PLANT NEMATOLOGY NOTES

PLANT NEMATOLOGY WORKSHOPS

NORTH CAROLINA STATE COLLEGE
RALEIGH, N. C.
1954

ALABAMA POLYTECHNIC INSTITUTE
AUBURN, ALA.
1955

Sponsored By The
SOUTHERN REGIONAL NEMATODE PROJECT
(S-19)

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FOREWORD TO 1955 EDITION

These lecture notes from the first Plant Nematology Workshop, held at Raleigh, North Carolina, September 7 to 18, were prepared for the use of the students at the Workshop and not for publication.

Instructors at the Workshop were: E. J. Cairns, Alabama Polytechnic Institute; J. N. Sasser, North Carolina State College; and A. L. Taylor, Section of Nematology, U.S.D.A.

This Workshop was a function of the Southern Regional Nematode Project and was held to provide training in nematology for professional plant pathologists.

Facilities were furnished by the Division of Plant Pathology, North Carolina State College.

The services of Mr. A. L. Taylor were made possible through the co-operation of Dr. G. Steiner of the Section of Nematology, U.S.D.A.

Drawings of nematodes for these notes were made by Dr. Hedwig Hirschmann, North Carolina State College.

Reproduction of the notes was made possible through a grant from the Rockefeller Foundation.

J. N. Sasser, Chairman
Technical Committee (S-19)

FOREWORD TO 1958 EDITION

Requests for copies of these Plant Nematology Notes soon depleted the first and second printings of it. Therefore, a final printing has been prepared and at the same time, extra sets of the new and revised portions have been made for distribution to owners of the earlier edition.

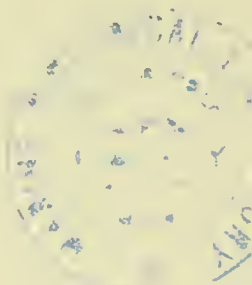
This manual was intended as a stopgap measure due to lack of up to date and available texts; we hope it has served this purpose well. Gratifying as the demand for the manual has been it is even better to note that need for it should soon be over. A book dealing with the plant-parasitic nematodes, the diseases they cause, and their control is soon to be released by Dr. J. R. Christie. A text, which is to be more taxonomic in its approach, is being written by Mr. G. Thorne. The out of print book, Plant Parasitic Nematodes, by T. Goodey is to revised by J. B. Goodey. Soil and Freshwater Nematodes, also by T. Goodey and out of print, is to be reissued. In addition, the

number of recent workshops in nematology that have been held and the release of their notes have reduced the need for increasing the scope or making an extensive revision of Plant Nematology Notes.

Appreciation is again acknowledged to Dr. Hedwig Hirschmann for her work in preparing the new plates for the section on morphology. Thanks are also due to the various authors from whose works we have collected information for these Notes.

As before, reproduction of the Notes was made possible through a grant from the Rockefeller Foundation. The services rendered by this Foundation to the southeastern regional plant-nematology program have set a pattern of activities for all the regions. The result is an arousal of interest and action in phytonematology, rewarding to all who are interested in this important subject and who have so generously supported it with money and active personal participation.

E. J. Cairns, Chairman
Technical Committee (S-19)



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EQUIPMENT AND MATERIALS

I. Equipment and Materials for the Laboratory

A. Optical Equipment:

1. Binocular sterescopic microscope of a type possessing a pedestal base or otherwise mounted so as to permit transmitted illumination of the specimens. A range of magnifications from low to high powers is desirable.

2. Monobjective microscope equiped with a mechanical stage, substage condenser, and objectives ranging from low to high magnifications (oil immersion). A set of oculars with powers from about 5X to 15X and interchangeable binocular body tube are useful additions. Achromatic lenses are satisfactory for most work, but an apochromatic oil immersion objective with compensating eyepieces is much to be preferred for critical examinations requiring maximum resolution at high magnification.

3. Illumination from an electric illuminator of a condenser type capable of providing the Koehler system of illumination is desirable, if detailed microscopic examinations of the nematodes are to be made.

4. Measurements of nematodes and their parts will require an ocular micrometer disc. A filiar micrometer eyepiece is a useful accessory for very accurate measurements, but it is not a necessity. Calibration of the ocular disc or the filiar micrometer will require access to a stage micrometer.

5. Camera lucida apparatus is also useful, if not necessary. Most nematologists dispense with the small mirror provided and instead use a large-sized, front-surfaced mirror. A source of these mirrors, which are made to size and stocked just for this purpose, is the Pancro Mirrors Co. Inc., 2958 Los Feliz Blvd., Los Angeles 39, California. The item is listed as camera lucida mirror, 6X9 inches, 1/8 inch thick mirror-quality glass, front-surfaced Pancro coating. The mirror is mounted on a piece of wood, and a very satisfactory assembly for the mirror can be made from standard Flexiframe supports obtainable from scientific supply houses.

B. Slide Making Materials:

1. Microscope slides of the 3X1 inch size are most used. The best quality, non-corrosive slides should be used for permanent slides. Clinical grade or other less highly finished or selected grades are satisfactory for routine work. A special metal slide which holds the nematode specimens mounted between two coverglasses is obtainable. This device permits study of both sides of the

mounted specimen. Equipment for making these slides is available on loan from various laboratories. Contact one of the authors for more information.

2. Cover-glasses of best quality are to be preferred. They should be of #0 thickness for routine work. Circles of 3/4 inch diameter are generally used. Square cover-slips may be satisfactory and are less expensive.

3. Slide sealing materials are of various kinds. An ideal sealing cement ZUT was devised by G. Thorne (1935) and is widely used by phytonematologists. It is used to seal water, formalin, T A F, lactophenol, and glycerine mounts. It dries quickly and resists action of solvents used for removing immersion oil from the cover-slips. ZUT is obtainable in pint and quart amounts from: Bennett's, 65 West First South Street, Salt Lake City 10, Utah. The recommended thinner for ZUT is butyl acetate. Ethyl acetate has been found to be a satisfactory substitute, and acetone may also be used, but the latter may produce small bubbles in the applied ZUT seal.

   Lactophenol gum is a cement used to seal lactophenol mounts. The directions for its preparation (Davis, 1924) are as follows: Dissolve 38 grams of pure gum arabic in 50 ml, of distilled water, add 5 grams of glucose and 6 grams of lactophenol. The solution is then filtered through glass-wool. Lactophenol consists of a solution made by mixing 3 parts melted phenol, 1 part lactic acid, 2 parts glycerine, and 1 part water.

   Other slide sealing cements include: Clearcol, used to seal lactophenol mounts, obtainable from H. W. Clark, 5419 - 32nd Street, N. W., Washington, D. C.; paraffin-vaseline mixture, 50-50, for temporary water and formalin mounts; gold-size varnish and bakelite resin varnish and other materials can be used, provided that they are not effected by the mounting medium or by the solvents used to remove immersion oil.

   A slide-ringing turntable is not necessary. When used for making neat seals of round cover-glasses, extra precaution should be taken to provide a seal of sufficient thickness, particularly if the ringing compound is thinned for easier application. Seals applied freehand are likely to be thicker, and round as well as square cover-glasses can be safely sealed in this manner, even if somewhat less neatly. Sources of turntables are the Will Corp., Rochester 3, N. Y.; and the Southern Scientific Co., Inc., Atlanta 3, Georgia.

4. Cover-glass supports are necessary to prevent distortion of the nematode specimens by the pressure of the cover-glass. Whatever the kind of support used, it is imperative that it be only slightly thicker than the cross-sectional diameter of the nematodes.

This is necessary in order that the oil immersion objective, which has a very limited working distance, may be focused on and within the nematode without contacting the cover-glass. An excellent means of supporting the cover-glass is by use of short lengths of glass-wool fibers. Glass-wool is readily obtained and will be found to have strands of various diameters, so that supports of the optimum thickness can be selected. Thick supports can be made from pieces of cover-glasses which come in various thicknesses, pieces of slides, pieces of drawn glass rod or tubing, plastic, or from numerous other materials.

One method of support is to apply a ring of ZUT or other inert material to a slide, making a shallow well of desired depth. This involves some practice in getting support of the correct thickness. This method is worthy of consideration and refinement to a more controlled degree, as it offers certain advantages, particularly to the beginner, in the preparation of good slides.

C. Additional Special Laboratory Equipment:

1. Manipulation of nematodes, individually and without harm, can be done in either of two ways. They can be picked up on needles of one kind or another or drawn up into a fine-tipped pipette, provided with a rubber bulb or connected to a tube and mouthpeice for oral operation. The use of a needle is usually preferred, because liquid is unavoidably carried over with the nematodes, when a pipette is used, which may not be desired.

   Ordinary sewing needles of fine size can be used when inserted in a suitable holder. They are improved by oxidizing in an open flame which roughens the needle surface. Cacti needles are excellent, although not always readily obtained. Bristles, strands, or loops of hair mounted in a holder have also been used.

   Long a favorite with nematologists has been the use of a needle whittled from bamboo. Using a razor blade and observing progress under the dissecting microscope, it is possible to get a very fine-tipped needle which is probably unsurpassed for picking such tiny objects as nematode eggs out of water. Leaving the outer hard covering of the bamboo piece as the actual needle end gives added resilience and moisture resistance to the needle.

   A very durable and satisfactory needle for manipulating larval and adult nematodes has been found in the use of dental pulp canal files. These spring metal, needle-like files can be further improved by grinding or filing the tips to a fine point and by slightly bending the last eighth of an inch or so of the tip. A local dentist may have such pulp canal files in stock or may be able to procure them from his supplier. The manufacturer of these files is the Kerr Manufacturing Co., Detroit 8, Mich. The W. A. Lockwood Dental Co., 1722 "I" St., N.W., Washington, D. C.,

is one source for this item which is designated as Kerr pulp canal files, style D, No. 1, and are packaged one-half dozen per box.

2. Slide making can be much easier if one has forceps having fine pointed tips and weak spring tension. Such forceps are best for handling the glass-wool strands used for cover-glass supports and for handling the cover-slips. Low-cost forceps, having the desired weak spring tension, are obtainable from scientific supply houses as "analytical weight" forceps. Stainless steel is preferable to brass. The tips of these forceps can be filed or ground to the desired shape.

   Surgical eye-knives are used for excising nematode heads and tails for special slides. They are also of use in cutting the body of root-knot females for identification to species by perineal pattern study. These knives are made in Europe, and, according to the manufacturer, they are now available in this country, but only through medical supply houses.

   The company will be glad to advise of surgical distributors for any locality upon writing to the Kny-Scheerer Corp., 35 East 17th St., New York 3, N. Y. One source which has been advised of the special use of these knives and stocks the item is McKessen & Robbins, Inc., Surgical Dept., 1706 First Ave., Birmingham 3, Ala. The description of the knife is No. 3224 Wheeler's discission knife.

   The general usefulness of this tool warrants purchasing two, one of which should be used only for cutting nematodes. The cutting edges can be renewed on a fine hone, and these knives, although expensive, have a useful life of many years. Cutting nematodes placed on a piece of celluloid or plastic will prolong the blade's keen edge.

3. Observation dishes for working with the nematodes should be stocked in good numbers. The most useful type of dish is the Syracuse watch glass; a few dozen will be needed. These dishes are very convenient in that they can be stacked, thus preventing evaporation of the water in which nematodes are almost invariably maintained for observation, counting, and other purposes. A similar type of dish, but of much smaller size, is the watch glass, U. S. Bureau of Plant Industry Model, which was developed for nematological work. A dozen or so of these will be found useful. When a small dish, having a flatter bottom surface, is needed, as when counting nematodes, the 60mm. "Petri" dish may be used. The Arthur H. Thomas, Co., West Washington Square, Philadelphia 5, Penn., is a source of the "B.P.I." watch glasses. Syracuse watch glasses and small "Petri" dishes are obtainable at all laboratory supply companies.

D. Sample Processing Equipment:

Processing plant or soil samples to recover the nematodes present involves separating the nematodes from the soil particles or plant tissues, getting the nematodes into suspension in water, and then concentrating the nematodes into a small volume of water for examination and for other purposes. Various means of accomplishing these three steps have been devised and have been used in different combinations. The equipment involved can be very simple, as is described here, or it may take the form of machines, usually utilizing the same principles, but requiring more description than space herein will allow.

1. A blendor, such as the Waring blendor or its equivalent, is used for facilitating the rapid recovery of nematodes from plant tissues, which are reduced to small fragments by the blendor.

2. Pails or pans for containing soil and water are used for the decantation-sieving method. Galvanized pails which nest together are more durable and space-saving than enameled pails. The 12 quart size is satisfactory. Four to six containers should be obtained with two as the minimum. Plastic pails are suitable.

3. Sieves in a series of meshes of 25, 60, 200, and 270 should be acquired. The addition of a 100 and a 325 mesh sieve may be desirable, but it is not necessary. The eight-inch diameter, nest-type sieve is stocked by all scientific supply companies. A smaller five-inch type is available from some companies, but will not be as generally useful as the larger size. A set of small sieves of about two or three-inch diameter for use in rapid removal of nematodes from small samples is useful to have but is not commercially stocked. Such a set is easily made by soldering, fusing, or cementing sieve screening to small tin or plastic dishes of suitable size and shape.

4. Sieve supports will eliminate the need for holding a sieve by hand while pouring the nematode-soil suspension in water through it. A simple, effective support can be made by shaping aluminum clothesline wire into the form of a ring the size of the sieve, with three or four lugs twisted outwards around the border. In use, the sieve fits in the ring which is then placed over the pail or pan and is supported there by the lugs. Similar sieve holders can be made of wood or metal strips. Another possibility is to make a stand to hold the sieve over the container.

5. Funnels are used for isolation of nematodes from soil and plant materials; this much used item of equipment is referred to as the Baermann funnel. In its simplest form, the Baermann funnel consists of a funnel with a short piece of flexible tubing attached to the stem and a clamp to close the tubing. The sample is either supported on or enclosed in a porous material and immersed in water with which the funnel is filled. Separation of the

nematodes from the sample occurs when the nematodes, moving about in the water-soaked sample, eventually reach and pass through the porous boundry which, however, holds back the soil or plant particles. The nematodes sink to the sides of the funnel, and gradually move or drift to the apex of the funnel and accumulate in the funnel stem and rubber tubing just above the clamp. Releasing the clamp at intervals over a period of hours or days permits collection of the nematodes, concentrated in a relatively small volume of water and reasonably free from soil and plant debris.

There are many variations in actual practice, all utilizing the principle of the Baermann funnel; there are more variations than it is possible to describe here. However, a few generalizations are offered which will be helpful in understanding the significance of the results obtained and in devising improvements.

Most of the nematodes which come through the porous barrier do so only because of their own activities. Nematodes which are normally not very active or which are sluggish because of insufficient oxygen, starvation, excessive bacterial contamination, or too cold a temperature are not likely to be recovered in high percentages. Random movement is probably the rule rather than tropistic movement, so the larger the surface area to volume ratio of the sample, the greater the chances of recovery of nematodes. The shorter the distance from the sample to the clamped-end of the funnel and the steeper the walls of the funnel, the higher the collection rate. It is, of course, obvious that the sample itself should be crumbled or cut into small fragments to facilitate freeing the nematodes.

In practice, funnels of about five to six inches in diameter are satisfactory for small samples or for residues from the sieves placed in the funnel for further cleaning. Funnels of about eight inches in diameter are good for moderate sized soil or plant samples which are placed directly in the funnel without previous sieving. Verylarge funnels made of sheet metal can be used to recover nematodes from large samples put to soak in the funnel. Funnels may be of glass, metal, or plastics.

The steeper the angle of the conical section, the larger the bore of the stem, and the shorter the stem, the better; both as an aid to recovery of more nematodes and for ease of cleaning after each use. Guard against using funnels designed for filtering purposes which have a constriction in the bore of the stem at the point of its union with the conical part of the funnel. Cut the stem short, leaving support for attaching the tubing. Spring-action hose clamps are preferable to the screw-type, in order to have adequate control over the small amounts of liquid to be drawn from the funnel.

Supports for the soil or plant samples, when placed in the funnel,

may be of various materials used in different ways. Unbleached muslin or similar cloth serves the purpose well. Small pieces of cloth can be fastened in the top of the funnel by means of clothespins, by lapping the covers over a ring of suitable diameter, or by cutting and sewing into the shape of a skull-cap with a ring sewn in its border. Cloth is re-usable after thorough washing in hot water as a safeguard against carry-over of nematodes from one sample to the next.

Aluminum clothesline wire is excellent for making supporting rings, as it is easily formed and does not rust. Paper, when properly supported, can also be used to hold the sample in the Baermann funnel. Toilet tissue, paper toweling, facial tissues, or paper handkerchiefs (some of which are water resistant) can be used. A disc cut from plastic or non-rusting metalic screening serves as a support for the paper, which, of course, is discarded after each use.

6. Funnel racks are a necessity if a considerable number of Baermann funnels are to be set up in the laboratory. A simple manner of holding the funnels is to insert them into holes of about one and one half inches diameter cut in pieces of wood shelving boards. A slot should be cut from the edge of the board to each hole and be wide enough to allow passage of the funnel stems. Tapering the sides of the holes will help to hold the funnels more stable. Use of a large sized pipe reamer is satisfactory for this purpose.

   The boards can be assembled or mounted in various ways as the laboratory space permits. Examples are: assemblies similar to bookshelves, mountings such as racks over a work table, or shelves along the wall. The one precaution to observe is that there is no chance for water dripping or leaking from funnels into others located below.

7. Soil processing sinks should be planned for, if one has the opportunity to do so. However, any sink can serve the purpose, if a few precautions are observed. The greatest hazard is the accumulation of soil in the plumbing system leading from the sink drain. Special traps are available for installation in place of the usual sink drain traps. These special traps retain the larger soil particles and are easily cleaned out. In some sink installations, the drainage can be piped directly to pits or settling tanks outside the laboratory.

   The sink itself should be large enough to accommodate two pails, and, if possible, have a swing-type faucet high enough to clear the tops of the pails. A faucet which provides for mixing of hot and cold water makes it possible to avoid use of water which may be too cold. Stone laboratory sinks are rugged and serve well for this soil processing work. Regular porcelained iron or steel sinks can be used, if protected from abrasion by rubber

sink-mats or by wooden-slat racks in the bottom.

A useful addition is to have either a separate water outlet, to which a piece of tubing can be fastened, or a spray-hose sink attachment. These are used in directing a stream or spray of water on the sieves and for flushing soil out of the pails and sink. It has been found that an inexpensive aerorator device on the faucet will result in stimulated activity of the nematodes and is, therefore, highly recommended for installation at any sink where samples are to be processed or where water for the Baermann funnels is obtained.

8. Soil disposal is a matter requiring special consideration, if there is a chance that nematodes which are new to the locality may be brought in and become dispersed. Soil processing arrangements having settling tanks, sumps, or pits can be treated periodically with a poisonous chemical. This will reduce the hazard of overflow or run-off water carrying viable nematodes. The accumulated soil can be treated by chemicals, including soil fumigants.

In laboratories where the processed sample residues are not passed into special drains, these materials can be accumulated in pails and carried out of the lab to special locations for contaminated materials. Nematodes in these residues can be killed by steam sterilization, fumigation, or, in some cases, by retaining in such a manner that plant-parasitic nematodes would be starved, dessicated, or subjected to lethal exposures of light, heat, or cold.

II. Equipment and Material for the Field

A. A shovel is needed for digging plants when field examinations of the root systems are necessary. The roots should be dug so that the small roots remain intact. Remove the plant and its adhering soil ball from the ground, and knock the soil from off the roots onto the shovel blade. This assures getting the soil which was in closest contact with the roots and thus having the highest concentration of nematodes of interest.

B. Soil sampling tubes and soil augers can be used to get samples from various depths in the ground and are an excellent means of quickly getting numerous, relatively small samples for consolidation into a larger, single sample, such as may be desired for survey work. The soil tube is preferable to the soil auger in most localities, as the soil sample is retained in the tube and can be readily stripped out of the slot in the side of the tube and caught in the sample container. Soil tubes which require tapping or a ramrod for removal of the soil core are not as satisfactory as the above mentioned slotted-type. Soil augers may have to be used where the soil is rocky or very hard. The disadvantages of the auger are its slowness in use

and the fact that dry or loose soils may fall off as the auger is withdrawn from the ground. Both soil tubes and soil augers should be obtained, however, in order to be able to obtain soil samples from any type or condition of the soil to be encountered. One source for soil sample tubes and soil augers is the National Agriculture Supply Co., Fort Atkinson, Wis.

It is important that, whenever possible, the soil samples should be taken from the rhizosphere of the plant. Soil sampling tubes or augers can serve to do this very well when it is not desired or possible to uproot an entire plant because of its special value or large size.

C. Sample containers can be of various sorts, the only requirement being that they are moisture resistant. The usual soil sample is about one pint to one quart in size. Satisfactory containers include: round ice-cream-type cartons; freezer-type boxes which can be folded flat, a space-saving feature; moisture-resistant lined paper bags; and plastic freezer-type bags. This latter type of container is ideal because of availability, low cost, durability, and compactness when empty.

Plant specimens must be kept from drying out before examinations. Roots can be left in the soil ball or replaced in the soil which had been knocked off for field checking of the roots. Plastic bags are particularly useful for packing plants, as they can be slipped over the roots and soil mass and tied securely about the plant's stem.

All samples should be labeled when taken, and the use of waterproof pencils and heavy-stock paper is recommended. This is particularly important, if there is to be an appreciable delay before processing the sample, and in all cases, if the label is placed inside the container. The moisture from condensation which develops in the closed containers can quickly obliterate the pencil or ink writing and disintegrate ordinary paper.

Samples, when prevented from drying out, can be kept for several days before being examined. If periods of extended delay before processing can be conducted are necessary, the samples, perhaps, are best stored under refrigeration, although this evidently is not true for all kinds of the nematodes.

D. Miscellaneous. Another recommended item of field equipment is a coarse brush which is used to clean soil from sampling tools and from the operator's shoes and pants cuffs. This is an important safeguard when collecting samples in locations where the likelihood of inadvertantly transporting and spreading a nematode pest must be considered. Examples of this are: where an apparently new occurrence of a nematode is being checked, where a quarantine is in effect, and where there are separate planting sites which may not have the same kinds of nematodes.

OUTLINE FOR PROCESSING SAMPLES

1. The complete and, therefore, the best sample consists of plants and soil from the plant's rhizosphere.

2. The samples should be reasonably representative of the condition involved, whether it is only a part of a single plant, a row or spot, or an extensive planting.

3. The samples should be maintained in such a manner that nematodes present will not be dead from overheating or dessication upon arrival at the laboratory or before processing.

Soil and Plant Samples

I. Soil with:

Cyst forming nematodes
- A. Dried
  - Recovery of "floaters"
- B. Not dried
  - Decanting & Sieving

Non-cyst forming nematodes
- A. Baermann funnel
- B. Decanting & Sieving
- C. Combination of both
- D. Direct observation
- E. Seinhorst Elutriator apparatus

II. Plant with:

Endo- and/or Ectoparasitic nematodes
- A. Direct examination
  - Roots and foliage for nematodes and symptoms of nematode damage.
- B. Isolation of nematodes
  1. Teasing out
  2. Soaking
  3. Baermann funnel
  4. Seinhorst extraction technique
  5. Incubation method
  6. Blendor and Sieving
- C. Staining in situ
  1. Osmic acid methods
  2. Lactophenol methods
  3. Bromphenol or bromthymol method
  4. Aceto-osmium method

ISOLATION OF NEMATODES FROM SOIL

Cyst-forming nematodes.

A. Dry cysts will float at the surface of the water when added to the soil sample. The material thus floating is poured, screened, ladled off, or in some other manner removed, and then placed on a 25 mesh sieve nested with a 60 mesh sieve. Both sieves are then thoroughly washed with water. The residue from the 60 mesh sieve is washed off and then distributed in shallow layers in Syracuse watch-glasses prior to searching for cysts under the binocular dissecting microscope.

   The soil may be deliberately permitted to dry out or force dried in order to get the cysts into the condition of being "floaters."

B. Non-dry cysts, whether they float or sink, may be recovered from non-dried soil by roiling the soil in a pail of water, allowing it to settle briefly, and then passing the decanted suspension through 25 and 60 mesh sieves. The process is repeated until only sand and gravel remain in the pail. The residue from the 60 mesh sieve is examined for the presence of nematode cysts.

   It has been found that differential stain which colors the debris and leaves the cysts unstained facilitates finding the cysts even in lightly infested samples. (Taylor, A. L., J. Feldmesser, and G. Fassuloitis, 1952) Stains suitable for this purpose are janus green, brilliant green, malachite green, and gentian violet. The time required for staining depends on the concentration of the stain. Staining times of one hour are satisfactory with one gram of janus green in 4,000 ml. of water, one gram of brilliant green or gentian violet in 32,000 ml. of water, or one gram of malachite green in 64,000 ml. of water. Staining overnight will require solutions of one half these concentrations.

Non-cyst forming nematodes in the soil should never be permitted to dry out, because some species are killed by dessication, and, in any case, the number of forms recoverable from the sample will be reduced by drying.

A. Baermann funnels will hold small volumes of soil from which the nematodes can be extracted, largely as a result of their own efforts, as explained previously. Small samples can, therefore, be put directly on the funnels without any previous treatment.

   Soil particles are most likely to settle into the funnel within the first few minutes after adding the water and the soil. Permit these particles to accumulate in the stem of the funnel, and after a short period, draw off in a small volume of water and replace at the top of the funnel. This practice will result in samples that are much

less obscured by debris, thus facilitating the examination for nematodes in the Syracuse watch-glasses into which the nematode suspensions have been drawn off from the funnels.

Small volumes of the water from the funnels should be withdrawn periodically over a period of 12 to 24 hours, or longer if desired. The water in the funnel should be maintained at a level submerging the sample.

B. Decanting and sieving is the method most used for samples ranging up to a pint or, perhaps, a quart in size. The principle of decanting involves washing the nematodes free from the soil particles by roiling the sample in water, allowing the heavier soil particles to settle momentarily, and then pouring off the supernatant liquid in which the nematodes are suspended.

Sieves operate in two ways in removing the various kinds and sizes of nematodes from the nematode suspension which is poured through them. If the diameter of a nematode is greater than that of the opening of the sieve, the nematode is retained by the sieve. However, if the diameter of the nematode is less than that of the sieve openings, the nematode will pass through, unless it is caught by hanging across the wires of the sieve. This latter condition exists with many of the typically eel-shaped plant-parasitic nematodes which have body diameters less than the 44mm. diameter openings of the finest, or 325 mesh screen. The following table illustrates the relationship of the sieve mesh number to the sieve's value in processing samples for nematodes:

Mesh numbers:	Effective for:	Manner of operation:
25	Retains debris and large soil particles	Too large to pass through sieve
50-60	Retains cyst-forming nematodes	Too large to pass through sieve
100	Retains eel-shaped nematodes of large size	Most of the nematodes are caught only when they hang across the wire mesh, with the exception of large nematodes which may not pass through the 270 or 325 sieve openings,
200	Retains nematodes of most sizes except the smallest forms	
270	Retains all nematode sizes; silted water flows through readily.	
325	Retains all nematode sizes; sizes; silted water flows flows through slowly.	(as above)

It will be apparent that more than one washing of the soil may be required to get most of the nematodes of a sample into suspension. Also, more than one passage through the finest mesh sieve used will be necessary to catch the nematodes which are caught only when they hang by chance over the wire mesh.

A good compromise between speed and efficiency in processing soil samples consists of two or three washings of the sample and passage of the supernatant suspension through the finest mesh sieve five separate times, removing and saving the residues from this sieve after each sieving. The choice of sieves will depend on the objective in mind, a good general series consists of: 25 mesh to remove large debris and soil particles; 60 mesh if cyst forms are being sought, otherwise, it is eliminated from the series; and 270 mesh for retention of all other nematodes. The 325 mesh clogs too readily with most soils other than sandy types. Five repeated sievings can usually be made with the 270 mesh sieve in the time required to sieve only once or twice with the 325 mesh sieve. It should be remembered that repeated sieving is necessary for higher rates of recovery of small nematode species which are caught only when they by chance hang over the fine mesh of the sieve. Thus, the advantage lies with the finest mesh sieve which permits rapid flow of silt laden water through it.

It may be worthwhile to note that sieves can be made of cloth, if it is necessary to improvise. In fact, some workers use fine silk bolting or flour miller's cloth for the fine-meshed series of sieves.

C. Combinations of Decanting and Sieving with the Baermann funnel technique. The value of combining these two methods of processing a soil sample (Christie and Perry, 1951) lies in the freedom from debris and soil particles which results when the residues from the sieves are placed on the Baermann funnel for 24 hours. A recent modification of this method (Feder and Feldmesser, 1954) substitutes a fritted glass Büchner funnel for the usual Baermann arrangement. This permits use of vacuum filtration. This method is also of general value in the handling of nematodes in suspension in fixatives, stains, disinfectants, wash solutions, and toxicants.

D. Direct observation of a very small soil sample soaked in water in a watch-glass is one manner of finding nematodes which may be of value under certain conditions. The time consuming, tedious nature of such a technique limits its general use. This method is, of course, the most accurate way of recovering all the nematodes from a sample, alive or dead. However, the very small size of the sample leaves doubt as to its representative value.

E. Seinhorst Elutriator apparatus. A recently developed method for quantitative extraction of nematodes was introduced into this country by Dr. J. W. Seinhorst during his visit from the Netherlands. A review of methods for determination of nematodes in soil samples

and a complete description of the elutriator apparatus is available (Seinhorst, 1956).

The elutriator apparatus was developed for work involving removal of a high percentage of nematodes from heavy clay soils; two of the most troublesome aspects of soil processing. The elutriator apparatus works well with all kinds of soil, resulting in a high rate of removal of the nematodes. The resulting samples will be found to be much freer from debris and silty water. Another noteworthy feature is that this can be a standardized technique, yielding more comparable results between any laboratories equipped with the elutriator apparatus.

The accompanying illustrations will assist in planning an installation of this apparatus in having the glassware and multiple sieves fabricated. One source for obtaining the glass items and suitable rubber stoppers is Mr. D. E. Sampson, Scientific Supply Room, University of North Carolina, Chapel Hill, North Carolina. If no special device is used for holding the rubber stopper at bottom of the glass column, difficulty in keeping an ordinary rubber stopper in place against the weight of the water and soil may result. A special kind of stopper, stocked by Arthur H. Thomas Company, will hold in place well. (Semi-solid type, Catalog No. 8806, HR-108, size $10\frac{1}{2}$.) The elutriator glassware parts diagrammed have to be made by a glassworker but represent a design suitable for standard glass materials available in this country.

The diagram of an installation consisting of a bank of five elutriators illustrates a convenient arrangement, utilizing readily available materials and incorporating design features suggested by Dr. Seinhorst. The photographs are used with the permission of Dr. Seinhorst and illustrate two types of multiple sieves which will be of use to anyone who uses sieving whether as a part of the Seinhorst elutriator method or in the other methods described. Having the sieves stacked in this manner obviously results in a great saving of time and labor while retaining the desired feature of several passages of the nematode suspension through the fine-mesh final sieve. One person using the bank of five elutriators as illustrated can routinely process at least forty-five samples per work day.

It is advisable to purchase a duplicate set of the flasks and the stems which fit the flasks so that a second lot of samples can be prepared while one set is being processed in the elutriator. Also, these portions of the apparatus are the most subject to breakage.

Another aspect of this method that should be noted by all who apply the Baermann funnel method for a final cleaning and concentrating step is the utilization of petri dishes in place of the funnels. A simplification of the procedure discribed by Seinhorst that has been tested and found very satisfactory is to pour the accumulated washings of the final sieves directly on to a double thickness of Scott

facial tissues supported in a shallow wire dish formed from window screen. The screen dish and its tissue paper filter are then placed in a covered petri dish containing just enough water to keep the tissue wet. Very few, if any, of the nematodes are poured through the tissue, but once in the petri dish where conditions for aeration and locomotion are ideal, the nemas work through the tissue and screen to the water beneath. Most of the nematodes will be in water of the petri dish by 4 hours, but routinely a 24 hour time is allowed for convenience and for maximum removal of nematodes. Tests have shown this procedure to be superior to use of the Baermann funnel in cleanliness of the sample, numbers and viability of the nematodes, and rapidity.

LOCATION AND ISOLATION OF NEMATODES FROM PLANT TISSUES

A. Direct examination of plant tissues is often an important part of processing the plant sample in order to find nematodes and to discover evidence of their activities. The latter is frequently the only clue to the presence of nematodes of the external-feeding or ectoparasitic type, particularly when an inadequate soil sample accompanies the specimen. The use of the binocular, steroscopic, dissecting microscope aids in examination of the suspected plant parts. Immersing the material in a shallow dish of water is usually necessary when fine root structures are being examined or if looking for nematodes at the plant's surface.

The typical eel-shaped nematode is usually seen as a glistening, white or translucent form, often first noted because of its body movement. Swollen, sedentary females and cysts are usually opalescent, but may range in colors from white or yellow to brown. Some forms may be obscured by the matrix of their egg masses or by aggregations of small soil particles adhering to them. Teasing the plant tissues apart is usually necessary. Tissues in advance of lesions may harbor more parasitic forms than in obviously decayed areas.

Nematode disease symptoms are for the most part not to be considered as having exact diagnostic value because other organisms and conditions do cause similar plant responses. The best rule is to make the diagnosis on the basis of the nematode forms recovered from the host tissues or, in the case of root ectoparasites, on the basis of nematodes recovered from the rhizosphere. However, there is considerable value in using plant symptoms as clues to the possibility of plant-parasitic nematodes being involved. Roots are therefore checked for galls, lesions, cortical sloughing, stunting, dead root-tips, and distortions. Foliar parts are checked for lesions, discolorations, and distortions.

B. Isolation of nematodes from plant tissues can be done in a number of ways, the choice depending upon the kind of nematode involved, the type and size of the sample, and the quantity of nematodes required.

1. Teasing of suspected diseased plant parts in a watch-glass containing water is usually the manner in which plant samples are first examined and nematodes recovered for identification.

2. Prolonged soaking of small fragments of plant parts in water may be necessary for the recovery of nematodes from light infections or from tissues which have become dry. Breaking or cutting the plant parts into small pieces expedites the release or movement of nematodes from the tissues in all of the methods suggested here. A harmless wetting agent such as Triton may also have value when added to water used for washing or soaking of samples in any of these methods.

3. The Baermann funnel technique is applicable to plant tissues in the same manner as with soil samples. The plant parts are reduced to small pieces and are submerged in water in the funnel. As decay of the tissues may quickly develop and is detrimental to the nematodes, small portions of the liquid should be drawn frequently from the funnel and clean aerated water added to them. If a prolonged retention of the plant parts in the funnel is necessary, it is better to transfer the tissues to another funnel containing clean water every day or two.

4. Seinhorst extraction technique. Seinhorst (1950) has devised an extraction apparatus which overcomes the above mentioned defect of excessive bacterial contamination and the resulting reduction of oxygen in the water. Infested material is put in funnels in the usual manner and sprayed with a mist of water from nozzles located above. The excess water not entering the funnels is caught on trays and flows away. The spray water slowly passing around and through the tissues in the funnels gently flows out the stem of the funnel into shallow trays below. The extracted nematodes settle to the bottom of these collection trays and the excess water passes through overflow outlets. The nematodes can be collected and concentrated and are ready for use or examination.

5. The incubation method proposed by Young (1954) was found to be more efficient than the Baermann funnel technique for recovery of burrowing and meadow nematodes from avocado roots. In this method, washed roots are collected in a covered container, such as a Mason jar, with a small amount of water, to maintain a humid atmosphere, and are incubated at room temperature.

   Within a few hours, nematodes start accumulating with the small amount of wash water which drains from the roots to the bottom of the container. These nematodes are collected for examination in Syracuse watch-glasses. Water in the jar is maintained by occasional additions from a wash bottle or pipette which, when applied to the roots, washes down more nematodes. The roots can be kept for periods up to several weeks, if care is taken to keep the amount of standing water in the container small. If cultures become overly contaminated, they may be flushed out while in the jars with several changes of water. Nematodes can be recovered from this wash water by use of the sieve-Baermann funnel combination process.

   Some suggested refinements of this technique include splitting the roots lengthwise, stripping back the cortex of larger roots, and the use of antibiotics or other fungal and bacterial inhibitors, which are known to be harmless to stylet-bearing nematodes. Examples of these include penicillin, calcium propionate, and the common sulfa drugs. These materials may also have value for retarding contaminant growth in water held in the Baermann funnel.

6. The blendor and sieving method developed by Taylor and Loegering (1953) is essentially a means of freeing the nematodes from the plant tissues so that they can be recovered by sieving. The method is rapid because it does not depend upon the nematodes moving out of the tissues by their own activity. The method should be used with caution if infections are light, as some of the specimens may be destroyed by the action of the blades or may not be freed from the plant tissues. In practice, the plant parts are cut into short pieces and placed in the blendor in a small volume of water. The blendor is operated for about 10-20 seconds or until the plant pieces are reduced to small fragments. The suspension is then washed simultaneously through a 25 mesh sieve to remove large debris particles, through a 60 mesh to remove cyst-forming and female root-knot nematodes, and through a 200 to 325 mesh sieve to remove other sizes and types of nematodes.

   This method is also useful for getting pieces of the cuticle of female root-knot nematodes free from internal contents for examination and photomicrographs of perineal patterns. The residue from the 60 mesh sieve is searched for these fragments which will be numerous and recognizable if heavily infected roots have been used. The differential stains previously mentioned for use with the cyst-nematode recovery techniques may also have value in this process.

C. Staining in situ provides a means for detecting and studying nematodes within plant tissues without the necessity of making serially sectioned slides with the aid of a microtome. Plant parts treated by the methods described may retain enough stain to be more or less opaque if the tissues are too thick. The remedy for this is to split the tissues.

   Several different methods for in situ staining of root and foliar parts for nematodes are presented.

FIXATION FOR NEMATIZED ROOTS

(Arzberger technique; Flemming Medium fixative; acetic acid-glycerine clearing; pure glycerine mounting.)

Section of Nematology, Beltsville, Maryland

Flemming Medium fixative:
1% chromic acid solution----------375 cc.
glacial acetic acid---------- 25 cc.
distilled water---------- 50 cc.
1 gram osmic acid (in sealed vial)

Mix the chromic acid solution, the glacial acetic acid, and the distilled water in a graduate. Pour about 25 to 50 cc. of the solution into a double-stoppered bottle that has been thoroughly cleaned. Avoid getting any oil—even finger marks—where the fixative will touch. Remove the labels from the outside of the osmic acid vial and clean its surface with alcohol, again avoiding any finger marks. Drop vial into the double-stoppered bottle, and break it by stamping down with a cleaned heavy glass rod, thereby releasing the osmic acid. Immediately pour on the rest of the chromic acid-acetic acid solution.

Store bottle in a dark container, away from light.

Place washed roots in the fixative, using only as much fixative as will cover the roots, in a small, well-corked bottle. Allow to stand 1, 2, or 3 days, depending on size of roots and color desired.

Wash in running water 1, 2, or 3 days.

Transfer by steps (20%, 30%, 40% alcohol, several hours to 1 day each) to 50% alcohol. For this a bottle or closely-covered glass dish may be used.

Transfer roots to an open Syracuse watch-glass or Petri dish, depending on the quantity of roots in the lot.

Make up a solution of ½ glacial acetic acid and ½ pure glycerine; keep in a drop-bottle.

Drop acetic-glycerine solution into dish containing roots gradually during the period of a week or less, depending upon temperature and humidity conditions, allowing the alcohol to evaporate at the same time that the acetic-glycerine is penetrating the roots. For a small quantity of roots, well covered by the 50% alcohol, in a Syracuse watch-glass, the process may be begun with 5 to 7 drops of acetic-glycerine in the morning, the same amount in the afternoon, then gradually increasing the amount dropped during the next few days.

As each quantity of acetic-glycerine is added, agitate or stir the dish to facilitate uniform mixing.

Keep dish uncovered during daytime to promote evaporation. Cover at night or when preparation cannot be watched. Depending on atmospheric conditions, adjust the cover to increase or decrease evaporation and differentiation, so that after the 4 to 7 day period the roots lie in a pure acetic-glycerine solution.

Roots may now be mounted in pure gylcerine. Nematodes within roots should be black, root tissue pale yellow and clear.

Roots treated with Flemming strong fixative

Flemming Fixative, strong formula, consists of:

1% chromic acid	15 parts
2% osmic acid	4 parts

Roots are washed free of soil and immersed in water heated to 70-80° C for 1-2 minutes in order to kill the nematodes within. The roots are then transferred to the fixative for 10-15 minutes, the degree of staining can be controlled by observation under the binocular stereoscopic microscope. Wash roots in running water for several hours or overnight. Dehydrate by passage through a graded alcohol series to absolute. Clear in clove oil and, if permanent slides are desired, mount in Canada balsam.

Foliar parts treated with Flemming strong fixative

In order to get satisfactory *in situ* staining of tissues containing chlorophyll it is necessary to pretreat before staining. Godfrey (1935) recommended treatment with hot acetone prior to soaking in the Flemming fixative.

Drop small pieces of the plant material into boiling acetone in a small conical flask held in a water bath. Boil for a few minutes and leave in the slowly cooling acetone until the green color is removed. Wash in several changes of water and then transfer into the Flemming fixative. Observe the process under the microscope and remove from the stain when the nematodes are sufficiently blackened. Wash in running water several hours or overnight. Dehydrate, clear, and mount in balsam, euparal, or diaphane, if permanent preparations are desired.

Osmic acid is quite expensive, and although it gives excellent results in the methods cited, other techniques of *in situ* staining have been developed.

Rapid method of staining for nematodes in roots

A modification of the original lactophenol plus cotton blue or acid

fuchsin method (Goodey 1937) was developed (McBeth, Taylor, and Smith 1941) in order to eliminate the necessity for dehydrating the tissue.

Goodey's formula for staining is used as follows:

Phenol, pure crystals	20 g.
Lactic acid	20 g.
Glycerine	40 g.
Distilled water	20 ml.
Acid fuchsin or cotton blue	5 ml. (1 g. to 100 ml. water)

The washed roots are boiled in the staining solution for one minute. Wash in tap water to remove excess stain and place in lactophenol solution until cleared. The same lactophenol formula is used for clearing that is used for staining, except that no stain is added. Clearing requires from one to several days, depending upon the type of roots and intensity of the stain. After clearing, the roots can be left in lactophenol or in glycerine for examination. If permanent mounts are desired, the material can be mounted directly in glycerine.

In some cases, better results are obtained by using a weaker stain concentration, $\frac{1}{2}$ ml. of stain solution in 100 ml. of clear lactophenol. Boil the plant parts for two minutes in clear lactophenol, then for one minute in the stain, or for two minutes in the stain.

Leaves stained in cotton blue lactophenol (Franklin 1949)

Prepare a 0.1 per cent solution of cotton blue in lactophenol (stock lactophenol for this purpose consists of phenol, 20 g.; lactic acid, 20 g.; glycerine, 40 g.; and water, 20 g.).

Plunge leaves into boiling 0.1 per cent cotton blue lactophenol and keep submerged in the gently boiling stain for 3-5 minutes. Leave in the stain, which is permitted to cool. Remove tissues and wash in running water or in 50 per cent alcohol to eliminate excess stain. Clear in lactophenol or in concentrated phenol. Concentrated phenol acts more quickly and removes the chlorophyll. More or less permanent mounts can be made by transferring the plant material through 50 and 70 per cent alcohol to iso-butyl alcohol and mounting in euparal. If euparal gets cloudy before mounting is completed, warm the slide gently until cloudiness disappears. The American equivalent for euparal is diaphane. Mounting in this medium may be from either 95 per cent or absolute alcohol, but one should work quickly to avoid clouding.

Roots of some plants, particularly of trees, are not satisfactorily stained by the preceeding methods because of oils and other materials present in the roots. These types of roots will require staining and sectioning by standard methods. The procedures recommended for demonstrating fungi and bacteria within plant tissues are also useful to demonstrate nematodes. These methods are to be found in other references (e.g. Rawlins 1936).

NEMATODE STAINING IN DEAD LEAVES (MINDERMAN 1956)

A method whereby nematodes even in opaque brown leaves can be observed. The leaves are bleached with hydrogen peroxide and made transparent by immersion in lactophenol. Nematodes and other organisms are stained by appropriate stains dissolved in lactophenol.

Damp leaves or pieces of them are covered with the following bleaching mixture:

Water	3 parts by volume
20% ammonia solution	1 part " "
30% hydrogen peroxide	1 " " "

Leave from 1 to 24 hours, depending upon the material to be bleached. Wash in water. Stain by pouring a hot stain solution (± 65° C.) over the leaves. After about 5 minutes, pour off dye and replace with pure lactophenol until no appreciable amount of dye comes out of the leaves. The specimens are mounted in lactophenol and sealed with "Zut" if permanent slides are desired. Acid Fuchsin and Cottonblue in 0.05% solutions were found most suitable for nematodes from a wide selection of stains tested for this purpose.

ACETO-OSMIUM IN SITU METHOD FOR ROOTS (TARJAN AND FORD 1957)

This method was developed for observing nematodes in citrus feeder roots which do not stain satisfactorily using conventional methods because of the presence of suberin and other unsaturated compounds.

The staining schedule found most effective was as follows: Insert washed roots into the fixing and staining solution at 52° C. for 2 hours. (Water, 16 parts; 10% acetic acid, 10 parts; and 2% aqueous osmium tetroxide, 2 parts.) Wash stained roots for 1 hour in running water. Bleach in 10-30% hydrogen peroxide at 32° C. until the darkened tissues lighten perceptibly, then wash several times with water. Pass through 70%, 90%, and 100% ethanol at 52° C., 1/2 hour in each. Immerse in methyl salicylate at 52° C. until tissues clear, after which the roots may be stored indefinitely in this liquid at room temperatures. Although other clearing agents such as clove oil are just as effective, methyl salicylate (synthetic oil of wintergreen) may be preferred due to its inoffensive odor.

Tissues that have been treated but still are opaque can be transferred back through 95% ethanol directly into the bleach and then passed up through the alcohol series to the clearing agent again. Excess exposure to hydrogen peroxide will result in excessive destaining of the nematodes and, in some cases, will cause disintegration of the roots.

BROMPHENOL OR BROMTHYMOL IN SITU STAIN FOR NEMATODES

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Clearing is by means of a bleach which makes possible the use of a wide variety of stains. In the procedure described using pH indicator dyes the color development and contrast obtained between plant and nematode tissue apparently is due to the greater buffer capacity of the nematode tissue. These maintain the absorbed dye in the pH range in which it is most highly colored. The nematode tissues apparently absorb considerable amounts of the dye. These two factors combined result in good color contrast.

Bromphenol blue gives a deep blue to green color to the nematodes and eggs contrasted with a very light yellow or clear background of plant tissue. Orange or light green color contrasted with a very light yellow background are obtained when using bromthymol blue or bromcresol green.

Kill fresh material with F.P.A. (formalin, 5 ml.; propionic acid, 5 ml.; and 50% ethanol, 90 ml.). Rinse in 50% ethanol and then place in water. In the above steps higher alcohol concentrations dehydrate, twist, and deform the nematodes; xylol destroys them. Drain water from the material and then transfer to 1% sodium hypochlorite until "cleared." Drain, rinse 3-4 times with water to remove excess $NaOCl_2$, rinse in 50% ethanol in which it can be stored. Transfer to 1% bromphenol blue in 50% ethanol. and leave for at least 4 hours (0.05-0.1% in 50% ethanol for 12-16 hours if more detail is required). Staining time is not critical and can be extended; some destaining is possible by leaving material in 50% ethanol for several days.

Drain off stain, rinse with 50% ethanol until wash is faintly yellow, drain briefly and transfer to a petri dish containing 0.2% v/v acetic acid in 50% ethanol. (Too much acid will destroy the buffer capacity of the tissues.) Observe the material for nematodes and eggs while the material is in the petri dish. For semi-permanent mounts the tissues can be transferred to water for several minutes and then mounted in glycerol. For permanent mounts the tissues can be transferred through a series consisting of 50% ethanol, 70% ethanol, and 70% isobutanol (1-2 minutes in each) and mounted in diaphane (euparal). If greater detail is desired of the plant cell walls, the tissue segments are placed in an aqueous solution of 1:50,000 safranin for 10 minutes, or until the desired degree of staining is obtained. Do this after removal of the tissue from the acetic acid step and before processing into semi-permanent or permanent mounts. Store all mounts away from bright light.

Smaller rootlets may be ruined by the "clearing" times required for the larger roots. The bleach appears to disintegrate nematodes that were dead prior to sampling and fixing. Prolonged immersion in the bleach will dissolve swollen female root-knot and cyst nematodes, but the eggs persist much longer. The stain, which is expensive, can be used repeatedly when saved and filtered through cloth.

PREPARATION OF NEMATODE SLIDE MOUNTS

Accurate identification of plant and soil nematodes usually requires the preparation of microscope slide mounts suitable for examination of the specimens with the aid of the oil immersion objective lens. The reader is referred to the section listing laboratory equipment and materials for details and discussions pertaining to manipulation of nematodes and supplies needed for slide making.

Usually the nematodes recovered from the soil or plant samples are collected in Syracuse watch-glasses, in water and reasonably free from soil and plant debris. Processed materials preserved in 5% formalin and kept in vials may be emptied into watch-glasses in preparation for slide making. The nematode samples are then examined with the aid of the binocular dissecting microscope and the desired nematodes selected, removed, and made into slide mounts. It should be noted that adult specimens are required for identification to species and that it is always worthwhile to mount several specimens of each kind of nematode present. Usually considerable numbers of individual nematodes can be mounted on a single slide, although it may be necessary to mount nematodes with relatively thick bodies separately from thinner nematodes.

Nematodes may be examined while alive, but usually it is necessary to inactivate them for detailed study. The very delicate complex structure of the nematodes requires appropriate means for killing, fixing, and preserving; and the methods found satisfactory have been few. The following table summarizes the types of slide preparations presented in this manual.

Type of Nematode Mount	Temporary	Semi-permanent	Permanent
Cyst forms (*Heterodera*) Root-knot (*Meloidogyne*)	water	lactophenol 5% formalin TAF	lactophenol
Eel-shaped forms	water	5% formalin TAF	glycerine
Head, tail and cross-section mounts	methyl-cellulose		glycerine-jelly

Relaxing the nematodes prior to killing and fixing them is usually done to reduce the likelihood of distortion of the nematode in the fixative. Heat is most generally used for this purpose. The nematodes can be placed in a drop of water on a slide or put into a depression slide and gently heated until activity ceases. Some workers maintain an incubator at 52° C. for standardizing this heat treatment. Larger samples of nematodes can be heated by adding a small amount of hot water or by placing the sample, concentrated by centrifugation, in a hot water bath at 65° C. for at least two minutes.

Fixation of the nematodes after relaxing is accomplished either by transferring the nematodes to the fixative or by removing the excess water from about the nematodes and adding the fixative. Nematodes concentrated in the bottom of a centrifuge tube or settled in a vial of water are fixed by the addition of a volume of double strength fixative equal to the volume of water present in the tube or vial. Mix the contents promptly by shaking.

Formulae for various commonly used fixatives and mounting media are given with notations on their use.

Formalin Fixative

5% formaldehyde is the concentration most frequently used and is prepared from:

Stock formalin	5 ml.	133 ml.
(37.5% formaldehyde)	or	
Water	32.5 ml.	to make one liter

The nematodes can be transferred directly to a drop of this fixative on a slide, glass-wool supports added, and the coverglass sealed carefully. This type of slide mount may last for as long as several months or longer. Distortion of the cuticle and of the oesophagus may be noted for some species when fixed and preserved in this material. This has for many years been the fixative used for routine identification work.

TAF Fixative

(Courtney, Polley, and Miller 1955):

Water distilled	91 ml.
40% commercial formaldehyde solution	7 ml.
Triethanolamine	2 ml.

This new fixative, called TAF (triethanolamine formalin), has given excellent fixation and preservation of specimens which have lasted for more than two years. It is said to eliminate the objectionable features of the ordinary 5% formaldehyde solution.

Living nematodes may be placed directly in the cold fixative, or they may be first relaxed. Although it is still too soon to know how long permanent slides of nematodes fixed in TAF will last, good slides have been obtained by the modified process of Baker, which is included in this section of the manual.

Formalin - Aceto - Alcohol

Numerous variations of this popular type of fixative have been used for fixation of nematodes, one recommended proportion is:

95% ethyl alcohol	15 or 20 ml.
Glacial acetic acid	1 ml.
Formalin (40% formaldehyde)	6 ml.
Distilled water	40 ml.

After relaxing by gentle heat, the nematodes are covered with the fixative and left there for 24-48 hours. A slight staining of the nematodes can be obtained if a few drops of saturated picric acid are added to the fixative.

Lacto-phenol Solution

Melted phenol	3 parts
Lactic acid	1 part
Glycerine	2 parts
Water	1 part

Cyst-forms and female root-knot nematodes can be mounted directly from water or formalin into lactophenol solution for examination of cuticular details. The cover-glass need not be sealed, as this material evaporates at a very slow rate.

Permanent Slides

Slides of soil and plant nematodes capable of lasting many years can be made but are usually, and perhaps wrongly, considered to involve too much trouble for routine work. However, specimens of taxonomic value should be made into permanent mounts. Two methods for making such slides are presented. The first method requires a lengthy lapse of time to allow dehydration of the nematodes to occur and is the method developed by G. Thorne of the Section of Nematology. Nematodes on slides prepared in this manner over 30 years ago are still in an excellent state of preservation. The second method is popular because of being quicker, due to a different manner of dehydration.

Special slides include mounts made of nematodes for examining the en face aspect of nematodes, cross-sectional views, and tail mounts, The method of Buhrer given in this manual can be used for making permanent mounts of cross-sections, nematode tail sections, as well as for head mounts. The methylcellulose method is used only for rapid preparation of mounts which are not permanent.

Miscellaneous Staining Methods

Intra-vital stains of various kinds are of value in water mount slides of most of the nematodes, except those of the Tylenchoidea and Aphelenchoidea groups. Anilin blue WS (Syn: cotton blue, water blue, china blue, Poirrier's blue) is a very useful stain which aids in the resolution of cuticular structures and markings and for locating amphidial and phasmidial openings. A small drop of saturated stain in water is added directly to living or fixed specimens on the slide.

DIRECTIONS FOR MAKING FACE VIEWS AND SECTIONS OF NEMATODES

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Satisfactory face views cannot be made from distorted, shrunken specimens, especially spear-bearing species of Dorylamoidea, Tylenchoidea, etc., in which spears are extruded if the specimens are not properly killed.

1. Kill and relax specimens by placing them in a hollow ground slide filled with water and hold over a small flame. Do not overheat and cook them.

2. Fix in F.A.A. or dilute Flemmings: The latter gives a slight stain which aids in locating the portions severed.

3. Place in 1½% glycerin in 30% alcohol and evaporate to pure glycerine in a small desiccator, a process which requires at least two weeks.

4. Prepare slide with a drop of hard glycerine jelly. Do this by placing a small piece of jelly on the slide and carefully warming it into a drop over a very small flame. Do not heat too much or bubbles will form. Spread drop as thin as possible with bamboo splinter.

5. Select the tip of a slender hair from a brush and imbed in jelly drop while warm. This will act as a marker for the head and should reach about half-way across the drop.

6. Place nematode in a drop of glycerine on a thin piece of celluloid and decapitate with an "eye knife." This is done under the binocular with the hand held firmly on the hand rest.

7. Pick up head with a very fine bamboo splinter and hold in hand while performing the next operation.

8. Insert head into glycerine jelly at end of pointer hair and stand on end, holding it in place with the bamboo splinter just touching the surface of the jelly above the face. The jelly sets in a few seconds.

9. Apply a very thin cover-glass to the drop, carefully centering it before it touches.

10. Heat the dissecting needle and carefully touch the cover-glass, melting the jelly underneath until the cover-glass is lowered to near the face. Do not use too much heat in this operation or the whole drop of jelly will become warm and the head fall out of place and migrate.

11. Place two small drops of candle wax just under the edge of the cover-glass on opposite sides, lightly cementing it to the slide.

12. Bring the head to perfect perpendicular by carefully pushing on the edge of the cover-glass. This is first done under the binocular and later, if necessary, perfected under the high power. The wax will hold the cover-glass in place, yet allow for the necessary slight movements necessary to bring the head into perpendicular. (Staining the nematode before cutting off the head aids in locating and orienting the face view. Acid Fuchsin, either in Goodey's formula or in fixative, may be used. The stain usually fades out withing 24 hours.)

RAPID PREPARATION OF TEMPORARY HEAD-MOUNTS

The desirability of a rapid method using a viscous mounting medium into which a specimen could be transferred directly from fixative or water led to the use of methylcellulose. (Cairns and Tarjan 1955).

Slides made by this method are of a temporary nature and are satisfactory mainly for determining the surface structures and the internal sclerotized parts of the nematodes' heads. These structures, unlike the rest of the body, apparently are not distorted by this treatment and are the anatomical elements usually considered of diagnostic value in en face preparations.

The viscous, clear mounting medium is prepared by heating 50 ml. of distilled water in a beaker to about 80 to 90° C. Enough methylcellulose of one of the higher viscosity types (e.g. 400-4,000 cps.) is immersed in the water so that it appears well soaked. After 30 minutes the mixture is cooled by partial immersion of the beaker in cold water. If the material fails to dissolve completely, the beaker is immersed in ice water. If the material becomes too viscous during or after its preparation, a small quantity of distilled water is added. The material becomes slightly more fluid after a few days and bubbles made by stirring disappear.

The specimen is transferred into a drop of the medium on a glass slide, its head is severed, and a cover-slip then placed directly on the drop. Manipulation of the cover-slip will bring the nematode head into the desired vertical position. The location of the head is indicated in the usual manner by placing three dots on the cover-slip with India ink in a triangular fashion around the specimen. Application of molten paraffin-petroleum jelly mixture to the edges of the cover-slip cements it firmly in place so that shifting of the specimen will be greatly reduced. The specimen is now ready for immediate examination.

PERMANENT MOUNTS IN GLYCERINE (THORNE'S METHOD)

1. Heat specimens in water in a depression slide very gently until they are killed. Frequent checking under the dissecting microscope is required if heating is done over a small flame or on a slide warming table. A better method is to put the specimens in a small amount of water in a "B.P.I." watch-glass and place in an incubator at 110° F. for about 20 minutes or at 120° F. for about 5 minutes.

2. Drain off the water with a fine pipette, add formalin-aceto-alcohol fixative. After a few minutes, draw off the liquid and add fresh fixative. Leave for 24 to 48 hours, the longer time is preferred.

3. Transfer the nematodes to a solution of 1¼% glycerine in 30% ethyl alcohol contained in a "B.P.I." watch-glass.

4. Place the watch-glass in a small desiccator. The desiccant is kept in a small, screw-capped vial with a hole of about one millimeter in diameter bored in the cap. Two grams of desiccated calcium carbonate is the amount of desiccating agent used. Calcium chloride is not used, because its use in this manner results in too rapid uptake of the moisture in the small desiccator. Allow three weeks for dehydration of the alcohol-glycerine solution; complete dehydration in less time is not desired.

5. After the water has thus been removed, after the three or more weeks required, transfer the watch-glass with the nematodes to a large calcium chloride desiccator for three days. Transfer specimens to pure glycerine, the supply of which is kept in a calcium chloride desiccator.

6. Mount specimens in a drop of pure desiccated glycerine on a slide cleaned with acidulated alcohol. Add glass-wool supports which have been kept in dehydrated glycerine. Orient supports and specimens and add a clean cover-glass that was preheated slightly. The cover-glasses used are selected for freedom from defects, #00 in thickness, and cleaned in acidulated alcohol.

7. Seal the cover-glass with ZUT and label slides.

The advantage of using the special metal slides, by means of which nematodes can be mounted between two cover-glasses for observation of both sides, will be appreciated for permanent slides of scarce specimens needed for taxonomic purposes.

MODIFICATION OF RAPID METHOD FOR MOUNTING NEMATODES IN GLYCERIN

Dr. A. D. Baker, Department of Agriculture, Ottawa, Canada

*** F O R M U L A E ***

Series -	I	II	III	IV	V
Glycerin	55	70	82	90.0	100.0
Lactic Acid	15	10	5	2.5	
Phenol (crystals)	15	10	5	2.5	
Distilled Water	15	10	5	2.5	
Formol	0	0	3	2.5	

With Lactophenol the proportions would be as follows:

Series -	I	II	III	IV	V
Lactophenol	75	50	25	12.5	
Glycerin	25	50	72	85.0	
Formol	0	0	3	2.5	

From fixative to Lactophenol and then through the above series to pure Glycerin. About 10 minutes in each of these mixtures at a temperature of 50 - 55° F.

In preparing these mixtures, it is convenient to have a stock solution of equal parts of Lactic Acid and Phenol (crystals) available. This should be kept in an amber bottle. Lactophenol and the above mixtures should also be stored in amber bottles.

Stain may be included with the Lactophenol, as described for cotton blue staining (0.01 per cent) by Franklin & Goodey in 1949. In making up these mixtures, add the stain to the distilled water used in making up your Lactophenol, or dissolve the dry stain in Glycerin (with heat). Remember that most stains are soluble in Glycerin. Thus, if some fading of the color is not to occur, it is sometimes necessary to faintly "tint" the mixtures, as well as the final mounting medium. It is also important to note that all nematodes do not stain equally well with the same stain. Cotton blue is a useful stain, but one has to be careful not to overstain. Other stains that may be tested for particular needs

include Chlorazol Black, Fast Green, Bismark Brown, Purpurin R, and B. Scarlet. Orange G is a good general stain. A stain that the writer has found useful is Van Giesen's Picro-Fuchsin. Some very interesting differential staining may often be obtained with this medium, with bright colors and very little danger of overstaining.

Controlling temperatures while using this mounting method may appear to be a problem if constant temperature equipment is not available. If possible, reduce the peak temperature of your hot plate below the boiling point of water, (preferably around 60 - 70° C.). Five drops of one of the mixtures are about sufficient to fill the shallow depression of a hanging-drop slide. Transfer the specimens to this slide. Place the slide on the hot plate with the tip of your index finger on top of the slide at one end. When the temperature is too hot for your finger, take the slide off the plate.

There is no present indication that this method gives better results than original Rapid Method (Baker 1953), but its main value may lie in the fact that fewer transfers are necessary.

PERMANENT NEMATODE MOUNTS IN GLYCERINE
Based on a procedure of Dr. J. W. Seinhorst

The following instructions are based on a procedure for making permanent mounts as described by Dr. Seinhorst during his visit in 1957 to the southeastern states as a function of the Southern Regional Nematology Project (S-19). Complete details of this method are to be published in the near future. The instructions given here are to be considered only as preliminary and not necessarily according to Dr. Seinhorst's technique in all details. The method, or modifications of it, are in use in several laboratories in the South and proving quite satisfactory for mounting most nematode species.

A. Relax and kill the nematodes with gentle heat (50-52° C.). The progress of this step can be observed under the dissecting scope by using a cavity slide containing a small quantity of water and the specimens.

B. Transfer the nematodes to a Bureau of Plant Industry watch glass or other suitable dish containing fixative. FA, FAA, Kahle's solution, TAF, or 5% formaldehyde can be used as fixatives. Cover dish to retard evaporation.

   1. If fixative used is at 50-52° C., keep nematode in it for 12-24 hours.

   2. Or, if fixative used is at room temperature, keep nematodes for 24-48 hours or longer.

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   2. Or, if fixative used is at room temperature, keep nematodes for 24-48 hours or longer.

C. Transfer fixed nematodes into methyl alcohol solution (glycerin 5% in methyl alcohol) in a Bureau of Plant Industry watch glass or other container. Place on hot plate regulated to 60-64°C.

D. Keep containers on a hot plate at 60-64°C. until the methyl alcohol has evaporated (about 30 minutes). Collapsing, if it occurs in some specimens, can be corrected by leaving container on the hot plate for a longer time.

E. Transfer containers with the specimens to a dessicator for at least one day.

F. Mount specimens in anhydrous glycerin.

ADDITIONAL TECHNIQUES AND SOURCES OF INFORMATION

General Methods

Goodey, J. Basil, 1957. Laboratory methods for work with plant and soil nematodes. Tech. Bul. 2 Ministry of Agriculture, Fisheries and Food. This third edition of a very useful manual is recommended to all workers in phytonematology and particularly should be used by those interested in the techniques of working with the cyst-forming nematodes, a topic which is not dealt with in any great detail in the Plant Nematology Notes. The bulletin can be obtained in the U.S. from the British Information Services, 45 Rockefeller Plaza, New York 20, New York, for $.70 (last quotation). In Canada, copies can be obtained from: United Kindgom Information Offices, 275 Albert Street, Ottawa; 119 Adelaide Street, West, Toronto; 1111 Beaver Hall Hill, Montreal.

* * *

Additional useful information concerning survey procedures and processing techniques for cyst-forming nematodes may be obtained upon request in the form of a handbook prepared for its field and laboratory by the Plant Pest Control Branch, Agricultural Research Service, U. S. Dept. of Agriculture, Washington, D. C.

* * *

Kevan, D. Keith McE. (Ed.), 1955. Soil Zoology. Butterworth, London, 512 pp. This is an excellent reference source for anyone interested in methods and ecology of soil fauna. The contents are presented as papers by individual contributors and discussions which resulted at the presentation of the papers at a symposium. A considerable portion of the book deals with nematodes. Many other items of interest to those concerned with the soil as an environment for animal life.

Processing of Samples

Tarjan, A. C., W. A. Simanton, and E. E. Russell. 1956. A labor saving device for the collection of nematodes. Phytopathology 46 (12): 641-644. A workable and inexpensively constructed device for extraction of nematodes from soil and comminuted plant tissue. It uses spray nozzles for washing and agitating the soil and a system of inclined, graded sieves for selective trapping of different sized nematodes.

* * *

Miller, Patrick M., 1957. Cheap, disposable filters for nematode surveys. Plant Dis. Reptr. 41:192.

Stoller, B. B., 1957. An improved test for nematodes in the soil. Plant Dis. Reptr. 41(6):531-532.

The above two methods, although described for use with small-sized soil or plant samples, can be used for cleaning residues containing nematodes washed from sieves. Both methods feature use of expendable

tissue filter materials and final containers for the nematodes; the first using paper cups, the second using tubes formed from polyethylene films.

* * *

Caveness, Fields E., and Harold J. Jensen, 1955. Modification of centrifugal-flotation technique for isolation and concentration of nematodes and their eggs from soil and plant tissues. Proc. Helm. Soc. Wash. 22(2):87-89.

Miller, Patrick M., 1957. A method for the quick separation of nematodes from soil samples. Plant Dis. Reptr. 41(3):194. The centrifugation-flotation technique as described is applicable to rather small-sized soil and plant samples but is of particular interest as a means for recovery of nematode eggs. The further modification of the technique by Miller consists of screening the nematodes from the separating sugar solution instead of allowing them to settle, this reduces the overall time of the process considerably.

* * *

Minderman, G., 1956. New techniques for counting and isolating free-living nematodes from small soil samples and from oak forest litter. Nematologica 1(3):216-226. Describes two techniques for use in investigating nematodes in small-sized soil samples. One involves staining of the nematodes in samples where total counts of nemas are desired. The other technique is a combination of centrifugation and flotation of the nematodes using magnesium sulphate solution. This method may have application for recovery of eggs from samples and a way for mechanization of the processing is illustrated.

Manipulation of Nematodes

Ford, Harry W., 1957. A source of controlled vacuum for pipetting nematodes. Plant Dis. Reptr. 41(2):89-90. A simply constructed device for applying a very weak suction to a pipette for removing individual nematodes from a dish. One ingenious feature of the device is that pipetting action will stop automatically when the tip is lifted above the surface of the water because surface tension of the water at the tip of the pipette is greater than the vacuum applied to the pipette.

Pathogenicity Experimental Work

Mountain, W. B., 1955. A method of culturing plant parasitic nematodes under steril conditions. Proc. Helm. Soc. Wash. 22(1):49-52. A technique for rearing plant-parasitic nematodes under aseptic conditions on root cultures.

* * *

Fenwick, D. W., 1956. The production of sterile viable larvae of the potato root eelworm, Heterodera rostochiensis. Nematologica 1(4):331-336. A simplified technique for obtaining eggs and larvae which may be adaptable to Meloidogyne and other plant-parasitic forms.

Lownsbery, B. F., and J. W. Lownsbery, 1956. A procedure for testing the sterility of large numbers of nematodes after treatment with various sterilants. Plant Dis. Reptr. 40(11):989-990.

* * *

Holdeman, Q. L., 1954. Value of greenhouse tests in evaluating the host range of nematodes. Plant Dis. Reptr. Supplement 227:81-82. Consideration of some of the factors in evaluating tests for the host range of nematodes. Should be read by all who contemplate work of this type.

* * *

Pitcher, R. S., 1957. A critical review of current techniques for the study of migratory root nematodes as etiological agents. Nematologica 2 (Supplement):413-423S. An excellent review paper which will be of use to anyone planning work on pathogenicty of plant-parasitic nematodes.

Miscellaneous

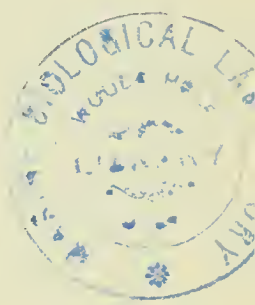
Korsten, L. H. J., J. W. Sieben, and L. Voskuyl, 1953. A colorimetric determination of the number of eelworms in a suspension. Euphytica 2: 135-138. A quick method of determining the nematode concentration in a suspension used for inoculation. The percentage absorption of a quantity of nematodes in an aqueous solution of carboxymethlcellulose is determined and, by comparison to a standard curve, the number of nematodes per ml. is found. A suspension of a given concentration can be made.

* * *

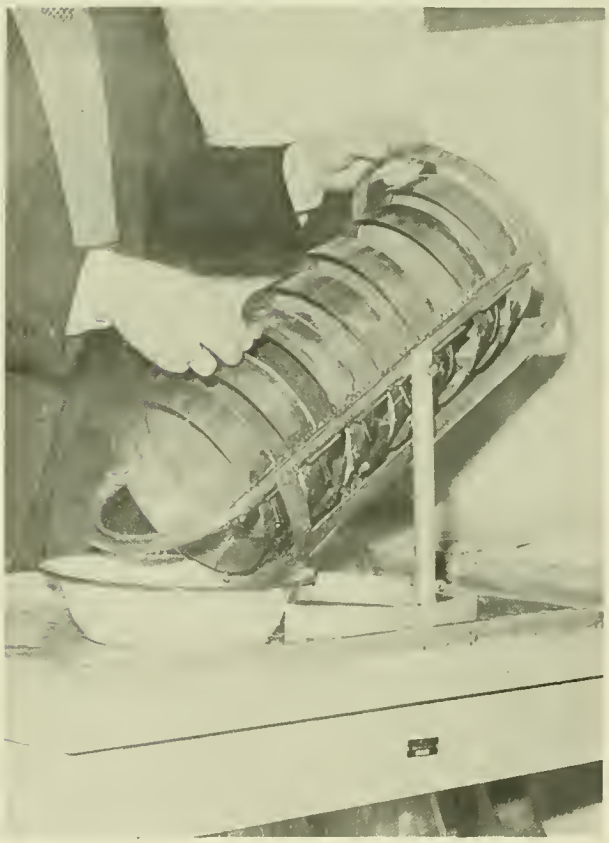
Feldmesser, J., and W. A. Feder, 1954. Maintaining and determining viability of nematodes in vitro. Soil Sci. Soc. Fla. 15:154-156. Discussion of a problem of obvious interest when viability of the nematodes must be determined. Various techniques are discussed for determining viability and for maintaining in vitro populations of nematodes under ideal conditions for examination.

* * *

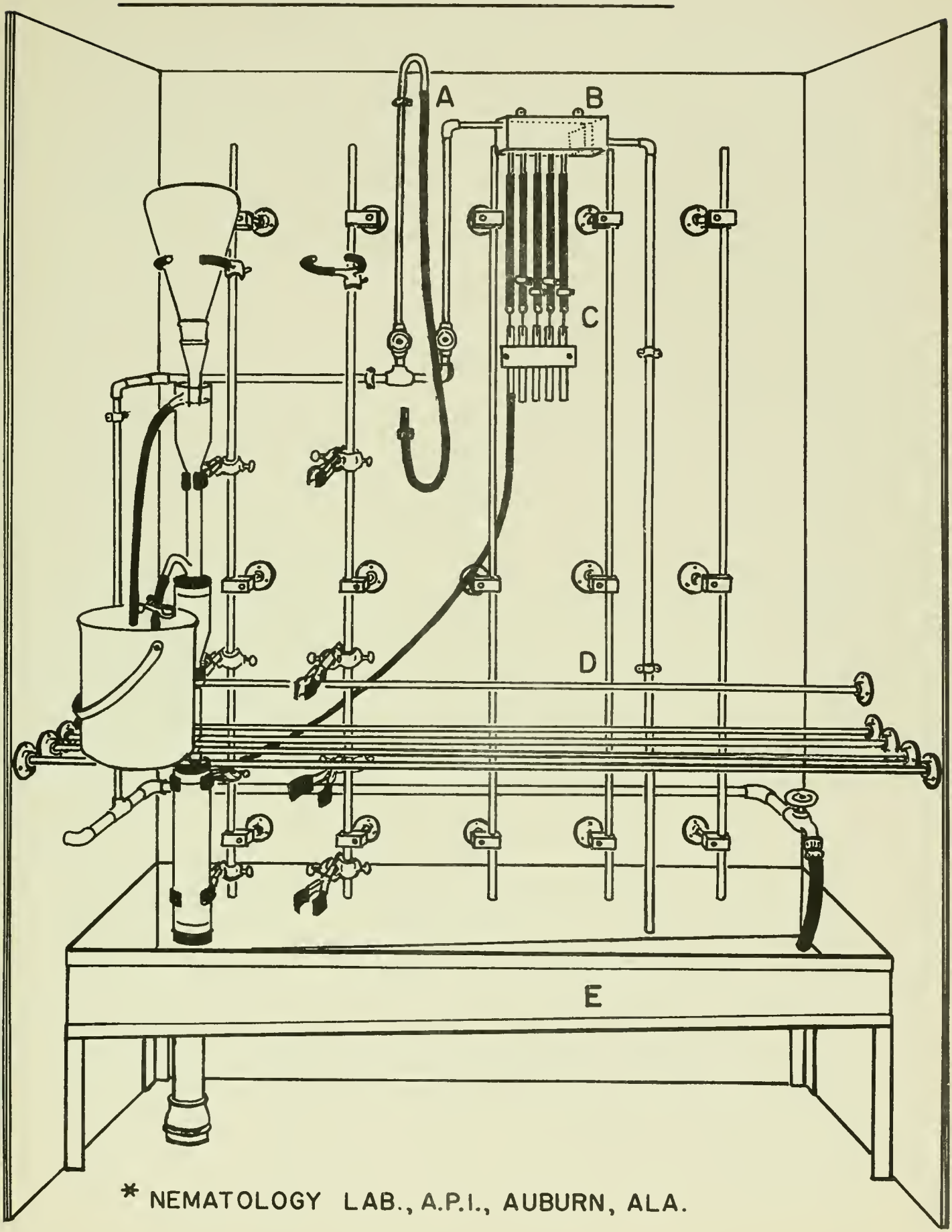
Jensen, H. J., F. E. Caveness, and R. H. Mulvey, 1954. A modification of Thorne's technique for examining soil diffusion patterns of nematodes. Plant Dis. Reptr. 38(9):680-686. Detailed description of a method using standard materials which can be applied to other situations in which nematode distribution or profiles are involved.



MULTIPLE SIEVES



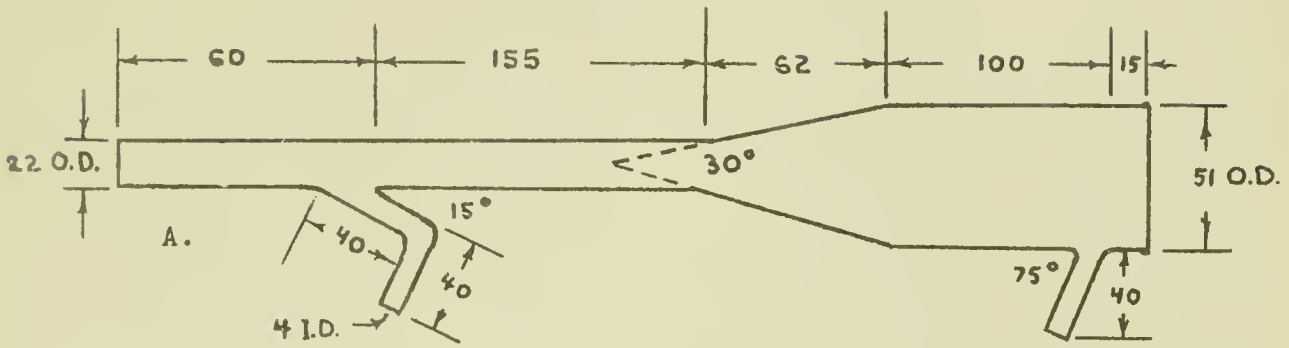
ELUTRIATOR INSTALLATION*

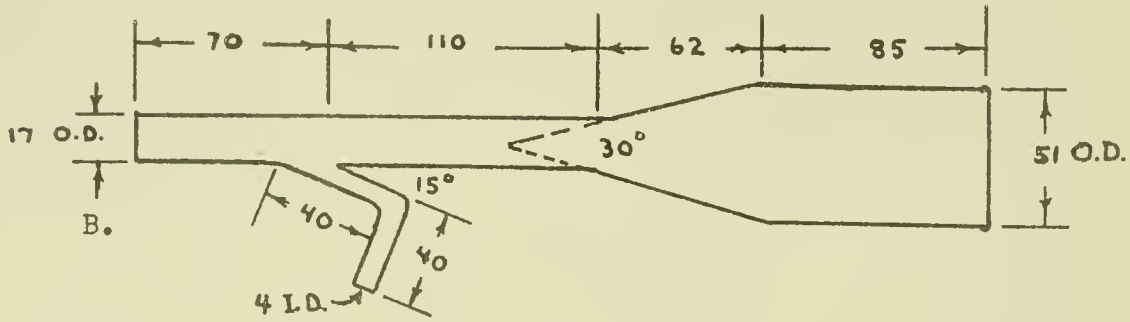


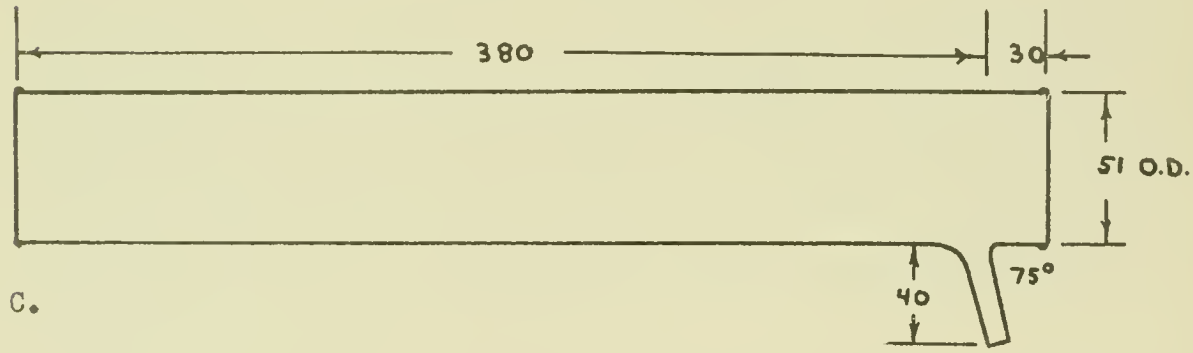
* NEMATOLOGY LAB., A.P.I., AUBURN, ALA.

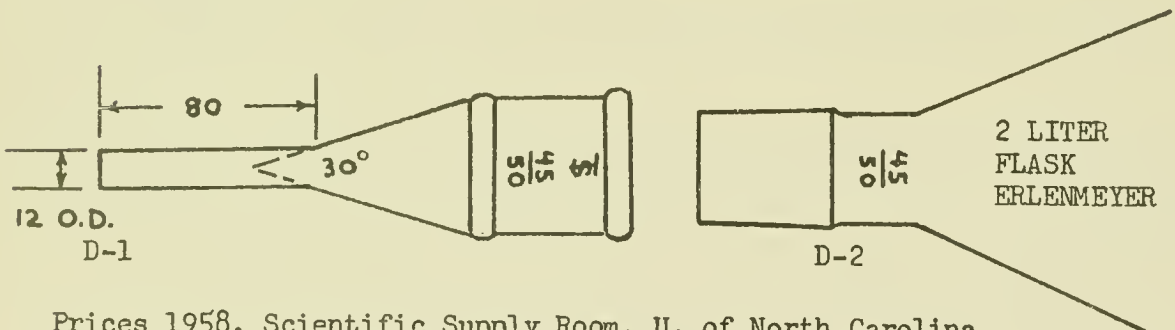
SEINHORST ELUTRIATOR

Glassware parts, measurements in mm.









Prices 1958, Scientific Supply Room, U. of North Carolina, Chapel Hill. Prices include the proper rubber stoppers. It is necessary only to specify the parts as designated, as Mr. D. E. Sampson at the above address knows the construction details.

A.	B.	C.	D-1	D-2
$6.00 ea.	$5.34 ea.	$4.86 ea.	$5.50 ea.	$7.35 ea.

NEMATODE MORPHOLOGY

General Structure of a Nematode

Nematodes are triploblastic, bilaterally symmetric, unsegmented, non-coelomate animals. Their shape is more or less cylindrical, sometimes fusiform, pear-shaped or otherwise modified, particularly in the adult female. The mouth opening is generally anterior and is usually surrounded by lips bearing sensory organs. The mouth is followed by a mouth cavity or stoma, an esophagus, intestine, and a rectum terminating in a ventral terminal or subterminal anus in females, or a cloacal opening in males. The body is covered with cuticle. There are usually no external appendages, but appendages do occur in rare forms. The body wall is composed of a hypodermis or epithelium which is situated beneath the cuticle, and a single layer of muscles. Sexes are usually separate. The male reproductive system opens directly into the rectum forming a cloaca, while the reproductive system of the female has a separate opening, the ventrally situated vulva. Excretory and nervous systems are present, but there are no specialized organs of circulation or respiration.

External Characters

The nematodes, or roundworms, are generally vermiform animals of long cylindrical shape, circular in cross section. There are two general types of body form, the fusiform and the filiform, the latter being less common. The fusiform is that of an elongated spindle, widest through the middle and tapering toward the blunt or pointed ends; the posterior end is generally more tapering and pointed than the anterior end and in some species is very slender. Filiform is thread-like with a uniform body diameter throughout. Other variations are the short, plump, pyriform or oval shape assumed by females of Heterodera and Meloidogyne. Most attain a length of 3 to 4 mm. Males are nearly always smaller than the females (Plate I).

Nematodes in general lack coloration, being transparent or of a whitish or yellowish tint conferred on them by the cuticle.

The body is not divisible into definite regions and lacks a distinct head, although this term is sometimes applied to the anterior end. The ventral surface of nematodes is identifiable by the presence in the mid-ventral line of the excretory pore (Plate I, Fig. 1, G), the gonopore in the female, and the anus. In most forms, the excretory pore is located about opposite the base of the esophagus. The vulva (Plate I, Fig. 1, Q) is usually situated in the posterior body half. The anus or cloacal opening of the male (Plate I, Fig. 1 and 2, U) lies near the posterior end. The body region behind the anus is commonly called the tail.

The oral opening is surrounded by six lips (Plate I, Fig. 3, B) in

many genera, but others show great modifications from this pattern. There may be fusion of pairs of lips giving rise to three lips or reduction of some and enlargement of others. When there are six lips, the submedial lips carry three papillae each, one apical and two sublateral in position. The two lateral lips carry two papillae, but along with the alteration or modification in lip structure there may also go modification in the number and arrangement of the papillae. The lips not infrequently bear, or are surrounded by, various cuticular protuberances. In certain terrestrial nematodes, such as Acrobeles, peculiar structures termed probolae, three or six in number, project forward; they vary from simple rounded, conical, or forked eminences to branched projections resembling antlers. When six are present, they are arranged in two circlets of three each.

In the region of each lateral lip, but behind the anterior face, there is a pair of sense organs very characteristic of nematodes, the amphids (Plate I, Fig. 3, A). The openings of the amphids are most conspicuous and best developed in the Aphasmidia, but are also present as minute pores in the Phasmidia. The amphidial openings of the Aphasmidia are cuticular depressions of three general shapes: cyathiform (Plate II, Fig. 1, A, B), spiral (Plate II, Fig. 1, D), and circular (Plate II, Fig. 1, C). The amphids consist of a gland and nerve endings and are presumably chemoreceptors. In most plant parasitic forms, the pore-like opening of the amphid cannot be seen except by a study of en face preparations.

The general body surface may be smooth, but very often is marked by a regular series of transverse striations (Plate I, Fig. 1, 2, T). These striations are often interrupted by the lateral fields (Plate I, Fig. 1, K and Fig. 4, A) which are quite prominent in some forms but are rather inconspicuous in others. In addition to the transverse striae, there may also be longitudinal striations (Plate I, Fig. 4, B). In the tail region of males of phasmidia, extentions of the cuticle often form lateral alae (caudal bursa; Plate I, Fig. 2, Y) which are employed in copulation and generally bear genital papillae (Plate IV, Fig. 1, G).

Another type of cuticular marking is termed punctation. Punctations are minute dots or ovals which may occur in transverse or longitudinal rows and often are arranged in patterns.

Near the posterior end of many nematodes there occurs a pair of cuticular pouches resembling the amphids. These are called phasmids (Plate I, Fig. 1, 2, V) and are probably sensory in function. They are located in the lateral fields, generally in the tail region or just above it. Each consists of a short duct opening on the surface of the cuticle, and leading inward to a small unicellular gland. Often a surface papilla is associated with the phasmids. In some cases the gland and duct may degenerate, leaving only the surface papillae as evidence of their former existance.

Body Wall

The body wall of nematodes consists of cuticle, hypodermis (epidermis, subcuticle), and muscle layer. The cuticle (Plate II, Fig. 5, F) is a non-cellular layer extended inward at the mouth, excretory pore, vulva, and anus. It is intimately connected with and, undoubtedly, is a product of the hypodermis. Histologically, it consists of several layers which are reducible to three kinds of material: the cortex, the matrix, and the fiber layers. The cortical layer consists of a dense material of the nature of a keratin and is resistant to solvents and to digestion. The matrix layer, according to Chitwood (1936), consists of, or contains, a fibroid named matricin, rich in sulphur. The innermost part of the cuticle consists of two or three fiber layers of very dense connective tissue running in different directions in adjacent layers. These fiber layers consist chiefly of collagen.

The hypodermis (Plate II, Fig. 5, G) is a syncytial layer that bulges into the pseudocoel at four places to form four longitudinal ridges termed the longitudinal chords (Plate II, Fig. 5, A, D, I), middorsal, midventral, and lateral in position. The nuclei of the hypodermis are confined to the chords. The nerves and excretory canals (when present) are in these chords. Some forms may have more than four chords.

The musculature of the body may be divided into two general types, the somatic musculature and the specialized muscles. The somatic musculature is the general muscular layer of the body wall and is composed of a single layer of obliquely arranged, more or less spindle-shaped cells attached to the hypodermis throughout their lengths (Plate II, Fig. 5, H). Specialized muscles, apparently of the same origin as the somatic musculature, are limited to some particular part of the body, such as labial muscles, somato-esophageal muscles, somato-intestinal muscles, rectal muscles, and copulatory muscles.

The Digestive System

The mouth leads into the buccal capsule, very variable in size, shape, and degree of differentiation in different nematodes. The buccal capsule and its stiffenings often exhibit a triradiate arrangement, corresponding with that of the esophagus. In some nematodes, especially rhabditoids, the buccal capsule is divisible into three sections: an anterior chamber enclosed by the lips, the vestibule or cheilostom; a middle and longest and most sclerotized portion, the protostom; and a small terminal chamber, the telostom (Plate II, Fig. 2). The walls of the various parts of the stoma are called rhabdions and are termed cheilorhabdions, protorhabdions, and telorhabdions, respectively.

In certain groups of nematodes, the buccal capsule is armed with a conspicuous protrusible spear or stylet, used to puncture plants and animal prey. This may be formed, as in tylenchoids, by the coming

together of the sclerotizations of the buccal capsule, so that it constitutes a buccal stylet (Plate II, Fig. 3). This is necessarily hollow and forms the path of food intake. This structure is called a stomatostylet. In other forms, as in dorylaimoids, the spear represents an enlarged tooth that originates in the esophagus wall. This type is called ondontostylet (Plate II, Fig. 4).

The structure of the esophagus varies in different nematode groups and is, therefore, an important taxonomic character (Plate III). It is a tube lined by a thin cuticle and covered by a membrane. The esophagus lumen is triradiate, being extended into three symmetrically arranged longitudinal grooves that partially divide the esophagus wall into three sectors, one dorsal and two ventrolateral (Plate II, Fig. 5, B, J). Three salivary glands are typically imbedded in the esophageal wall, one dorsal and two ventrolateral. In most plant parasitic nematodes, the esophageal glands are single cells, often with conspicuous nuclei (Plate III). The main cuticularized duct of each gland opens, often by way of an ampulla, into the lumen of the esophagus. It is usual for the dorsal gland to open much farther forward than the ventrolateral glands. In some tylenchoids, the glands protrude from the esophagus wall into the pseudocoel (Plate III, Fig. 7,8).

The esophagus commonly has one or more muscular swellings known as bulbs. Bulbs provided with a valvular apparatus are called true bulbs. Those lacking such an apparatus are termed pseudobulbs. The true bulbs are the chief pumping and sucking structures of the nematode esophagus. Bulbs may be situated near mid-length and spoken of as median, or may occur at the end of the esophagus and termed posterior, cardiac, or end bulbs. With regard to shape and the presence of bulbs, esophagi of plant parasitic and soil nematodes are classifiable as follows (Filipjev and Stekhoven, 1941): cylindrical, when of nearly the same diameter throughout (Plate III, Fig. 1); dorylaimoid, when slender anteriorly and wider posteriorly (Plate III, Fig. 2); bulboid when provided with an end bulb (Plate III, Fig. 3); rhabditoid, with an anterior wide region (corpus), usually leading into a mediam pseudobulb, followed by a narrowed region (isthmus), succeeded by an end bulb with valvular apparatur (Plate III, Fig. 4); diplogasteroid, with an anterior muscular region terminating in a median bulb, succeeded by a posterior glandular region forming a distinct bulb without valvular apparatus (Plate III, Fig. 5); tylenchoid, having a very narrow esophageal tube attached to the base of the stylet and enclosed in a larger thin-walled tube. In most genera there is a muscular median bulb with an ovoid valve (Plate III, Fig. 6, 7), but this bulb is much reduced or absent in the neotylenchidae (Plate III, Fig. 8). The posterior portion of the esophagus is glandular, and the glands may form a distinct basal bulb (Plate III, Fig. 6) or a lobe overlapping the anterior part of the intestine (Plate III, Fig. 7). The duct of the dorsal esophageal gland empties into the esophageal tube well anterior of the median bulb, usually very near the base of the stylet (Plate III, Fig. 6, 7); aphelenchoid, similar to the tylenchoid, except that the dorsal esophageal gland empties into the lumen of the esophagus in

the median bulb (Plate III, Fig. 9).

Esophagi may be quite diversified due to feeding habit. Certain things, however, are constant: (1) triradiate lumen; (2) cuticular lining, and (3) glands.

The esophagus may be connected with the intestine through a short structure termed the esophago-intestinal valve, which often projects some distance into the lumen of the intestine. This functions as a valve which impedes the flowing back into the esophagus of food contained in the intestine. Like the esophagus, it is lined with cuticle.

The intestine is composed of a single layer of epithelial cells (Plate II, Fig. 6, A). It is usually a straight tube in contrast to the reproductive organs which may be reflexed or coiled.

Posteriorly, the intestine leads into the rectum, which is lined by an invagination of the body cuticle, and opens at the anus. The rectal glands, generally three in number, open into the rectum, one dorsal and two subventral. In the male, the sperm duct enters the ventral wall of the rectum. The rectum is thus in whole, or in part, a cloaca in male nematodes. In both sexes, the rectum empties posteriad on the ventral surface of the body.

The Reproductive System

Nematodes, as a rule, are dioecious, existing as separate males and females. Males are readily distinguished externally from females by the presence of copulatory spicules (Plate I, Fig. 2, W). Other distinguishing features which may or may not be present are smaller size, curvature of the posterior end, and presence of bursae, genital papillae, and other accessory copulatory structures. In marine and most parasitic nematodes, the sexes occur in about equal proportions, but this is not the case with terrestrial and fresh-water forms. In the terrestrial and fresh-water forms, the female predominates. The scarcity or absence of males indicates a tendency toward hermaphroditism or parthenogensis in nematodes from these types of habitats. The hermaphroditism is usually of the protandric type; the gonad first produces sperm that are stored and later fertilize the eggs subsequently developed by the same gonad. The occurrence of parthenogenesis has been proved for several terrestrial nematode , e.g. Mermis subnigrescens (Christie, 1929), and in the root-knot nematode Meloidogyne sp. (Tyler, 1933), although males are known in both groups.

Intersexes are known in some nematode genera (Mermithid, Meloidogyne, Ditylenchus). An intersex is an individual which exhibits a blending of male and female characters. In most cases, the intersexes are females which show secondary male characters. They may copulate with males and lay viable eggs. The cause of intersex formation is not quite understood.

Nematode gonads are of tubular shape, varying greatly in length, and may be straight, sinuous, reflexed, or coiled back and forth (Plate IV). The male gonad consists of a single testis or paired testes. A single testis is usually present, and this extends anteriorly (Plate IV, Fig. 1, A). However, two testes occur in many nematodes, and these are oppositely oriented, except in Meloidogyne, where they are parallel. The terms diorchic and monorchic are convenient for referring to the two-testes or one-testis condition, respectively. When two testes are present, one usually extends forward and the other backward in the body cavity, but they join medially into the vas deferens, which runs posteriorly ventral to the intestine and finally narrows down to an ejaculatory duct which opens into the rectum to form a cloaca (Plate IV, Fig. 1, C).

Male nematodes, with few exceptions (example, Trichinella), are provided with copulatory spicules lodged in and secreted by spicule pouches (Plate IV, Fig. 1, D). Often the spicules are accompanied by an accessory piece, the gubernaculum (Plate IV, Fig. 1, E), which is a sclerotization of the dorsal wall of the spicule pouch.

The female reproductive system may be paired (Plate IV, Fig. 3, 4, and 5) or single (Plate IV, Fig.2), the organ lying in the body cavity along-side the intestine being either outstretched (Plate IV, Fig. 2, 3) or reflexed (Plate IV, Fig. 4, 5). The terms monodelphic and didelphic are convenient for indicating the single or double condition of the female tract, respectively. Each gonad consists essentially of an ovary, containing developing eggs, and a tubular portion which, in many forms, is dividible into an oviduct and a uterus. The uterus joins on to the vagina which opens on the ventral body surface at the vulva. Variations of the female reproductive system are shown in Plate IV.

Nervous System

The most easily recognizable part of the nervous system is the nerve ring which encircles the isthmus region of the esophagus (Plate III). Associated with it are a number of ganglia, and, according to Chitwood and Chitwood (1937), there are six nerves which are directed anteriad from the nerve ring. The four submedial have three chief distal branches, The two lateral have only two branches. These branches innervate sensory organs of the anterior extremity (sensory papillae or setae). The amphids are innervated from the amphidial nerves which originate in the lateral ganglia of the nerve ring. There are a dorsal, a ventral, four submedian, and one, two or three pairs of lateral nerves situated in the chords of the hypodermis, which proceed posteriorly from the nerve ring. The paired postanal lateral sensory organs (phasmids), the ventral supplementary organs of males, and the genital papillae are all innervated by branches from one or the other of the main nerves. The nerves themselves and their branches cannot be seen without special methods for demonstrating them or by means of sections. In routine microscopic examinations, the only part of the nervous system observed

is the nerve ring which surrounds the esophagus, and this is often difficult to see.

The Excretory System

The excretory system presents a varied picture in the phylum as a whole. It is simplest in the Class Aphasmidia where there is a single ventral excretory cell, or renette, which opens through an excretory pore on the midventral line in the region of the esophagus by way of a short to long duct. In the Class Phasmidia, there are two lateral excretory canals imbedded in the lateral chords of the hypodermis throughout most of the body length. They are connected anteriorly and ventrally by a transverse canal, thus forming an H or U shape. A duct, variable in length, connects the transverse duct with the excretory pore. The terminal excretory duct is cuticularly lined in the Phasmidia and can be observed in routine microscopic examinations. In the Aphasmidia, the terminal excretory duct is not lined with cuticle (except in some plectinae), thus making it difficult to see. There may or may not be two special cells (the renette) associated with the transverse duct.

In a few genera, including Dorylaimus, no excretory system has been found. Considerable excretion through the digestive tract may occur in all nematodes.

Circulatory and Respiratory Systems

Circulatory and respiratory systems are not known; the movement of the fluids of the body cavity apparently serving these purposes.

Plate I

Fig. 1-4. Tylenchorhynchus claytoni Steiner, 1937.

Fig. 1. Female
A - lip region
B - stylet
D - median bulb of esophagus
G - excretory pore
H - basal bulb of esophagus
I - cardia
K - lateral field
L - intestine
M - ovary
N - seminal receptacle
O - uterus
P - vagina
Q - vulva
S - sperms
T - annulation of the cuticle
U - anus
V - phasmids

Fig. 2. Male
A - lip region
B - stylet
C - dorsal esophageal gland orifice
D - median bulb of esophagus
E - subventral esophageal gland orifice
F - nerve ring
G - excretory pore
H - basal bulb of esophagus
I - cardia
L - intestine
R - testis
S - sperms
T - annulation of the cuticle
U - cloacal opening
V - phasmids
W - spicule
X - gubernaculum
Y - caudal bursa

Fig. 3. Face view
A - amphid
B - lips

Fig. 4. Cuticle detail
A - lateral field with four incisures forming three ridges
B - annules subdivided by longitudinal striations

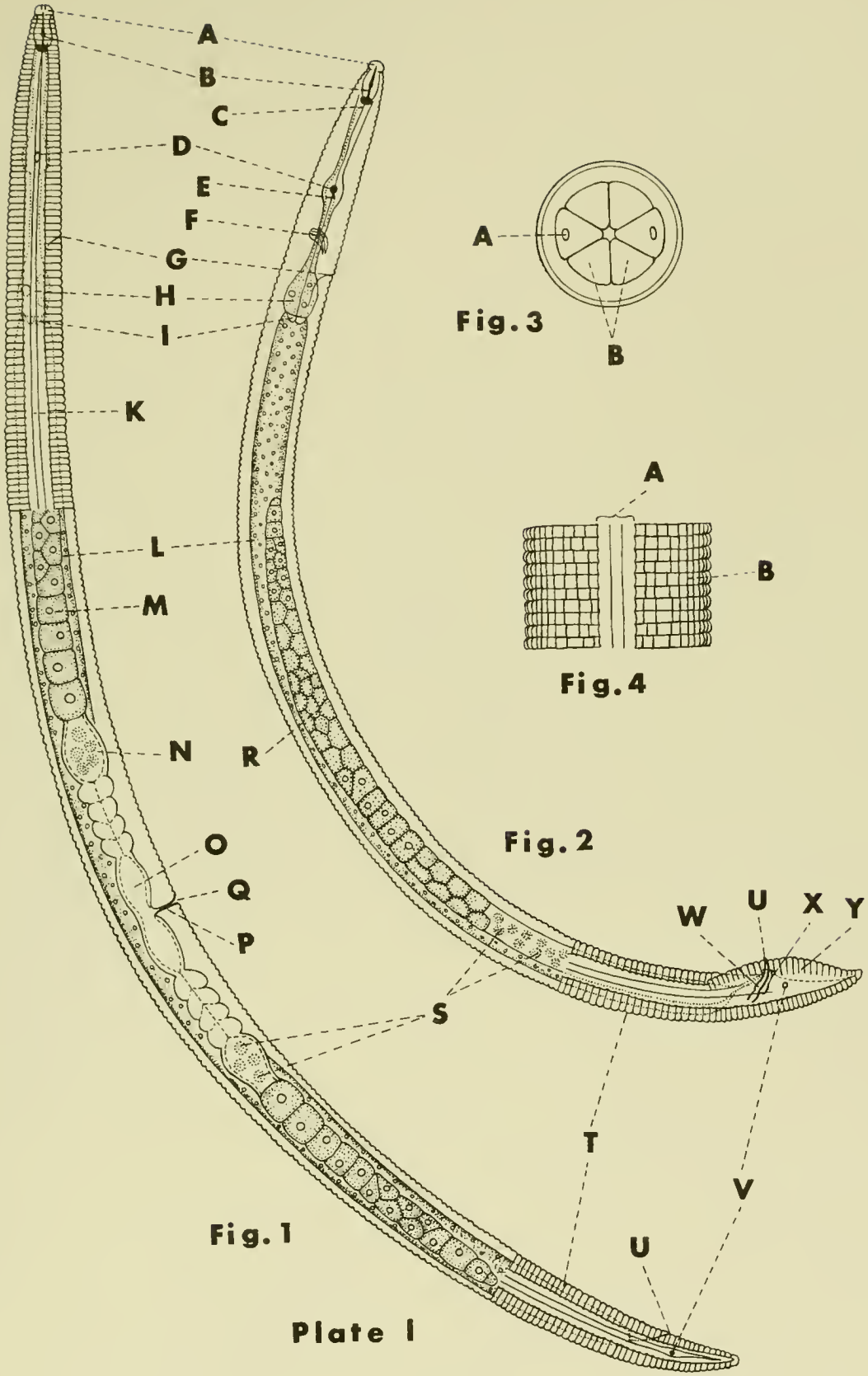


Fig. 1

Fig. 3

Fig. 4

Fig. 2

Plate I

Plate II

Fig. 1. Amphids
A - cyathiform type (Trilobus)
B - cyathiform type (Dorylaimus)
C - circular type (Monhystera)
D - spiral type (Choanolaimus)

Fig. 2-4. Variations of the buccal capsule (See also Plate III).

Fig. 2. Cylindrical buccal capsule (Rhabditis)

Fig. 3. Stomatostylet (Rotylenchus)

Fig. 4. Odontostylet (Dorylaimus)

Fig. 5. Cross section through esophageal region
A - dorsal chord
B - dorsal sector of esophagus
C - esophagus lumen
D - lateral chord
E - lateral field
F - cuticle
G - hypodermis
H - muscle layer
I - ventral chord
J - the two ventrolateral sectors of esophagus

Fig. 6. Cross section through reproductive region of female
A - intestine
B - ovary with unfertilized egg (Oocyte)

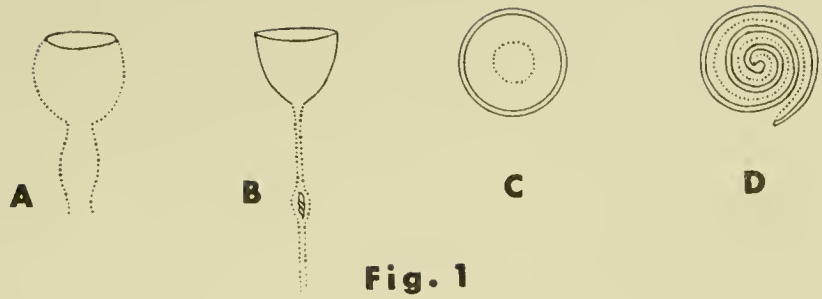


Fig. 1

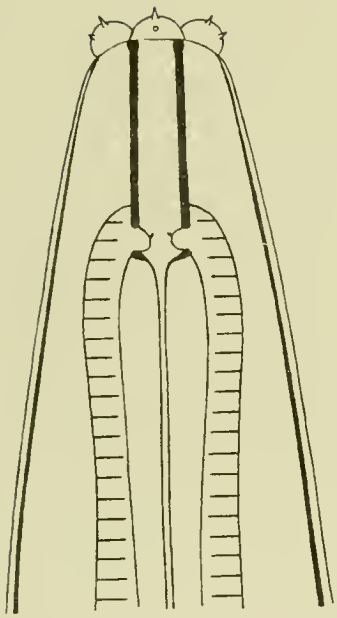


Fig. 2

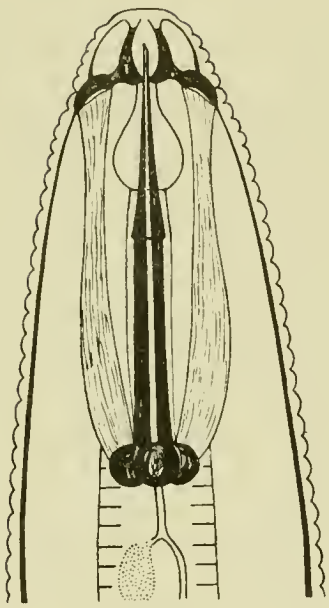


Fig. 3

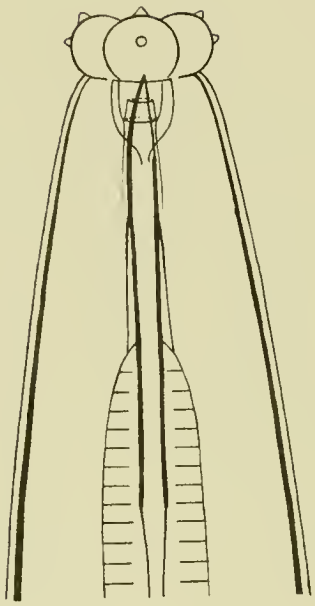


Fig. 4

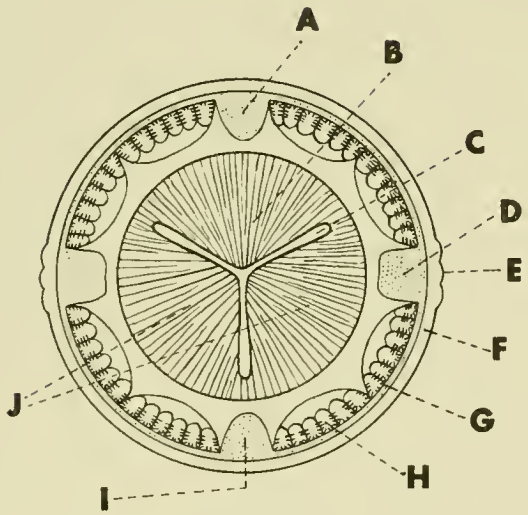


Fig. 5

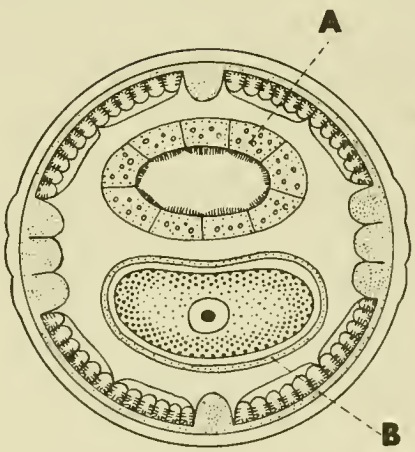


Fig. 6

Plate II

Plate III

Fig. 1-9. Different types of oesophagi.

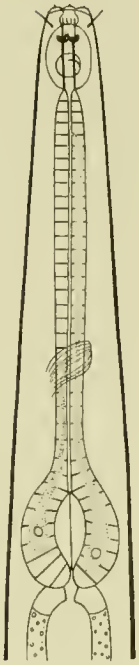
Fig. 1

CYLINDRICAL
(MONONCHUS)

Fig. 2

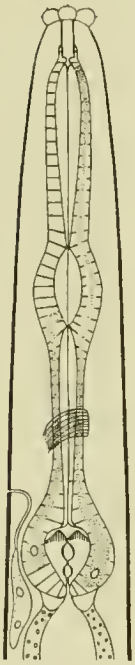
DORYLAIMOID
(DORYLAIMUS)

Fig. 3



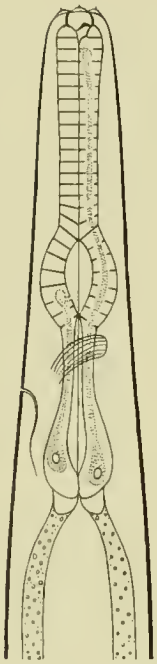
BULBOID
(ETHMOLAIMUS)

Fig. 4



RHABDITOID
(RHABDITIS)

Fig. 5



DIPLOGASTEROID
(DIPLOGASTER)

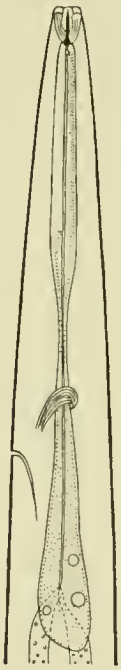
Fig. 6

(TYLENCHORHYNCHUS

Fig. 7

TYLENCHOID
HELICOTYLENCHUS

Fig. 8



NEOTYLENCHUS)

Fig. 9

APHELENCHOID
(APHELENCHUS)

Plate III

Plate IV

Fig. 1-5. Reproductive Systems.

Fig. 1. Male reproductive system (Rhabditis)
A - single testis (monorchic)
B - intestine
C - cloaca
D - spicule
E - gubernaculum
F - bursa
G - genital papillae

Fig. 2-5. Female Reproductive Systems.

Fig. 2. Single (monodelphic), outstretched (Ditylenchus)

Fig. 3. Paired (didelphic), outstretched (Tylenchorhynchus)

Fig. 4. Paired (didelphic), reflexed (Rhabditis)

Fig. 5. Paired (didelphic), reflexed (Meloidogyne)

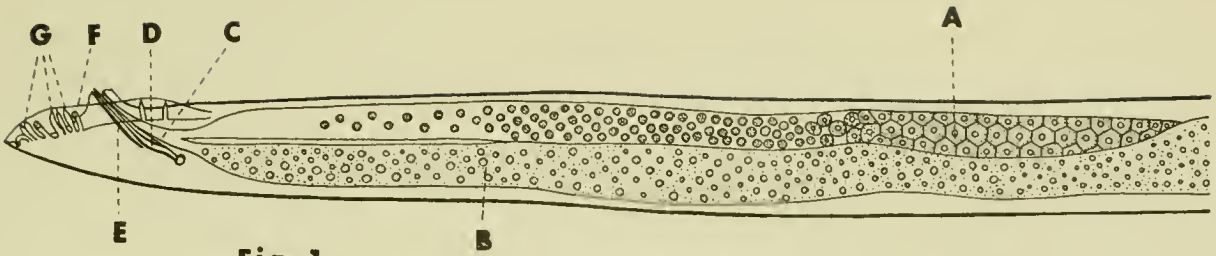


Fig. 1

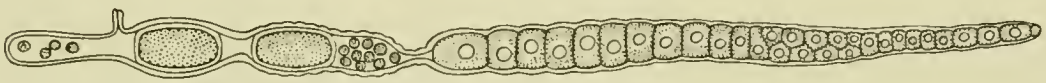


Fig. 2

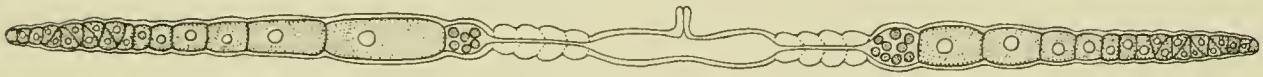


Fig. 3

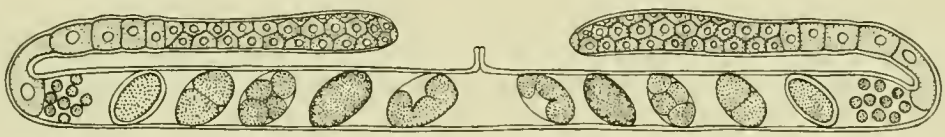


Fig. 4

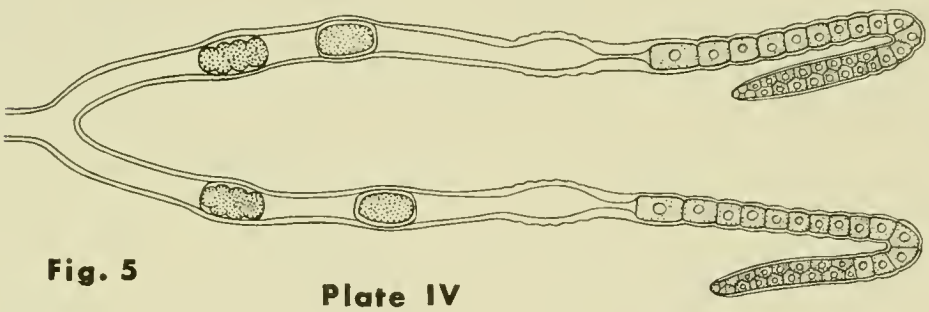


Fig. 5

Plate IV

NEMATODE SYSTEMATICS

According to Mayr et al. (1953), nematodes belong to the Kingdom Animalia, Subkingdom Metazoa, and Phylum Nematoda. Chitwood and Chitwood (1950) use "Nemathelminthes" as the designation of the phylum. Goodey (1951) places the nematodes in the Subkingdom Vermes, Phylum Aschelmintha, and Class Nematoda.

Whether the nematodes are considered a class or a phylum, there is fairly general agreement on the classification of Chitwood (1933). According to this scheme, all nematodes are divided into two large groups (classes or subclasses) called Phasmidia and Aphasmidia. The classes are divided into orders with names derived from the type genus of the order and ending in -ida (e.g., Rhabditida). Names of suborders end in -ina (Rhabditina); of superfamilies in -oidea (Rhabditoidea); of families in -idae (Rhabditidae); and of subfamilies in -inae (Rhabditinae). The subfamilies are composed of genera and the genera of species. In addition, a few subspecies have been named.

As with plants and other animals, the various taxa are composed of forms having a greater or lesser degree of resemblance and presumably have evolved from common ancestry. Species in the same nematode genus may differ from one another in only a few characters, and are usually easily recognizable as belonging to the same genus. Genera in the same subfamily differ to a larger degree, and so on through the higher categories.

Nematode names are subject to the International Rules of Zoological Nomenclature, and some knowledge of these rules should form part of the basic information of the nematologist. At present, the best available explanation of the rules is to be found in the book, "Methods and Principles of Systematic Zoology," by Mayr, Linsley, and Usinger. These rules provide for the changing of names under certain circumstances, and this is often done with nematode names. Consequently, desired information on a given species of nematode may be found in literature files under several different names. As examples, the root-knot nematodes have been transferred from the genus Heterodera to the genus Meloidogyne; many genera of the Tylenchida were formerly grouped in the genus Tylenchus and later in the genus Anguillulina; and the genus Hemicycliophora was formerly known as Procriconema. The table of contents of Goodey's "Soil and Freshwater Nematodes" is the best guide to general classification of the soil and plant parasitic forms available at present. However, it will be found lacking in several respects since several important changes have been made since the book was prepared about 1949.

It should also be remembered that nematode taxonomy is in a fairly early stage of development. New species are constantly being described and new genera created, either through the discovery of new forms, the splitting of old genera, or the correction of errors of the past. As concepts change,

new species are studied and better working methods are developed, the taxonomy must change.

In this course, emphasis has been placed on the most common soil and plant parasitic nematodes. Two keys for identification of these forms are presented. The first of these, the "Key to the Most Common Nematodes of Agricultural Soils and Plants," permits identification to family or subfamily. Once this identification has been made, identification to genus can usually be made by reference to the illustration and descriptions in the appropriate section of Goodey more quickly than by the use of additional keys. The second "Key to the Mature Females of the Tylenchoidea" was designed to facilitate identification to genus of the greater part of the plant parasites. Both keys have been simplified by omission of rare forms. While this simplifies construction and use of the keys, it also implies that rare forms will not "key out."

In use of the keys, it should be kept in mind that keys are imperfect tools at best and that verbal descriptions are very apt to be misleading. Consequently, the use of the keys should be supplemented by frequent reference to the illustrations in Goodey or other available publications. Also, it is suggested that time and effort will be saved if no attempt is made to identify larval forms (without sexual organs). When possible, several specimens should be studied. Visibility of the key characters varies with specimens, and structures which are obscure on one may be plainly visible on another. When a tentative identification has been made, available illustrations should be carefully checked.

KEY TO THE MOST COMMON NEMATODES OF AGRICULTURAL SOILS AND PLANTS

1. Oesophagus rhabditoid (with valve in basal bulb as in Pl. III, 4), diplogasteroid (without valve in basal bulb as in Pl. III, 5), tylenchoid (Pl. III, 6, 7, 8), or aphelenchoid (Pl. III, 9). If tylenchoid or aphelenchoid, always with stylet. Males of some genera have a distinct bursa (Pl. I, Fig. 2). Never with setae or conspicuous amphids. Phasmids always present, but most often difficult to locate. Mature females of some genera have a much enlarged body.----------Phasmidia--------2.

   Oesophagus bulboid (Pl. III, 3), cylindrical (Pl. III, 1) or dorylaimoid (Pl. III, 2). Amphids often conspicuous, appearing as circles, spirals, stirrup forms, etc. (Pl. II, A, B, C, D). Often with setae. Males without bursa. Always with elongate, vermiform body.----------Aphasmidia-------6.

2. Stylet always present,usually, though not always, with well-developed, rounded knobs. Shapes and proportions of stylets vary greatly with genera and species, but are usually recognizable as variations of the form shown in Pl. II, Fig. 3. Attached to the stylet is a thin oesophageal tube which may be straight or coiled. ----------Tylenchida-------3.

   Stylet absent. Anterior portion of oesophagus muscular (striated) (Pl. III, 4, 5).----------Rhabditida-------4.

3. Dorsal oesophageal gland orifice near base of stylet, or at most, not more than one stylet length posterior to stylet knobs. The oesophageal tube often has an abrupt bend at this point (Pl. II, Fig. 3). Stylet mostly with well-developed knobs. If stylet knobs are absent, tail is long and thin.----------Tylenchoidea

   Dorsal oesophageal gland orifice in median bulb and difficult to locate. Oesophageal tube without abrupt bends. Stylet knobs small or absent. Median oesophageal bulb occupying nearly full width of body (Pl. III, 9). Tail rounded or conical.----------
   ----------Aphelenchoidea

4. Oesophagus rhabditoid, with valve in basal bulb (Pl. III, 4)-------5.

   Oesophagus diplogasteroid, without valve in basal bulb (Pl. III, 5)----------Diplogasteridae

5. Stoma cylindrical, usually much longer than wide (Plate II, 2).

Lips plain, except in one genus (Diploscapter), which has hooked projections on lips. Usually with two ovaries----Rhabditidae

Stoma not cylindrical, or if nearly so, about as long as wide. Lips of some genera have distinct projections ranging in shape from rounded to elaborately ornamented. With one ovary, this being reflexed so distal end is posterior to vulva--Cephalobidae

6. Oesophagus plectoid (with basal bulb, Pl. III, 3). Tail tip with a small projection (spinneret)----------Plectinae

Oesophagus cylindroid (Pl. III, 1) or dorylaimoid (Pl. III, 2)-----7.

7. Oesophagus cylindroid. Mouth cavity large, subglobular, usually with one or more large teeth (Pl. III, 1)---------Monochidae

Oesophagus dorylaimoid (Pl. III, 2). Stylet often as shown in Pl. II, 4. Some with much longer stylet, others with a tooth ----------Dorylaimoidea

KEY TO THE MATURE FEMALES OF THE TYLENCHOIDEA

1. Without median oesophageal bulb, stylet length never more than 3 times width of lip region (Pl. III, 8)----------Neotylenchidae

   With median oesophageal bulb (Pl. III, 6, 7)----------2.

2. Body of mature female pear-shaped, lemon-shaped, or enlarged and saccate. Found in roots of plants, either embedded or attached by neck, some as cysts in soil----------3.

   Body of mature female vermiform (much longer than wide)----------7.

3. Body of mature female pear- or lemon-shaped----------4.

   Body of mature female saccate----------5.

4. Body of mature female pear-shaped, white. Found completely or almost completely embedded in roots or other plant tissue, nearly always in distinct knots or galls----------Meloidogyne

   Body of mature female pear-shaped or lemon-shaped, white, yellowish, or brown according to age, attached to root by neck only, or found as a brown cyst in soil----------Heterodera

5. Mature female embedded in plant root, often in knot, body shape ovoid to spheroid with elongated "tail," vulva nearly terminal, ----------Nacobbus

   Mature female attached to root by neck, body more or less kidney-shaped----------6.

6. Vulva at 90% of body length, parasites of citrus and olives------ ----------Tylenchulus

   Vulva at 72% of body length, parasites of numerous plants, mostly annuals----------Rotylenchulus

7. Tail long and thin, more than 6 times anal body diameter----------8.

   Tail not long and thin, but rounded, or conical and more or less pointed----------9.

8. Tail tip frequently clavate, one or two ovaries, distance from anterior end to center of median oesophageal bulb equal to, or greater than distance from center of bulb to base of oesophagus. ----------Psilenchus

Tail tip usually pointed, often curved ventrally. Distance from anterior end to middle of median oesophageal bulb less than distance from this point to base of oesophagus. One ovary-----Tylenchus

9. With one ovary----------10.

With two ovaries----------18.

10. Body more or less sausage-shaped, i.e., short and stout (a= 6-10), length seldom more than about 0.6mm----------11.

Body slender (a= 20 or more), length usually more than 0.6mm------13.

11. Body with prominent retrorse (directed posteriorly) annules, usually longer than 0.3mm, a- 10 or more. Found in soil----------12.

Body without prominent annules, very stout (a= 6-8). Length about 0.3mm. Found in roots----------Cacopaurus

12. Annules with prominent spines or scales----------Criconema

Annules of mature female without spines or scales-------Criconemoides

13. Mature female more than 2mm, often 3-5mm long. Found in galls in leaves, or in inflorescence of grains and grasses---------Anguina

Body length less than 2mm. Found in soil, or in roots and tubers; sometimes in bulbs, leaves and stems----------14.

14. Stylet longer than 3 times width of lip region----------15.

Stylet shorter or about twice width of lip region----------16.

15. Posterior portion of body strongly curved ventually-----Paratylenchus

Body usually nearly straight, usually covered by loose cuticle of fourth molt----------Hemicycliophora

16. Body long and slender (a= 40 or more), tail conical, pointed. Endoparasitic in bulbs, stems, leaves and tubers, from mushroom compost, or sometimes in soil----------Ditylenchus

Tail tip rounded----------17.

17. Lip region distinctly set off from body; oesophageal glands forming a lobe overlapping intestine; knobs of stylet closely joined. A common genus endoparasitic in roots and tubers, also found in soil----------Pratylenchus

Lip region not distinctly set off from body; oesophageal gland forming a distinct bulb; knobs of stylet separated like an inverted Y. An apparently very rare genus from soil---Chitinotylenchus

18. At least 2mm long, slim (a= 45 or more). Stylet very long, six or more times as long as width of lip region----------19.

Less than 1.5mm long, length stylet not more than 5 times width of lip region----------20.

19. Tail pointed, oesophagus with distinct basal bulb--------Dolichodorus

Tail rounded, base of oesophagus a lobe overlapping intestine----------Belonolaimus

20. Lip region flattened, stylet length about twice width of lip region----------Radopholus

Lip region convex conoid, stylet length 2 or more times width of lip region----------21.

21. Tail 2 or more times as long as anal body diameter, tapering------22.

Tail shorter than anal body diameter, rounded----------23.

22. Tail tip rounded----------Tylenchorhynchus

Tail tip nearly pointed----------Tetylenchus

23. Lip region distinctly set off from body, divided into minute plates. Body at rest or fixed lying only slightly curved-Hoplolaimus

Annulated lip region continuous with body contour, body usually

lies in loose spiral when fixed or at rest----------24.

24. Dorsal gland orifice about one-third stylet length or more posterior to stylet knobs----------Helicotylenchus

Dorsal gland orifice much less than one third stylet length posterior to stylet knobs----------Rotylenchus

CYST NEMATODES (*HETERODERA* SPP.)

The genus *Heterodera* was placed in the family Heteroderidae of the Tylenchoidea by Thorne (1949) with the genus *Meloidogyne* (root-knot nematodes). Prior to 1949, species of both these genera were placed in the genus *Heterodera*. Prior to 1940, there was a strong tendency to refer all of the cyst-forming nematodes to a single species, *H. schachtii* Schmidt, 1871, though some effort was made to differentiate races, strains, or varieties according to the host plants attached. However, little was done toward study of morphology, and identification was difficult unless the host plants were known. Some progress has been made in recent years, though the situation is yet far from satisfactory.

Morphology

The adult females and cysts of *Heterodera* are the forms most commonly encountered. Adult females or cysts will be found on roots of various plants if these are carefully removed from the soil and washed. The nematodes are attached to the roots by the neck only, with most of the body outside the root. The females are white or yellowish in life, and the cysts are light to dark brown. Average size is about 0.5mm by 0.75mm. Some species are lemon-shaped, others are pear-shaped. Cysts are very highly resistant to decay and may be found in soil in which infected plants have grown, even many years afterward.

The males are slender worms shaped very much like *Meloidogyne* males. That is, they are about 1.25 to 1.75mm long, slender (a = 35-40), taper slightly anteriorly, and have a short rounded tail (Goodey, 1951, Fig. 70). Males will be found in abundance at certain times of the year, but may be very scarce at other times. The larvae have an average length of about 0.5mm. They differ from root-knot nematode larvae in that the stylet is 20 to 30 microns long (*Meloidogyne*, 10-11 microns) and in the shape of the anterior end.

Excellent drawings of the larvae and other stages of *H. schachtii* will be found in "The Life History and Morphology of the Sugar-Beet Nematode, *Heterodera schachtii* Schmidt," by D. J. Raski (Phytopath. 40(2): 135-152, 1950). Larvae are seldom found free in soil, but can easily be obtained from the cysts.

The cysts are important contaminants of imported plant material and also are searched for in soil in connection with quarantine and rotation programs in various countries. Consequently, they have been intensively studied, and most of the present information on identification of species is based on characters of the mature cysts and their contents.

A key to aid in identification of cysts is presented. Key characters will be found on the mature cysts.

Life History

Larvae in the soil enter the roots of plants near the root tips and begin to feed on the developing tissues. Here they undergo three moults, breaking through the outer root tissue at the last one. Females remain attached to the root by the neck. Males leave the larval cuticle and go in search of females. Apparently all of the females of the H. schachtii and H. göttingiana groups deposit some eggs in a jelly-like substance, forming an egg mass or "egg sac." However, these species also retain eggs in the body so that by the end of the life of the female, the body is tightly packed with eggs. Females of the H. rostochiensis and H. cacti groups do not deposit any eggs, retaining all in the body. The female finally dies and her cuticle becomes transformed into a cyst filled with eggs. Eggs in the cyst develop to the first larval stage, then moult once, becoming second stage larvae. Apparently hatching may then take place immediately, or the larvae can remain in the eggs in the cyst indefinitely.

It has been shown that root excretions of various plants can stimulate hatching. Most work on this subject has been done with H. rostochiensis. When cysts of this species are placed in leachings from a growing potato plant the rate of hatching of their eggs is enormously increased. However, it is seldom that all the eggs in a cyst hatch even under the most favorable conditions. In the absence of a host plant only a few eggs hatch each year. Hatching after 17 years has been reported in the literature. Probably the maximum time under most conditions is less than half of that. Once hatched, the larvae make their way to a host plant completing the life cycle.

Because of the limited host range of most of the Heterodera species, it is usually easy to devise crop rotation methods for control. On the other hand, delayed hatching of the eggs means that the rotations must be very long; in heavily infested sugar beet fields, as long as 5 or 6 years must be allowed between beet crops. Since it has been shown that larvae die within 12 to 18 months after hatching if they do not reach a host plant, efforts are being made to analyze the "hatching factor" in root leachings in the hope that it can be synthesized and used in control. Some progress has apparently been made.

The literature on Heterodera is voluminous, European workers having studied the sugar beet nematodes and other species of this genus since around 1860. A summary to about 1938 will be found in "A Manual of Agricultural Helminthology," by I. N. Filipjev and J. H. Schuurmans Stekhoven (1941). A later and shorter summary, "The Cyst-forming species of Heterodera," by Mary T. Franklin, was published by the Commonwealth Agricultural Bureaux, Farnham Royal, Bucks, England, in 1951. "The Golden Nematode of Potatoes," by B. G. Chitwood, (USDA circular No. 875) summarizes information to about 1951 on H. rostochiensis in this country. There are several host lists, but because of the confusion as to identity of species, most are apt to be misleading. Probably the best information on hosts is found in reports of experiments by F. G. W. Jones, "Observations on the beet eelworm and other cyst-forming species of Heterodera." (Annals of Applied Biology 37(3):

407-440), of 1950.

Available names of the genus Heterodera are listed in Table 1. Identification of some of these by characters of the cysts and contained eggs and larvae is doubtful, and it is possible that some of the species are not valid. The final answer to this question must await careful study and description of the males, females, and larval stages. It should also be mentioned that while the writer is certain there are a considerable number of undescribed species, very few cysts have been seen which could not be placed in one of the species groups described below.

Species formerly referred to this genus are as follows:

Heterodera radicicola (Greef, 1872) Miller, 1884, was shown by Goodey (1932) to be a species of another genus and is now known as Ditylenchus radicicola (Greef, 1872) Filipjev, 1936.

Heterodera vitis Phillipi, 1884, was shown by Giard (1894) to be an insect Margarodes vitium (Phillipi, 1884) Giard, 1894.

Heterodera javanica Treub, 1885, is a root-knot nematode, Meloidogyne javanica (Treub, 1885) Chitwood, 1949.

Heterodera exigua (Goldi, 1887) Loos and Foaden, 1902, is also a root-knot nematode, Meloidogyne exigua Goldi, 1887. (The species name was misspelled exiqua by Loos and Foaden.)

Heterodera marioni (Cornu, 1879) Goodey, 1932, is a root-knot nematode, Meloidogyne marioni (Cornu, 1879) Chitwood, 1952.

Heterodera lupuli Filipjev and Schuurmans-Stekhoven, 1941, is an obvious error, H. humuli having been intended.

Heterodera viale (Lavergne, 1901) Chitwood, 1949, is also an obvious error of transcription for Anguillula viale.

Special attention should be called to the fact that several additional names to be found in Cooper's paper published in 1955 have no nomenclatorial standing, having been specifically designated a provisional name by the author.

Table 1

The Species of Heterodera with Type Hosts and Localities

The Species:	Type Host:	Type Locality
H. schachtii Schmidt, 1871	Beta vulgaris	Halle, Germany
H. gottingiana Liebscher, 1892	Pisum sativum	Gottingen, Germany
H. rostochiensis Wollenweber, 1923	Solanum tuberosum	Rostock, Germany

Species	Type Host	Type Locality
H. punctata Thorne, 1928	Triticum vulgare	Saskatchewan, Canada
H. major (Schmidt, 1930) Franklin, 1940*	Avena sativa	Halle, Germany
H. trifolii (Goffart, 1932) Oostenbrink, 1949	Trifolium pratense	Schleswig-Holstein, Germany
H. humuli Filipjev, 1934	Humulus lupulus	Kent, England
H. galeopsidis (Goffart, 1936) Filipjev & Schuurmans-Stekhoven, 1941	Galeopsis tetrahit	Lauscha, Germany
H. cacti Filipjev & Schuurmans-Stekhoven, 1941	Epiphyllum ackermanni	Haartensdyk, Holland
H. cruciferae Franklin, 1945	Brassica oleracea	England
H. weissi Steiner, 1949	Polygonum pennsylvanicum	Beltsville, Maryland, U.S.A.
H. carotae Jones, 1950	Daucus carotae	Isle of Ely, England
H. glycines Ichinohe, 1952	Glycine max	Tokachi Province Hokkaido, Japan
H. leptonepia Cobb and Taylor, 1953	Unknown	Probably Peru
H. tabacum Lownsbery and Lownsbery, 1952	Nicotiana tabacum	Hazardsville, Conn. U.S.A.
H. fici - Kirianova, 1954	Ficus sp.	U.S.S.R

*/There is some confusion as to the proper name for this species. H. avenae (Mortensen, Rostrup, and Kolpin Ravn, 1908) Filipjev, 1934, has been used in some recent literature. However, as pointed out by Franklin (1957), this name was never accompanied by an adequate "indication" as required by the Rules of Zoological nomenclature, and is therefore invalid.

The Cysts

Cysts of Heterodera are of two general types which are easily distinguished by examination of the lower end. 1/This part of the cysts of H. rostochiensis and H. punctata has a smooth rounded contour (figs. 1 and 2). The cysts of all other known species is shaped somewhat like the end of a lemon, that is, the vulva is located on a definite protuberance. This is shown in figures 3 to 6, the variety of forms illustrated being representative not only of the species shown, but of the other known species as well. The first type of cyst is conveniently referred to as "round" and the second type as "lemon-shaped", or it might be said that the vulva does not or does protrude. At the upper end of the cyst is a distinct neck

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1/For convenience here and in the following parts of this paper, the cysts will be described as viewed laterally with the vulva at the lowest point. The "lower end" of the cyst would then refer to the region around the vulva, the vertical axis would extend from the vulva along the center line of the cysts, horizontal lines would be at right angles to the center line, etc.

which varies in length, shape, and position with reference to the vertical axis of the cyst. Shape, size, and proportions of the cysts are highly variable; and while it can be shown statistically that averages of these dimensions or of the relations between them differ between species, such averages are of little value for identification of small lots of cysts.

As has been illustrated by Chitwood (1951) for H. rostochiensis, the cysts of all species are made up of several distinct layers. When the cysts of some species are fresh, they may be covered or partly covered by the "subcrystalline layer." This is a waxy, translucent substance which apparently persists for only a short time in the soil.

The color of mature cysts is always some shade of brown. The cuticle of living females may appear white, colorless, or yellow.

The outermost layer of cysts is marked by grooves and ridges which form distinctive "patterns." These vary in detail with individuals, but are sufficiently constant within groups of species to be of value in identification. The pattern is visible on immature females and can nearly always be seen on part or all of cysts even when these are very old. In certain species, the basic element of the pattern at the middle of the cyst is a short zig-zag line which may appear as light on a dark background or dark on a light background, according to the focus of the microscope (figs. 7, 8, and 26). The segments of the line are straight, and the angles between them are well defined. Usually these lines near the middle of the cyst show no trace of regular arrangement. Near the base of the neck and around the vulva there may be parallel lines (fig. 9) or wavy lines (fig. 10). The size of the elements of the pattern may vary greatly, being small as shown in figure 8, relatively large as shown in figure 26, or intermediate between these two. A variation which seems rare is the network pattern shown in figure 12. An occasional cyst with partly zig-zag and partly network pattern has been seen. So far as is known at present, there is no constant difference in pattern between species in the H. schachtii group, though it is possible that fine, coarse, or network patterns will be found more frequently in some species than in others.

A second type of pattern is found in the group of species which includes H. weissi, H. cacti, and probably a number of undescribed species. This pattern has as its basic element parallel lines running around the cyst at right angles to the vertical axis (fig. 13). These may be interrupted at intervals by short vertical or oblique lines (figs. 14 and 15). Sometimes this pattern may appear somewhat like the zig-zag pattern but differs in that some trace of the parallel lines always remains.

The cysts of H. rostochiensis and H. punctata have a third type of pattern. Around the vulva it is made up of wavy lines (fig. 19. On the lower portion of the cyst, there are short, crooked lines, sometimes in horizontal rows (fig. 22). On the upper part of the cyst, the lines tend toward the vertical, sometimes appearing as nearly vertical striae (fig. 23).

Some of the species with zig-zag patterns have in the lower end a striated object shaped somewhat like a sheaf of grain, apparently the cuticular lining of the vagina (fig. 11). This is nearly always accompanied by a number of dark bodies of irregular, though never angular shape. These may be few or numerous. No constant number or arrangement has been observed. These are absent in cysts of other species having zig-zag patterns and can be used for separation of these cysts into two groups.

Punctation is found on a layer of the cyst below that which carries the pattern. According to Franklin (1939) punctation is minute pits in one of the layers of the cyst. Under high magnification these appear as round dots of uniform size, either light or dark, according to the focus of the microscope.

Punctation is usually very prominent in H. rostochiensis and H. punctata, with the dots often being arrayed in distinct parallel horizontal rows (figs. 20 and 24). In cysts of the H. schachtii group, punctation is of several types. One of these is a prominent feature of most H. avenae cysts, but also occurs on other species. The dots are about one-half micron in diameter, and there is little or no trace of regular arrangement (figs. 25 and 26). This is called "coarse irregular" punctation.

Cysts of H. trifolii have dots of about the same size as those found on cysts of H. avenae, but these are often arranged in parallel lines on part of the cyst at least. This is shown in figure 27 with the lines running diagonally from lower left to upper right across the photograph, but the rows are seldom as long as those shown.

In other species of the H. schachtii group, fine irregular punctation occurs. The dots of fine irregular punctation are much smaller than those of the coarse type, being difficult to see even with the oil immersion objective of the microscope. Unfortunately, punctation is a somewhat variable character, being easy to see on some cysts and difficult or impossible to find on others. Its presence is therefore a useful character, but its absence cannot be taken to indicate that a given specimen does not belong to a species for which punctation is described.

Punctation has not been seen on H. weissi or H. cacti, though it may occur on some of the undescribed species of this group. But cysts of these species often have a grainy appearance (figs. 17 and 18) due to the presence of dots of somewhat irregular size and shape on the outer layer of the cyst.

The anus of H. cacti is shown in figure 17. All lemon-shaped cysts have the anus located in about this same relationship to the vulva. The anus of H. rostochiensis is shown near the upper edge of figures 19 and 20. The pattern runs around the vulva, but the anus is marked only by a slight irregularity. The anus of H. punctata is located at a thin spot on the cyst, which is about the same size as the vulvar opening. Figure No. 21 shows this clearly, though the cyst wall was split in the process

of preparing the slide for photographing. This difference, together with the round shape of the cysts, permits the identification of H. rostochiensis and H. punctata from examination of the cysts alone.

The Larvae

Larval characters used in the key are average length, relation of body length to breadth, shape of stylet knobs, location of the dorsal gland orifice, and relation of the tail terminal to the stylet length.

Identification by average length of the larvae has distinct limitations due to the fact that variation in length within a species might be as much as 20% of the total length. Relation of larval length to width is useful for separation of only one species, H. leptonepia.

Stylet knobs are of two general types, concave anteriorly and convex anteriorly. With most species, there is no doubt as to the type of knobs, since the concavity or convexity is distinct, but some forms have stylet knobs intermediate in type and difficult to distinguish.

Location of the dorsal gland orifice is used mostly to distinguish between H. trifolii and H. glycines. As was pointed out by Hirschmann (1956), the dorsal gland orifice in larvae of H. glycines is located 3.0 to 5.2 microns posterior to the stylet knobs; in H. trifolii, the location is 5.6 microns posterior to the stylet knobs.

The tail terminal is defined as the hyaline portion of the tail posterior to the body cavity. This portion of the tail is usually clearly defined, since the contents of the body cavity are more or less granular. In poorly preserved specimens, the body contents may be shrunken, making the tail terminal appear longer than it is in reality.

Key to The Mature Cysts of Species of Heterodera

Note: This key is designed to facilitate identification of the species of Heterodera, using only characters of the mature cysts and their contents, that is, eggs with second stage larvae. Certain characters used in the key may not be visible on other than fully mature cysts.

Measurements of larvae are from Fenwick and Franklin (1951) for most species, from Jones (1951) for H. carotae, from Ichinohe (1952) for H. glycines, and from Kirianova for H. fici.

1. Body of cyst ovoid to globular, that is with posterior portion rounded and vulva not located on a distinct protuberance (figs. 1 and 2)----------Heterodera rostochiensis group 4.

   Body of cyst lemon-shaped, that is, with vulva located on a distinct protuberance (figs. 3 and 6)----------2.

2. Basic element of pattern of outer layer of cyst wall at middle portion of cyst short zig-zag lines with little or no trace of regular transverse arrangement (figs. 7 and 8) sometimes modified to appear as network (fig. 12)----------3.

   Basic element of pattern at outer layer of cyst wall at middle portion of cyst straight or wavy lines (figs. 14 and 16) lines at right angles to axis of cyst; sometimes broken by short oblique or vertical lines; outer layer of cyst may have grainy appearance (fig. 18)----------Heterodera cacti group----------7.

3. Mature cysts with dark bodies (brown knobs) and often sheaf-shaped object (lining of vagina) at posterior end (fig.11). On immature cysts, these seldom visible, and then do not appear dark----------Heterodera schachtii group--------8.

   Mature cysts without brown knobs or sheaf-shaped object at posterior end----------Heterodera göttingiana group-----11.

4. H. rostochiensis group. Cyst often ovoid, anus located at a transparent spot on cyst so that anal and vulvar openings appear to be about the same size when seen by transmitted light (fig. 21). Hyaline portion of larval tail much longer than stylet----------Heterodera punctata

   Cyst ovoid to globular; anal opening appears much smaller than vulva opening (figs. 19 and 20). Hyaline portion of larval tail about the same length as stylet----------5.

5. Larvae very slender; length about 39 times greatest width; orifice of dorsal oesophageal gland about two-thirds stylet length posterior to stylet knobs--Heterodera leptonepia

   Length of larvae about 22 times greatest width; orifice of dorsal oesophageal gland about one-fourth stylet length posterior to stylet----------6.

6. Distance between vulva and anus about one and one-half times diameter of vulva----------Heterodera rostochiensis

   Distance between anus and vulva about two and one-half times diameter of vulva----------Heterodera tabacum

7. H. cacti group. Hyaline portion of larval tail about as long as stylet; stylet knobs concave anteriorly----------
   ----------Heterodera weissi

   Hyaline portion of larval tail usually shorter than stylet; stylet knobs convex anteriorly--Heterodera cacti

8. H. schachtii group. Cyst always with distinct punctation consisting of dots of uniform size but not in rows (fig. 25);

brown knobs closely clustered around vulva. Hyaline portion of larval tail at least one and one-half times longer than stylet----------Heterodera major

Cyst with or without punctation, mostly in rows if present; brown knobs not closely clustered around vulva. Hyaline portion of larval tail about as long as stylet----------9.

9. Average length of larvae 480 μ or more----------10.

Average length of larvae about 460 μ----Heterodera schachtii

10. Average length of larvae 484 μ ----------Heterodera glycines

Average length of larvae 502 μ ----------Heterodera trifolii

Average length of larvae 518 μ ----------Heterodera schachtii galeopsidis

11. H. göttingiana group.

Average length of larvae 414 μ ----------Heterodera cruciferae

Average length of larvae 454 μ ----------Heterodera carotae

Average length of larvae 474 μ ----------Heterodera göttingiana

Average length of larvae 405 μ ----------Heterodera humuli

Average length of larvae 406 μ ----------Heterodera fici

Host Plants

Many lists of host plants of Heterodera species have been published, but it seems probable that many of these are inaccurate in that they include plants which are not hosts of the species discussed. This is especially true of the older lists and of those based on information compiled from the literature rather than from host tests. In order to avoid this particular error, the following list of hosts includes only the type host of each species and an indication of the other plants which it attacks so far as there is general agreement or information on host tests available. That is, the list is not intended to be complete, but is believed to be accurate so far as what is included is concerned.

The species of Heterodera and their principal hosts are:

H. schachtii. Type host, sugar beet (Beta vulgaris L.). Also other Chenopodiaceae, many species of Cruciferae (Oostenbrink, 1950 and Jones, 1951) and various species of other plant families (Thorne 1932). It seems possible that H. schachtii attacks a wider variety of plants than any

other known species of Heterodera.

H. göttingiana. Type host, garden peas (Pisum sativum L.). Also other Leguminosae, but according to Oostenbrink (1951), this species does not attack beans (Phaseolus vulgaris L.), clover (Trifolium spp.), alfalfa (Medicago sativa L.), or soybeans (Soya max Piper).

H. trifolii. Type host, red clover (Trifolium pratense L.). Also other Leguminosae, including beans (Phaseolus vulgaris L.), but not peas (Pisum sativum L.), alfalfa, or soybenas. (Oostenbrink, 1951).

H. glycines. Type host, soybean (Glycine max L.). Also snap bean (Phaseolus vulgaris L.), Adzuki Bean (P. angularis), vetch (Vicia sp.), Annual lespedeza (Lespedeza stipulacea Maxim.), Henbit (Lamium sp.).

H. major. Type host, oats (Avena sativa L.). Also other Gramineae (Oostenbrink, 1950).

H. cruciferae. Type host, cabbage (Brassica oleracea L.). Also other Cruciferae.

H. carotae. Type host, carrot (Daucus carotae L.). Wild carrot (Daucus carotae) is the only other known host (Jones, 1950b).

H. humuli. Type host, hops (Humulus lupulus L.). Also other Urticaceae.

H. galeopsidis. Type host, hemp nettle (Galeopsis tetrahit L.). Also other Labiatae and some species of Chenopodiaceae and Carophyllaceae (Jones, 1950b).

H. fici. Type host, rubber plant (Ficus sp.)

H. weissi. Type host, knotweed (Polygonum pensylvanicum L.). No other hosts known.

H. cacti. Type host, Phyllocactus (Epiphyllum ackermanni). Also other Cactaceae.

H. rostochiensis. Type host, potato (Solanum tuberosum L.). Also tomato (Lycopersicon esculentum Mill.) and a few other species of Solanaceae (Oostenbrink, 1950), but not tobacco (Nicotiana tabacum L.) (Taylor, 1952).

H. tabacum. Type host, tobacco (Nicotiana tabacum L.) and tomato.

H. punctata. Type host, wheat (Triticum vulgare Vill.). Also other Gramineae.

Species of other plant families than those mentioned above have been reported as infected by nematodes of the genus Heterodera. Some of these may be attacked by known species, but it is highly probable that others are attacked by species as yet undescribed. Among these should be mentioned the species found attacking sea marram grass (Ammophila arenaria

(L.) Link) by Triffitt (1929), one found by Thorne on Shadscale (Atriplex confertifolia (Torr. and Frem.) S. Wats.), one found by Chitwood (1949) in soil from North Dakota, and several found by Oostenbrink (1950) attacking plants of various species.

Captions for Illustrations

Plate I. Shapes of cysts of Heterodera species. Fig. 1. H. rostochiensis. Fig. 2. H. punctata. Fig. 3. H. schachtii. Fig. 4. H. avenae. Fig. 5. H. weissi, Fig. 6. H. cacti.

Plate II. Cyst patterns of Heterodera schachtii and related species. Figs. 7 and 8. Zig-zag line pattern near middle of cysts of H. trifolii and H. schachtii respectively. Fig. 9. Pattern at junction of neck and body of cysts of H. schachtii. Fig. 10. Pattern near vulva of cyst of H. gottingiana. Fig. 11. Sheaf-shaped object and dark bodies at lower end of cyst of H. schachtii. Fig. 12. Network pattern, a variation of that shown in Figs. 7 and 8. Magnification of figure 11 is about 200X, all other about 410X.

Plate III. Cyst markings of Heterodera weissi and H. cacti. Fig. 13. Lower part of cyst of H. cacti. Fig. 14. Pattern near middle of cyst of H. weissi. Fig. 15. Pattern at junction of body and neck of H. weissi. Fig. 16. Pattern near middle of cysts of H. cacti. Fig. 17. Lower end of cysts of H. cacti showing anus. Fig. 18. Grainy appearance of cyst of H. cacti. All about 410X.

Plate IV. Cyst patterns and punctation of Heterodera rostochiensis and H. punctata. Fig. 19. Pattern at vulva and anus of H. rostochiensis. Fig. 20. Same as Fig. 19, but with deeper focus to show punctation. Fig. 21. Anal and vulvar openings of cyst of H. punctata. (Cyst split in process of preparation). Fig. 22. Pattern at about middle of cyst of H. rostocniensis. Fig. 23. Pattern of upper part of cyst of H. punctata. Fig. 24. Punctation of H. rostochiensis. All about 410X.

Plate V. Cysts and eggs of Heterodera species. Fig. 25. Punctation of cyst of H. avenae. Fig. 26. Punctation and pattern of cyst of H. humuli. Fig. 27. Punctation of cyst of H. trifolii. Fig. 28. Punctation of egg shell of H. cacti.

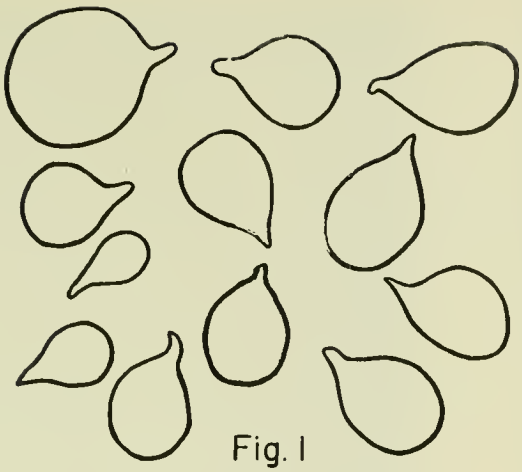


Fig. 1

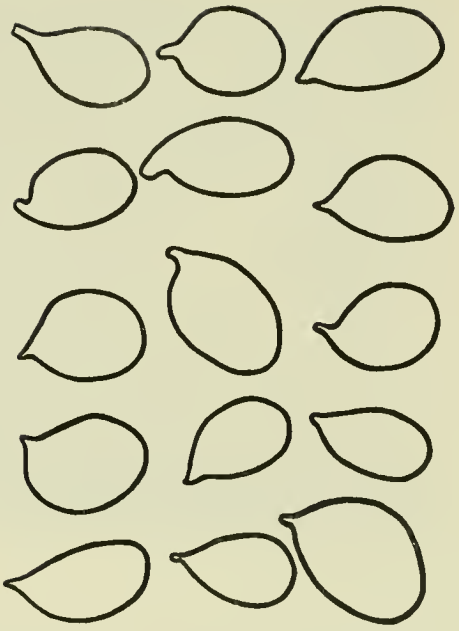


Fig. 2

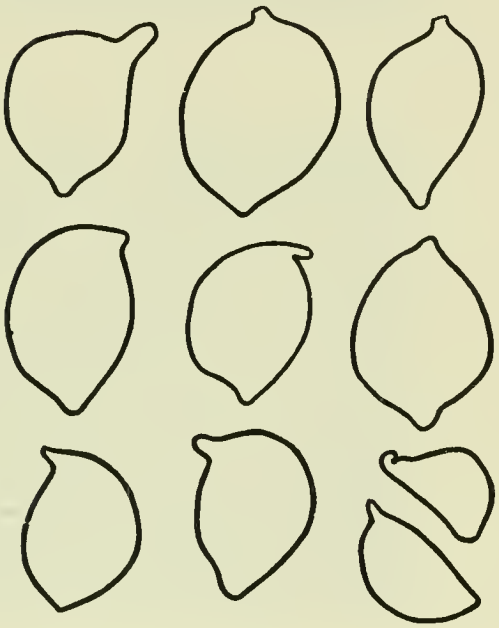
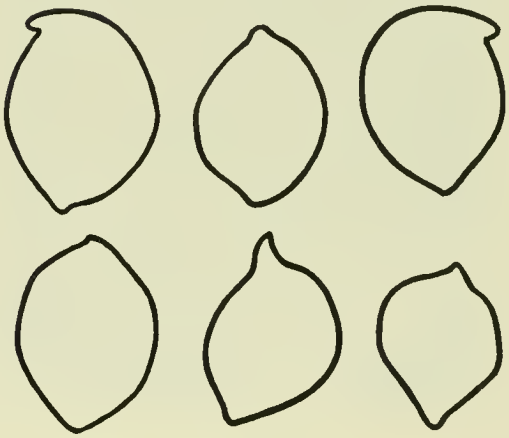


Fig. 3



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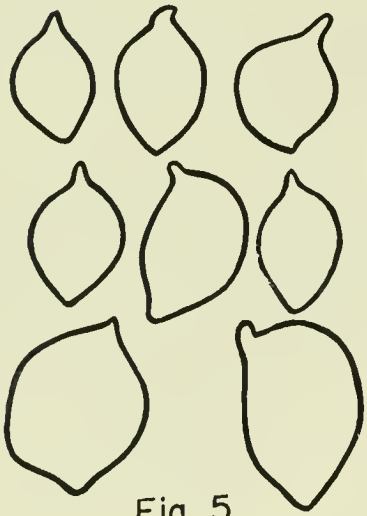


Fig. 5

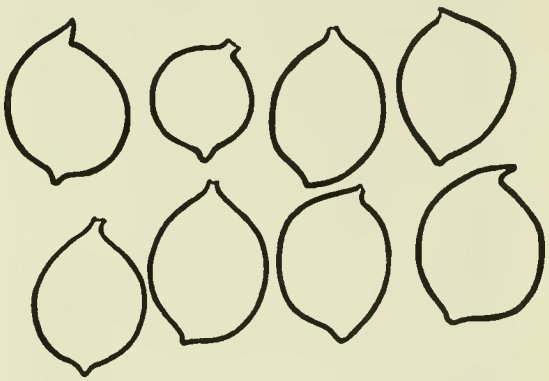
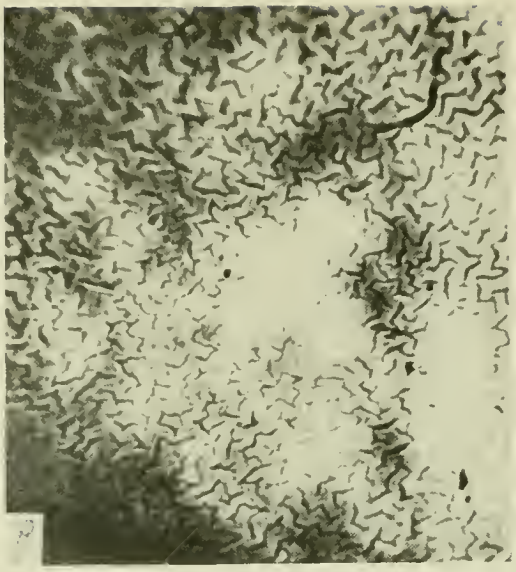
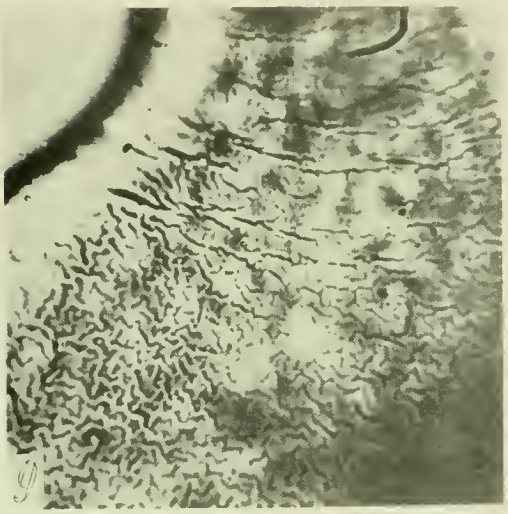
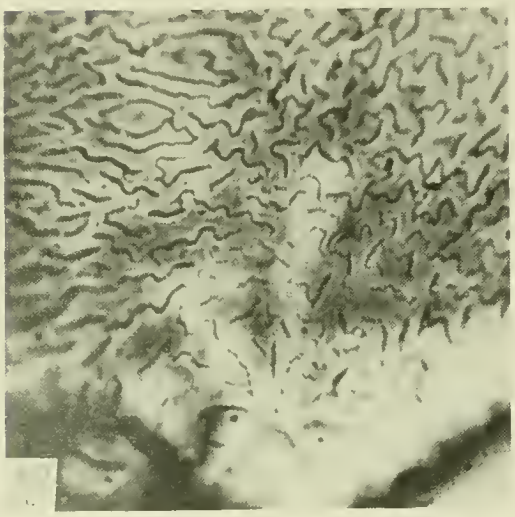
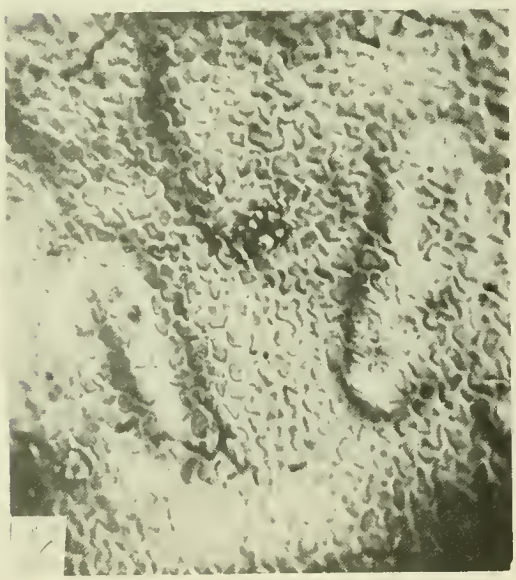


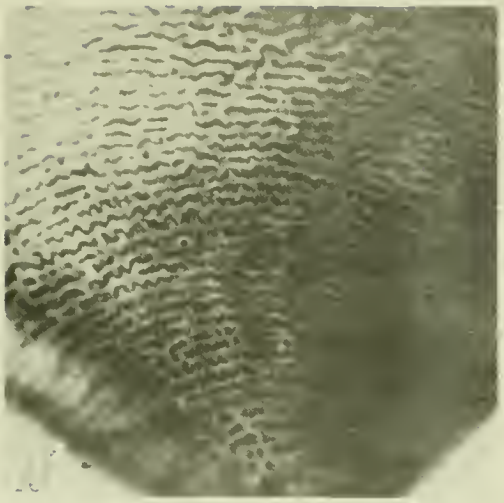
Fig. 6

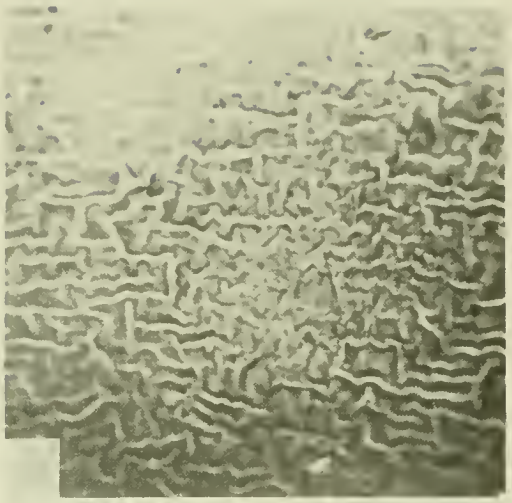


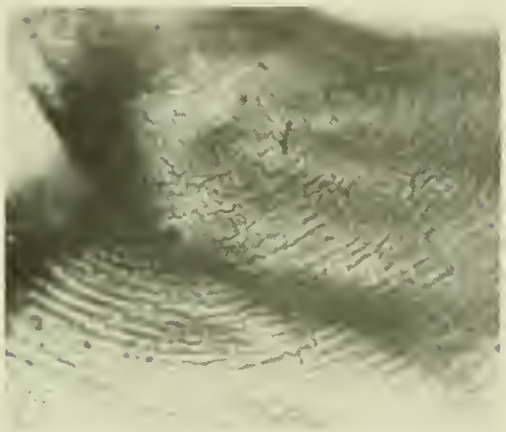


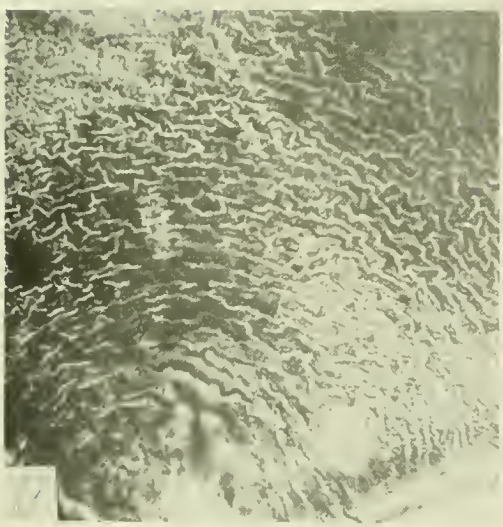


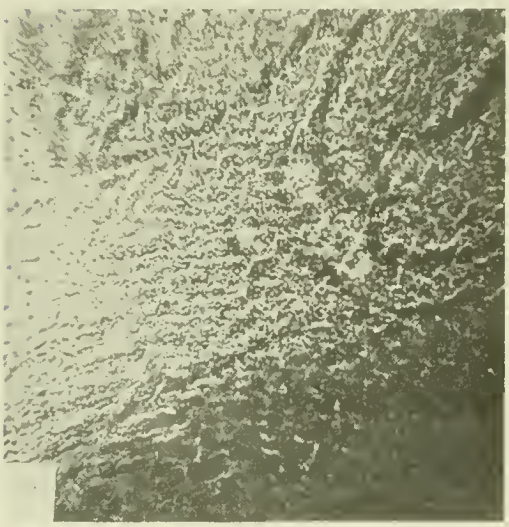


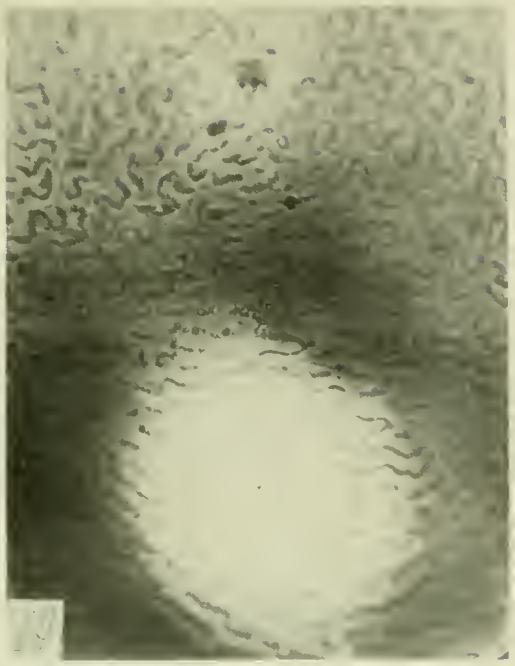


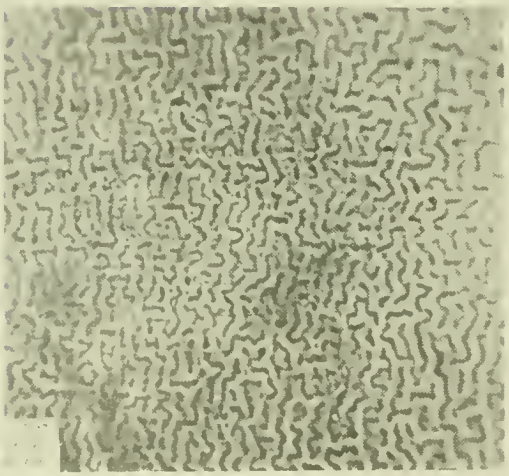


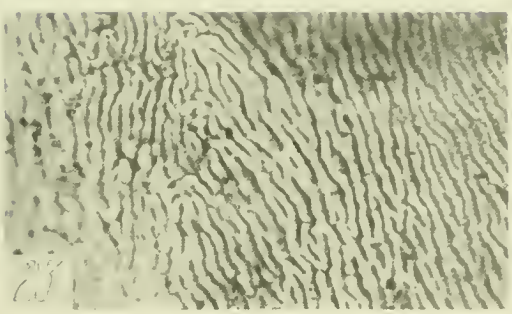


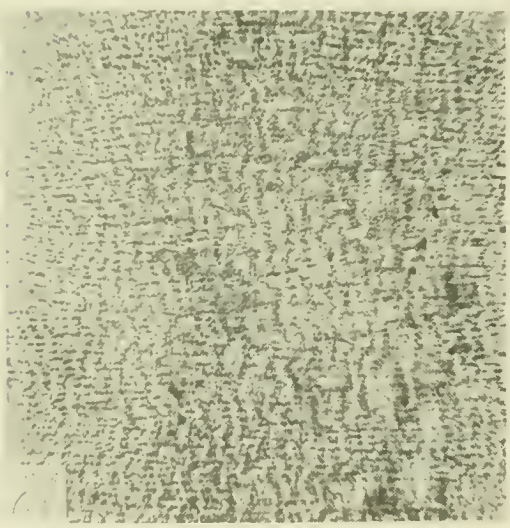


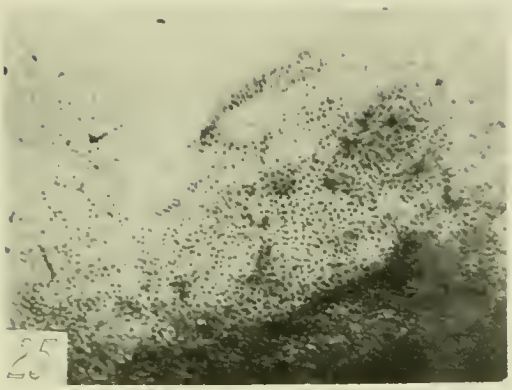


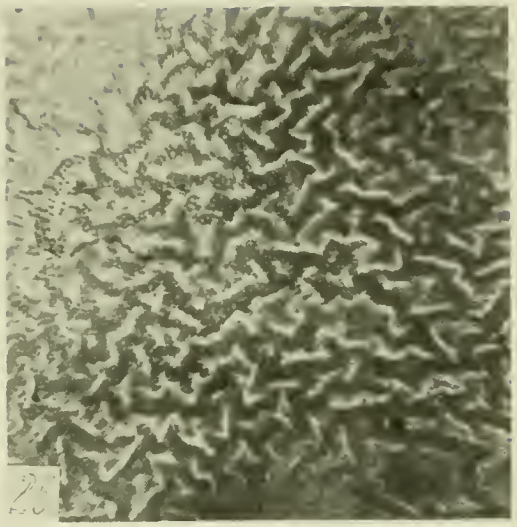


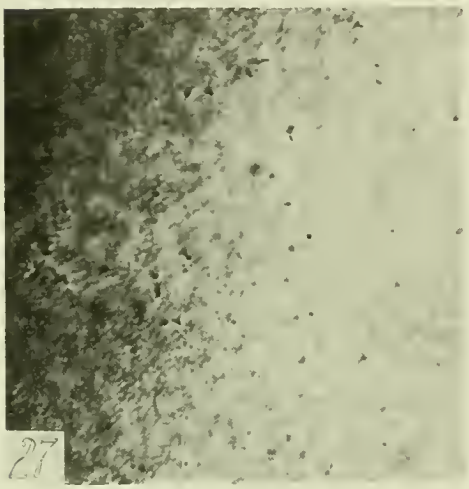


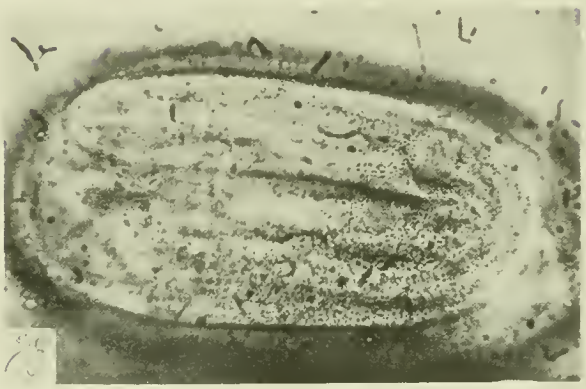












ROOT-KNOT NEMATODES (*MELOIDOGYNE* SPP.)

The genus *Meloidogyne* was placed in the family Heteroderidae of the Tylenchoidea by Thorne (1949) with the genus *Heterodera* (cyst-forming nematodes). Prior to 1949, all the root-knot nematodes were included in *Heterodera* and considered as one species, *Heterodera marioni*. Chitwood (1949), after making a morphological study of the root-knot nematodes, removed them from the genus *Heterodera* and reassigned them to *Meloidogyne*, since this was the earliest valid generic name for this group, having been used by Goeldi in 1887 for a nematode causing root galling of coffee trees in Brazil. Five species and one subspecies were described by Chitwood at this time and later (1952) he described another subspecies.

The root-knot nematodes differ in many respects from the cyst-forming group and the following comparisons might be made: *Meloidogyne* females disintegrate soon after death; the *Heterodera* female body turns into a tough, durable cyst which may remain in the soil for several years. The *Meloidogyne* female never retains eggs but instead deposits them in a mucoid fibrous mass outside the body; the *Heterodera* female always retains eggs in the body which acts as a protective cyst. *Meloidogyne* males possess two lateral cheeks on the lip region and sometimes have two testes; *Heterodera* males have no cheeks but the lip region has ridges dividing the labial region into six sectors, and only one testis is present. The lip region of *Meloidogyne* larvae is not definitely set off from the body, and the stylet is 10 to 15 microns long; the lip region of *Heterodera* larvae is set off from body by a definite constriction and the stylet is 20-33 microns long. *Meloidogyne* species characteristically cause root-swellings or knots on suitable hosts with females tending to remain inside roots at maturity; *Heterodera* species usually do not cause gall formation and females tend to be located on the external surface of root at maturity. *Meloidogyne* species have a rather wide host range while *Heterodera* species are usually rather restricted in host range. Other differences between these two large and important groups are known but those listed above are the easiest to recognize.

At the present time, eight species and three subspecies are known and no doubt more will be described. A listing of these will be of use because some of the original articles are not likely to be encountered without library facilities.

Meloidogyne exigua Goeldi, 1887

M. javanica (Treub, 1885) Chitwood, 1949

M. javanica bauruensis Lordello, 1956

M. hapla Chitwood, 1949

M. incognita (Kofoid and White, 1919) Chitwood, 1949

M. incognita acrita Chitwood, 1949

M. arenaria (Neal, 1889) Chitwood, 1949

M. arenaria thamesi Chitwood, 1952

M. inornata Lordello, 1956

M. brevicanda Loos, 1953

M. acronea Coetzee, 1956

At the present time, the following species and subspecies are known to occur in the United States: Meloidogyne incognita, M. incognita acrita, M. hapla, M. arenaria, M. arenaria thamesi, and M. javanica. Detailed descriptions of these forms are given by Chitwood (1949). Sasser (1954) studied the host-parasite relationships of certain of the root-knot nematode species and proposed a method of identification by host reaction. Taylor, Dropkin, and Martin (1955) discuss in detail the identification of the various known species. This paper is very useful because of its illustrations and a key to the species reported up to 1955 (which includes the known species in the United States up to the present).

The reader is referred to a recent review of the genus Meloidogyne by Franklin (1957) which deals with some aspects of the taxonomy of these nematodes. The taxonomy of these forms is not easy and it involves an appreciation of the fact that one is not dealing with identical specimens, but rather with specimens which fall within relatively consistent morphological and host ranges. Separation of the species is of practical importance because the host ranges of the different species are not identical and thus offer a means of control.

It is of interest to note that a new genus, Meloidodera, has been reported (Chitwood et al, 1956). As its name suggests, it has features of both Meloidogyne and Heterodera. It is intermediate between them in that, unlike root-knot nematodes, no gall is formed in the plant, the female body wall is tough, and there is retention of eggs within the body. It differs from the cyst-forming nematodes in that no distinct cyst stage exists and there is a distinct pattern form of the annules. There are other definite morphological distinctions which clearly set the Meloidodera apart. The original description should be studied, because this nematode is already being found in various places in the United States.

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MEADOW OR ROOT-LESION NEMATODES (PRATYLENCHUS SPP.)

The genus Pratylenchus is made up of several species, all of which are known plant parasites. As a group, these nematodes are widely distributed, have an extensive host range, and rank as one of the most important plant-parasitic genera. All old host lists should be used with caution because considerable changes have been taking place in the taxonomy of this group. A monograph (Sher and Allen, 1953) revising the genus and adding three new species to it has marked the beginning of more attention being paid to the taxonomy of these important parasites in various parts of the world. Additional new species were described along with a study of morphological variation within certain of the species (Taylor and Jenkins, 1957) in a paper. It also contains some very worthwhile suggestions regarding consideration of morphological variation and their expression which ought to be considered by anyone doing taxonomic work, particularly with economically important genera which will be intensively studied for many years to come.

The useful, but now outdated, key to Pratylenchus species from Sher and Allen (1953) is furnished in these Notes. In addition there is given a listing of the Pratylenchus spp. to date with citations where information can be obtained. It is suggested that forms which may not be identified readily be sent to workers who are specializing in the taxonomy of this genus.

The Pratylenchs are primarily root-parasites and may be endo- or ectoparasitic in habit. Infections may give rise to disease symptoms of various kinds according to the hosts involved. In general, the damage resulting is what would be expected of a plant debilitated by increasing amounts of root injury and loss. In boxwoods, a characteristic bronze coloration of the foliage has been noted, and foliar discolorations in other kinds of plants may also suggest the below-ground presence of these nematodes.

Meadow nematodes in various stages of their life cycle are to be found in soil samples. These nematodes are known to evacuate root tissues which are decaying, and thus may be overlooked if root examination alone is relied upon. Infected roots may be stunted and show spots, streaks, or encircling bands of discolored necrotic tissue. These symptoms are not exclusive for Pratylenchus, although they are usually found when these nematodes are the parasite involved. The nematodes can enter into the root tissues, migrate within the root, and complete their life cycle. The external feeding sites, places of entry, and other damaged areas of the root may serve as portals of entry for other infective soil microorganisms.

It is important to remember that these nematodes usually leave roots when decay sets in and that most of the species are reported to be unable to withstand drying. Both of these factors can effect chances of finding the nematode in older infections or in samples of soil and plants which become dry before being processed.

LISTING OF SPECIES OF PRATYLENCHUS

Sher, S. A., and M. W. Allen. 1953. Revision of the genus Pratylenchus (Nematoda: Tylenchidae). Univ. Calif. Public. in Zool. 57(6):441-470.

P. brachyurus (Godfrey); Syn. P. leiocephalus Steiner

P. coffeae (Zimmerman); Syn. P. mahogani, P. musicola

P. goodeyi n. sp.

P. minyus n. sp.

P. penetrans (Cobb)

P. pratensis (DeMan); (Tylenchus gulosus, Aphelenchus neglectus

P. scribneri (Steiner)

P. thornei n. sp.

P. vulnus Allen & Jensen

P. zeae Graham (P. zeae Steiner, nomen nudum)

Species Inquirendae

P. sacchari (Soltivedel)

Dolichodorus heterocercus Kreis

Taylor, D. P., and W. R. Jenkins. 1957. Variation within the nematode genus Pratylenchus, with the descriptions of P. hexincisus, n. sp. and P. subpenetrans, n. sp.

P. hexincisus n. sp.

P. subpenetrans n. sp.

Also information on morphology of:

P. zeae

P. penetrans

Merzheyevskaya, O. I. 1951. New species of nematodes. (In Russian). Akademiia Nauk Belaruskaia, Minsk. Instytut Biialogii. Sbornik

Nachnykh Trudovä. 2:112-120. Fig. 1-6.

P. tumidiceps n. sp.

Meyl, A. H. 1953. Beitrage zur Kenntnis der Nematoden fauna vulkanisch erhitzter Biotope I. Die terrikolen Nematoden in Bereich von Fumarolen auf der Insel Ischia. Z. Morph. Okol. Tiere. 42:67-116.

P. pratensis var. tenuistriatus n. sp.

Meyl, A. H. 1953. Die bisher in Italien gefundenen frei lebenden Erd- und Süsswasser-Nematoden. Arch. zool. Italiano 39:161-264.

P. pratensis var. bicaudatus n. sp.

Lordello, L. G. E. 1956. Sobre um nematodeo do genero Pratylenchus, parasito das raizes de Allium cepa. Revista de Agricultura 31(3): 181-188.

P. coffeae brasiliensis n. subsp.

Loof, P. A. A. 1957. Was ist Aphelenchus neglectus Rensch? Nematologica 2 (Supplement):348.

P. neglectus (Rensch, 1924) n. comb.

KEY TO THE SPECIES OF PRATYLENCHUS
Sher-Allen, 1953

1. Lip region with 2 annules (1 striation)---------- 2
   Lip region with 3 or 4 annules (2 or 3 striations)--------- 5

2. Lateral margin of lips angular--------- brachyurus (Godfrey)
   Lateral margin of lips rounded ---------- 3

3. Body long and slender (L=0.4-0.7, A=25-40), males numerous
   coffeae (Zimmerman)
   Body short and stout (L=0.3-0.67, A=18-26), males rare ---- 4

4. Vulva (=V) at 75-80 per cent ---------- scribneri Steiner
   Vulva at 80-88 per cent ---------- minyus n. sp.

5. Striations around terminus of tail--------- pratensis (de Man)
   No striations around terminus of tail ---------- 6

6. Outer margins of cephalic framework prominent, extending posteriorly about two body annules, tail bluntly rounded ----
   ---------- thornei n. sp.
   Outer margins of cephalic framework normal ---------- 7

7. Vulva 68-76 per cent, tail tapering to narrow rounded terminus ---------- 8
   Vulva 78-84 per cent ---------- 9

8. Three annules on lip region, males absent -------- zeae Graham
   Four annules on lip region, males numerous goodeyi n. sp.

9. Postuterine branch short, length about equal to body width at vulva, tail broadly rounded ---------- penetrans (Cobb)
   Postuterine branch long, two or three times body width at vulva, tail tapering to narrow rounded terminus ----------
   ---------- vulnus Allen and Jensen

BUD AND LEAF NEMATODES (APHELENCHOIDES SPP.)

The genus Aphelenchoides consists of numerous species, is widespread, and specimens are frequently found in soil and plant samples. This genus has a diversity of biological associations. There are species that are parasitic on and in plant roots, buds, and leaves. Some species feed on fungi and possibly on soil algae, others are parasites or associates of insects, and still others are predators of nematodes and other soil microorganisms.

As the common name of this genus suggests, the plant parasitic species of this group are probably best known because of the foliar diseases they cause. Symptoms of disease vary, depending upon the host plant and the nematode species involved. Plants may show varying degrees of deformation of stems, leaves, flowers, and buds may remain rudimentary. "Dwarf" or "crimp" are descriptive terms applied to such conditions as, for example, in strawberries. In chrysanthemums, dahlias, ferns, and other plants leaves invaded by the nematodes show discolorations and eventually necrotic areas appear. these spots are often bounded by the larger leaf veins and appear as distinctly angular in outline. Aphelenchoides may lodge in the leaf sheathes of grasses or between the developing leaves or petals in the buds. These nematodes feeding as ectoparasites, as they are not within the plant tissues, can cause symptoms of disease in the parts noted, such as abnormal foliar color, distortion of the leaves, and in some cases lead to decay of the structures.

An important example of a disease caused by a species of Aphelenchoides, in which the stem of the host is affected, is the red ring disease of coconuts. The nematodes may initiate invasion of the host by penetrating at the terminal growing point, being carried there in some cases by an insect. Eventually the entire parenchyma of the host is penetrated by the migrating nematodes, and a red-collored ring which characterizes the disease is found in the parenchyma of the tree's trunk.

It is not possible to assign a definite role to all of the Aphelenchoides species that are recovered from about plants. The fact that some of the plant-parasitic species can thrive on cultures of fungi suggests parasitism on the higher plants may be only incidental or of a facultative nature. It is possible that incidental feeding on the root surfaces by some of the often quite numerous Aphelenchoides and similar forms could be of importance. Numerous minute wounds in the presence of other soil microorganisms may be related to establishment of disease complexes. The ability of Aphenlenchoides to survive on fungi and perhaps algae, plus an ability to withstand periods of inactivation in conditions of adversity, must be considered when developing control practices for this genus.

The taxonomy of the genus is difficult, not because of a lack of reports in the literature, but rather because of so many accounts without

adequate descriptions and which are occasionally in conflict. Differences in hosts, rather than morphological differences of the nematodes, were sometimes the criteria used for setting up a species. The textbook by Filipjev and Stekhoven (1941) is a good reference for this genus and is provided with a taxonomic key, descriptions, and bionomics for many of the species. A recent paper (Allen 1952) presents a reconsideration of important foliar species which is based entirely upon the comparative morphology of the nematodes. The result is a synonymizing of numerous species previously regarded as distinct on the basis of the host involved.

Literature Cited

1. Allen, M. W. 1952. Taxonomic status of the bud and leaf nematodes related to Aphelenchoides fragariae (Ritzema Bos 1891). Proc. Helminth. Soc. Washington 19(2): 108-120.

KEY TO THE APHELENCHIDAE

The superfamily, Aphelenchoidea, has a single family, Aphelenchidae, which is divided into two subfamilies, Aphelenchinae and Paraphelenchinae. A key is presented for distinguishing genera of these subfamilies which are found in the soil in association with plant life.

1. Posterior portion of esophagus not a distinct glandular bulb, esophageal glands considerably overlapping the beginning of the intestine.----------Aphelenchinae-----2.

   Posterior portion of esophagus a distinct structure containing the esophageal glands and not overlapping the intestine. ----------Paraphelenchinae-----4.

2. Head possessing a shallow, sclerotized frontal-disc. (Lateral field with 3 incisures, male unknown.)-------Anomyctus

   Head not possessing a shallow, sclerotized frontal-disc.--------3.

3. Isthmus leading to a more or less distinct posterior portion region about 1/2 to 1/3 length of precorpus and blending with the beginning of the intestine. Lateral field with 10-12 incisures, spicules slender, gubernaculum present, bursa present. Spear without basal knobs.------Aphelenchus

   Isthmus comparatively short and blending with beginning of intestine. Longitudinal striae present, characteristic rose-thorn shaped spicules, no gubernaculum, bursa absent. Spear with or without basal knobs.----------Aphelenchoides

4. Post-bulbar region of esophagus set-off from intestine by a constriction. Absence of longitudinal striae in lateral fields.----------Paraphelenchus

   Post-bulbar region of esophagus usually not set-off from intestine by a constriction. Lateral field with 10-12 incisures with a bulge ventrally on tail region.-------- ----------Metaphelenchus

KEY TO THE SPECIES OF BUD AND LEAF NEMATODES
(From Allen, 1952)

1. Head swollen, wider than neck, 4 lines in wing area.----------2.

   Head not swollen, 2 lines in wing area.----------A. fragariae

2. Length of posterior-uterine branch 5 or more times body width.-------3.

   Length of posterior-uterine branch less than 4 times body width.-----
   ----------A. Besseyi

3. Tail bluntly rounded, armed by a single ventral spine.-A. subtenuis

   Tail terminus peg-like, armed with 4 small mucrons.-A. ritzema-bosi

Aphelenchoides fragariae (Ritzema Bos, 1891) Christie, 1932

Synonyms:

Aphelenchoides olesistus, Ritzema Bos, 1893

Aphelenchoides olesistus, Steiner, 1932

Aphelenchoides olesistus var. longicollis, Schwartz, 1911

Aphelenchoides olesistus var. longicollis, Goodey, 1933

Aphelenchoides longicollis, Filip. and Stek., 1941

Aphelenchus psuedolesistus, Goodey, 1928

Aphelenchoides psuedolesistus, Goodey, 1933

Aphelenchus omerodis, Ritzema Bos, 1891, in part.

Aphelenchoides besseyi, Christie 1942

Synonym:

Aphelenchoides oryzae, Yokoo, 1948

Aphelenchoides subtenuis (Cobb, 1926) Steiner and Buhrer 1932

Synonym:

Aphelenchoides hodsoni, Goodey, 1935

Aphelenchoides ritzema-bosi (Schwartz, 1912) Steiner, 1932

Synonyms:

Tylenchus ribes, Taylor, 1917

Aphelenchus ribes, Goodey, 1932

Aphelenchoides ribes, Goodey, 1933

Aphelenchus phyllophagus, Stewart, 1921

STEM AND BULB NEMATODE (DITYLENCHUS SPP.)

This genus represents one of the major plant-parasitic groups and is reported in association with many kinds of host plants and from many countries. There are about 30 species described, the best known being Ditylenchus dipsaci. As perhaps is to be expected from a genus with many species and having an extensive host range and wide geographic distribution, there exists difficulty in the exact taxonomy of the group. There has long been recognition of "races" and application of existing host lists must take into consideration this matter of our not presently being able to accurately distinguish between certain closely related species on the basis of morphology. Mixed infections are also known to occur and must be guarded against in host range studies. The book by Filipjev and Stekhoven (1941) is a good reference for this genus and gives a taxonomic key, descriptions of the species, and considerable information regarding the biology and control of the more important species.

Symptoms of plant disease caused by these nematodes vary widely, depending upon the plant and nematode species involved. The diseased parts of the plants may be the roots, leaves, stems, flowers, and seeds. In the case of the teasle, the entire plant may be invaded by the nematode, Ditylenchus dipsaci, More usually, only particular parts of the host plants are involved, for example, the foliar parts of alfalfa or the tubers of potatoes. Almost all row crop plants, forage plants, grains, and many ornamentals and weeds have been reported as hosts for nematodes of this genus. Some of the forms parasitic on higher plants can be cultured on fungi, and it is possible that all the species may be incidental feeders on the surface of roots, whether or not they ever become endoparasitic in habit. Members of the genus which are regarded as parasites of known importance, for the most part, are found within the diseased host tissues.

Plants exhibiting irregularity of growth form and distortions of foliar parts should be checked for the presence of Ditylenchus. Lesions of stems and underground parts of plants showing a loose, granular, brown-colored condition of the cells should also be checked carefully for these nematodes which are likely to be located in advance of the lesions. A galled condition of the roots similar to root-knot is also known to occur on some plants as a result of invasion by Ditylehchus. The damage caused by these nematodes is by chemical in addition to mechnaical action, as evidenced by what appears to be dissolution of the middle lamella of the hosts' cells and abnormal hypertrophy of cells and tissues in the vicinity of the nematodes.

Some members of this genus have the ability to withstand adverse environmental conditions by going into a state of dormancy called anabiosis. This capacity for survival under a wide range of circumstances is a matter of significance in dissemination and control of such nematodes.

SPIRAL NEMATODES (HELICOTYLENCHUS SPP. AND ROTYLENCHUS SPP.)

These organisms are commonly called spiral nematodes because of the orientation of the body into a spiral when the animals are inactive or dead. As a group, these nematodes are found associated with the roots of numerous kinds of plants from many countries. They may be found with heads embedded to shallow depths in lesions of roots and other underground plant parts or, in some cases, penetration may be deeper. In most instances of lesions other microorganisms are present. There is no report of successful cultivation of these nematodes in cultures with fungi, so it is likely that they are obligate parasites of the higher plants. The spiral nematodes do not appear to be as harmful to their hosts as other parasitic nematodes such as root-knot or meadow nematodes. Recent experimental work (Sledge, 1955) with Helicotylenchus nannus Steiner, 1945, indicates that these nematodes, although obligate parasites, are highly successful parasites in that their hosts are not quickly rendered unsuitable as sources of food and sites for nematode reproduction. Such nematodes, however, may have significance as wounding agents providing portals of entry for other soil microorganisms, some of which may be harmful in their effects. It is too soon to do much generalizing about the sprial nematodes, because intensive consideration of them as plan-parasites has only recently been started.

Despite the present unsettled state of the taxonomy of the genera of the Hoplolaiminae, which includes the spiral nematodes, the worker is advised to make careful observation of these forms because of the increasing appreciation of their distribution and potential importance as plant pests. As should be done with the other nematode species, when reporting work with these nematodes, cite the complete scientific name with the authors and revisors of it. This is particularly important with the spiral nematodes because of the state of flux of the taxonomy. Considerable experimental work may be reduced to lesser value, if in the future it is uncertain as to which spiral nematode the results pertain. The complete listing of synonyms as taken from Andrassy (1958) is presented in the notes to assist in applying exact names.

Two important papers dealing with the taxonomy of the group are available (Golden, 1956, and Andrassy, 1958). The first paper also includes a study of the developmental stages and host-parasite relationships of Rotylenchus buxophilus Golden, 1956. Taxonomic keys are copied from both of these papers as that of Andrassy gives a synopsis of the subfamily Hoplolaiminae and makes certain transfers of species and sets up two new genera. The paper by Golden gives a key to the genera and species of spiral nematodes (Helicotylenchus spp. and Rotylenchus spp.) Both papers have excellent literature reviews and should also be referred to for the illustrations. The book by Filipjer and Stekhover (1941) gives a key to the Rotylenchus, but will be more useful now because of the illustrations and descriptions. If one follows the taxonomy as set forth by Andrassy, the key to species in the paper by Golden will also be found useful.

Literature Cited

Andrassy, I. 1958. Hoplolaimus tylenchiformis Daday, 1905 (Syn. H. coronatus Cobb, 1923) und die Gattungen der unterfamilie Hoplolaiminae Filipjev, 1936. Nematologica 3(1):44-46.

Filipjev, I. N. and J. H. Schuurmans Stekhoven, Jr. 1941. A manual of agricultural helminthology. E. J. Brill, Leiden, Holland. pp. 213-229.

Golden, A. M. 1956. Taxonomy of the spiral nematodes (Rotylenchus and Helicotylenchus) and the developmental stages and host-parasite relationships of R. buxophilus, n.sp., attacking boxwood. Univ. of Md. Bul. A-85. 28 pp.

Sledge, E. B. 1955. Pathogenicity of the spiral nematode, Helicotylenchus nannus Steiner, 1945, in relation to selected varieties of corn. Thesis. Ala. Polytech. Inst., Alabama. 60 pp.

A KEY TO THE GENERA AND SPECIES OF SPIRAL NEMATODES
(From Golden, 1955)

1. Opening of dorsal esophageal gland usually less than 1/3 of stylet length from base of stylet; phasmids either small, dot-like or large, round or oval structures (scutellum).
Rotylenchus Filipjev, 1934------4

Opening of dorsal esophageal gland 1/3 or more of stylet length from base of stylet; phasmids always small, dot-like structures.----------Helicotylenchus Steiner, 1945------2

2. Tail generally rounded; stylet approximately 22 μ.
H. multicinctus (Cobb, 1893) n. comb.

Tail not rounded, with most of the curvature being on the dorsal side; stylet about 25-28 μ.----------3

3. Tail usually with long ventral terminal process; well-developed spherical spermatheca present.
H. erythrinae (Zimmermann, 1904) n. comb.

Tail with or without short, blunt ventral terminal process; well-developed spherical spermatheca absent.
H. nannus Steiner, 1945

4. Phasmids large, round or oval structures (scutellum).----------7

Phasmids small, dot-like structures.----------5

5. Tail with distinct ventral terminal process; stylet 23-25 μ.
R. melancholicus Lordello, 1955

Tail without a ventral terminal process; stylet 28 μ or over.------6

6. Phasmids at about latitude of anus; stylet approximately 28 μ.
R. robustus (de Man, 1880) Filipjev, 1945

Phasmids well forward of anus; stylet about 33 μ.
R. buxophilus n.sp.

7. Phasmids not opposite each other, location of anterior one being at average of 79% of body length and posterior one at an average of 86%; lips of vulva protruding.
R. christiei Golden and Taylor, 1956

Phasmids opposite each other and located on tail or in vicinity of anus; lips of vulva not protuding.----------8

8. Tail as long as or longer than anal body diameter; head with seven or more annules.----------10

Tail much shorter than anal body diameter; head with five annules or less.----------9

9. Lateral field aerolated* in esophageal region; five annules on head.----------R. brachyurus Steiner, 1938

Lateral field not aerolated in esophageal region; three annules on head, rarely four.----------R. coheni Goodey, 1952

10. Stylet knobs anteriorly flattened or slightly concave.
R. bradys (Steiner and LeHew, 1939) Filpjev, 1936

Stylet knobs ovoid, anteriorly convex.
R. blaberus Steiner, 1937

Species of undetermined status:
Rotylenchus pararobustus (Schuurmans-Stekhoven and Teunissen, 1938) Filipjev and Schuurmans-Stekhoven, 1941.
Rotylenchus africanus (Micoletzky, 1915) Filipjev and Schuurmans-Stekhoven, 1941.

*Transverse body striae extending into or across the lateral field.

A KEY TO THE GENERA OF HOPLOLAIMINAE
(From Andrassy, 1958)

1. Phasmids normal, small, pore-like: Rotylenchus genus - group 2

   Phasmids extremely large, scutellum-like: Hoplolaimus genus - group 4

2. Opening of dorsal esophageal gland about 1/2 spear length from base of knobs of spear: Helicotylenchus Steiner

   Opening of dorsal esophageal gland not more than 1/3 spear length from base of knobs of spear: 3

3. Lip region marked by annules divided into numerous small sections; gubernaculum bearing titillae: Rotylenchus Filipjev

   Lip region with transverse annules only; gubernaculum without titillae: Gottholdsteineria n. gen.

4. All three fields of lateral field aerolated by transverse striae; spear knobs anteriorly furcate and pointed: Hoplolaimus Daday

   Center field of lateral field smooth, without transverse striae;. spear knobs rounded: Scutellonema n. gen.

I. Rotylenchus genus-group

A. Rotylenchus Filipjev, 1936

R. robustus (de Man, 1876) Filipjev, 1936 (Type for the genus)

syn: Tylenchus robustus de Man, 1876
Tylenchorhynchus robustus (de Man, 1876) Micoletzky, 1921 partim
Hoplolaimus uniformis Thorne, 1949

B. Helicotylenchus Steiner, 1945

H. africanus Micoletzky n. comb.

syn: Tylenchus africanus Micoletzky, 1915
Tylenchorhynchus africanus (Micoletzky, 1915) Schuurmans-Stekhoven and Teunissen, 1938
Rotylenchus africanus (Micoletzky, 1915) Filipjev and Schuurmans-Stekhoven, 1941

H. erythrinae (Zimmermann, 1904) Golden, 1956

syn: Tylenchus erythrinae Zimmermann, 1904
Tylenchus psuedorobustus Steiner, 1914
Aphelenchus dubius var. peruensis Steiner, 1920
Tylenchus spiralis Cassidy, 1930

Tylenchorhynchus robustus var. erythrinae (Zimmermann, 1904) Bally and Reydon, 1931
Anguillulina multicincta in Goodey, 1932 nec Cobb, 1893
Tylenchorhynchus multicinctus in Schuurmans-Stekhoven and Teunissen, 1938 nec Cobb, 1893
Anguillulina erythrinae (Zimmermann, 1904) Goodey, 1940
Rotylenchus erythrinae (Zimmermann, 1904) Goodey, 1951 partim

H. iperoiguensis (Carvalho, 1956) n. comb.
syn: Rotylenchus iperoiguensis Carvalho, 1956
H. melancholicus (Lordello, 1955) n. comb.
syn: Rotylenchus melancholicus Lordello, 1955
H. multicinctus (Cobb, 1893) Golden, 1956
syn: Tylenchus multicinctus Cobb, 1893
Rotylenchus multicinctus (Cobb, 1893) Filipjev, 1936
Anguillulina multicinta (Cobb, 1893) Goodey, 1940
H. nannus Steiner, 1945 (Type for the genus)

C. Gottholdsteineria n. gen.
G. buxophila (Golden, 1956) n. comb.
syn: Rotylenchus buxophilus Golden, 1956
G. goodeyi (Loos and Oostenbrink, 1958) n. comb. (Type for the genus)
syn: Tylenchorhynchus robustus in Micoletzky, 1921 partim nec de Man, 1876
Rotylenchus robustus in Filipjev and Schuurmans-Stekhoven, 1941 and in Thorne, 1949 nec de Man, 1876
Rotylenchus goodeyi Loof and Oostenbrink, 1958
G. pararobusta (Schuurmans-Stekhoven and Teunissen, 1938) n. comb.
syn: Tylenchorhynchus robustus in Schuurmans-Stekhoven, 1936 nec de Man, 1876
Tylenchorhynchus pararobustus Schuurmans-Stekhoven and Teunnisen, 1938
Rotylenchus pararobustus (Schuurmans-Stekhoven and Teunnisen, 1938) Filipjev and Schuurmans-Stekhoven, 1941
G. quarta Andrassy, 1958

II. Hoplolaimus genus-group
A. Hoplolaimus Daday, 1905
H. proporicus J. B. Goodey, 1957
H. tylenchiformis Daday, 1905 (Type for the genus)
syn: Hoplolaimus coronatus Cobb, 1923

B. Scutellonema n. gen.
S. blaberum (Steiner, 1937) n. comb. (Type for the genus)
syn: Rotylenchus blaberus Steiner, 1937
S. boocki (Lordello, 1957) n. comb.
syn: Rotylenchus boocki Lordello, 1957

S. brachyurum (Steiner, 1938) n. comb.
syn: Rotylenchus brachyurus Steiner, 1938
S. bradys (Steiner and LeHew, 1933) n. comb.
syn: Hoplolaimus bradys Steiner and LeHew, 1933
Anguillulina bradys (Steiner and LeHew, 1933) Filipjev, 1936
S. christiei (Golden and Taylor, 1956) n. comb.
syn: Rotylenchus christiei Golden and Taylor, 1956
S. coheni (J. B. Goodey, 1952) n. comb.
syn: Rotylenchus coheni J. B. Goodey, 1952

STING NEMATODES (BELONOLAIMUS SPP.)

The sting nematode, Belonolaimus gracilis, was described by Steiner in 1949 ("Plant Nematodes the Grower Should Know." Soil Science Society of Florida Proceedings IV-B pp. 72-117). This genus was not described in time to be included in either Goodey's 1951 "Soil and Fresh-water Nematodes" or Thorne's "On the Classification of the Tylenchida" (Proc. Helm. Soc. Wash. 16(2): 37-73, of 1949). However, Steiner has an excellent illustration and description. The fairly large size (about 2mm.), the very long stylet, and the other distinctive features illustrated make identification easy. It should be pointed out that only one species of the genus has been described, and it is quite probable that other species exist. If so, these will differ in details from B. gracilis.

Belonolaimus is an ectoparasite and will, therefore, be found in soil collections, though occasional specimens, usually young individuals, may be found in the root tissues. (Christie, J. R., A. N. Brooks and V. G. Perry, 1952. Phytopath. 42(4): 173-176).

Christie et al. (loc. cit.) gives an excellent discussion of the sting nematode and its effects. The following excerpts are taken from this paper:

"The principal above ground symptom of attack by sting nematodes is poor growth of the crops, with severe stunting in patches in the field. As with other nematodes, plants will show symptoms of nutrient deficiencies. Roots of attacked plants may be injured at the tips with consequent development of short, stubby branches. Necrotic lesions may be produced along the sides of the roots. On celery growing in soil heavily infested with Belonolaimus, most of the roots are apt to be in the upper two inches of soil where the nematodes are least numerous. Final diagnosis of the trouble depends on finding the nematodes in the soil. It should be kept in mind that Belonolaimus, like most other plant parasitic nematodes, is an obligate parasite. That is, it feeds only on living roots. For this reason, it is often difficult to establish a correlation between apparent damage to the plant and the number of nematodes which can be found in the adjacent soil. In fact, just the opposite might be found. There may be more nematodes around a root system which is still healthy than around one which has already been mostly destroyed by the nematodes."

Christie et al. reported a series of experiments which demonstrated injury to strawberry, celery, corn, and other plants. In some of these experiments they used a system which is well worth considering for similar work. This system was devised to meet the objection that damage observed on plants exposed to nematodes extracted from the soil might be due to associated organisms rather than to the nematodes. They say: "While it may be impossible to obtain nematodes free from contaminating

organisms, it is neither impossible nor especially difficult to obtain these same organisms free from nematodes. One can remove the nematodes from the water in which they are suspended when drawn from the funnels, and this fluid can be placed around the roots of control plants or around the roots of a third set of plants provided for the purpose." If a third set of plants is provided, comparisons can be made between the three sets of plants: (1) Controls in sterilized soil; (2) In sterilized soil inoculated with nematodes and associated organisms; and (3) In sterilized soil inoculated with associated organisms without nematodes. If growth in (1) and (3) is equal, while growth in (2) is depressed, the logical assumption is that the decrease in growth is either due to the nematodes alone or to the combination of nematodes and associated organisms. For practical purposes, it makes little difference as it has been shown that the nematodes are an essential member of the complex, and control might be expected to be obtained by their elimination.

The fact that sting nematode may be an important factor in cotton wilt was shown by Holdeman and Graham (Phytopath. 42(5): 283-284, of 1952). They collected infested field soil and eliminated sting nematodes from a portion of it by air drying, while a second portion was kept moist. Cotton Fusarium inoculum was added to each lot of soil and wilt-resistant and wilt-susceptible cotton varieties were planted. With the sting nematodes, the resistant variety averaged 52% wilt, compared with no wilt where the nematode was absent. The susceptible variety had an average of 88% wilt with the sting nematode and only 10% without it.

Information on hosts of Belonolaimus can be obtained from Christie et at. (loc. cit.) and from Holdeman and Graham (Plant Dis. Reporter 37(10): 497-500, of 1953). Apparently, sting nematodes are capable of feeding on a large variety of plant species, including both crop plants and weeds. As would be expected, certain other species appear to be unsuitable hosts, among them being tobacco, watermelon, and crotalaria. However, establishment of a host list for sting nematodes is still in the early stages, and it should be kept in mind that there may be species, subspecies, populations, or races with varying host habits, as have been found with other plant-parasitic nematodes.

Control of sting nematodes by crop rotation or by soil fumigation appears to present no special problems. Miller (Phytopath. 42(9): 470, of 1952) reports excellent control with ethylene dibromide at moderate rates of application. When adequate host lists are available, control rotations can be devised.

RING NEMATODES (CRICONEMOIDES SPP.)

Ring nematodes are fairly common in agricultural soils and sometimes occur in great numbers. However, because of their rather odd shape and sluggish movements, they may sometimes be overlooked. Shape is typically plump, since they are usually only about ten to fifteen times as long as wide. Often the body is strongly curved, as shown in Fig. 75 of Goodey's "Soil and Freshwater Nematodes." Once found, identification to genus presents no special difficulty. The cuticle is strongly annulated, and the annules are retrorse (directed backward). Apparently this feature is connected with their locomotion, which is of a somewhat different type than the serpentine motion of longer and slimmer nematodes. Ring nematodes move through the soil by alternate contraction and expansion of the body, the retrorse annules acting somewhat in the fashion of a ratchet, catching on soil particles when the body is extended, and slipping past when the body is contracted. The common rame, "ring nematodes," is applied to two genera, Criconema and Criconemoides. These are closely related, differing mostly in that the annules of the latter are smooth, while those of the former are fringed with scale-like projections, the shape and arrangement of which varies with species (Fig. 76, Goodey). Criconema appears to be rare in agricultural soils, but is often found in forest soils.

Ring nematodes are usually about one-half millimeter long. They have stylets which are often-one-tenth of the body length and may be as long as one-third of the body length. The stylet knobs are well developed and are to be seen in the anterior part of the median oesophageal bulb when the stylet is retracted.

An aid to identification to species is "On the Morphology of Criconemoides, etc.," by Dewey J. Raski (Proc. Helmin. Soc. Wash. 19(2):85-99 of 1952). This paper has a key to the species and is copied for inclusion in these Notes.

Very little information, either on the biology or pathogenicity of ring nematodes, is available. It is quite certain that they are plant-parasites. Steiner (Plant Nematodes the Grower Should Know) presents a photograph of a ring nematode partially embedded in a root, though it is probable that most feeding is done from the outside of the root. It is a fair assumption that this feeding kills or injures cells and that the dead tissue might provide an entrance for pathogenic bacteria or fungi which could not otherwise attack the plant.

Present evidence that ring nematodes may be a cause of important losses in crops is mostly limited to observations of great numbers of ring nematodes associated with roots of plants which were growing poorly.

KEY TO THE SPECIES OF CRICONEMOIDES
(From Raski, 1952)

1. Spear length 100 μ or more----------2

   Spear length 90 μ or less----------5

2. Total body annules 95 or more----------3

   Total body annules 58-61----------annulifer (de Man)

3. Length 0.450 mm. or more; spear not very long and thin (less than 1/3 of body length)----------4

   Length 0.270-0.300 mm.; spear very long and thin (more than 1/3 of body length)----------macrodorum Taylor

4. Spear 105 μ; total body annules 140; length 0.880-1.000 mm.
   annulatum Taylor

   Spear 122 μ; total body annules 95-103; length 0.456 mm.
   sphagni (Micoletzky)

5. Tail pointed----------6

   Tail rounded----------11

6. Total body annules 110 or more----------7

   Total body annules less than 80----------8

7. Length 0.700 mm.; vulva on 16-17th annule from terminus
   komabaensis (Imamura)

   Length 0.550-0.590 mm.; vulva on 8th annule from terminus
   morgense (Hofmanner and Menzel)

8. Total body annules 70 or more----------9

   Total body annules 65----------heideri (Stefanski)

9. Vulva located on 12-15th annule from terminus; total body annules 70-76----------10

Vulva located on 7th annule from terminus; total body annules 79
peruense (Steiner)

10. Length 0.700 mm.; first annule larger than second annule
crotaloides (Cobb)

Length 0.400-0.485 mm.; first annule smaller than second annule
demani (Micoletzky)

11. Joints on lateral line except on anterior end of body----------12

No joints on lateral line, annules unbroken except occasional anastomosis----------13

12. Lateral line zig-zag; spear 57 μ----------sphaerocephalum Taylor

Lateral line with simple breaks; spear 50 μ---------citri Steiner

13. Total body annules 115 or less; spear 48 μ or more----------14

Total body annules 142 or more; spear 38-41 μ--------parvum Raski

14. Total body annules more than 73----------15

Total body annules 60-65----------informe (Micoletzky)

15. Spear length 70-86 μ----------16

Spear length 48-67 μ----------18

16. Sublateral lobes absent----------17

Sublateral lobes present----------xenoplax Raski

17. Total body annules 106-113; length 0.338-0.420 mm.----teres Raski

Total body annules 73; length 0.532 mm.
congolense (Stekhoven and Teunissen)

18. Sublateral lobes not prominent or flattened anteriorly----------19

Sublateral lobes prominent, flattened anteriorly presenting a truncated head----------lobatum Raski

19. First annule not well set off; cuticle of larvae smooth or with delicate fringe----------20

    First annule well set off; cuticle of larvae provided with rows of spines----------*mutabile* Taylor

20. Length 0.303-0.452 mm.; head and tail not blunt—truncate (tail of *cylindricum* somewhat truncate)----------21

    Length 0.600 mm.; head and tail both blunt—truncate *rusticum* (Micoletzky)

21. Anterior flap of vulva forming 2 definite points; larvae with longitudinal culticular fringes----------*cylindricum* Raski

    Anterior flap of vulva bilobed, rounded; larvae without cuticular markings----------*curvatum* Raski

STYLET NEMATODES (TYLENCHORHYNCHUS SPP.)

The genus Tylenchorhynchus Cobb, 1913, is comprised of many species, some of which are important plant pathogens. Thorne (1949) places this genus in the subfamily Tylenchinae, of the family Tylenchidae. Nematodes of this genus are medium in size ranging from .6 to 1.7 mm in length. The cuticle is coarsely annulated and in at least one species, T. claytoni, longitudinally subdivided (Morphology section, Plate I, Fig. 4). Lateral fields are marked by four to six incisures. The esophagus is of the tylenchoid type with a well developed basal bulb connected with the intestine by a large cardia. Females have double ovaries, outstretched, with the vulva near the middle of the body. Female tail blunt, rounded. Male tail slightly arcuate and enveloped by the bursa.

At least two species, and undoubtedly there are others, have been shown to cause considerable damage to crop plants. Reynolds et. al. (1953) have demonstrated that T. dubius can cause moderate stunting of cotton under both greenhouse and field conditions. Steiner (1937) described T. claytoni from tobacco in South Carolina and at the time considered it to be an apparently rare parasite of tobacco. Subsequent to that time, Graham (1954) reports that T. claytoni was present in 67 per cent of 175 soil samples collected from fields where tobacco was stunted (1951 through 1953). It was also found in cotton and corn samples. He demonstrated its pathogencity on tobacco in both greenhouse and field trials. Effects on tobacco are stunted top growth and a much retarded root system. Graham further reports (1954) that root lesions are absent and root decay does not occur, although the roots are seriously retarded in their growth and do not elongate normally.

In North Carolina, T. claytoni is one of the most serious nematode parasites to tobacco and is widespread in the state, having been identified from 27 counties. It apparently has a wide host range, although few studies have been made to date to determine this. Aside from tobacco, high populations have been found in soil samples from cotton, corn, milo, alfalfa, strawberry, oats, soybeans, peanuts, and various ornamentals. It appears to be primarily an ectoparasite.

A monograph dealing with the taxonomy of the genus has been prepared by Allen (1955). The key to the species known to that date is copied from the monograph for inclusion with these Notes. At the present time, two additional species have been described and are not included in the monograph or key by Allen. These are as follows:

> Tylenchorhynchus martini Fielding, 1956. Resembles T. claytoni Steiner, 1937, but differs in having simple body annulations; female tail blunt with distinctive, finger-like shape, that is, having parallel outline instead of being tapered; and presence of a slight constriction setting off the lip region. Males absent.

Tylenchorhynchus lenorus Brown, 1956. T. lenorus keys to T. ornatus, according to the key (Allen, 1955), but differs from it in having a set-off lip region, a conoidobtuse tail, and fewer longitudinal striae. T. lenorus differs from T. quadrifer in having fewer longitudinal striae and from T. tessalatus in being smaller in size and having no annulations around terminus of tail.

Three other new species' descriptions have been submitted to Nematologica by Bruce E. Hopper. It is likely that additional taxonomic work will be done by others as this plant-parasitic group is investigated more fully.

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4. Graham, T. W. 1954. The tobacco stunt nematode in South Carolina. (Abs.) Phytopathology 44:332.

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6. Reynolds, Harold W. and Milton M. Evans. 1953. The stylet nematode, Tylenchorhynchus dubius, a root parasite of economic importance in the southwest. U. S. Dept. Agr. Plant Dis. Rptr. 37:540-544.

7. Steiner, G. 1937. Tylenchorhynchus claytoni, an apparently rare nemic parasite of the tobacco plant. Proc. Helminthol. Soc. Wash. 4(1):33-34.

8. Thorne, Gerald. 1949. On the classification of the Tylenchida, a new order (Nematoda, Phasmidia). Proc. Helminthol. Soc. Wash. 16(20):37-73.

KEY TO THE SPECIES OF TYLENCHORHYNCHUS
(From Allen, 1955)

Females

1. Cuticle marked by longitudinal striae----------2

   Cuticle not marked by longitudinal striae----------6

2. Lateral field marked by 4 lines----------3

   Lateral field marked by 6 lines----------4

3. Lip region bearing 3 or 4 annules----------claytoni Steiner

   Lip region bearing 6 or 7 annules----------lamelliferus (de Man)

4. Annules extending around terminus of tail
   tessellatus J. B. Goodey

   Annules not extending around terminus of tail----------5

5. About 32 longitudinal striae at middle of body------ornatus n. sp.

   About 60 longitudinal striae at middle of body-quadrifer Andrassy

6. Lateral field marked by 4 lines----------7

   Lateral field marked by 5 lines----------19

   Lateral field marked by 6 lines----------20

7. Annules extending around terminus of tail----------8

   Annules not extending around terminus of tail----------12

8. Lip region set off by constriction----------dubius (Bütschli)

   Lip region continuous with body contour----------9

9. Lip region bearing 4 or 5 annules; tail tapering conoid
   eremicolus n. sp.

Lip region bearing 6 or 7 annules; tail cylindrical, bluntly rounded----------10

10. Lip sclerotization heavy, conspicuous---------*magnicauda* (Thorne)

Lip sclerotization faint, inconspicuous----------11

11. Tail less than 3 x anal body diameter----------*parvus* n. sp.

Tail more than 3 x anal body diameter----------*maximus* n. sp.

12. Lip region continuous with body contour----------13

Lip region set off by constriction or depression----------18

13. Lip region bearing 2 annules----------*nudus* n. sp.

Lip bearing more than 2 annules----------14

14. Lip sclerotization faint, inconspicuous----------15

Lip sclerotization strong, conspicuous----------16

15. Tail bearing 10 to 15 annules----------*clarus* n. sp.

Tail bearing 20 to 27 annules----------*striatus* n. sp.

16. Spear less than 20 μ long----------*manubriatus* Litvinova

Spear more than 20 μ long----------17

17. Spear not more than 31 μ long, tail bearing 50 annules
kegenicus Litvinova

Spear more than 31 μ long, tail bearing 45 annules
galeatus Litvinova

18. Spear more than 20 μ long, lips rounded, sclerotization conspicuous----------*cylindricus* Cobb

Spear less than 20 μ long, lips broadly rounded, sclerotization faint----------*latus* n. sp.

19. Tail about 2 x anal body diameter, bearing less than 25 annules
acutus n. sp.

Tail about 3 x anal body diameter, bearing more than 25 annules
capitatus n. sp.

20. Annules extending around terminus of tail----------21

Annules not extending around terminus of tail----------25

21. Lip region set off by constriction or depression-----leptus n. sp.

Lip region not set off by constriction or depression----------22

22. Lip sclerotization well developed, conspicuous--macrurus (Goodey)

Lip sclerotization faint, inconspicuous----------23

23. Spear more than 23 μ long----------obscurus n. sp.

Spear less than 22 μ long----------24

24. Spear more than 15 μ long, tail less than 3 x anal body diameter
nothus n. sp.

Spear not more than 15 u long, tail more than 3 x anal body diameter----------nanus n. sp.

25. Lip region continuous with body contour----------26

Lip region set off by constriction or depression----------28

26. Tail short, conoid, terminus pointed-----brachycephalus Litvinova

Tail subcylindrical, bluntly rounded----------27

27. Spear less than 20 μ long----------brevidens n. sp.

Spear more than 20 μ long----------affinis n. sp.

28. Lip sclerotization heavy, conspicuous----------29

Lip sclerotization faint, inconspicuous----------31

29. Tail more than 3 x anal body diameter----------*alpinus* n. sp.

    Tail less than 3 x anal body diameter----------30

30. Spear more than 35 μ long----------*macrodens* n. sp.

    Spear less than 35 μ long----------*grandis* n. sp.

31. Spear less than 35 μ long----------*lineatus* n. sp.

    Spear more than 35 μ long----------32

32. Spear more than 55 μ long, tail less than 2 x anal diameter *superbus* n. sp.

    Spear less than 55 μ long, tail more than 2 x anal diameter *conicus* n. sp.

STUBBY-ROOT NEMATODES (TRICHODORUS SPP.)

"Stubby-root" and "stubby-root nematode" have been suggested as common names for the disease and the causal organism, respectively, by Christie and Perry (1951). Since this report of Trichodorus being the causative agent of diseases of various crop plants in Florida, considerable attention has been given to the genus. Now that a monograph of the genus has been prepared by Allen (1957) progress can be expected in the study of this interesting plant-parasitic genus. The Taxonomic key from the monograph is included for use in these Notes.

These nematodes are not in the Class Phasmidia as have been the previously mentioned genera; rather, they are in the Class Aphasmida, Dorylaimoidea. The species are small (0.5 to 1.5 mm. long), thick-bodied, cylindrical nematodes, tapering at the anterior end. Fixed specimens often appear as though they had a swollen condition of the cuticle or as though they had retained the last molted cuticle. Allen (1957), who made studies of cross sections of these nematodes, points out that the spear or stylet should be referred to as a dorsally located tooth. This onchiostyle is hollow but not throughout its entire length; and, although it may be used for puncturing cells, the actual feeding is by a somewhat different mechanism than in nematodes having a hollow axial stylet.

These nematodes have been found associated with the roots of a diversity of plants and from many different localities. Perhaps, primarily external feeders, thorough examination of the soil rather than of the roots may be necessary for finding these nematodes. Allen (1957) suggests that it may be preferable to confine the use of the name "stubby-root nematode" to Trichodorus christiei, since no other species of the genus is known to produce the type of symptoms that are associated with the feeding of this species. T. christiei appears at the present to be the most widely distributed species of this genus in the United States and is reported from the root zones of quite a number of economic and ornamental plants. Old host listings or reports of T. primitivus prior to 1957 should be used with caution because, until that time, only this one species had been described.

Literature Cited

Allen, M. W. 1957. A review of the nematode genus Trichodorus with descriptions of ten new species. Nematologica 2(1):32-62.

Christie, J. R. and V. G. Perry. 1951. A root disease of plants caused by a nematode of the genus Trichodorus. Science 113:491-493.

KEY TO THE SPECIES OF TRICHODORUS
(From Allen, 1957)

Males and Females

1. Females with one ovary (monodelphic)----------monohystera n. sp.

   Females with two ovaries (amphidelphic)----------2

2. Spear more than 130 microns long----------elegans n. sp.

   Spear less than 110 microns long----------3

3. Excretory pore posterior to base of the esophagus, spear less than 30 microns long, males not known----------nanus n. sp.

   Excretory pore anterior to end of esophagus, spear more than 30 microns long, males known----------4

4. Females with ventro-median or ventro-submedian pores near vulva, males with 2 or 3 supplementary papillae----------5

   Females without ventro-median or ventro-submedian pores near vulva, males with one or 3 supplementary papillae----------6

5. Females with ventro-median pores, 2 anterior and 2 posterior to the vulva, male with caudal alae, 2 supplementary papillae
   porosus n. sp.

   Females with 2 ventro-submedian pores posterior to the vulva, males with caudal alae, 3 supplementary papillae
   atlanticus n. sp.

6. Females without caudal pores or lateral hypodermal pores, males with a single supplementary papilla, caudal alae present
   christiei n. sp.

   Females with caudal pores and lateral hypodermal pores, males with or without caudal alae, more than one ventral supplement------7

7. Females with three lateral hypodermal pores posterior to vulva, males with caudal alae and three ventral supplements
   pachydermus Seinhorst

   Females with one lateral hypodermal pore posterior to vulva, males without caudal alae----------8

8. Females with three lateral hypodermal pores, 2 anterior and one posterior to vulva, males with 3 or 4 ventro-median papillae anterior to excretory pore----------primitivus (de Man)

   Females with not more than two lateral hypodermal pores, males with 0-2 ventro-median papillae anterior to excretory pore---------9

9. Females with one lateral hypodermal pore posterior and one anterior to vulva, 1st supplement in male about opposite proximal end of spicules----------10

   Females with one lateral hypodermal pore at level of vulva, males with 1st supplement near distal end of spicules----------11

10. Excretory pore in female slightly posterior to middle of esophagus, males with 2 ventro-median esophageal papillae anterior to the excretory pore----------aequalis n. sp.

    Excretory pore in female near base of esophagus, male with one ventro-median papilla anterior to excretory pore proximus n. sp.

11. Male with one ventro-median papilla anterior to excretory pore----------californicus n. sp.

    Male without ventro-median esophageal papilla-----obscurus n. sp.

DAGGER NEMATODES (XIPHINEMA SPP.)

The genus Xiphinema Cobb, 1913, is comprised of several species and is a member of the subfamily Longidorinae and family Dorylaimidae of the Class Aphasmidia.

The status of this genus as a plant-parasitic group has been recently reviewed by Schindler (1957 a), who has done work demonstrating the pathogenicity of X. diversicaudatum (Micoletzky, 1927) Thorne, 1939, on various hosts (Schindler, 1954, 1957; Schindler and Braun, 1957). X. americanum is a form commonly encountered in the southeastern United States. It is found, sometimes in large numbers, in soil from such crops as tobacco, cotton, oats, vetch, peach, boxwood, and azaleas. This species is believed to also be an ectoparasite of roots and to cause damage under certain conditions. Although not yet demonstrated under controlled conditions to be pathogenic, various species have been found under circumstances strongly indicating the likelihood of a pathogenic role. Biennial and perennial plants would appear to be hosts most likely to show eventual damage because of large numbers of the nematodes are required to produce appreciable damage.

Galling of rose, peanut, and strawberry root tips has been reported (Schindler, 1954) for X. diversicaudatum and is characteristic enough on these hosts to be given the name of "curly-tip." Characteristically, an enlargement of the tip and a curling of the end of the root is produced with apparent necrosis and shriveling of the proximal portion. These galls are somewhat similar to those caused by root-knot nematodes but differ in that they almost invariably involve the distal portion of the root. There are some other aspects concerning the pathogenicity of X. diversicaudatum which Schindler, 1957, points out as being worthy of further investigation.

A steadily growing list of species exists for this genus; forms being reported from various countries and in association with various kinds of plants. There are two recent taxonomic studies of the group (Lordello, 1955, and Luc, 1958). Keys from both of these papers are taken for inclusion in the Notes because the later paper by Luc does not contain all the forms contained in the paper by Lordello. Another important earlier paper containing illustrations of species of Xiphinema known up to that date is the monograph by Thorne (1939).

Literature Cited

Lordello, L. G. E. 1955. Xiphinema krugi n. sp. (Nematoda, Dorylaimidae) from Brazil with a key to the species of Xiphinema. Proc. Helminth. Soc. Wash. 22(1):16-21.

Luc, M. 1958. Xiphinema de l'ouest Africain: Description de cinq nouvelles especes (Nematoda:Dorylaimidae). Nematologica 3(1): 57-72.

Schindler, A. F. 1954. Root galling associated with the dagger nematode, Xiphinema diversicaudatum (Micoletzky, 1927) Thorne, 1939. (Abs.) Phytopathology 44:389.

Schindler, A. F. 1957. Parasitism and pathogenicity of Xiphinema diversicaudatum, an ectoparasitic nematode. Nematologica 2(1): 25-31.

Schindler, A. F. and A. J. Braun. 1957. Pathogenicity of an ectoparasitic nematode, Xiphinema diversicaudatum, on strawberries. Nematologica 2(1):91-93.

Thorne, G. 1939. A monograph of the nematodes of the superfamily Dorylainoidea. Capita Zool. Vol. 8, Part 5, 261 pp.

KEY TO THE SPECIES OF THE GENUS XIPHINEMA COBB, 1913
(From Lordello, 1955)

As several new forms of "dagger nematodes" have been described since the publication of Thorne's monograph (1939), the writer organized the following key for the separation of the species known to date. Only female characters were used in the key, as the Xiphinema males are frequently rare and unknown in some species. Unfortunately, it was not possible to place in the key the species described by Schuurmans Stekhoven in 1951 (X. brevicaudatum, X. effilatum, and X. digiticaudatum) as they were based on larvae.

In previous publications, the writer emended the names of three species of the genus, because they were established either by a printing error or a lapsus calami. The writer's procedure was based on the 19th article of the International Rules of Zoological Nomenclature (apud Amaral, 1950). These three forms are: X. grande Steiner, 1914; X. pratense Loos, 1949; and X. insigne Loos, 1949; formerly referred to as X. grandis, X. pratensis, and X. insignis.)

1. Ovary one----------2

   Ovaries two----------5

2. Tail rounded and short-----X. ensiculiferum (Cobb, 1893) Thorne, 1939

   Tail elongated, conoid or digitate----------3

3. Two pairs of caudal papillae present----------4

   Three pairs of caudal papillae present---X. radicicola Goodey, 1936

4. Tail elongate, amphids narrow and long----X. chambersi Thorne, 1939

   Tail distinctly digitate, amphids wide and short
   X. brasiliense Lordello, 1951

5. Tail short and rounded----------6

   Tail elongate, conoid, subconoid or digitate----------9

6. Lip region set off by constriction----------7

   Lip region continuous----------8

7. Small species (1.616 mm.), V = 40.0%, oesophagus short (b = 6)
X. grande Steiner, 1914

Large species (4.0 mm.), V = 60.0%, oesophagus very long (b = 2)
X. makrodorum (Vanha, 1893) Thorne, 1939

8. Small species (0.8 mm.)----------X. obtusum Cobb, 1939

Large species (4.1 mm.)
X. rotundatum Sch. Stekhoven & Teunissen, 1938

9. Lip region expanded----------X. lineum (Grube, 1849) Thorne, 1937

Lip region not expanded----------10

10. Head truncate----------X. truncatum Thorne, 1939

Head more or less rounded, not truncate----------11

11. Length 3.4-4.0 mm. or much larger (8.94 mm.)----------12

Length 2.60 mm. or less----------14

12. V = 38.9%----------X. index Thorne & Allen, 1950

V = 47.8-48.0%----------13

13. Length 8.94 mm.
X. cylindricaudatum Sch. Stekhoven & Teunissen, 1938

Length .40 mm.----X. diversicaudatum (Micoletzky, 1927) Thorne, 1939

14. V = 51.0-54.0%----------X. americanum Cobb, 1913

V = 29.8-47.0%----------15

15. Tail subconoid or digitate, not ventrally arcuate----------16

Tail distinctly elongated, more or less deeply ventrally arcuated-17

16. Tail distinctly digitate (55.5 μ long), V = 39.4%
X. mammillatum Sch. Stekhoven & Teunissen, 1938

Tail not digitate, subconoid (32 u long), V = 33.4-34.2%
X. krugi n. sp.

17. Four pairs of caudal papillae present--*X. campinense* Lordello, 1951

    More than 4 pairs of caudal papillae present----------18

18. Combined length of spear and spear extension shorter (123.4 µ)
X. italiae Meyl, 1953

    Combined length of spear and spear extension longer (142.0-158.0 µ)----------19

19. Six pairs of caudal papillae present, spear apparently consisting of two parts--*X. elongatum* Sch. Stekhoven & Teunissen, 1938

    Seven pairs of caudal papillae present, spear consisting of only one part, as is usual----------20

20. Tail long (77.0-102.0 µ), V = 29.8-31.6%, with a cuticular triangular-shaped structure in the anterior slender part of the oesophagus----------*X. insigne* Loos, 1949

    Tail short (48.0-57.0 µ), V = 39.0-42.0%, without such a triangular-shaped structure in the anterior portion of the oesophagus----------*X. pratense* Loos, 1949

KEY TO THE SPECIES OF XIPHINEMA COBB, 1913
(After Luc, 1958)

Females

1. One ovary----------2

   Two ovaries (the anterior one reduced and obscure in X. krugi)------5

2. Tail long (more than four times body width at anus)
   X. chambersi Thorne, 1939

   Tail short (less than four times body width at anus)----------3

3. Tail hemispherical-----X. ensiculiferum (Cobb, 1893) Thorne, 1939

   Tail mammillate (digitate)----------4

4. Two pairs of caudal papillae--------X. brasiliense Lordello, 1951

   Three pairs of caudal papillae---------X. radicicola Goodey, 1936

5. Tail long (more than four times body width ar anus)----------6

   Tail short (less than four times body width at anus)----------7

6. V = 29-32%; seven pairs of caudal papillae--X. insigne Loos, 1949

   V = 47%; three to five pairs of caudal papillae--X. hallei n. sp.

7. Tail hemispherical or mammillate (digitate)----------8

   Tail conical----------13

8. Tail hemispherical----------9

   Tail mammillate (digitate)----------10

9. L = 0.8 mm.; lip region not set off by constriction, i. e. continuous----------X. obtusum Thorne, 1939

   L = 3 mm.; lip region set off by distinct constriction
   X. yaoense n. sp.

10. Lips distended, separated one from another
X. mamillatum Schuurmans Stekhoven & Teunissen, 1938

Lips smooth, united----------11

11. Lips flattened; lip region not set off by a contriction
X. index Thorne & Allen, 1950

Lips rounded; lip region set off by distinct constriction---------12

12. L = 2 mm.; "organ Z"* in the females----------*X. ebriense* n. sp.

L = 3-4 mm.; without "organ Z" in the females
X. diversicaudatum (Micholetzky, 1927) Thorne, 1939

13. L = at least 8 mm.
X. cylindricaudatum Schuurmans Steckhoven & Teunissen, 1958

L = at most 3 mm.----------14

14. V = 54%----------*X. americanum* Cobb, 1913

V = at most 47%----------15

15. Length of tail one to one and one-half body with at anus; anterior ovary reduced----------*X. krugi* Lordello, 1955

Length of tail longer than twice body width at anus; anterior ovary normal----------16

16. Head truncate----------*X. truncatum* Thorne, 1939

Head rounded----------17

*"Organ Z" in the females has reference to a structure seen in the genital tract of *X. ebriense*. This organ lies between the spermatheca and the uterus. It is golbular in shape; very muscular; the lumen, which opens wider in the center of the bulb, is bounded by a cuticularized lining and possesses two to four moveable cuticularzied "teeth." Generally, three "teeth" are seen, but it is difficult to distinguish the exact number because of the thickness of the musculature of the organ. Because of the unknown function of this organ M. Luc proposed the name "organ Z."

17. Two-three pairs of caudal papillae----------18

    At least four pairs of caudal papillae----------20

18. Two pairs of caudal papillae----------19

    Three pairs of caudal papillae----------*X. pratense* Loos, 1949

19. A = 50-55; ventral pores----------*X. setariae* n. sp.

    A = 70; without ventral pores----------*X. parasetariae* n. sp.

20. Four pairs of caudal papillae--------*X. campinense* Lordello, 1951

    More than four pairs of caudal papillae----------21

21. V = 40-42%; six pairs of caudal papillae
    X. elongatum Schuurmans Steckhoven & Teunissen, 1938

    V = 43-47%----------*X. italie* Meyl, 1953

IDENTIFICATION AND BIOLOGY OF FREE-LIVING NEMATODES

Free-living nematodes, as the term will be used here, are defined as nematodes found in soil, in decaying plant tissue, and in similar habitats, but which do not feed on living plants. Discussion is limited to the most common genera.

Free-living nematodes are found in all agricultural soils without exception, and also are found in other locations where food is available, as in manure piles, compost heaps, and other places where access can be had to dead plant or animal material. While not quite so ubiquitous as bacteria or fungi, they can still be found in many odd places where any sort of food has been present for a considerable time. This includes some very strange habitats indeed; one of the oddest being in the felt mats which were used in German taverns for parking beer steins between drinks. Another form is found in paperhangers' paste; another in pitchers of pitcher plants; and another in vinegar, etc. Occasionally they may be something of a pest. In a shipment originating in Argentina, Dr. Steiner found nematode bodies in great abundance in canned tomato paste. Undoubtedly, they also occur in other food processing operations where the sanitation is not all that might be desired.

In agricultural soils, free-living nematodes are often very numerous, counts of from several to hundreds per gram of soil not being unusual. For the most part, little is known of food habits. However, it is a fair assumption, with some evidence to support it, that many live on bacteria. Others are predators, feeding by killing other nematodes. Possibly others may feed on dead plants directly, though the evidence for this is scanty.

The nematologist working on agricultural problems will encounter free-living forms in the soil, in decaying plant tissues, and at times in apparently healthy plant tissues. Nearly every sample of alfalfa stems and leaves collected on a field trip in Virginia and examined for stem nematodes had a number of specimens of Panagrellus.

With practice, it is easy enough to identify free-living nematodes to genus, but identification to species is difficult or often impossible.

For the Cephalobidae, an aid to identification is Thorne's paper, "A Revision of the Nematode Family Cephalobidae, Chitwood and Chitwood, 1934." (Proc. Helminthological Soc. Washington. 4(1): 1-16 of 1937).

Genera of the Aphasmidia most common in soil samples are Plectus, Mononchus (and subgenera) and various Dorylaimina. The Dorylaimina include a large number of genera which are best separated by the use of "A Monograph of the Nematodes of the Superfamily Dorylaimoidea," by G. Thorne (Capita Zoologica 8(5): 1-261 of 1939). "A Monograph of the Nematode Genera Dorylaimus Dujardin, Aporcelaimus n.g., Dorylaimoides

n.g., and Pungentus n.g." (Ibid, 6(4):1-223 of 1936) is indispensible for identification of Dorylaims to species. Fortunately, these two large works which had been out of print can now be obtained again. (Martinus Nijhoff, Publisher, Post Office Box #269, The Hague, Netherlands, or through other booksellers. Price at last listing (May, 1959) was 38 guilders each (approx. $10.10).)

Though Dorylaims are often found in large numbers around the roots of plants and many have stylets which would seem to be well adapted for feeding on plants, there is little or no evidence that they are ever serious plant parasites, with the exceptions of species of the genera Xiphinema, Trichodorus, and Longidorus. Except for these genera, one seldom finds large numbers of a single species of Dorylaims around the roots of plants, as would be expected if they are dangerous plant parasites. Some are believed to be predators, feeding on nematodes and other small soil animals.

Mononchus species are easy to recognize, since they have a cylindrical oesophagus and a somewhat globular mouth cavity, usually with one or more teeth. Mononchus species are predatory.

CHEMICAL CONTROL OF NEMATODES

Historical

Almost as soon as it was realized that nematodes in the soil were capable of damaging crop plants, attempts were made to kill them by means of chemicals. The literature contains a long list of substances which have been tested for this purpose. As early as 1881, Kühn attempted to kill nematodes with carbon bisulphide, though without conspicuous success. Carbon bisulphide was also recommended by Bessey (1911) for killing nematodes, his methods being copied from those used for control of Phylloxera, an insect pest of grapes, in France. The method was essentially that used today; application of measured amounts in holes punched at intervals in the soil. Shortly after the first World War, chloropicrin was tested for nematode control in England with good results. About ten years later, Johnson and Godfrey again tested chloropicrin and developed methods and machinery for field application in pineapple fields in Hawaii. By 1937, this work was sufficiently advanced so that Innis, Speiden & Co. started commercial development of chloropicrin, finding most of their customers among greenhouse owners. About 1940, methyl bromide was found to be a good nematocide and shortly thereafter, Dow Chemical Company entered the nematocide business, again finding most of their customers among greenhouse owners.

During the World War II years, the value of a mixture of dichloropropane (D-D) was tested by Carter in Hawaii and ethylene dibromide (EDB) was tested by the Dow Chemical Company, with good success in both cases. These being much less expensive than chloropicrin and methyl bromide, they could be used on a large scale. Intensive efforts to develop a market for them started in 1946. In 1950, Shell Chemical Corporation started development of Chlorobromopropene (CBP). This company soon followed this with the development of another chemical first referred to as OS1897, now trademarked Nemagon, and chemically designated as 1,2-dibromo-3-chloropropane.

The Virginia-Carolina Chemical Corporation, at about this same time, released a commercially available chemical called V-C13 Nemacide, the active ingredient of which is O-2-4-dichlorophenyl O-O-diethyl phosphorothioate. This was sold as a water emulsifiable form to be applied to the soil as a pre- or post-planting treatment and was one of the first of the new nematocides to be recommended for treatment of soil around living plants.

About 1955, an experimental soil fumigant, formerly called compound N-869, was released for commercial use as Vapam, a liquid manufactured by the Stauffer Chemical Company. Its active ingredient, sodium methyl dithiocarbamate, is lethal not only to nematodes but to various soil fungi and insects and weeds. Thus, it is considered as a temporary soil sterilant.

Another recent nematocide, also in the class of temporary soil sterilants and commercially available, is Mylone 85W under the Crag brand of the Carbide and Carbon Chemicals Company, a Division of the Union Carbide and Carbon Corporation. Mylone is marketed as a dust or as a wettable powder and is identified chemically as 3,5-dimethyltetrahydro-1,3-5,2H thiadiazine-2-thione.

Some other recent developments have been initiated by the Dow Chemical Company. The introduction to commercial use in 1957 of Telone, the trademark name for a liquid fumigant composed of undiluted technical dichloropropenes and containing no bromine materials. Also, there was commercial use at this same time of Dorlone, the trademark name for a mixture of two nematocides (Telone 75.2% and Dowfume W-85 (EDB) 24.8% by weight). This was in response to the observations that under various soil and environmental conditions the present day soil fumigants exhibit varying degrees of effectiveness in controlling different species of plant-parasitic nematodes. At the present time, several other promising chemicals are under test, but have not been placed on the market yet.

Thus, commercial soil fumigation in the United States began in 1937, and the greater part of the development to its present status as a multi-million dollor business has taken place since 1945. The gradual development of new materials, permitting new ways of application, broader lethalness for use as temporary soil sterilants or limited phytotoxicity for use on and around living plants, is greatly expanding the usefulness of chemicals for nematode control.

Basic Principles

Most nematocides are used for control of nematodes in soil before planting, and this situation will be discussed first. Since plant-parasitic nematodes feed only on living plants, they must be killed by "contact" rather than "stomach" poisons. Also, movements of nematodes in soil are slow and limited in extent, which means that the poison must be distributed throughout the soil. Where annual crops have been grown, the nematodes will be in the upper 15 to 18 inches of soil, with the greater part of the population in the upper 12 inches. Nematodes in the soil may be in the form of eggs, larvae, or adults; but, in any case, will be surrounded by a thin film of water. In certain cases, they may be further protected as cysts (Heterodera), or the gelatinous medium of egg masses (Meloidogyne), or may be in more or less decomposed plant material.

Nematodes have a cuticle which is of a lipoidal nature and impermeable to many substances.

When these various facts are considered, it is evident that the requirements for a soil nematocide are: 1) It must thoroughly permeate or be mixed with the soil at least as deep as 12 inches and perhaps as deep as 18 inches. 2) It must be able to penetrate the various barriers surrounding the nematodes, cysts, plant material, or at the very least,

the film of water and the nematode cuticle. 3) It must kill or at least disable the nematodes. 4) Since the ultimate object of treating the soil is the growing of crop plants, no residue toxic to plants must be left in the soil after a reasonable waiting period.

The first requirement is best met by gases. These are difficult to confine in large scale work, though this can be done on a small scale. The next most favorable material is vapors or fumes of volatile liquids. If very large amounts of water are available, it is possible to distribute a water soluble chemical through the upper layers of the soil. Thorough mixing of a solid (powder or granule) with the soil is possible only on a small scale or to a depth of not more than 6 or 8 inches with ordinary farm machinery.

The water film around the nematode will be penetrated by any substance even slightly soluble in water. The lipoidal cuticle is best penetrated by fat solvents.

The fourth requirement (lack of phytotoxicity) may be inherent in the nematocide used. However, if the substance is at all toxic, it must either: 1) be dissipated into the atmosphere; 2) decompose, forming harmless compounds; 3) be leached out of the soil.

Materials which are gases at ordinary temperatures must be confined by an impervious cover over the soil. Methyl bromide (boiling point 4° C) is used in this way for seedbeds and other comparatively small areas.

The most successful nematocides are volatile materials. They may be applied as solutions, emulsions, powders, or granules, but the active ingredient spreads in the soil as a volatile agent. Distributed at intervals through the soil, the chemicals diffuse evenly in all directions—downward, laterally, and upward. As they diffuse, the vapors pass over soil particles and soil organisms, each surrounded by a thin film of water. As conditions are ideal for solution of the chemical vapors in the water, they make a nearly saturated solution until equilibrium is reached.

It is evident that this simple and automatic process is superior to anything that can be done with a non-volatile nematocide. It would be difficult to get as thorough a distribution of such a chemical even if it were water soluble. Obviously, the mixing of a non-volatile solid nematocide as thoroughly in the soil would be far more laborious, even if machinery capable of doing such a job were available.

Procedure in application of liquid and solid-carrier soil fumigants is designed to make the best possible use of the fumes. For large scale work, tractor applicators which deliver the fumigant in continuous streams spaced at intervals in the soil are used. Generally, lines of fumigant are spaced at about 12-inch intervals, though 10-inch intervals are used in some soils.

In experimental work, spacing and application rates are varied syste-

matically to determine the most favorable combination. In ordinary farm practice, manufacturers' directions are followed.

To date, no one has devised a method which is demonstrably better than the one described. There is, for example, no evidence that distributing the fumigant over a plane several inches wide (spraying over the whole width of a flow furrow, for example) is any better than simply dribbling the fumigant in lines. And most certainly, emulsions, powders, and granules applied to the soil surface are wasteful, since some fumes are lost into the atmosphere. In addition, the great bulk of the chemical is likely to be retained in the upper few inches of the soil where the fumes are too readily dissipated. Instructions regarding application of sufficient quantities of water to obtain adequate leaching of the chemicals, and recommendations to working solid forms into the soil must be carefully followed.

At best, soil fumigants are still expensive and the usual reason for the commercial grower to use them is to increase profits from the projected crop. The profits may come as a result of increased yield, better quality, in savings on some other operation, or from a combination of all three. The tobacco farmer gets more pounds per acre and is content if quality is not appreciably reduced. The sweet potato grower may increase both yield and quality. The carrot grower eliminates culls. The celery grower who fumigates his seedbeds with methyl bromide saves expensive hand weeding. But whatever the advantage, it can be reduced to a dollars and cents basis so that the cost of fumigating an acre can be balanced against returns due to fumigation.

It is this comparison which is of interest to the practical farmer, rather than a comparison of nematode control in treated and untreated areas, or one based on general appearance of the field. Thus, the optimum application of soil fumigant is that which produces the greatest cashreturn for each dollar expended for fumigation. Considered from the purely monetary angle, row treatments may be better than solid treatment and a light application better than a heavy one under certain conditions. A fact which has become apparent as soil fumigation has progressed is that it is not necessary to kill all of the nematodes to make a very great difference in the crop. In many cases, all that is necessary is to kill enough nematodes so that the plants are not subjected to an overwhelming attack in the early stages of growth when they are most vulnerable.

It should be recognized that the considerations and techniques may be different for certain special commercial growing enterprises, such as where bienniel and perennial plants are concerned and for the home gardener determined to grow good home garden and ornamental plants. The point is, due consideration should be given to the intentions and needs of the grower, particularly when the cost factor is involved.

In addition to control of nematodes in soil before the crop is planted, there are several other situations where nematocides can or might be used.

One of the most important is for control of ectoparasites or endoparasites in or around the roots of living plants, particularly perennials, such as orchard trees and ornamentals. Two of the presently available nematocides may be generally recommended for this purpose (1,2-dibromo-3-chloropropane and V-C13 Nemacide). Most of the nematocides currently available are more or less toxic to plants and might do more harm than good. There are some recent experiments which point out that even with a phytoxic material (D-D) a dosage applied to one side of a tree one year and the other side the following year can be an overall beneficial treatment. However, a degree of balance between plant injury and nematode control must be obtained. This kind of application is rather an exception, not the general rule. An intensive search is being made for nematocides which are less toxic to plants.

There is also a specialized but important need for nematocides which will kill nematodes on bare-rooted plants to be transplanted, but at this time no material is being generally recommended for this purpose. Search is also being made for suitable chemicals for this purpose, because of the hazard of nematode transport in infected plant stocks.

It has been shown that foliar nematodes in chrysanthemums and certain other plants can be killed by nematocides which apparently have a systemic action. One of these is sodium selenate, which is applied to the soil. However, it should be remembered that selenium taken up by edible plants can be very poisonous to man and to domestic animals. Where selenium occurs naturally in soils, serious trouble with livestock has been encountered. Selenium, once added to the soil, might remain for a long time, so its use is discouraged. It has also been shown that repeated spraying with Parathion will control foliar nematodes on chrysanthemums and other plants. Amounts used are 0.25 to 0.50 lbs. active ingredient (as wettable powder) to 100 gallons of water, and control is obtained by 4 or more applications by spraying at weekly intervals.

White tip of rice is caused by Aphelenchoides oryzae carried on the seed. Control can be obtained by seed treatment with several compounds, the most satisfactory of which seems to be 3-p-chlorophenyl-5-methyl rhodanine (N-244, made by Stauffer Chemical Company).

Space fumigation is also mentioned because it has been used for many years generally for disinfecting non-living materials. The generally used chemical is methyl bromide which, although highly phytoxic, can be safely used for a few special problems involving nematodes on living plant materials. Examples are fumigation of onion and clover seed infested with the stem or bulb nematode.

Materials and Methods

Selection of a nematocide for any particular purpose involves consideration of the area to be fumigated, the crop to be grown, and other factors, including economic ones.

In general, D-D, Telone, EDB, or 1,2-dibromo-3-chloropropane will be used for large scale work on soils to be planted to crops of fairly high value. Seedbeds or other small areas will be fumigated with methyl bromide, chloropicrin, Vapam, or Mylone where the broader lethal spectrum of a soil sterilant is desired. In greenhouses, methylbromide or chloropicrin will be used, though use of the latter should be avoided in greenhouses where plants are growing, since small concentrations in the air are highly poisonous to plants.

In case of doubt, it is well to inquire of the manufacturer before using nematocides. If informed of the exact circumstances possible hazards to avoid will be pointed out. Exact descriptions of methods are also best obtained from the literature of manufacturers of soil fumigants and applicators. Discussion here will be confined to generalities.

Improvised equipment can be used for small jobs. Perhaps the easiest way is to use a fruit jar with two nail holes punched in the lid, one for the fumigant to run out, the other to admit air. The fumigant is poured from the jar into an open furrow which is promptly covered. Dosage is adjusted by trial and error, changing size of nail hole until the proper amount is delivered at a steady walking pace. The availability now of water soluble materials, emulsifiable formulations, wettable powders, and impregnated granular carriers open up a wide range of simple methods of application of the chemicals. The chart on the following page illustrates various methods suitable for treating small areas of soil.

If commercial fumigation of small areas is a job which has to be done often, as in a greenhouse or nursery, a hand applicator or injector may be a good investment. Where topical applications may be preferred, a rotory tillage device is recommended and can be rigged for distribution of the chemicals at the time of tilling them thoroughly into the soil.

For work involving greater area, it is usually best to use some sort of continuous flow applicator. These can be made in any size desired, from a single shank on a garden tractor to six or eight shanks on a regular farm tractor. Kits of parts to make a machine for delivery of liquids are readily available, and regular distributor devices for granular insecticides and fertilizers are available, or such equipment on hand may be adapted. No matter what the size of the machine, it must be capable of delivering accurately measured amounts of the chemical and placing them usually at a depth of at least six to eight inches.

In all fumigation jobs, except for experimental work, the manufacturers' directions should be followed exactly. Read the label and any circular you can get. Observe all safety precautions. It is also an excellent idea to observe procedures used by others in your area.

Effect of Soil Nematocides

The effect of soil nematocides depends on the kind and amount of chemical used. There are now available materials with different ranges of toxicity to living organisms. Materials with a broad spectrum of toxicity are conveniently termed soil sterilants. For example, methyl bromide at one pound or more per 100 square feet does a fairly effective sterilization job in the upper 12 inches or so of soil; killing weed seeds, many bacteria, and fungi as well as the nematodes and soil insects. Chloropicrin at high rates (400-500 pounds per acre) has a similar effect. Vapam and Mylone are sold as being able to control weeds and fungi in addition to nematodes. The other nematocides are intended principally for control of nematodes and do not have as wide a spectrum of toxicity as the soil sterilants, and these materials fall into two groups depending on whether or not the chemical is toxic to plants. This is a very important distinction because phytotoxic nematocides require a waiting period before planting to permit the chemical to be lost from the soil or to be changed to a non-phytotoxic form.

Two of the most widely-used nematocides are fumigants requiring a waiting period before planting. These are ethylene dibromide (EDB) and some form of dichloropropane-dichloropropene (D-D and Telone) or a mixture of both (Dorlone). With the standard application of D-D (20 gallons per acre) or EDB (54 pounds of active ingredient per acre), the usual result is the killing of 80 to 95% of the nematodes. Effect on weed seeds, bacteria, and fungi seems to be small, though not entirely absent. That there is some effect on bacteria is indicated by reports that the proportion of ammonia nitrogen to nitrate nitrogen remains high for some weeks after fumigation, presumably due to destruction of some bacteria.

Less is known as yet about the toxicity spectrum of the two generally available nematocides reported to be non-toxic to some plants and having low or moderate toxicity to many others. These chemicals are 1,2-dibromo-3-chloropropane (Nemagon, Fumazone and V-C13 Nemacide).

If the soil was heavily infested with nematodes, elimination of a large proportion of the population permits increased root growth with a consequent improvement in vigor of the plants. There is often a noticeable increase in uniformity of growth over the field. Yield increases of 25% to 50% or more, often with improvement in quality, are obtained.

However, the increased root growth of the plants provides nearly ideal conditions for increase of the remaining nematodes, and the final result may be that there are more nematodes at the end of the season than there were at the start. Thus the effect of soil fumigation lasts only for one season, as a rule, though if conditions are particularly favorable, some effect may be seen the next season.

The Economics of Soil Fumigation

At present prices, cost of fumigating an acre of soil with the usual amount of D-D or EDB is about 35 dollars, including an allowance for application. The prudent farmer sees little point in making this expenditure unless he has a fair prospect of returns which will amount to about four times the investment. In other words, the value of the crop, over and above that which would have been obtained without fumigation, must be four times 35 dollars, or about 140 dollars. This is not an excessive demand, but about the same sort of return the farmer expects from fertilizer, etc.

For example, the tobacco farmer producing 1,200 lbs. of tobacco per acre without fumigation will sell his crop for an average of 55 cents per pound, or a total of 660 dollars. By fumigating his soil he can increase his crop 25% to a total of 1,500 lbs., and sell it for 825 dollars, an increase of 165 dollars, which is more than four times his investment in fumigation.

The peanut farmer who grows 1,200 lbs. of peanuts per acre and sells them for 12 cents a pound, or 144 dollars, would need to increase his crop nearly 100% to realize four times his investment. Fumigation of peanut soil with prospects of no more than 25% increase would be a very poor investment.

For purposes of rough calculation, the prospective selling price of the crop from fumigated soil should be about 16 times the cost of fumigation if an average increase of 25% in crop value can be expected. With fumigation at 35 dollars per acre, this works out to a total crop value of 560 dollars per acre.

In calculating total crop values, an allowance can sometimes be made for an increase in average quality as well as for increase in yield.

About the same ratio between fumigation cost and total value of the crop applies equally to the less expensive row fumigation. If row fumigation can be done for 10 dollars per acre, it will be a good investment on crops selling for 160 dollars per acre.

Another cost cutting possibility being explored is to spread the cost of a single soil treatment by getting the benefits of nematode control extended to two different crops planted in succession on the treated land. Experiments indicate this is feasible.

The experiment station worker or soil fumigant salesman is often asked whether or not a particular field should be fumigated. In answering this question, the first consideration should be the value of the crop to be planted. Knowing approximate yields and selling prices for the crop, it is possible to calculate whether or not there is any prospect that fumigation will be a profitable investment. If the crop is not of sufficient value, the farmer should be advised against the use of fumigants.

Where a high value crop is planted, prospective returns from the fumigation must be considered. Since this depends on the nematode population of the field, information on this point is desirable, but often difficult to obtain.

If the nematodes are one of the root-knot species and a susceptible crop is growing in the field, it is easy to get a fairly accurate idea of the size of the population by examination of the roots of the plants. But if symptoms of attack are not so readily recognized, or no crop is growing on the field, the task is more difficult.

Theoretically, it should be possible to estimate the nematode population of a field by examination of soil samples. In practice, attempts to apply this method reveal a number of difficulties. The first of these is obtaining and examining an adequate sample of the field. Examination of a sufficient number of soil samples simply requires more time than is usually available. And, even if an accurate estimation of the nematode population can be made, the background information necessary to correlate such findings with subsequent crop growth is largely lacking.

In case of doubt, the grower should be encouraged to experiment on his own farm. That is, he should be advised to use soil fumigants on a trial basis, in such a way that easy comparisons between fumigated and unfumigated soil are possible.

METHOD OF CONTROL OTHER THAN CHEMICAL

The purpose of this section is to stress that in phytonematology the general principles of plant disease control are applicable and should not be overlooked.

I. Exclusion. Exclusion of any parasitic nematode from the farm, nursery, greenhouse, or garden is probably the least expensive and is certainly the most effective control available to the plant grower. Prohibition of the introduction into an area of parasitic nematodes and the additional safeguard of Interception are methods of nematode control being conducted on international and interstate levels. The grower should, whenever possible, take advantage of the benefits of these government services, as for example, purchasing inspected and certified nematode-free plants and propagules. Elimination of nematodes from infected plants or from infested shipping and packing materials is conducted in a number of ways, depending upon the nematodes, plants, and carriers involved. Some examples are sorting, fumigation, hot water treatments, and disinfesting dips or washes. It should be remembered that every new planting, large or small, offers the chance to apply the common-sense methods which can prevent a nematode problem from developing. Simply try to keep parasitic nematodes from being brought in on plants, propagules, and with their soil, packing, or containers.

II. Eradication. The phytonematologist and the plant pathologist are usually sought for only after a nematode problem has been found to exist, and getting rid of the troublesome little animals then becomes the aim of control.

Removal of the nematodes in one way or another is a means of eradication which is applicable in a surprisingly large number of instances and usually at no greater cost than that of the plants involved. Removal of nematized individual plants is practiced in plantings of all sizes and of almost all kinds. Removal of nematized plant parts may be all that is necessary in some cases, for example, removal of the diseased lower leaves of chrysanthemums, or sorting out of infected seed. Removal of nematode infested soil is feasible and effective for greenhouse operations, flower beds, and potted plants.

Destruction of the nematodes in place is the objective of most control efforts. Whether one is dealing with nematodes on or in viable hosts or carriers, or else dealing with nematodes in the non-living environment determines how drastic the control treatment can be made. Nematodes on or very near to the outer surfaces of plant parts can, in some cases, be removed by washing or killed by toxic wash or dip solutions, fumigation, and other chemical treatments. Examples of non-chemical controls are heat treatments of one kind or another, depending upon the thermal tolerance of the plant part and whether or not surface or deep

penetration of the heat is required. Other, as yet, little investigated possibilities include the use of ultrasonics, high frequency heating, and irradiation treatments. Destruction of nematodes in the environment, whether this be the soil, the greenhouse bench, the flat or flower pot, is done by various techniques usually based on one of the following principles: a. Heat - hot water drenches or soaks, steam, flame, electrical resistance, heated inclosures such as ovens and pressure containers; b. Exposure - exposure to the effects of sunlight and desiccation; c. Electricity - probably effective only by production of heat, rather than by the electrical effects alone; and d. Chemical - as is covered in the section dealing with chemical control of nematodes.

Attrition is a form of eradication which has taken many forms in actual practice, but which has as its goal "making life too tough" for the nematodes to thrive. The principles involved include inactivation, starvation, and antibiosis. Removal of carry-over and weed hosts, fallowing, flooding, tillage, and crop rotations are possibilities used when feasible. Antibiosis has been used in some instances as a nematode control in commercial plant production and is a fertile field for further research. Antibiosis involves the application and manipulation of the biological factors of the environment in such ways as to be detrimental to the plant-parasitic nematodes present. Examples are the use of trap crop plants such as Crotolaria, and green manure to promote development of nematode-trapping fungi. Other possibilities include the use of antibiotics, predacious mites and nematodes, and introduction of the various bacterial, fungal, and protozoan pathogens causing diseases of nematodes. Isolation, identification, and synthesis of nematode effecting root exudates is now being done and may be effective for some kinds of nematodes.

III. Protection. Protection is a low-cost form of nematode control which is applied in situations where parasitic nematodes are thought to be present. In its simplest forms it may be nothing more than manipulation of the environment in such a way that nematodes are not blown, washed, splashed, or transported from infested to non-infested sites. Control of nematode dissemination may also be had by choice of planting sites, plant spacing, and planting dates. The use of protectant materials has not been very much explored, and although this is in the realm of chemical control, the concept bears mentioning here. One should keep in mind that for many of the annual plants, a satisfactory economical control is attained if the plants can be protected from the nematodes long enough to get a vigorous root system well started. After that, the plant may be able to support the nematodes which attack it and yet prove to be a worthwhile plant.

IV. Resistance. This is probably the ultimate goal in the development of control for all plant diseases. There are nematode resistant varieties of some crop plants, and others are being developed. The problems in producing nematode resistant plants are difficult because of the numerous kinds of plant-parasitic nematodes, the fact that usually more than one species is present, and the interrelationship of nematodes with other pathogenic organisms in disease complexes.

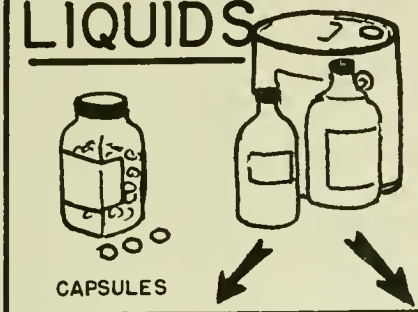
Resistance, due to heredity, and its development, by means of selection and hybridization, is the task of the plant breeder; and although the results may be lasting, their attainment is necessarily a slow process. Resistance or tolerance to nematodes due to non-hereditary factors is becoming of interest with the present-day development of so many new chemicals and the extensive screening programs to test for possible applications. Non-hereditary resistance or increased tolerance to plant nematodes, perhaps, could result from nutritional therapy to compensate for the losses due to the activities of these parasites, or from the application of counteractants or inhibitors of the enzymatic secretions, and to the excretory products of the nematodes. Instances of immunity, tolerance, and inhibition of plant nematodes are known in nature; therefore, development of non-hereditary resistance is considered possible. It is already almost axiomatic to recommend providing ample water and nutrients to valuable nematized plants to compensate for root damage sustained.

The use of resistance as a practical control measure always calls for careful consideration of the nematode situation in which the resistant plants are to be used, because of the diversity of the nematodes and their relationships as mentioned above.

CHEMICAL TREATMENT ON SMALL SCALE

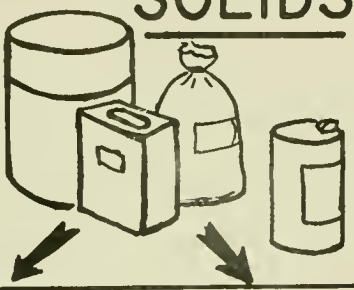
NEMATOCIDES

LIQUIDS



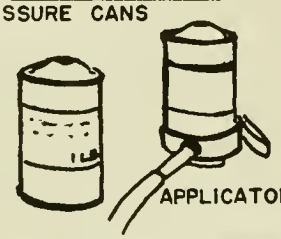
CAPSULES

SOLIDS



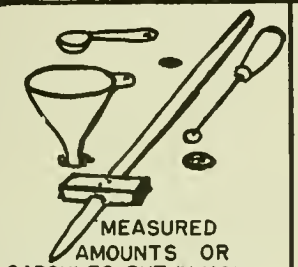
GASES

PRESSURE CANS

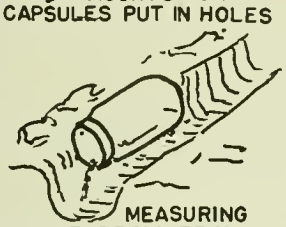


APPLICATOR

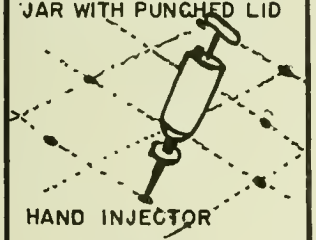
APPLICATION OF LIQUIDS



MEASURED AMOUNTS OR CAPSULES PUT IN HOLES



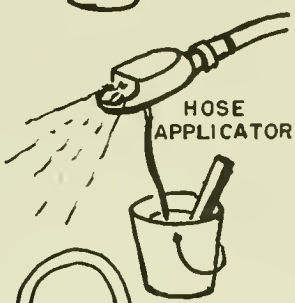
MEASURING INTO FURROW FROM JAR WITH PUNCHED LID



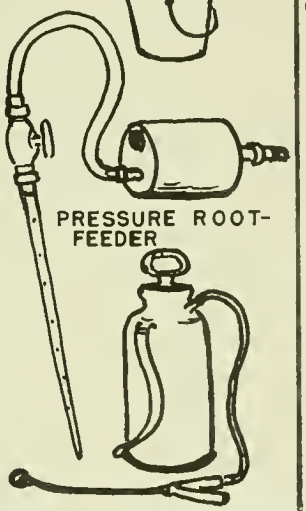
HAND INJECTOR

GRAVITY-FLOW KIT ON PLOW OR HARROW

EMULSIFIABLE & WETTABLE FORMS MIXED IN WATER



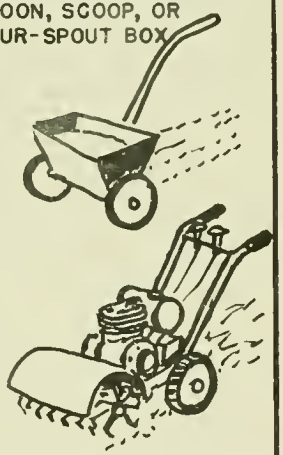
HOSE APPLICATOR



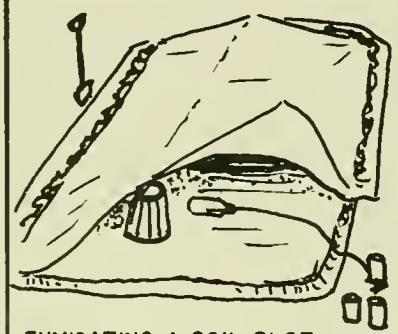
PRESSURE ROOT-FEEDER

APPLICATION OF DRY GRANULES

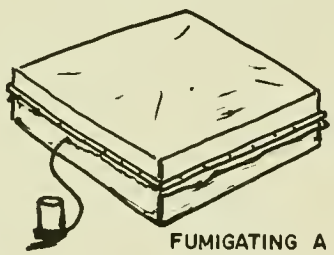
MEASURED INTO HOLES OR FURROWS WITH SPOON, SCOOP, OR POUR-SPOUT BOX



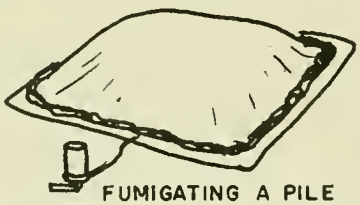
GAS RELEASED UNDER CONFINING COVERS



FUMIGATING A SOIL PLOT

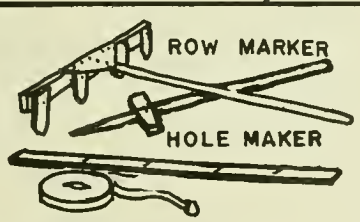


FUMIGATING A SOIL BIN



FUMIGATING A PILE OF SOIL, POTS, OR FLATS

OVERALL SURFACE APPLICATIONS MUST BE FOLLOWED PROMPTLY BY ROTOTILLAGE &/OR WASHING INTO SOIL WITH AMPLE WATER

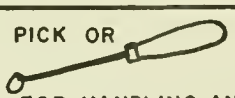


ROW MARKER

HOLE MAKER

MEASURES

ICE PICK OR



AWL FOR HANDLING AND PERFORATING CAPSULES

TIGHTLY COVERED CONTAINERS FOR TREATING SMALL LOTS OF SOIL OR POTS

USEFUL ITEMS FOR FUMIGATION

USEFUL REFERENCES

Journals

In the United States papers dealing with plant-parasitic nematodes are published most often in:

Proceedings of the Helminthological Society of Washington
Phytopathology
The Plant Disease Reporter

In England:

Annals of Applied Biology
Nature
Journal of Helminthology
Plant Pathology
Euphytica
Empire Journal of Experimental Agriculture

International:

Nematologica

Books and Bulletins

Chitwood, B. G. and W. Birchfield. 1956. Nematodes, their kinds and characteristics. Vol. 2. Bul. 9. State Plant Board of Florida. Liberally illustrated and brief descriptions of the principal plant-parasitic nematodes with a useful outline of control measures with specific recommendations.

Chitwood, B. G. and M. B. Chitwood. 1950. An introduction to nematology. Section 1. Anatomy. Revised ed. The most comprehensive source for detailed information on the anatomy and morphology of nematodes of all kinds.

Dollfus, Robert Ph. 1946. Parasites (animaux et vegetaux) des helminthes. Encyclopedie Biologique. Paul Lechevalier, Editeur. Paris. 482 pp. Probably the most complete compilation of work on the parasites of the helminths, including nematodes. Information and illustrations of the bacterial, fungal, protozoan, and metazoan parasites of nematodes. Many illustrations.

Duddington, C. L. 1957. The friendly fungi. A new approach to the eelworm problem. Faber and Faber, London. 188 pp. An interestingly written and informative book concerning the role and application of fungi in the control of plant-parasitic nematodes. Information on how to collect, culture, and observe predacious fungi. Illustrated.

Filipjev, I. N. and J. H. Schuurmans Stekhoven. 1941. A manual of agricultural helminthology. E. J. Brill Co., Leiden, Holland.

878 pp. A comprehensive source of information concerning diseases caused by nematodes and nematode taxonomy as reported in the extensive literature up to about 1939.

Franklin, M. T. 1951. The cyst-forming species of Heterodera. Commonwealth Agricultural Bureaux, England, 147 pp. An extensive review of this group of nematodes, their history, the diseases they cause, control, and taxonomy. Illustrated.

Goffart, H. 1951. Nematodes der kulturpflanzen Europas. Paul Parey, Berlin. 144 pp. Information on diagnosis of the nematode parasites of various crops and plants, primarily those of Europe. Numerous illustrations.

Goodey, J. Basil. 1957. Laboratory methods for work with plant and soil nematodes. Tech. Bul. 2. Ministry of Agric., Fisheries and Food, London. An excellent source for methods, particularly for work with the cyst-forming nematodes. Illustrated.

Goodey, T. 1933. Plant parasitic nematodes and diseases they cause. E. P. Dutton and Co., New York. 306 pp. Considers each parasite according to historical, morphology, life-history, pathology, hosts, and control topics. Illustrated.

Goodey, T. 1940. (Revised 1956 by J. B. Goodey and M. T. Franklin) The nematode parasites of plants cataloged under their hosts. Imp. Bur. Agric. Parasit., St. Albans. A very useful publication for host range considerations with an extensive bibliography.

Goodey, T. 1951. Soil and freshwater nematodes. Methuen and Co., Ltd., London. 390 pp. The latest compilation of nematodes of these kinds with descriptions down through the various taxonomic levels to genotype. Illustrated.

Hyman, Libbie Henrietta. 1951. The invertebrates. Vol. 3. McGraw-Hill Book Co., New York. 572 pp. A good consideration of the entire Nematoda as a group of the invertebrates. Covers such topics as anatomy, embryology, ecology, physiology, host-parasite relations (plant and animal hosts), and presents general taxonomic and life history information for the various genera within the nematode orders. Illustrated.

Kevan, D. K. McE. (Editor) 1955. Soil Zoology. Butterworth's Scientific Publications, London. 512 pp. Collection of a diversity of papers dealing with the soil animals, including nematodes. Highly recommended for broadening ones concept of the soil as an environment. A source for techniques for study of the soil and the soil animals. Contains a useful key to the orders and suborders of soil and litter inhabiting animals. Illustrated.

Pennak, R. W. 1953. Fresh-water invertebrates of the United States. Ronald Press Co., New York. 769 pp. The chapter dealing with

nematodes has an excellent key supplemented with illustrations of representatives of the common genera encountered in soils and moist habitats.

Steiner, G. 1942. Plant nematodes the grower should know. Proc. Soil Sci. Soc. Fla. 4-B:72-117 (Issued 1949) Well illustrated and informative description of the major types of plant-parasitic nematodes, the diseases they cause, and host-parasite relations.

Steinhaus, Edward A. 1949. Principles of insect pathology. McGraw-Hill Co., New York. p. 633-664. A general introduction to the nematodes parasitic on insects; life histories, host-parasite relations, and applications. Illustrated.

Stewart, M. A. 1945. A laboratory manual of helminthology. U. of Calif. Syllabus Series ZN, Univ. of Calif. Press, Berkeley. 194 pp. (Third printing 1954) A useful compilation of methods and taxonomic keys for the study of Platyhelminthes, Annelida (Hirudinea), Acanthocephala, Nematoda, Nematomorpha (Gordiacea) of importance as human and animal parasites.

Thorne, Gerald. 1939. A monograph of the nematodes of the superfamily Dorylaimoidea. Capita Zoologica 8 (Part 5), Martinus Nijhoff, The Hague, Netherlands. 261 pp. A basic reference in taxonomy provided with descriptions, drawings, and keys. Recently reprinted with the addition of a listing of the new species in the Dorlyaimoidea for the interval between the first printing and about 1955.

Thorne, Gerald and Helen Swanger. 1936. A monograph of the nematode genera Dorylaimus Dujardin, Aporcelaimus, n. g., Dorylaimoides n. g., and Pungentus n. g. Capita Zoologica 6 (Part 4), Martinus Nijhoff, The Hague, Netherlands. 223 pp. A basic reference book provided with descriptions, drawings, and keys. Recently reprinted with the addition of the new species reported for the interval between the first printing and about 1955.

Chemical Control Recommendations

A. Dow Chemical Company, Midland, Michigan.
ACD Information Bulletin No. 110, Nov. 29, 1957.
Factors Influencing diffusion and nematode control by soil fumigants. Cleve A. I. Goring. 57 pp.
ACD Information Bulletin No. 112, Feb. 1958.
Dow soil fumigants. 15 pp.

B. Shell Chemical Corp., Agricultural Chemical Sales, New York 22, N. Y. Specimen labels for Shell agricultural products. (A plastic bound book permitting revisions as needed.)

SCIENTIFIC AND COMMON NAMES FOR PLANT-PARASITIC NEMAS*

This cross-reference listing of the scientific and common names is based on the largest compilations of common names as yet available. The first reference (Buhrer, E. M. 1954. Common names of some important plant pathogenic nematodes. Pl. Dis. Reptr. 38(8):535-541.) should be carefully read by all who are concerned with this useful but sometimes controversial aspect of phytonematology. The second reference is a chart issued by the Florida State Plant Board which, for the most part, follows the listing of the reference just mentioned but, in addition, lists a number of new common names to species for which no common names have been designated. Perhaps the compilation presented in these Notes will further promote the use of reasonably standardized common names.

*The words "nematode(s)" and "nema(s)" have been used interchangeably in the text of these Notes. For a review of the origins of these words, derivations from them, and suggestions concerning future usage, see: Chitwood, B. G. 1957. The English word "Nema" revised. Systematic Zoology 6(4):184-186.

I. SCIENTIFIC NAMES

No.	Nematode	Common Name No.
1	Anguina	152
2	A. agrostis	83
3	A. tritici	212
4	Aphelenchoides	14, 80
5	A. besseyi	131,196
6	A. cocophilus	40
7	A. fragariae	180
8	A. olesistus	8, 77
9	A. ritzema-bosi	29
10	Belonolaimus	185
11	B. gracilis	124
12	Cacopaurus	154
13	C. epacris	155
14	C. pestis	156
15	Criconema	166
16	C. civellae	167
17	C. decalineatum	168
18	C. spinalineatum	169
19	Criconemoides	132
20	C. citri	133
21	C. simile	134
22	Ditylenchus	15
23	D. angustus	129
24	D. dipsaci	199
25	D. destructor	126
26	D. myceliophagus	108
27	Dolichodorus	3
28	D. heterocephalous	4
29	Dorylaimus	165
30	Gottholdsteineria	170
31	G. buxophila	172
32	Helicotylenchus	(?)170
33	H. africanus**	171
34	H. erythrinae	179
35	H. multicinctus	176
36	H. nannus	177
37	Hemicriconemoides	160
38	H. biformis	161
39	H. floridensis	162
40	H. wessoni	163
41	Hemicycliophora	157
42	H. similis	158
43	H. parvana	159
44	Heterodera	48
45	H. cacti	50
46	H. carotae	52
47	H. cruciferae	49, 51
48	H. glycines	62
49	H. goettingiana	60
50	H. humuli	57
51	H. major	53, 59
52	H. punctata	56
53	H. rostochiensis	55
54	H. schachtii	63
55	H. tobacum	64
56	H. trifolii	54
57	H. weissi	58, 61
58	Hoplolaimus	93
59	H. coronatus	94
60	H. uniformis	95
61	Longidorus	109
62	L. sylphus	110
63	Meloidodera	65
64	M. floridensis	66
65	Meloidogyne	136
66	M. arenaria	144
67	M. arenaria thamesi	147
68	M. brevicauda	141
69	M. exigua	137
70	M. hapla	143
71	M. incognita	145
72	M. incognita acrita	140
73	M. javanica	142
74	Nacobbus	75
75	N. batatiformis	76

No.	Nematode	Common Name No.
76	Paratylenchus	118
77	P. elachistus	121
78	P. hamatus	120
79	P. dianthus	119
80	Pratylenchus	96
81	P. brachyurus	103
82	P. coffeae	100
83	P. leiocephalus	105
84	P. minyus	98
85	P. musicola	97
86	P. penetrans	99
87	P. pratensis	102
88	P. scribneri	104
89	P. thornei	106
90	P. vulnus	107
91	P. zeae	101
92	Radopholus	
93	R. oryzea	130
94	R. similis	16
95	Rotylenchulus	
96	R. reniformis	128
97	Rotylenchus (see also: Gottholdsteineria & Scutellonema)	170
98	R. blaberus**	178
99	R. brachyurus	174
100	R. buxophilus	172
101	R. christiei	175
102	R. coheni	173
103	R. robustus	95
104	Scutellonema	(?)170
105	S. blaberum**	178
106	S. brachyurum	174
107	S. christiei	175
108	S. coheni	173
109	Trichodorus	186
110	T. christiei	187
111	T. obtusus	188
112	T. pachydermis	189
113	Turbatrix	
114	T. aceti	208
115	Tylenchorhynchus	191
116	T. claytoni	190, 194
117	T. martini	192
118	Tylenchulus	
119	T. semipenetrans	30
120	Xiphinema	67
121	X. americanum	68
122	X. chambersi	70
123	X. diversicaudatum	71
124	X. index	69
125	X. radicicola	72

** It is suggested that Helicotylenchus africanus be called the "African spiral nema" rather than Scutellonema blaberum syn. Rotylenchus blaberus. As the latter was discovered in West Africa, the common name "West African spiral nematode" seems appropriate.

No.	Nematode	Scientific Name No.
1	African spiral nema**	98
2	American dagger nema	121
3	Awl nemas	27
4	Cobb's awl nema	28
5	Banana nema	85
6	Banana meadow nema	85
7	Beet nema	54
8	Begonia leaf nema	8
9	Boxwood spiral nema	31, 100
10	Brassica-root nema	47
11	Brazilian root-knot nema	69
12	British spiral nema	102
13	Bud nemas	4
	Bud nema	7
14	Bud and leaf nemas	4
15	Bulb or stem nema	22
16	Burrowing nema	94
17	Cabbage cyst nema	47
	Cabbage-root nema	
18	Cactus cyst nema	45
19	California dagger nema	124
20	California meadow nema	84
21	California sessile nema	13
22	Carnation pin nema	79
23	Carolina spiral nema	99
24	Carrot cyst nema	46
	Carrot-root nema	
25	Cereal-root nema	51
26	Chamber's dagger nema	122
27	Christie's spiral nema	101
28	Christie's stubby root nema	110
29	Chrysanthemum nema	9
	Chrysanthemum leaf nema	
30	Citrus nema	119
	Citrus-root nema	
31	Citrus ring nema	20
32	Citrus spine nema	16
33	Clover cyst nema	56
34	Cobb's awl nema	28
35	Cobb's lance nema	59
36	Cobb's meadow nema	86
37	Cobb's ring nema	21
38	Cobb's spiral nema	35
39	Cobb's stubby root nema	111
40	Coconut palm nema	6
	Coconut nema	
	Cocopalm nema	
41	Coffee meadow nema	82
42	Coffee root-knot nema	69
43	Confused root-knot nema	72
44	Corn meadow nema	91
45	Cotton root-knot nema	72
46	Crimp nema	7
47	Currant nema	9
48	Cyst nemas	44
49	Brassica-root nema	47
50	Cactus cyst nema	45
51	Cabbage cyst nema	47
52	Carrot cyst nema	46
53	Cereal-root nema	51
54	Clover cyst nema	56
55	Golden nema	53
56	Grass cyst nema	52
57	Hops cyst nema	50
58	Knotweed cyst nema	57
59	Oat cyst nema	51
60	Pea cyst nema	49
61	Polygonum cyst nema	57
62	Soybean cyst nema	48
63	Sugar beet nema	54
64	Tobacco cyst nema	55
65	Cystoid nemas	63
66	Pine cystoid nema	64
67	Dagger nemas	120
68	American dagger nema	121
69	California dagger nema	124
70	Chamber's dagger nema	122
71	European dagger nema	123
72	Pacific dagger nema	125
73	De Man's meadow nema	87
74	European dagger nema	123
75	False root-knot nemas	74
76	False root-knot nemas of sugar beets	75
77	Fern nemas	8
78	Fig pin nema	78
79	Fig spine nema	17
80	Foliar nemas	4

No.	Nematode	Scientific Name No.
81	Gallworm	65
82	Garden nema	65
83	Grass nema	2
84	Grass cyst nema	52
85	Godfrey's meadow nema	81
86	Golden nema of potato	53
87	Grass sheath nema	42
88	Hops cyst nema	50
	Hops-root nema	
89	Indian root-knot nema	68
90	Javanese root-knot nema	73
91	Kidney-shaped nema	96
92	Knotweed cyst nema	57
93	Lance nemas	58
94	Cobb's lance nema	59
95	Thorne's lance nema	60, 103
96	Meadow nemas (see: Root-lesion nemas)	80
97	Banana meadow nema	85
98	California meadow nema	84
99	Cobb's meadow nema	86
100	Coffee meadow nema	82
101	Corn meadow nema	91
102	De Man's meadow nema	87
103	Godfrey's meadow nema	81
104	Scribner's meadow nema	88
105	Smooth-headed "nema	83
106	Thorne's meadow nema	89
107	Walnut meadow nema	90
108	Mushroom spawn nema	26
109	Needle nemas	61
110	Thorne's needle nema	62
111	Northern root-knot nema	70
112	Oak sheathoid nema	38
113	Oat cyst nema	51
	Oat nema	
	Oat-root nema	
114	Pacific dagger nema	125
115	Pea cyst nema	49
	Pea-root nema	
116	Peanut root-knot nema	66
117	Persian sessile nema	14
118	Pin nemas	76
119	Carnation pin nema	79
120	Fig pin nema	78
121	Ramie pin nema	77
122	Pine cystoid nema	64
123	Pine sheathoid nema	39
124	Pine sting nema	11
125	Polygonum cyst nema	57
126	Potato rot nema	25
	Potato tuber nema	
127	Ramie pin nema	77
128	Reniform nema	96
129	Rice nema	23
130	Rice root nema	93
131	Rice white tip nema	5
132	Ring nemas	19
133	Citrus ring nema	20
134	Cobb's ring nema	21
135	Root-gall nema (see: Root-knot nemas)	65
136	Root-knot nemas	65
137	Brazilian root-knot nema	69
138	Confused root-knot nema	72
139	Coffee root-knot nema	69
140	Cotton root-knot nema	72
141	Indian root-knot nema	68
142	Javanese root-knot nema	73
143	Northern root-knot nema	70
144	Peanut root-knot nema	66
145	Southern root-knot nema	71
146	Tea root-knot nema	68
147	Thames' root-knot nema	67
148	Root-lesion nemas (see: Meadow nemas)	80
149	Root nemas	80
150	Root rot nemas	80
151	Scribner's meadow nema	88
152	Seed gall nemas	1
153	Seinhorst stubby root nema	112
154	Sessile nemas	12
155	California sessile nema	13
156	Persian sessile nema	14
157	Sheath nemas	41
158	Grass sheath nema	42
159	Tarjan's sheath nema	43
160	Sheathoid nemas	37

No.	Nematode	Scientific Name No.
161	Oak sheathoid nema	38
162	Pine sheathoid nema	39
163	Wesson's sheathoid nema	40
164	Soybean cyst nema	48
165	Spear nemas	29
166	Spine nemas	15
167	Citrus spine nema	16
168	Fig spine nema	17
169	Zoysia spine nema	18
170	Spiral nemas	(?)30, 32, 97, (?) 104
171	African spiral nema**	33, 98, 105
172	Boxwood spiral nema	31, 100
173	British spiral nema	102, 108
174	Carolina spiral nema	99, 106
175	Christie's spiral "	101, 107
176	Cobb's spiral nema	35
177	Steiner's spiral nema	36
178	West African spiral nema**	98, 105
179	Zimmermann's spiral nema	34
180	Spring dwarf nema	7
181	Steiner's spiral nema	36
182	Stem or bulb nemas (see: Bulb or Stem nemas)	
183	Strawberry bud nemas	5, 7
184	Strawberry dwarf nemas	5, 7
185	Sting nemas	10
186	Stubby root nemas	109
187	Christie's stubby root nema	110
188	Cobb's stubby root nema	111
189	Seinhorst's stubby root nema	112
190	Stunt nemas	115
	Tobacco stunt nema	116
191	Stylet nemas	115
192	Sugar cane stylet nema	117
193	Tesselate stylet nema	116
194	Tobacco stylet nema	116
195	Sugar beet nema	54
196	Summer dwarf nema	5
197	Tarjan's sheath nema	43
198	Tea root-knot nema	68
199	Teasel nema	24
200	Tesselate stylet nema	116
201	Thames' root-knot nema	67
202	Thorne's lance nema	60, 103
203	Thorne's meadow nema	89
204	Thorne's needle nema	62
205	Tobacco cyst nema	55
206	Tobacco stunt nema	116
207	Tobacco stylet nema	116
208	Vinegar eelworm	113
	Vinegar eels	
209	Walnut meadow nema	90
210	Wesson's sheathoid nema	40
211	West African spiral nema**	33
212	Wheat nema	3
	Wheat gall nema	
	Wheat eelworm	
213	Zimmermann's spiral nema	34
214	Zoysia spine nema	18

** It is suggested that Helicotylenchus africanus be called the "African spiral nema" rather than Scutellonema blaberum syn. Rotylenchus blaberus. As the latter was discovered in West Africa, the common name "West African spirl nema" seems appropriate.