Post-Infection Development of Heterodera lespedezae[1]

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Abstract: Morphological changes occurring during post-infection development and the influence of temperature on the life cycle of *Heterodera lespedezae* are reported. Morphological development was similar to that of *H. schachtii*. Each post-infection stage had a distinct stylet, and fed actively. Detailed observations were made of cuticle formation and markings, esophageal glands, and reproductive system. Certain developmental phases, such as matrix deposition and oviposition, appeared to be correlated with color changes of the adult female body. The effect of temperature on nematode development was observed in a phytotron at day/night temperatures of 18/14, 18/18, 22/18, 26/22, 26/26, and 30/26 C; the optimum was 26/26 C. More time was required to complete the life cycle at the three lower temperatures than at the three higher temperatures. *Key words:* morphology, lespedeza, temperature effect, phytotron.

The lespedeza cyst nematode, *Heterodera lespedezae* Golden and Cobb, was found in North Carolina in 1960 (5) and later in Illinois (3) and Tennessee (9). It may play an important role in the decline of annual lespedezas (*Lespedeza striata* Hook. and Arn., and *L. stipulacea* Maxim.). Life cycle studies demonstrated that at greenhouse temperatures of approximately 24 C, lemon-shaped adult females developed in 20-22 days following infection, and egg deposition occurred after 36-38 days (6). Little else is known about the biology of this nematode.

Our investigations were undertaken to characterize morphologically the developmental stages, and to determine the influence of

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temperature on development of *H. lespedezae* in a susceptible host.

MATERIALS AND METHODS

Stock cultures of *H. lespedezae* were obtained from infested field soil and maintained on 'Kobe' lespedeza (*L. striata*) in a greenhouse at 25-30 C. Cyst-infested soil from these cultures was stored up to 6 weeks in a cold chamber at 10 C, then held at 26/22 C for 24 hr before being used as a source of inoculum.

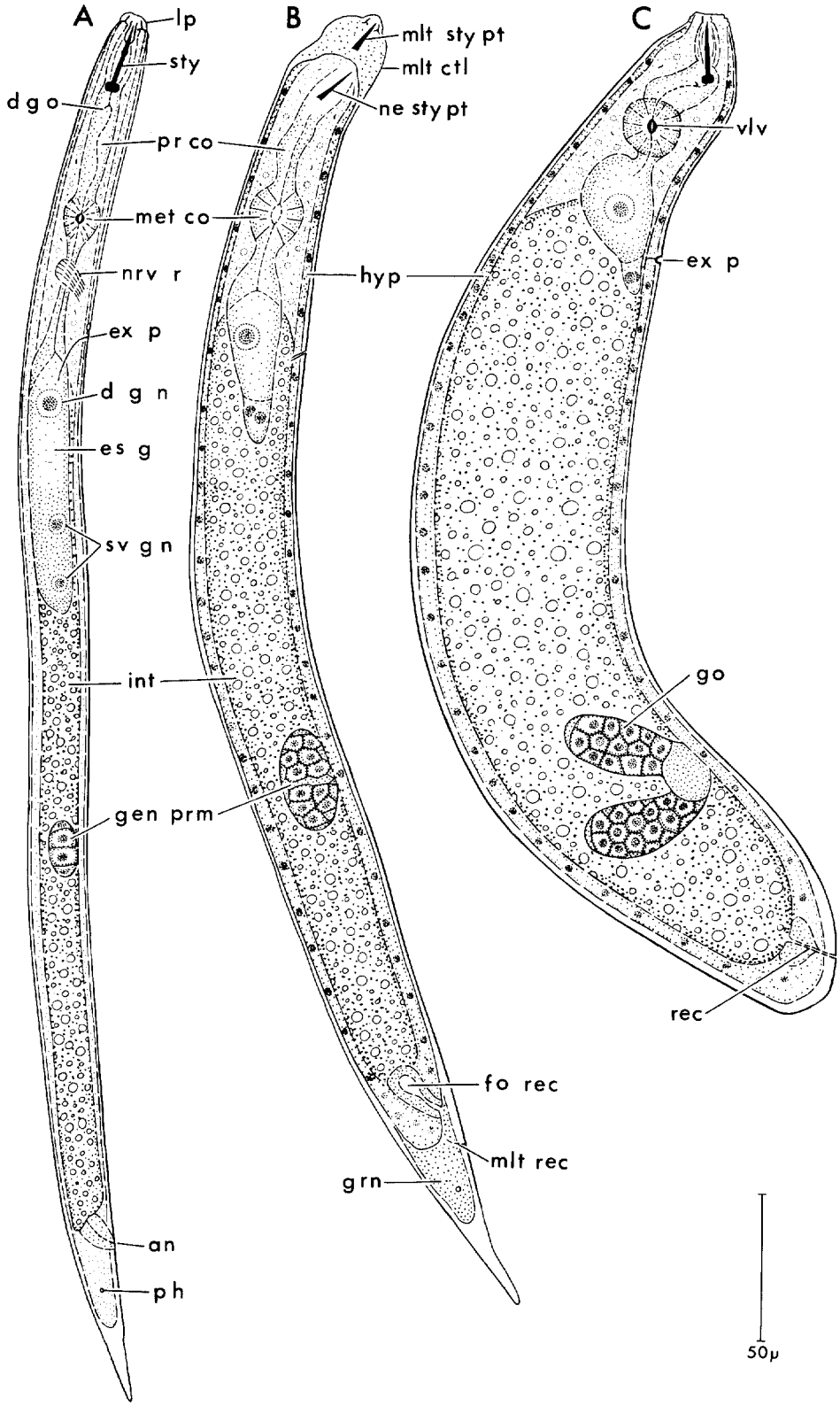
Nematode development was studied at day/night temperatures of 18/14, 18/18, 22/18, 26/22, 26/26, and 30/26 C in the Southeastern Plant Environment Laboratories, North Carolina State University Unit (phytotron). Plants were grown under 9-hr days (4000-4500 ft-c), and the dark periods were interrupted by 3 hr of weak light (300 ft-c) to prevent flowering. Four-week-old 'Kobe' lespedeza seedlings were transplanted into 6.5-cm pots filled with cyst-infested soil and kept at 26/22 C. Ten hours later, the seedlings were washed free of soil, transplanted into sterilized fine sand in 10-cm pots, and transferred to temperature chambers in the phytotron. Three plants each were sampled daily from 26/22, 26/26, and 30/26 C, and every 3 days from 18/14, 18/18, and 22/18 C for 38 and 72 days, respectively. Roots were washed free of sand and fixed and maintained in FAA (40% formaldehyde, 6.5 ml; glacial acetic acid, 2.5 ml; 50% ethanol, 100 ml) for 5-11 months. Then they were washed in water for 1 hr, stained with acid fuchsin in lactophenol in an oven at 80 C for 15-25 min (depending on root size and age), and cleared in lactophenol for 2-3 days. The stage of development was determined by studying 50 nematodes dissected from each sample and mounted on slides in lactophenol.

RESULTS

MORPHOLOGY: *Second-stage larva.* Since the morphology of second-stage larvae (Fig. 1-A) has been described by Golden and Cobb (5), only organs and structures undergoing changes during development will be emphasized here. The body is marked by coarse transverse annulations interrupted at the lateral fields. The hypodermis is inconspicuous. The robust stylet has three well-developed basal knobs, slightly concave anteriorly. The elongated esophageal gland lobe overlaps the intestine ventro-laterally, and dorsal and subventral glands differ in texture. The dorsal gland appears clear and light, whereas the subventral glands are granular and dark. The excretory pore is located adjacent to the end of the isthmus. The genital primordium, comprising two central germinal cells bordered by two somatic cells, lies a short distance posterior to the middle of the body. Rectum and anus are distinct. The tail tapers gradually and ends in a point. The small phasmids are located slightly anteriad of the middle of the tail. The hyaline tail terminus begins at about 17-18 annulations posteriad of the anus, and has a slight to conspicuous constriction at six to seven annulations from its beginning.

Second molt. At the beginning of the second molt (Fig. 1-B), the stylet shaft and knobs disappear. The hypodermis and hypodermal nuclei become prominent. The cuticle in the lip region separates, and the conical part of the stylet is shed with it. The area between the old and newly forming cuticle encloses coarse granules. The esophagus shortens; the valve of the metacorpus becomes faint, and the gland lobe is compressed anteriorly. The dorsal gland enlarges, whereas the subventral glands diminish. As molting proceeds, the entire old cuticle is separated from the newly forming cuticle. The conical part, shaft, and knobs of the stylet of the developing third-stage larva are formed. The genital primordium becomes multicellular and oval-shaped, and gradually migrates posteriad. Toward the end of the second molt, the flatly rounded tail of the third-stage larva is formed completely. The esophagus shortens further. The genital primordium elongates and moves posteriad to about three-fourths of the body length and becomes oriented toward the ventral side. Finally, the second-stage larval cuticle is shed.

Third-stage larva. The third-stage larval body increases in size, particularly in width (Fig. 1-C). No cuticular markings are present, except for annulations in the head region. The multi-nucleate hypodermis increases in thickness, particularly posteriorly. The stylet is robust, with distinct knobs. The esophagus further shortens and is pushed forward. The excretory pore located adjacent to the subventral glands is more prominent than in second-stage larvae. It is funnel-shaped and distinctly cuticularized. The genital primordium continues to move posteriad and curves slightly in the middle, resulting later in a "V" shape of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the two developing oval-shaped gonad branches. A well-developed rectum and anus are present.

Third molt. The third molt proceeds in similar manner to the second (Fig. 2-A). The new cuticle has distinct longitudinal striae (Fig. 2-C) which extend over the entire body and cause a serrate appearance of the posterior body. The esophagus remains reduced in length, and the median bulb enlarges. The gonads elongate and migrate posteriorly, approaching the forming rectum. The central part of the gonads lies close to the hypodermis on the ventral side, and ovaries and gonoducts can be differentiated morphologically. Near the completion of the molt, the two gonads increase in length and extend anteriorly more than half the body length. Posteriorly, the central part of the gonads makes contact with the thickened hypodermis just short of the forming rectum.

Fourth-stage larva. The fourth-stage larva increases in size and assumes a flask shape (Fig. 2-B). Cuticle and hypodermis are thicker than in the preceding stages, and the longitudinal cuticular striae begin to break into zigzag lines in the future vulval area (Fig. 2-C). The stylet is robust, with well-developed knobs. The enlarged muscular metacorpus of the esophagus has a strongly sclerotized valve. The gonads further elongate, approach the esophageal gland lobe, and coil slightly before the onset of the fourth molt. Posteriorly, a vaginal primordium (vaginal chamber) becomes distinct, assuming a spherical shape (Fig. 2-B). The well-developed rectum opens at the slightly subterminally located anus, resulting in the disappearance of the tail.

Fourth molt. The main and most rapid change occurring during the fourth molt (Fig. 3) is the growth and coiling of the gonads. At the beginning of the fourth molt, only one coil is formed in each gonad. Near the completion of the molt the gonads coil several times, tending to fill the entire body. The vagina gains access to the exterior by formation of the terminal vulva. The rectum shifts dorsally and completes development. The new cuticle is folded, and the reticulate cuticular pattern begins to form. The old cuticle generally ruptures near mid-body, and parts of it adhere to the adult female for considerable time. Stylet formation is similar to that of previous molts.

Adult female. The female body posteriad to the neck region enlarges considerably and assumes the typical lemon shape with protruding vulval cone (Fig. 4). The cuticular zigzag pattern becomes well defined. The hypodermis is prominent and multinucleate. Stylet and metacorpus are larger than in the larval stages. The number of coils of each gonad increases, and soon the whole body is packed with gonadal convolutions. Egg formation follows shortly. The vagina is long, enlarged anteriorly, and tapers posteriorly towards the vulva. The rectum opens at the distinct anus dorsal and anteriad of the vulva. A gelatinous matrix is extruded through the vulva, and adheres to the vulval cone. The number of eggs deposited in this matrix varies from a few to more than 100. Adult females pass through three color phases; i.e., white, yellow, and brown, and eventually turn dark brown.

TIME REQUIRED FOR DEVELOPMENT: At the three higher temperature combinations of 26/22, 26/26, and 30/26 C, larval development, egg formation, and matrix deposition were uniform and quite rapid, whereas they were less uniform and delayed at the three lower temperatures of 18/14, 18/18, and 22/18 C (Table 1). Striking differences were observed in the time required for the white females to turn yellow and to start egg deposition, as well as for the beginning of egg hatching. These stages were completed much earlier at the 26/22 and 26/26 C temperature combinations than at higher or lower temperatures. Optimum temperature for development of *H. lespedezae* is 26 C, at which

FIG. 1. *Heterodera lespedezae.* A. Second-stage larva. B. Second molt. C. Third-stage larva. Abbreviations for figures: an = anus; ctl = cuticle; d g n = dorsal gland nucleus; d g o = dorsal gland orifice; es g = esophageal gland; ex p = excretory pore; fo rec = forming rectum; fo vag = forming vagina; gen prm = genital primordium; go = gonad; go du = gonoduct; grn = granules; hyp = hypodermis; int = intestine; lp = lip or lip region; lu = lumen; met co = metacorpus of esophagus; mlt ctl = molted cuticle; mlt rec = molted rectum; mlt sty pt = molted conical part of stylet; n = nucleus; ne ctl = new cuticle; ne sty pt = new conical part of stylet; nrv r = nerve ring; ocy = oocyte; ovy = ovary; ph = phasmid; pr co = procorpus of esophagus; rec = rectum; sty = stylet; sty kn = stylet knobs; sv g n = subventral gland nucleus; ut = uterus; vag = vagina; vag ch = vaginal chamber; vlv = valve of metacorpus; vu = vulva.

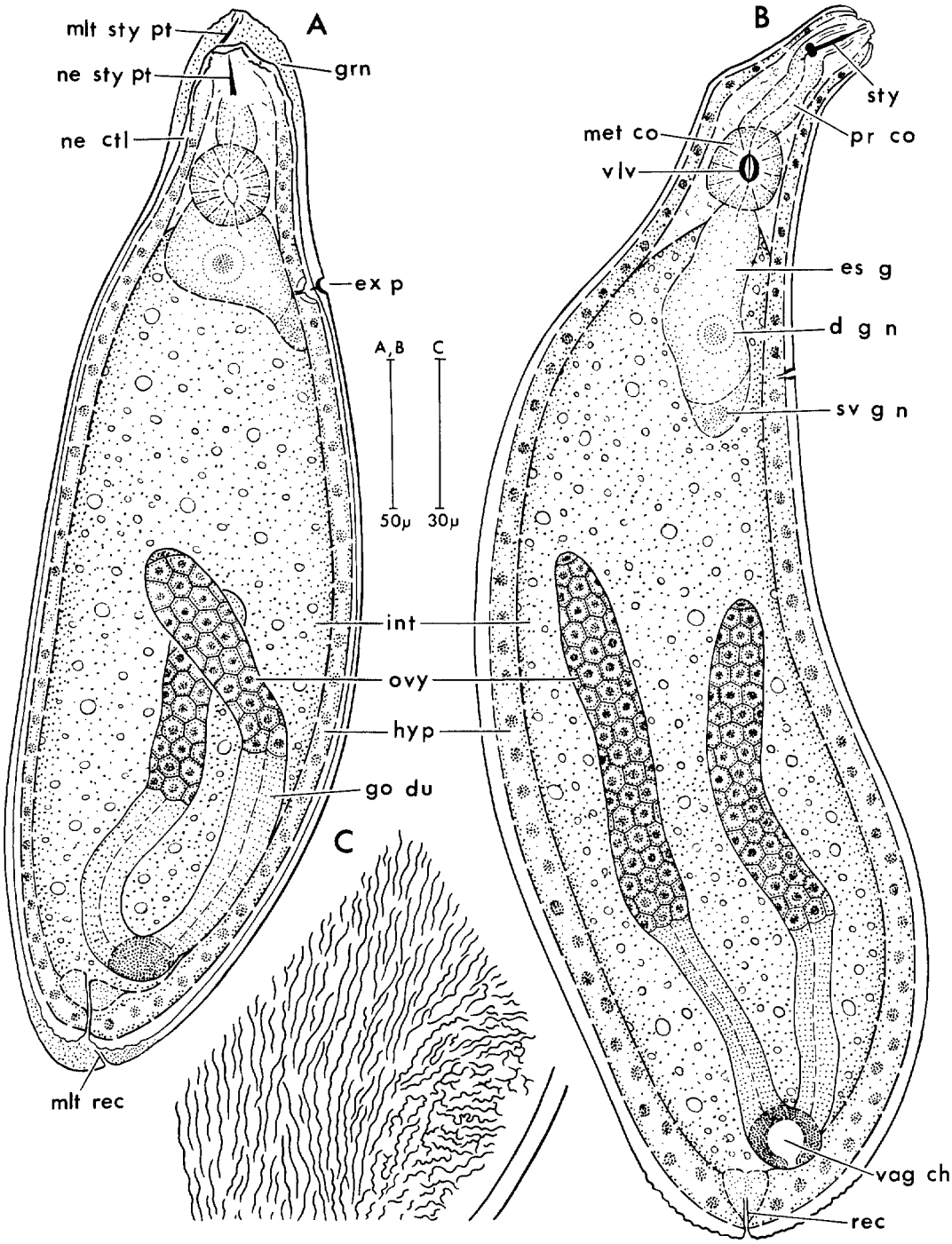

FIG. 2. *Heterodera lespedezae.* **A.** Third molt. **B.** Fourth-stage larva. **C.** Section of longitudinal cuticular striation near posterior end of fourth-stage larva. For definition of abbreviations, see Fig. 1.

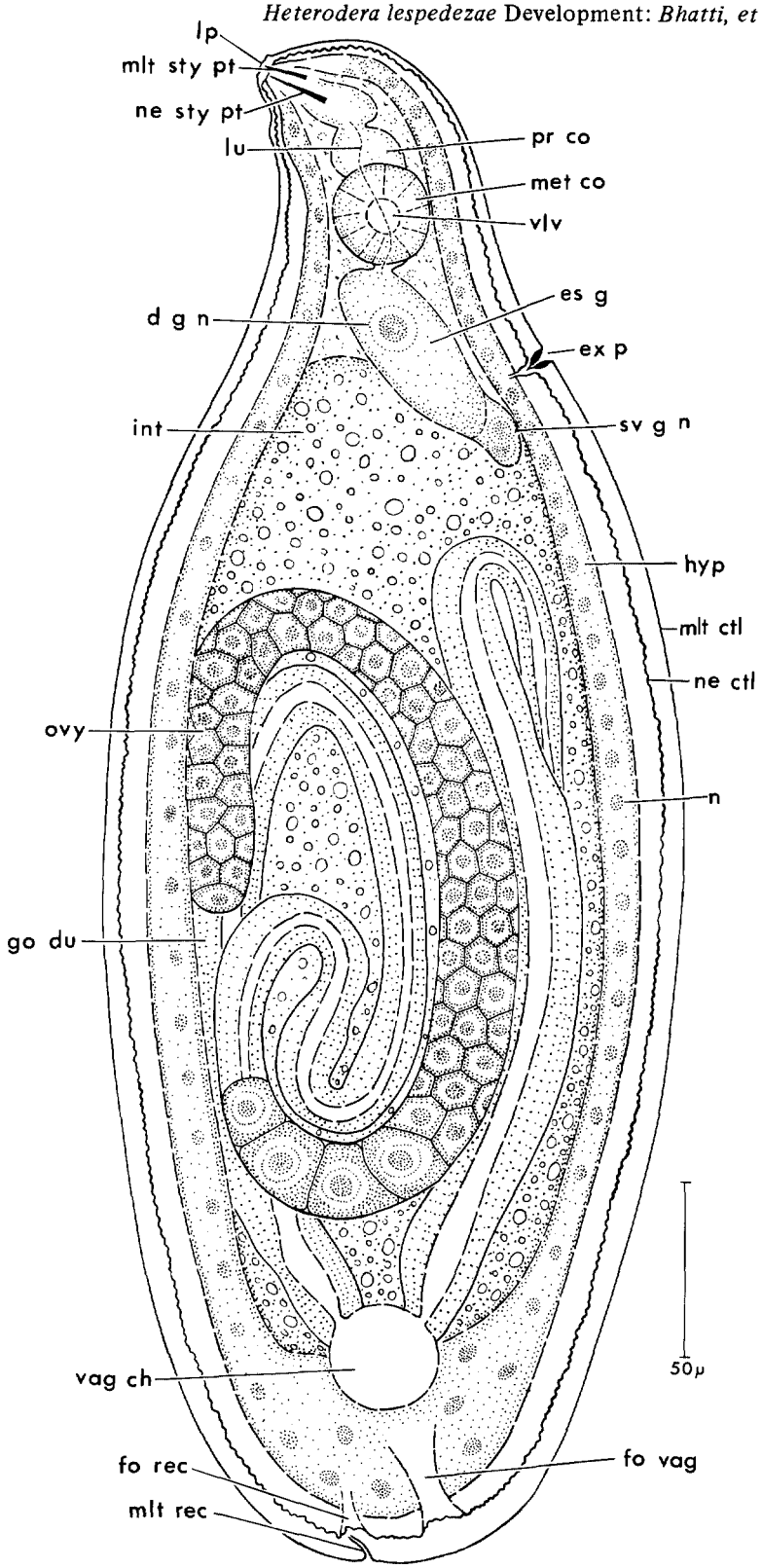

FIG. 3. *Heterodera lespedezae.* Fourth molt. For definition of abbreviations, see Fig. 1.

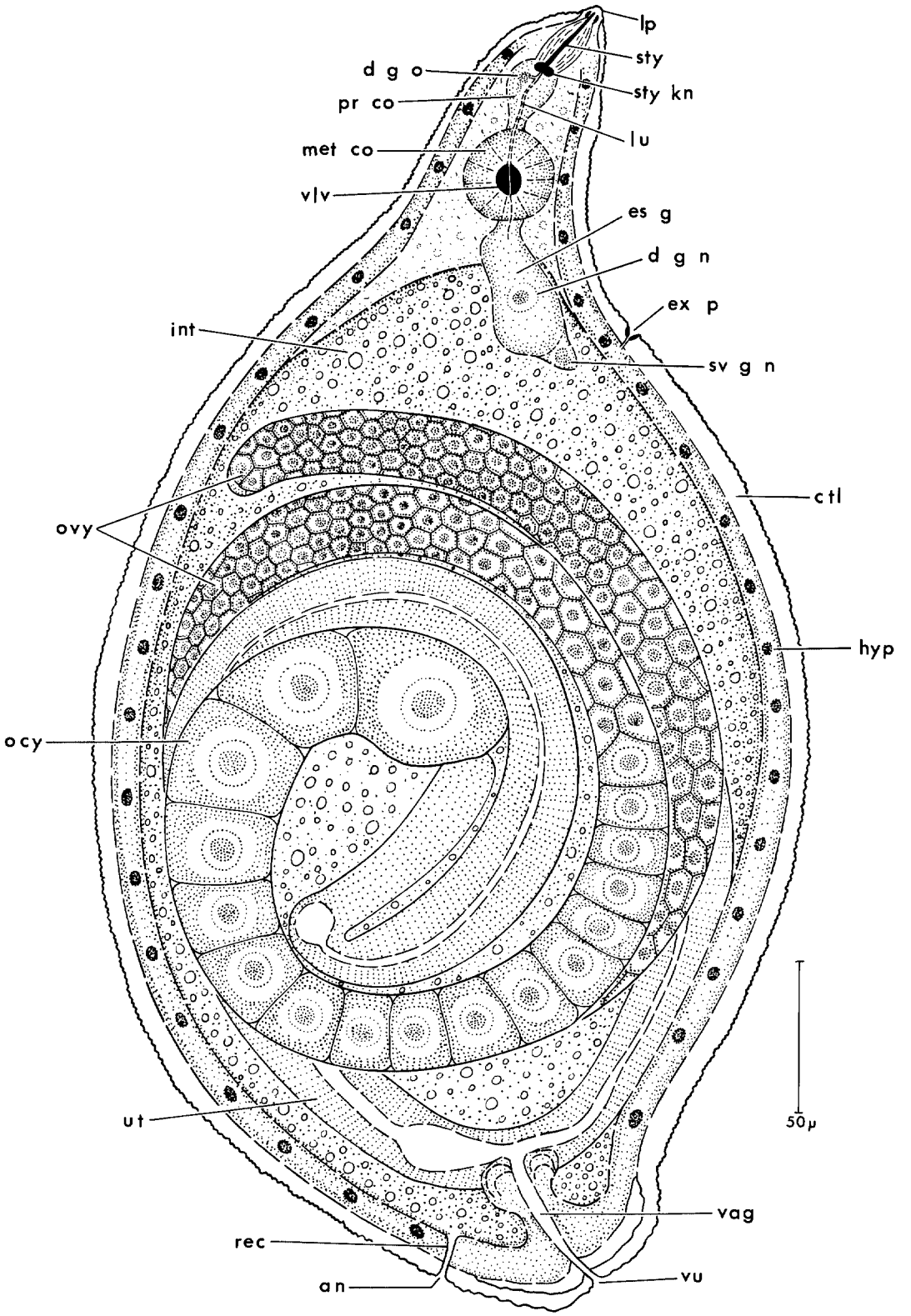

FIG. 4. *Heterodera lespedezae.* Adult female. For definition of abbreviations, see Fig. 1.

TABLE 1. Effect of temperature on development of *Heterodera lespedezae.*

Stages of development	Day/night temperatures (C)					
	18/14	18/18	22/18	26/22	26/26	30/26
Second molt	9-18[a]	9-12	6-9	5-7	5-7	5-7
Third molt	18-21	12-15	9-12	8-9	8-9	8-9
Fourth molt	24-30	18-21	15-18	13-15	12-14	13-17
Egg formation[b]	30	21	21	16	15	17
Matrix deposition[b]	33	24	21	18	16	18
Yellow females[b]	42	30	27	21	21	30
Oviposition[b]	48	33	30	24	21	31
Hatching[b]	51	36	36	26	25	36
Brown cysts[b]	72	60	54	36	36	38

[a]Days after penetration of roots by second-stage larvae.
[b]First observed.

temperature the life cycle was completed in 25 days.

DISCUSSION

The space between the newly forming cuticle and the molted cuticle of *H. lespedezae* contained granular particles which may be similar to those encountered by Bird and Rogers (2) during the cuticle formation in *Meloidogyne javanica* (Treub). These particles may be associated with the breakdown and re-absorption of the innermost layers of the old cuticle.

The progressive atrophy of the subventral glands in *H. lespedezae* following larval penetration of the roots is similar to that reported by Bird (1) for *Meloidogyne javanica.* Secretions of the subventral glands are perhaps needed for the penetration of the roots and the commencement of the parasitic mode of life, and subsequent atrophy of the glands may be due to functional disuse.

The longitudinal cuticular striation present in fourth-stage larvae of *H. lespedezae* exhibited a distortion of the striae in the vulval area, and probably gave rise to the formation of the zigzag pattern of the cuticle in adult females. Raski (8) observed similar longitudinal markings in fourth-stage larvae of *H. schachtii* Schmidt. Franklin (4) and Wieser (10), however, reported that the reticulate cuticular pattern in *Heterodera* spp. may have developed from transverse larval striations.

Observations with regard to excretory pore, tail, rectum, growth of reproductive system, and vulva formation during the development of *H. lespedezae* were in close agreement with those of Raski (8) for *H. schachtii.* The various developmental stages could be distinguished on the basis of gonad size and structure.

Growth behavior of 'Kobe' lespedeza in the phytotron in the absence of nematodes indicated that this plant can grow well over the temperature ranges tested. Therefore, the differences in time required for completion of the nematode's life cycle under six different temperature combinations appear to be due primarily to a direct effect of the temperature on the nematode rather than to an indirect effect through the host plant.

The development of *H. lespedezae* at 26/22 C was slightly faster than that reported in greenhouse studies at 24 C by Hung (6). He found fourth-stage larvae 16 days after inoculation, and lemon-shaped adults 20 to 22 days after inoculation. Egg deposition occurred between 36 and 38 days after larval penetration of roots. These differences were probably due to fluctuating temperature conditions in the greenhouse as compared to the precise conditions maintained in the phytotron. Considering temperature conditions, the development of *H. lespedezae* at 26/22 C seems to compare quite closely with that of *H. trifolii* Goffart at greenhouse temperatures of 21-27 C. In *H. trifolii,* adults developed 17 days after inoculation and eggs were formed after 22 days (7). With reference to color change of females from white to yellow to brown, cessation of oviposition, retention of eggs in the cysts, and subsequent hatching of larvae inside the cysts, *H. lespedezae* is similar to *Heterodera schachtii* (8). We observed, however, that certain developmental phases of *H. lespedezae* appear to be correlated with color changes of the adult female body; e.g., production of the gelatinous matrix begins when the females are white; and oviposition starts during the yellow phase and ceases when females turn brown.

Since *H. lespedezae* can complete its life

cycle in 25 days at the optimum temperature of 26 C, several nematode generations could be completed between the beginning of spring and end of fall in North Carolina if temperatures were uniformly 26 C. Under field conditions, however, temperatures fluctuate greatly, and fewer than the optimum number of generations should be expected.

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