5	4.2
After 5 weeks	0	5.0	4.6	5.5	5.6	4.2
After 6 weeks	1.1	7.0	6.5	6.6	6.3	5.5
Temperature averages (L.S.D. = 0.68)	0.3	5.7	5.6	5.3	5.0	

Analysis of variance:

	P values
M. incognita (V)	
F temperature = 58.25	**
F time = 7.75	**
Interaction = 5.83	**
M. incognita (N)	
F temperature = 72.49	**
F time = 10.35	**
Interaction = 6.13	**

TABLE 18. Inoculation of *Meloidogyne incognita* (V) and (N) onto tomato in climatic cells. Free larvae in the soil 2, 4, 5 and 6 weeks after inoculation of three egg masses per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the natural logarithm $^e\log(x+1)$ of the sum of the number of larvae in four replicate tubes (x) plus one.
L.S.D. = least significance difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leqslant 0.01$), * = significant ($0.01 < P \leqslant 0.05$), (*) = nearly significant ($0.05 < P \leqslant 0.10$), – = non-significant ($P > 0.10$).

Climatic cell temperatures / Evaluation time	*M. incognita* (V) 10°	15°	20°	25°	30 °C	Time averages (L.S.D. = 2.32)
After 2 weeks	4.6	5.2	5.3	5.2	3.7	4.8
After 4 weeks	0.0	5.3	4.8	4.5	0.0	2.9
After 5 weeks	0.0	0.0	0.0	3.0	5.6	1.7
After 6 weeks	2.4	3.7	4.3	3.7	6.1	4.0
Temperature averages (L.S.D. = 2.54)	1.8	3.6	3.6	4.1	3.9	

Climatic cell temperatures / Evaluation time	*M. incognita* (N) 10°	15°	20°	25°	30 °C	Time averages (L.S.D. = 1.63)
After 2 weeks	5.5	5.5	5.8	4.6	0.0	4.3
After 4 weeks	4.6	5.1	4.1	4.4	3.0	4.3
After 5 weeks	6.0	5.3	3.0	3.0	6.2	4.7
After 6 weeks	5.3	3.7	4.1	4.6	6.4	4.8
Temperature averages (L.S.D. = 1.79)	5.4	4.9	4.3	4.2	3.9	

Analysis of variance:

		P values
M. incognita (V)		
F temperature	= 0.84	–
F time	= 2.16	–
Interaction	= 0.01	–
M. incognita (N)		
F temperature	= 0.48	–
F time	= 0.15	–
Interaction	= 0.40	–

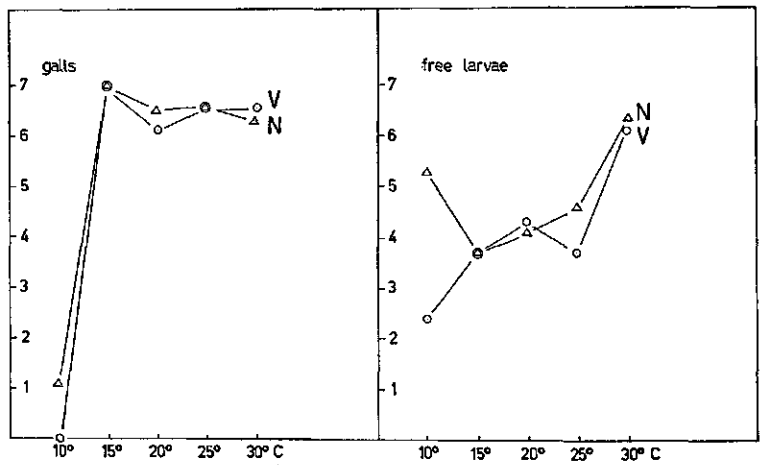

FIG. 7. Comparison of the (V) and (N) populations of *Meloidogyne incognita* with respect to infestation and with respect to free larvae in the soil, 6 weeks after inoculation of 3 egg masses to tomato growing in 300 ml tubes in climatic cells (cf. Tables 17 and 18).
Ordinate: lefthand graph: natural logarithm $^e\log(x + 1)$ of the total number of nematode-inhabited galls of four replicate plants (x) plus one; righthand graph: of the total number of free larvae in four tubes (x) plus one.
Abscissa: temperatures in the various climatic cells.

the greenhouse could have occurred there owing to temperature fluctuations but in the climatic cells the temperature is rigidly maintained at 10 °C. The minimum temperature for the (N) population probably lies somewhere between 10° and 15 °C and is at any rate lower than for the (V) population.

At all temperatures of 15 °C and higher, penetration and gall formation in both populations were generally visible already after two weeks. Penetration and galling increased consistently with time. There was, however, no difference between 15 °C and higher temperatures in this experiment. This may be due to the fact that all plants were kept at 25 °C for three days before being exposed to different temperatures. This result suggests that the temperature at the moment of penetration is determinant for the degree of gall formation and possibly also for nematode development within a wide temperature range.

The numbers of free larvae in the soil (Table 18) are more erratic in this experiment than in the greenhouse and Wisconsin tanks experiments. Statistical analysis of the data of Table 18 indicates no significance for temperature effect, time effect or interaction. This may partly be due to variability in the results, but there is also another cause. The free larvae in the soil lump survivors of the inoculum and newly-formed larvae of the new generations, and this obscures the real relations in this experiment. The number of inoculated larvae was 3 egg masses per tube, against one in the greenhouse and Wisconsin tank experiments, and the first evaluation date was after 2 weeks against 4 weeks in the other experiments. Survivors of the inoculum were noticed at 15 °C after 4 weeks in the

(N) population of the earlier experiments. It may therefore be assumed that the larvae found here after 2 weeks at perhaps all temperatures and after 4 weeks at the lower temperatures are survivors of the inoculum. Most if not all of the larvae found in the higher temperature range after 4 weeks, and certainly after 6 weeks, must be newly formed larvae. Despite the non-significance of temperature and time effects as a whole the data suggest that at 10 °C the (N) population shows better survival of inoculated and perhaps already penetrated larvae, and also earlier and stronger reproduction than the (V) population. In the range of 15 °C and higher there is again little influence of temperature which in the case of root galling may be explained by the fact that infestation started at 25 °C for all series and maintained its level at all temperatures from 15 ° to 30 °C.

Figure 7 suggests that at the end of the experiment, i.e. after 6 weeks, the number of root galls was low at 10 °C and consistently high at all other temperatures, and that the number of free larvae in the soil was lower at 10 °C than at the other temperatures for the (V) population but not for the (N) population.

6.2.3. *Morphology*

The difference in temperature requirements between the (V) and (N) populations of *M. incognita* (cf. 6.2.2) raises the question whether the populations are morphologically identical.

Characters of the posterior perineal pattern of adult *Meloidogyne* females are generally used for identification of species (CHITWOOD 1949, DROPKIN 1953, SASSER 1954). The pattern is characteristic and stable, although difficult to describe. It is evidently under control of heredity (DROPKIN 1953) although early experiments by Allen (1952) and recent works by TRIANTAPHYLLOU (1963) and NETSCHER (1965) suggest that some of the present 'species' are possible polyploids of one species. The characters of the perineal pattern of the (V) and (N) populations used here were studied in details.

M. incognita (V) and (N) grown from single egg masses on tomato for three months in the greenhouse at 20 °–25 °C were available. Females from each population were removed directly into water without fixing them in lactophenol. The posterior part of the female was dissected and the body contents were removed by gentle pressure which left the cuticle free of odd parts. Small portions of the cuticle were removed from the perineal patterns in water which were then stained in cotton blue lactophenol. 25 perineal patterns of each population were prepared. Measurements were taken according to SANTOS (1968), plus an extra measurement of the distance between the striae (cf. Figure 8). Three striae per specimen, therefore 75 per population, were taken at the right top side of the perineal pattern.

The results of these measurements are summarized in Table 19. They were statistically treated in the computer and t-values were calculated. It appears that the two *M. incognita* populations, (V) and (N) do not show significant differences in any of the 6 characters measured, and that for this reason they have to be considered conspecific on the basis of their morphology.

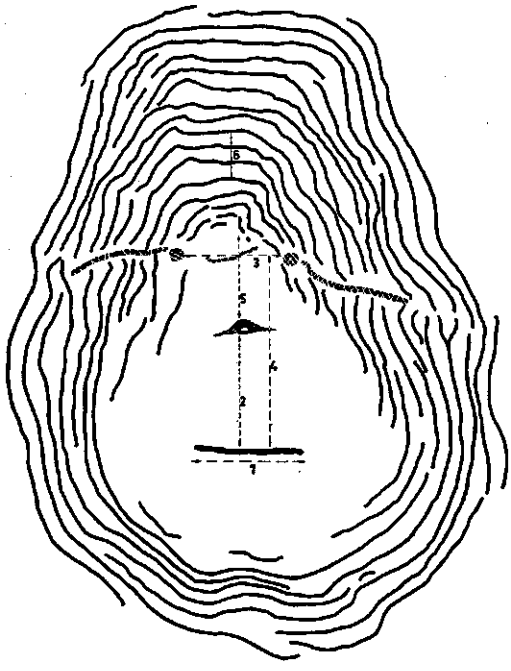

FIG. 8. Perineal pattern of *Meloidogyne incognita* with indication of the different measurements used on the statistical analysis; cf. Table 19.

TABLE 19. Measurements of perineal patterns of *Meloidogyne incognita* from Venezuela (V) and the Netherlands (N) expressed as ocular scale units (1 unit = 1.25 u). Averages of 25 patterns. The number of the characters used correspond with the indications in Figure 8.

Characters measured	Populations		t values (- = non significant)
	(V)	(N)	
1. Vulva width	22.1	22.9	0.98 –
2. Distance from anus to vulva	18.1	18.0	1.19 –
3. Distance between phasmids	24.9	24.6	0.32 –
4. Distance from vulva to imaginary line between phasmids	28.2	27.2	1.05 –
5. Distance from anus to tail terminus	14.9	15.8	1.37 –
6. Distance between striae	6.2	5.7	1.74 –

6.2.4. *Discussion*

Figures 3–7 and Tables 12–18 leave no doubt that the *M. incognita* populations from the Venezuelan tropical plains and from greenhouses in the Netherlands are both thermophil, in accordance with the common name of this nematode in the USA southern root-knot nematode. Hatching, gall formation and reproduction were abundant and rapid at the temperatures 20°–25°–30°C. They are distinctly less at 15°C (except when penetration had already taken place at a higher temperature, cf. Table 17), and they drop to a low value at

10 °C. Exposure of eggs to 5 °C for 15 days is evidently noxious although some of the eggs survive (Table 12). The same holds for the temperature of 35 °C. This temperature is too high for the nematode in water, but it is remarkable that galling and reproduction on tomato were not less than at lower favourable temperatures (Tables 15 and 16). This may be explained by the fact that growing plants have a lower temperature than the surrounding soil at high soil temperatures (cf. also REYNOLD & O'BANNON 1963).

Despite the fact that the (V) and (N) populations were morphologically indentical (Fig. 8, Table 19) and that the gross temperature requirements were much the same, there appear to be physiological differences which may be significant for the geographic distribution of the nematodes in different climatic zones.

All experimental data indicate that the (N) population was somewhat more thermophob and markedly less cryophob, than the (V) population. The (N) population hatched and survived in a somewhat lower degree in water at 35 °C and 40 °C (Fig. 3 and 4, Table 12). However, the main difference between the two populations shows up at low temperatures in the degree of plant infestation and nematode reproduction (Tables 13–18, Fig. 5–7). Penetration, gall formation and reproduction of the (N) population is markedly better than of the (V) population at 15 °C (again, unless penetration had already taken place at a higher temperature). At 10 °C (± 1 °C) the difference is very great since infestation and reproduction were practically nil in the (V) population, and considerable in the (N) population (Tables 13 and 14, Fig. 5). At exactly 10 °C, however, the (N) population was also inactivated (Tables 17 and 18, Fig. 7). All data together indicate that the minimum temperature for the (N) population to start infestation and reproduction is about 5 °C lower than for the (V) population, and also that the larvae of the (N) population inoculated in soil may survive at 15 °C for at least 4 weeks whereas the (V) population does not survive that long.

It therefore appears that there are strains with different temperature requirements within *M. incognita* which could be indicated as thermotypes. These differences are fixed in that they did not disappear when the nematodes were propagated on the same plant, in the same environment, for more than a year. It is not known whether these characters are genetically determined.

6.3. MELOIDOGYNE HAPLA (V) and (N)

Temperature range experiments comparable to those described for *M. incognita*, were carried out with the (V) and (N) populations of *M. hapla* and one additional experiment was conducted to test lethality at low temperature. The experiments included one on hatching (6.3.1), three on penetration and development, root galling and reproduction (6.3.2a, b, c), one on survival at low temperature (6.3.3) and one on morphology (6.3.4). They are followed by a final discussion of the species (6.3.5).

6.3.1. *Hatching*

Breeding, manipulation, incubation and evaluation of the populations (V) and (N) of *M. hapla* took place in exactly the same way with the same intervals and at the same temperatures, as in the case of *M. incognita*. The data are summarized in Figures 9 and 10, and in Table 20.

The Figures 9 and 10 indicate that hatching in both populations was strong between 20° and 30°C, with an optimum at 25°C. The (N) population seems to have a little wider range than the (V) population; some hatching occurred at 5°C and at 35°C and the (N) population hatched somewhat better at 30°C than the (V) population.

It appears from Figure 10 that hatching started in both populations between 3 and 6 days and that it was initially much stronger at 25° and 30°C than at the other temperatures. Most of the eggs were hatched after 9 days for the (V) population, but only after 12 days for the (N) population. The graphs of 30°C fell below the graph of 20°C in the (V) population between the 6th and the 9th

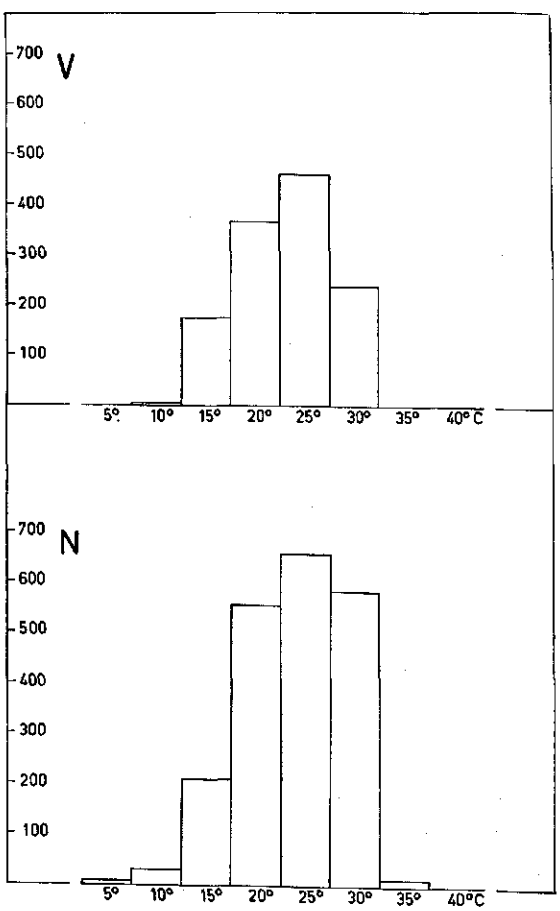

FIG. 9. Hatching thermograms of *Meloidogyne hapla* from Venezuela (V) and the Netherlands (N) after 15 days.
Ordinate: final number of hatched larvae per egg mass; average of four single egg masses.
Abscissa: temperatures in the various thermostat compartments (5° to 40°C).

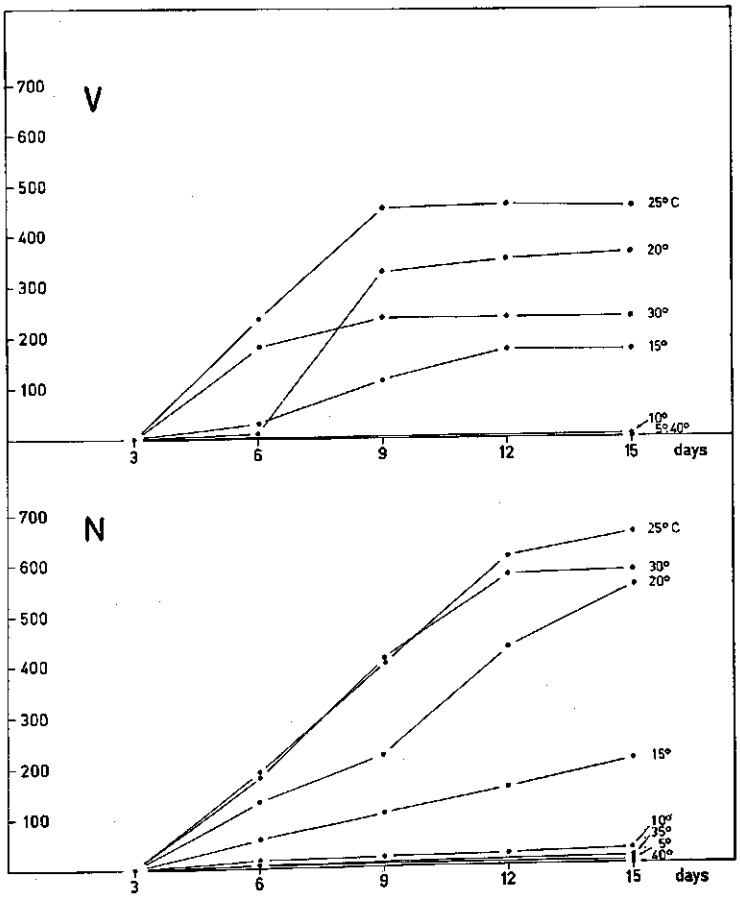

FIG. 10. Rate of hatching of larvae from egg masses of *Meloidogyne hapla* from Venezuela (V) and from the Netherlands (N) at eight different temperatures with time.
Ordinate: number of hatched larvae per egg mass; average of four single egg masses.
Abscissa: duration of exposure to distilled water at different temperatures from 5° to 40 °C as indicated in the graphs.

day, whereas the 30 °C graph stayed above the 20 °C graph in the (N) population.

Table 20 indicates that none of the two populations suffered much from the 15 days treatment at 5°; 35° and 40 °C, however, were lethal for both.

6.3.2. *Penetration and development, root galling, reproduction*

a. Experiments in greenhouse compartments

An inoculation experiment with the (V) and (N) populations of *M. hapla* comparable to the experiment described under 6.2.2a, was started in greenhouse compartments on 9th March 1967 for *M. hapla* (N) and on 10th July 1968 for *M. hapla* (V). The results are described and summarized in Tables 21 and 22, and in Figure 11.

TABLE 20. Number of hatched larvae per four egg masses of *Meloidogyne hapla* from Venezuela (V) and the Netherlands (N) after 15 days in water at different temperatures and after a further 15 days in water at 25 °C.

Temperatures °C	*M. hapla* (V)		*M. hapla* (N)	
	After 15 days at the temperatures indicated	After an additional 15 days at 25 °C	After 15 days at the temperatures indicated	After an additional 15 days at 25 °C
5	0	1251	10	1597
10	17	947	121	1202
15	705	1609	829	1098
20	1482	1707	2210	2290
25	1826	1951	2644	2644
30	970	970	2348	2348
35	0	0	22	22
40	0	0	7	7

Tables 21 and 22 show that root galling as well as free larvae in the soil increased highly significant in both populations according to a linear trend. Interaction between temperature and time was significant for free larvae in the soil. This is due to the fact that only at low temperatures, 10 °C for the (V) and (N) and 15 °C for the (V) population, did the initial density present after 5–6 weeks first decrease before rising again, thus indicating good survival and retarded reproduction at 10 ° and 15 °C.

Galls with developing females were not found at 10 °C before the 8th week in the (V) and before the 10th week in the (N) population; later on slight galling was caused by both populations at these temperatures. At 15 °C and higher galls were visible from the 4th week on. The gall indexes 4–6 weeks after inoculation as well as the average gall indexes indicate, that the favourable temperatures for the (V) population are 20 °–30 °C and for the (N) population 25 °–30 °C (Table 21). These data are confirmed by the numbers of free larvae in the soil (Table 22). Despite the fact that in the first 6 weeks inoculated specimens obscure the evaluation of newly formed larvae, the average numbers per temperature suggest again, that the optimum for the (V) population is somewhat lower (20 °–25 °C) than for the (N) population (25 °–30 °C).

The difference between the populations can also be seen from Fig. 11 which summarizes the situation after 12 weeks at the end of the experiment. The (V) population causes more galling and more nematode reproduction at the lower temperatures, but it was surpassed by the (N) population at the higher temperatures. It should be noted that the experiment with the (V) and (N) populations of *M. hapla* were not made simultaneously, and comparison is therefore less safe than in the case of the two *M. incognita* populations.

b. Experiments in Wisconsin tanks

Parallel to the experiment described under 6.2.2b similar inoculations with the (V) and (N) populations of *M. hapla* were made in the Wisconsin tanks. The results are summarized in Tables 23 and 24 and in Fig. 12.

TABLE 21. Inoculation of *Meloidogyne hapla* (V) and (N) onto tomato in greenhouse compartments. Root gall index (0 = no galling, 4 = 100% of the root system transformed to galls; cf. 2.5.2.) 4, 6, 8, 10 and 12 weeks after inoculation of one egg mass of about 600 eggs per tube of 300 ml with one plant, at 5 different temperatures. Each figure is the root knot index per plant (x) plus one and transformed to the natural logarithm, according to the formula $^e\log(x + 1)$, average of two replicates. L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), x = significant ($0.01 < P \leq 0,05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non-significant ($P > 0.10$).

Greenhouse temperatures / Evaluation time	*M. hapla* (V). Experiment started at 10.7.1968					
	10°	15°	20°	25°	28–30°C	Time averages (L.S.D. = 0.45)
After 4 weeks	0.00	0.35	0.69	1.10	0.90	0.61
After 6 weeks	0.00	0.55	0.90	0.90	1.24	0.72
After 8 weeks	0.69	1.10	1.39	0.90	1.50	1.11
After 10 weeks	0.35	1.10	1.04	1.39	1.61	1.10
After 12 weeks	0.69	1.10	1.39	1.50	1.50	1.23
Temperature averages (L.S.D. = 0.45)	0.35	0.84	1.08	1.15	1.35	

Greenhouse temperatures / Evaluation time	*M. hapla* (N). Experiment started at 9.3. 1967					
	10°	15°	20°	25°	28–30°C	Time averages (L.S.D. = 0.46)
After 4 weeks	0.00	0.35	0.00	0.69	1.10	0.43
After 6 weeks	0.00	0.35	0.90	0.90	1.24	0.68
After 8 weeks	0.00	0.69	0.69	1.39	1.39	0.83
After 10 weeks	0.35	0.90	0.35	1.50	1.24	0.87
After 12 weeks	0.35	0.69	1.04	1.50	1.61	1.04
Temperature averages (L.S.D. = 0.46)	0.14	0.60	0.60	1.19	1.32	

Analysis of variance:

	P values
M. hapla (V)	
F temperature = 21.00	**
F time = 10.65	**
Interaction = 0.90	–
M. hapla (N)	
F temperature = 32.19	**
F time = 7.22	**
Interaction = 1.29	–

TABLE 22. Inoculation of *Meloidogyne hapla* (V) and (N) onto tomato in greenhouse compartments. Free larvae in the soil 4, 6, 8, 10 and 12 weeks after inoculation of one egg mass of about 600 eggs per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the natural logarithm $^e\log(x+1)$ of the number of larvae per tube (x) plus one; average of two replicates.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), x = significant ($0.01 < P \leq 0.05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non-significant ($P > 0.10$).

Greenhouse temperatures / Evaluation time	*M. hapla* (V). Experiment started at 10.7.1968					
	10°	15°	20°	25°	28–30°C	Time averages (L.S.D. = 0.33)
After 4 weeks	3.24	3.24	3.43	3.04	4.30	3.45
After 6 weeks	1.72	3.04	3.38	7.24	8.81	4.84
After 8 weeks	1.20	1.72	9.74	5.48	7.46	5.12
After 10 weeks	1.52	4.79	3.66	10.19	10.02	6.04
After 12 weeks	3.91	7.49	8.33	8.32	6.46	6.90
Temperature averages (L.S.D. = 0,33)	2.32	4.06	5.71	6.85	7.41	

Greenhouse temperatures / Evaluation time	*M. hapla* (N). Experiment started at 9.3.1967					
	10°	15°	20°	25°	28–30°C	Time averages (L.S.D. = 3.02)
After 4 weeks	0.00	4.23	1.20	1.97	1.86	1.85
After 6 weeks	5.70	4.89	3.91	5.99	9.02	5.90
After 8 weeks	0.00	5.16	1.52	10.19	8.17	5.01
After 10 weeks	4.25	2.78	5.92	10.61	10.74	6.86
After 12 weeks	3.39	4.05	9.83	10.63	11.34	7.85
Temperature averages (L.S.D. = 3.02)	2.67	4.22	4.48	7.88	8.22	

Analysis of variance:

	P values
M. hapla (V)	
F temperature = 11.76	**
F time = 4.56	**
Interaction = 2.64	*
M. hapla (N)	
F temperature = 18.94	**
F time = 16.81	**
Interaction = 3.62	*

The increase of galling and of free larvae in the soil with time was again highly significant for both populations; the increase with temperature was less significant for the (V) than for the (N) population. Both populations infested and reproduced at all temperatures from 15° to 35°C. As was the case in the experiment under a., galling and reproduction for the (V) population are absolutely and relatively higher at 15° and lower at 35°C than for the (N) popula-

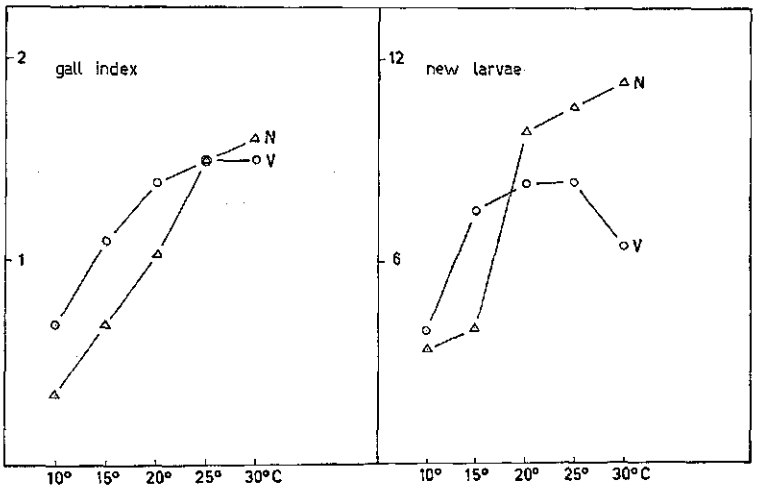

FIG. 11. Comparison of the (V) and (N) populations of *Meloidogyne hapla* with respect to penetration and root gall formation and with respect to free larvae in the soil, 12 weeks after inoculation to tomato growing in 300 ml tubes in the greenhouse experiments (cf. tables 21 and 22).
Ordinate: lefthand graph: natural logarithm °log (x + 1) of root gall index per plant (x) plus one, righthand graph: of number of free larvae per tube (x) plus one, in both cases average of two replicates.
Abscissa: temperatures in the various greenhouse compartments.

tion (Tables 23 and 24). This causes the significant increase with temperature according to a linear trend found for the (N) population. The same is also illustrated in Fig. 12, which reflects the situation at the end of the experiment. The (V) population from the Venezuelan mountains is apparently less thermophil than the population from the Netherlands.

Survival of the inoculated larvae, also at 15 °C, seemed to be less marked than in the former experiment and the same was true for gall formation at 15 °C after 4 weeks.

It was interesting to note that the (V) population of *M. hapla* comprised males in addition to the free larvae in the soil at 35 °C only, namely 5% after 4 weeks, 22% after 8 weeks and 20% after 10 weeks. No males were found at the lower temperatures in the (V) population, nor at any temperature in the (N) population.

The fact that 35 °C, which was unfavourable for hatching in water (cf. 6.3.1), obtained the highest scores for gall formation and nematode reproduction on plants grown in Wisconsin tanks, earlier may be due to lower temperatures in the roots of the plants than in the surrounding soil as was suggested previously for *M. incognita*.

TABLE 23. Inoculation of *Meloidogyne hapla* (V) and (N) onto tomato in Wisconsin tanks. Root gall index (0 = no galling, 4 = 100% of the root system transformed to galls; cf. 2.5.2) 4, 6, 8, 10 and 12 weeks after inoculation of one egg mass per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the root knot index per plant (x) plus one, and tranformed to the natural logarithm, according to the formula $^{e}\log(x+1)$; average of two replicates.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leqslant 0.01$), * = significant ($0.01 < P \leqslant 0.05$), (*) = nearly significant ($0.05 < P \leqslant 0.10$), – = non-significant ($P > 0.10$).

Wisconsin tanks temperatures / Evaluation time	*M. hapla* (V) 15°C	20°C	25°C	30° C	35°C	Time averages (L.S.D. = 0.63)
After 4 weeks	0.00	0.00	0.69	1.10	0.90	0.54
After 6 weeks	0.55	0.35	0.35	0.90	1.50	0.73
After 8 weeks	0.69	1.04	0.69	0.69	0.69	0.76
After 10 weeks	0.90	0.69	0.55	0.35	1.04	0.70
After 12 weeks	1.10	0.90	1.04	0.35	1.04	0.88
Temperature averages (L.S.D. = 0.63)	0.65	0.60	0.66	0.68	1.03	

Wisconsin tanks temperatures / Evaluation time	*M. hapla* (N) 15°C	20°C	25°C	30°C	35°C	Time averages (L.S.D. = 0.53)
After 4 weeks	0.00	0.69	0.69	0.00	0.69	0.42
After 6 weeks	0.69	0.69	0.90	0.69	0.69	0.73
After 8 weeks	0.90	0.90	0.69	1.24	1.50	1.05
After 10 weeks	0.69	0.55	1.10	0.55	1.61	0.90
After 12 weeks	0.69	1.10	1.39	1.61	1.61	1.28
Temperature averages (L.S.D. = 0.53)	0.60	0.79	0.95	0.82	1.22	

Analysis of variance:

		P values
M. hapla (V)		
F temperature	= 1.68	(*)
F time	= 10.42	**
Interaction	= 1.44	–
M. hapla (N)		
F temperature	= 30.94	**
F time	= 51.80	**
Interaction	= 2.65	*

TABLE 24. Inoculation of *Meloidogyne hapla* (V) and (N) onto tomato in Wisconsin tanks. Free larvae in the soil 4, 6, 8, 10 and 12 weeks after inoculation of one egg mass per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the natural logarithm °log (x + 1) of the number of larvae per tube (x) plus one; average of two replicates.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), * = significant ($0.01 < P \leq 0.05$), (x) = nearly significant ($0.05 < P \leq 0.10$), – = non-significant ($P > 0.10$).

Wisconsin tanks temperatuers / Evaluation time	*M. hapla* (V) 15°	20°	25°	30°	35 °C	Time averages (L.S.D. = 3.00)
After 4 weeks	0.35	0.69	0.00	0.80	1.52	0.67
After 6 weeks	1.52	0.00	2.61	4.14	6.35	2.92
After 8 weeks	3.57	5.83	4.23	2.59	3.53	3.95
After 10 weeks	4.95	4.40	1.72	2.01	5.37	3.69
After 12 weeks	6.67	4.32	6.03	4.85	6.29	5.63
Temperature averages (L.S.D. = 3.00)	3.41	3.05	2.92	2.88	4.61	

Wisconsin tanks temperatures / Evaluation time	*M. hapla* (N) 15°	20°	25°	30°	35 °C	Time averages (L.S.D. = 2.12)
After 4 weeks	0.00	0.00	0.00	0.00	2.31	0.46
After 6 weeks	1.52	2.06	4.77	3.04	8.29	3.94
After 8 weeks	4.92	3.88	4.44	5.42	11.80	6.09
After 10 weeks	6.57	5.26	6.86	4.28	9.58	6.51
After 12 weeks	4.11	5.08	7.85	9.57	11.10	7.54
Temperature averages (L.S.D. = 2.12)	3.42	3.25	4.78	4.46	8.61	

Analysis of variance:

	P values
M. hapla (V)	
F temperature = 1.68	*
F time = 10.42	**
Interaction = 1.42	–
M. hapla (N)	
F temperature = 30.94	**
F time = 51.80	**
Interaction = 2.65	*

c. Experiments in climatic cells

Using the same procedure as described under 6.2.2c experiments with *M. hapla* (V) and (N) were made in climatic cells. After keeping the inoculated plants for three days at 25 °C they were placed in the temperature cells on 26th December 1968. Tables 25 and 26 and Figure 13 summarize the results.

TABLE 25. Inoculation of *Meloidogyne hapla* (V) and (N) onto tomato in climatic cells. Number of nematode-inhabited galls 2, 4, 5 and 6 weeks after inoculation with three egg masses per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the total number of the nematode-inhabited root galls in four replicate plants (x) plus one and transformed to the natural logarithm, according to the formula $^{e}\log(x+1)$.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leqslant 0.01$), * = significant ($0.01 < P \leqslant 0.05$), (*) = nearly significant ($0.05 < P \leqslant 0.10$), – = non-significant ($P > 0.10$).

Climatic cell temperatures / Evaluation time	*M. hapla* (V) 10°	15°	20°	25°	30°	Time averages (L.S.D. = 0.86)
After 2 weeks	0	2.8	4.8	5.2	4.1	3.4
After 4 weeks	0	5.1	5.2	5.6	4.0	4.0
After 5 weeks	0	5.2	4.9	4.9	5.3	4.1
After 6 weeks	3.7	6.6	6.1	6.5	6.4	5.9
Temperatures averages (L.S.D. = 0.94)	0.9	4.9	5.2	5.5	4.9	

Climatic cell temperatures / Evaluation time	*M. hapla* (N) 10°	15°	20°	25°	30 °C	Time averages (L.S.D. = 1.10)
After 2 weeks	0	4.1	5.0	2.4	3.6	3.0
After 4 weeks	0	5.7	4.6	3.3	3.1	3.3
After 5 weeks	0	5.3	1.6	2.1	1.8	2.2
After 6 weeks	4.4	6.5	6.2	6.4	6.2	5.9
Temperature averages (L.S.D. = 1.20)	1.1	5.4	4.4	3.5	3.7	

Analysis of variance:

		P values
M. hapla (V)		
F temperature	= 25.85	**
F time	= 10.24	**
Interaction	= 3.09	*
M. hapla (N)		
F temperature	= 10.86	**
F time	= 14.25	**
Interaction	= 1.01	–

TABLE 26. Inoculation of *Meloidogyne hapla* (V) and (N) onto tomato in climatic cells. Free larvae in the soil 2, 4, 5 and 6 weeks after inoculation with three egg masses per tube of 300 ml of soil with one plant, at 5 different temperatures. Each figure is the natural logarithm $^e\log(x+1)$ of the sum of the number of larvae in four replicate tubes (x) plus one.
L.S.D. = least significance difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leqslant 0.01$), * = significant ($0.01 < P \leqslant 0.05$), (*) = nearly significant ($0.05 < P \leqslant 0.10$), – = non-significant ($P > 0.10$).

Climatic cell temperatures / Evaluation time	*M. hapla* (V) 10°	15°	20°	25°	30 °C	Time averages (L.S.D. = 2.13)
After 2 weeks	4.8	4.1	5.1	0	3.0	3.4
After 4 weeks	5.5	0	3.0	5.1	0	2.7
After 5 weeks	5.4	4.6	3.0	0	6.0	3.8
After 6 weeks	5.8	0	3.7	0	6.5	3.2
Temperature averages (L.S.D. = 2.33)	5.4	2.2	3.7	1.3	3.9	

Climatic cell temperatures / Evaluation time	*M. hapla* (N) 10°	15°	20°	25°	30 °C	Time averages (L.S.D. = 1.78)
After 2 weeks	5.7	5.4	6.6	0	0	3.5
After 4 weeks	5.7	5.4	3.0	0	4.9	3.8
After 5 weeks	5.5	3.0	3.7	5.6	3.7	4.3
After 6 weeks	4.9	5.4	5.6	3.7	4.4	4.8
Temperature averages (L.S.D. = 1.95)	5.5	4.8	4.7	2.3	3.3	

Analysis of Variance:

		P values
M. hapla (V)		
F temperature	= 1.862	–
F time	= 0.192	–
Interaction	= 0.246	–
M. hapla (N)		
F temperature	= 1.733	–
F time	= 0.411	–
Interaction	= 0.236	–

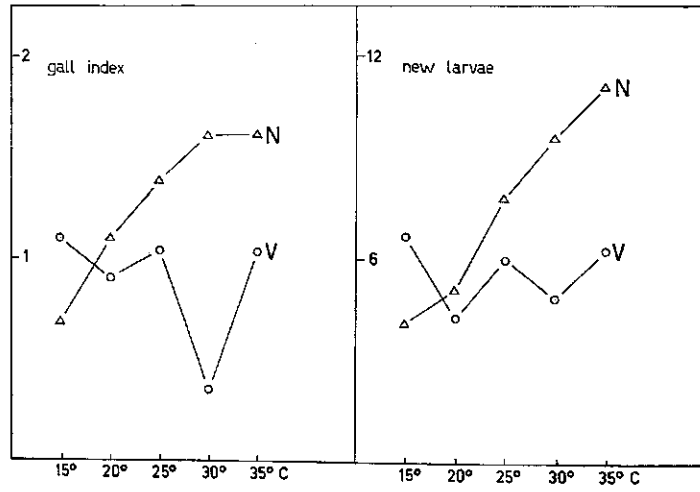

FIG. 12. Comparison of the (V) and (N) populations of *Meloidogyne hapla* with respect to penetration and root gall formation and with respect to free larvae in the soil, 12 weeks after inoculation to tomato growing in 300 ml tubes in the Wisconsin tank experiments (cf. Tables 23 and 24).
Ordinate: lefthand graph: natural logarithm ^{e} log (x + 1) of root gall index per plant (x) plus one, righthand graph: of number of free larvae per tube (x) plus one, in both cases average of two replicates.
Abscissa: temperatures in the various Wisconsin tanks.

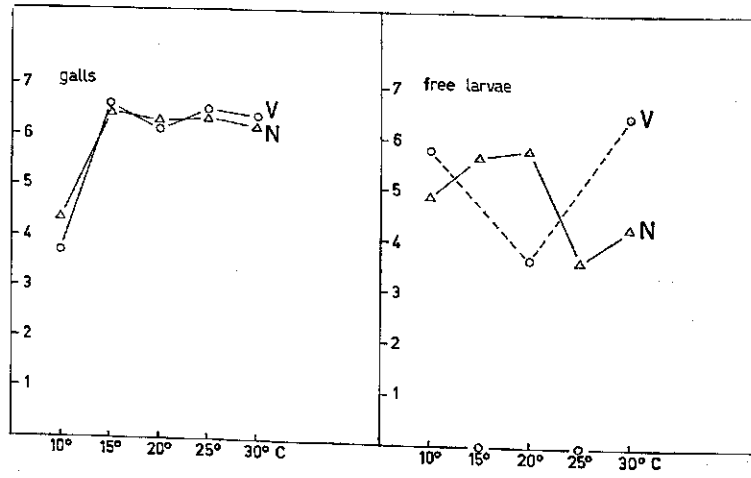

FIG. 13. Comparison of the (V) and (N) populations of *Meloidogyne hapla* with respect to infestation and with respect to free larvae in the soil, 6 weeks after inoculation of 3 egg masses to tomato growing in 300 ml tubes in the climatic cells (cf. Tables 25 and 26).
Ordinate: lefthand graph: natural logarithm ^{e}log (x + 1) of the total number of nematode-inhabited galls of four replicate plants (x) plus one, righthand graph: of the total number of free larvae in four tubes (x) plus one.
Abscissa: temperatures in the various climatic cells.

Although all plants were kept at 25 °C for 3 days after inoculation and more replicates were available, the results of the climatic cell experiments were more variable than those of the greenhouse and Wisconsin tank experiments. This was also noticed under 6.2.2c. It is possibly due to erratic extraction of the nematodes in the climatic cell experiments, for zero counts were found for tubes with galled plants. Despite this variability some conclusions can be drawn.

As in the experiments with *M. incognita* gall formation was weak and delayed at 10 °C for the (V) and the (N) populations of *M. hapla*. At all higher temperatures penetration and galling was generally present in both populations after 2 weeks; it increased with time but there was no difference between the temperatures from 15 °C upwards. The homogeneous infection may be caused in the pre-treatment method of 3 days when all plants were kept at 25 °C. Free larvae were present right from the start and their numbers did not increase with temperature and time. This is explained, as for the corresponding experiments with *M. incognita*, by the presence of surviving inoculated larvae during the first weeks and of newly formed larvae at later evaluation dates.

The situation at the end of the experiment, after 6 weeks, is summarized in Figure 13, that illustrates that root galling was equal at all temperatures, except at 10 °C at which temperature it was lower. The number of free larvae, comprising surviving inoculum and new-generation larvae, was about equal over the entire range of 10° to 30 °C for the (N) population and probably also for the (V) population (the zero counts at 15° and 25 °C must be due to erratic extraction and are omitted in the graph; the plants were infested, and they must have been surrounded by free larvae both on the earlier evaluation dates and at the lower and higher temperatures. Population and temperature may have had little influence in this experiment because a uniform infection was established at 25 °C in the pre-treatment period of three days. This suggests a determinant significance of larval penetration considering the degree of damage and reproduction (cf. also 6.2.2c).

6.3.3. *Survival at low temperature*

The (V) and (N) populations of *M. hapla* were inoculated in soil and exposed to low temperatures for different periods and their survival was tested in a bioassay.

Egg masses, free eggs and active larvae were collected from the stock population on tomato roots originally infected with a single egg mass. Egg masses were picked from the roots. Free eggs were obtained by tearing egg masses into pieces in a 1:10 Chlorix solution in order to dissolve the matrix and thus to release the eggs which were then washed repeatedly with water to remove the Chlorix. Larvae were obtained by placing egg masses on moist filter paper in a petri dish at 25 °C so that hatching occurred. The three types of inoculum material were inoculated into plastic tubes each containing 300 ml of a mixture of riversand and sterilized garden soil. Per tube 1 egg mass, or 400 eggs, or 400 larvae were inoculated. For each nematode population there were 60 tubes

containing each type of inoculum; 12 tubes were placed at each of the following five temperatures –10°, –5°, 0°, 4° and 10°C; 4 replicate tubes were taken from these temperatures after 3, 7 and 10 days and transferred to a greenhouse cabinet of 25°C. All tubes were subsequently planted simultaneously with a tomato seedling in the four-leaf stage. One month later the plants were taken from the the tubes, washed free from soil, fixed in boiling cotton blue lactophenol and evaluated for galling. All the galls were counted, including small ones with only one or two female nematodes. The results are summarized in Tables 27 and 28.

TABLE 27. Survival of *M. hapla* (V) and (N) at different low temperatures after different exposure periods, according to the number of nematode-inhabited galls, due to subsequent inoculation of the nematodes to tubes of 300 ml of soil with one tomato plant. Each figure is the total number of nematode-inhabited galls in four replicate plants (x) plus one and transformed to the natural logarithm which is divided by 3, according to the formula $1/3^{e}\log(x+1)$.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), * = significant ($0.01 < P \leq 0.05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non-significant ($P > 0.10$).

Exposure times \ Treatment temperatures	*M. hapla* (V)					
	–10°	–5°	0°	4°	10°	Time averages (L.S.D. = 0.51)
3 days	0.6	1.6	4.4	4.9	4.9	3.3
7 days	0.0	1.5	5.4	5.0	4.9	3.4
10 days	0.0	1.1	5.2	5.2	5.5	3.4
Temperature averages (L.S.D. = 0.66)	0.2	1.4	5.0	5.0	5.1	

Exposure times \ Treatment temperatures	*M. hapla* (N)					
	–10°	–5°	0°	4°	10°	Time averages (L.S.D. = 0.66)
3 days	1.8	3.2	4.2	3.6	4.2	3.4
7 days	0.5	2.9	3.7	4.0	4.1	3.1
10 days	1.0	2.7	3.3	4.1	4.6	3.2
Temperature averages (L.S.D. = 0.86)	1.1	3.0	3.7	3.9	4.3	

Analysis of variance:

		P values
M. hapla (V)		
F temperature	= 116.26	**
F time	= 0.12	–
Interaction	= 1.03	–
M. hapla (N)		
F temperature	= 19.46	**
F time	= 0.61	–
Interaction	= 1.38	–

TABLE 28. Survival of egg masses, free eggs and free larvae of *M. hapla* (V) and (N) at different low temperatures according to the number of nematode-inhabited galls due to subsequent inoculation of the nematodes to tubes of 300 ml of soil with one tomato plant. Each figure is the total number of nematode-inhabited galls in four replicate plants (x) plus one and transformed to the natural logarithm which is divided by 3, according to the formula $1/3 * \log(x + 1)$.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), * = significant ($0.01 < P \leq 0.05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non-significant ($P > 0.10$).

Types of inoculum \ Treatment temperatures	*M. hapla* (V)					
	–10°	–5°	0°	4°	10°	Inoculum averages (L.S.D. = 0.51)
Egg mass	0.0	2.5	5.4	5.2	5.0	3.3
Free eggs	0.6	0.0	4.1	4.2	4.7	3.4
Free larvae	0.0	1.7	5.6	5.7	5.6	3.4
Temperature averages (L.S.D. = 0.66)	0.2	1.4	5.0	5.0	5.1	

Types of inoculum \ Treatment temperatures	*M. hapla* (N)					
	–10°	–5°	0°	4°	10°	Inoculum averages (L.S.D. = 0.66)
Egg mass	0.3	3.5	3.5	4.3	4.9	3.4
Free eggs	0.9	2.3	3.7	3.2	3.7	3.1
Free larvae	2.2	3.0	4.1	4.3	4.2	3.2
Temperature averages (L.S.D. = 0.86)	1.1	3.0	3.7	3.9	4.3	

Analysis of variance:

	P values
M. hapla (V)	
F temperature = 116.23	**
F inoculum = 10.21	**
Interaction = 3.07	*
M. hapla (N)	
F temperature = 19.46	**
F inoculum = 3.38	*
Interaction = 1.38	–

Tables 27 and 28 summarize the data independent of type of inoculum and of exposure time respectively. The statistical treatment of the results, however, covered all data. The number of nematode-inhabited galls on the test plants, and therefore of surviving nematodes, in the treated soils was significantly influenced by temperature and type of inoculum, but not by exposure time within the periods applied in the experiment. The survival, based on the galling effect of the population, was reduced considerably at –10 °C, perceptibly at –5 °C, already after 3 days exposure. Three days of exposure reduced survival by

about as much as 7 and 10 days (Table 27). The influence of time at the low temperatures would probably have made itself felt more significantly if very short or very long exposure periods had been applied. It is clear that the nematodes of both populations survived at 0°as well as 4° or 10°C. However, at −5° and −10°C the Venezuelan population survived less well than the (N) population. The (V) population seems to be slightly more susceptible to low temperatures than the (N) population as long as the nematodes are outside the plants, but not when they have penetrated, for this greater susceptibility to cold was only found in the present direct treatment and in the hatching experiments (cf. 6.3.1).

For both populations free eggs, i.e. eggs in an unnatural state, were slightly less infective than eggs in egg masses or free larvae. This was true on an average, and not especially at the low temperatures. The difference between the types of inoculum can therefore not be explained as a temperature effect.

6.3.4. *Morphology*

A comparison of the morphology of the perineal patterns of the (V) and (N) populations of *M. hapla* was made in exactly the same way as *M. incognita* (cf. 6.2.3). The results are summarized in Table 29.

TABLE 29. Measurements of perineal patterns of *M. hapla* from Venezuela (V) and the Netherlands (N) expressed as ocular scale units (1 unit = 1.25 microns). Averages of 25 patterns. The numerical indications of the characters used correspond to the indications in Figure 8. t values: ** = highly significant, at 1%; * = significant, at 5%; − = non significant.

Characters measured	Populations		t values
	(V)	(N)	
1. Vulva width	19.2	18.6	0.74 *
2. Distance from anus to vulva	17.7	16.6	1.27 −
3. Distance between phasmids	19.9	22.4	2.94 **
4. Distance from vulva to imaginary line between phasmids	25.4	23.9	1.46 −
5. Distance from anus to tail terminus	11.1	8.6	3.69 **
6. Distance between striae	7.8	7.5	1.04 −

It appears that the *M. hapla* populations were different in three of the six characters measured. The distance between phasmids was smaller and the distance from anus to tail terminus as well as the vulva width were larger in the (V) than in the (N) population. It is possible therefore that the (V) and (N) populations of *M. hapla* are not the same species.

Comparison of the data from Table 29 and Table 19 indicates that all characters measured of *M. hapla* populations were markedly smaller than those of the *M. incognita* populations, except for the distance between striae, which was larger in *M. hapla*. This could be expected for different species and it demonstrates the usefulness of the selected characters.

6.3.5. *Discussion*

The *M. hapla* populations from the Venezuelan mountains (V) and from outdoors in the Netherlands (N) are both more resistant to low temperatures than *M. incognita*. *M. hapla* (V) and (N) showed good survival at 5 °C in water (Table 20) and at 0 °C in soil, whereas there was partial survival in soil at – 10 °C for several days (Tables 27, 28). This is in accordance with the common name of the nematode in the USA, northern root-knot nematode, and also with the fact that it is, with *M. naasi*, the only *Meloidogyne* species which maintains itself with success outside greenhouses in temperate regions (BROWN 1955) or at high altitudes in warm areas (KRUSBERG & HIRSCHMANN 1958, LOOF 1964).

Both *M. hapla* populations could start some activity at 10 °C, but for the rest they were certainly not less thermophil than the *M. incognita* populations with respect to their activities. This appears from the hatching thermograms (Figures 9 and 10) and also from the experiments about gall formation and reproduction which indicate a very broad temperature range from 15 ° to 35 ° in which *M. hapla* can thrive, with 30 °C and on growing plants 35 °C being even more favourable than lower temperatures. This is clearly shown in Figures 11, 12 and 13, derived from Tables 21–26. *M. hapla*, therefore, is not at all thermophob and could thrive under tropical conditions as far as optimum temperature requirements are concerned. This is in agreement with BERGESON's (1960) unexpected record that larvae and eggs of *M. hapla* survive better, not less, at 32 °C than the species *M. incognita* and *M. javanica*, which are considered tropical species. *M. hapla* does occur in tropical areas and in greenhouses, but is rare there as compared with other species, and this must have some other reason than its thermopreferendum.

The two *M. hapla* populations differed in their morphology and in the formation of males and there were slight differences in their temperature requirements. The (N) population hatched slightly better at extreme temperatures (Figure 9, Table 20) and survived slightly better in the inactive state at extreme temperatures (Tables 20, 27, 28). Galling and nematode reproduction, however, were a little less at the lower temperatures, 10 °–20 °C, and certainly better at the higher temperatures, 30 °–35 °C (Tables 21–24, Figures 11 and 12). In general the (N) population seems a little more resistant to adverse temperature extremes and more thermophil in its biological activities. The differences between the two *M. hapla* populations are small compared to the differences between the *M. hapla* populations at one side and the two *M. incognita* populations at the other.

The fact that *M. hapla* populations may thrive at relatively low temperatures (although they thrive better at higher temperatures) and that they can survive spells of low temperatures are evidently the key factors in explaining the occurrence of this nematode in cool climatic areas.

6.4. APHELENCHUS AVENAE (V) AND (N)

Experiments were conducted in temperature gradients with the (V) and (N) populations of *A. avenae* concerning reproduction, including the minimum temperature for reproduction of the (V) population (6.4.1.), adaptation to lower temperature (6.4.2.), sex ratio as affected by temperature (6.4.3.), influence of contaminating fungi (6.4.4.), and morphology as affected by temperature (6.4.5.), with a final discussion on the species (6.4.6.).

6.4.1. *Reproduction*

a. Inoculation experiments

Young adult females of *A. avenae* (V) and (N) were inoculated 5th April 1967 into culture dishes with 10 ml oat meal agar with colonies of about 4 cm in diameter of *Alternaria solani*. Eight females were inoculated per dish; there were 8 petri dishes at each temperature; the temperatures selected in the series thermostat were approximately 5°–10°–15°–20°–25°–30°–35° and 40°C. The cultures were evaluated after 2, 4, 6 and 8 weeks. The results are summarized in Figures 14 and 15.

Figure 14 shows that after 4 weeks optimum reproduction had occurred at

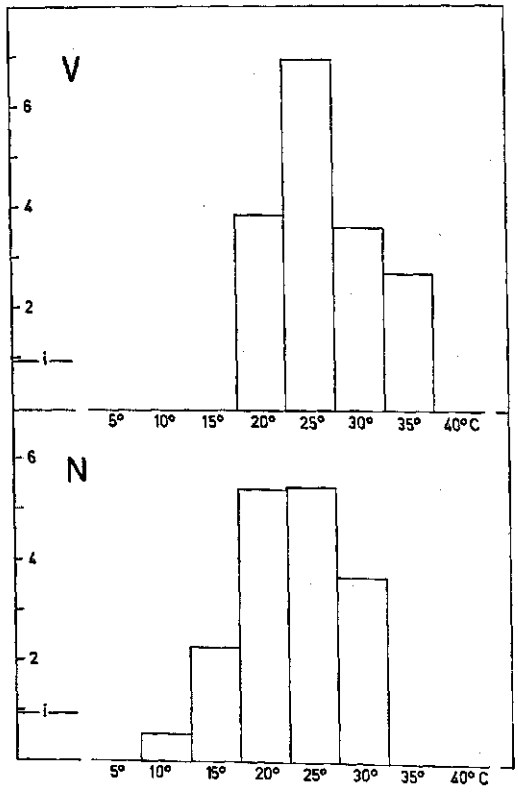

FIG. 14. Reproduction thermograms of the (V) and (N) populations of *Aphelenchus avenae:* evaluation 4 weeks after inoculation of 8 females per plate with *Alternaria solani* on 10 ml of oat meal agar. Ordinate: logarithm $^{10}\log(x + 1)$ of the number of nematodes as average of two replicate plates (x) plus one. The inoculated density is indicated by i.
Abscissa: temperatures in the thermostat compartments.

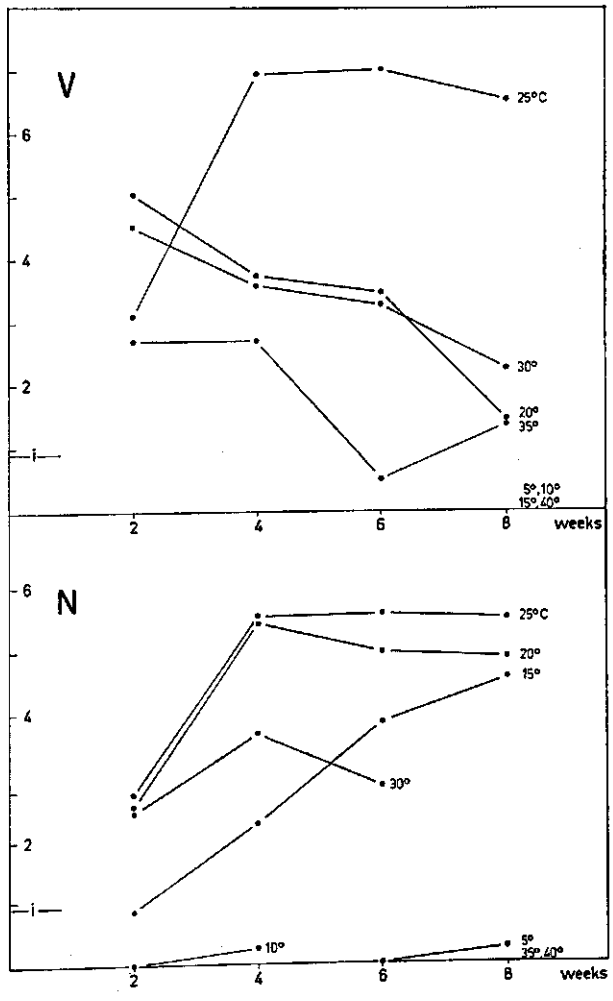

FIG. 15. Densities of *Aphelenchus avenae* (V) and (N) with time after inoculation onto *Alternaria solani* on agar plates of 8 females per plate. Ordinate: logarithm $^{10}\log(x+1)$ of the number of nematodes as average of two replicate culture plates (x) plus one. The inoculated density is indicated by i. Abscissa: breeding time in weeks at the different thermostat temperatures, varying from 5° to 40 °C, as indicated in the graphs.

25°; 20° and 30 °C were somewhat less favourable. The (V) population did not reproduce at 15 °C or lower, contrary to the (N) population which reproduced at 15° and probably at 10 °C (and after 8 weeks even at 5 °C; cf. Figure 15). At the other side the (V) population reproduced rather well at 35 °C, whereas the (N) population did not; 40 °C was too high for reproduction and survival by any of the two populations. The (V) population, therefore, was more cryophob, the (N) population more thermophob than the other.

The graphs of Figure 15 confirm that the favourable temperature range for reproduction was 20°–30 °C or possibly 35 °C for the (V) and 15°–25 °C or possibly 30 °C for the (N) population. Reproduction at favourable temperatures was already noticeable afer 2 weeks. The (N) population showed its first measurable reproduction at the very low temperatures with delay, namely after 4 weeks at

10 °C and after 8 weeks at 5 °C. The final nematode numbers at 5° and 10 °C were lower than the inoculated numbers. It is therefore uncertain whether the nematodes caught were surviving inoculum or newly formed nematodes.

b. Single female cultures

One young adult female of *A. avenae* (V) and (N) was inoculated per culture dish, with ten replicates per population and per temperature. The series thermostat temperatures selected were 15°–20°–25° and 30 °C. The nematode populations were evaluated after 15 days. The results are recorded in Table 30 and statistical analysis of the data was made.

TABLE 30. Reproduction of *Aphelenchus avenae* from Venezuela (V) and from the Netherlands (N), in single female cultures kept for 15 days at four different temperatures. Each figure is the logarithm $^{10}\log (x + 1)$ of the number of nematodes per culture dish (x) plus one; average of 10 replicates. Least significant difference of the figures at 1 % = 0.837.

Populations \ Thermostat temperatures	15°	20°	25°	30 °C
(V)	0	2.209	2.531	3.206
(N)	1.316	2.642	3.981	3.456

At all temperatures reproduction of the (N) population was stronger than of the (V) population. The (V) population did not reproduce at 15 °C contrary to the (N) population as in experiment a. The optimum temperature found for the (N) population was again 25 °C, but it was 30 °C for the (V) population in this experiment. All data confirm that the range of suitable temperatures for reproduction is higher for the (V) than for the (N) population. It was interesting to note that males were present at 30 °C in both populations, although more numerous in the (N) than in the (V) population.

c. Minimum temperature

This experiment was carried out to find the minimum temperature for *A. avenae* (V) to start reproduction. As in experiment b. single female cultures were started and ten replicate dishes were placed at each of the temperatures 16°–17°–18°–19° and 20 °C in precision thermostats. After 15 days the nematode populations were evaluated; the results are recorded in Table 31.

The results show, that reproduction dropped significantly from 20° to 16 °C, but also that there was still a fair reproduction at 16 °C. The real minimum temperature for reproduction of the (V) population is therefore lower than 16 °C and according to the experiments a. and b., probably somewhat higher than 15 °C.

TABLE 31. Reproduction of *Aphelenchus avenae* (V) from a single female in cultures kept for 15 days at five temperatures near the minimum for reproduction. Each figure is the logarithm $^{10}\log (x + 1)$ of the number of nematodes per culture dish (x) plus 1; average of 10 replicates.
Significance of differences: ** = highly significant, at 1%; * = significant, at 5%; – = non significant

Temperature °C	Nematode figures	Standard error	Significance of differences				
			16°	17°	18°	19°	20 °C
20	2.214	0.0030	**	**	–	–	–
19	2.253	0.0128	**	**	–	–	–
18	1.794	0.0080	**	**	–	–	–
17	1.004	0.0200	–	–	**	**	**
16	0.883	0.0190	–	–	**	**	**

6.4.2. *Adaptation to low temperature*

The (V) population of *A. avenae* did not reproduce at 15 °C (± 1 °) in the series thermostat experiments (Figures 14 and 15, Table 30), but reproduction did occur at 16 °C in the experiment described under 6.4.1c (Table 31). The average numbers of offspring per nematode after one month divided by the number of days were lower at 16° than at 18 °C, viz. 4.2 and 5.9 respectively in this experiment. In order to study the possibility of adaptation to lower temperature of the (V) population an experiment was started which ran continuously for two years from May 1967 on. Nematodes used in the experiment had been grown at exactly 25 °C for 8 months. From this population 20 single female cultures were started on oat meal agar plus *Alternaria solani* at 18 °C and left in the same dish for one month, which period is sufficient for the nematode to develop more than 4 generations at 25 °C. Each month new single female cultures were started in fresh culture dishes from that culture which showed the greatest density and therefore the highest rate of reproduction. This was continued for 24 months, during which period the temperature was exactly maintained at 18 °C. In the first month a parallel experiment with the (N) population ran under exactly the same conditions.

Figure 16 shows the gradual increase of the fecundity of the (V) population. The (V) population produced an average of 5.4 offspring during the first month as compared with 108 of the (N) population at 18 °C.

In the first two months the (V) population had an offspring reproduction average of about 5, in the third month reproduction was three times higher and it increased gradually up to about 25 offspring in the 21st – 24th month, however, with at least two deep inclinations down to the original level. These inclinations may be due to variability introduced by the choice of one mother population for breeding of all cultures for the next month, but they can not be explained with certainty.

The increase in fertility achieved during the two years' exposure of the reproducing nematode population to a constant temperature of 18 °C indicates that

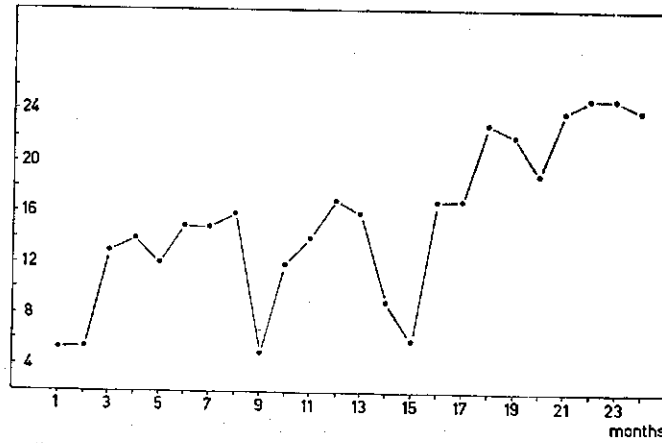

FIG. 16. Fecundity of the (V) population of *Aphelenchus avenae* in 24 successive generations reared at 18 °C. Each month 20 new single female cultures were started from the one culture which showed the highest rate of reproduction.
Ordinate: final number of offspring per nematode after 1 month divided by the number of days; average of 20 single female cultures.
Abscissa: number of successive cultures, each of which took one month.

adaptation to low temperature or selection of less cryophob nematodes may have occurred.

A further experiment was carried out in order to determine whether the nematodes had maintained their ability to thrive at higher temperatures after being exposed to 18 °C for 2 years on end. 20 single female cultures were started with nematodes grown for two years at 18 °C and 20 others were started with nematodes which had always reproduced at 25 °C.

Both series were placed at 25 °C and after one month the nematodes were extracted and counted. The nematodes adapted to 18 °C had an average offspring of 170 and those adapted to 25 °C of 125. The differences were not significant. It therefore appears that the *A. avenae* population adapted to the lower temperature had fully maintained its potency to thrive at 25 °C.

6.4.3. *Sex ratio*

A. avenae is generally considered to be a monosexual species; males have incidentally been found but they are considered very rare in temperate climates. Males were regularly noticed in our cultures placed at high temperatures. To study the influence of temperature on the production of males, and therefore on the sex ratio, single female cultures of both populations (cf. 6.4.1) were placed at 25 ° and 32°–33 °C, and of the (N) population also at 29 °C. The cultures at 25° and 29 °C were evaluated after one month; those at 32°–33 °C had to be examined already after 15 days because the fungus culture *Alternaria solani* became highly contaminated. The results are summarized in Table 32.

TABLE 32. Sex ratio in populations of *Aphelenchus avenae* (V) and (N) from single female cultures grown at different thermostat temperatures

Culture numbers \ Temperature treatments	*A. avenae* (V)					
	25 °C, one month			32–33 °C, 15 days		
	males	females	ratio	males	females	ratio
1	0	2,460	0.0	9	4	2.3
2	0	12,220	0.0	23	2	12
3	0	2,800	0.0	70	5	14
4	0	15,000	0.0	34	7	4.9
5	0	8,000	0.0	6	0	∞
6	0	10,400	0.0	14	7	2.0
7	0	16,000	0.0	17	1	17
8	0	6,000	0.0	41	10	4.1
9	0	9,000	0.0	17	9	1.9
10	0	5,800	0.0	11	7	1.6
Total	0	87,680	0.0	242	52	4.7

Culture numbers \ Temperature treatments	*A. avenae* (N)								
	25 °C, one month			29 °C, one month			32–33 °C, 15 days		
	males	females	ratio	males	females	ratio	males	females	ratio
1	0	10,900	0.0	1,300	1,900	0.7	78	2	39
2	0	8,900	0.0	100	1,600	0.1	64	2	32
3	0	56,100	0.0	1,400	2,500	0.6	170	1	170
4	0	8,900	0.0	1,200	7,500	0.2	24	5	4.8
5	0	72,000	0.0	1,500	200	7.5	160	57	2.8
6	0	2,800	0.0	1,200	3,000	0.4	50	9	5.6
7	0	1,500	0.0	1,600	2,300	0.7	91	8	11
8	0	26,700	0.0	900	1,100	0.8	15	1	15
9	0	2,200	0.0	900	600	1.5	8	11	0.7
10	0	51,000	0.0	300	2,100	0.1	60	8	7.5
Total	0	241,000	0.0	10,400	22,800	0.5	720	104	6.9

It appears from the table that no males are formed at 25 °C, the optimum temperature for both populations. Males are about as numerous as females in the (N) population at 29 °C; variability is considerable and the male/female ratio varies from 0.06 to 7.5, but males are rare in none of these cultures. The temperature of 32°–33 °C allows only poor reproduction, but nearly all cultures of both populations comprise more males than females; in one culture all specimens are males and in several others the male/female ratio exceeds 10. It is clear that temperatures determine the formation of males and that this holds for both populations.

Since *A. avenae* reproduces on the living fungus *Alternaria solani* in the cultures it cannot be decided whether the influence of temperature is on the nematodes direct or indirect via the fungus. Inoculation of adult females onto the fungus-agar cakes, already use dat 32°–33 °C in the above experiment, freed from nematodes by extraction in water and then recounted in clean petri dishes, showed that strong reproduction without formation of males occurred when they were placed at 25 °C. Table 33 shows that a few males were found. However, they were present in one out of ten culture dishes of the (V) and (N) population, and it is likely that they were overlooked when the nematodes were extracted from the agar cake after the first experiment. As in all other experiments with nematodes on living hosts the results do not completely exclude the possibility of indirect temperature influence but they make it improbable. They show that the fungus present on the oat meal agar in the dish was a suitable substrate at 25 °C and that the dish did not comprise persistent toxins. An extra identification of the fungus at the end of the second experiment confirmed, that *A. solani* was the sole fungus in the cultures throughout the experiments.

TABLE 33. Sex ratio in 10 populations of *Aphelenchus avenae* (V) and (N) from single female cultures grown for one month at 25 °C in used culture plates on which mainly males were formed in the earlier experiment at 32–33 °C (cf. table 32).

Culture numbers	*A. avenae* (V)			*A. avenae* (N)		
	males	females	ratio	males	females	ratio
1	5	3,900	0.001	0	2,860	0
2	0	2,300	0	0	3,020	0
3	0	2,600	0	0	4,730	0
4	0	7,200	0	0	3,420	0
5	0	1,950	0	2	5,920	0.0003
6	0	2,300	0	0	3,600	0
7	0	4,300	0	0	3,690	0
8	0	8,000	0	0	3,700	0
9	0	1,000	0	0	4,000	0
10	0	2,700	0	0	3,800	0
Total	5	36,250	0.0001	2	38,740	0.0001

6.4.4. *Influence of contaminating fungi*

It has been observed throughout the experiments with aseptic cultures of *A. avenae*, that the culture plates with *Alternaria solani* seldom become contaminated with other fungi. When contamination occurs, however, the numbers of nematodes in the cultures decrease. The main contaminating fungus in our cultures appeared to be *Trichoderma koningi*. The decrease in the nematode density may be caused by nematicidal substances in the cultures. To check this possibility an experiment was set up, in which nematodes were exposed to the extract of contaminated plates for different periods. A culture plate contaminated with *T. koningi* was squeezed in cheese cloth to extract about 25

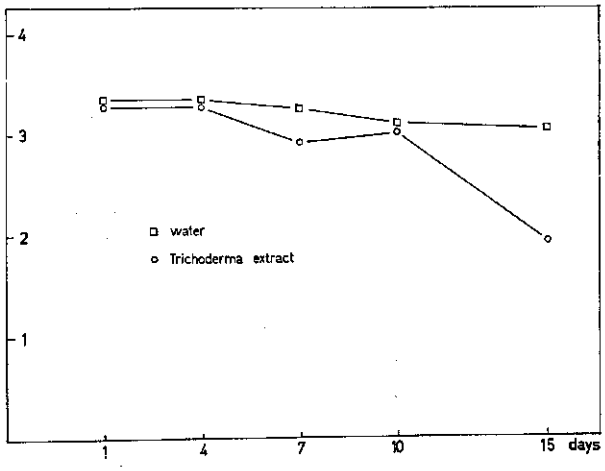

FIG. 17. Effect of extract from *Trichoderma koningi*-grown culture plates on motility of *Aphelenchus avenae*, as compared with water. 2500 nematodes per watch glass.
Ordinate: number of nematodes after different exposure periods passing a cotton wool filter, on normal logarithmic scale.
Abscissa: exposure periods in days.

ml of liquid. Five batches of 2500 larvae and adult females were placed in clean 5 cm wide watch glasses with about 5 ml of the extract. One of the glasses was evaluated for activity of the nematodes after 1–4–7–10 and 15 days. There was a control series with nematodes kept in water. All watch glasses with nematodes were kept at room temperature which was about at 22 °C. The criterium used to assess the vitality and therefore the mobility of the nematodes was their passage through a cottonwool filter in water within 24 hours after the suspension had been poured onto the filter.

Figure 17 indicates that the extract of *T. koningi* contaminated culture plates decreased the motility of the nematodes, as opposed to water, and that the effect increased with time. It is therefore probable that *T. koningi* releases a nematicidal substance and that this is the cause of the population decline in cultures contaminated with this fungus.

6.4.5. *Morphology*

As in the earlier experiments, *A. avenae* (V) and (N) were reared as single female cultures at different temperatures for three months; after each month the culture was re-established from one single female. The temperatures chosen were 18 °–20 °–25 ° and 30 °C for the (V) population, and 15 °–20 °–25 ° and 30 °C for the (N) population. Adult female nematodes of the populations available after three months were used for morphological studies.

Eight characters which are commonly used in nematode taxonomy were measured or calculated from measurements on a number of nematodes of each population which were fixed and mounted in glycerin. They are indicated by the symbols L, α, β, β', γ, T/ABW, S and V, which are explained in Figure 18. Table 34 gives the results. All characters indicated are averages of measurements on a number of specimens; the significance of differences between populations and between temperatures is also calculated. The influence of temperature on these characters is graphically represented for both populations in Figure 19, which also indicates which differences are statistically significant.

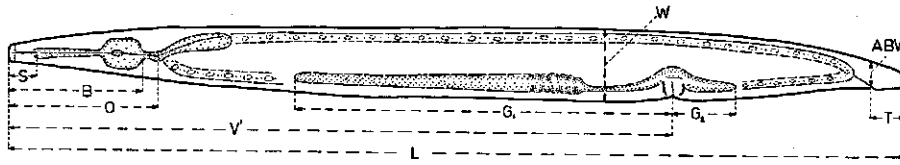

FIG. 18. Definition and illustration of morphometric characters used in the studies on *Aphelenchus avenae* (Table 34, Figure 19) and *Ditylenchus dipsaci* (Table 38, Figure 28).

L	= body length in microns
α	= body length divided by maximum body width (= L/W)
β	= body length divided by distance from head end to junction of oesophagus and intestine (L/O)
β'	= body length divided by distance from head end to posterior end of median oesophageal bulb (L/B)
γ	= body length divided by tail length (= L/T)
T/ABW	= tail length divided by anal body width
S	= stylet length in microns
V	= vulva position from head end as percentage of body length (V'/L × 100)
g_1, g_2	= length of anterior and posterior ovary, respectively, as percentage of body length ($G_1/L \times 100$, $G_2/L \times 100$)

Table 34 and Figure 19 show that temperature caused significant differences for all eight characters in both populations except for S in the (V) population. This should convey a warning against the common practice of describing new species on the basis of these characters without determining the influence of temperature and possibly other environmental factors e.g. host plant (cf. also LOOF 1960b). The only character which was hardly influenced was the stylet length S, which therefore may be of special value for the description of species closely related to *A. avenae*. It is noteworthy that all characters show significant differences at one or more temperatures between the (V) and (N) populations. This was so for S, T/ABW and γ at all temperatures. This is a strong indication that these two populations may be different species.

The following remarks can be made with respect to the characters individually.

L, body length, reached an optimum for the (V) and the (N) populations at 20 °C. Temperatures caused differences of about 25 % in both populations. The (V) population is smaller than the (N) population at all temperatures; this difference was greatest at 25 °C, namely 18 %.

FIG. 19. Influence of rearing temperatures on eight morphological characters of *Aphelenchus avenae* populations from Venezuela (●) and from the Netherlands (★). Solid line (—) between two neighbouring points means that the difference is statistically significant at 5 % level, whereas broken line (- - -) or no connection indicate non-significance. Ordinate: L and S are expressed as microns, V as a percentage, and α, β, β', γ and T/ABW are ratios; cf. Figure 18 for explanation of the symbols and Table 34 for the number of specimens from which the average values indicated are determined. Abscissa: temperatures at which the nematode populations were reared, °C.

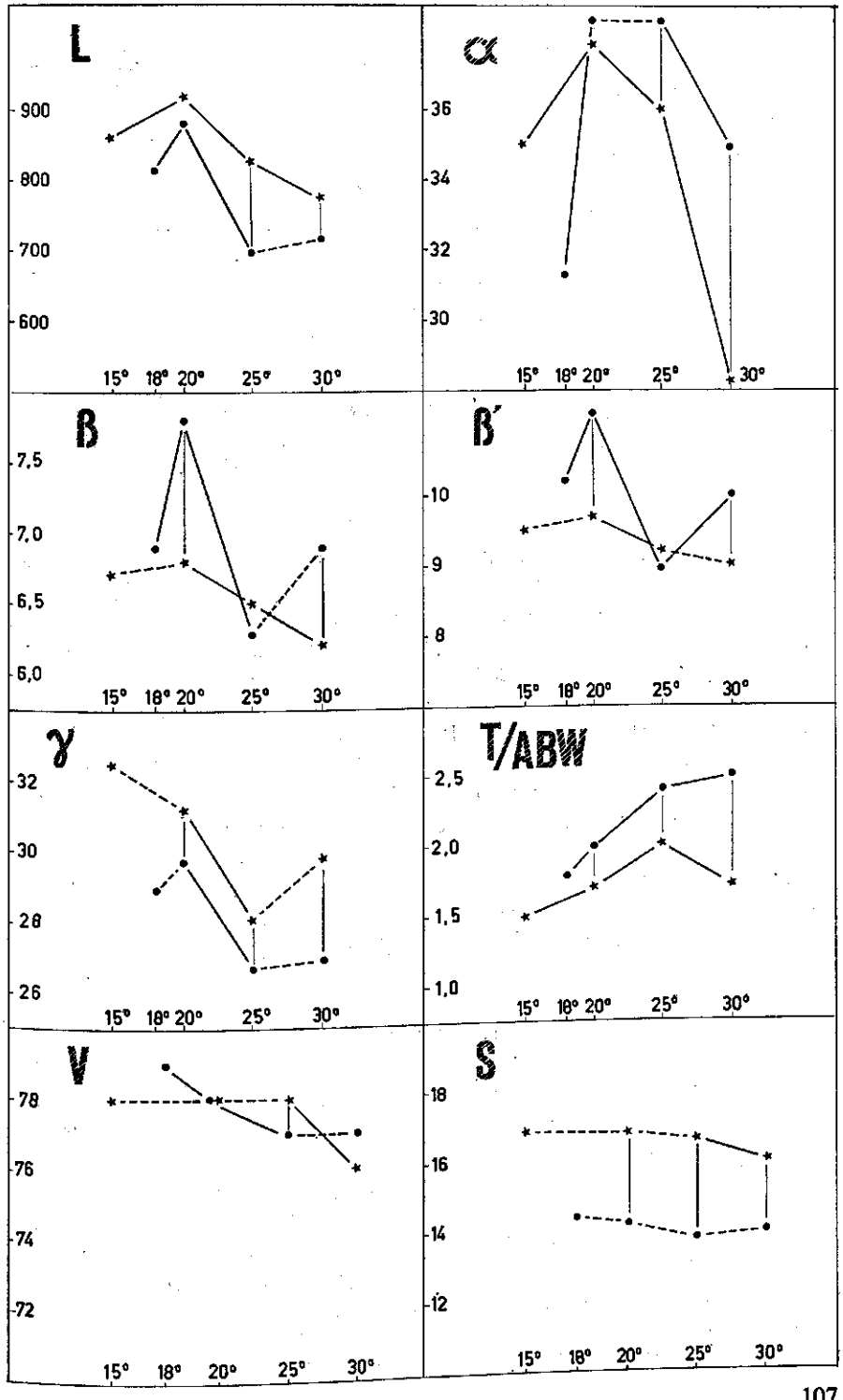
L
900
800
700
600
15° 18° 20° 25° 30°
α
36
34
32
30
15° 18° 20° 25° 30°
ß
7,5
7,0
6,5
6,0
15° 18° 20° 25° 30°
ß'
10
9
8
15° 18° 20° 25° 30°
γ
32
30
28
26
15° 18° 20° 25° 30°
T/ABW
2,5
2,0
1,5
1,0
15° 18° 20° 25° 30°
V
78
76
74
72
15° 18° 20° 25° 30°
S
18
16
14
12
15° 18° 20° 25° 30°

TABLE 34. Morphometric characters of adult females of *Aphelenchus avenae* populations from Venezuela (V) and from the Netherlands (N), both cultivated at different temperatures for three months. Cf. Figure 18 for definition and illustration of the characters used.

Population and rearing temperature in °C	Number of specimens measured	*L*	α	β	β'	γ	*T/ABW*	*S*	*V*
(V) 18°	25	822	31.3	6.9	10.2	29.0	1.8	14.4	79
(V) 20°	25	887	38.6	7.8	11.2	29.8	2.0	14.3	78
(V) 25°	25	700	38.6	6.3	9.0	26.7	2.4	13.9	77
(V) 30°	25	719	35.0	6.9	10.0	27.0	2.5	14.1	77
(N) 15°	8	859	35.0	6.7	9.5	32.5	1.5	16.9	78
(N) 20°	25	922	37.9	6.8	9.7	31.2	1.7	16.9	78
(N) 25°	25	827	36.0	6.5	9.2	28.1	2.0	16.7	78
(N) 30°	11	779	28.3	6.2	9.0	29.8	1.9	16.1	76

α, body length divided by width, also reached an optimum for both populations at 20 °C; the differences caused by temperature were up to 23 % in the (V) and up to 33 % in the (N) population. The nematodes at low temperatures (15° or 18 °C) and at high temperatures (30 °C) were apparently not only shorter, but also thicker than at 20°–25 °C. At the high temperatures 25° and 30 °C, opposed to the low temperatures between 15 and 18 °C, the (V) population was significantly more slender than the (N) population.

β and β' also reached an optimum for both populations at 20 °C, and there were significant differences between the (V) and (N) populations at 20° and at 30 °C. The (V) population scored an aberrant low β and β' value at 25 °C. These nematodes were somewhat smaller (cf. *L*), whereas the oesophagous and the tail were fully developed, as is generally the case in young specimens. Maybe the specimens selected were relatively young. Anyway the shorter length at 25 °C is reflected in the relative values of β, β' and also γ.

γ is significantly higher at 20° than at 25 °C in both populations. This may, however, be due to the aberrant low value at 25 °C, as indicated before. It is possible that γ would not have been influenced significantly by temperature if the value at 25 °C would not have been aberrant, and it is therefore possible that γ is a stable character as is *S*. There was a significant difference between the (V) and (N) populations at 25 °C, but also at 20° and 30 °C which makes this result dependable.

T/ABW, therefore the shape of the tail end, is strongly influenced by temperature in both populations. The values are low at low temperatures, indicating that the tail is relatively short (cf. also γ) or the anal body width is relatively great (cf. also α) at low temperatures. The (V) population has significantly greater *T/ABW* values than the (N) population, over the whole temperature range tested.

V, vulva position, varies irregularly with population or with temperature.

S, stylet length, is very little influenced by temperature, but the (V) population has considerably shorter stylets than the (N) population at all temperatures.

6.4.6. *Discussion*

A. avenae is one of the most common soil-inhabiting nematodes in warm regions and it also occurs in temperate regions. It is frequently found associated with subterranean plant parts, but it is not considered an important plant parasite (STEINER 1936, MANKAU & MANKAU 1963). It thrives very well on fungi and may cause damage to cultivated mushrooms (CHOLEVA 1966). On the other hand it may feed and reproduce on mycorrhyzal fungi and retard fungal growth and in this way promote plant growth (SOUTHERLAND & FORTIN 1968, COOKE & PRAMER 1968). It can easily be reared in artificial media with a suitable fungus, as was done in our experiments. The data collected in Table 2 already indicate that the species is rather thermophil; the optimum recorded by different authors for hatching, development and egg production are usually in the range from 25–35 °C. TAYLOR (1962) found that *A. avenae* hatched over a wide range of temperatures, but not at 5 ° and 42 °C.

Our data confirm the thermophily of *A. avenae*, but also that the (V) and (N) populations differ significantly in several respects, as far as biological and morphological characters are concerned.

Reproduction occurs from 16 °–35 °C in the (V) and probably from 5 °–30 °C in the (N) population, with an optimum for both populations at about 25 °C, apparently somewhat higher for the (V) and somewhat lower for the (N) populations (Figures 14 and 15, Tables 30 and 31).

The more cryophob (V) population appears to reproduce gradually better, although subject to long-term fluctuation, when kept for 24 months at 18 °C (Figure 16). It dit not lose its potency to reproduce strongly at 25 °C. Some adaptation or selection may have occurred but the treated (V) population did not reach the same reproduction rate at low temperature as the (N) population did by nature. Males appear to be absent or very rare in both (V) and (N) populations when these are grown at 25 °C, but a less numerous population consisted largely of males in both cases when grown at 32 °–33 °C. The temperature of 29 °C caused an intermediate result in the (N) population. It appears that temperature determined the formation of males in *A. avenae* and this holds for both populations. This definitely adds high temperature to a number of environmental influences known to be potential factors in determining the sex ratio of nematodes. Other recorded influences which increase the percentage of males are crowding in members of the family *Mermithidae* (CHRISTIE 1929) and in Heterodera rostochiensis (TRUDGILL 1967), resistance of the potato variety and the age of the roots in the case *H. rostochiensis* since relatively more males were formed on resistant varieties and on the finer side rootlets of susceptible plants (ELLENBY 1954).

TRIANTAPHYLLOU (1960) indicates that the sex ratio varies during the year, perhaps as a function of environmental factors. HANSEN & CRYAN (1966) found that the sex ratios of *Panagrellus redivivus* in axenic cultures were changed

from 1:1 by the stress of low protein content of the culture medium and by a high temperature of 28 °C. DAVIDE & TRIANTAPHYLLOU (1967) found that a high density of penetrated larvae increased the percentage of males in *Meloidogyne spp.*, and that low soil temperature increased the number of males in *Meloidogyne incognita.* It appears therefore, that temperature, crowding and food or host plant characters in addition to chromosomal mechanisms can influence the sex ratio. The high temperature in our experiment probably worked direct and not via the host fungus *A. solani,* because no changes were observed in the fungus colonies and because the same colonies yielded monosexual populations after being transmitted to the lower temperature of 25 °C. The possibility of an indirect influence should, however, not be excluded.

MANKAU & MANKAU (1963) already demonstrated that certain phytopathogenic fungi are the preferred food for *A. avenae,* while pythiaceous fungi do not support the nematode and are toxic, probably as a result of the production of antibiotics which do not occur in the plant parasitic species tested by them. Figure 17 demonstrates that the main contaminant in our cultures, *Trichoderma koningi,* also released a noxious agent for the nematode. The number of active nematodes was reduced by 4% after one day and by 94% after 15 days compared to water.

Figure 19 demonstrates that the morphology of both *A. avenae* populations is significantly influenced by temperature in respect of all characters studied, and also that there are significant differences between the (V) and (N) populations at one or more temperatures for all morphological characters studied. The notable differences between the (V) and (N) populations with respect to temperature requirements and morphology indicate that the populations are probably different species.

It is noteworthy that the longest nematodes of both populations occur at an intermediate temperature of 20 °C and not at low or high temperatures, and also that at 20 °C the (V) population is longer than the (N) population at 15 °C. Vertebrates of the same species grow bigger when they live at lower temperatures, according to BERGMAN's well known climatic rule (1847). PREVOST (1955) also accepted a correlation between the geographic range of animal species and their size. GIJSELS (1964) reported that Bergman's rule seems to hold for nematodes. He found that larvae of *Trichodorus* species grow bigger at high temperatures and he referred to authors who found very small nematodes in hot water springs and to the observation that nematodes from tropical seashores are usually smaller than those from arctic regions and from the deep sea. Our experimental data about *A. avenae,* however, indicate that Bergman's rule does probably not hold for nematodes. This conclusion is supported by the observations reported later about *Ditylenchus dipsaci* (cf. 6.5.9).

6.5. DITYLENCHUS DIPSACI (N) AND (V)

Several experiments were made with one (V) population from garlic, and three (N) populations from onion, tulip and narcissus, of *D. dipsaci* in temperature ranges or gradients. Reproduction of all four populations was studied in onion bulbs or in phlox plants (6.5.1–6.5.4). Also studied was the survival of the tulip and narcissus strains when treated in water at high temperatures (6.5.5–6.5.6), and special experiments were conducted about conditioning of the same strains (6.5.7). Finally, temperature-induced morphometric variations in a (V) and an (N) population were studied (6.5.8). All data concerning the species are discussed under 6.5.9.

6.5.1. *Reproduction of the (V) garlic and the (N) onion populations in onion bulbs*

Onion bulbs were each inoculated with 50 adult females of *D. dipsaci* (V) garlic, i.e. originating from garlic grown at high altitude in Venezuela, and (N) onion, i.e. originating from onion grown in the Netherlands.

The nematodes were pipetted into a cylindrical hole of 0.5 cm wide and 2 cm deep bored in the bulb. The hole was subsequently filled with a moist cotton plug and closed with paraffin paper in order to avoid dessiccation. 60 onion bulbs were used for each population, 10 of them being placed in the series thermostat at each of the following temperatures: 10°–15°–20°–25°–30°–35 °C. The experiment was started in October, 1967. The populations were assessed after 4–6–8–10 and 12 weeks. The results of this experiment are transformed to the normal 10-logarithms. They are illustrated in Figures 20 and 21 and described below.

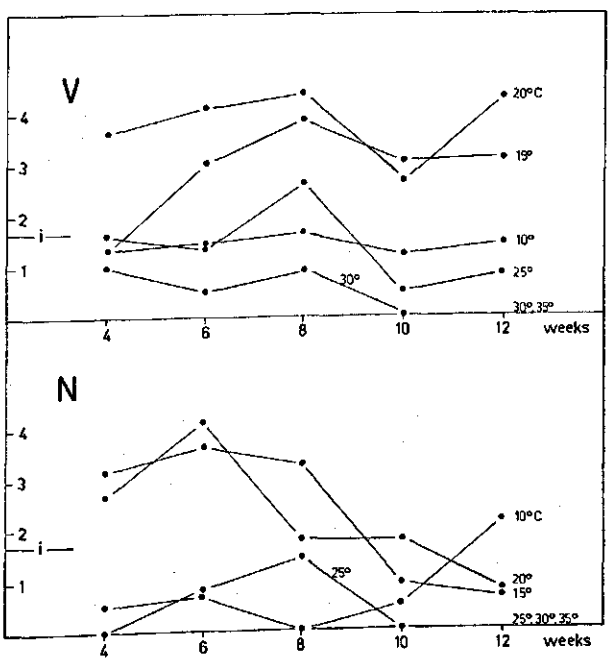

FIG. 20. Densities of *Ditylenchus dipsaci* (V) garlic and (N) onion with time after inoculation into onion bulbs of 50 females per bulb.
Ordinate: logarithm $^{10}\log (x + 1)$ of the number of nematodes as an average of two replicate bulbs (x) plus one. The inoculated density is indicated by i.
Abscissa: breeding time in weeks at different thermostat temperatures, varying from 5° to 35 °C, as indicated in the graphs.

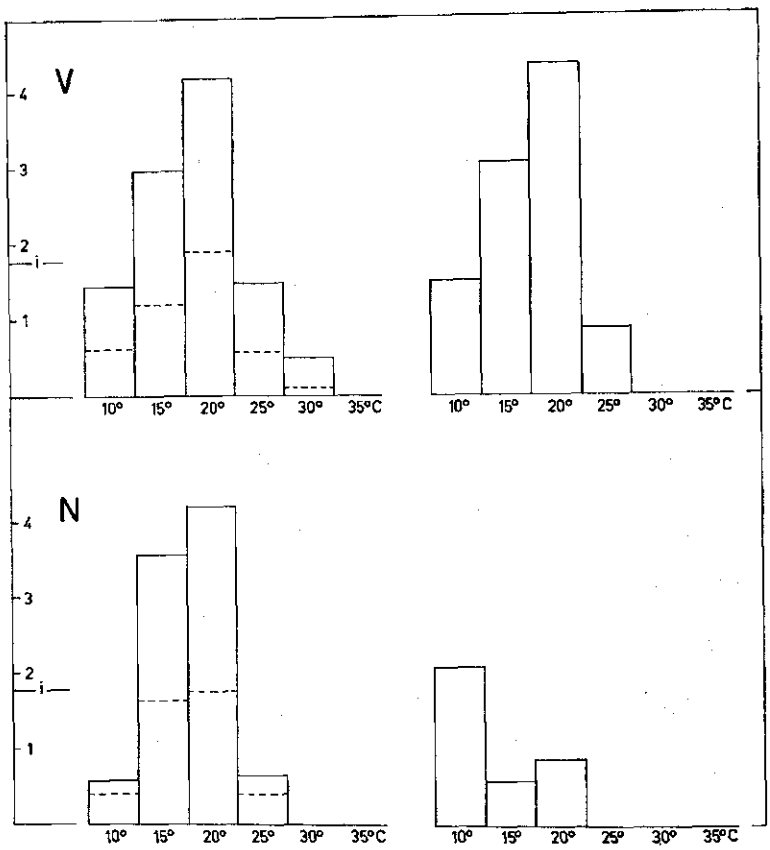

FIG. 21. Reproduction thermograms of the (V) garlic and (N) onion populations of *Ditylenchus dipsaci* in onion bulbs, 6 weeks after inoculation of 50 females per bulb (left-hand graphs) and 12 weeks after inoculation (righthand graphs).
Ordinate: logarithm $^{10}\log (x + 1)$ of the number nematodes as an average of two replicate bulbs (x) plus one (———) or when the average was corrected to 5 g bulb tissue (x) plus one (- - -). The inoculated density is indicated by i.
Abscissa: Temperatures in the thermostat compartments.

Figure 20 shows that both populations reproduced well in onion bulbs at the suitable temperatures of 15° and 20°C. This was already visible after 4 weeks. The densities were highest after 8 weeks for the (V) and after 6 weeks for the (N) population, after which they declined considerably, except for the (V) population at 20° and the (N) population at 10°C, which increased markedly again between 10 and 12 weeks. Complete collapses after 10 weeks of the (V) population at 30°C and of the (N) population at 25°C, apparently here the highest temperatures at which the populations multiplied, were associated with fungal decay and deterioration of the corresponding bulbs. The influence of temperature was great and differed slightly between nematode populations.

The thermograms of Figure 21 indicate that after 6 weeks the (V) population was present at temperatures from 10°–30°C, and had at any rate reproduced at 15° and 20°C with an optimum at 20°C. The (N) population differed in that it reproduced at 15° nearly as much as at 20°C that no active nematodes occurred at 30°C. It is therefore more thermophob than the (V) population. Figure 21 also indicates that the nematode number per average bulb and per 5 g of bulb tissue gives similar results. As appears from the right-hand thermograms in Figure 21, the final nematode densities after 12 weeks, differ from the densities after 6 weeks. After 12 weeks measurable densities of the (V) population were present at 10°–25°C with an optimum at 20°C, whereas measurable densities of the (N) population were only present at 10°–20°C, this time with an optimum at 10°C.

The influence of temperature and time, without much interaction, is evident from the graphs for both populations, and statistical treatment of the data was therefore omitted.

6.5.2. *Reproduction of the (V) garlic and the (N) onion populations in phlox plants*

In this experiment the *D. dipsaci* populations (V) garlic and (N) onion were inoculated to garden phlox.

Phlox was sown in wooden boxes and transplanted after 3 weeks in clay pots 8 centimeters in diameter. After transplanting, the top of each plant was cut down to about 0.5 cm above two side branches on the main stem, which was then inoculated. A superficial scratch was cut with a needle along two opposite sides of the stem stump down to the base of the axillary buds. A glass tube 1.5 cm long and of suitable width, was loosely fitted around the stem and filled with loose cottonwool. 50 adult females of *D. dipsaci* were pipetted into each tube (WINOTO 1969). The cottonwool was kept moist for one week by adding several drops of water twice a day. After inoculation, the plants were placed in the greenhouse compartments at temperatures of 10°–15°–20°–25° and 30° (28°–30°C). Ten plants were placed in each temperature compartment; two of them were taken as samples 4–6–8–10 and 12 weeks later; whole plants were cut to pieces and each of them was put in a funnel-spray apparatus for nematode extraction after their fresh weight had been determined for correction of differences in weight; cf. Figures 22 and 23.

It appears from Figure 22 that both populations produced well on phlox at suitable temperatures. Noticeable reproduction to a density higher than the inoculated density occurred at all temperatures on one or more dates. At suitable temperatures the densities were high after 6 or 8 weeks, wherefrom they generally increased further although with much variability. The incidental decrease in the (V) population at 30°C after 10 weeks, and in the (N) population at 20°C after 10 weeks and at 25° and 30°C after 12 weeks are probably due to experimental variability, since the two replicate plants were relatively poor at all these observation points. Again influence of temperature was great. Both populations temporarily reproduced at all temperatures from 10 to 30°C, with an optimum at 15°C, but then was one great difference between the two. Unlike

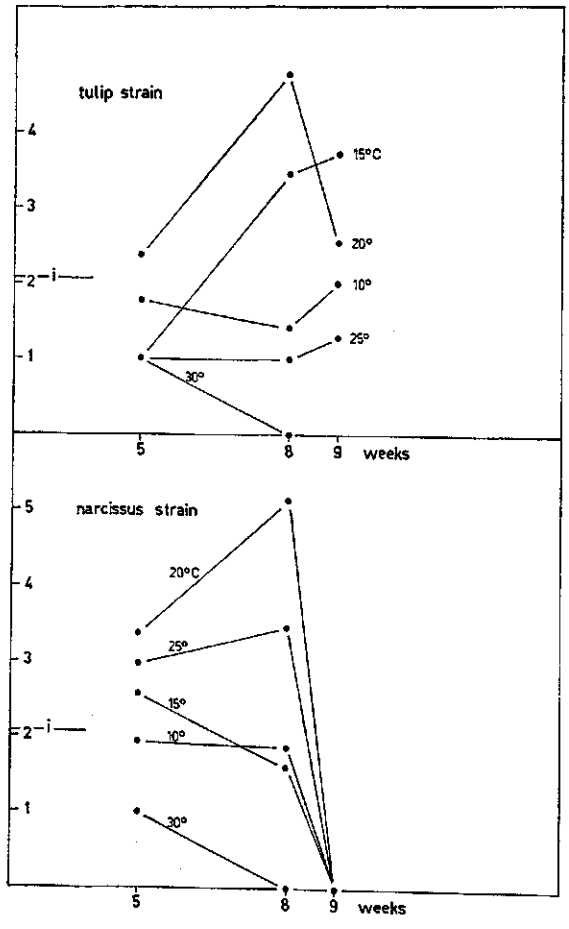

FIG. 24. Densities of the (N) tulip and narcissus populations of *Ditylenchus dipsaci* with time, after inoculation into onion bulbs of 50 females per bulb.
Ordinate: logarithm $^{10}\log (x + 1)$ of the total number of nematodes in three replicate bulbs (x) plus one. The inoculated density is indicated by i.
Abscissa: breeding time in weeks at thermostat temperatures, varying from 10° to 30°C, as indicated in the graphs.

zero. This may be due to extensive decay of the bulbs which may be caused or initiated by the narcissus strain itself.

The thermograms of Figure 25 show that both strains reproduced optimally at 20°C after 5 weeks and also after 8 weeks. For both strains 30°C was evidently too high for reproduction. The strains were present in measurable densities after 5 weeks, but had both disappeared after 8 weeks. At any rate the narcissus strain was not less thermophil than the tulip strain.

Statistical analysis shows a highly significant effect for temperature for the tulip strain ($P < 0.01$) but non-significant for time and interaction, in both cases ($P > 0.05$); for the narcissus strain, there was a highly significant effect for temperature, time and interaction ($P < 0.01$).

6.5.4. *Reproduction of the (N) tulip population in phlox plants*

Following the procedure described under 6.5.2 phlox plants were inoculated with 50 females each. There were 30 inoculated plants, for each of the green-

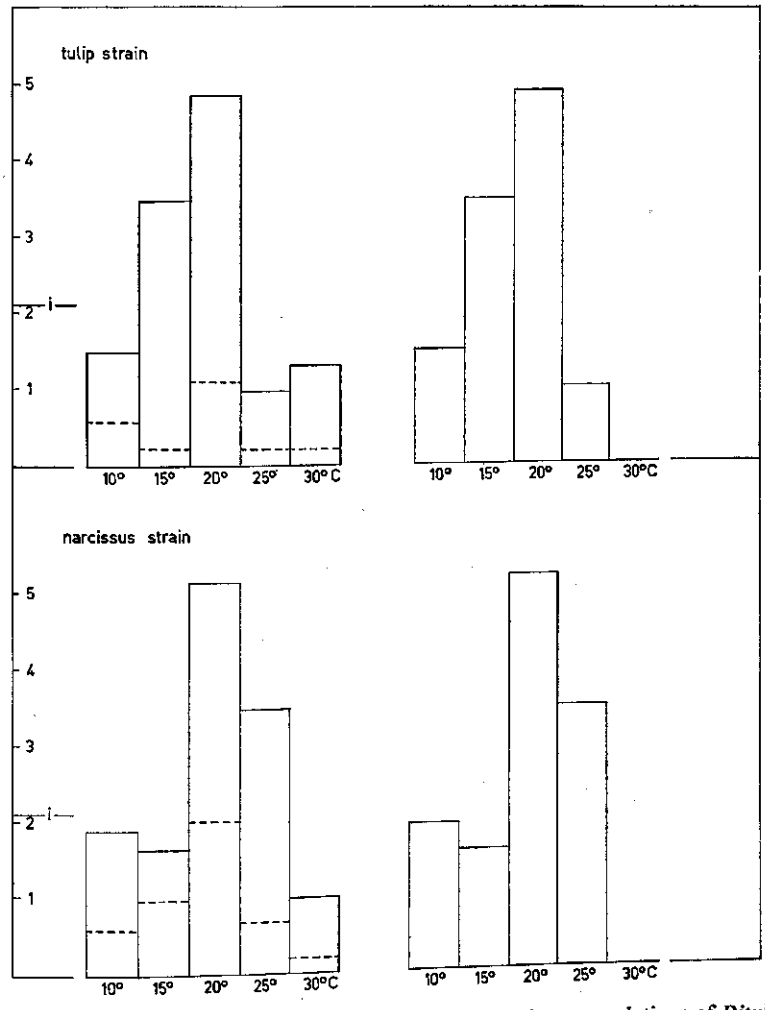

FIG. 25. Reproduction thermograms of the (N) tulip and narcissus populations of *Ditylenchus dipsaci* in onion bulbs, 5 weeks after inoculation of 50 females per bulb (lefthand graphs) and 8 weeks after the inoculation (righthand graphs).
Ordinate: logarithm $^{10}\log (x + 1)$ of the total number of nematodes in three replicate bulbs (x) plus one (—), or calculated for totally 10 g of bulb tissue (- - -). The inoculated density is indicated by i.
Abscissa: temperatures in the thermostat departments.

house compartments at temperatures of 10°–15°–20°–25° and 30° (28–30°C); 2 plants from each temperature compartment were harvested after 7, 9 and 11 weeks. The plants were weighed and nematode populations were evaluated as indicated. The nematode numbers were transformed to ^{10}logarithms and are presented in Figures 26 and 27.

The (N) tulip population reproduced on phlox at all temperatures throughout

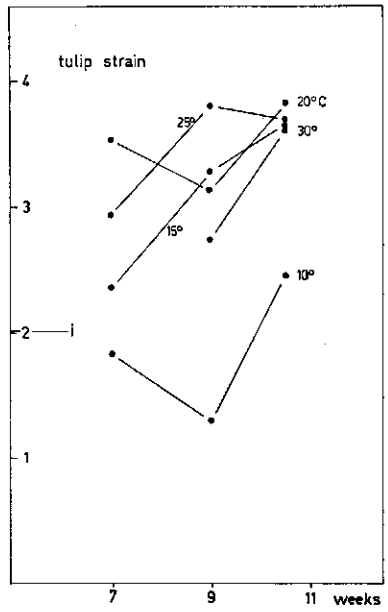

FIG. 26. Densities of the (N) tulip population of *Ditylenchus dipsaci* with time, after inoculation to phlox plants of 50 females per plant.
Ordinate: logarithm $^{10}\log (x + 1)$ of the total number of nematodes in two replicate plants (x) plus one. The inoculation density is indicated by i.
Abscissa: breeding time in weeks at greenhouse temperatures, varying from 10° to 30°C, as indicated in the graphs.

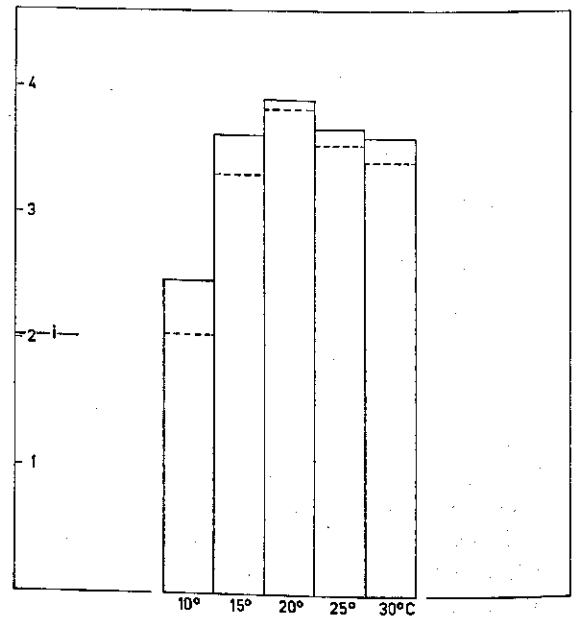

FIG. 27. Reproduction thermograms of the (N) tulip population of *Ditylenchus dipsaci* in phlox plants, 11 weeks after inoculation of 50 females per plant.
Ordinate: logarithm $^{10}\log (x + 1)$ of the total number of nematodes in all fresh tissues of two replicate plants (x) plus one (——) or calculated per 10 g of tissue of two replicate plants (x) plus one (- - -). The inoculated density is indicated by i.
Abscissa: temperatures in the greenhouse compartments.

the observation period. The first evaluation date, after 7 weeks, was apparently too late to see the initial rise of the population, and the population had probably not yet reached its equilibrium density after 11 weeks (Figure 26). The thermogram of figure 27 shows a broad optimum at 15–25 °C for nematode reproduction. It appeared that conclusions based on transformed nematode numbers are the same if they are corrected for weight of the plants. The plants were not much heavier but they were more luxuriant at 10° and 15 °C than at 25°–30 °C; at high temperatures the plants had small and thin leaves of a lighter colour. This may be due to a direct influence of temperature. There were symptoms of nematode infestation but they were more pronounced at 10–15 °C than at higher temperatures. The intrinsic temperature requirements and reactivity of the host is a disturbing influence in studies with obligatory plant parasites. It is nevertheless safe to conclude that the (N) tulip populations can reproduce very well on phlox at all temperatures from 10 to 30 °C and also that temperatures from 15° to 25 °C, and possibly up to 30 °C, are about equally favourable. The effect of temperature and time was highly significant ($P < 0.01$) but interactions were non-significant ($P > 0.05$). This population, often indicated as the tulip race, is apparently more thermophil than the (N) onion population (Figure 23) which had its optimum at 15 °C and did not reproduce well at 25° and 30 °C.

6.5.5. *Motility of the (N) tulip and narcissus populations after heat treatments*

The (N) narcissus and tulip populations of *D. dipsaci*, reared at different temperatures, were placed in water and exposed to high temperature to determine their potencies to survive such treatment. The nematodes were pipetted into glass tubes of 0.5 × 5 cm containing about 0.1 ml water, which tubes were then placed in a water bath for heat treatment. Four experiments, a–d, were made in which the criterium for survival was the motility of the treated nematodes on being touched with a hair needle. Each nematode which showed motility upon touch was considered a survivor. All four experiments were made with recently reared populations because an introductory experiment had shown that (N) tulip populations stored in dry filter paper for 6 years and for 1 year, which were subsequently reactivated in water, survived a 5-minutes heat treatment at 48 °C up to 40 % and 99 % respectively. To avoid loss of viability during storage recently reared populations were always used in the following experiments.

a. The narcissus population, cultured for 5 weeks in onion bulbs at 10°–15 °C, was compared with the nematodes cultured at 25 °C. There was a period of about two weeks between the culturing period and the heat treatment, during which time nematodes were extracted and stored in the refrigerator at about 3 °C. Four batches of 50 unselected nematodes of each origin were treated at 48 °C for 15 minutes. The average percentage survival for the nematodes reared at 10°–15 °C was 27, against 39 for the batches reared at 25 °C. Analysis of the data shows a just significant difference ($P = 0.05$) by the Wilcoxon's test.

b. Tulip populations reared at 15 °C and at 25 °C in phlox plants were compared in the same way as in experiment a. by exposure to 46 °C for 20 minutes. The average survival percentage was 33% for the nematodes reared at 15 °C and 43% for those reared at 25 °C. There was a significant difference ($P < 0.05$) between the temperatures for survival.

c. Tulip populations reared at 15 °C and 30 °C in phlox plants and exposed for 20 minutes to 46 °C showed a survival percentage of 13% and 89% respectively. This was a highly significant difference ($P < 0.01$) between the two temperatures.

The results of experiments a – c indicate that high rearing temperatures, which are themselves not lethal, increase the resistance of the nematodes to heat treatments. This resistance is not lost during a few days extraction at room temperature followed by a few weeks storage at 3 °C. Comparative experiments about heat resistance therefore require nematodes which are previously cultured and kept under controlled conditions.

d. The tulip and narcissus populations, both reared at 20 °C in onion, were compared by exposing them in tubes to water bath temperatures of 46 °C (two experiments) and 47 °C for different periods, viz. 5–10–20–40 and 80 minutes. Four batches of 50 unselected nematodes were used for each temperature-time combination. The percentage survivors are recorded in Table 35.

The results of Table 35 indicate that the tulip strain is significantly less susceptible to high temperature treatment. The difference, however, is small and

TABLE 35. Survival of the (N) tulip and narcissus strains of *Ditylenchus dipsaci*, both cultures from the same inoculum at 20 °C in onion, after exposure of batches of 50 in tubes with water to temperatures of 46 °C (two experiments) and 47 °C for different periods. The criterium for survival was motility of the nematodes upon touch. Each figure is a survival percentage as an average of four batches.

Heat treatments	*D. dipsaci* (N)	
	tulip strain	narcissus strain
5 minutes at 46 °C	100	100
10 minutes at 46 °C	100	96
20 minutes at 46 °C	65	44
40 minutes at 46 °C	27	26
80 minutes at 46 °C	5	3
5 minutes at 46 °C	98	100
10 minutes at 46 °C	95	82
20 minutes at 46 °C	65	54
40 minutes at 46 °C	28	18
80 minutes at 46 °C	7	2
5 minutes at 47 °C	96	60
10 minutes at 47 °C	78	27
20 minutes at 47 °C	15	10
40 minutes at 47 °C	12	7
80 minutes at 47 °C	.	.

becomes difficult to measure at very low survival percentages. The survivors of heat treatments are usually fourth stage larvae, which are therefore more resistant to lethal high temperatures than other stages.

6.5.6. *Reproduction of the (N) tulip and narcissus populations after heat treatments*

The survival of the (N) tulip and narcissus strains after exposure to 46 °C for different times was also studied in two experiments, a and b, by inoculation of the treated nematodes into host plant bulbs. For each temperature-time treatment 5 treated batches and 2 untreated control batches of fifty nematodes were each inoculated into a separate bulb.

a. Tulip and narcissus populations treated at 46 °C for 20 minutes and the corresponding controls were inoculated into onion bulbs. The bulbs were then placed at 20 °C for seven weeks which is a suitable period for measuring reproduction (cf. Figures 24 and 25). Extraction revealed that none of the bulbs inoculated with treated nematodes yielded living nematodes, whereas the bulbs inoculated with untreated nematodes yielded three times the amount of the inoculated nematodes.

b. Tulip and narcissus populations treated at 46 °C for 20 minutes and at 47 °C for 15 minutes and the corresponding controls were inoculated into tulip and narcissus bulbs respectively. The bulbs were again placed at 20 °C for seven weeks and then extracted. The results are recorded in Table 36.

TABLE 36. Survival of the (N) tulip and narcissus strains of *Ditylenchus dipsaci* according to reproduction of batches of 50 nematodes treated at 46 °C for 20 minutes and at 47 °C for 15 minutes when they were inoculated into tulip and narcissus bulbs respectively, which were then kept at 20 °C. Five replicate bulbs of each kind were inoculated with the correspondingly treated populations per temperature – time combination, two control bulbs of each kind with untreated nematodes. Each figure is the number of extracted nematodes per bulb 7 weeks after inoculation.

Treatments	*D. dipsaci* (N)	
	tulip strain in tulip bulbs	narcissus strain in narcissus bulbs
20 minutes at 46 °C	0–0–0–0–0	0–0–0–0–0
untreated (control)	1260–700	12400–800
15 minutes at 47 °C	0–0–0–0–0	0–0–0–0–0
untreated (control)	4250–300	8700–12270

The treated populations did not yield any living nematodes after 7 weeks breeding time in the bulbs of their main hosts, contrary to the untreated populations which reproduced in all replicates. As in experiment a, it appears that both treatments, at 46 °C for 20 minutes and at 47 °C for 15 minutes, were sufficient to check reproduction. The experiment 6.5.5 indicated that a fairly high percentage of the nematodes of both populations were still motile after

such treatments. It now appears that they must nevertheless have been damaged to such an extent that they could not survive and reproduce in their hosts.

6.5.7. *Conditioning of the (N) tulip population*

The experiments were made in addition to those described under 6.5.5 to determine whether the susceptibility of the tulip strain to heat treatment was influenced by preceding temperature treatments, therefore whether conditioning or acclimatization occurred.

a. Batches of 50 unselected nematodes in tubes with water as described before, were submitted for one hour to 3°–20° and 35°C, and then exposed in the water bath to 47°C for 20 minutes. There were two replicates per conditioning temperature. After the heat treatment survival was determined by the percentage of nematodes which showed motility upon touch with a hair needle. The results are recorded in Table 37A. It appears that the pre-treatment at 35°C for 1 hour made the nematodes less susceptible to subsequent heat treatments at 47°C.

b. A similar experiment was made, four-replicate with batches of 100 nematodes, pre-treated for 16 hours at 3°–15°–25° and 35°C; and subjected to a further heat treatment at 47°C for 20 minutes. Table 37B. The results are similar to those of experiments a. Pre-treatment at 35°C made the nematodes markedly less susceptible to further heat treatment. The figures suggest that pre-treatment at 35°C for 16 hours was slightly more effective than for 1 hour, although this difference is not significant. The lower temperatures from 3° to 25°C, show no influence on the condition of the nematodes.

c. A four-replicate experiment with batches of 100 nematodes included pre-treatments of 17 hours at 20°C, and of 1 hour at 37°C + 16 hours at 20°C. The further heat treatment was given at 46° for 30 minutes. Table 37C. The results indicate that survival was high at this heat treatment, but also that the pre-treatment at 20°C for 16 hours probably destroyed the increased heat resistance obtained at the earlier pre-treatment at 37°C.

d. A two-replicate experiment with batches of 50 nematodes included pre-treatments of 15 hours at 20°C, 1 hour at 35°C + 14 hours at 20°C, and 1 hour at 35°C + 14 hours at 3°C. The further heat treatment was now given at 46°C for 60 minutes, Table 37D. It appears that the increased heat resistance obtained at 35°C is largely lost again when the nematodes are kept for 14 hours at 20°C, but not when they are kept for the same period at 3°C.

The fact that heat resistance can be built up by a pre-treatment at 35°C and is lost at 20°C in 14 hours but not at a very low temperature suggests that a principle for heat resistance, possibly of chemical nature, is rapidly built up in the nematode body and is removed or broken down again in only 14 hours when the nematodes are not in cold stupor. In practice the data of these experiments should be taken into account when treating bulbs infected with *D. dipsaci*.

TABLE 37. Conditioning of the (N) tulip strain of *Ditylenchus dipsaci* by different temperature treatments prior to further exposure to high water bath temperatures. The criterium for survival was motility upon touch. Each figure is a survival percentage, based on batches of 50 (A and D) or 100 (B and C) nematodes.

Preceding temperature treatments	Further heat treatment	Percentage survival		
		Replicates	Average	Significance test Chi-square at 0.05[1])
A				
3 °C for 1 hour	47 °C for 20 minutes	50–52	51	
20 °C for 1 hour	47 °C for 20 minutes	43–43	43	*
35 °C for 1 hour	47 °C for 20 minutes	79–67	73	
B				
3 °C for 16 hours	47 °C for 20 minutes	46–56–56–59	54	
15 °C for 16 hours	47 °C for 20 minutes	34–34–50–50	42	
25 °C for 16 hours	47 °C for 20 minutes	40–50–50–42	46	**
35 °C for 16 hours	47 °C for 20 minutes	80–87–78–71	79	
C				
20 °C for 17 hours	46 °C for 30 minutes	85–87–83–84	85	
37 °C for 1 hour + 20 °C for 17 hours	46 °C for 30 minutes	78–80–72–69	74	–
D				
20 °C for 15 hours	46 °C for 60 minutes	37–31	34	
35 °C for 1 hour + 20 °C for 14 hours	46 °C for 60 minutes	45–36	41	**
35 °C for 1 hour + 3 °C for 14 hours	46 °C for 60 minutes	69–57	63	

[1] ** = highly significant, * = significant, – = non-significant.

6.5.8. *Morphology*

The (V) garlic and the (N) onion populations of *D. dipsaci* were reared in onion bulbs for 6 weeks at 15 °C and at 20 °C; 25 nematodes of each population grown at each temperature were killed, fixed and mounted in glycerine as permanent slides (SEINHORST 1962), and measured. The eight characters already used before and listed and explained in Figure 18, were measured and calculated. The results are summarized and illustrated in Table 38 and Figure 28. Figure 28 also indicates which differences between populations and between temperatures are significant.

Temperature caused significant differences for four characters in the (V) population, viz. β, β', γ and V, and for four characters in the (N) populations which were only partly the same, viz. L, α, β' and V. Six of the eight characters also showed significant differences between the (V) and (N) populations at one of the temperatures; β' differed at both temperatures but not in the same direction. The only two characters which did not show any significant differences are S, stylet length, and T/ABW, tail length divided by anal body width.

The following remarks may be made with respect to some of the characters.

L, body length, of the (V) population was somewhat shorter and of the (N)

TABLE 38. Morphological characters of adult females of the (V) garlic and (N) onion populations of *Ditylenchus dipsaci*, both reared in onion bulbs at 15 °C and at 20 °C for six weeks. Cf. figure 18 for definition and illustration of the morphometric characters used. Figures are averages of 25 specimens.

Population and rearing temperature in °C	L	α	β	β'	γ	T/ABW	S	$(g_1)\ V\ (g_2)$
(V) 15°	1290	48.9	6.9	16.1	14.7	5.5	12.9	(51) 81 (6)
(V) 20°	1326	47.6	7.4	17.0	15.6	5.3	12.8	(62) 82 (6)
(N) 15°	1420	47.0	7.3	17.0	15.6	5.5	12.8	(63) 81 (7)
(N) 20°	1324	41.7	7.3	16.0	16.1	5.2	12.6	(50) 79 (6)

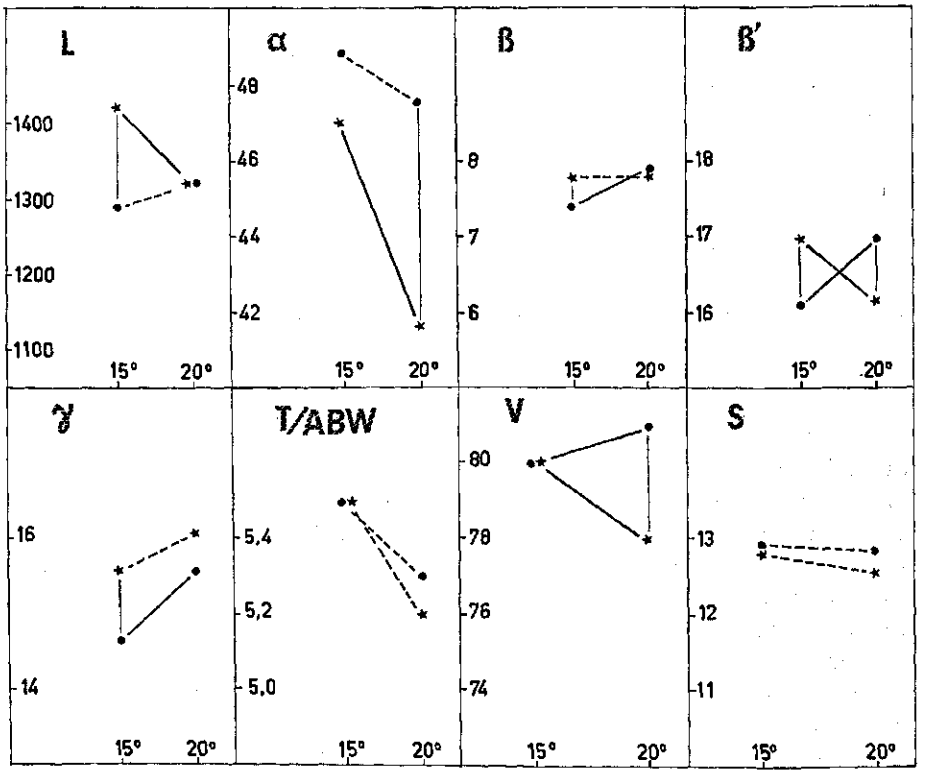

FIG. 28. Influence of rearing temperature on eight morphological characters of *Ditylenchus dipsaci* (V) garlic (●) and (N) onion (★). Solid line (——) between two neighbouring points means that the difference is statistically significant at 5% level, whereas broken line (- - -) or no connecting line indicate non-significance.
Ordinate: L and S are expressed as microns, V as a percentage, and α, β', β, γ and T/ABW are ratios; cf. figure 18 for explanation of the symbols.
Abscissa: temperatures at which the nematodes were reared as °C.

populations significantly longer at 15 °C than at 20 °C. The shortest nematodes occurred at (V) 15 °C and the longest at (N) 15 °C; the difference was significant and was about 10%. This may support the conclusion made under 6.5.1 that the (V) population has a higher thermopreferendum than the (N) population.

α, body length divided by body width, was exceptionally low at 20 °C in the (N) population. These nematodes were therefore not only rather short, but also very thick. This may again indicate that 20 °C is too high for the (N) population to thrive well.

β and γ were both exceptionally low in the (V) population at 15 °C. This indicates that the nematodes, which were short (cf. *L*) nevertheless had a well developed oesophagus and tail. This is often the case with young or poorly growing specimens, and confirms that 15 °C was too low for the (V) population.

β' and g_1 were both lower in the nematodes grown at the unsuitable temperatures, 15 °C for the (V) and 20 °C for the (N) population. This may indicate that in well-developed nematodes the length of the anterior part of the oesophagus (β') and the length of the anterior ovary (g_1) are greater in well-developed nematodes. The fact that *S*, stylet length, and *T/ABW*, tail length divided by anal body width, were not influenced by the population or by the temperature may indicate that these characters are particularly useful for identification of *Ditylenchus* species.

Thus several characters showed significant differences related to both temperature and nematode origin as was the case with *Aphelenchus avenae* (6.4.5). The differences for *D. dipsaci*, however, were not coherent and do not give the impression that the (V) and (N) populations differ markedly or that nematode morphology was markedly changed by temperature as was the case with *A. avenae*.

6.5.9. *Discussion*

D. dipsaci comprises many populations which differ in their host affinities (SEINHORST 1956, HESLING 1966). Such populations are sometimes indicated as strains, races, biotypes or pathotypes. OOSTENBRINK (1968) proposed the term trophotypes to indicate populations which differ in feeding and reproduction. STURHAN (1964a) proved, that different strains can be interbred and that the offspring may comprise new strains with host affinities differing from both parent strains. He postulates that the differences are genetic and can be explained by the Mendel's laws. Several authors have observed small morphological differences between strains (STEINER 1956) and some specific names are proposed for such strains, e.g. *D. allii* Beyerinck, 1883 and *D. phloxidis Kirjanova*, 1951.

It is, however, accepted by most nematologists that we have to do with one species comprising many physiologically different strains. In our experiments three strains from the Netherlands were used, indicated as (N) onion, (N) tulip and (N) narcissus, and one population from Venezuela indicated as (V) garlic, which may possibly be identical to the firstmentioned strain.

D. dipsaci is widespread as a noxious plant parasite which is recorded mainly from places with a moist, temperate climate. In Venezuela it is only reported

from garlic and onion at high altitudes. These crops are also grown in the tropical lowlands at 400 m above sea level, but no infestation occurs there. These observations fit in with the data by different authors collected in Table 2 which suggest that the optimum temperatures for infection, reproduction and other activities are generally between 10° and 20°C. The marked specialization in strains with different host affinities, however, suggests that specialization in strains with different temperature requirements may also exist. Our experiments with *D. dipsaci* populations in temperature ranges and gradients confirm this supposition.

(N) onion, reared in onion bulbs, was less thermophil than (V) garlic since it reproduced better at 10° and 15°C and not at all at 30°C (Figure 21). The difference was also noticeable when these populations were grown in phlox plants. Both populations had an optimum at 15°C, but the (N) onion reproduced better at 10°C and (V) garlic showed an aberrant strong increase at 30°C (Figure 23).

(N) tulip and (N) narcissus, reared in onion bulbs, reproduced equally well with an optimum at 20°C. (N) narcissus was not less, maybe slightly more thermophil than (N) tulip as far as reproduction in onions concerned (Figure 25). (N) tulip was also reared in phlox plants and reproduced well at all temperatures from 10° to 30°C, with a broad optimum around 20°C. Comparison of Figure 27 with Figure 23 reveals that (N) tulip is more thermophil than (N) onion, which population did not reproduce well at 25° and 30°C and had its optimum at 15°C.

Heat treatments of nematodes in water baths revealed that (N) tulip was significantly more resistant to high temperatures than (N) narcissus (Table 35) and that both populations grown at 25°–30°C were more resistant to heat than when grown at 10°–15°C. It appeared that a percentage of nematodes from (N) tulip and (N) narcissus were motile after treatments at 46°C for 20 minutes and at 47°C for 15 minutes, but that these nematodes could not reproduce in onion bulbs and in tulip or narcissus bulbs respectively, contrary to untreated nematodes (Table 36). The greater resistance to heat treatments found for (N) tulip as compared with (N) narcissus is in agreement with the difficulty to obtain satisfactory control of (N) tulip by hot water treatments of flower bulbs in practice.

It appears that (N) tulip is significantly more resistant to heat treatments not only when reared at 25°–30°C instead of at 10°–15°C, but also when it is given a pre-treatment for 1–16 hours at 35°C (Table 37). This must be due to conditioning or acclimatization of the nematodes, as was recorded for the narcissus strain by GREEN (1964). Another acclimatization result was reported by CROLL (1967). The acclimatization effect seems to increase with time, since a pre-treatment of 16 hours at 35°C caused more survival than 1 hour. Such a cumulative conditioning effect was found by BRUN (1966) with *Caenorhabditis elegans*. It further appears that the increased heat resistance obtained at 35°C is lost again when the nematodes are kept for 14 hours at 20°C, but not at 3°C. Heat resistance may well be associated with a chemical principle.

The morphology study revealed that the (V) garlic and the (N) onion populations differed in respect of several characters at one of the two rearing temperatures, 15° or 20°C, but not in the same sense. Temperature also caused significant differences for several characters in (V) garlic and (N) onion, but the characters influenced and the influence itself varies. Moreover there were two characters which did not show any significant differences at all, either for temperature or for population. This makes it more doubtful than in the case of *Aphelenchus avenae* that the two populations are different species, despite the differences in temperature requirements and the differences which are noticed in some morphological characters of (V) garlic and (N) onion. Crossing between nematodes of the two populations are needed to decide here. The thermophil (V) population is smaller than the (N) population at 15°C, but not at 20°C. This supports the conclusion already drawn for *A. avenae*, that the size of the nematodes of the species *D. dipsaci* is greatest at the most favourable temperature, and not at the lowest temperature at which they may exist, a conclusion which therefore runs counter to Bergman's climatic rule.

6.6. Helicotylenchus dihystera (v)

Two experiments were made, one in greenhouse compartments and one in Wisconsin tanks, to study the reproduction of *H. dihystera* at different temperatures (6.6.1 and 6.6.2).

6.6.1. *Reproduction in greenhouse experiment*

Hand-picked *H. dihystera*, washed from soil with a stock culture of *H. dihystera* from Venezuela (V) were inoculated onto three weeks old tomato plants in plastic tubes with 300 ml of sterilized potting soil. Fifty nematodes were inoculated per tube; 8 tubes were placed in each of the greenhouse compartments at the following temperatures: 10°–15°–20° and 25°C; two plants from each compartment were evaluated after 8–10–12 and 14 weeks to make a series with time. The number of nematodes in the soil and in the roots was determined separately according to the methods described in Chapter 2. The data are collected in Table 39 A and B and in Figure 29G.

Table 39 shows that reproduction occurred at all temperatures in the range from 10 to 25°C. The population density in the soil as well as in the roots increased highly significant according to a linear trend with temperature, and was highest at 25°C. There was also a highly significant increase with time for the population in soil, but not for the population in the roots which apparently had passed its highest density after 14 weeks.

The thermogram of Figure 29G illustrates the total number of nematodes in soil and roots at the end of the experiment, e.g. after 14 weeks, in relation to temperature. It shows that *H. dihystera* prefers high temperatures and that its optimum temperature may well be higher than 25°C.

TABLE 39. Inoculation of *Helicotylenchus dihystera* (V) onto tomato plants in greenhouse compartments. Nematodes in the soil (A) and in the roots (B) 8, 10, 12 and 14 weeks after inoculation of 50 nematodes per tube of 300 ml with one plant, at 4 different temperatures. Each figure is the natural logarithm *log (x + 1) of the number of nematodes per tube or per plant as sum of two replicates (x), plus one. L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), * = significant ($0.01 < P \leq 0.05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non-significant ($P > 0.10$).

Greenhouse temperatures / Evaluation time	*A. Soils* 10°	15°	20°	25 °C	Time averages (L.S.D. = 0.88)
After 8 weeks	2.3	0.0	3.8	3.6	2.4
After 10 weeks	3.7	3.0	3.9	4.3	3.7
After 12 weeks	4.2	3.9	5.8	6.1	5.0
After 14 weeks	4.0	5.1	5.8	6.7	5.4
Temperature averages (L.S.D. = 0.88)	3.5	3.0	4.8	5.2	

Greenhouse temperatures / Evaluation time	*B. Roots* 10°	15°	20°	25 °C	Time averages (L.S.D. = 0.13)
After 8 weeks	0.0	2.3	2.0	3.6	2.0
After 10 weeks	0.0	3.4	2.3	3.4	2.3
After 12 weeks	1.3	3.0	3.7	4.6	3.1
After 14 weeks	1.7	0.0	3.7	4.8	2.5
Temperature averages (L.S.D. = 0.13)	0.7	2.2	2.9	4.1	

Analysis of variance:

	P values
A. Soil	
F temperature = 7.74	*
F time = 12.93	*
B. Roots	
F temperature = 6.78	*
F time = 0.83	–

6.6.2. *Reproduction in Wisconsin tank experiment*

A similar experiment as described under 6.6.1 was made in Wisconsin tanks, this time at five different temperatures, namely 15°–20°–25°–30° and 35 °C. The results are summarized in Table 40 A and B and Figure 29 WT.

In this experiment the populations in soil and in roots also increased highly significant with temperature and time. The results of Table 40 as well as of

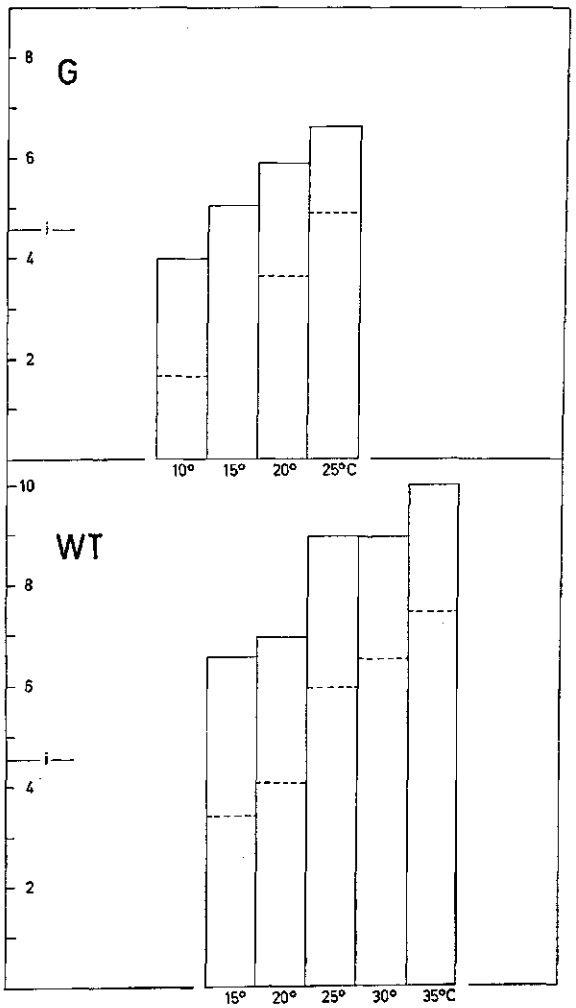

FIG. 29. Temperature thermograms of *Helicotylenchus dihystera* (V) for populations in the soil and in roots, 14 weeks after inoculation of 50 nematodes per tube of 300 ml of soil with one tomato plant.
G = Greenhouse experiment.
WT = Wisconsin tank experiment.
Ordinate: Natural logarithm $^{e}\log (x + 1)$ of the total number of nematodes of two replicate tubes, soil (——) or roots (- - -), (x), plus one
Abscissa: temperatures in the greenhouse compartments and in the Wisconsin tanks.

Figure 29 WT indicate that there was also strong reproduction at 35 °C, although the optimum found was 30 °C. From the earlier experiments in Wisconsin tanks it should be borne in mind that the temperature inside the plant may have been somewhat lower.

6.6.3. *Discussion*

H. dihystera from Venezuela (V) is evidently capable of reproducing at temperatures varying from 10 to 35 °C with its optimum at 30 °C or possibly higher. This nematode represents an example of a tropical nematode although it may thrive in somewhat cooler regions. It is normally found in the lowland of Venezuela, but it also occurs at high altitudes (1800 m above sea level).

TABLE 40. Inoculation of *Helicotylenchus dihystera* (V) onto tomato plants in Wisconsin tanks. Nematodes in the soil (A) and in the roots (B) 8, 10, 12 and 14 weeks after inoculation of 50 nematodes per tube of 300 ml with one plant, at 5 different temperatures. Each figure is the natural logarithm $^e\log(x+1)$ of the number of nematodes per tube or per plant as sum of two replicates (x) plus one.
L.S.D. = least significant difference at 5%, F = variance ratio, P = probability, ** = highly significant ($P \leq 0.01$), * = significant ($0.01 < P \leq 0.05$), (*) = nearly significant ($0.05 < P \leq 0.10$), – = non-significant ($P > 0.10$).

Wisconsin tanks temperatures / Evaluation time	*A. Soil* 15°	20°	25°	30°	35°C	Time averages (L.S.D. = 0.92)
After 8 weeks	5.6	4.7	4.8	6.2	6.1	5.5
After 10 weeks	4.6	6.5	7.3	7.6	6.9	6.6
After 12 weeks	5.3	6.6	7.1	8.8	8.5	7.3
After 14 weeks	6.6	6.9	8.9	8.9	10.0	8.3
Temperature averages (L.S.D. = 0.10)	5.5	6.2	7.0	7.9	7.9	

Wisconsin tanks temperatures / Evaluation time	*B. Roots* 15°	20°	25°	30°	35°C	Time averages (L.S.D. = 0.15)
After 8 weeks	3.4	1.7	0.0	4.0	3.5	2.5
After 10 weeks	3.2	0.0	3.4	5.4	4.5	3.3
After 12 weeks	2.3	1.7	5.0	6.0	5.3	4.1
After 14 weeks	3.4	4.1	5.9	6.5	7.4	5.5
Temperature averages (L.S.D. = 0.14)	3.1	1.9	3.6	5.5	5.2	

Analysis of variance:

	P values
A. Soil	
F temperature = 8.06	*
F time = 12.40	*
B. Roots	
F temperature = 5.87	*
F time = 5.30	*

6.7. PRATYLENCHUS CRENATUS AND ASSOCIATES (N)

Two experiments were made, one in greenhouse compartments and the other in Wisconsin tanks, to study the reproduction of these nematode species at different temperatures. The nematodes used in these experiments were a natural community occurring in a sandy loam soil of a plot at the Plantenziektenkundige Dienst, Wageningen. This plot was continuously under maize for several years

and the soil harboured large numbers of *P. crenatus*, *Tylenchorhynchus dubius* and *Rotylenchus robustus*, as well as other species.

The initial density was determined by extraction and appeared on an average to be 750 *P. crenatus* + 870 *T. dubius* + 1350 *R. robustus* per 300 ml soil.

6.7.1. *Reproduction in a greenhouse experiment*

Fifty 300 ml plastic tubes as used in earlier experiments were filled with the naturally infested soil and planted with maize; 10 tubes were placed in each of the greenhouse compartments with the following temperatures: 10°–15°–20°–25° and 30° (28°–30°C) in January 1967; two plants from each compartment were harvested after 38–53–68–83 and 98 days. The nematodes were extracted from the soil and the number of *P. crenatus*, *T. dubius* and *R. robustus* were counted. The roots were also extracted to determine the number of the endoparasitic *Pratylenchus* inside the roots. The results are described below or summarized in Table 41A.

TABLE 41. Populations of *Pratylenchus crenatus* (P), *Tylenchorhynchus dubius* (T) and *Rotylenchus robustus* (R) as a natural community in soil under maize, at different temperatures in greenhouse compartments (A) and in Wisconsin tanks (B).
Nematodes per two replicate tubes of 300 ml after 98 days. Each figure is the natural logarithm $^e\log(x + 1)$ of the number of nematodes per two replicate tubes or root systems (x) plus one.
F = variance ratio of analysis of variance; ** = highly significant ($P \leqslant 0.01$), * = significant ($0.01 < P \leqslant 0.05$), (*) = nearly significant ($0.05 < P \leqslant 0.10$).

Nematodes Between brackets the original figures		A. Greenhouse temperatures 10°	15°	20°	25°	28°–30°C	F temperature
P in soil	(7.31)	7.9	8.3	7.0	6.6	6.1	8.03**
P in soil + in roots		8.6	8.4	7.4	7.1	7.0	
T in soil	(7.46)	7.6	6.1	6.6	7.0	6.2	3.17*
R in soil	(7.90)	6.9	6.4	4.3	4.3	5.6	6.70**

Nematodes Between brackets the original figures		B. Wisconsin tanks temperatures 15°	20°	25°	30°C	F temperature
P in soil	(7.31)	8.3	8.2	7.7	6.6	16.08**
P in soil + in roots		8.6	8.4	8.2	7.4	
T in soil	(7.46)	7.7	7.6	6.3	5.9	3.47*
R in soil	(7.90)	7.7	7.4	6.5	7.6	2.84(*)

Analysis of variance of the logarithmically transformed nematode numbers showed that the densities of all three species in the soil decreased linearly with temperature. This indicates that all three species are cryophil or thermophob. The optimum temperatures were apparently 10°–15°C, and there was some increase in the density with time at the low but not at the high temperatures.

This result is marked when we take into account that plant growth was better at the higher temperature: root weight at 28°–30°C was 2.44 g against 0.61 g at 10°C. The nematode numbers per tube at the end of the experiments, after 98 days, are recorded in Table 41A, with an indication for the significance of the influence of temperature calculated from all data. It cannot be determined with certainty how much of the final population belongs to the initial populations and how much is due to multiplications.

The density of *P. crenatus* in the soil at 10° and 15°C is higher than the original density, but at the high temperatures it is lower. This holds when the nematodes in the roots were taken into account. The original densities of *T. dubius* and *R. robustus* dropped slightly at 10°C and much more at higher temperatures.

6.7.2. *Reproduction in Wisconsin tank experiment*

A similar experiment as described under 6.7.1 was made in Wisconsin tanks, but with temperatures 15°–20°–25° and 30°C. The results were treated in the same way as described under 6.7.1 (see also Table 41B). It appears also from this experiment that the lowest temperature 15°C, is more favourable to the nematodes than higher temperatures, despite the fact that plant growth was better at higher temperatures (root weight per tube at 10° and 30°C was 0.98 and 4.80 g respectively).

6.7.3. *Discussion*

P. crenatus, *T. dubius* and *R. robustus* are widespread on sandy soils in Western Europe; *P. crenatus* is found in Venezuela only at high altitudes. They are considered to be nematodes of cool climates. Our experiments confirm that 10°–15°C is probably optimal, and was at any rate more favourable than higher temperatures for the reproduction of these nematodes despite less roots at low temperature. It is difficult to separate the effect of reproduction from the effect of conservation, which may also have been higher at low temperature. If we consider the increase of populations with time within each temperature, than we find also more increase at the lower temperatures. This confirms the conclusion that they must be considered cryophil nematodes. Inoculation experiments with these nematodes should therefore take place at low temperatures. This result may help to explain controversial results about the effects of these nematodes in inoculation experiments recorded in literature.

6.8. Natural nematode community in soil (N)

Most nematode inoculations are effected by the involuntary spread of soil comprising a natural nematode community. The fate of such a community in soil when exposed to different temperatures for different periods was determined.

6.8.1. *Survival in soil*

An amount of sandy clay soil was collected from around willow trees in a

meadow at Beesd, the Netherlands. It was freed from roots and coarse objects and thoroughly mixed. Then a metal tunnel of 192 × 10 × 5 cm, fitted along a series thermostat and connected with the temperature elements, was filled with the soil. Eight successive sections of the soil bar, each 24 cm long, were exposed to temperatures of about 5°–10°–15°–20°–25°–30°–35° and 40°C. At regular intervals after the beginning of the experiment, viz. after 1–1½–2–2½–3 and 3½ months, 100 ml of soil was taken from each temperature section and the nematode population was quantitatively extracted and analysed. The main nematode species or groups found to be present before the experiment started, with the numbers per 100 ml of soil indicated between brackets, were: *Trophurus imperialis* (100), *Helicotylenchus pseudorobustus* (470), *Rotylenchus fallorobustus* (10), *Criconemoides morgensis* (380), *Paratylenchus straeleni* (42), other Tylenchida (1000) and saprozoic nematodes (440). The results of the experiment are given in the 7 diagrams, one for each nematode species or group of Figure 30. The density of each nematode category is indicated by a colour shade, for each temperature-time combination.

The visual presentation of the results in Figure 30 indicates a few results, which are marked and convincing.

All nematodes or nematode groups maintained their density at low temperatures from 5 to 15°C. Throughout the experiment the numbers for *Rotylenchus* and *Paratylenchus* were higher and those for the other Tylenchida were lower than was the case in the sampling before the experiment started. It was realised later that larvae of *Rotylenchus*, which was a scarce species, and larvae of *Paratylenchus*, which have an aberrant shape, may not have been properly identified at the preliminary sampling and were probably shifted to the other Tylenchida.

At 40°C all Tylenchida were extinct after a month and did not reappear later. Only the saprozoic nematodes maintained a high density, although they were less numerous at 40°C than at lower temperatures.

At 35°C *T. imperialis* was extinct after a month, this was true for *Criconemoides morgensis* after 2 months, for *H. pseudorobustus* and for *Pa. straeleni* after 2½ months, for *R. fallorobustus* after 3 months and all other Tylenchida after 3½ months, whereas the saprozoic nematodes became less numerous after 3 months.

At 30°C all specifically mentioned tylenchid species disappeared gradually and were extinct after 3½ months; as were the other Tylenchida.

At 25°, even at 20°C, there are indications that the tylenchid species, including the other tylenchids were finally reduced to low densities, lower than at the temperature range from 5°–15°C.

6.8.2. *Discussion*

The marked suppression of all active Tylenchida at temperatures from 20°C upwards and their complete removal from their natural community in soil within 3½ months at temperatures from 30°C upwards is a noteworthy result. It indicates that none of the phytophagous nematodes of this community from a temperate zone would have survived the transmission of the infested soil to a

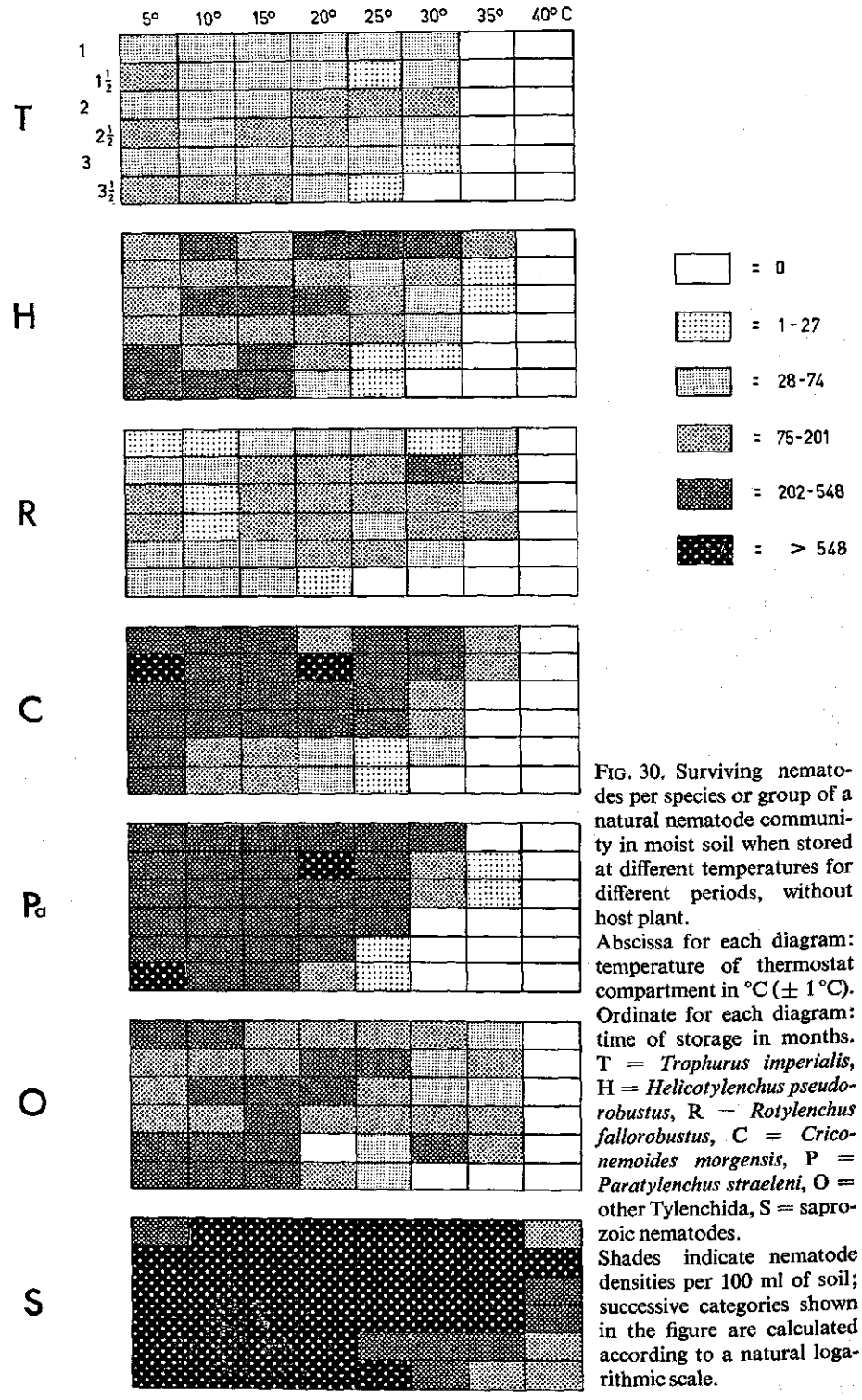

FIG. 30. Surviving nematodes per species or group of a natural nematode community in moist soil when stored at different temperatures for different periods, without host plant.
Abscissa for each diagram: temperature of thermostat compartment in °C (± 1 °C).
Ordinate for each diagram: time of storage in months.
T = *Trophurus imperialis*, H = *Helicotylenchus pseudorobustus*, R = *Rotylenchus fallorobustus*, C = *Criconemoides morgensis*, P = *Paratylenchus straeleni*, O = other Tylenchida, S = saprozoic nematodes.
Shades indicate nematode densities per 100 ml of soil; successive categories shown in the figure are calculated according to a natural logarithmic scale.

tropical region where the temperature is normally 25°–35°C, even if the soil is kept moist. The studied nematode community was randomly chosen and it is probably that other populations are equally susceptible. Good persistence of phytophagous nematodes for 3½ months at 5°C (the normal storage temperature for samples in nematology laboratories) and for 3½ months at 10° to 5°C or for 2½ months at 20°C (the normal soil temperatures in temperate climates) could be expected from other studies (OOSTENBRINK 1960a, 1966b). The experiments show that temperature has a determinant direct influence on the fate of nematode infections, as is indicated in the scheme of Figure 2.

The result applies to nematodes in moist soil without a host plant; our data do not give information about the fate of nematodes in dried soil or in moist soil when a host can be reached. It is well known however that most phytophagous nematodes are susceptible to drought and that drought periods cause heavy population losses even among nematodes in their natural soils (WALLACE 1963, ADHAMI thesis, *in litt.*). Some endoparasites of aerial plant tissues developed extreme drought resistance, but they can not become active either unless moisture becomes available. It is therefore unlikely that phytophagous nematodes transmitted in dry soil to tropical regions stand a better chance of surviving than phytophagous nematodes in soil which stays moist.

The chance that plant nematodes from a temperate zone would have survived longer than in the experiment if host plants had been available, is not excluded. Most plant nematodes in natural populations are polyphagous and could probable thrive on tropical plants, as far as plant characters are concerned (SHARMA 1968). Plants under warm conditions may also be somewhat cooler than the rest of the environment, as was indicated by some of our Wisconsin tanks experiments. It was, however, clear from the foregoing experiments that nematodes from temperate regions thrive less well at high temperatures. And it is also clear from the faunitic lists made (Table 2) that plant nematodes from temperate regions do not occur in the tropical lowlands of Venezuela. It seems reasonably certain now that this is determined by the influence of temperature, directly on the nematodes in the soil or in and around the host plants.

The saprozoic nematodes, as a group, were less susceptible to high temperatures than the plant nematodes. This fits in with the data of section 3.4 which indicate that few possible ubiquists were nearly all saprozoic or microphagous nemades. At the other hand, it is possible that some species of this heterogeneous group have disappeared and that some others were more thermophil and multiplied on the micro-organisms in the soil during the incubation period.

SUMMARY AND CONCLUSION

General introduction and literature review

The purpose of this study has been to evaluate geographic variation of the nematofauna, to analyse important influences, and to determine the influence of climate, and how it works. The experimental part of this study is restricted to soil and plant nematodes with emphasis on the nematofaunas in Venezuela and in the Netherlands. The introduction and literature includes also freshwater and marine nematodes.

The extensive literature survey summarizes data about occurrence and main characteristics of nematodes. They are the most ubiquitous group of the animal kingdom, constituting more than 80% of all multicellular animals (Table 1). Despite numerous inventories made for faunistic and agricultural purposes all over the world, pertinent knowledge about the geographic distribution of well defined species is scarce and data to explain the situation are fragmentary. This is due to incomplete coverage of the sampling areas and of the habitats within an area, to erratic collection and poor identification of the nematodes, and to the primitive state of study on nematode biology and physiology.

An attempt is made to review faunistic nematode studies in three sections. The initial surveys (1.2.1), from BORELLUS' discovery of the first freeliving nematode in 1656 to DE MAN's classic faunistic studies in 1884, are discussed in detail. They aroused the interest of many zoologists and stimulated activity. The zoological surveys and expeditions (1.2.2), numerous from 1884 up to World war II and later more incidental, are fully recorded and briefly discussed where necessary, to obtain a near-complete review of this period. The results ascertained the widespread occurrence and the pluriformity of nematode communities in widely varying habitats and furnished some ecological observations on relation of nematodes to food, moisture, soil type and climate. The inventories for agricultural purposes (1.2.3), rapidly increasing in number up till now, are already far too numerous to be cited here. The relevant publications are listed in a separate publication (DAO *et al* 1970). The information is brought together in sections on general surveys per area (in which the areas covered are listed), general surveys per plant (in which the plants or plant groups studied are recorded), and surveys for a specific plant nematode or group of nematodes. The distribution pattern of the best studied species, *Heterodera rostochiensis*, is mapped (Figure 1). The inventories for agricultural purposes reveal the widespread occurrence of dense populations of plant nematodes, omnipresent in all soils and all crops, and suggest strong influences of plant species, soil type and geographic situation.

The main environmental influences on a nematode population in the soil are listed and are synthesized into a qualitative scheme which is descriptive, functional and causal with respect to the relations between nematodes and their environment (Figure 2). Essential modifications from similar schemes for other animals are the acceptance of a direct influence of climate, soil and other physic-

al and chemical components on nematode populations, and the omitting of active migration due to limited locomotion. The passive spread of nematodes, however, is extremely efficient. Together with their great persistence, their polyphagy and lack of interspecific competition, this potency may account for the widespread occurrence of polyspecific nematode mixtures in soil. Nematodes are normally carried passively but alive into a habitat, after which the environment becomes operative. They have to survive and reproduce before the population becomes measurable and can be related to the geographic environment. As soon as a population is measurable it is already certain that the habitat affords suitable conditions for survival and propagation of the species.

Climate, soil and plant factors are recorded as determinant for the development of nematode populations. Because it will appear later that soil, plant and other local factors cannot account for the geographic variation of the nematofauna, further attention is focussed on climate. The influence of climate and weather, average and momentary effects of atmospheric conditions, is usually reflected in the main density and the density fluctuation of a population. Most nematode infections carried into unsuitable climate and soil environments become extinct. It is stressed that most population-dynamic studies have been on existing, and therefore successful combinations of species and environment. KLOMP's statement (1962) that climate or weather cannot regulate density directly, because too many populations would soon become extinct, is meant for such established, and therefore selected populations. Innumerable nematode inoculations, however, must become extinct due to the direct influence of climatic, soil and other environmental influences, immediately or after a number of generations. This direct influence may be called destruction or determination rather than regulation, but it must be a main governing process in determining the borders of the range of nematode species and it is therefore included in the scheme of Figure 2.

Temperature will appear as the main climatic factor related to geographic distribution. Therefore literature data about temperature are collected. Nematological thermograms show the normal type of biological optimum curve, demonstrating kill, cold stupor, limited activity, optimum, limited activity again, heat stupor and kill successively in a gradient from low to high temperature.

Complete data on any one species are not available, but bits of information on more than 140 different cases of a nematode's activity related to temperature could be listed (Table 2). Most soil and plant nematodes are active and thriving between 15° and 30°C and become motionless at 5°–15°C and at 30°–40°C.

Minimum temperatures for hatch and emergence, invasion and activity and optimum temperatures for reproduction and other processes are apparently low for plant parasites in temperate climates (e.g. *Heterodera avenae, H. trifolii, H. rostochiensis, H. schachti, Ditylenchus dipsaci, Meloidogyne hapla*), whereas they are high for plant nematodes from warmer regions (e.g. *Hemicycliophora arenaria, Tylenchulus semipenetrans, Trichodorus christiei, Scutellonema brachyurum, Heterodera glycines, Meloidogyne* and some other species). The

maximum temperatures for activity, generally between 30° and 35°C, do not seem to be correlated with the geographic range of the nematodes. Temporary tolerance to extreme temperatures, and to drought, may be determinant for survival, and for the geographic range of a species, and diurnal and seasonal fluctuations may increase or decrease certain nematode activities and their susceptibility to extreme temperatures. The literature already suggests a strong influence of temperature on the distribution pattern of nematodes.

Materials and methods

The materials and methods regularly used in the experimental work are described in Chapter 2. The faunistic studies and the evaluation of population densities in soil, plant tissues or other substrates were based on standard, known sampling techniques. The nematodes for faunistic and taxonomic studies were put into permanent slides and stored in the collection of the Landbouwhogeschool. The nematode populations for experimental work were some natural communities in their original soil, and also a number of selected species from Venezuela and the Netherlands reared and kept as monospecific populations in greenhouse compartments: *Meloidogyne incognita* from Venezuela (V) and from the Netherlands (N), *M. hapla* (V) and (N), *Ditylenchus dipsaci* (V) and (N), *Aphelenchus avenae* (V) and (N) and *Helicotylenchus dihystera* (V). The main plants used for nematode propagation and for experiments were tomato, maize, phlox and bulbs of onion, tulip and narcissus, as well as agar cultures of *Alternaria solani*. The soil was usually sterilized potting soil. Apart from clay pots and petri dishes the usual containers for nematode studies on plants were plastic tubes of 4 × 4 × 20 cm. Different facilities or apparatuses were used to control temperature, namely greenhouse compartments and climatic cells, Wisconsin tanks, a series thermostat and special incubators.

Estimation of nematode densities in soils, plant tissues and agar cultures was made according to methods described by OOSTENBRINK (1960) and S'JACOB & VAN BEZOOIJEN (1967), with some modifications for extraction of *Aphelenchus avenae* from agar fungus cultures. Most experiments were replicated and the results were statistically treated, after transformation of the nematode and plant figures as indicated in the tables if this was considered desirable.

Analysis of the nematofaunas in Venezuela and the Netherlands

Table 3 lists the nematode species recorded from Venezuela and from the Netherlands, 176 and 425 species respectively. These countries were chosen as test areas for comparison of their nematofaunas and for the selection of suitable test species because: a) the countries represent a tropical and a temperate climate, however with temperate conditions on the Venezuelan mountains and with tropical conditions in greenhouses in the Netherlands; b) the nematofaunas of both countries are fairly well studied; c) the nematodes of both these countries have largely been identified and could be checked at one place, namely the Landbouwhogeschool, Wageningen; d) the author was personally involved in nematode studies in both countries.

Table 3 shows that the nematode faunas of the two countries differ markedly. The difference is evident at the generic level and certainly in the species range of genera with many species. Scrutiny of the data reveals that of the 63 species reported from both countries only 21 may safely be considered to occur in the tropical and the temperate climate, whereas 7 were incorrectly identified for one country or the other, 13 appeared to be uncertain for poor state of taxonomy of the genus in question or for insufficient species diagnosis and 22 were found at high altitudes in Venezuela or in greenhouses in the Netherlands only. The 21 species which may live in both climatic zones, against at least 362 in the Netherlands only and 113 in Venezuela only, are microphagous or predatory, with only two possible plant parasites: *Macroposthonia sphaerocephala* and *Nothocriconema mutabile*. Ubiquitous species, therefore, are rare.

Transmission and inoculation experiments

The differences in nematofauna cannot be related to the geographic situation of the two countries as such or be due to insufficient transportation of inoculum, for many tropical species are found in Dutch greenhouses and many temperate species on Venezuelan mountains. The known records of plant parasites are brought together in Table 4. Environmental factors must therefore be instrumental. The influence of climatic factors was studied in inoculation and transmission experiments in which the influence of soil, host and other organisms were excluded as far as possible.

Inoculation experiments at a lowland station of Maracaibo, followed by transport of half the pots to an altitude of 2800 m after two months, revealed that subsequently the lowland species *Meloidogyne incognita, Pratylenchus zeae* and *Helicotylenchus dihystera* developed better at 0 m and the mountain species *M. hapla* developed better at 2800 m (Table 5). *Aphelenchus avenae* developed at both altitudes in this experiment.

Transmission of a natural lowland nematode community in its original soil to the mountain and of a mountain community to the lowland, with control pots at the original locality, confirmed this result of the inoculation experiments. Most of the lowland species declined at high altitudes and the mountain species at low altitudes, except the composite group of saprozoic nematodes (Table 6).

Transplantation experiments with tropical *Meloidogyne* species in the Netherlands revealed that these species cannot maintain themselves out of doors (4.4). Infested plant material planted outdoors is nematode-free after one to three seasons.

The direct influence of locality, soil, plants and other growth factors was excluded here. This leaves temperature, and for the experiments in Venezuela possibly air pressure, as instrumental influences which have to be studied further.

Air pressure effects

Experiments in exsiccators showed, that the (V) and (N) populations of *A. avenae* and *D. dipsaci* (V) and (N) did not reproduce less at a low air pressure

equivalent to the situation at 3000 m above sea level than at the normal atmospheric air pressure at sea level (Tables 8–11). Low air pressure as at high altitudes therefore does not determine the reproduction of these nematodes, and it is unlikely that it influences the geographic range of nematodes.

It was found in these experiments that the reproduction of *A. avenae* on cultures of *Alternaria solani* and of *Ditylenchus dipsaci* in onion bulbs were both suppressed when too many fungal cultures or too many onion bulbs were placed together in a closed exsiccator. The fungus grew poorly and the onion bulbs decayed. It is probable that these materials released nematicidal agents, which could not be fully identified by gas chromatography.

Temperature effects

It appeared from the Chapters 3, 4 and 5 that climatic influences on nematode communities are great, and by exclusion of the other influences the conclusion seems justified that temperature is the key factor. Therefore the influence of temperature ranges and gradients on biological activities of one or more populations of six nematode species was extensively studied.

Meloidogyne incognita. Comparative experiments with the (V) and (N) populations were made on hatching (Figures 3 and 4, Table 12), on penetration, reproduction and gall formation after inoculation onto tomato plants in greenhouse compartments (Tables 13 and 14, Figure 5), in Wisconsin tanks (Tables 15 and 16, Figure 6) and in climatic cells (Tables 17 and 18, Figure 7), and about the morphology (Figure 8, Table 19). Both populations appear to be thermophil. Hatching, gall formation and reproduction was abundant and rapid at 25° and 30°C and for the (N) population also at 20°C. They were less at 15°C, except for nematodes that had already penetrated at a higher temperature, and they dropped to a low value at 10°C. A 15-day exposure of eggs in water at 5° or 35°–40°C killed more than 80% of both populations (Table 12). The temperature of 35°C was too high for the eggs in water, but galling and reproduction on tomato occurred at 35°C, probably because a growing plant is cooler than the surrounding soil at high temperatures. The (V) and (N) populations were morphologically the same, but the (N) population was somewhat more thermophob and markedly less cryophob than the (V) populations which may be significant for the geographic range of the nematodes. All data together indicate that the minimum temperature for the (N) populations to start infection and reproduction is about 10°C which is about 5°C lower than for the (V) populations, and also that the larvae of the (N) population inoculated in soil may survive 15°C for at least 4 weeks whereas the (V) population cannot survive that period. It therefore apears that there are strains with different temperature requirements in the morphological species *M. incognita* which were still present after propagation of the nematodes on the same plant in the same environment for more than one year. We propose to indicate strains with a fixed difference in temperature requirements as thermotypes.

Meloidogyne hapla. Experiments similar to those described for *M. incognita* were made with the (V) and (N) populations of *M. hapla*, and one experiment

was added on survival at low temperature (Figures 9–13, Table 20–29). Both *M. hapla* populations appear more resistant to low temperature than *M. incognita*. Their survival was good at 5 °C in water (Table 20) and at 0 °C in soil whereas there was partial survival in soil at–10 °C for several days (Tables 27 and 28). Both populations could start some activity at 10 °C, but for the rest they were thermophil and could thrive at 30° and 35 °C like *M. incognita*. The fact that *M. hapla* is widespread out of doors in temperate climates is therefore explained, for the species can thrive at low temperatures and can survive spells of low temperature, but unexplained is the fact that it is rarer in tropical regions and in greenhouses than *M. incognita*, for its thermopreferendum is as high or higher. The two *M. hapla* strains differ in morphology (Table 29) and in development of males (males at 35 °C only in the (V) population but not at all in the (N) population) and there were slight differences in their temperature requirements. The (N) population appeared somewhat more resistant to adverse low and high temperatures and was somewhat more thermophil in its biological activities than the (V) population. These differences are small compared to the differences between *M. hapla* and *M. incognita*.

Aphelenchus avenae. Experiments were in temperature ranges and gradients with the (V) and (N) populations grown on agar plates with *Alternaria solani*. They were on reproduction (Figures 14 and 15, Tables 30 and 31), adaptation to lower temperature (Figure 16), sex ratio (Tables 32 and 33), influence of contaminating fungi (Figure 17) and morphology (Figures 18 and 19, Tables 34). Both populations are apparently thermophil; the differences between the two populations in several biological and morphological aspects, however, suggest that they may not be the same species. Reproduction occurs from 16 °–35 °C in the (V) and probably from 5 °–30 °C in the (N) population, with an optimum for (V) slightly above and for (N) slightly below 25 °C. The cryophob (V) population gradually reproduced somewhat better, although with temporary depressions, when grown for 24 months at 18 °C (Figure 16) without losing its potency to reproduce strongly at 25 °C. Males are absent at 25 °C, but a less numerous population consisted mainly of males when populations were grown at 32 °–33 °C. Temperature appears to determine the formation of males in *A. avenae* and this holds for both populations (Table 32). The influence of temperature is probably direct and not indirect via the fungus (Table 33). The main contaminating fungus in the *A. avenae* cultures on *Alternaria solani* was *Trichoderma koningi*, which released a nematicide into the cultures (Figure 17). Temperature influenced the morphology of *A. avenae* in all eight characters studied (Figure 18) and there were significant differences between the (V) and (N) populations at one or more temperatures for all morphological characters studied (Table 34, Figure 19). The two populations are probably different species. BERGMAN's climatic rule that vertebrates of the same species grow bigger when they live at lower temperatures does not apparently hold for nematodes.

Ditylenchus dipsaci. Several experiments were made in temperature ranges or gradients with (V) garlic and with (N) onion, (N) tulip and (N) narcissus populations. The experiments were on reproduction in onion bulbs and phlox

plants (Figures 20–27), on survival and conditioning of the narcissus and tulip strains in hot water (Tables 35–37), and on morphometric variation (Table 38, Figure 28). The optimum temperatures for infection, reproduction and other activities of *D. dipsaci* are generally between 10° and 20°C (Table 2) but it appears that populations differ significantly. The (N) onion population, grown in onion bulbs and in phlox plants, was less thermophil than the (V) garlic population although the last mentioned population is restricted to high altitude in Venezuela. Temperatures of 15°–20°C were favourable for both populations, but (N) onion thrived relatively better at 10°–15°C, and (V) onion was more numerous at 25–30°C; in fact (V) garlic showed an aberrant strong increase at 30°C in phlox (Figures 21 and 23). (N) tulip and (N) narcissus showed a relatively broad temperature spectrum with the same optimum of 20°C for reproduction; they are apparently more thermophil than (N) onion. Although the difference is small, (N) tulip appeared to be significantly more resistant to destruction by heat in water than (N) narcissus. Nematodes of both populations grown at the high temperature of 25°–30°C were more resistant to heat than populations grown at 10°–15°C; this difference was maintained for some weeks when the nematodes were stored at 3°C (6.5.5, Table 35). The (N) tulip population was also more resistant to heat after a pre-treatment at 35°C for 1–16 hours. This resistance is lost again when the nematodes are kept at 20°C for 14 hours, but not at 3°C (6.5.7, Table 37). It is postulated that this temporary resistance induced by high rearing temperature or by pre-treatment at 35°C is associated with a chemical principle. It was further noticed that nematodes may be motile after certain heat treatments, but nevertheless must be damaged to such an extent that they cannot stay alive and reproduce in their hosts (6.5.6). The data collected may help in understanding and in improving the results of commercial treatment of flower bulbs with hot water.

Some morphological characters showed differences between the (V) garlic and (N) onion populations at one of the two rearing temperatures but the characters influenced and the influence itself varies. And there are characters which did not show differences at all, either for population or for temperature (Table 38, Figure 28). This makes it unlikely that the two populations (V) garlic and (N) onion are different species although the significant differences noticed in some morphological characters and in the temperature requirements suggest that the possibility should not be excluded. Crossing between nematodes of the two populations are needed to decide this.

Helicotylenchus dihystera. The influence of temperature on reproduction of a tropical (V) population on tomato was studied in inoculation experiments in greenhouse compartments and Wisconsin tanks (Tables 39 and 40, Figure 29). Reproduction occurred at all temperatures tested in the range from 15° to 25° or 35°C in the greenhouse and Wisconsin tanks respectively. The population density, in the soil and in the roots, increased linearly with temperature up to the highest temperatures, 25° and 35°C in the experiments mentioned. *H. dihystera* therefore is a tropical nematode, although it may perhaps thrive in somewhat cooler regions.

Pratylenchus crenatus (N) and associates. Soil with a natural community of *Pratylenchus crenatus, Tylenchorhynchus dubius* and *Rotylenchus robustus* was grown with maize at different temperatures in greenhouse compartments and Wisconsin tanks (Table 41 A and B). The population of all three nematodes were optimum at 10°–15 °C and declined at the higher temperatures, although root growth increased much with temperature. After 98 days the populations at 25°–30 °C were normally half to one twentieth of the original populations or of the populations at 10 °C.

Treatment of a natural (N) nematode community in moist sandy clay soil without a host plant at different temperatures for periods up to 3½ months yielded interesting results (Figure 30). All phytophagous Tylenchida were suppressed from 20 °C upwards and they were eradicated at 25°–30 °C or higher within 3½ months. Temperature apparently has a determinant influence on the course of plant nematode infections taken in soil from temperate zones into tropical regions; the same may hold for tropical nematodes taken to a temperate climate. The saprozoic nematodes as a group were less susceptible to heat than the plant nematodes. These data are in accordance with the faunistic data of Chapter 2 which indicate that the few possible ubiquists are usually saprozoics.

Conclusion

The nematofaunas of the two climatic zones, tropical Venezuela and the Netherlands, differ conspicuously in the species. The few possible ubiquists found in both climatic zones do not comprise known or suspected plant parasites except two criconematid species. The fact that many tropical plant nematodes are common in Dutch greenhouses and temperate species are found in Venezuelan mountains indicates the far spread of inoculum. The results may be of value for plant quarantine.

Experimental exclusion of the direct influence of locality, soil, plant and other growth factors shows that the distribution range of nematodes must be determined to a large extent by climate, especially by temperature, as could be expected from the literature.

Effects of temperature on survival and thrift of a nematode differs markedly between species and between populations of the same species. The climatic or geographic range of a nematode appears closely related to lethal temperatures, or optimum temperatures for activity, or diverse combinations.

Temperatures of 25 °C and higher were lethal to plant nematodes in unplanted soil from the temperate zone, and temperatures of 5 °C or lower were lethal to nematodes from the tropical zone within ½–3½ months. This excludes hibernation in the other climate in the absence of growing hosts. Also established populations on growing host plants placed in the other unsuitable climate decline or disappear, although this takes apparently much longer than in the absence of hosts.

The temperature influences found to be instrumental for the populations studied are summarized in Table 42. The populations of the tropical zone do

not start their activity under 15°C and thrive at 25°–35°C, e.g. *M. incognita* (V), *A. avenae* (V) and *H. dihystera* (V). *M. incognita* (N) from Dutch greenhouses is intermediate. The nematodes from the temperate climate, the Netherlands and the Venezuelan mountains, survive spells of very low temperature and become active at about 10°C. The temperature range at which they thrive varies; it may be low, i.e. 10°–20°C for *D. dipsaci* (N) and *P. crenatus* (N), moderate i.e. 15°–30°C for *A. avenae* (N) and *D. dipsaci* (V), or even as high as for the tropical species, i.e. 20°–35°C for *M. hapla* (V) and (N). *M. hapla* (N) and (V) do not differ from *M. incognita* (N) except for the fact that the last species cannot survive very low temperatures. The populations which were studied appear to be well temperature-adapted to their climate, but the mechanisms vary.

TABLE 42. Summary of critical temperatures in °C for the nematode populations studied in Chapter 6.

Nematodes studied	Minimum for activity	Favourable for activity	Nearly lethal
Meloidogyne incognita (V) – lowland	15°	25°–35°	15° for 2 weeks
Aphelenchus avenae (V) – lowland	15°/20°	20°–35°	16° for 15 days
Helicotylenchus dihystera (V) – lowland	15°	25°–35°	
Meloidogyne incognita (N) – greenhouse	10°	20°–35°	15° for 4 weeks
Meloidogyne hapla (V) – mountain	10°	20°–35°	– 5° for 10 days
Meloidogyne hapla (N)	10°	20°–35°	–10° for 10 days
Aphelenchus avenae (N)	5°/15°	15°–30°	5° for 8 weeks
Ditylenchus dipsaci (V) – mountain	10°	15°–30°	
Ditylenchus dipsaci (N)	10°	10°–20°	
Pratylenchus crenatus (N)	< 10°	10°–20°	

In the same morphological species the temperature requirements of populations differ. These differences are stable and therefore probably genetic (thermotypes). Some adaptation or selection occurred when *A. avenae* (V) was grown at a sub-lethal low temperature for 24 months. Rearing and storage temperatures induced a principle for heat resistance in *D. dipsaci* which was not stable and may be of chemical nature. At the other hand temperature influenced the morphology of *A. avenae*, *D. dipsaci* and *M. hapla* (with maximum size specimens at the optimum temperatures) and determined the formation of males in *A. avenae*).

All data together illustrate the value of the morphological species to characterize the difference between nematofauna and between individual species approximately. The profound influence of temperature on morphology and the marked physiological specialisation within the species studied, however, underline the great value of the biological species concept in work with nematodes.

RESUMEN Y CONCLUSION

Introducción y revisión bibliográfica

El propósito de este estudio ha sido la evaluación de la variación geográfica de la nematofauna, analizar las influencias importantes del clima y qué factor climático es determinante y como opera. La parte experimental de este estudio se restringe a los nemátodos de las plantas y del suelo, con especial énfasis en las nematofaunas de Venezuela y Holanda. En la introducción y bibliografia se consideran además los nemátodos de aguas dulces y marinas. Se hace un estudio extensivo de las referencias sobre la ocurrencia y principales características de los nemátodos. Se consideran como el grupo más disperso del reino animal, constituyendo el 80% de todos los animales multicelulares (Cuadro 1.). A pesar de los numerosos inventarios efectuados con propósitos agrícolas y faunísticos, en todo el mundo, conocimientos precisos sobre la distribución geográfica de bien definidas especies es escasa, y los datos para explicar esta situación son muy fragmentarios. Esto es debido a que no se cubren completamente el área y los diferentes habitats dentro del área; a toma de muestras viciadas y por último a una identificación no precisa, además de la escasa información existente en los estudios biológicos y fisiológicos de los nemátodos.

Se ha tratado de hacer una revisión de los estudios faunísticos sobre los nemátodos, en tres capítulos de este trabajo. Los más antiguos (1.2.1.) desde el descubrimiento por BORELLUS en 1656 del primer nemátodo de vida libre, hasta los clásicos estudios faunísticos por DE MAN en 1884, son discutidos en detalle. Estos estudios causaron gran interés dentro del campo de la zoología, estimulando esta actividad. Los estudios y expediciones zoológicas (1.2.2.), muy numerosos desde 1884 hasta la segunda guerra mundial, y más tarde otros, se catalogan y comentan brevemente, de acuerdo a la necesidad, con el objeto de tener una casi completa revisión de dicho período. Los resultados manifiestan la enorme distribución y abundancia de las comunidades donde se encuentran los nemátodos en los diversos y variados habitats y dan algunas observaciones ecológicas en relación a alimentos, humedad, tipo de suelo y clima.

Los inventarios con propósitos agrícolas (1.2.3.), han incrementado rápidamente en cantidad hasta hoy día, siendo tan numerosos que no pueden ser citados en el presente trabajo. Una información más detallada ha sido publicada separadamente (DAO *et al*, 1970). Dicha información es combinada en grupos concernientes a: inventarios generales por áreas (en los cuales se especifican las áreas cubiertas), por plantas (en los cuales las plantas o grupos de plantasse mencionan), y por nemátodos especificos o por grupos de parásitos de plantas. Uno de los más estudiados en cuanto a su distribución es *Heterodera rostochiensis* (Fig. 1), del cual se han señalado los sitios de distribución en el mundo. Los inventarios con propósitos agrícolas revelan la distribución y la densa población de las nemátodos parásitos de plantas, su presencia en todos los suelos y cultivos, sugiriendo influencia de la especies de plantas, tipo de suelo y situación geográfica.

Los principales factores del medio ambiente sobre las poblaciones de nemátodos en el suelo, se catalogan en un esquema cualitativo el cual es, descriptivo, funcional y causal con respecto a la relación nemátodo ambiente (Fig. 2). Modificaciones esenciales, de esquemas similares para otros animales, es la aceptación de la influencia directa del clima, suelo y otros factores físico-químicos, influyentes en las poblaciones de nemátodos y la omisión de la migración, debido a lo limitado de ésta. La dispersión pasiva de los nemátodos, es extremadamente eficiente. Unido a su gran persistencia, a ser polífagos y a la falta de competencia interespecífica, este poder es de gran importancia en la ocurrencia de varias especies en el suelo. Los nemátodos son llevados en una forma pasiva pero en estado viviente, a un habitat, después de lo cual el ambiente comienza a ser funcional. Ellos tienen que sobrevivir y reproducirse antes de que la población pueda ser medida y relacionada al ambiente geográfico. Tan pronto la población se pueda medir, sin lugar a dudas el habitat opera en condiciones favorables para la propagación y sobrevivencia de las especies.

Los factores clima, suelo y planta, son reconocidos como principales determinantes para el desarrollo de las poblaciones; pero como se indica posteriormente, el suelo, planta y otros factores locales, no pueden contarse para la variación geográfica de la nematofauna y por lo tanto una mayor atención se dedica al clima. Este y sus estados atmosféricos se reflejan usualmente en la densidad promedio y fluctuaciones de una población. La mayoría de las infestaciones por nemátodos llevados a un ambiente de clima y suelo no apropiados, tienden a desaparecer. Se hace énfasis en la mayoría de los estudios de dinámica de poblaciones, concernientes a las ya existentes, en tal forma combinaciones convenientes de especies y ambiente. KLOMP (1962), analiza los factores clima y estados atmosféricos y dice que éstos no pueden regular la densidad directamente, porque muchas poblaciones desaparecen, esto significa, para tales y por lo tanto a poblaciones selectas. Un sinnúmero de inoculaciones de nemátodos, sinembargo, pueden desaparecer debido a la influencia directa de clima, suelo y otros factores del ambiente, inmediatamente o después de una serie de generaciones. Esta influencia directa puede llamarse destrucción o determinación, más que regulación, pero debe existir un proceso que regule la determinación y límite de las especies de nemátodos y por esto se incluye en el esquema de la Figura 2. La temperatura parece ser el principal factor relacionado con la distribución geográfica, por lo tanto datos bibliográficos sobre la temperatura han sido registrados. Termogramas sobre nemátodos, muestran las curvas normales biológicas, óptimas, señalando área de muerte por efectos del frío, actividad limitada, óptima, actividad limitada, entorpecimiento por calor y muerte, sucesivamente, en una escala de baja a alta temperatura.

Datos completos sobre una determinada especie, son escasos, pero información sobre más de 140 diferentes casos de actividad en los nemátodos relacionados con temperatura han sido señalados en el Cuadro 2. La mayoría de los nemátodos en el suelo y plantas son activos y prosperan a temperaturas entre 15° y 30°C y se tornan sin movimientos a 5°–15° 30°–40°C. Temperaturas

mínimas para eclosión, emergencia, invasión, actividad, y temperaturas óptimas para reproducción y otros procesos son aparentemente bajas para los parásitos de plantas en los climas templados *(Heterodera avenae, H. trifolii, H. rostochiensis, H. schachti, Ditylenchus dipsaci, Meloidogyne hapla)* mientras que éstas son altas para los de regiones tropicales *(Hemicycliophora arenaria, Tylenchulus semipenetrans, Trichodorus christiei, Scutellonema brachyurum, Heterodera glycines, Meloidogyne incognita, M. javanica* y algunas otras especies). Las temperaturas máximas para la actividad de los nemátodos, generalmente entre 30° y 35°C, parecen no estar correlacionadas con sus límites geográficos. Las fluctuaciones diurnas y de estaciones pueden incrementar o reducir algunas de sus actividades y su suceptibilidad a temperaturas extremas. Los datos bibliográficos sugieren una fuerte influencia de la temperatura en la forma de distribución de los nemátodos. Tolerancia temporal a temperaturas extemas y sequedad, pueden ser determinantes para la supervivencia y los límites geográficos de las especies.

Materiales y métodos

Los materiales regularmente empleados y los métodos usados en el trabajo experimental están descritos en el Capítulo 2. Los estudios faunísticos, como también la evaluación de densidades de poblaciones en el suelo, tejidos y otras partes de plantas, se basan en conocidas y usadas técnicas de muestreos. Los nemátodos para los estudios faunísticos y taxonómicos han sido llevados a colecciones permanentes en láminas de montaje y archivadas en la colección de la Universidad Agrícola de Wageningen. Las poblaciones de nemátodos para los trabajos experimentales, constituyeron algunas comunidades naturales en su suelo original, y también un número de especies seleccionadas de Venezuela y Holanda, cultivadas y conservadas como poblaciones monoespecíficas en invernaderos, p.e.: *Meloidogyne incognita* de Venezuela (V) y de Holanda (N), *M. hapla* (V) y (N), *Ditylenchus dipsaci* (V) y (N), *Aphelenchus avenae* (V) y (N), *Helicotylenchus dihystera* (V). Las principales plantas usadas en la propagación y experimentación fueron: tomate, maíz, flox, bulbos de cebolla, tulipanes y narcisos, como también cultivos en agar para el hongo *Alternaria solani.* El suelo fué de tipo esterilizado para potes de invernaderos. Aparte de potes de arcilla y cajas de petri, los otros tipos de recipientes usados en la siembra de las plantas fueron tubos plásticos de 4 × 4 × 20 cm. Se controló la temperatura usando diferentes tipos de equipos o aparatos, principalmente cubículos en invernaderos con temperaturas regulables, células climáticas, tanques de Wisconsin y una serie de termostatos e incubadoras especiales.

La estimación de las densidades de nemátodos en el suelo, tejidos y partes de plantas y cultivos de agar, fueron procesados de acuerdo a los métodos descritos por OOSTENBRINK (1960) y s'JACOB y VAN BEZOOIJEN (1967), con algunas modificaciones para la extracción de *Aphelenchus avenae* de los cultivos de hongos. La mayoría de los experimentos fueron con repeticiones y los resultados fueron analizados estadísticamente, después de transformar los datos como se indica en los cuadros.

Análisis de las nematofaunas en Venezuela y Holanda

El Cuadro 3 indica las 176 especies de nemátodos encontrados en Venezuela y 425 de Holanda. Estos países fueron escogidos como áreas experimentales para la comparación de sus nematofaunas y para la selección de especies convenientes por las razones siguientes: a) los países representan, uno de clima tropical y otro de clima templado, sinembargo con condiciones de clima templado en las cordilleras de Venezuela y con condiciones tropicales en los invernaderos de Holanda; b) la nematofauna de ambos países ha sido relativamente bien estudiada; c) la identificación de los nemátodos de ambos países ha sido efectuada y revisada en un solo lugar, principalmente la Universidad Agrícola de Wageningen; d) el autor ha estado comprometido personalmente en los estudios nematológicos de ambos países.

En el Cuadro 3 se observa que los nemátodos de los dos países, difieren marcadamente. La diferencia es evidente al nivel de género y ciertamente al nivel de especies y de géneros con varias especies. Los escrutinios de los datos revelan que de las 63 especies reportadas para ambos países, de acuerdo a la lista del Cuadro 3, sólo 21 puede asegurarse que ocurran en climas tropicales y templados, sinembargo 7 han sido identificadas incorrectamente para un país u otro, 13 parecen ser inciertas por la pobre taxonomía del género tratado o por insuficiente diagnosis y solamente 22 fueron encontradas en las montañas elevadas en Venezuela o en los invernaderos de Holanda. Las 22 especies que viven en ambas zonas climáticas, en comparación con casi 363 en Holanda, y 113 en Venezuela, son micrófagos predatores, con sólo dos posibles parásitos de plantas, viz. *Macroposthphonia sphaerocephala* y *Nothocriconema mutabile*, especies omnipresentes.

Experimentos de inoculación y transmisión

Las diferentes nematofaunas no deben relacionarse con la situación geográfica de los dos países, como tales, o debido al insuficiente transporte de inoculum, ya que muchas especies tropicales se encuentran en los invernaderos de Holanda y muchas especies de clima templado en las montañas de Venezuela. Los nemátodos considerados como parásitos de plantas se resumen en el Cuadro 4. Los factores del ambiente deben ser por lo tanto instrumentales. La influencia de los factores climáticos ha sido estudiada en experimentos con inoculaciones y en diferentes localidades, en los cuales la influencia del suelo, huésped y otros organismos se excluyen lo más posible.

Los experimentos fueron inoculados en Maracaibo, zona baja, al nivel del mar. La mitad de los potes inoculados fueron transportados después de dos meses a 2.800 m. de altura; los resultados demuestran que las especies de las zonas bajas *Meloidogyne incognita*, *Pratylenchus zeae* y *Helicotylenchus dihystera*, se desarrollan mejor al nivel del mar (0 m.), que la especie *M. hapla* de la zona montañosa, que se desarrolla mejor a 2.800 m. (Cuadro 5). *Aphelenchus avenae* se desarrolló igualmente en ambas latitudes en este experimento.

El paso de una comunidad natural de las zonas bajas en su suelo original a la región de montaña y viceversa, con potes control de la localidad original,

refuerzan los resultados de los ensayos de inoculación. La mayor parte de las especies de las zonas bajas declinan en las montañas y a la inversa, con la excepción de los grupos de nemátodos zaprozoicos (Cuadro 6).

Ensayos de transmisión con la especie tropical *Meloidogyne*, en Holanda revelaron que éstas no pueden subsistir externamente (4.4); partes de plantas sembradas fuera del invernadero se encontraron libres de nemátodos después de una a tres estaciones.

La influencia directa de localidad, suelo, planta y otros factores del crecimiento, se excluyen. Esto nos deja sólo con la temperatura y para los experimentos en Venezuela, posiblemente con la presión atmosférica como influencia instrumental, que luego es estudiada.

Efectos de la presión atmosférica

Los experimentos efectuados en desecadores, muestran que las poblaciones (V) y (N) de *A. avenae* y *D. dipsaci*, no son afectadas por la baja presión de aire equivalente a 3000 metros sobre el nivel del mar y la presión atmosférica normal (Cuadros 8–11). Baja presión atmosférica y altitudes geográficas no son por lo tanto determinantes en la reproducción de estos nemátodos, y por ende no parecen tener influencia en su distribución geográfica.

Se ha encontrado en estos experimentos que la reproducción de *A. avenae* en cultivos de *Alternaria solani* y de *Ditylenchus dipsaci* en bulbos de cebolla fueron suprimidos, cuando varios cultivos de hongos ó muchos bulbos de cebolla fueron colocados juntos en el desecador. Se observó mal desarrollo de los hongos y pudrición de los bulbos, siendo probable que estos materiales produzcan agentes nematicidas, los cuales no pudieron ser plenamente indentificados por medio de cromatografía de gases.

Efectos de la temperatura

Se asume de los capítulos 3, 4 y 5 que las influencias climáticas son importantes y por exclusión de otras influencias, la conclusión parece ser justificada de que el factor principal es la temperatura. Por lo tanto la influencia de niveles de temperaturas en las actividades biológicas de una y más poblaciones de seis especies de nemátodos fueron extensamente estudiadas.

Meloidogyne incognita: Experimentos comparativos con las poblaciones (V) y (N), fueron efectuados para eclosión (Figuras 3 y 4, Cuadro 12), penetración, reproducción y formación de agallas después de la inoculación, en plantas de tomate, en compartimentos en invernaderos (Figura 5, Cuadros 13 y 14), en tanques de Wisconsin (Figura 6, Cuadros 15 y 16) y en células climáticas (Figura 7, Cuadros 17 y 18) y también en morfología (Figura 8, Cuadro 19). Ambas poblaciones parecen ser termófilas. Eclosión, formación de agallas y reproducción fueron abundantes y rápidas en las temperaturas de 25° y 30°C y para la población (N) también a 20°C. Sucedieron en menor cuantía a 15°C, con excepción de los casos en que penetraron en altas temperaturas y bajaron a un valor menor a 10°C. Una exposición de los huevos en agua durante 15 días, a 5° ó 35°–40°C, eliminó más del 80% de los huevos de ambas poblaciones

(Cuadro 12); pero la formación de agallas y reproducción en tomate ocurrió a 35 °C, lo cual es explicable probablemente por el hecho de que en una planta en crecimiento, la temperatura es más baja que la del suelo alrededor de ella, cuando la temperatura es alta. Las poblaciones (V) y (N) fueron morfológicamente iguales, pero la población (N) fué más termofóbica y marcadamente menos criofóbica que la población (V), lo cual puede ser significativo por el área geográfica de este nemátodo. Todos los datos en conjunto indican que le temperatura mínima para comenzar la infección y reproducción de la población (N) es cerca de 10 °C, la cual es 5 °C más baja que la de la población (V) y también que las larvas de la población (N) inoculada en el suelo puede sobrevivir 15 °C por cerca de 4 semanas, mientras que la población (V) no puede sobrevivir este período. Parece ser que existen razas o tipos con diferentes requerimientos de temperatura especies morfológicas en *M. incognita*, los cuales continúan todavía presentes después de la propagación de los nemátodos en la misma planta, en el mismo ambiente por más de un año. Se propone indicar tipos con una diferencia fija en requerimientos de temperatura como termotipos.

Meloidogyne hapla: Similares experimentos a aquellos descritos para *M. incognita*, fueron efectuados con las poblaciones (V) y (N) de *M. hapla* y se añadió un experimento en cuanto a sobrevivencia a temperaturas bajas (Figuras 9–13, Cuadros 20–29). Ambas poblaciones de *M. hapla*, parecen ser más resistentes a las bajas temperaturas que *M. incognita*. Su supervivencia fué buena a 5 °C, en agua (Cuadro 20) y a 0 °C en suelos y parcial a –10 °C por varios días (Cuadros 27 y 28). Las dos poblaciones pudieron comenzar algunas actividades a 10 °C, pero para el resto de estas fueron termófilas y pudieron prosperar a 30° y 35 °C, al igual que *M. incognita*. El hecho de que *M. hapla* ocurra externamente en climas templados hace por esta razón explicable que esta especie prospere a bajas temperaturas y pueda sobrevivir cortos períodos a muy bajas temperaturas, pero es más extraño el hecho de que es más rara en los países tropicales y en invernaderos que *M. incognita*, por su termopreferencia tan alta o más alta.

Los dos tipos de *M. hapla* difieren en su morfología (Cuadro 29), en la producción de machos estos estuvieron presentes a 35 °C, solamente en la población (V) pero no del todo en la población (N); y también en pequeñas differencias en los requerimientos de temperatura. La población (N) parece ser más resistente a condiciones adversas de bajas y altas temperaturas y ser además más termófila en sus actividades biológicas, que la población (V). Estas diferencias son pequeñas comparadas con las diferencias entre *M. hapla* y *M. incognita*.

Aphelenchus avenae: Se efectuaron experimentos en diferentes gradientes de temperatura con las poblaciones (V) y (N) cultivadas en agar con *Alternaria solani*. Concernientes a reproducción (Figuras 14 y 15, Cuadros 30 y 31), adaptación a bajas temperaturas (Figura 16). relación hembras-machos (Cuadros 32 y 33), la influencia de hongo contaminante (Fugura 17) y morfología (Figuras 18 y 19, Cuadro 34). Ambas poblaciones aparentemente son termófilas, las diferencias entre las dos poblaciones, en varios aspectos biológicos y mor-

fológicos, sugiere, sinembargo, que son las mismas especies. La reproducción occurre de 16°–35°C en (V) y de 5°–30°C en la (N), con un óptimo para (V) un poco por encima y para (N) un poco por debajo de 25°C. La población (V) es criofoba, se reproduce gradualmente en una mejor forma, a pesar de varias declinaciones, cuando se cultiva durante 24 meses a 18°C (Figura 16), sin perder su potencia de reproducción a 25°C. Los machos están ausentes a 25°C. Una población menor consistente sólo de machos, se obtiene cuando ésta se cultiva a 32° y 33°C. La temperatura parece ser determinante en la formación de machos en *A. avenae* y esto se refleja en ambas poblaciones (Cuadro 32). La influencia de la temperatura parece ser directa y no indirecta vía el hongo (Cuadro 33). El principal hongo contaminante en los cultivos de *A. avenae* sobre *Alternaria solani* fué *Trichoderma koningi*, que libera un agente nematicida en los cultivos, (Figura 17). La temperatura tiene influencia en la morfología de *A. avenae* con respecto a los ocho caracteres estudiados (Figura 18) y hubo diferencias significativas entre las poblaciones (V) y (N) a una ó más temperaturas, para todos los caracteres morfológicos estudiados (Figura 19, Cuadro 34). El principio de BERGMAN, que establece que los vertebrados de la misma especie, crecen más cuando viven a bajas temperaturas, aparentemente no puede aplicarse a los nemátodos.

Ditylenchus dipsaci: Varios experimentos fueron efectuados con diversos gradientes de temperaturas, con poblaciones de ajo (V), de cebolla (N), narciso y tulipan, también (N). Los experimentos efectuados fueron sobre reproducción en bulbos de cebollas y en plantas de flox (Figuras 20 y 27); otro en la supervivencia y acondicionamiento de los tipos de narcisos y tulipanes en agua caliente (Cuadros 35–37) y variaciones morfométricas (Figura 28, Cuadro 38). Las temperaturas óptimas para infección y reproducción y otras actividades de *D. dipsaci*, están generalmente entre 10° y 20°C (Cuadro 2), pero parece ser que las poblaciones difieren significativamente. La población (N) de cebolla, cultivada en bulbos de cebollas y plantas de flox, fueron menos termófilas que las de ajo (V), sinembargo, esta última población está restringida a las zonas montañosas en Venezuela. Temperaturas de 15°–20°C fueron favorables para ambas poblaciones, pero la de cebolla (N) prosperó relativamente mejor a 10°–15°C y la de cebolla (V) fué más numerosa a 25°–30°C. De hecho la población de ajo (V) mostró un anormal incremento a 30°C en flox, (Figuras 21 y 23). Las poblaciones de tulipan y narciso (N), mostraron relativamente un amplio espectro con el mismo óptimo de 20°C para la reproducción, ellas son aparentemente más termófilas que la de cebolla (N). Sinembargo, la diferencia es pequeña, la población (N) de tulipan parece ser significativamente más resistente a la destrucción por calor en agua que la población (N) de narciso. Los nemátodos de ambas poblaciones, cultivados a altas temperaturas de 25°–30°C, fueron más resistentes al calor que aquellas cultivadas a 10°–15°C; esta diferencia fué mantenida por varias semanas cuando los nemátodos fueron almacenados a 3°C (6.5.5. Cuadro 35). La población (N) de tulipan fué también más resistente contra el calor después de un pre-tratamiento a 35°C por 1–16 horas. Esta resistencia se pierde otra vez cuando los nemátodos se con-

servan a 20 °C por 14 horas, pero no a 3 °C (6.5.7. Cuadro 37). Ha sido postulado que la resistencia temporaria obtenida por cultivos a altas temperaturas o por pre-tratamientos a 35 °C es asociada con un principio químico. Se observó que los nemátodos presentan estado de movilidad después de ciertos tratamientos de calor, pero sinembargo estaban afectados a tal punto que no podían permanecer vivos ni reproducirse en el huésped (6.5.6.). Los datos presentados pueden servir para entender y mejorar los resultados de los tratamientos con agua caliente a los bulbos de flores, que hoy día se practican.

Algunos caracteres morfológicos presentaron differencias entre la población de ajo (V) y de cebolla (N), a una de las dos temperaturas de cultivo, pero los caracteres influenciados y la influencia varían. Hay también caracteres que no muestran del todo diferencias, tanto como para población como para temperatura (Cuadro 35, Figura 28). Esto hace pensar que las dos poblaciones, la de ajo (V) y la de cebolla (N), sean diferentes especies, y por lo tanto las diferencias significativas sugieren que esta posibilidad no debe ser excluída. Cruzamiento entre nemátodos de ambas poblaciones son necessarios para dilucidar dichos puntos.

Helicotylenchus dihystera: La influencia de temperatura sobre la reproducción de una población tropical en tomate (V), fué estudiada con experimentos de inoculación, en invernaderos y en tanques de Wisconsin (Figura 29, Cuadros 39 y 40). La reproducción tuvo lugar en todas las temperaturas estudiadas, desde 10° a 25 °C en el invernadero y desde 15° a 35 °C en los tanques de Wisconsin. La densidad de población, en el suelo, también como en las raíces, incrementaron en línea recta con la temperatura, hasta la más elevada, 25 °y 35 °C, en los dos experimentos respectivamente. *H. dihystera,* es por lo tanto un nemátodo tropical, sinembargo, puede tener posibilidades de prosperar en regiones frías.

Pratylenchus crenatus (N) y asociados: Un suelo con una comunidad natural de *Pratylenchus crenatus, Tylenchorhynchus dubius* y *Rotylenchus robustus,* fué sembrado con maíz a diferentes temperaturas, en invernaderos con temperatura controlada y en tanques de Wisconsin (Cuadros 41 A y B). Las poblaciones de estos tres nemátodos fueron óptimas a 10°–15 °C y bajaron a altas temperaturas, a pesar de que, el crecimiento radical aumentó. Después de 98 días las poblaciones a 25°–30 °C constituyeron generalmente 1/2–1/20 de la población original y también de las poblaciones a 10 °C.

Tratamientos de una comunidad natural en un suelo areno-arcilloso, sin planta hospedera y a diferentes temperaturas por períodos hasta de $3\frac{1}{2}$ meses condujeron a resultados bastante satisfactorios (Figura 30). Todos los nemátodos fitófagos pertenecientes a Tylenchida fueron suprimidos a partir de 20 °C y fueron completamente erradicados de 25° a 30 °C ó mayores temperaturas dentro de los $3\frac{1}{2}$ meses. La temperatura aparentemente tiene un efecto determinante en el curso de las infecciones que puedan causar los parásitos de plantas de regiones templadas en regiones tropicales y viceversa. Los nemátodos zaprozoicos como un grupo, fueron menos susceptibles a las altas temperaturas que los parásitos de plantas. Estos resultados están de acuerdo con los datos faunís-

ticos del Capítulo 3, que indica que los posibles nemátodos que habitan todas las áreas son mayormente zaprozoicos.

Conclusion

Las nematofaunas de las dos zonas climáticas, Venezuela país tropical y Holanda, difieren claramente en las especies. Las pocas encontradas ubicuamente en ambas zonas climáticas, no comprenden supuestos ó conocidos parásitos de plantas, con excepción de dos especies de criconematide. El hecho de que muchos nemátodos parásitos de la región tropical sean comunes en invernaderos Holandeses y especies de zona templada sean encontradas en las regiones montañosas de Venezuela, nos indica la amplia dispersión del inóculo. Los resultados pueder ser de utilidad en las regulaciones para servicio de cuarentena vegetal.

La exclusión experimental de la influencia directa de localidad, suelo, planta y otros factores de crecimiento, demuestra que los límites de distribución de los nemátodos deben ser determinados a largo grado por el clima, principalmente por la temperatura, como puede deducirse del análisis bibliográfico.

Efectos de la temperatura en la supervivencia y desarrollo de los nemátodos difiere marcadamente entre especies y entre poblaciones de la misma especie. El clima y límites geográficos de un nemátodo parecen estar estrechamente relacionados a las temperaturas letales o a las óptimas para actividad u otras combinaciones.

Temperaturas de 25°C y más fueron letales a nemátodos de plantas en suelos no sembrados de zonas templadas y temperaturas de 5°C ó menos fueron letales a nemátodos de las zonas tropicales dentro de $\frac{1}{2}$ mes a $3\frac{1}{2}$ meses. Esto exluye hibernación en los otros climas en ausencia de plantas. También poblaciones establecidas sobre plantas en crecimiento en climas no favorables, declinan o desaparecen, sinembargo, esto aparentemente toma mucho más tiempo que en la ausencia de huéspedes.

Las influencias de temperatura que fueron encontradas instrumentales para las poblaciones estudiadas, se sumarizan en el Cuadro 42. Las poblaciones de la zona tropical no comienzan su actividad bajo 15°C y prosperan a 25°–35°C; p.e.: *M. incognita* (V), *A. avenae* (V), y *H. dihystera* (V). *M. incognita* de los invernaderos Holandeses adopta una posición intermedia. Los nemátodos de los climas templados, Holanda y las montañas de Venezuela, sobreviven períodos a muy bajas temperaturas y comienzan su actividad cerca de 10°C. Los límites de temperatura a los cuales ellos prosperan, varían, pueden ser bajos, p.e.: 10°–20°C para *D. dipsaci* (N) y *P. crenatus* (N); moderados, p.e.: de 15°–30°C para *A. avenae* (N) y *D. dipsaci* (V) ó aún tan altos como para las especies tropicales, p.e.: 20°–35°C para *M. hapla* (V) y (N). *M. hapla* (V) y (N) no difieren de *M. incognita* (N) con excepción del hecho de que esta última especie no puede sobrevivir a muy bajas temperaturas. Las poblaciones que fueron estudiadas parecen ser bien adaptadas a sus ambientes climáticos por la temperatura, aún cuando sus mecanismos varían.

En la misma especie morfológica los requerimientos de temperatura difieren.

Estas diferencias son estables y por lo tanto probablemente termotipos genéticos. Solamente alguna adaptación o selección ocurre cuando *A. avenae* (V) fué cultivada a una temperatura sub-letal por 24 meses. El cultivo y almacenamiento a diferentes temperaturas, producen ciertos principios de resistencia al calor en *D. dipsaci*, los cuales no fueron estables y posiblemente sean de naturaleza química. Por otra parte la temperatura tuvo influencia en la morfología de *A. avenae*, *D. dipsaci* y *M. hapla* (con especímenes de máximo tamaño a las temperaturas óptimas) y determinó la formación de machos en *A. avenae*.

Todos los datos ilustran el valor del concepto especie morfológica para caracterizar aproximadamente las diferencias entre las nematofaunas y entre individuos de la especie. La profunda influencia de la temperatura sobre la morfologia y la marcada especialización fisiológica dentro de las especies estudiadas, sinembargo, subraya el gran valor del concepto de especies biológicas en trabajos con nemátodos.

SAMENVATTING EN CONCLUSIE

Algemene inleiding en literatuuroverzicht

Het doel van dit onderzoek was het bepalen van geografische verschillen in de aaltjesfauna, van de belangrijke oorzaken, van de invloed van het klimaat en zijn werking. Het eigenlijke onderzoek was beperkt tot aaltjes uit grond en uit planten, in het bijzonder die van Venezuela en van Nederland; de inleiding heeft ook betrekking op zoetwater en mariene nematoden.

De literatuur verschafte gegevens over voorkomen en voornaamste eigenschappen van aaltjes. Zij vormen de meest algemeen voorkomende diergroep: 80% van de meercellige dieren zijn aaltjes (Tabel 1). Ondanks de op vele plaatsen verrichte faunistische en landbouwkundige surveys is van geen soort het verspreidingsgebied en de verklaring van de situatie goed bekend. Dit komt door onvolledige bemonstering en extractie en gebrekkige determinatie van de aaltjes en door de geringe kennis van hun biologie en fysiologie.

De faunistische studies over aaltjes zijn zo volledig mogelijk weergegeven in drie secties, die betrekking hebben op inleidende explorations (1.2.1), zoölogische surveys en collecties (1.2.2) en surveys voor landbouwkundige doeleinden (1.2.3). Het verspreidingsgebied van de best bestudeerde soort, *Heterodera rostochiensis*, vastgesteld in 1950, 1961 en 1968, is op een kaart aangeduid (Figuur 1). De totale faunistische resultaten bevestigen het wijd verspreid voorkomen van veelsoortige aaltjesgemeenschappen, ook van plantenaaltjes, en wijzen op een sterke invloed van voedsel (plant), bodemtype en geografische situatie.

De voornaamste invloeden op een aaltjespopulatie in de grond zijn in een qualitatief schema verwerkt, dat beschrijvend, functioneel en causaal is met betrekking tot de relaties tussen aaltjes en hun omgeving (Figuur 2). Actieve migratie van de weinig beweeglijke aaltjes is onbelangrijk, maar zij worden passief zeer efficiënt verspreid. De meeste aaltjesinfecties die in een ongunstig milieu terecht komen gaan te gronde door de directe invloed van klimaat en andere abiotische milieu componenten, die daardoor mede het verspreidingsgebied van een soort bepalen.

Deze directe invloed is eerder destructie dan regulatie in de zin van Nicholson (1954) of Klomp (1962) die betrekking heeft op meetbare en dus geselecteerde, bij het milieu passende populaties. Elke ingebrachte populatie moet de inoculatie overleven en zich vermeerderen voordat zij meetbaar wordt en met het volledige geografische milieu in verband kan worden gebracht. Dit omvat invloeden van het klimaat, de grond, het voedsel, andere organismen en intraspecifieke invloeden.

Van de milieucomponenten zal het klimaat het belangrijkste blijken voor de geografische verbreiding van aaltjes, en van de klimaatcomponenten vooral de temperatuur. De literatuurgegevens hierover zijn verzameld. Van geen aaltjessoort is een exact en volledig thermogram beschikbaar, maar enkele gegevens over ruim 140 gevallen van een aaltjesactiviteit in verband met de temperatuur konden worden bijeengebracht (Tabel 2). De meeste grond- en plantenaaltjes

zijn actief tussen 15° en 30° en worden bewegingloos bij 5°–15° en 30°–40°C. Voor planteparasitaire aaltjes van gematigde streken worden lagere minimum temperaturen voor verschillende activiteiten gemeld dan voor plantenaaltjes uit warmere streken. Daarnaast zullen optimum temperaturen, tijdelijke tolerantie voor extreme temperaturen en temperatuurschommelingen van invloed zijn. De literatuur suggereert dus reeds een nauw verband tussen temperatuur en verspreidingspatroon van aaltjes.

Materialen en methoden

De hierna vermelde faunistische studies en de bepaling van populatiedichtheden in grond, planteweefsels en andere substraten zijn gebaseerd op gestandaardiseerde bemonsteringstechnieken. De aaltjes voor faunistisch en taxonomisch onderzoek zijn in permanente preparaten gebracht en geborgen in de Landbouwhogeschool Collectie. Voor het experimentele onderzoek werden enkele natuurlijke aaltjesgemeenschappen in hun oorspronkelijke grond en een aantal geselecteerde populaties uit Venezuela en Nederland als monospecifieke populaties gekweekt: *Meloidogyne incognita* uit Venezuela (V) en uit Nederland (N), *M. hapla* (V) en (N), *Ditylenchus dipsaci* (V) en (N), *Aphelenchus avenae* (V) en (N) en *Helicotylenchus dihystera* (V).

De voornaamste planten voor aaltjesvermeerdering en voor proeven waren tomaat, mais, phlox en bollen van ui, tulp en narcis, en ook agarcultures van *Alternaria solani*. De gebruikte grond was meestal gesteriliseerde potgrond van een vaste samenstelling. Naast potten en petri-schalen werden vaak doorzichtige plasticbuizen van 4 × 4 × 20 cm gebruikt voor aaltjesonderzoek op planten.

Om reeksen gecontroleerde temperaturen te verkrijgen werd gebruik gemaakt van kassen en klimaatcellen, Wisconsin tanks, een seriethermostaat en speciale nauwkeurige thermostaten.

Het bepalen van aaltjesdichtheden in grond, planteweefsels en agar geschiedde volgens de methoden beschreven door OOSTENBRINK (1960) en s'JACOB en VAN BEZOOIJEN (1967), met modificaties voor de extractie van *Aphelenchus avenae* uit agar cultures. Bij vrijwel alle proeven waren herhalingen aanwezig en werden de resultaten wiskundig bewerkt, na transformatie van de aaltjes- en plantecijfers indien dit is aangegeven in de tabellen.

Analyse van de aaltjesfauna van Venezuela en van Nederland

Tabel 3 bevat de namen van alle aaltjessoorten die bekend zijn uit Venezuela en uit Nederland, respectievelijk 176 en 425 soorten. Deze landen werden als toetsgebieden gekozen omdat a) zij een gematigd en een tropisch klimaat vertegenwoordigen, evenwel met een gematigd klimaat ook op de bergen in Venezuela en met een tropisch klimaat in de kassen in Nederland, b) de aaltjesfauna's van beide landen vrij goed bekend zijn, c) de aaltjes van beide landen grotendeels gedetermineerd waren op één plaats en daar gecontroleerd konden worden, namelijk bij de Landbouwhogeschool te Wageningen, d) de schrijver persoonlijk betrokken was bij aaltjesonderzoek in beide landen.

Tabel 3 toont aan dat de aaltjesfauna van de twee landen sterk verschilt. Dit

is zichtbaar aan de geslachten, maar duidelijker nog aan de soorten van geslachten met veel vertegenwoordigende soorten. Een nauwkeurige analyse van de gegevens toont aan dat van de 63 soorten die van beide landen vermeld zijn er slechts 21 geacht mogen worden in het tropische en het gematigde klimaat voor te komen, terwijl 7 onjuist bleken te zijn geïdentificeerd, 13 onzeker waren in verband met de gebrekkige taxonomische kennis van het betreffende geslacht of met onvolledige soortdiagnose, en 22 alleen gevonden werden op grote hoogte in Venezuela of in kassen in Nederland. De 21 soorten die in beide klimaten kunnen leven, tegenover 362 alleen in Nederland en 113 alleen in Venezuela, zijn microphaag of predatoor, met slechts twee mogelijke planteparasieten, namelijk *Macroposthonia sphaerocephala* en *Nothocriconema mutabile*. Ubiquisten zijn dus zeldzaam.

Overbrengings- en inoculatieproeven

Het genoemde verschil in aaltjesfauna kan geen verband houden met de geografische situatie op zichzelf of met het ontbreken van inoculum, want veel tropische soorten leven in Nederlandse kassen en veel gematigde soorten op de Venezolaanse bergen (Tabel 4). Milieufactoren moeten dus van doorslaggevende invloed zijn. De invloed van klimaat werd onderzocht in inoculatie- en overbrengingsproeven waarbij de directe invloed van grond, planten en andere organismen zoveel mogelijk werd uitgeschakeld.

Inoculatieproeven te Maracaibo dat op zeeniveau ligt en overbrenging na twee maanden van de helft van de potten naar het op 2800 m hoogte gelegen Mucuchies, toonden aan, dat de tropische soorten van het lage land, *M. incognita*, *P. zeae* en *H. dihystera*, zich beter ontwikkelen op 0 m hoogte terwijl de soort *M. hapla* uit de bergen zich op 2800 m hoogte beter ontwikkelde (Tabel 5). Overbrenging van grond met een natuurlijke aaltjesgemeenschap uit het tropische lage land naar de bergen en van besmette berggrond naar het lage land, met contrôlepotten op het oorspronkelijke station, bevestigden de resultaten van de inoculatieproeven. De meeste tropische soorten van het lage land kwijnden op grote hoogte en de soorten van de bergen op zeeniveau, behalve de veel soorten omvattende groep van de saprofagen.

Overbrengingsproeven met tropische *Meloidogyne* soorten in Nederland toonden aan dat deze soorten zich buiten niet kunnen handhaven en dat uit warme streken afkomstig besmet plantgoed in de loop van één tot drie seizoenen aaltjesvrij wordt (4.4).

De directe invloed van plaats, grond, planten en andere groeifactoren was bij deze proeven vrijwel uitgeschakeld. Temperatuur, en voor de proeven in Venezuela mogelijk luchtdruk, zijn de enige factoren die de verschillen kunnen hebben veroorzaakt en die dus nader onderzocht moeten worden.

Luchtdrukeffecten

Proeven in exsiccatoren toonden aan, dat *A. avenae* (V) en (N) en *D. dipsaci* (V) and (N) zich niet slechter vermenigvuldigen bij een lage luchtdruk, zoals die op 3.000 m hoogte voorkomt, dan bij de normale atmosferische druk op zee-

niveau (Tabellen 8–11). Het is daarom onwaarschijnlijk dat de lage luchtdruk op grote hoogte de oorzaak is van de verschillen in aaltjespopulatie tussen het bergland en het lage land.

Uit de proeven bleek terloops dat aaltjes gedood worden wanneer teveel *Alternaria solani* cultures of teveel uiebollen bijeen worden geplaatst in gesloten exsiccatoren. De schimmel groeide dan slecht en de uiebollen verrotten. Het is waarschijnlijk dat deze materialen vluchtige nematicide stoffen afscheiden; deze konden door gaschromatografie niet volledig geïdentificeerd worden.

Temperatuureffecten

Uit het voorgaande is gebleken dat de invloed van het klimaat op aaltjesgemeenschappen groot is, en door uitsluiting van andere factoren kon worden geconcludeerd dat de temperatuur de belangrijkste bepalende factor moet zijn. Daarom is uitvoerig onderzoek verricht over de invloed van temperatuurreeksen op enkele biologische activiteiten van een of meer populaties van een zestal aaltjessoorten.

Meloidogyne incognita. Parallelproeven met de (V) en (N) populaties werden gedaan over het uit het ei komen van de larven (Figuren 3 en 4, Tabel 12), over binnendringing, reproductie en galvorming op tomaat in kassen (Tabellen 13 en 14, Figuur 5), in Wisconsin tanks (Tabellen 15 en 16, Figuur 6) en in klimaatcellen (Tabellen 17 en 18, Figuur 7), en over de morfologie (Figuur 8, Tabel 19). Beide populaties zijn blijkbaar thermofiel. Het uitkomen en de galvorming en reproductie geschiedde rijkelijk en snel bij 25° en 30 °C en voor de (N) populatie ook bij 20 °C, minder bij 15 °C behalve voor de aaltjes die bij hogere temperatuur reeds in de wortels waren gedrongen, en zeer weinig bij 10 °C. Het blootstellen gedurende 15 dagen van eieren in water aan temperaturen van 5° of van 35°–40 °C doodde ruim 80% van de eieren van beide populaties (Tabel 12). Hoewel 35 °C te hoog was voor eieren in water, bleek galvorming en reproductie bij die temperatuur mogelijk, waarschijnlijk omdat een groeiende plant dan koeler is dan de omgevende grond. De (V) en (N) populaties waren morfologisch gelijk, maar de (N) populatie was iets meer thermofoob en duidelijk minder cryofoob, hetgeen van betekenis kan zijn voor de geografische verbreiding. Bij de (N) populatie blijken infectie en reproductie bij ongeveer 10 °C te beginnen, hetgeen bij de (V) populatie ongeveer 15 °C is. De in grond geïnoculeerde larven van de (N) populatie blijven ook langer leven dan de (V) populatie. Er zijn dus stammen met verschillende temperatuurbehoeften binnen de morfologische soort *M. incognita* die aanwezig bleven na vermeerdering van de populaties onder dezelfde omstandigheden gedurende een jaar. Voorgesteld wordt om zulke stammen met een vast verschil in temperatuurbehoeften aan te duiden als thermotypen.

Meloidogyne hapla. Proeven als beschreven voor *M. incognita* werden ook gedaan met de (V) en (N) populaties van *M. hapla*, en een proef werd toegevoegd over de overleving bij lage temperatuur (Figuren 9–13, Tabellen 20–29). De *M. hapla* populaties zijn beide beter bestand tegen lage temperaturen dan *M. incognita*, want zij overleefden een verblijf bij 5 °C in water (Tabel 20) en bij

0 °C in grond en zelfs een verblijf gedurende verscheidene dagen bij −10 °C in grond (Tabellen 27 en 28). Beide populaties ontwikkelden zich echter evengoed als *M. incognita* bij 30° en 35 °C. Het buiten wijd verspreid voorkomen van *M. hapla* in gematigde klimaten zal samenhangen met het vermogen om bij lage temperaturen te leven en om koudeperiodes te doorstaan. Niet verklaard is het feit dat *M. hapla* in tropische streken en in kassen minder algemeen voorkomt dan *M. incognita*, want het temperatuuroptimum is minstens even hoog. De twee *M. hapla* populaties verschilden enigszins in vorm, en tevens in de ontwikkeling van mannetjes bij hoge temperatuur (geen ♂♂ bij de (N) populatie, wel bij 35 °C bij de (V) populatie). Daarnaast was de (N) populatie iets meer bestand tegen schadelijke lage en hoge temperaturen en iets meer thermofiel in de biologische activiteiten. De verschillen tussen de twee *M. hapla* populaties zijn klein vergeleken met de verschillen tussen *M. hapla* en *M. incognita*.

Aphelenchus avenae. Er waren verschillende proeven in temperatuurreeksen met de (V) en (N) populaties, gekweekt op agar schalen met *Alternaria solani*. De grote verschillen tussen beide populaties in biologie en morfologie maakt het waarschijnlijk dat de populaties tot verschillende soorten behoren. Beide populaties zijn blijkbaar thermofiel (Figuren 14 en 15, Tabellen 30 en 31). Reproductie vindt bij de (V) populatie plaats bij 16°–35 °C en bij de (N) populatie bij 5°–30 °C, met optima voor (V) iets hoger en voor (N) iets lager dan 25 °C. De cryofobe (V) populatie vermeerderde zich geleidelijk iets beter, hoewel met tijdelijke terugvalperiodes, toen zij gedurende 24 maanden voortdurend bij 18 °C werd gekweekt (Figuur 16), zonder daarbij het vermogen te verliezen om zich sterk te vermeerderen bij 25 °C. Er waren geen mannetjes aanwezig in bij 25 °C gekweekte populaties, maar de minder dichte, bij 32°–33 °C gekweekte populaties bestonden hoofdzakelijk uit mannetjes. Temperatuur blijkt een doorslaggevende invloed te hebben op de vorming van mannetjes bij *A. avenae*, en dit geldt voor beide populaties (Tabel 32). Deze temperatuurinvloed werkt waarschijnlijk direct op de aaltjes en niet via de waard-schimmel (Tabel 33). De schimmel die in de *A. avenae* cultures op *A. solani* het vaakst als verontreiniging voorkwam was *Trichoderma koningi*, en deze produceerde een nematicide stof in de cultures (Figuur 17). Alle acht onderzochte morfologische kenmerken van *A. avenae* (zie Figuur 18) bleken significant door temperatuur te worden beinvloed en ook waren er voor alle acht kenmerken bij een of meer temperaturen significante verschillen tussen de (V) en (N) populaties (Tabel 34, Figuur 19). BERGMAN's klimaatregel voor vertebraten, dat dezelfde soort groter wordt wanneer zij bij lagere temperatuur leeft, geldt blijkbaar niet voor nematoden.

Ditylenchus dipsaci. Verscheidene temperatuurproeven werden gedaan met een Venezolaanse populatie van knoflook ('(V) garlic') en met Nederlandse populaties van ui, tulp en narcis ('(N) onion', '(N) tulip', '(N) narcissus'). Temperaturen van 10° tot 20 °C zijn volgens de literatuur (Tabel 2) gunstig voor infectie, reproductie en andere activiteiten van *D. dipsaci* in het algemeen, maar er blijkt een duidelijk verschil tussen de populaties te bestaan. '(N) onion' was minder thermofiel dan '(V) garlic' hoewel de laatste toch beperkt is tot grote

hoogte in Venezuela. Temperaturen van 15°–20°C waren gunstig voor beide populaties, maar '(N) onion' vermenigvuldigde zich relatief sterker bij 10°–15° en '(V) garlic' bij 25°–30°C, met een afwijkende top voor '(V) garlic' in phlox bij 30°C (Figuren 20–23). '(N) tulip' en '(N) narcissus' zijn blijkbaar wat meer thermofiel dan '(N) onion' (Figuren 24–27). Hoewel het verschil gering is blijkt '(N) tulip' significant meer resistent tegen vernietiging door warmwaterbehandeling van '(N) narcissus'. Aaltjes van beide populaties gekweekt bij 25°–30°C waren warmtebestendiger dan aaltjes gekweekt bij 10°–15°C; dit verschil bleef enkele weken aanwezig wanneer de aaltjes bewaard werden bij 3°C (6.5.5, Tabel 35).

De '(N) tulip' populatie was ook meer warmtebestendig na een voorbehandeling gedurende 1–16 uur bij 35°C. Deze resistentie verdween weer wanneer de aaltjes gedurende 14 uur bewaard werden bij 20°C, maar niet bij 3°C (6.5.7, Tabel 37); mogelijk is zij van chemische aard. Verder werd geconstateerd dat aaltjes vaak nog wel beweeglijk zijn na bepaalde warmwaterbehandelingen, maar toch in hun waardplanten niet in leven blijven of tot vermenigvuldiging in staat zijn (6.5.6.) Deze gegevens kunnen helpen de resultaten van de practische warmwater-behandeling van bloembollen te interpreteren en te verbeteren. Bij enige morfologische kenmerken werden verschillen geconstateerd tussen '(V) garlic' en '(N) onion' en ook de kweektemperatuur had soms invloed. (Tabel 38, Figuur 28). De morfologische eigenschappen die beinvloed werden en de richting varieerde, en er waren ook eigenschappen die noch door de populatie noch door de temperatuur beinvloed werden. Ondansks de significante verschillen die voorkwamen bij sommige morfologische kenmerken en bij de temperatuurbehoeften, lijkt het voorlopig niet verantwoord '(V) garlic' en '(N) onion' als verschillende soorten aan te duiden. Kruising tussen aaltjes van de beide populaties zullen nodig zijn om dit uit te maken.

Helicotylenchus dihystera. De invloed van temperatuur op de vermenigvuldiging van de tropische (V) populatie op tomaat werd nagegaan door inoculatieproeven in kassen en Wisconsintanks (Tabellen 39 en 40, Figuur 29). Vermeerdering bleek plaats te vinden bij alle onderzochte temperaturen vanaf 15° (en mogelijk zelfs 10°C). De aaltjesdichtheid in de grond en in de wortels steeg lineair met de temperatuur tot de hoogste temperaturen, 25° in de kas en 35° in de Wisconsin tanks. *H. dihystera* is dus een tropische nematode met een zeer hoge optimum temperatuur die zich mogelijk ook zou kunnen handhaven in wat koeler streken.

Pratylenchus crenatus (N) en geassocieerde soorten. Grond met een natuurlijke gemeenschap van *P. crenatus, Tylenchorhynchus dubius* en *Rotylenchus robustus* werd met mais beteeld bij verschillende temperaturen in kassen en in Wisconsin tanks (Tabel 41A en B). De populaties van alle drie soorten waren het hoogste bij 10° of 15°C en waren lager bij hogere temperaturen, hoewel daar duidelijk meer wortelgroei voorkwam. Na 98 dagen waren de populaties bij 25°–30° meestal 1/2 tot 1/20 van de uitgangsdichtheid of van de dichtheid bij 10°C.

Plaatsing van vochtige kleigrond met een natuurlijke (N) gemeenschap zon-

der waardplant bij temperaturen van 5° tot 40 °C gedurende 1 tot 3½ maand gaf interessante resultaten (Figuur 30). Alle fytofage Tylenchida werden in aantal gedrukt van 20 °C opwaarts en zij werden uitgeroeid bij 25°–30 °C of hoger binnen 3½ maanden. De temperatuur heeft blijkbaar een beslissende invloed op het lot van plantenaaltjesinfecties die met grond van gematigde klimaten naar de tropen worden gebracht; waarschijnlijk geldt hetzelfde voor tropische aaltjes die in grond naar een gematigd klimaat worden verplaatst. De saprofagen waren als groep minder gevoelig voor hoge temperaturen dan de fytofagen. Dit is in overeenstemming met de faunistische gegevens van hoofdstuk 3 die er op wijzen dat de weinige ubiquisten als regel saprofagen zijn.

Conclusie

De aaltjesfauna's van de twee klimaatgebieden, tropisch Venezuela en Nederland, verschillen sterk. Bij de weinige ubiquisten of mogelijke ubiquisten die in beide klimaten werden gevonden waren geen bekende of verdachte fytofagen behalve twee criconematide soorten. Het feit dat vele tropische plantenaaltjes voorkomen in Nederlandse kassen en dat verscheidene in gematigde streken endemische aaltjes op de Venezolaanse bergen gevonden zijn, wijst op de ruime verspreiding van inoculum. Deze gegevens worden van belang geacht voor de plantenquarantaine.

Uitsluiting door proeven van de directe invloed van standplaats, grond, plant en andere groeifactoren toont aan dat het verspreidingsgebied van aaltjes grotendeels bepaald moet zijn door het klimaat, vooral door de temperatuur, zoals ook op grond van literatuuronderzoek verwacht mocht worden.

Het effect van temperatuur op overleving en groei van een aaltjespopulatie variëert sterk met de soort en met de populatie binnen de soort. Het klimatologische of geografische verspreidingsgebied van een nematode blijkt nauw verbonden te zijn met letale temperaturen, of optimum temperaturen voor biologische processen, of diverse combinaties.

Temperaturen van 25 °C of hoger waren binnen½–3½ maand letaal voor plantenaaltjes in onbeteelde grond afkomstig van de gematigde klimaatzone, en temperaturen van 5 °C of lager waren letaal voor aaltjes van de tropische zone. Dit maakt hibernatie in het andere klimaat bij afwezigheid van waardplanten onmogelijk. Ook gevestigde populaties op groeiende waardplanten die geplaatst werden in het andere, ongeschikte klimaat kwijnen of verdwijnen, hoewel dit blijkbaar veel langzamer gaat dan bij afwezigheid van waardplanten.

De temperatuurinvloeden die van belang bleken voor de onderzochte populaties zijn samengevat in Tabel 42. De populaties uit de tropische zône beginnen hun activiteit niet beneden 15 °C en gedijen bij 25°–35 °C, bijvoorbeeld *M. incognita* (V), *A. avenae* (V), en *H. dihystera* (V). *M. incognita* (N) uit de Nederlandse kas neemt een tussenplaats in. De aaltjes van de gematigde klimaatzones, Nederland en het Venezolaanse berggebied, overleven perioden van zeer lage temperaturen en beginnen hun activiteit bij ongeveer 10 °C. Het temperatuurtraject waarin zij gedijen varieert; deze temperaturen kunnen laag zijn, bijvoorbeeld 10°–20 °C voor *D. dipsaci* (N) en *P. crenatus* (N), of matig hoog,

bijvoorbeeld 15°–30°C voor *D. dipsaci* (V) en *A. avenae* (N), of zelfs even hoog als voor vele tropische soorten, bijvoorbeeld 20°–35°C voor *M. hapla* (V) en (N). De beide *M. hapla* populaties verschillen niet van *M. incognita* (N), afgezien van het feit dat de laatstgenoemde soort zeer lage temperaturen niet overleeft. De onderzochte populaties blijken goed bij hun klimaat te zijn aangepast door hun temperatuur-relaties, maar het mechanisme varieert.

Binnen een morfologische soort verschillen de temperatuurbehoeften van de populaties. Deze verschillen zijn stabiel en waarschijnlijk genetisch bepaald (thermotypen). Slechts een geringe aanpassing of selectie werd geconstateerd bij het kweken van *A. avenae* gedurende 24 maanden bij een bijna letale temperatuur. Kweek- en bewaartemperaturen induceerden een duidelijke warmtebestendigheid in *D. dipsaci* die niet stabiel was en chemisch van aard kan zijn. Anderzijds beinvloedde temperatuur de afmeting en de vorm van *A. avenae*, *D. dipsaci* en *M. hapla* (waarbij de grootste dieren voorkwamen bij de optimale temperaturen) en bepaalde de temperatuur de vorming van mannetjes bij *A. avenae*.

Alle gegevens bijeen illustreren de betekenis van de morfologische soort om het verschil tussen aaltjesfauna's en tussen individuele soorten in grote lijnen weer te geven. De sterke invloed van de temperatuur op de morfologie en de opvallende fysiologische specialisatie binnen de onderzochte soorten onderstrepen evenwel de grote waarde van het biologische soortconcept voor de nematologie.

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REFERENCES

ALLEN, M. W. (1952). Observations on the genus *Meloidogyne* Goeldi, 1887. Proc. helminth. Soc. Wash. **19**: 44–51.

ALLEN, M. W. (1957). A review of the nematode genus *Trichodorus* with descriptions of ten new species. Nematologica **2**: 32–62.

ALLEN, M. W. (1960). Systematic concepts. In: Nematology, ed. J. N. Sasser and W. R. Jenkins, Univ. N. Carolina Press, Chapel Hill: 229–230.

ALLEN, M. W. and NOFFSINGER, E. M. (1968). Revision of the genus *Anaplectus* (Nematoda: Plectidae). Proc. helminth. Soc. Wash. **35**: 77–91.

ALLGÉN, C. A. J. (many articles on marine, brackish water, freshwater, terrestrial and moss nematodes in the period 1921–1960, notably 1929, 1933a, b, 1934a, b, 1950, 1951a, b, c, 1952, 1953, 1954a, b, c, 1955a, b, c, 1956a, b, c, 1957a, b, 1958, 1959). Cf. BOB bibliography*).

ALTHAUS, B. (1954/1955). Beitrag zur Kenntnis des süssen Sees bei Mansfeld und seiner Fauna unter besondere Berücksichtigung der Litoralzone. Wiss. Z. Univ. Greifswald **4** (1).

ALTHERR, E. (1938, 1950a, b, 1952, 1953, 1954a, b, 1955, 1958, 1960, 1963a, b, c, 1965, 1968). Cf. BOB bibliography.

ALYAVDINA, L. P. (1929). Free-living nematodes as food of Volga fish (Russian text). Russ. gidrobiol. Zh. **8**: 139–140.

AMICI, A. (1966). Free-living and plant-parasitic nematodes from the shore of Lake Trasimeno (Italy). Riv. Idrobiol. **5** (1–2): 39–47.

ANDRÁSSY, I. (1952, 1953a, b, 1954, 1956a, b, 1958a, b, 1959a, b, c, d, e, f, 1960a, b, c, d, 1961, 1962a, b, c, d, e, 1963a, b, 1964a, b, c, 1965, 1966a, b, 1967a, b, c, 1968a, b). Cf. BOB bibliography.

ANDRÁSSY, I. (1956). Die Rauminhalts- und Gewichtsbestimmung der Fadenwürmer (Nematoden). Acta zool. hung. **2**: 1–15.

ANONYMUS, (1966). Potato root eelworm (*Heterodera rostochiensis* Woll.) in Europe and the Mediterranean Basin, 1964, A. Rep. EPPO 1965/66, **56**, Ser. B.: 19 p.

ANONYMUS, (1968). Intercepted plant pests 1966–1967. Pl. Prot. Div., Can. Dep. Agric.: 24 p.

ANONYMUS, (1969). U.S.D.A. list of intercepted plant pests, 1968. Agric. Res. Serv. U.S. Dep. Agric.: 66 p.

ARROLD, N. P. and BLAKE, C. D. (1967). Some effects of *Ditylenchus myceliophagus* and *Aphelenchoides composticola* on the growth on agar plates of the cultivated mushroom, *Agaricus bisporus*. Nematologica **12** (1966): 501–510.

BAINES, R. C. (1950). Nematodes on citrus. Soil fumigation and resistant citrus varieties promising as controls. Calif. Agric. **4** (8): 7.

BAKER, A. D., OOSTENBRINK, M. and BERKUM, J. A. VAN (1967). Bibliography on plant, soil and freshwater nematodes (with supplements). Brill. Leiden.

BAKKER, K. (1964). Backgrounds of controversies about population theories and their terminologies. Z. angew. Ent. **53**: 187–192.

BARANOWSKAYA, I. A. (1958). Contribution to the knowledge of the genus *Paraphelenchus* (Micoletzky, 1922) Micoletzky, 1925 (Nematoda: Aphelenchidae) (Russian text). Zool. Zh. **37**: 13–19.

BARKER, K. R. (1959). Studies on the biology of the stem nematode (Abstr.). Phytopathology **49**: 315.

BASTIAN, H. C. (1865). Monograph on the Anguillulidae, or free nematoids, marine, land, and freshwater; with descriptions of 100 new species. Trans. Linn. Soc. Lond. **25**: 73–184.

BASTIAN, H. C. (1866). On the anatomy and physiology of the nematoids, parasitic and free; with observations on their zoological position and affinities to the echinoderms. Phil. Trans. R. Soc. **156**: 545–638.

BELLO P., A., ALVARADO, R. and JIMÉNEZ, M., F. (1965). Estudio de los nematodos de

* BOB bibliography refers to BAKER, OOSTENBRINK and VAN BERKUM (1967) in this reference list.

cultivos de platanera de las Canarias Occidentales. Bol. R. Soc. esp. Hist. nat. 63, Secc. Biol: 33–46.

BELYAEVA, K. V. (1959a). The nematode fauna of the principal soil types of Kara-kalpakia (Russian text). Trudȳ gel'mint. Lab. 9: 49.

BELYAEVA, K. V. (1959b). The nematode fauna of the principal soil types of Kara-kalpakia (Russian text). Trudȳ sred.-aziat. gos. Univ., Tashkent 123: 45–61.

BELYAEVA, K. V. (1961). Materials for a nematode fauna of some soils of the Kashka-Dar'ya basin (Russian text). In: Voprosȳ Fitogel'mintologii, Gel'mintologicheskaya Lab. Akad. Nauk SSSR, Mosk.: 38–50.

BENTHEM JUTTING, W. S. S. VAN (1951). Dr. Johannes Govertus de Man. Biol. Jaarb. 18: 130–259.

BERGESON, G. B. (1960). The influence of temperature on the survival of some species of the genus *Meloidogyne*, in the absence of a host. Nematologica 4 (1959): 344–354.

BERKUM, J. A. VAN (1970). Geographic distribution of workers engaged in the field of nematology. Proc. 9. int. Nematology Symp. (Warsz. 1967).

BIRCH, L. C. (1957). The role of weather in determining the distribution and abundance of animals. Cold Spring Harb. Symp. quant. Biol. 22: 203–215.

BIRD, A. F. and WALLACE, H. R. (1966). The influence of temperature on *Meloidogyne hapla* and *M. javanica*. Nematologica 11 (1965): 581–589.

BIRD, G. W. and MAI, W. F. (1967). Factors influencing population densities of *Trichodorus christiei*. Phytopathology 57: 1368–1371.

BLAKE, C. D. (1962). Some observations on the orientation of *Ditylenchus dipsaci* and invasion of oat seedlings. Nematologica 8: 177–192.

BORELLUS, P. (1656). Observationum microscopicarum centuria. Hagae – Comitum: 45 p.

BOSHER, J. E. and MCKEEN, W. E. (1954). Lyophilization and low temperature studies with the bulb and stem nematode *Ditylenchus dipsaci* (Kühn, 1858) Filipjev. Proc. helminth. Soc. Wash. 21: 113–117.

BRAKENHOFF, H. (1914). Beitrag zur Kenntnis der Nematodenfauna des nordwestdeutschen Flachlandes. Abh. naturw. Ver. Bremen 22: 266–311.

BRAND, T. VON (1960). Influence of temperature on life processes. In: Nematology, ed. J. N. Sasser and W. R. Jenkins, Univ. N. Carolina Press, Chapel Hill: 257–266.

BRESSLAU, E. L. and STEKHOVEN, J. H. SCHUURMANS. (1940). Marine freilebende Nematoda aus der Nordsee. Mus. r. Hist. nat. Belg.: 74 p.

BROWN, E. B. (1955). Occurrence of the root-knot eelworm, *Meloidogyne hapla*, out of doors in Great Britain. Nature, Lond. 175: 430–431.

BROWN, H. D. (1928). Free-living nematodes in North China soils. China med. J. 42: 207–209.

BRUIJN, N. DE and STEMERDING, S. (1968). *Nacobbus serendipiticus*, a plant parasitic nematode new to The Netherlands. Neth. J. Pl. Path. 74: 227–228.

BRUN, J. L. (1966). L'adaptation aux températures élevées chez un nématode: *Caenorhabditis elegans* Maupas, 1900. Annls Biol. anim. Biochim. Biophys. 6: 127–158, 267–300, 439–466.

BRYANT, W. E. and WYLLIE, T. D. (1968). Effect of temperature and variety on nematode development and gall production on soybeans infected with *Meloidogyne incognita acrita* (Abstr.). Phytopathology 58: 1045.

BRZESKI, M. (1961a, b, 1962, 1963a, b, 1964). Cf. BOB bibliography.

BUANGSUWON, D. K. and JENSEN, H. J. (1966). A taxonomic study of Mononchidae (Enoplida: Nemata) inhabiting cultivated areas of Thailand. Nematologica 12: 259–274.

BUNT, J. S. (1954). The soil-inhabiting nematodes of Macquarie Island. Aust. J. Zool. 2: 264–274.

BURKHALTER, M. (1928). Die Verbreitung der freilebenden Erdnematoden in verschiedenen Geländearten im Massif der Rochers de Naye. Revue suisse Zool. 35: 389–437.

BÜTSCHLI, O. (1873). Beiträge zur Kenntnis der freilebenden Nematoden. Nova Acta Acad. Caesar. Leop. Carol. 36 (5): 1–144.

BÜTSCHLI, O. (1874). Zur Kenntnis der freilebenden Nematoden, insbesondere der des Kieler Hafens. Abh. senckenb. naturforsch. Ges. 9: 237–292.

BYARS, L. P. (1920). The nematode disease of wheat caused by *Tylenchus tritici*. Bull. U.S. Dep. Agric. **842**: 40 p.
CAIRNS, E. J. (1954). Effects of temperature upon pathogenicity of the mushroomspawn nematode, *Ditylenchus* sp. Mushr. Sci. **2**: 164–167.
CALAWAY, W. T. (1963). Nematodes in wastewater treatment. J. Wat. Pollut. Control Fed. **35**: 1006–1016.
CAPSTICK, C. K. (1959). The distribution of free-living nematodes in relation to salinity in the middle and upper reaches of the river Blyth estuary. J. anim. Ecol. **28**: 189–210.
CARTER, H. J. (1859). On *Dracunculus* and microscopic Filaridae in the island of Bombay. Ann. Mag. nat. Hist. **4**, Ser. 3: 28–44, 98–116.
CASSIDY, G. H. (1926). Remarks on the genera of spearbearing nematodes found in Hawaii, with a table for their identification. Hawaii. Plrs' Rec. **30**: 454–459.
CASSIDY, G. H. (1930). Note regarding a Java collection of nematodes. J. Parasit. **16**: 168–169.
CAYROL, J. C. (1967a). Contribution à l'étude de la fauna nématologique de quelques grottes du sud de la France. Annls Spéléol. **22**: 298–309.
CAYROL, J. C. (1967b). Etude du cycle évolutif d'*Aphelenchoides composticola*. Nematologica **13**: 23–32.
CAYROL, J. C. (1967c). Les conditions de culture et de milieu et le développement des principaux nématodes nuisibles aux champignons de couche. Mushr. Sci. **6**: 475–482.
CHANDLER, A. C. (1954). Nematoda. Fish. Bull., Fish. Wildl. Serv. U.S. **89**: 357–358.
CHATIN, J. C. M. (1885). Helminthes de l'île Campbell et de la Nouvelle – Zélande. Bull. Soc. philomath. Paris **9**: 36–43.
CHITWOOD, B. G. (1934, 1936, 1937, 1938, 1951, 1954, 1960). Cf. BOB bibliography.
CHITWOOD, B. G. (1949). 'Root-knot nematodes'. 1. A revision of the genus *Meloidogyne* Goeldi, 1887. Proc. helminth. Soc. Wash. **16**: 90–104.
CHITWOOD, B. G. and BIRCHFIELD, W. (1957). A new genus, *Hemicriconemoides* (Criconematidae: Tylenchina). Proc. helminth. Soc. Wash. **24**: 80–86.
CHITWOOD, B. G. and BUHRER, E. M. (1945). The life history of the golden nematode of potatoes, *Heterodera rostochiensis* Wollenweber, under Long Island, New York conditions. Phytopathology **36**: 180–189.
CHITWOOD, B. G. and BUHRER, E. M. (1964). Further studies on the life history of the golden nematode of potatoes *Heterodera rostochiensis* Wollenweber, season 1945. Proc. helminth. Soc. Wash. **13**: 54–56.
CHITWOOD, B. G. and CHITWOOD, M. B. (1950). An introduction to nematology. Sect. 1. Anatomy. Monum. Print. Co., Baltimore, Md: 213 p.
CHITWOOD, B. G. and TIMM, R. W. (1954). Freeliving nematodes of the Gulf of Mexico. Fish. Wildl. Serv. U.S. **89**: 313–323.
CHODOROWSKA, W. (1961). Free-living nematoda fauna in small pools in the Kampinos Forest. Polskie Archwm. Hydrobiol. **9**: 265–285.
CHODOROWSKA, W. (1963). Note on the nematode-fauna from the caverns of the Pyrenees (Polish text). Biul. Inst. Ochrony Roślin **21**: 125–127.
CHOLEVA, B. (1966). The nematode fauna of cultivated mushrooms (Bulgarian text). Rastenievdni nauki, Sofia **3** (7): 97–102.
CHRISTIE, J. R. (1929). Some observations on sex in the Mermithidae. J. exp. Zool. **53**: 59–76.
CHRISTIE, J. R. (1959). Plant nematodes, their bionomics and control. Agric. Exp. Stations Univ. Fla, Gainesville: 256 p.
CILLIS, M. I. O. DE (1917). Nuovi generi e nuove specie de nematodi liberi d'acqua dolce. Monitore zool. ital. **28**: 57–62.
CLAPHAM, P. A. (1931). On variations in size of the nematode worm *Rhabditis succaris* n. sp., produced by different culture media. J. Helminth. **8**: 211–222.
CLARK, W. C. (1960/1). New Zealand Mononchidae (Enoplida, Nematoda). 1, 2, 3, 4. Nematologica **5**: 199–214, 260–274, 275–284; **6**: 1–6.
CLARK, W. C. (1963). Notes on the Mononchidae (Nematoda) of the New Zealand region with descriptions of new species. N.Z. Jl Sci. **6**: 612–632.

CLAUS, C. F. W. (1862). Ueber einige im Humus lebende Anguillulinen. 2. Wiss. Zool. **12**: 345–359.

COBB, N. A. (1889, 1890, 1893a, b, 1898, 1904, 1906, 1913, 1914a, b, 1915a, b, c, 1916, 1917a, b, 1918, 1919, 1920, 1921a, b, 1923, 1924, 1926a, b, 1927, 1929, 1930, 1935). Cf. BOB bibliography.

COBB, N. A. (1915). Nematodes and their relationships. Yb. U.S. Dep. Agric. 1914: 457–490.

COCHRAN, W. G. and COX, G. M. (1964). Experimental designs. John Wiley & Sons Inc., N.Y., & Chapman & Hall Ltd, Lond.: 611 p.

COETZEE, V. (1967). Species of the genus *Mylonchulus* (Nematoda; Mononchidae) occurring in southern Africa. Nematologica **12** (1966): 557–567.

COHN, E. (1966). Observations on the survival of free-living stages of the citrus nematode. Nematologica 12: 321–327.

COLES, J. W. (1958). Nematodes parasitic on sea weeds of the genera *Ascophyllum* and *Fucus*. J. mar. biol. Ass. U.K. **37**: 145–155.

CONINCK, L. A. P. DE. (1930a, b, 1932, 1935, 1939, 1940, 1943, 1944, 1962a, b, 1964, 1965). Cf. BOB bibliography.

CONINCK, L. A. P. DE. (1968). Les bases morphologiques de la systématique des nématodes. Rep. 8. int. Symp. Pl. Nematol. (Antibes 1965): 19–24.

COOKE, R. C. and PRAMER, D. (1968). Interaction of *Aphelenchus avenae* and some nematode-trapping fungi in dual culture. Phytopathology **58**: 659–661.

COOMANS, A. (1962). Systematisch-ecologisch onderzoek van de vrijlevende bodemnematoden in België. De vrijlevende nematodenfauna van weideland. 1. Natuurw. Tijdschr. **43** (1961): 87–132.

COOMANS, A. (1966). Some nematodes from Congo. Revue Zool. Bot. afr. **74**: 287–312.

COOMANS, A. and GERAERT, E. (1963). Some species of Dorylaimoidea found in Belgium. Nematologica **8** (1962): 233–241.

COURTNEY, W. D. and LATTA, R. (1934). Some experiments concerning the revival of quiescent *Anguillulina dipsaci*. Proc. helminth. Soc. Wash. **1**: 20–21.

CROLL, N. A. (1967). Acclimatization in the eccritic thermal response of *Ditylenchus dipsaci*. Nematologica **13**: 385–389.

DADAY, J. (1893a, b, 1894, 1896, 1897a, b, c, 1899, 1901a, b, 1902, 1903a, b, c, 1904, 1905a, b, 1906a, b, 1908, 1910a, b, 1911, 1913, 1914). Cf. BOB bibliography.

DAO, F., OOSTENBRINK, M. and VIETS, H. A. (1970). A preliminary literature list of nematode surveys made for agricultural purposes up to 1969. Versl. Meded. plziektenk. Dienst Wageningen sep. Ser. 415.

DASGUPTA, D. R; RASKI, D. J. and SHER, S. A. (1968). A revision of the genus *Rotylenchulus* Linford and Oliveira, 1940 (Nematoda: Tylenchidae). Proc. helminth. Soc. Wash. **35**: 169–192.

DASGUPTA, D. R. and RASKI, D. J. (1968). The biology of *Rotylenchulus parvus*. Nematologica **14**: 429–440.

DAVIDE, R. G. and TRIANTAPHYLLOU, A. C. (1967). Influence of the environment on development and sex differentiation of root-knot nematodes. Nematologica **13**: 102–110.

DAVIS, R. A. (1959). Nematodes associated with roses and the root injury caused by *Meloidogyne hapla* Chitwood, 1949, *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939, and *Helicotylenchus nannus* Steiner, 1945. Diss. Univ. Md.

DAVIS, R. A. (1960). Nematodes associated with roses and the root injury caused by *Meloidogyne hapla* Chitwood, 1949, *Xiphinema diversicaudatum* (Micoletzky, 1927) Thorne, 1939, and *Helicotylenchus nannus* Steiner 1945. Am. Rose A. **45**: 34–47. Bull. Md. Agric. Exp. Stn **A-106**: 16 p.

DECKER, H. (1963). Pflanzenparasitäre Nematoden und ihre Bekämpfung. VEB dt. Landwirtschaftsverlag, Berl.: 374 p.

DITLEVSEN, H. (1911, 1919, 1921, 1922, 1923, 1926, 1928a, b, c, 1930). Cf. BOB bibliography.

DIXON, H. N. (1905). Nematode galls on mosses. J. Bot., Lond. **43**: 251.

DOLLIVER, J. S.; HILDEBRANDT, A. C. and RIKER, A. J. (1962). Studies of reproduction of *Aphelenchoides ritzemabosi* (Schwartz) on plant tissues in culture. Nematologica **7**: 294–300.

DROPKIN, V. H. (1953). Studies on the variability of anal plate patterns in pure lines of *Meloidogyne* spp.; the root-knot nematode. Proc. helminth. Soc. Wash. **20**: 32–39.
DROPKIN, V. H. (1955). The relations between nematodes and plants. Expl Parasit. **4**: 282–322.
DUJARDIN, F. (1845). Histoire naturelle des helminthes ou vers intestinaux. Librairie Encyclopédique de Rozet, Paris, **6**: 16 + 654 + 15 p.
EBERTH, C. J. (1863). Untersuchungen über Nematoden. Leipzig: 77 p.
ELIAVA, I. YA. (1966). Contribution to the knowledge of the nematode fauna of mosses of Georgia (Russian text). Mater. Faune Gruzii **1**: 5–10.
ELLENBY, C. (1954). Environmental determination of the sex ratio of a plant parasitic nematode. Nature, Lond. **174**: 1016–1017.
FAULKNER, L. R. and BOLANDER, W. J. (1967). Occurrence of large nematode populations in irrigation canals of South Central Washington. Nematologica **12** (1966): 591–600.
FAULKNER, L. R. and BOLANDER, W. J. (1969). Interaction of *Verticillium dahliae* and *Pratylenchus minyus* in Verticillium wilt of peppermint: effect of soil temperature. Phytopathology **59**: 868–870.
FENWICK, D. W. (1951). The effect of temperature on the development of the potato-root eelworm, *Heterodera rostochiensis*. Ann. appl. Biol. **38**: 615–617.
FERRIS, J. M. (1957). Effect of soil temperature on the life cycle of the golden nematode in host and non-host species. Phytopathology **47**: 221–230.
FILIPJEV, I. N. (1916, 1917, 1918a, b, 1921, 1922a, b, 1924, 1926, 1927, 1928, 1929, 1930, 1931, 1933, 1934a, b, 1936a, b, 1946). Cf. BOB bibliography.
FILIPJEV, I. N. (1934a). The classification of the freeliving nematodes and their relation to the parasitic nematodes. Smithson. misc. Collns. **89**: 63 p.
FILIPJEV, I. N. (1934b). Harmful and useful nematodes in rural economy (Russian text). OGIZ – Sel'khozgiz., Mosk. and Leningrad: 440 p.
FILIPJEV, I. N. (1936a). On the classification of the Tylenchinae. Proc. helminth. Soc. Wash. **3**: 80–82.
FILIPJEV, I. N. (1936b). Über freilebende und pflanzenparasitische Gattungen der Tylenchinen. Trudȳ zool. Inst. Akad. Nauk SSSR **3**: 537–550.
FILIPJEV, I. N. and STEKHOVEN, J. H. SCHUURMANS. (1941). A manual of agricultural helminthology. Brill, Leiden: 878 p.
FISHER, J. M. (1966). Observations on moulting of fourth-stage larvae of *Paratylenchus nanus* Aust. J. biol. Sci. **19**: 1073–1079.
FISHER, J. M. (1967). Effect of temperature and host on *Paratylenchus neoamblycephalus* and effect of the nematode on the host. Aust. J. agric. Res. **18**: 921–929.
FISHER, J. M. (1969). Investigations on fecundity of *Aphelenchus avenae*. Nematologica **15**: 22–28.
FISHER, K. D. (1968). Population patterns of nematodes in lake Champlain (Abstr.). Nematologica **14**: 7.
FLEGG, J. J. M. (1969). The effects of temperature on the embryogeny of *Xiphinema diversicaudatum*. Nematologica **15**: 285–286.
FRANKLIN, M. T. (1951). The cyst-forming species of *Heterodera*. Tech. Commun. Commonw. Bur. Helminth.: 147 p.
FRANZ, H. (1950). Bodenzoologie als Grundlage der Bodenpflege. Mit besonderer Berücsichtigung der Bodenfauna in den Ostalpen und im Donaubecken. Akad.-Verlag, Berl.: 10 + 316 p.
FRENZEL, G. (1936). Untersuchungen über die Tierwelt des Wiesebodens. Jena: 130 p.
FRIEND, H. (1911). The nematodes of the Thames. Nature, Lond. **87**: 551, **88**: 244.
FUCHS, A. G. (1915). Die Naturgeschichte der Nematoden und einiger anderer Parasiten. Zool. Jb. **38**, Abt. Syst.: 109–222.
FUSHTEY, S. G. and JOHNSON, P. W. (1966). The biology of the oat cyst nematode, *Heterodera avenae* in Canada. Nematologica **12**: 313–320.
GADEA, E. (1952a, b, c, 1953a, b, c, d, 1954a, b, c, d, 1955a, b, 1956a, b, 1957, 1958, 1960a, b, c, 1961, 1962a, b, c, 1963a, b, 1964a, b, c, d, e, f, 1965, 1968). Cf. BOB bibliography.

GERAERT, E. (1965). The genus *Paratylenchus*. Nematologica **11**: 301–334.
GERLACH, S. A. (many faunistic and related studies on marine nematodes in the period 1948–1964). Cf. BOB bibliography.
GHILAROV, M. C. (1967). Abundance, biomass and vertical distribution of soil animals in different zones. Proc. Working Meet. **2** (Jabtonna 1966): 611–629.
GIJSELS, H. (1964a). Invloed van de temperatuur op de lichaamsgrootte van de vrijlevende nematode *Panagrellus silusiae*. Biol. Jaarb. **32**: 336–344.
GIJSELS, H. and BRAKE, E. (1964b). Invloed van de temperatuur op de allometrische groei van de vrijlevende nematode Panagrellus silusiae (de Man, 1913) Goodey, 1945. Natuurw. Tijdschr. **46**: 17–33.
GISIN, H. (1947). Es wimmelt im Boden von Unbekannten. Prisma **2** (5/6): 144–147, 184–187.
GODFREY, G. H. (1926). Effect of temperature and moisture on nematode root knot. J. agric. Res. **33**: 223–254.
GOFFART, H. (1925). Die Bedeutung der Moore für die zoologische Forschung. Biol. Zbl. **45**: 369–373.
GOFFART, H. (1928). Beitrag zur Kenntnis der Fauna westfälischer Hochmoore, unter besonderer Berücksichtigung der aquatilen Fauna. Beitr. NatDenkmPflege **12**: 237–285.
GOFFART, H. (1949). Zur Nematodenfauna unterirdischer Gewässer. Verh. dt. Zool. (Kiel 1948) **49**: 308–312.
GOFFART, H. (1950). Nematoden aus unterirdischen Gewässern. Dt. zool. Z. **1**: 73–78.
GOFFART H, (1951). Nematoden der Kulturpflanzen Europas. Paul Parey, Berl.: 144 p.
GOODEY, J. B., FRANKLIN, M. T. and HOOPER, D. J. (1965). T. Goodey's: The nematode parasites of plants catalogued under their hosts (3. Edn). Commonw. agric. Bur., Farnham Royal, Buckinghamshire, Engl.: 214 p.
GOODEY, T. (1928). The species of the genus *Apelenchus*. J. Helminth. **6**: 121–160.
GOODEY, T. (1932). The genus *Anguillulina* Gerv. and v. Ben., 1859, vel *Tylenchus* Bastian, 1865. J. Helminth. **10**: 75–180.
GOODEY,T. (1933). Plant parasitic nematodes and the diseases they cause. Methuen and Co. Ltd, Lond.: 306 p.
GOODEY, T. (1963). Soil and freshwater nematodes (2. Edn). Rewritten by J. B. Goodey. Methuen and Co. Ltd, Lond., J. Wiley and Sons Inc., N.Y.: 544 p.
GRASSÉ, P. P. (1965). Traité de zoologie. Anatomie, systématique, biologie. Masson et Compagnie, Paris **4** (2/3): 1497 p.
GREEFF, R. (1870). Ueber die frei im Wasser und in der Erde lebenden Nematoden, namentlich die Meeresbewohner. Sber. niederrhein. Ges. Nat.-u. Heilk.: 87–90.
GREEN, C. D. (1964). The effect of high temperatures on aqueous suspensions of stem eelworm, *Ditylenchus dipsaci* (Kühn) Filipjev. Ann. appl. Biol. **54**: 381–390.
GRIFFIN, G. D. and BARKER, K. R. (1966). Effects of soil temperature and moisture on the survival and activity of *Xiphinema americanum*. Proc. helminth. Soc. Wash. **33**: 126–130.
GRIFFIN, G. D. (1968). The pathogenicity of *Ditylenchus dipsaci* to alfalfa and the relationship of temperature to plant infection and susceptibility. Phytopathology **58**: 929–932.
GRIFFIN, G. D. (1969). Effects of temperature on *Meloidogyne hapla* in alfalfa. Phytopathology **59**: 599–602.
GRIFFIN, G. D. and JORGENSON, E. C. (1969). Effect of soil temperature on the pathogenicity and reproduction of *Meloidogyne hapla* on Russet Burbank potato (Abstr.). Phytopathology **59**: 11.
GRISSE, A. DE. (1968). Bijdrage tot de morfologie en de systematiek van Criconematidae (Taylor, 1936) Thorne, 1949 (Nematoda). Deel 1 & Platenatlas. Diss. Univ. Gent.
GUNHOLD, P. (1952). Über die in Kompost lebenden Nematoden. Bonn. zool. Beitr. **3**: 151–166.
GUSHANSKAYA, L. Kh. (1951). On the soil nematode fauna of Uzbekistan (Russian text). Trudy zool. Inst. Akad. Nauk SSSR **9**: 658–660.
HANSEN, E. L. and CRYAN, W. S. (1966). Variation in sex ratio of *Panagrellus redivivus* in response to nutritional and heat stress. Nematologica **12**: 355–358.
HEINDL-MENGERT, H. (1956). Die Nematodenfauna im Schleimfluss lebender Laubbäume. Sber. phys.-med. Soz. Erlangen **77**: (1954) 158–176.

HEINIS, F. (1908). Beitrag zur Kenntnis der Moosfauna der Kanarischen Inseln. Zool. Anz. **33**: 711–716.
HEINIS, F. (1914). Die Moosfauna Columbiens. Mém. Soc. Neuchât. Sci. nat. **52**: 675–730.
HEINIS, F. (1929). Über die Mikrofauna alpiner Polster- und Rosettenpflanzen. Festschr. Zschokke **6**: 1–30.
HESLING, J. J. (1966). 3. Biological races of stem eelworm. Rep. Glasshouse Crops Res. Inst. (1965): 132–141.
HEYNS, J. (1965). On the morphology and taxonomy of the Aporcelaimidae, a new family of dorylaimoid nematodes. Entomology Mem. Dep. Agric. Un. S. Afr. 10; 51 p.
HEYNS, J. (1966). Studies on South African *Xiphinema* species, with descriptions of two new species displaying sexual dimorphism of the tail (Nematoda: Dorylaimoidea). Nematologica **12**: 369–384.
HEYNS, J. (1968). A monographic study of the nematode families Nygolaimidae and Nygolaimellidae. Entomology Mem. Dep. Agric. Un. S. Afr. **19**: 144 p.
HIJINK, M. J. and OOSTENBRINK, M. (1968). Vruchtwisseling ter bestrijding van planteziekten en -plagen. Versl. Meded. plziektenk. Dienst Wageningen sep. Ser. **368**: 7 p.
HIRSCH, J. (1941). Comfort and disease in relation to climate. Yb. Agric. U.S. Dep. Agric. 1941: 237–245.
HIRSCHMANN, H. (1952). Die Nematoden der Wassergrenze mittelfränkischer Gewässer. Zool. Jb. **81**: Abt. Syst.: 314–407.
HNATEWYTSCH, B. (1929). Die Fauna der Erzgruben von Schneeberg im Erzgebirge. Zool. Jb. **56**, Abt. Syst.: 173–261.
HOEPPLI, R. J. C. et al. (1925, 1926, 1932a, b, c). Cf. BOB bibliography.
HOESTRA, H. (1968). Replant diseases of apple in the Netherlands. Meded. LandbHogesch. Wageningen **68-13**: 105 p.
HOFMÄNNER, B. (1913a). Beiträge zur Kenntnis der freilebenden Nematoden. Zool. Anz. **42**: 413–418.
HOFMÄNNER, B. (1913b). Contribution à l'étude des nématodes libres du Lac Léman. Revue suisse Zool. **21**: 589–658.
HOFMÄNNER, B. and MENZEL, R. (1914). Neue Arten freilebender Nematoden aus der Schweiz. Zool. Anz. **44**: 80–91.
HOFMÄNNER, B. and MENZEL, R. (1915). Die freilebenden Nematoden der Schweiz. Revue suisse Zool. **23**: 109–243.
HOOPER, D. J. (1962). Three new species of *Trichodorus* (Nematoda: Dorylaimoidea) and observations on *T. minor* Colbran, 1956. Nematologica **7**: 273–280.
HOPKINS, A. D. (1938). Bioclimatics – a science of life and climate relations. Misc. Publs U.S. Dep. Agric. **280**: 188 p.
HOPPER, B. E. (1961a, b, 1962, 1963, 1967a, b). Cf. BOB bibliography.
HORN, P. (1909). Beitrag zur Kenntnis der Moosbewohnenden *Tylenchus*-Arten. Arch. Ver. Freunde Naturg. Mecklenb. **63**: 67–77.
HUMPHREY, H. B. (1941). Climate and plant disease. Yb. Agric. U.S. Dep. Agric. 1941: 499–502.
HYMAN, L. H. (1951). The invertebrates 3. McGraw-Hill, N.Y., Toronto, Lond.: 197–445.
HYSLOP, J. A. (1941). Insects and the weather. Yb. Agric. U.S. Dep. Agric. 1941: 503–507.
ICHINOHE, M. (1955). Studies on the morphology and ecology of the soybean nematode, *Heterodera glycines*, in Japan. Rep. Hokkaido natn. agric. Exp. Stn **48**: 64 p.
INGLIS, W. G. (1966). Marine nematodes from Durban, South Africa. Bull. Br. Mus. nat. Hist. **14** (Zool.): 79–106.
JÄGERSKIÖLD, L. A. K. E. (1894, 1901, 1909, 1913). Cf. BOB bibliography.
JANIK, J. (1962, 1963). Cf. BOB bibliography.
JATALA, P. and MORRISON, L. S. (1967). Effect of temperature on the development of *Meloidogyne incognita* in sweet-potato (Abstr.). Phytopathology **57**: 816.
JENKINS, W. R. and TAYLOR, D. P. (1967). Plant nematology. Reinhold Publishing. Corp., N.Y., Amst. and Lond.: 270 p.
JIMÉNEZ, M., F.; ARIAS, M.; BELLO, A. and LOPEZ P., J. N. (1965). Catálogo de los nemato-

dos fitoparásitos y peri-radiculares encontrados en España. Boln R. Soc. esp. Hist. nat. **63**, Secc. Biol.: 47–104.

JOHNSON, G. E. (1912). The free-living marine nematodes. Nature, Lond. **89**: 320–321.

JOHNSON, R. N. and VIGLIERCHIO, D. R. (1969). Sugar beet nematode *(Heterodera schachtii)* reared on axenic *Beta vulgaris* root explants. 1. Selected environmental factors affecting penetration. Nematologica **15**: 129–143.

JONES, F. G. W. (1959). Ecological relationship of nematodes. In: Plant Pathology. Problems and Progress 1908–1958, C. S. Holton et al., Univ. Wis. Press, Madison: 395–411.

JONES, F. G. W. (1961). The potato root eelworm *Heterodera rostochiensis* Woll. in India. Curr. Sci. **30**: (5): 187.

JOSEPH, G. (1879). Über die in den Krainer Tropfsteingrotten freilebenden Rundwürmer. Zool. Anz. **2**: 276–277.

KÄMPFE, L. (1955). Die Aktivität von Kartoffel- und Rübennematoden bei verschiedenen Temperaturen und ihre Bedeutung für die Mittelprüfung. Mitt. biol. BundAnst. Ld-u. Forstw. **83**: 139–142.

KÄMPFE, L. (1959). Physiologische Befunde zur Arttrennung und zum Herkunftsnachweis in der Gattung *Heterodera* Schmidt (Nematodes). Verh. dt. zool. Ges. (Frankfurt 1958) **25**: 383–391.

KANNAN, S. (1960). Soil nematodes from Madras City. J. zool. Soc. India **12**: 40–50.

KANNAN, S. (1961). Soil nematodes of Madras City. 2. J. zool. Soc. India **13**: 56–61.

KHERA, S. (1965, 1966, 1967). Cf. BOB bibliography.

KIRJANOVA, E. S. and KRALL, E. (1969). Parasitic nematodes of plants and their control. (Russian text). Nauka, Leningrad: 447 p.

KIRKPATRICK, J. D.; VAN GUNDY, S. D. and TSAO, P. H. (1965). Soil pH, temperature, and citrus nematode reproduction (Abstr.). Phytopathology **55**: 1064.

KISCHKE, U. (1956). Die Nematoden aus der Torf-Zone der Hochmoore des Oberharzes nebst Bemerkungen über gewisse Gruppen der terricolen Begleitfauna (Rotatoria, Acarina, Collembola). Arch. Hydrobiol. **52**: 210–277.

KISTLER, E. (1922). Die Würmer des Chiemsee - Moorgebietes. Arch. Naturgesch. **87** (12) (Abt. A): 220–232.

KLAUSENER, C. (1908). Die Blutseen der Hochalpen. Eine biologische Studie auf hydrographischer Grundlage. Int. Revue ges. Hydrobiol. Hydrogr. **1**: 359–424.

KLEIJBURG, P. and OOSTENBRINK, M. (1959). Nematodes in relation to plant growth. 1. The nematode distribution pattern of typical farms and nurseries. Neth. J. agric. Sci. **7**: 327–343.

KLEIJBURG, P. (1960). Soil sample examination as a basis for advisory work against stem eelworms, *Ditylenchus dipsaci* (Kühn). Nematologica Suppl. **2**: 22–27.

KLOMP, H. (1962). The influence of climate and weather on the mean density level. The fluctuations and the regulation of animal populations. Archs néerl. Zool. **15**: 68–109.

KÖPPEN, W. (1923). Die Klimate der Erde. Grundrisz der Klimakunde. De Gruyter & Co., Berl. and Leipzig: 369 p.

KÖRNER, H. (1954). Die Nematodenfauna des vergehenden Holzes und ihre Beziehungen zu den Insekten. Zool. Jb. **82**, Abst. Syst.: 245–353.

KREIS, H. A. (1924, 1926, 1928a, b, 1929a, b, 1930, 1932a, b, c, 1933, 1936, 1938.). Cf. BOB bibliography.

KRNJAIČ, D. (1968). A contribution to the knowledge of the nematode fauna of Yugoslavia (Serbo-Croat text). Zašt Bilja **98**: 75–96.

KRUGLOVA, R. V. (1960). Investigation of nematode fauna of soil and tree fungi of the Gorki district. Mater. 5. vses. Soveshch. Izuch. Nematod. Tezisȳ Dokl. Samarkandskii gos. Univ. Samarkamd: 141–142.

KRUSBERG, L. R. and HIRSCHMANN, H. (1958). A survey of plant parasitic nematodes in Peru. Pl. Dis. Reptr. **42**: 599–608.

KRUSBERG, L. R. (1959). Investigations on the life cycle reproduction, feeding habits and host range of *Tylenchorhynchus claytoni* Steiner. Nematologica **4**: 187–197.

KUIPER, K. (1969). Enige bijzondere aaltjesaantastingen in 1968. Neth. J. Pl. Path. 75., 4; sep. Ser. 403.

KUX, M. and REMPE, H. (1954). Experience with nematodes in sawdust-compost. Proc. 2. int. Conf. Mushr. Grow., Gembloux (1953): 175–177.
LAUGHLIN, C. W.; WILLIAMS, A. S. and FOX, J. A. (1969). The influence of temperature on development and sex differentiation of *Meloidogyne graminus*. J. Nematol. **1**: 212–215.
LEIDY, J. (1851). Contributions to helminthology. Proc. natn. Acad. Sci. U.S.A. **5**: 205–209, 224–227, 239–244, 349–351.
LEIPER, R. T. and ATKINSON, E. L. (1914). Helminthes collected by the British Antarctic Expedition 1910–1913. Proc. zool. Soc., Lond.: 222–226.
LELLÁKOVÁ – DUŠKOVÁ. F. (1964). Beitrag zur Nematodenfauna einer feuchten Wiese in Westböhmen. Věst. čsl. Spol. zool. **28**: 117–133.
LEVASHOV, M. M. (1928, 1929, 1935). Cf. BOB bibliography.
LIEBERMANN, A. (1927, 1928a, b, 1931). Cf. BOB bibliography.
LINSTOW, O. F. B. VON (1876). Helminthologische Beobachtungen. Arch. Naturgesch. **42** (1) 1: 1–18.
LINSTOW, O. F. B. VON (1877). Helminthologica. Arch. Naturgesch. **43** (1) 1:1–18.
LINSTOW, O. F. B. VON (1901). *Dorylaimus atratus* n. sp. Boll. Musei Lab. Zool. Anat. comp. R. Univ. Genova **109**: 2 p.
LINSTOW, O. F. B. VON (1907a, b, c, 1909). Cf. BOB bibliography.
LOOF, P. A. A. (1960a). Miscellaneous notes on the genus *Tylenchorhynchus* (Tylenchinae: Nematoda). Nematologica **4** (1959): 294–306.
LOOF, P. A. A. (1960b). Taxonomic studies on the genus *Pratylenchus* (Nematoda). Tijdschr. PlZiekt. **66**: 29–90.
LOOF, P. A. A. and OOSTENBRINK, M. (1962a). Bijdrage tot de kennis van de aaltjesfauna van de Nederlandse bodem. Versl. Meded. plziektenk. Dienst Wageningen **136**: 176–184.
LOOF, P. A. A. and OOSTENBRINK, M. ((1962b). *Rotylenchulus borealis* n. sp. with a key to the species of *Rotylenchulus*. Nematologica **7**: 83–90.
LOOF, P. A. A. (1964). A review of the nematode genus *Leptonchus* (Enoplida). Nematologica **9** (1963): 507–520.
LOOF, P. A. A. (1964). Einige Aspekte der Systematik der bodenbewohnenden Nematoden. Wiss. Z. Univ. Rostock (Reihe Math. Naturw.) **13**: 317–322.
LOOF, P. A. (1964). Free-living and plant-parasitic nematodes from Venezuela. Nematologica **10**: 201–300.
LOOF. P. A. A. (1968). Taxonomy of *Hemicycliophora* species from West and Central Europe (Nematoda: Criconematoidea). Meded. LandbHogesch. Wageningen **68-14**: 43 p.
LOOF, P. A. A. and JAIRAJPURI, M. S. (1968). Taxonomic studies of the genus *Tylencholaimus* de Man, 1876 (Dorylaimoidea) with a key to the species. Nematologica **14**: 317–350.
LORENZEN, S. (1966). Diagnosen einiger freilebender Nematoden von der schleswigholsteinischen Westküste. Veröft. Inst. Meeresforsch. Bremerh. **10**: 31–48.
LOWNSBERY, B. F. (1950). Stimulation of golden nematode larvae by root leachings (Abstr.). Phytopathology **40**: 18.
LOWNSBERY, B. F..; HUANG, C. S. and JOHNSON, R. N. (1967). Tissue culture and maintenance of the root-lesion nematode, *Pratylenchus vulnus*. Nematologica **13**: 390–394.
LUC, M. and CONINCK, L. A. P. DE (1959). Travaux de la station biologique de Roscoff. 52. Nématodes libres marins de la région de Roscoff. Archs Zool. exp. gén. **98**: 103–165.
LUC, M. (1961). *Xiphinema* de l'Ouest Africain (Nematoda – Dorylaimoidea). Deuxième note. Nematologica **6**: 107–122.
LUC, M., MERNY, G. and NETSCHER, C. (1964). Enquête sur les nématodes parasites des cultures de la République Centrafricaine et du Congo – Brazzaville. Agron. trop. **19**: 723–746.
LUC, M. (1968). Nematological problems in the former French African tropical territories and Madagascar. In: Tropical Nematology, G. C. Smart and V. G. Perry, Univ. Fla Press, Gainesville: 93–112.
LUCKER, J. T. (1941). Climate in relation to worm parasites of livestock. Yb. Agric. U.S. Dep. Agric. 1941: 517–527.
LUDWIG, F. (1910). Baumälchen und andere pflanzenbewohnende Aale (Aguilluliden). Eine

Studie für den biologischen Unterricht. Der Natur **6**: 43–49.
MACFAYDEN, A. (1957). Animal Ecology. Aims and methods. Sir Isaac Pitman & Sons, Ltd., Lond.: 264 p.
MAESENEER, J. DE (1962). Occurrence of free-living plant parasitic nematodes in Belgium (Abstr.). Nematologica **7**: 13.
MAI, W. F. (1952). Temperature in relation to retention of viability of encysted larvae of the golden nematode of potato, *Heterodera rostochiensis* Wollenweber. Phytopathology **42**: 113.
MAI, W. F. and HARRISON, M. B. (1959). The golden nematode. Ext. Bull. Cornell agric. Exp. Stn **870**: 32 p.
MAI, W. F.; CRITTENDEN, H. W. and JENKINS, W. R. (1961). Distribution of stylet-bearing nematodes in the northeastern United States. Bull. New Jers. agric. Exp. Stn. **795**: 62 p.
MALAGUTTI, G. (1950). The presence of *Ditylenchus dipsaci* on garlic in Boconó, Trujillo, Venezuela. Personal communication.
MALEK, R. B. and JENKINS, W. R. (1964). Aspects of the host parasite relationships of nematodes and hairy vetch. Bull. New Jers. agric. Exp. Stn **813**: 31 p.
MALEK, R. B.; JENKINS, W. R. and POWERS, E. M. (1965). Effect of temperature on growth and reproduction of *Criconemoides curvatum* and *Trichodorus christiei* (Abstr.). Nematologica **11**: 42–43.
MAN, J. G. DE (1876, 1880, 1884, 1885. 1886, 1888, 1889, 1890, 1892, 1893, 1895, 1904, 1906, 1907a, b, 1908a, b, 1910, 1913, 1917, 1919, 1920, 1921, 1922a, b, 1923a, b, 1928, 1929). Cf. BOB bibliography or Van Benthem Jutting (1951).
MAN, J. G. DE (1876). Onderzoekingen over vrij in de aarde levende nematoden. Tijdschr. ned. dierk. Vereen. **2**: 78–196.
MAN, J. G. DE (1880). Die einheimischen, frei in der reinen Erde und im süssen Wasser lebenden Nematoden. Vorläufiger Bericht und descriptiv – systematischer Theil. Tijdschr. ned. dierk. Vereen. **5**: 1–104.
MAN, J. G. DE (1884). Die frei in der reinen Erde und im süssen Wasser lebenden Nematoden der niederländischen Fauna. Eine systematisch – faunistische Monographie. E. J. Brill, Leiden, The Netherlands: 6 + 206 p.
MANKAU, R. and MANKAU, S. K. (1963). The role of mycophagous nematodes in the soil. 1. The relationships of *Aphelenchus avenae* to phytopathogenic soil fungi. In: Soil Organisms, J. Doeksen and J. van der Drift, N. Holland Publishing Co., Amst.: 271–280.
MARCHANT, E. H. J. (1934). The estimated number of nemas in the soils of Manitoba. Can. J. Res. **11**: 594–601.
MARCINOWSKY, K. (1909). Parasitisch und semiparasitisch an Pflanzen lebende Nematoden. Arb. biol. BundAnst. Land- u. Forstw. **7**: 1–192.
MARION, A. F. (1870a). Recherches zoologiques et anatomiques sur les nématoïdes non parasites, marins. Annls Sci. *nat., Zool.* **13** (14): 100 p.
MARION, A. F. (1870b). Additions aux recherches sur les nématoïdes libres du Golfe de Marseille. Annls Sci. nat., Zool. **14** (1): 16 p.
MARION, A. F. (1872). Recherches sur les animaux inférieurs du Golfe de Marseille. Annls Sci. nat., Zool. **17** (6): 23 p.
MAUPAS, E. P. (1900). Modes et formes de réproduction des nématodes. Archs. Zool. exp. gén. **8**: 463–624.
MAWSON, P. M. (1953, 1956, 1957a, b, 1958a, b). Cf. BOB bibliography.
MCBETH, C. W. (1956). Some nematodes associated with Venezuelan agriculture. Shell Dev. Co., Techn. Rep. 9041.
MCELROY, F. D. and VAN GUNDY, S. D. (1966). Effect of temperature on in vitro egg production, hatch and motility of *Hemicycliophora arenaria* (Abstr.). Phytopathology **56**: 889.
MENZEL, R. (1912, 1914, 1920, 1922, 1925a, b, 1929, 1930, 1943). Cf. BOB bibliography.
MESCHKAT, A. (1934). Der Bewuchs der Röhrichten des Plattensees. Arch. Hydrobiol. **27**: 436–517.
MEYL, A. H. (1953a, b, 1954a, b, c, d, 1955a, b, 1957a, b, c, 1961a, b). Cf. BOB bibliography.
MICOLETZKY, H. (1912, 1913a, b, 1914, 1915, 1916, 1917, 1920, 1922a, b, c, d, 1923a, b, 1924, 1925a, b, 1927, 1929, 1930). Cf. BOB bibliography.

MICOLETZKY, H. (1922). Die freilebenden Erd-Nematoden mit besonderer Berücksichtigung der Steiermark und der Bukowina, zugleich mit einer Revision sämtlicher nicht mariner freilebender Nematoden in Form von Genusbeschreibungen und Bestimmungsschlüsseln. Arch. Naturgesch. **87** (8) (Abt. A.) (1921): 1–650.

MIHELČIČ, F. (1953a). Nemátodos de los suelos de humus. Contribución al conocimiento de la microfauna de los suelos de humus. An. Edafol. Fisiol. veg. **12**: 879–905.

MIHELČIČ, F. (1953b). Vorläufiger Bericht über die in den Wäldern um Göltschach (Maria Rain, Kärnten) festgestellten Tardigraden und Nematoden. Carinthia II 143: 115–117.

MÜLLER, O. F. (1786). Animalcula Infusoria fluviatilia et marina, quae detexit, systematice descripsit et ad vivum delineari curavit... sistit opus hoc posthumum quod cum tabulis aeneis L. in lucem tradit vidua ejus nobilissima cura Othonis Fabricii. Hauniae: 56 + 367 p.

MULVEY, R. H. (1961/67). The Mononchidae: a family of predaceous nematodes. 1, 2, 3, 4, 5, 6 and 7. Can. J. Zool. **39**: 665–696, 807–826; **40**: 65–81; **41**: 79–98, 763–774; **45**: 915–940, 941–953.

MULVEY, R. H. (1963). Some soil-inhabiting, freshwater, and plant-parasitic nematodes from the Canadian Arctic and Alaska. Arctic **16**: 203–204.

MULVEY, R. H. (1969). Soil-inhabiting nematodes of the orders Araeolaimida, Chromadorida, Enoplida and Monhysterida from the Canadian high Arctic. Can. J. Zool. **47**: 365–382.

MURAD, J. L. (1965). A study of nematodes from sewage filter beds and of some factors influencing nematode populations. Diss. Abstr. **26**: 558.

MURPHY, P. W. (1962). Progress in soil zoology. Butterworth & Co. Ltd. Lond.: 398 p.

MURPHY, D. G. (1965, 1966). Cf. BOB bibliography.

NEEDHAM, T. (1743). A letter from Mr. Turbevil Needham, to the president; concerning certain chalky tubulous concretions called malm: with some microscopical observations on the farina of the red lily, and of worms discovered in smutty corn. Phil. Trans. R. Soc. **42**: 634–641.

NETSCHER, C. (1965). Les nématodes du genre *Meloidogyne*. Parasites des cultures maraîchères en Afrique occidentale. C. r. Trav. Congr. Prot. Cult. trop. (Marseille 1965): 673–676.

NICHOLAS, W. L. (1962). A study of a species of *Acrobeloides* (Cephalobidae) in laboratory culture. Nematologica **8**: 99–109.

NICHOLSON, A. J. (1954). An outline of the dynamics of animal population. Aust. J. Zool. **2**: 1–64.

NIELSEN, C. OVERGAARD (1949). Studies on the soil microflora. 2. The soil inhabiting nematodes. Natura jutl. **2**: 1–131.

NIELSEN, C. OVERGAARD (1967). Nematoda. In: Soil Biology, A. Burges and F. Raw, Acad. Press Inc. Ltd, Lond. and N.Y.: 197–211.

O'BANNON, J. H.; REYNOLDS, H. W. and LEATHERS, C. R. (1967). Effects of temperature on penetration, development and reproduction of *Tylenchulus semipenetrans*. Nematologica **12** (1966): 483–487.

O'BANNON, J. H. (1968). Observations on seasonal population changes of *Tylenchulus semipenetrans* and the influence of temperature on egg hatch (Abstr.). Nematologica **14**: 12–13.

OERLEY, L. (1880). Az anguillulidák nagánrajza. Természetr. Füz. **4**: 16–177.

OERLEY, L. (1882). Report on the nematodes in the possession of the British Museum, with a review of the classification of the order. Ann. Mag. Hist. **9**: 301–318.

OKHOTINA, M. A. (1926). The distribution of the nematodes in the lake Valda (Russian text). Zap. Gidrolog. Inst. **1**: 177–184, 201–203.

OMER-COOPER, J. (1930). Dr. Hugh Scott's expedition to Abyssinia. A preliminary investigation of the freshwater fauna of Abyssinia. Proc. Zool. Soc., Lond.: 195–207.

OOSTENBRINK, M. (1950). Het aardappelaaltje (*Heterodera rostochiensis* Wollenweber) een gevaarlijke parasiet voor de eenzijdige aardappelcultuur. Versl. Meded. plziektenk. Dienst Wageningen **115**: 230 p.

OOSTENBRINK, M. (1954). Een doelmatige methode voor het toetsen van aaltjesbestrijdingsmiddelen in grond met *Hoplolaimus uniformis* als proefdier. Meded. LandbHoogesch. OpzoekStns Gent **19**: 377–408.

OOSTENBRINK, M. (1954/55). Nematologische waarnemingen 1–4. 1. Verschillende *Meloidogyne*-soorten in Nederland. Versl. Meded. plziektenk. Dienst Wageningen **127**: 231–242.
OOSTENBRINK, M. (1955). Nematologische waarnemingen. 1. Verschillende *Meloidogyne*-soorten in Nederland. Versl. Meded. plziektenk. Dienst Wageningen **127**: 231–234.
OOSTENBRINK, M. (1956). Over de resultaten van verschillende methoden voor het bepalen van vrij beweeglijke aaltjes in de grond. Versl. Meded. plziektenk. Dienst Wageningen **129**: 187–190.
OOSTENBRINK, M. (1957). Das Vorkommen von Artgemischen bei pflanzenparasitären Nematoden. Nematologica **2**, Suppl.: 342 S–346 S.
OOSTENBRINK, M. (1957). Der Transport von *Pratylenchus penetrans* (Nematoda) met Pflanzgut. Z. PflKrankh. PflPath. PflSchutz **64**: 484–490.
OOSTENBRINK, M.(1958). Enige bijzondere aaltjesaantastingen in 1957. Tijdschr. PlZiekt. **64**: 122.
OOSTENBRINK, M. (1959). Enige bijzondere aaltjesaantastingen 1958. Tijdschr. PlZiekt. **65**: 64.
OOSTENBRINK, M. (1960a). Enige bijzondere aaltjesaantastingen in 1959. Tijdschr. PlZiekt. **66**: 126–127.
OOSTENBRINK, M. (1960b). Estimating nematode populations by some selected methods. In: Nematology, J. N. Sasser and W. R. Jenkins; Univ. N. Carolina Press, Chapel Hill: 85–102.
OOSTENBRINK, M. (1961a). Enige bijzondere aaltjesaantastingen in 1960. Tijdschr. PlZiekt. **67**: 57–58.
OOSTENBRINK, M. (1961b). Reviews. 2. Problems in breeding nematode-resistant potatoes (Lectures for a symposium given from 14th to 16th July, 1958, at the Gross-Lüsewitz Plant Breeding Institute). Conference report no. 20 of the German Academy of Agricultural Science in Berlin, 1959. Eur. Potato J. **4**: 418–429.
OOSTENBRINK, M. (1966a). Major characteristics of the relation between nematodes and plants. Meded. LandbHogesch. Wageningen **66-4**: 46 p.
OOSTENBRINK, M. (1966b). Vermeerdering van plantenaaltjes op de geschade plant. Neth. J. Pl. Path. **72**: 208–209.
OOSTENBRINK, M. (1967). Studies on the emergence of encysted *Heterodera* larvae. Meded. Rijksfaculteit Landb.-Wet. Gent **32**: 503–539.
OOSTENBRINK, M. (1970). On the working hypothesis of phytonematology. Proc. 9. int. Nematology Symp., Warsaw. Agust, 1967.
ORR, C. C. (1965). Nematodes in native prairie soils of Kansas and the plants with which they are associated. Diss. Kansas St. Univ.: 105 p.
ORR, C. C. and DICKERSON, O. S. (1967). Nematodes in true prairie soils of Kansas. Trans. Kans. Acad. Sci. **69**: 317–334.
PAESLER, F. (1936, 1939, 1941, 1946, 1956, 1957a, b, 1959, 1962). Cf. BOB bibliography.
PAETZOLD, D. (1955). Untersuchungen an freilebenden Nematoden der Salzwiese bei Aseleben. Ein Beitrag zur Kenntnis der Nematodenfauna binnenländischer Salzbiotope. Wiss. Z. Martin-Luther-Univ. Halle-Wittenb. (Reihe Maeth. Naturw.) **4**: 1057–1090.
PAETZOLD, D. (1958). Beiträge zur Nematodenfauna mitteldeutscher Salzstellen im Raum von Halle. Wiss. Z. Martin-Luther-Univ. Halle-Wittenb. (Reihe Math. Naturw.) **8**: 17–48.
PARAMONOV, A. A. (1927, 1929, 1938, 1951, 1952, 1956, 1962, 1968). Cf. BOB bibliography.
PARAMONOV, A. A. (1962/1964). Osnovȳ fitogel'mintologii. 1 and 2. Izd. Akad. Nauk SSSR, Mosk.: 480 and 446 p.
PARAMONOV, A. A. (1968). Plant-parasitic nematodes. Vol. 1 Ed. K. Skrjabin. Israel Progm. scient. Translns, Jerusalem: 390 p.
PATTERSON, M. T. and BERGESON, G. B. (1967). Influence of temperature, photoperiod, and nutrition on reproduction, male-female-juvenile ratio, and root to soil migration of *Pratylenchus penetrans*. Pl. Dis. Reptr. **51**: 78–82.
PEARSE, A. S. (1946). Observations on the microfauna of the Duke Forest. Ecol. Monogr. **16**: 127–150.
PENNAK, R. W. (1953). Freshwater invertebrates of the United States. Ronald Press Co., N.Y.: 769 p.

PETERS, B. G. (1930). Some nematodes met within a biological investigation of sewage.J. Helminth. **8**: 165–184.
PETERS, B. G. (1955). Soil-inhabiting nematodes. Proc. 2. Easter Sch. agric. Sci. Univ. Nott., Soil Zoology **14**: 44–53.
PETZOLD, H. G. (1956). Zur Fauna des Küstengrundwassers der Insel Hiddensee. 2. Holotriche Ciliaten, Nematoden und Gastrotrichen aus dem Küstengrundwasser. Wiss. Z. Ernst Moritz Arndt-Univ., Greifswald **5**: 429–433.
PIECZYŃSKA, E. (1959). Character of the occurrence of free-living nematoda in various types of periphyton in Lake Tajty (Polish text). Ekol. pol. **7**, Ser. A: 317–337.
PHILLIPSON, J. Proceedings to the UNESCO IBP. Symposium on methods of study in soil ecology, Paris 1967 (in print).
PILLAI, J. K. and TAYLOR, D. P. (1968a). Biology of *Paroigolaimella bernensis* and *Fictor anchicoprophaga* (Diplogasterinae) in laboratory culture. Nematologica **14**: 159–170.
PILLAI, J. K. and TAYLOR, D. P. (1968b). Effect of temperature on the time required for hatching and duration of life cycle of five mycophagous nematodes. Nematologica **13** (1967): 512–516.
PLATONOVA, T. A. (1958). Contribution to the nematode fauna of the family Leptosomatidae from the Kerguelen Island (Russian text). Inf. Byull. sov. antarkt. Eksped. 1955–58: 59–61.
PLATONOVA, T. A. (1967). Free-living marine nematodes of the family Leptosomatidae from the European Arctics (Russian text). Zool. Zh. **46**: 828–839.
PLESSIS–GOURET, G. DU (1885). Essai sur la faune profonde des lacs de la Suisse. Neue Denkschr. allg. schweiz. Ges. ges. Naturw. **29**: 63 p.
PLOTNIKOV, V. (1899, 1901, 1906). Cf. BOB bibliography.
PRASAD, S. K. and WEBSTER, J. M. (1967). Effect of temperature on the rate of development of *Nacobbus serendipiticus* in excised tomato roots. Nematologica **13**: 85–90.
PREVOSTI, A. (1955). Geographical variability in quantitative traits in populations of *Drosophila subobcura*. Cold Spring Harb. Symp. quant. Biol. **20**: 294–299.
RAHM, G. F. (1925, 1928, 1929, 1932, 1937a, b, 1938). Cf. BOB bibliography.
RAHM, G. F. (1928). Wie überwintern die in Moos- und Flechtenrasen der Alpinen Region eingefrorenen bryophilen Tiere (Tardigraden, Nematoden und Rotatorien). Revue suisse Zool. **35**: 271–275.
RAPOPORT, E. H. and TSCHAPEK, M. (1967). Soil water and soil fauna. Revue Ecologie Biol. Sol **4**: 1–58.
RASKI, D. J. (1958). Nomenclatorial notes on the genus *Criconemoides* (Nematoda: Criconematidae) with a key to the species. Proc. helminth. Soc. Wash. **25**: 139–142.
RASKI, D. J. and JOHNSON, R. T. (1959). Temperature and activity of the sugar-beet nematode as related to sugar-beet production. Nematologica **4**: 136–141.
RASKI, D. J. and GOLDEN, A. M. (1966). Studies on the genus *Criconemoides* Taylor, 1936 with descriptions of eleven new species and *Bakernema variabile* n. sp. (Criconematidae: Nematoda). Nematologica **11** (1965): 501–565.
RATZ, I. H. VON (1900). Ujfonalférgek a magyar faunában. Természetr. Füz. **23**: 178–186.
REMANE, A. and SCHULZ, E. (1935). Die Tierwelt des Küstengrundwassers bei Schilksee. 1. Das Küstengrundwasser als Lebensraum. Schr. naturw. Ver. Schlesw.-Holst. **20**: 399–408.
RENAUD–DEBYSER, J. (1959). Contribution à l'étude de la faune interstitielle du Basin d'Arcachon. Proc. 15. int. Cong. Zool. (Lond. 1958): 323–325.
REUVER, J. (1959). Untersuchungen über *Paratylenchus amblycephalus* n. sp. (Nematoda: Criconematidae). Nematologica **4**: 3–15.
REYNOLDS, H. W. and O'BANNON, J. H. (1963). Factors influencing the citrus nematode and its control on citrus replant in Arizona. Nematologica **9**: 337–340.
RHOADES, H. L. and LINDFORD, M. P. (1959). Molting of preadult nematodes of the genus *Paratylenchus* stimulated by root diffusates. Science, N.Y. **130**: 1476–1477.
RICHTERS, F. (1908). Moosfauna – Studien. Ber. senckenb. naturf. Ges. 1907–1908: 14–30.
RIVERA, C., J. E. (1964). Pathogenic and biological aspects of sting and lance nematodes. Diss. Abstr. **25**: 19.

ROBERTSON, D. (1929). Free-living nematodes occurring in arable soil in the north of Scotland. Proc. R. phys. Soc. Edinb. **21**: 253–263.
ROHDE, R. A. and JENKINS, W. R. (1957). Effect of temperature on the life cycle of stubby-root nematodes (Abstr.). Phytopathology **47**: 29.
ROSS, J. P. (1960). The effect of soil temperatures on development of *Heterodera glycines* in soybeans (Abstr.). Phytopathology **50**: 652.
ROSSEN, H. VAN and LOOF, P. A. A. (1962). Notities over het voorkomen van enkele aaltjes-soorten in Zweden. Versl. Meded. plziektenk. Dienst Wageningen **136**: 185–192.
ROUVILLE, E. DE (1903a, b, 1905, 1907). Cf. BOB bibliography.
RUEHLE, J. L. (1967). Distribution of plant-parasitic nematodes associated with forest trees of the world. S.E. Forest Exp. Stn, U.S. Dep. Agric. Forest Serv., Asheville, N. Carolina: 156 p.
RÜHM, W. (1956). Die Nematoden der Ipiden. Parasit. SchrReihe **6**: 437 p.
SACHS, H. (1950). Die Nematodenfauna der Rinderexkremente. Eine ökologisch-systematische Studie. Zool. Jb. **79**, Abt. Syst.: 209–272.
SALMON, S. C. and HANSON, A. A. (1964). The principles and practice of agricultural research. Leonard Hill, Lond.: 384 p.
SANTMYER, P. H. (1955). A comparison of the thermal death time of two dissimilar species of nematodes: *Panagrellus redivivus* (Linn., 1767), Goodey, 1945, and *Meloidogyne incognita* var. *acrita*, Chitwood, 1949. Proc. helminth. Soc. Wash. **22**: 22–25.
SANTOS, M. S. N. DE. A. (1968). *Meloidogyne ardenensis* n. sp. (Nematoda: Heteroderidae), a new British species of root-knot nematode. Nematologica **13** (1967): 593–598.
SAVELJEV, S. N. (1912). Zur Kenntnis der freilebenden Nematoden des Kolaffords and des Relictensee Mogilnoje. Vorläufige Mitteilung. Trudy imp. S-peterb. Obshch. Estest. **43**: 108–126.
SASSER, J. N. (1954). Identification and host-parasite relationships of certain root knot nematodes (*Meloidogyne* spp.). Bull. Md. agric. Exp. Stn: 31 p.
SASSER, J. N. and JENKINS, W. R. (1960). Nematology. Fundamentals and recent advances with emphasis on plant parasitic and soil forms. Univ. N. Carolina Press, Chapel Hill: 480 p.
SAYRE, R. M. and MOUNTAIN, W. B. (1962). The bulb and stem nematode *(Ditylenchus dipsaci)* on onion in southwestern Ontario. Phytopathology **52**: 510–516.
SAYRE, R. M. (1964). Cold-hardiness of nematodes. 1. Effects of rapid freezing on the eggs and larvae of *Meloidogyne incognita* and *M. hapla*. Nematologica **10**: 168–179.
SCHNEIDER, A. F. (1866). Monographie der Nematoden. Georg Reimer, Berl.: 8 + 357 p.
SCHNEIDER, W. (1923a, b, 1924, 1925a, b, 1930, 1935, 1937, 1940, 1943). Cf. BOB bibliography.
SCHNEIDER, W. (1937). Freilebende Nematoden der deutschen Limnologischen Sunda-Expedition nach Sumatra, Java und Bali. Arch. Hydrobiol. **15**, Suppl.: 30–108.
SCHULZ, E. (1932, 1934, 1935a, b). Cf. BOB bibliography.
SCHÜTZ, L. and KINNE, O. (1955). Über die Mikro-und Makrofauna der Holzpfähle des Nord-Ostseekanals und der Kieler Förde. Kieler Meeresforsch. **11**: 110–135.
STEKHOVEN, J. H. SCHUURMANS (1937). Nematodes. In: Klassen und Ordnungen des Tierreichs, H. G. Bronn, Akad. Verlagsges., Leipzig **4**: 365–498.
STEKHOVEN, J. H. SCHUURMANS et al. (1929/1930, 1931a, b, 1933, 1935a, b, c, d, e, 1936, 1937, 1938, 1942a, b, c, 1943a, b, c, 1944a, b, 1946, 1950a, b, 1951, 1954, 1955). Cf. BOB bibliography.
SEINHORST, J. W. (1950). De betekenis van de toestand van de grond voor het optreden van aantasting door het stengelaaltje *(Ditylenchus dipsaci)* (Kühn) Filipjev. Tijdschr. PlZiekt. **56**: 289–348.
SEINHORST, J. W. (1956). Biologische rassen van het stengelaaltje *Ditylenchus dipsaci* (Kühn) Filipjev en hun waardplanten. 1. Reacties van vatbare en resistente planten op aantasting en verschillende vormen van resistentie. Tijdschr. PlZiekt. **62**: 179–188.
SEINHORST, J. W. (1961). Plant-nematode inter-relationships. A. Rev. Microbiol. **15**: 177–196.
SEINHORST, J. W. (1962). On the killing, fixation and transferring to glycerin of nematodes. Nematologica **8**: 29–32.
SESHADRI, A. R. (1965). Investigations on the biology and life cycle of *Criconemoides xeno-*

plax Raski, 1952 (Nematoda: Criconematidae). Nematologica **10** (1964): 540–562.
SEURAT, L. G. (1913). Sur quelques nématodes du Sud Tunisien. Bull. Soc. Hist. nat. Afr. N. **5**: 126–130.
SHARMA, R. D. (1968). Host suitability of a number of plants for the nematode *Tylenchorhynchus dubius*. Neth. J. Pl. Path. **54**: 97–100.
SHER, S. A. (1961–1966). Revision of the nematode subfamily Hoplolaiminae. Brill, Leiden. (Reprinted from Nematologica 1961–1966).
SHER, S. A. (1968a). Revision of the genus *Hirschmanniella* Luc and Goodey, 1963 (Nematoda: Tylenchoidea). Nematologica **14**: 243–275.
SHER, S. A. (1968b). Revision of the genus *Radopholus* Thorne, 1949 (Nematoda: Tylenchoidea). Proc. helminth. Soc. Wash. **35**: 219–237.
SIDDIQI, M. R. (1959). Studies on *Xiphinema* spp. (Nematoda: Dorylaimoidea) from Aligarh (North India), with comments on the genus *Longidorus* Micoletzky, 1922. Proc. helminth. Soc. Wash. **26**: 151–163.
SIDDIQI (1963). *Trichodorus* spp. (Nematoda: Trichodoridae) from Tunisia and Nicaragua. Nematologica **9**: 69–75.
SIDDIQI (1964). Three new species of Dorylaimoides Thorne and Swanger, 1936, with a description of *Xiphinema orbum* n. sp. (Nematoda: Dorylaimoidea). Nematologica **9** (1963): 626–634.
SINITZKII, N. N. (1932). On the question of the significance of the nematodes living in decaying beet (Russian text). Rab. eksp. entomo-nematodnoi Lab. UNIS, Kiev.
SKWARRA, E. (1922). Freilebende Nematoden Ostpreussens. Schr. phys.-ökon. Ges. Königsb. **63**: 107–112.
SKWARRA, E. (1924). Neuere Forschungen über freilebende Nematoden. Schr. phys.-ökon. Ges. Königsb. **64**: 62–63.
SLACK, D. A. and HAMBLEN, M. L. (1959). Factors influencing emergence of larvae from cysts of *Heterodera glycines* Ichinohe. Influence of constant temperature (Abstr.). Phytopathology **49**: 319–320.
SLACK, D. A. and HAMBLEN, M. L. (1961). The effect of various factors on larval emergence from cysts of *Heterodera glycines*. Phytopathology **51**: 350–355.
SNEDECOR, G. W. (1962). Statistical methods. Iowa St. Univ. Press, Ames, Iowa: 534 p.
SOÓS, A. (1940, 1941a, b, c, d, 1943). Cf. BOB bibliography.
SOUTHEY, J. F. (1965). Plant nematology (2. Edn). Techn. Bull. Minist. Agric. Fish. Fd **7**, Lond.: 282 p.
SPEARS, J. F. (1968). The golden nematode handbook. Survey, laboratory, control and quarantine procedures. Agric. Handb. agric. Res. Serv. U.S. Dep. Agric. **353**: 81 p.
STANTSCHEFF, W. (1944). Die Nematoden des Psammolitorals einiger holsteinischer Seen. Zool. Anz. **144**: 216–222.
S'JACOB, J. J. and BEZOOIJEN, J. VAN (1967). A manual for practical work in nematology. Mimeogrd Pap. Int. Postgraduate Nematology Course, Wageningen: 47 p.
STEFANSKI, W. (1913, 1914a, b, 1915a, b, 1916a, b, c, 1922, 1923, 1924a, b, 1926, 1927, 1933, 1937, 1938). Cf BOB bibliography.
STEINER, G. (1913, 1913/1914, 1914a, b, 1915, 1916a, b, c, d, e, f, 1918a, b, c, d, 1919, 1920, 1921a, b, c, 1925, 1926, 1929, 1930, 1931a, b, and incidental records up to 1960). Cf BOB bibliography.
STEINER, G. (1936). The status of the nematode *Aphelenchus avenae* Bastian, 1885, as a plant parasite. Phytopathology **26**: 294–295.
STEINER, G. (1953). Changes in basic concepts in plant nematology. Pl. Dis. Reptr. **37**: 203–205.
STEINER, G. and RAMÍREZ, C. T. (1964). Bibliography on agro- and plant nematodes of the American tropics. J. Agric. Univ. P. Rico **48**: 101–126.
STEMERDING, S. and KUIPER, K. (1968). Aaltjes in land- en tuinbouw. W. E. J. Tjeenk Willink, Zwolle, The Netherlands: 178 p.
STEWART, F. H. (1914). Report on a collection of free-living nematodes from the Chilka Lake on the east coast of India. Rec. Indian Mus. **10**: 245–254.
STÖCKLI, A. (1952, 1957a,b). Cf. BOB bibliography.

STÖCKLI, A. (1943). Über Methoden zur quantitative Bestimmung der im Boden freilebenden Nematoden. Ber. schweiz. bot. Ges. **53A**: 160–174.
STÖCKLI, A.(1946). Der Boden als Lebensraum. Vjschr. naturf. Ges. Zürich **91**: 1–18.
STRADOWSKI, M. (1964). Distribution of the free-living nematodes (Nematoda) in the emerged part of psammolitoral of the Mamry and Sniardwy lakes (Polish text). Fragm. faun. **11**: 273–285.
STURHAN, D. (1964a). Kreuzungsversuche mit biologischen Rassen des Stengelälchens *(Ditylenchus dipsaci)*. Nematologica **10**: 328–334.
STURHAN, D. (1964b). Zum Problem der biologischen Rassen bei *Ditylenchus dipsaci* unter besonderer Berücksichtigung des 'Rübenkopfälchens'. Mitt. biol. BundAnst. Ld-u. Forstw. **115**: 191–193.
SUDAKOVA, I. M. (1958). The plant nematode fauna of the Chuvash A.S.S.R. (Russian text). Zool. Zh. **37**: 134–139.
SUTHERLAND, J. R. and FORTIN, A. J. (1968). Effect of the nematode *Aphelenchus avenae* on some ectotrophic mycorrhizal fungi and on a red pine mycorrhizal relationship. Phytopathology **58**: 519–532.
TANI, Y. (1925). On the free living nematodes. Osaka Igakkwai Zasshi **24**: 1485–1497.
TANIGUCHI, R. (1935). Notes on the movement of the soil nema, *Rhabditis filiformis* Bütschli. Proc. imp. Acad. Japan **11**: 77–79.
TARJAN, A. C. (1952). The nematode genus *Hemicycliophora* de Man, 1921 (Criconematidae) with a description of a new plant-parasitic species. Proc. helminth. Soc. Wash. **19**: 65–77.
TARJAN, A. C. (1966). A compendium of the genus *Criconemoides* (Criconematidae: Nemata). Proc. helminth. Soc. Wash. **33**: 109–125.
TARJAN, A. C. (1967). Some plant nematode genera associated with citrus and other crops in Costa Rica and Panama. Turrialba **17**: 280–283.
TAYLOR, A. L. (1935). A review of the fossil nematodes. Proc. helminth. Soc. Wash. **2**: 47–49.
TAYLOR, A. L. (1936). The genera and species of the Criconematinae, a subfamily of the Anguillulinidae (Nematoda). Trans. Am. microsc. Soc. **55**: 391–421.
TAYLOR, A. L. (1968). Nematode problems of rice. In: Tropical Nematology, ed. G. C. Smart and V. G. Perry, Univ. Fla Press, Gainesville: 68–80.
TAYLOR, D. P. (1962). Effect of temperature on hatching of *Aphelenchus avenae* eggs. Proc. helminth. Soc. Wash. **29**: 52–54.
THOMAS, P. R. (1965). Biology of *Acrobeles complexus* Thorne, cultivated on agar. Nematologica **11**: 395–408.
THOMASON, I. J. and LEAR, B. (1961). Rate of reproduction of *Meloidogyne* spp. as influenced by soil temperature. Phytopathology **51**: 520–524.
THOMASON, I. J. and FIFE, D. (1962). The effect of temperature on development and survival of *Heterodera schachtii* Schmidt Nematologica **7**: 139–145.
THOMASON, I. J.; VAN GUNDY, S. D. and KIRKPATRICK, J. D. (1964). Motility and infectivity of *Meloidogyne javanica* as affected by storage time and temperature in water. Phytopathology **54**: 192–195.
THORNE, G. (1929). Nematodes from the summit of Long's Peak, Colorado. Trans. Am. microsc. Soc. **48**: 181–195.
THORNE, G. and SWANGER, H. H. (1936). A monograph of the nematode genera *Dorylaimus* Dujardin, *Aporcelaimus* n.g., *Dorylaimoides* n.g. and *Pungentus* n.g. Capita zool. **6**: (4) 223 p.
THORNE, G. (1937). A revision of the nematode family Cephalobidae Chitwood and Chitwood. Proc. helminth. Soc. Wash. **4**: 1–16.
THORNE, G. (1938). Notes on free-living and plant parasitic nematodes. 4. Proc. helminth. Soc. Wash. **5**: 64–65.
THORNE, G. (1939). A monograph of the nematodes of the superfamily Dorylaimoidea. Capita zool. **8**: 1–261.
THORNE, G. (1949). On the classification of the Tylenchida, new order (Nematoda, Phasmidia). Proc. helminth. Soc. Wash. **16**: 37–73.
THORNE, G. (1955). Fifteen new species of the genus *Hemicycliophora* with an emended des-

cription of *H. typica* de Man (Tylenchida Criconematidae). Proc. helminth. Soc. Wash. **22**: 1–16.
THORNE, G. (1961). Principles of nematology. McGraw-Hill Book Co Inc., N.Y., Toronto & Lond.: 553 p.
THORNE, G. (1964). Nematodes of Puerto Rico: Belondiroidea new superfamily, Leptonchidae, Thorne, 1935, and Belonenchidae new family (Nemata, Adenophorea, Dorylaimida). Tech. Pap. agric. Exp. Stn P. Rico **39**: 51 p.
THORNE, G. and MALEK, R. B. (1968). Nematodes of the northern Great Plains. Part I. Tylenchida (Nemata: Secernentea). Tech. Bull. S. Dak. agric. Exp. Stn **31**: 111 p.
TIMM, R. W. (1952, 1954, 1956, 1957a, b, 1958, 1959, 1962, 1964, 1965). Cf. BOB bibliography.
TORREALBA, P. A. (1967): Taxonomic and biological observations on some nematode genera. Diss. Univ. Wisc.: 118 p.
TRIANTAPHYLLOU, A. C. (1960). Sex determination in *Meloidogyne incognita* Chitwood, 1949 and intersexuality in *M. javanica* (Treub, 1885) Chitwood, 1949. Annls Inst. phytopath. Benaki, N.S. **3**: 12–31.
TRIANTAPHYLLOU, A. C. (1963). Polyploidy and parthenogenesis in the root-knot nematode *Meloidogyne arenaria*. J. Morph. **113**: 489–499.
TRUDGILL, D. L. (1967). The effect of environment on sex determination in *Heterodera rostochiensis*. Nematologica **13**: 263–272.
TULAGANOV, A. T. (1947). A review of the investigations on the nematode fauna of Central Asia (Russian text). Tezizy Dokl. Sessii Akad. Nauk uzbek. S.S.R.: 41–44.
TÜRK, F. (1903). Über einige im Golfe von Neapel freilebende Nematoden. Mitt. zool. Stn Neapel **16**: 281–348.
TYLER, J. (1933). Development of the root -knot nematode as affected by temperature. Hilgardia **7**: 392–415.
VALKANOV, A. (1934, 1936, 1937/1938, 1957). Cf. BOB bibliography.
VOLZ, P. (1949). Nematodensukzessionen bei der Fallstreuzersetzung im Walde. Verh. dt. zool. Ges. (Kiel 1948): 398–401.
VOLZ, P. (1951). Untersuchungen über die Mikrofauna des Waldbodens. Zool. Jb. **79**: Abt. Syst.: 514–566.
WALLACE, H. R. (1955). Factors influencing the emergence of larvae from cysts of the beet eelworm, *Heterodera schachtii* Schmidt. J. Helminth. **29**: 3–16.
WALLACE, H. R. (1958). Movement of eelworms. 2. A comparative study of the movement in soil of *Heterodera schachtii* Schmidt and of *Ditylenchus dipsaci* (Kühn) Filipjev. Ann. appl. Biol. **46**: 86–94.
WALLACE, H. R. (1962). Observations on the behaviour of *Ditylenchus dipsaci* in soil. Nematologica **7**: 91–101.
WALLACE, H. R. (1963). The biology of plant parasitic nematodes. Edward Arnold Ltd, Lond.: 280 p.
WALLACE, H. R. (1966). Factors influencing the infectivity of plant parasitic nematodes. Proc. R. Soc. **164**, Ser. B: 592–614.
WALLACE, H. R. (1969). The influence of nematode numbers and of soil particle size, nutrients and temperature on the reproduction of *Meloidogyne javanica*. Nematologica **15**: 55–64.
WARD, C. H. (1961). Occurrence, distribution and populations of plant parasitic nematodes associated with forage crops in New York State. Diss. Abstr. **21**: 1702.
WEHUNT, E. J. and EDWARDS, D. I. (1968). *Radopholus similis* and other nematode species on banana. In: Tropical Nematology, ed. G. C. Smart and V.G. Perry, Univ. Fla Press, Gainesville: 1–19.
WEINGÄRTNER, I. (1953). Die Nematoden des Kompostes. Sber. phys.-med. Soz. Erlangen **76**: 86–107.
WEISCHER, B. (1962). Überraschende Funde 'mariner' Nematoden im Binnenlande (Abstr.). Nematologica **7**: 16.
WEISCHER, B.(1963). Pflanzenschädigende Nematoden als natürlicher Bestandteil der Bodenfauna. Wein-Wiss. **18**: 257–260.

WEISER, W. (1953). Reports of the Lund University Chile Expedition 1948–1949. 10. Free-living nematodes 1. Enoploidea. Acta Univ. lund. **49**: 155 p.
WHITEHEAD, A. G. and GRISSE, A. DE (1962). Root-knot nematodes of Arabica coffee in relation to altitude on Mt. Kilimangaro, Tanganyika. Proc. 1. Inter-Afr. Pl. Nematology Conf. (Kikuyu 1960) (IAPSC Publ. 86): 8.
WHITEHEAD, A. G. (1968). Taxonomy of *Meloidogyne* (Nematoda: Heteroderidae) with descriptions of four new species. Trans. zool. Soc. Lond. **31**: 263–401.
WHITLOCK, L. S. and ZWET, T. VAN DER (1963). Survey of tung soils for presence of parasitic nematodes. Pl. Dis. Reptr **47**: 817–819.
WIESER, W. (1956, 1957, 1959a, b, 1960). Cf. BOB bibliography.
WIESER, W. and HOPPER, B. (1967). Marine nematodes of the East coast of North America. 1. Florida. Bull. Mus. comp. Zool. Harv. **135**: 239–344.
WILLIAMS, T. D. (1964). Plant and soil nematodes from the Orkney Islands (Abstr.). Nematologica **10**: 71.
WINOTO S., R. (1969). Studies on the effect of Tagetes species on plant parasitic nematodes. Publ. Fonds Landb. Export Bur. 1916/1918, Wageningen: 132 p.
WINSLOW, R. D. (1955). The hatching responses of some root eelworms of the genus *Heterodera*. Ann. appl. Biol. **43**: 19–36.
WINSLOW, R. D. (1960). Some aspects of the ecology of freeliving and plant parasitic nematodes. In: Nematology, J. N. Sasser and W. R. Jenkins, Univ. N. Carolina Press, Chapel-Hill: 341–415.
WU, H. W. and HOEPPLI, R. J. C. (1929). Free-living nematodes from Fookien and Chekiang. Arch. Schiffs-u. Tropenhyg. **33**: 35–43.
WUEST, P. J. and BLOOM, J. R. (1965). Effect of temperature and age of egg population on the in vitro hatching of *Meloidogyne hapla* eggs. Phytopathology **55**: 885–888.
WÜLKER, G. and STEKHOVEN, J. H. SCHUURMANS (1933). Nematoda (Allgemeiner Teil). In: Die Tierwelt der Nord- und Ostsee, Leipzig 5a.
YAMAGUTI, S. (1954). Helminth fauna of Mt. Ontake. Part 1. Nematoda and Acanthocephala. Acta med. Okayama **8**: 386–392.
YEATES, G. W. (1967a, b, c, d, e, f, g, h). Cf. BOB bibliography.
YOUNG, H. C. and STRUBLE, F. B. (1966). Effect of temperature on the development of *Trichodorus christiei* on weat (Abstr.). Phytopathology **56**: 907.
ZSCHOKKE, F. (1900). Die Tierwelt der Hochgebirgsseen. Neue Denkschr. allg. schweiz. Ges. ges. Naturw. **37**: 400 p.