THE ANATOMY AND BIOLOGY OF THE PARASITIC *APHELENCHI*.

By F. H. STEWART, M.A., D.Sc., M.D.,

Major, Indian Medical Service.

(From the Quick Laboratory, University of Cambridge.)

(With Figs. 1, 2 on Pl. VIII and Figs. 3–32 in Text.)

CONTENTS.

I. THE PARASITIC SPECIES OF THE GENUS *APHELENCHUS* AND THEIR DISCOVERY.

A. fragariae Ritzema Bos, 1891.

A. ormerodis R.B., 1891. A doubtful species, possibly the young of *A. fragariae* (Marcinowski, 1908).

A. olesistus R.B., 1893.

A. phyllophagus n. sp.

DISCOVERY OF THE PARASITE.

Aphelenchus fragariae was first described by Ritzema Bos (1891) as causing a disease among strawberry plants in Kent; the plants had been sent to him by Miss Ormerod.

Nematoid worms were observed to cause disease in the leaves of *Begonia* by Worthington Smith (1890), in plants which came from Dunstable. In the following year Klebahn (1891) found *Aphelenchi* in blotched leaves of two species of fern (*Asplenium diversifolium* and *bulbiferum*) growing in a nursery in Bremen, and sent specimens to Ritzema Bos. The latter authority, having found the same species in these specimens and in *Begonia* leaves from England, described it under the name of *A. olesistus*.

II. DISTRIBUTION OF THE PARASITES, AND THE DISEASES CAUSED BY THEM.

A. fragariae. The disease caused by this species has been named strawberry bunch by Cobb (1891) (Blumenkohlkrankheit der Erdbeerpflanze, Ritzema Bos, 1891). It is characterised by stunting of growth in the length of the stem, and hypertrophy in breadth; the flowers abort, and fruit is not formed. Complete failure of the crop consequently results. It has been recorded from England, Scotland, Germany, and Norway (Marcinowski, 1908), and is doubtless much more widely spread.

A. olesistus since its discovery in *Asplenium* and *Begonia* has been found in many species of ferns and flowering plants. It attacks the leaves, causing large, sharply defined, brown blotches; the leaves finally wilt and fall off. Louis Mangin (1895) recorded the disease "de la Rouille" in Everlasting (*Helichrysum*) cultivated in the districts of Ollioules, Bandol, and St Nazaire, near Toulon. It causes great loss, as affected plants are unsaleable. A similar disease has been recorded from chrysanthemums by Atkinson (1891), from the United States by Sorauer (1901), Hofer (1901), Osterwalder (1904), and Molz (1909) from Switzerland and Germany. Ritzema Bos identified Sorauer and Hofer's nematode as *A. olesistus*. Sorauer (1902) described a serious epidemic in *Begonia* "Gloire de Lorraine," in which the plants were unsaleable. Osterwalder (1902) found the disease in flowering plants cultivated in the open air in Wadenswil and Zürich, in *Anemone japonica* and *Sylvestris*, *Ranunculus montana*, *Atragena alpina*, *Eryngium alpinum*, *Scabiosa sileni folia*, *Spiraea astilboides*, *Epipactis palustris* and others. It also occurs in *Coleus* and *Salvia* (Hofer, 1901) and in orchids (Marcinowski, 1908). Among ferns *Pteris ouvardi* and *cretica* have been found affected in addition to *Asplenium*.

Material used in the present investigation.

I received (1) specimens of strawberry plants containing *A. fragariae* from a correspondent in Ayrshire, (2) leaves of *Lygodium dichotomum* and *Lomaria ciliata* containing *A. olesistus* from the Royal Botanic Gardens, Kew,

through the kindness of Sir David Prain and Mr Arthur Hill, and (3) *Chrysanthemum* leaves containing the new species *A. phyllophagus*, from Messrs Tacon and Horwood, Cheshunt, Hertfordshire.

Regarding this disease in chrysanthemums, Messrs Tacon and Horwood write, that they have had this trouble for ten years at least; in one batch of pot chrysanthemums (Cheshunt White) one half were affected; they have been compelled to abandon certain varieties entirely, as they used to lose all their leaves.

Strawberry bunch and *Aphelenchus* leaf disease are therefore two widespread and destructive plant diseases, which cause considerable loss to the growers of fruit and flowers.

III. SPECIFIC DISTINCTNESS OF *APHELENCHUS FRAGARIAE* R.B., *A. OLESISTUS* R.B., AND *A. PHYLLOPHAGUS* N.SP.

A. fragariae and *olesistus*. Ritzema Bos distinguished his two species by their pathological effects on their hosts. Several points of anatomical distinction must have been observed under low powers of the microscope, but they are unsatisfactory. The tail of *A. fragariae* narrows suddenly behind the anus, that of *A. olesistus* does not.

Marcinowski (1908) considers *A. fragariae* identical with *A. olesistus*, and possibly also with *A. helophilus* De Man. The differences in measurements she considers to be within the limits of individual variability. She performed experiments which she claims prove that the *Aphelenchi* of orchids and begonias can be transferred to strawberry plants.

Infected orchid leaves were placed in contact with (1) begonias, (2) strawberry plants. In (1) immediate infection occurred with typical leaf disease; in (2) on the other hand no *Aphelenchi* were found on the strawberry plants during the first month, thereafter only a few in one plant; leaf disease and not characteristic strawberry bunch resulted.

This experiment therefore so far from proving the identity of *A. olesistus* and *fragariae* tends to prove their distinctness.

A. phyllophagus. When numerous fresh and well-mounted specimens are compared with *A. fragariae* and *olesistus* the following differences are obvious: it is a larger and more robust animal, and the excretory pore is situated some distance behind the nerve ring, while in *A. fragariae* and *olesistus* it is on a level with the nerve ring.

The difference in size between *A. phyllophagus* and *A. olesistus* is not a mere temporary difference between individuals of the same species due to the occupation of different host plants. I transferred both species to cabbage seedlings, but they retained their differences of size in the new host. It should be noted that the stomata (through which the parasites enter the plants, *vide* p. 176, E) are larger in the normal hosts of *A. phyllophagus* (chrysanthemums) than in the normal hosts of *A. olesistus* (begonias and ferns). The two species are therefore adapted to their normal hosts.

PLATE VIII
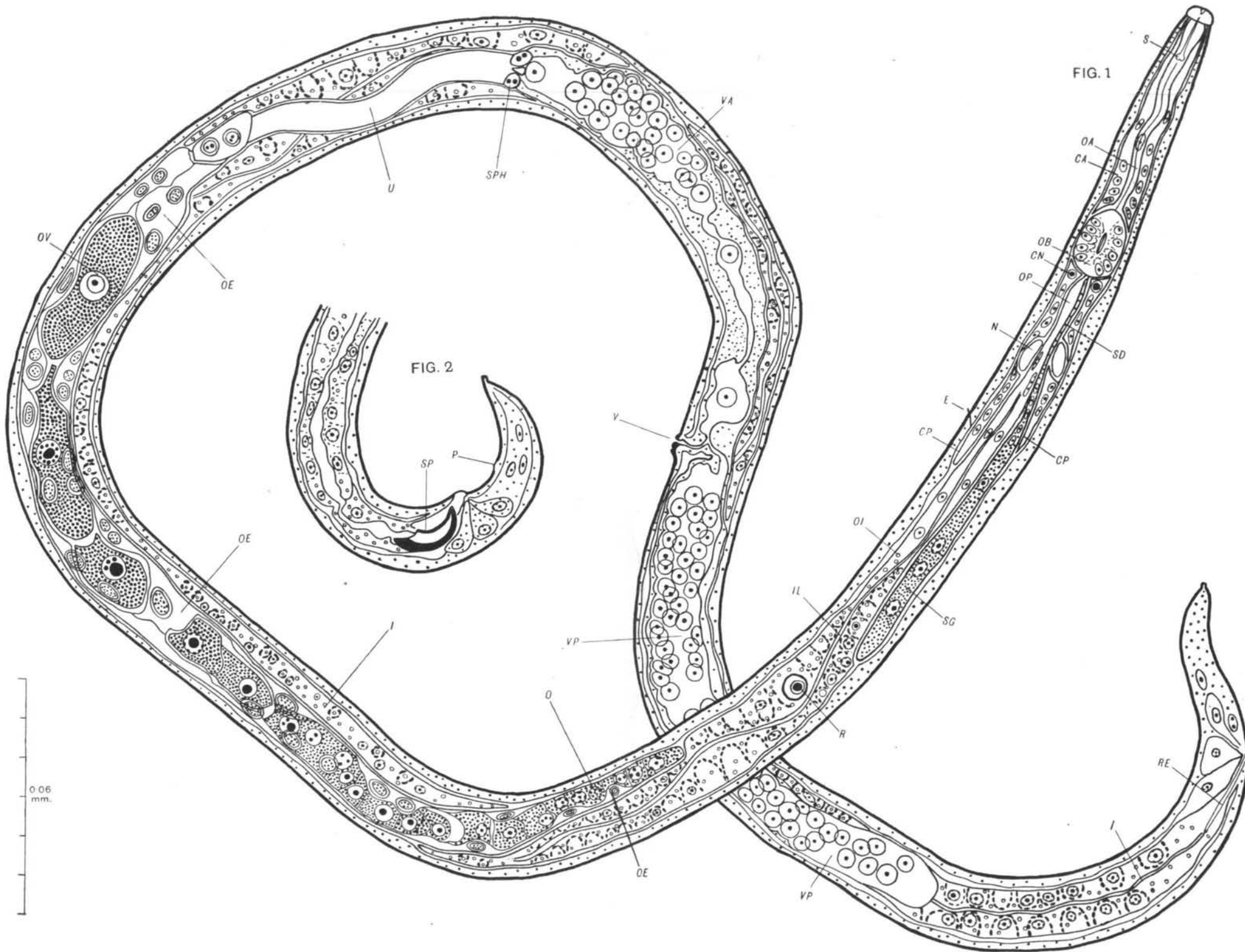
Figs. 1 and 2. **Aphelenchus phyllophagus** n.sp.

Fig. 1. Adult female from a chrysanthemum leaf; outlines drawn with a camera from a specimen fixed with 70 per cent. alcohol, and mounted in glycerine jelly, detail completed from specimens stained with haemalum, and mounted in balsam.   Fig. 2. Tail of male.

KEY TO THE GROUP OF THE SLENDER-BODIED *APHELENCHI*.

Aphelenchi of slender-body form. a^1, 45 and over.

(1) Excretory pore behind the nerve ring. Parasitic. L. 0·85–1 mm.
A. phyllophagus n.sp.

(2) Excretory pore at the level of the nerve ring.

    (*a*) Intra-uterine egg rounded oval. Free living. L. 1 mm. *A. helo-philus* De Man, 1886.

    (*b*) Intra-uterine egg elongated cylindrical. Parasitic.

        (i) Anterior lip of anus prominent. Causes hypertrophy of tissues of host. L. 0·07–0·08 mm. *A. fragariae* R.B., 1891.

        (ii) Anterior lip of anus not prominent. Does not cause hypertrophy. L. 0·05–0·06 mm. *A. olesistus* R.B., 1893.

IV. *A. PHYLLOPHAGUS* N.SP. ANATOMY OF THE ADULT.

Measurements. ♀, L. 0·845–0·92 mm.; *a*, 51–53 (β, 11·3–16·5)^2; γ, 20·5–21. ♂, L. 0·88–0·96 mm.; *a*, 48–52 (β, 12·6–13); γ, 16–19.

$$\text{Cobb's formula:} \quad ♀ \quad \frac{1\cdot3 \quad (6\cdot8) \quad 9 \quad ? \quad 70 \quad 95\cdot5}{1\cdot2 \quad (1\cdot6) \quad 1\cdot8 \quad ? \quad 2\cdot1 \quad 1\cdot3} \quad 0\cdot923;$$

$$♂ \quad \frac{1\cdot2 \quad (7\cdot4) \quad 9\cdot9 \quad ? \quad M \quad 93}{0\cdot8 \quad (1\cdot4) \quad 1\cdot5 \quad ? \quad 2 \quad 1\cdot5} \quad 0\cdot965.$$

Body-form (Figs. 1 and 2). The hemispherical *head* is marked off by a groove. *Lateral membranes* absent. *Transverse striae* of cuticle 0·0008 mm. in breadth. *Tail* ends in a mamilliform appendage. *Lateral lines* (Figs. 4–14, *LL*) occupy ⅙th of the circumference. *Muscle fields* each contain five cells in cross-section.

Alimentary system. The *spear* (Fig. 1, *S*), length 0·013 mm., thickens slightly to the knobbed base. The *oesophagus* is divided into anterior oesophagus, bulb, and posterior oesophagus (Fig. 1, *OA, OB, OP*); the bulb is conoid and muscular; the intrinsic nuclei form a layer in its surface; the centre is occupied by the usual oval chitinous structure; the posterior oesophagus[3] is swollen slightly behind the bulb, narrows as it traverses the nerve ring, then increases gradually in width to its junction with the intestine, which is marked only by the commencement of the intestinal droplets and granules (Fig. 2, *OI*); the wall of the oesophagus is eosinophil in staining reaction, its lumen circular in the posterior section. The *salivary glands*[3] (Figs. 1, 11 and 12, *S.G.*) lie dorsal to, and slightly to the right of the posterior oesophagus and the commencement of the intestine; they consist of four cells arranged in linear series: the duct (Figs. 1 and 6–10, *S.D.*), containing several nuclei, passes forward through the nerve ring, and enters the oesophageal bulb at its posterior end (Fig. 5, *S.O.*); both gland and duct are basophil.

[1] De Man (1886) employs the following useful contractions:

$$\frac{\text{Length}}{\text{Breadth}} \; a. \qquad \frac{\text{Length}}{\text{L. of oesophagus}} \; \beta. \qquad \frac{\text{Length}}{\text{L. of tail}} \; \gamma.$$

Length, L.   Maximum breadth, B.

[2] The length of the oesophagus is measured throughout to the posterior end of the bulb, owing to the indefiniteness of the function of the oesophagus and intestine. This measurement is given as the second figure in Cobb's formula—in brackets. The formula therefore runs:

Posterior end of spear, (Posterior end of bulb), Nerve ring, ?, Vulva, Anus.

[3] Cf. p. 169 (1).

The *intestine* (Figs. 1, 13 and 14, *I*) is thick and patent; walls without cell limits but containing numerous nuclei, droplets and granules; staining reaction basophil; the lumen is sinuous, flattened and slit-like, contrasting with the round lumen of the posterior oesophagus. The *rectum* (Fig. 1, *RE*) is one half to two-thirds as long as the tail.

Nerve ring and oesophageal collar. The *nerve ring* (Figs. 1, 9 and 10, *N*) is situated behind the oesophageal bulb at a distance rather greater than the length of the bulb. The *oesophageal cellular collar* (Figs. 1 and 6–12, *CA, C.P.*) is an ingrowth from the four longitudinal lines (Fig. 8, *C.P.*) and clothes the oesophagus throughout its whole length; it contains many nuclei, of which two or four situated immediately behind the bulb (Figs. 1 and 6, *CN*) are particularly large and prominent; the function of the collar is probably nervous, it, together with the nerve ring, representing the central nervous system of the animal.
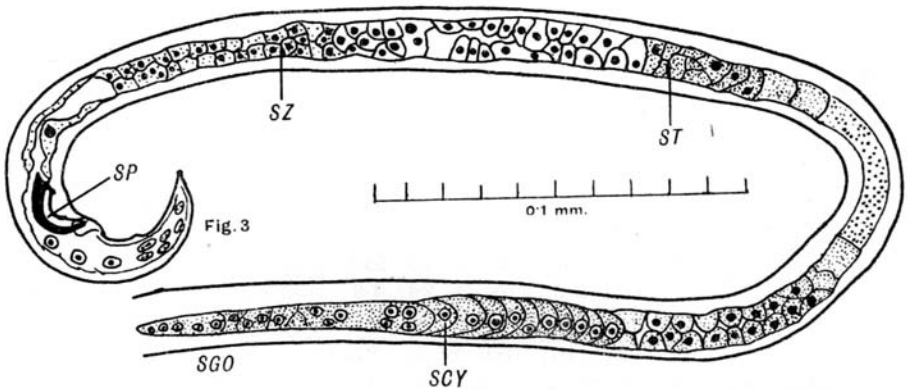
Fig. 3. **Aphelenchus phyllophagus** n. sp. Posterior two-thirds of male, showing gonads.
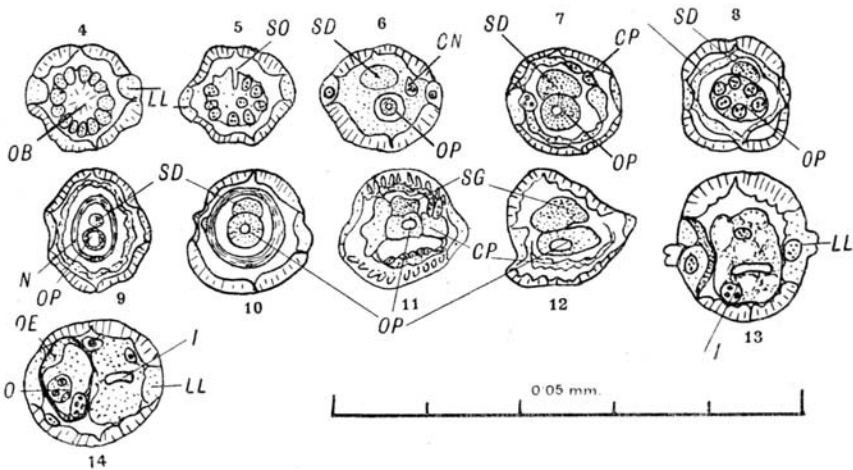
Figs. 4–14. **Aphelenchus phyllophagus** n. sp. Transverse sections in series.
Figs. 4, 5. Through the oesophageal bulb.
Figs. 6–8. Between the bulb and the nerve ring.
Figs. 9, 10. Through the nerve ring.
Figs. 11, 12. Between the nerve ring and the end of the oesophagus.
Fig. 13. Through the intestine in front of the gonad.
Fig. 14. At the commencement of the ovary.

Excretory system. The single *renette*[1] cell (Fig. 1, *R*) lies between the left lateral line and the intestine, at a level midway between the end of the salivary gland and the commencement of the gonad; it is somewhat difficult to recognise; in unstained preparations it has the appearance of a spherical space containing a small highly refringent sphere, which in stained specimens proves to be a chromatin mass. I have not succeeded in tracing the duct between the renette cell and the excretory pore; this aperture is situated behind the posterior margin of the nerve ring at a distance varying slightly in different specimens, but averaging 0·02 mm., equal to rather more than the length of the oesophageal bulb; this position markedly behind the nerve ring is of systematic importance (*vide supra*); the excretory duct contains a substance which stains with haematoxylin, giving a curious appearance, as if a spine were projecting through the cuticle.

There are no *caudal glands.*

The reproductive system in the female consists of two tubular sacs (Figs. 1 and 14, *OE*, *VA*, *VP*) extending forward and backward from the vulva; the anterior end of the anterior sac contains the ovary (Figs. 1 and 14, *O*); the ova (Fig. 1, *OV*), as they pass backward increase in size and become elongated oval in shape; the wall of this part of the sac consists of a fairly robust endothelium, which, in those places where it contains nuclei, bulges into the lumen and compresses or separates the ova (Fig. 1, *OE*); the nuclei have a characteristic circumvallate appearance. The structure of the *uterus* (Fig. 1, *U*) is difficult to analyse; it is closed at its posterior end where it joins the anterior vagina by a sphincter (Fig. 1, *SPH*). It is probable that fertilisation and formation of the shell occur in the *anterior vagina.* Marcinowski (1910) describes a shell gland in *Cephalobus*, but it should be remembered that Schneider (1866, p. 285) proved that the shell of nematode eggs is secreted by the egg itself, and not by the wall of the oviduct. The anterior vagina extends from the sphincter to the vulva; its walls are of irregular thickness, and do not contain the typical nuclei of the ovarian sac; the lumen is occupied by a mass of spherical spermatozoa. The retrovulvar portion of the reproductive tube consists of the *posterior vagina* alone, which resembles the anterior vagina in length and other respects, but is a caecum; the vaginae function as receptacula seminis. (For development in the larva, and early fertilisation, see Sect. V below: for comparative anatomy, p. 172 (3).) The *vulva* is a transverse slit situated $\frac{7}{10}$ths of the body-length from the head.

Male reproductive organs (Figs. 2 and 3). The single testicular tube has an endothelial wall, like the ovarian; posteriorly it joins the intestine to form the cloaca, and is here closed by a valve-like projection of the ventral wall. The *spicules* (Figs. 2 and 3, *SP*) are very broad, curved and hollow, the posterior margin is much thickened, the anterior margin is the structure described and figured by Ritzema Bos (1893) as the accessory piece; the two spicules are very closely apposed; there is no accessory piece. The *tail of the male* (Fig. 2) is sharply curved, the ventral surface flat, with one poorly-defined papilla, post-anal, median ventral (Fig. 2, *P*); in mounted specimens there is sometimes an appearance of a bursal membrane, which is probably artificial, but the flattening of the ventral surface must produce a ridge along each lateral line which forms the rudiment of a bursal membrane.

V. *A. PHYLLOPHAGUS.* THE EGG, AND LARVAL DEVELOPMENT.

The *egg* (Figs. 15 and 18) is sausage-shaped, 0·085 × 0·023 to 0·095 × 0·022 mm. The shell is thin and unsculptured, the ovum when first laid unsegmented. I have not observed segmentation or embryonic development.

Larvae. Molz (1909) gives the length of the newly-hatched larva as 0·16–0·18 mm. and its breadth 0·01 mm. (*a* 16–18). The youngest larva which I have seen (Fig. 17) measured 0·22 × 0·015 mm. (*a* 14·7), the spear 0·012 mm.; it was found in the leaf axil of a groundsel on the 28th day after infection, together with adults and eggs, and had recently hatched

[1] Cf. p. 172 (2).
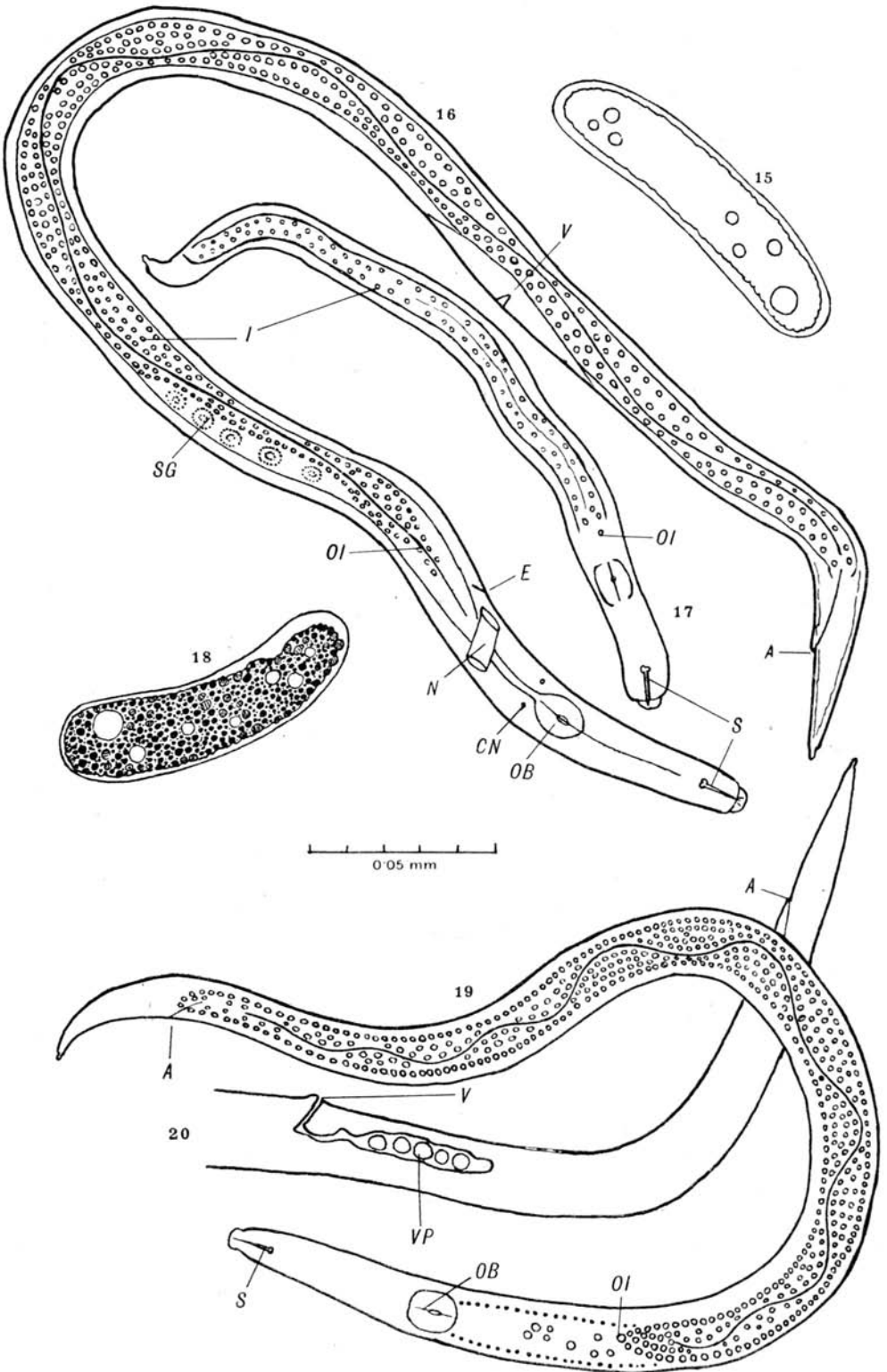
Figs. 15–20.

in that situation. An older larva (Fig. 19) measured 0·465 × 0·02 mm. (a 23), spear again 0·012 mm.; it was found in the tissue of a leaf of a groundsel on the 5th day after infection, together with larvae measuring 0·25, 0·3, and 0·32 mm. Being thus associated with recently hatched larvae it was probably derived from an egg laid within the previous five days in a leaf axil of the plant. In both the 0·22 mm. and the 0·465 mm. larva the oesophageal bulb was distinct; the intestine (Figs. 17 and 19, OS) commenced behind the bulb at a distance equalling head to bulb; the alimentary canal was patent.

A still older larva (Fig. 16) measured 0·65 × 0·018 mm. (a 36); spear still 0·012 mm.; the *salivary gland* (*SG*) was apparent, with five nuclei of which the anterior probably forms the duct; the gland was situated further back than in the adult, the whole of it lying behind *OI*; the nerve ring (*N*), and the two large nuclei of the posterior collar (*CN*), were also apparent, the latter as two black dots which I at first took for eye spots; the excretory pore was in the same situation as in the adult. The rudimentary vulva was situated three-quarters of the body-length from the head; it lay in a clear area in the midventral line, which represented the rudiment of the vaginae. This specimen was found in a leaf of a groundsel, ten days after infection, together with adults and other larvae. A young female (Fig. 20), from the same leaf, was probably also hatched in the same situation, not more than ten days previously; it measured 0·8 × 0·022 mm. (a 36).

The outstanding fact in this specimen was the condition of the developing genital ducts, the vulva and the posterior vagina only being present, the latter already containing spermatozoa! The rudiment of the ovary was not observed, but there was no trace of germinal cells at the fundus of the vagina; a condition of protandrous hermaphroditism was therefore not present, such as occurs in *Rhabditis* (*Leptodera*) (Schneider, 1866, p. 316).

Length of time required for embryonic and larval development, and by one generation from egg to egg.

We have seen above that recently hatched larvae, measuring 0·25–0·46 mm., have been found in the leaf of a plant exposed to infection for only five days, older larvae, 0·65, and young adults after only ten days. We may therefore conclude that embryonic development does not occupy more than five days, complete embryonic and larval development not more than ten, and that a generation from egg to egg could be completed in fourteen days, the conditions of temperature being those of a European spring, summer or autumn, with the day temperature not falling below 15° C. (60° F.). This agrees with the observations of Ritzema Bos (1892) on *Tylenchus dipsaci* (*devastatrix*) Kühn. As in this species, and in contrast to *T. tritici*, many generations can thus succeed each other in the course of one year, enormous multiplication may occur in a short period.

Figs. 15, 16. **Aphelenchus phyllophagus** n. sp. Specimens from *Senecio vulgaris*.
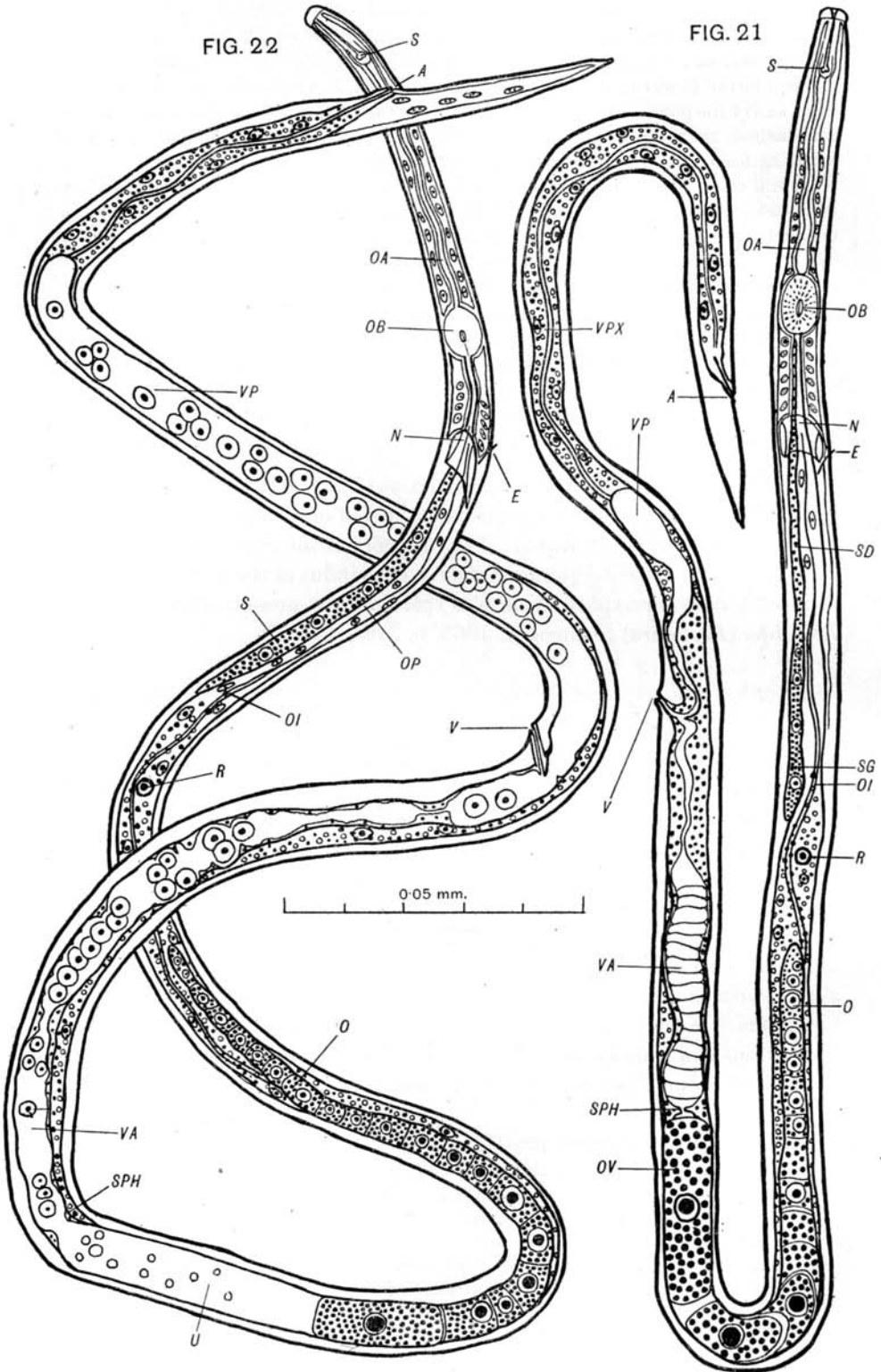
Fig. 15. Egg from leaf axil, 28th day. Outline.

Fig. 16. Larva, 0·65 mm., from mesophyll, 10th day.

Fig. 17. Larva, 0·22 mm., from leaf axil, 28th day.

Fig. 18. Egg from leaf axil, 28th day.

Fig. 19. Larva, 0·465 mm., from mesophyll, 5th day.

Fig. 20. Young female, 0·8 mm., posterior third of body, from mesophyll, 13th day.

FIG. 22

FIG. 21

0·05 mm.

VI. *A. OLESISTUS* R.B. 1893. ANATOMY. (Figs. 21, 23, 24.)

Measurements. ♀ L. 0·529 mm., B. 0·011 mm., *a* 48, (β 8), γ 20. ♀ L. 0·574 mm., *a* 44, (β 11), γ 19. ♂ L. 0·5 mm., *a* 50, (β 8·87), γ 15.

Cobb's formula: ♀ $\dfrac{1\cdot8 \quad (9) \quad 11\cdot8 \quad ? \quad 70 \quad 95}{1 \quad (1\cdot8) \quad 2 \quad ? \quad 2\cdot3 \quad 1\cdot2}$ 0·574.

In general anatomy, this species, as well as *A. fragariae*, so strongly resembles *A. phyllophagus*, that it is necessary to refer to a few points only. *Cuticular striae* are not visible. *Spear*, 0·01 mm. *Excretory system*, the renette cell (*R*) lies in the same position on the left side; the excretory pore (*E*) is however at the level of the nerve ring. *Reproductive system*: female, the structure is naturally more difficult to distinguish than in the larger species; in the specimen figured the uterus is occupied by a large cylindrical egg without a shell; the sphincter at the junction of uterus and anterior vagina is well developed; the spermatozoa occupying the anterior vagina are arranged in a rouleau. (The posterior vagina extends further back than is shown in Fig. 21, to the point marked *VPX*.)

Male spicules (Fig. 24). Length, 0·013 mm.; no accessory piece, no caudal papilla.

VII. *A. FRAGARIAE* R.B. 1891. ANATOMY. (Figs. 22 and 25–29.)

Measurements. ♀ L. 0·723 mm., B. 0·012 mm., head to posterior margin of bulb, 0·065 mm.; tail, 0·038 mm., *a* 60, (β 11), γ 19.

Cobb's formula: ♀ $\dfrac{1\cdot6 \quad (9) \quad 11 \quad ? \quad 69 \quad 93}{0\cdot8 \quad (1) \quad 1\cdot1 \quad ? \quad 1\cdot6 \quad 1}$ 0·723.

This is the most slim of the three species, especially as regards the oesophageal region of the body. Compare breadth at the nerve ring, 1·1 per cent. with 2 per cent. in *A. olesistus*.

Spear, 0·01 mm. *Oesophageal bulb* conoid to cylindrical (Figs. 25, 26). *Rectum* (Fig. 27), long, nearly equal to the length of the tail. The anterior lip of the *anus* is prominent (Figs. 27, 28). *Excretory pore* at the level of the anterior margin of the nerve ring. *Female reproductive system*: the uterus is well-defined (Fig. 22, *U*), and is marked off from the anterior vagina by the sphincter (*SPH*); the anterior and posterior vaginae are of equal length. *Spicules* of the male (Fig. 29), 0·014 mm.; no accessory piece, the accessory of Ritzema Bos being again the anterior margin of the spicule.

VIII. COMPARATIVE ANATOMY OF (1) THE OESOPHAGUS AND SALIVARY GLANDS, (2) THE RENETTE, (3) THE VAGINAE, IN THE GENUS *APHELENCHUS*.

1. THE OESOPHAGUS AND SALIVARY GLANDS. In this genus the *oesophagus* is usually described as terminating at the bulb, and as joining the intestine at that point. The following facts, however, prove that the section of the alimentary canal between the bulb and the point of commencement of the intestinal droplets and granules (Figs. 1, 16–19, 21 and 22, *OI*) is morphologically the posterior oesophagus: (1) it is embraced by the nerve ring; (2) it is

Fig. 21. **A. olesistus** R.B., adult female from cabbage seedling infected 14 days previously from *Lomaria ciliata*. Fixed in Bouin's sol., mounted in glycerine jelly. (Note: The posterior vagina extends to the point *VPX*.)

Fig. 22. **A. fragariae** R.B., adult female from bud of strawberry plant; fixed in Bouin's sol., stained with haemalum, and mounted in balsam.

Parasitic Aphelenchi
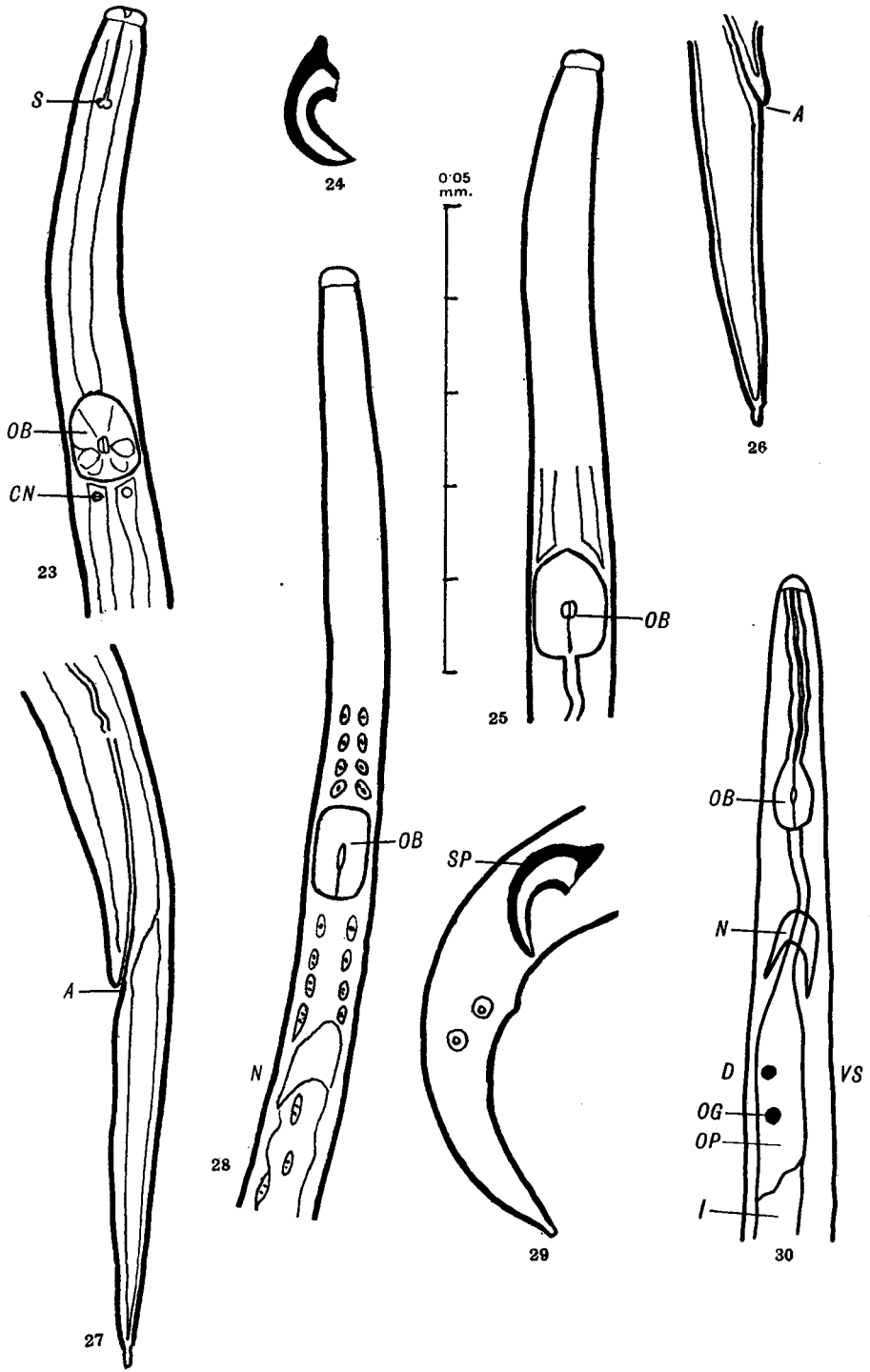
Figs. 23–30.

clothed by the oesophageal cellular collar; (3) its walls are eosinophil in contrast to the basophil intestine; (4) its lumen is cylindrical, that of the intestine is flattened. The lack of a definite line of demarcation from the intestine is due to the separation of the oesophageal glands from the body of this portion of the oesophagus, which will be considered in the next paragraph.

The *salivary glands* have been described in *Tylenchus similis* Cobb, by that writer (1915). They resemble those of *Aphelenchus phyllophagus, olesistus,* and *fragariae.* (Cobb has, however, traced their duct through the substance of the bulb and anterior oesophagus to an opening at the base of the spear.) The posterior oesophagus in *Tylenchus similis* also resembles that of the *Aphelenchi,* and differs from the corresponding organ of other *Tylenchi, e.g. T. dipsaci* Kühn, in which it is thick, club-shaped, and glandular, and is sharply marked off from the intestine (Fig. 30). In *T. dipsaci* this posterior bulb contains several large nuclei in its dorsal wall, while no salivary glands are present. It is therefore a reasonable supposition that the posterior oesophageal bulb of the *Tylenchi* (less *T. similis*) represents, morphologically and physiologically, the combined posterior oesophagus and salivary glands of *Aphelenchus* and *T. similis;* in other words, the oesophageal glands of *Tylenchus,* which are situated in the dorsal sector of the posterior bulb, have, in *Aphelenchus* and *T. similis,* separated themselves from the oesophagus to form the salivary glands.

Considering further the anatomy of the oesophagus and its glands, in other nematode genera, we find three glands, one in each longitudinal sector of the organ, which open by three ducts into the alimentary canal (*Thoracostoma* and *Cylicolaimus* Jägerskiöld, 1901; *Oncholaimus* Stewart, 1906; *Ascaris,* adult, Jägerskiöld; *Agchylostoma* Looss, 1911). The dorsal gland is, however, always more important than the two subventrals, is longer, and stains more deeply (Jägerskiöld, 1901, p. 14). Finally on examining the larva of *Ascaris lumbricoides* (Stewart, 1921), we find that these three glands originate from a single giant nucleus in the hind end of the dorsal sector, which, as growth proceeds, expands downward into the subventral sectors. We therefore have in series (1) *Aphelenchus* and *T. similis* with the glands separate from the oesophagus, on its dorsal surface, as the "salivary glands"; (2) *Tylenchus* (less *T. similis*) and the larvae of *Ascaris* to the seventeenth day, with

Figs. 23, 24. **A. olesistus** R.B.
Fig. 23. Male, outline of oesophageal region.
Fig. 24. Male, spicule.
Figs. 25–30. **A. fragariae** R.B.
Fig. 25. Male, outline of oesophageal region.
Fig. 26. Female, outline of tail.
Fig. 27. Female, outline of tail.
Fig. 28. Female, outline of oesophageal region.
Fig. 29. Male, outline of tail.
Fig. 30. **Tylenchus dipsaci (devastatrix)** Kühn, from clover, fixed in 70 per cent. alcohol, mounted in glycerine jelly.

the glands in the dorsal sector; (3) *Ascaris* larva of the nineteenth day with glands growing from the dorsal sector into the two subventrals; (4) the great majority of adult nematodes with the typical three oesophageal glands.

2. THE RENETTE lies between the left lateral line and the intestine in *Tylenchus similis* Cobb (1915), and the three species under consideration. In the development of *Ascaris lumbricoides*, it originates in the same position, and thence gives rise to the apparently bilateral organ of the adult Ascarids. This origin of the excretory cell from the left lateral line is striking, and may prove to be of very general occurrence. Without laying too much stress on the homology, it may be recalled that the great, unicellular, skin glands of both lateral lines are the excretory organs of *Cylicolaimus magnus* Villot, *Thoracostoma acuticaudatum* Jägerskiöld (Jägerskiöld, 1901), and of the adult female of *Oncholaimus vulgaris* Bast. (Stewart, 1906).

3. THE VAGINAE are derived, as we have seen (p. 167) from a superficial cell group, which gives rise to them only, and not to the remainder of the reproductive tube—the gonad proper. They are probably ectodermal, their walls are not endothelial, and their junction with the uterus is marked by a sphincter. The corresponding sphincter is situated at the junction of the vagina and uterus in *Cylicolaimus*, *Thoracostoma* (Jägerskiöld, 1901), and *Oncholaimus* (Stewart, 1906), in which genera the vaginae are much shorter than in *Aphelenchus*. In *Cylicolaimus* and *Thoracostoma* sperm is permitted to pass the sphincter, and the uteri function as receptacula; in *Aphelenchus* the vaginae are large enough to accommodate the whole sperm mass; in *Oncholaimus*, where sperm is not allowed to pass into the uterus, although the vagina is too small to contain it, the surplus is drained off into the intestine by the gonenteric canals (Stewart, 1906).

The posterior vagina is the only portion of the posterior reproductive tube (of *e.g. Tylenchus*) which persists in *Aphelenchus*.

IX. BIOLOGY OF THE PARASITIC *APHELENCHI*.

The following experiments and observations were made principally on the species *A. phyllophagus* and *olesistus*. It may, however, be assumed that the three species are indentical in their general mode of life.

A. DEFINITIVE HABITAT OF THE PARASITE.

Aphelenchus fragariae. The strawberry plants received by Ritzema Bos (1891) were not fresh, and he was therefore not able to make satisfactory observations on the situation of the parasite. Marcinowski (1908) found them to be mainly ectoparasitic, in the leaf axils, and in the flowers among the stamens, during the month of May; only a few were endoparasitic, in brown patches of leaf sheaths. In June, however, they were chiefly endoparasitic, in leaves and stem. She notes that apparently healthy runners contained *Aphelenchi* in the bud.

In specimens sent to me in January I found adult and larval *A. fragariae*, in large numbers as ectoparasites under the scales of the bud. In serial sections (Fig. 32), they are seen lying among the hairs on the surface of the growing point.

The definitive habitat of *A. olesistus* and *phyllophagus*, that in which feeding and reproduction proceed most actively, and in which their pathological effects are manifest, is in the mesophyll spaces of leaves; they are never
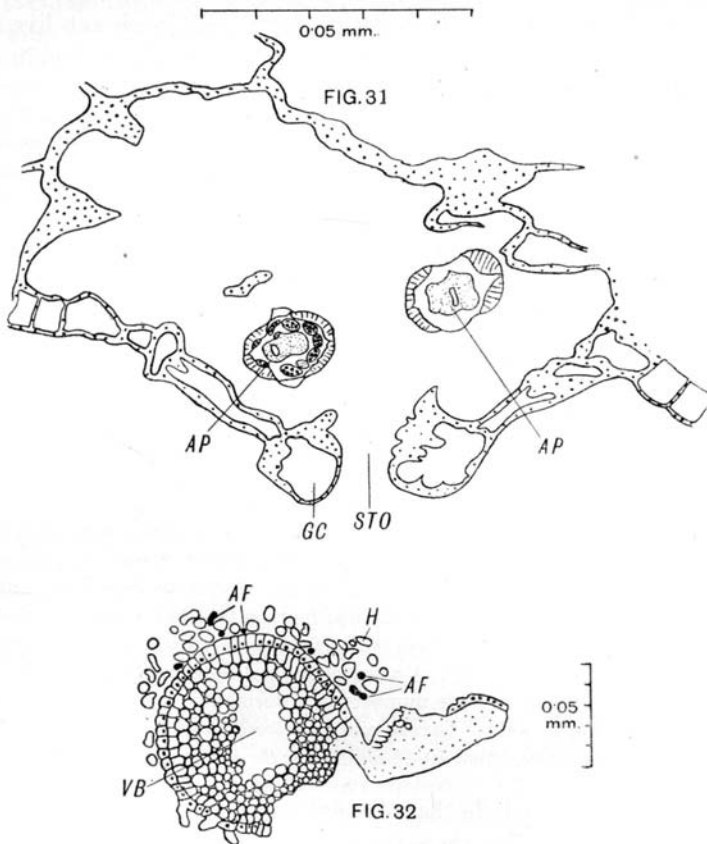
Fig. 31. Chrysanthemum leaf, transverse section through a stoma, showing **A. phyllophagus** in a mesophyll space.

Fig. 32. Strawberry plant, transverse section through the bud, showing **A. fragariae** on the surface.

intracellular, and avoid tissues without spaces (Ritzema Bos, 1893; Molz, 1909); they occur frequently close to the stomata.

MIGRATION IN THE TISSUES OF THE LEAF. Ritzema Bos (1893) believes that *A. olesistus* travels in or along the outside of the nerves. Molz (1909) denies this, and asserts that migration only occurs through the spaces of spongy tissue, and on the surface; he finds the vessels of chrysanthemum leaves to be too narrow to admit the parasite; the animal wanders out of the

diseased patches in search of fresh nourishment for itself and its progeny; eggs are found laid only in healthy tissue, consequently the gravid female has the greatest impulse to travel.

Adults and larvae *reach the soil* by the fall of the diseased leaves, bringing the life cycle of the race[1] to the resting stage in the soil.

B. THE RESTING STAGE IN THE SOIL.

Marcinowski (1910) asserts that the parasitic *Aphelenchi* can live only for a very short period in the soil, in fact that they only enter the soil in order to pass directly from a fallen leaf to a new host plant.

She placed portions of infected leaves on the earth of a pot, which was kept damp, and examined the earth from the second week onward—result negative. On the other hand, earth in which an infected *Begonia* had grown, proved infectious when placed on leaves of begonias.

Ritzema Bos (1892) showed that *Tylenchus dipsaci* can persist in the soil only on the surface, where it can undergo partial drying, and so enter a dormant condition. I found that adult *A. phyllophagus* can live actively in water for at least six days. Early death in damp soil may be due to mycosis.

Molz (1909) apparently believes that *A. phyllophagus* resides in the soil.

I have made the following observations to test the power of survival in earth:

Portions of infected leaves of *Chrysanthemum* and *Lomaria ciliata* were placed on earth in pots, which were watered daily, the surface of which, however, remained dry except for a period not exceeding two minutes each day. On the *7th and 9th days* many adults, male and female, were found on the surface of the leaf fragments, a few actually in their tissues.

On the *16th, 17th, 20th* and *46th days the surface layer of earth* was examined. It was quite dry at the time, and had been at a temperature below the freezing point at night. Adult *Aphelenchi* were found in large numbers in a dormant condition, but they resumed activity after immersion in water. Larvae and eggs were looked for on the 46th day, but were not found. *In earth taken from 10–15 mm. below the surface* no *Aphelenchi* were found.

We can therefore conclude that *A. phyllophagus* and *olesistus* reaching the soil in fallen leaves, can live there for at least 46 days. Like *T. dipsaci*, they collect on the surface of the earth, where they suffer partial desiccation, and pass into a condition of suspended vitality. In the case of *T. dipsaci*, it is the larvae only which survive in this manner, while in *Aphelenchus* on the contrary the adults survive, and it is doubtful whether the larvae do so (they probably grow to adults before abandoning the fallen leaves).

---

[1] It should be clearly realised that the life cycle in *Aphelenchus* and *T. dipsaci* is that of the race, while in *T. tritici* it is that of the individual; in other words in the cycle from (*a*) the definitive habitat, through (*b*) the resting stage in the earth, and (*c*) the stage of immigration, many generations are completed in the former, only one generation in the latter; also many cycles can be completed in one year in the former, only one in the latter. Hence the enormously greater power of multiplication in the former than in the latter.

We now proceed to the consideration of:

C. THE ROUTE TAKEN BY THE PARASITE ON THE HOST DURING IMMIGRATION FROM THE SOIL.

There are two views on this subject: (1) that the parasite, having entered the host plant below the level of the ground, ascends through the tissues of the stem to the leaves (Ritzema Bos, 1893, and Marcinowski, 1908–1910); (2) that it enters through the surface of the leaf, having reached this situation by travelling over the surface of the plant.

(1) Ritzema Bos (1893) found *Aphelenchi* in apparently healthy leaf-stalks of *Begonia* and *Asplenium*. In an experiment with an infected *Pteris*, he cut off all the parts of the plant above ground; the young fronds which grew up contained *Aphelenchi*, and he concludes that they came from the interior of the rhizome; it is, however, equally probable that they came from the surface of the soil. Marcinowski (1908) found a few *Aphelenchi* in the stem of a plant. The two observations of *Aphelenchi* in the stem appear to have been made by dissection or teasing; in such a method it must be borne in mind, that worms lurking on the surface may appear in the medium, and it may be thought that they have issued from the tissues. I have found a few *Aphelenchi* on teasing up portions of flower stalk, but on cutting serial sections have never found them actually in the stalk or stem; in one case I divided a groundsel stem longitudinally in halves, one half was teased out in water, and numerous adults and larvae found, the other half was cut into serial sections, but no *Aphelenchi* were found in the tissues, although several appeared on the cut surface.

The following observations also are offered to this view: the parts of an infected plant below the leaves are healthy (Klebahn, 1891); plants placed among infected leaves, if protected by a ring of vaseline around the stem, remain free of the parasite (Marcinowski, 1908); if all diseased leaves are removed from a plant, the buds growing out of the axils remain free (Molz, 1909).

We can therefore conclude that *Aphelenchi*, in their invasion of the host plant, do not traverse the tissues of the stem.

APHELENCHI *in the soil, revived by moisture, and attracted by a suitable plant, wander on to it, and may live for some time as ectoparasites in the leaf axils before reaching their definitive habitat in the mesophyll.*

In order to trace the course of immigration, I placed a number of young plants of groundsel (*Senecio vulgaris*) in pots of earth, on the surface of which infected leaves of *Chrysanthemum* and *Lomaria* had been strewn. The pots were kept in a room at a temperature of 10–15° C. (50–60° F.), were freely exposed to the sun, and were watered once daily. Entire plants or leaves were examined at intervals with the following results:

2nd day. Four entire plants negative.

3rd day Three plants examined. Plant (*a*): on syringing out the leaf axils with water three adult males and four females were found; in a leaf touching the ground—one adult female. The earth in this case had been infected 44 days previously, and had been bare of vegetation for some hours before the planting of plant (*a*). Plant (*b*): in a leaf axil one adult, on a leaf one larva, 0·305 mm. long. The earth had been infected 43 days previously, a chrysanthemum which bore ectoparasitic *Aphelenchi* was growing in the same pot. Plant (*c*): negative.

5th day. Lower leaves from four plants. (*a*), (*b*) and (*c*) were negative. (*d*): in the tissues of one lower leaf four larvae found. 0·25, 0·3, 0·32, 0·465 mm. long; soil infected 45 days previously; the leaf axils were unfortunately not examined, but it is probable that they contained adults which were the source of these larvae, a chrysanthemum was however also growing in the pot. *10th day.* In a low growing leaf many adults and larvae found; the pot had been infected 41 days previously, and had been bare of vegetation for three days before the planting of this specimen, the adults must therefore have lived at least three days, and almost certainly 31 days in the soil before attacking this plant. *8th, 13th and 26th days.* Adult *Aphelenchi* in the leaf axils or lower leaves. *28th day.* Adults, larvae and eggs found in the leaf axils of the lower 20 mm. of the stem, adults and larvae also in the tissues of the lower leaves.

Marcinowski (1910) with begonias planted in infected soil, found that leaves in contact with the earth were invaded.

We can therefore conclude that adults invading a plant from the soil may take up ectoparasitic life in the leaf axils up to a height of at least 2 cm. above ground, and may here deposit eggs; the larvae hatched from them then proceed to the more extended invasion, and to their definitive habitat in the mesophyll. On the other hand, leaves close to the ground level may be invaded direct from the soil, the worms becoming entoparasites forthwith.

This brings us to the passage from ecto- to entoparasitic life, and the mode of entry into the spaces of the mesophyll:

D. *APHELENCHI* HAVING REACHED THE LEAF SURFACE ENTER THE
MESOPHYLL SPACES THROUGH THE STOMATA.

This statement rests on the following observations: (1) *Aphelenchi* from orchid leaves, dropped in water on the ventral surface of leaves of an inverted *Begonia*, entered the leaves; application of infected to healthy leaves gave the same result (Marcinowski, 1908). (2) *Aphelenchi* have been observed traversing the stomata (Osterwalder, 1902 [quoted by Marcinowski], Marcinowski, 1908).

Molz (1909) held that the worms did not enter through the stomata, but through wounds of the plant surface, on the ground that (1) the stomata were too small to admit the parasites, and (2) leaf to leaf infection succeeded only with infected leaves. On the other hand, (1) my measurements of *Chrysanthemum* stomata give larger apertures (viz. 0·02 × 0·01 mm., and the shorter diameter can be increased by the forcible abduction of the guard cells), and the size of the parasite varies with the size of the stomata (*vide* bottom of p. 162); and (2) the negative result of Molz's leaf to leaf infection is explained by conditions of atmospheric humidity (Marcinowski, 1910, *vide infra* (E)).

E. INFLUENCE OF ATMOSPHERIC HUMIDITY, AND OF PREVIOUS DISEASE
OR INJURY OF THE HOST PLANT, IN ASSISTING MIGRATION
OF THE PARASITE. IMMUNITY.

Marcinowski (1908, 1910) records three important experiments on the influence of damp on the migration of *Aphelenchi*. (1) A portion of infected chrysanthemum leaf was mounted, ventral surface uppermost, in water on a slide; many worms were observed to creep out through the stomata. (2) If infected plants are placed in a humid atmosphere the parasites wander out, and adopt ectoparasitic life in the leaf axils. (3) Leaf to leaf infection experiments in begonias succeed in a humid atmosphere under a bell jar, but fail in the open air.

When chrysanthemum leaves are moistened by heavy dew, adult *Aphelenchi* can be observed (under a binocular microscope) wandering freely over their hairy surface (Molz, 1909).

High humidity assists migration by approximating the conditions of life on the plant surface to those prevailing in the mesophyll spaces. It is not, however, essential to migration (*vide* p. 175, and since *Aphelenchus* disease prevails in the open air).

Sorauer (1902) maintains that *Aphelenchi* cannot invade healthy plants, that a disease of the vascular bundles is produced in pot plants grown at a temperature above the optimum, and that *Aphelenchi* enter such diseased plants only. Marcinowski (1908), on the other hand, infected healthy begonias in a natural manner.

Aphelenchi are attracted by cell sap. I found that they collect in considerable numbers in plant wounds. Molz's theory of entry through wounds is doubtless true in a limited sense; the injuries to which he refers are caused by insect parasites, and by the eruption of axillary buds.

Certain races of many species of flowering plants are immune to the parasite (see *litt. passim* and Molz, 1909).

X. METHODS OF COMBATING THE DISEASE.

Chemical treatment is of use only in such plants as Everlasting (Mangin, 1895), if the blotching appears after the harvest, when the flowers are stored in warehouses. Exposure to a dry atmosphere saturated with carbon bisulphide for 24–48 hours, kills the parasites without affecting the appearance of the plants. In no other plants has chemical treatment of any kind been found of avail, since chemicals of sufficient strength damage the appearance of the plant.

Treatment by heat. Marcinowski (1910)—immersion of the plant in water at 50–52° C. for five minutes, kills the parasite but not the plant.

Prophylaxis based on the habitat of the parasite in the surface of the soil. Earth for filling pots or boxes should be dug from a pit, surface earth being rejected. For open air culture deep ploughing of infected fields may prove useful. All infected leaves and plants should be burned. If it is necessary to use infected stock plants, they should be treated by the hot-water method, or by burying to a depth of at least three inches—after daily watering for at least a week, the surface earth should be removed, after which it is unlikely that any *Aphelenchi* will remain on the plant.

XI. TECHNIQUE.

Fixatives. Carnoy's chloroform-acetic-alcohol mixture, Schaudinn's or Bouin's fluid, and boiling 70 per cent. alcohol are satisfactory; the first-named gives the most clear definition.

Stains for mounting *in toto* haemalum differentiated with acid 70 per cent. alcohol, following Carnoy or Schaudinn is best; after Bouin, picrocarmine gives the best stain.

Mounting. Looss' alcohol-glycerine evaporation method, followed by glycerine jelly for unstained specimens. For stained specimens transfer to xylol, and add canada balsam very gradually on several successive days.

Embedding in paraffin. The tissue should be transferred to chloroform, paraffin (60° C. M.P. for vegetable tissues) added to saturation, the chloroform slowly evaporated in an incubator for 24 hours. Transfer to two baths of melted paraffin for 5 and 10 minutes respectively.

Staining sections. Thionin and eosin give the best results.

<div align="center">SUMMARY.</div>

There are three species of true parasites in the genus *Aphelenchus*, viz.: *A. fragariae* R.B., 1891, *A. olesistus* R.B., 1893, and *A. phyllophagus* n.sp. *A. ormerodis* R.B., 1891, is a doubtful species, possibly a young form of *A. fragariae* (Marcinowski, 1908).

A. fragariae causes the disease strawberry bunch; *A. olesistus* and *phyllophagus* cause leaf disease in flowering plants and ferns. The diseases are widely distributed and of considerable economic importance.

From the study of the anatomy of the three species certain points of general importance emerge: (1) The salivary glands of *Aphelenchus* represent the oesophageal glands of other nematode genera, which have separated from the body of the oesophagus. This separation causes the reduction in size of the posterior portion of the oesophagus, and its lack of clear demarcation from the intestine. (2) The excretory organ of *Aphelenchus*, and of some other nematode genera, originates from the left lateral line. (3) The vaginae are ectodermal organs distinct in origin from the gonads proper.

The life cycle of the parasitic *Aphelenchi* is divided into three definite stages: (A) That of residence in the definitive habitat, which for *A. fragariae* is in the stem and leaves of the strawberry plant, for *A. olesistus* and *phyllophagus* in the mesophyll spaces of the leaves of many plants. Nutrition and reproduction are most actively carried on in this situation, several or many generations succeeding each other, and here the pathological effects are manifest. (B) The resting stage in the soil. The worms reach the soil in fallen leaves, and the adults survive in a partially dried, dormant condition on the surface of the soil for prolonged periods. (C) The stage of immigration into the host plant. When revived by moisture the *Aphelenchi* may be attracted by a suitable plant, and wanders on to it; they may live as ectoparasites in the leaf axils, breeding in this situation, the larvae migrating to the definitive habitat; or they may enter the definitive habitat direct. They do not traverse the tissues of the stem during immigration. *A. olesistus* and *phyllophagus* enter the mesophyll spaces through the stomata.

It should be noted that this life cycle is that of the race, not of the individual. It comprises many generations, and in this respect resembles that of *Tylenchus dipsaci* and differs from that of *T. tritici*, which includes one generation only.

In the life of the individual *Aphelenchus*, embryonic development occupies not more than five days, embryonic and larval development not more than ten, and a complete generation not more than fourteen days.

High atmospheric humidity assists the migration of the worms, previous disease or injury of the host plant may do so. Some races of various species of plants are immune.

Treatment of the affected plants by chemical methods is of limited applicability. Treatment by immersion in water at 50–52° C. for five minutes is recommended by Marcinowski (1908). Prophylaxis should be based on burning of infected plants and leaves, and on the avoidance of infected surface soil in the filling of pots and boxes.

REFERENCES.

ATKINSON (1890). Nematode Leaf Disease. *Insect Life*, IV. 31.

CATTIE, THEODOR (1901). Kleiner Beitrag zur Kenntnis der Älchenkrankheit der Farnkräuter. *Zeitschr. f. Pflanzenkrankh.* XI. 34.

CHIFFLOT (1902). Sur l'origine de certaines maladies des Chrysanthèmes. *C.R. Acad. Sci.* Paris, CXXXIV. 196.

COBB, N. A. (1891). Strawberry bunch. *Agric. Gaz. N. S. Wales*, II. 390.

—— (1915). *Tylenchus similis* in Sugar Cane and Bananas. *Journ. Agric. Res.* IV. 561.

HOFER, J. (1901). Nematodenkrankheit bei Topfpflanzen. *Zeitschr. f. Pflanzenkrankh.* XI. 34.

JÄGERSKIÖLD, L. A. (1901). Weitere Beiträge zur Kenntniss der Nematoden. *K. Svensk· Vetensk. Akad. Handl.* XXXV.

KLEBAHN, H. (1891). Zwei vermutlich durch Nematoden erzeugte Pflanzenkrankheiten. *Zeitschr. f. Pflanzenkrankh.* I. 321.

LOOSS, A. (1911). The anatomy and life history of *Agchylostoma duodenale* Dub. Pt II. *Rec. School of Med. Cairo.*

MAN, J. G. DE (1884). *Nematoden der Niederländischen Fauna.*

MANGIN, L. (1895). La Maladie de la Rouille. *C.R. Soc. Biol.* ser. 10, II. 749.

MARCINOWSKI, K. (1908). Zur Kenntnis von *Aph. Ormerodis* R.B. *Arb. der Kais. Anst. f. Land u. Forstw.* Berlin, VI. 407.

—— (1910). *Ibid.* VII. 1.

MOLZ, E. (1909). *Aph. olesistus* und Älchenkrankheit der Chrysanthemum. *Centr. Bakt. u. Parasit.* Orig. 2 Abt. XXIII. 656.

OSTERWALDER, A. (1901). Nematoden als Feinde des Gartenbaues. *Gartenflora*, L. 337.

—— (1902). Nematoden an Freilandpflanzen. *Zeitschr. f. Pflanzenkrankh.* XII. 338.

—— (1904). *Ibid.* XV. 43.

RITZEMA Bos (1891). L'Anguillule de la Tige. *Tylenchus devastatrix* Kühn. *Arch. du Musée Teyler.*

SCHNEIDER, A. (1866). *Monographie der Nematoden.*

SORAUER, P. (1901). Die Älchenkrankheit bei *Chrysanth. indicum. Gartenflora*, L. 35.

—— (1902). Zur Wurmkrankheit der Begonien. *Zeitschr. f. Pflanzenkrankh.* XII. 189.

SMITH, WORTHINGTON (1890). *Gardeners' Chronicle*, II. 298.

STEWART, F. H. (1906). Anatomy of *Oncholaimus vulgaris* Bast. *Quart. Journ. Micr. Sci.* L. 101.

—— (1921). Life history of *Ascaris lumbricoides* L. *Parasitology*, XIII. 37.

REFERENCE LETTERS.

A, anus; *AF*, *Aphelenchus fragariae*; *AP*, *A. phyllophagus*; *CA*, anterior section of oesophageal collar; *CN*, coilar nucleus; *CP*, posterior limit of collar; *D*, dorsal surface; *E*, excretory pore; *GC*, guard cell of stoma; *H*, hair; *I*, intestine; *IL*, intestinal lumen; *LL*, lateral line; *N*, nerve ring; *O*, ovary; *OA*, anterior oesophagus; *OB*, oesophageal bulb; *OE*, endothelium of gonad tube; *OG*, oesophageal gland; *OI*, junction of oesophagus and intestine; *OP*, posterior oesophagus; *OV*, ovum; *P*, papilla; *R*, renette cell; *RE*, rectum; *S*, spear; *SCY*, spermatocyte; *SD*, salivary duct; *SG*, salivary gland; *SGO*, spermatogonium; *SO*, opening of salivary duct into bulb; *SP*, spicule; *SPH*, sphincter; *ST*, spermatid; *STO*, aperture of stoma; *SZ*, spermatozoon; *U*, uterus; *V*, vulva; *VB*, vascular bundle; *VA*, anterior vagina; *VP*, posterior vagina; *VS*, ventral surface.