BIOLOGY OF COLUMBIA LANCE NEMATODE (*HOPLOLAIMUS COLUMBUS* SHER) ON SOYBEAN EXCISED ROOT CULTURE

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ABSTRACT

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Feeding, egg development and oviposition, embryology, and postembryonic development of *Hoplolaimus columbus* were observed on soybean (cultivar Hutcheson) excised root culture in Gamborg's B5 medium (pH 5.7, 1.3% agar). Petri plate sterile cultures were placed inverted on a heated microscope stage (30°C) and observations were conducted using a color video camera and a time lapse digital videocassette recorder. Oöcytes were observed in each gonad 38 hours after feeding commenced and an egg possessing a terminal stalk was laid 96 hours later. Eggs were deposited in the one-celled stage and a total of 217 hours (9 days) was required for a newly laid egg to develop into a hatching second-stage juvenile at 30°C. Postembryonic development consisted of three juvenile stages and an adult female with prior feeding occurring before molts and oviposition. The nematode fed ectoparasitically on the subepidermal cells and endoparasitically in the cortex and outer vascular tissue and remained at the infection site for 2-9 days.

Key words: Columbia lance nematode, embryology, excised root culture, feeding behavior, *Hoplolaimus columbus*, life cycle, oviposition, postembryonic development, soybean.

RESUMEN

Supramana, S., S. A. Lewis, J. D. Mueller, B. A. Fortnum, y R. E. Ballard**.** 2001**.** Biologia del nematodo lanceta de Columbia (*Hoplolaimus columbus* Sher) en cultivo de raíces de soya. Nematrópica 3131:283-290.

Se estudio el hábito alimenticio, desarrollo de huevo y oviposición, embriología, y desarrollo post-embriónico de *Hoplolaimus columbus* en secciones de raíces de soya (cultivar Hutcheson) cultivadas en el medio de Gamborg B5, (pH 5.7, 1.3% agar). Cultivos estériles en placas Petri se colocaron invertidos en un microscopio cuya temperatura se mantvo a 30°C. Las observaciones se realizaron a través de una cámara de video por el lapso de tiempo correspondiente a la duración de la cinta. Treinta y ocho horas después del inicio de la alimentación, se observaron oocitos en cada gonada, y un huevo con un eje terminal fue puesto 96 horas después. Los huevos fueron puestos en estado unicelular y un total de 217 horas (9 días) fue necesario para que un huevo recién puesto se desarrollara en el segundo estado juvenil a 30°C. El desarrollo post-embriónico consistió de tres estados juveniles, y una hembra adulta con una alimentación antes de las mudas y oviposición. El nematdo se alimenta ectoparasíticamente de las células epidermales y endoparasíticamente de la corteza y tejidos vasculares externos y permanece en el sitio de infección por 2-9 días.

Palabras claves: Ciclo de vida, conducta alimenticia, cultivo de secciones de raíces, desarrollo post-embriónico, embriología, *Hoplolaimus columbus*, nematodo lanceta de Columbia, oviposición, soya.

INTRODUCTION

The Columbia lance nematode, *Hoplolaimus columbus* Sher, was first reported by Q. L. Holdeman in a field of declining soybean [*Glycine max* (L.) Merr.] and cotton (*Gossypium hirsutum* L.) near Eastover, South Carolina (Fassuliotis *et al.*, 1968). The nematodes grew poorly in monoxenic and greenhouse cultures (Fassuliotis, 1974; Fassuliotis and Nelson, 1988; Lewis and Smith, 1976). Experiments requiring large numbers of monospecific nematodes have been limited, but success in culturing the nematode (Supramana and Lewis, 1999) offers great opportunities to conduct research on this species. The growth of the nematode on petri plate culture allows for the observation of individual nematodes and their development over time. The present study used *in vitro* observations on the biology of the nematode to determine the time associated with different nematode stages, the nematode activity at different stages, and specific sequence of events that occur during the nematode's development.

MATERIALS AND METHODS

The nematodes were obtained originally from soil samples at the Edisto Research and Education Center, Blackville, South Carolina and maintained on alfalfa excised root culture. Soybean seeds were sterilized in 95% ethanol, followed by dipping in 1.3% sodium hypochlorite for 10 minutes, and were then placed on 1.3% water agar for germination. A single root tip was excised and transferred onto solidified Gamborg's B5 medium (pH 5.7-6.0) (Gamborg *et al.*, 1976) with 1.3% agar in 9-cm plastic petri plates. A cube of agar containing approximately 20 *H. columbus* from alfalfa root culture was placed at a distance of 2 cm from roots. The plate was double sealed with parafilm strips, and the culture was placed inverted on a heated microscope stage (Olympus Optical Co., Japan) that was adjusted to 30°C. Nematode behavior was observed using an Olympus compound microscope with standard optics. Embryonic and postembryonic development and feeding behavior were recorded using a Hitachi model VK-C370 (Hitachi, Ltd., Tokyo, Japan) color video camera and a Panasonic model AG-6540P (Panasonic, Tokyo, Japan) time-lapse, digital, videocassette recorder. The video recorder was adjusted to record a total of 960 hours, with 30-second recording times every 3 minutes. In addition, 24-hour-continuous recordings of several nematodes were made to confirm the egg hatching mechanism and observe nematode feeding.

RESULTS

Feeding: The nematodes migrated toward roots soon after inoculation of plates with excised roots containing nematodes. All of the juveniles and adults migrated from the agar cube within 3 hours. The root cap and maturation zone were more attractive to *H. columbus* than other portions of the root. The nematodes reached the root surface in 3-11 hours and migrated along the length of the root for 0.5-3 hours. While migrating along the root surface, *H. columbus* pressed its lips against the root surface and intermittently thrust the stylet (8-30 times per minute) causing epidermal cells to slough off. At a suitable infection site, where probing migration ceases and penetration begins, the nematode stylet pierced the epidermis 55-71 times per minute. An infection site was established approximately 3 hours later and the nematode inserted its body as deep as the median bulb into the root. During feeding, the median bulb pulsated at 200-255 beats per minute for 9-10 minutes, rested for 9-16

minutes, and resumed pulsation. The nematode stayed in one infection site in a sustained 60-hour observation period. Other observations on feeding juveniles revealed that *H. columbus* stayed in one infection site as long as 9 days.

Egg development and oviposition: A newly molted female was viewed feeding 8 hours after ecdysis. Oöcytes were first observed in the germinal zone of the anterior gonad 38 hours after feeding began and were easily distinguished after 56 hours. As many as six oöcytes were observed in each gonad. The developing egg was observed in the columella of the anterior gonad 48 hours later and moved into the uterus after 72 hours. Immediately prior to oviposition, the female withdrew from the infection site and became very active. The nematode undulated intensely, and pseudocoelomic fluid circulated rapidly around the uteri and vagina. The egg moved directly adjacent to the vagina. At first the eggshell appeared as a bud through the vulva. The ovum then flowed into the shell as it proceeded to emerge, until the egg was laid. Oviposition lasted approximately 3 minutes. The second egg laid was from the posterior gonad, and the third from the anterior gonad. A total of 15 eggs were counted that could be directly attributed to a single female. It took at least 6 days for newly molted females to deposit the first egg after feeding commenced. The nematode resumed feeding and a female with dark intestine, indicating significant food intake, was observed 48 hours later.

Embryology: Eggs of *H. columbus* were deposited in the one-celled stage (Fig. 1a). The eggs are oblong, slightly kidney-shaped, and possess a stalk (Fassuliotis, 1975) on the anterior end of the egg. Video recorded observation showed that eggs started to develop right after oviposition and the first cleavage occurred 8 hours later. At 30°C, it took an average of 9 days (217 hours) for the newly laid egg to develop into a hatching second-stage juvenile (Table 1). The first cleavage was equatorial and unequally holoblastic; the anterior cell was slightly smaller than the posterior cell (Fig. 1b). The second and third transverse cleavages divided the anterior and posterior blastomeres consecutively, resulting in the formation of three and four blastomeres arranged in tandem (Fig. 1c, d). The fourth cell division was by transverse cleavage of the anterior cell (Fig. 1e) resulting in the formation of the 5-cell stage and this was followed by three or more quick cell divisions, the exact sequence of which could not be followed. Thereafter, a series of rapid cell divisions led to the formation of a multi-celled stage (Fig. 1f). The gastrula stage (Fig. 1g) was observed 75 hours after oviposition, and within 14 hours the cells had differentiated into two zones of differing cytoplasm densities (Fig. 1h). Twenty-four hours afterward the light zone, animal pole, developed into the esophageal region while the dark zone, vegetal pole, became the intestine and the posterior part of the first-stage juvenile (Fig. 1i). There was evidence that the stylet developed during the first-stage juvenile and the differentiated stylet was observed during the first molt (Fig. 1k). The cuticle of the first-stage juvenile was smooth, while that of the second-stage juvenile was annulated. Immediately prior to hatching, the second-stage juvenile started regular head and body movements inside the eggshell. The juvenile emerged by pressing and thrusting its head against the eggshell (Fig. 1m, n), and the rupture of the eggshell occurred on the terminal end (Fig. 1o).

Post Embryonic Development: Postembryonic development of *H. columbus* consisted of three juvenile stages and an adult. Development of all stages outside the egg was observed only after feeding. On soybean excised root culture, the nematode

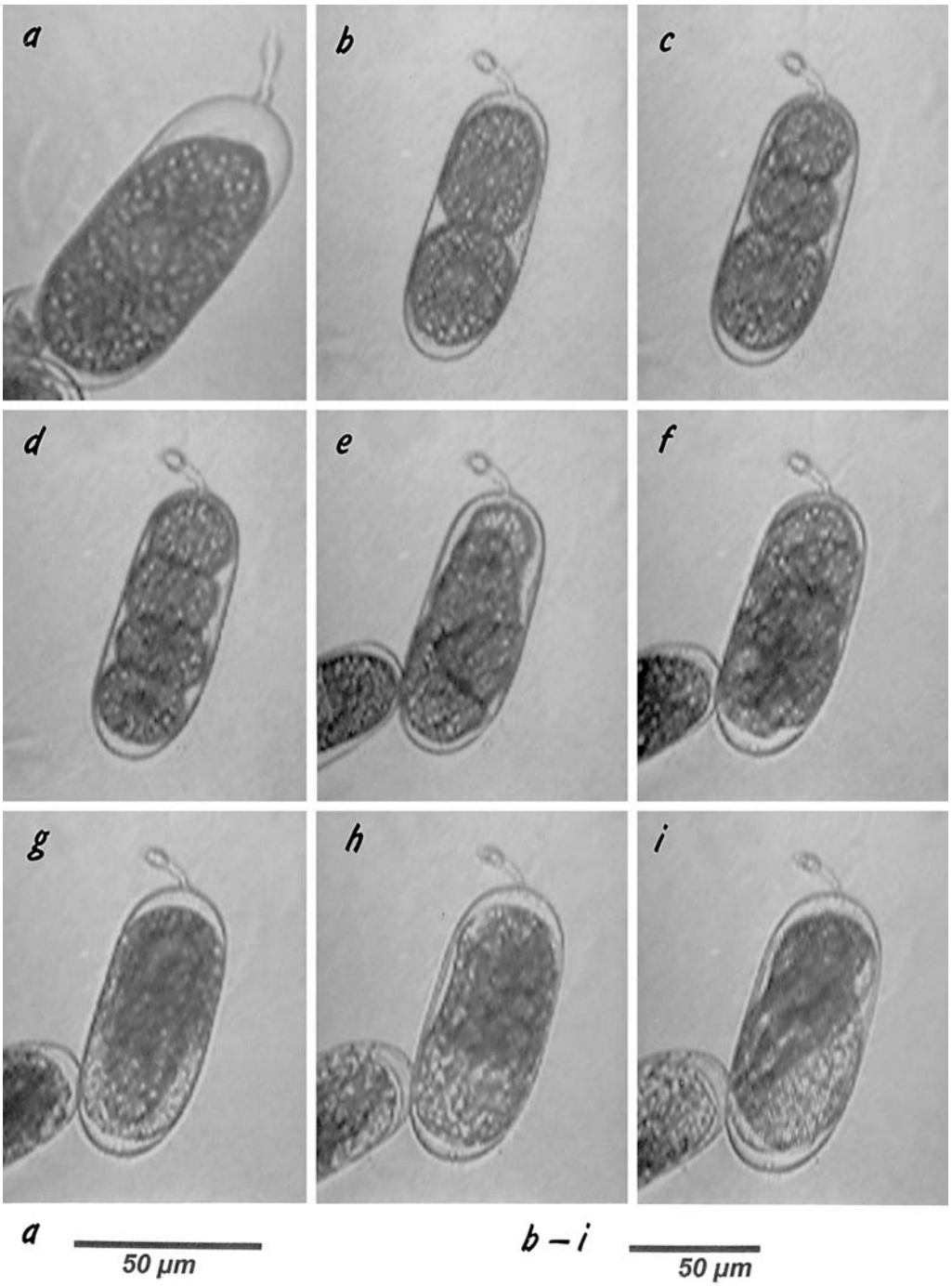

Fig. 1. Egg of *Hoplolaimus columbus* in various stages of embryonic development; (a) one-celled stage, (b) two-celled stage, (c) three-celled stage, (d) four-celled stage, (e) five-celled stage, (f) multiple-celled stage, (g) gastrula, (h) tadpole stage, (i) first-stage juvenile.

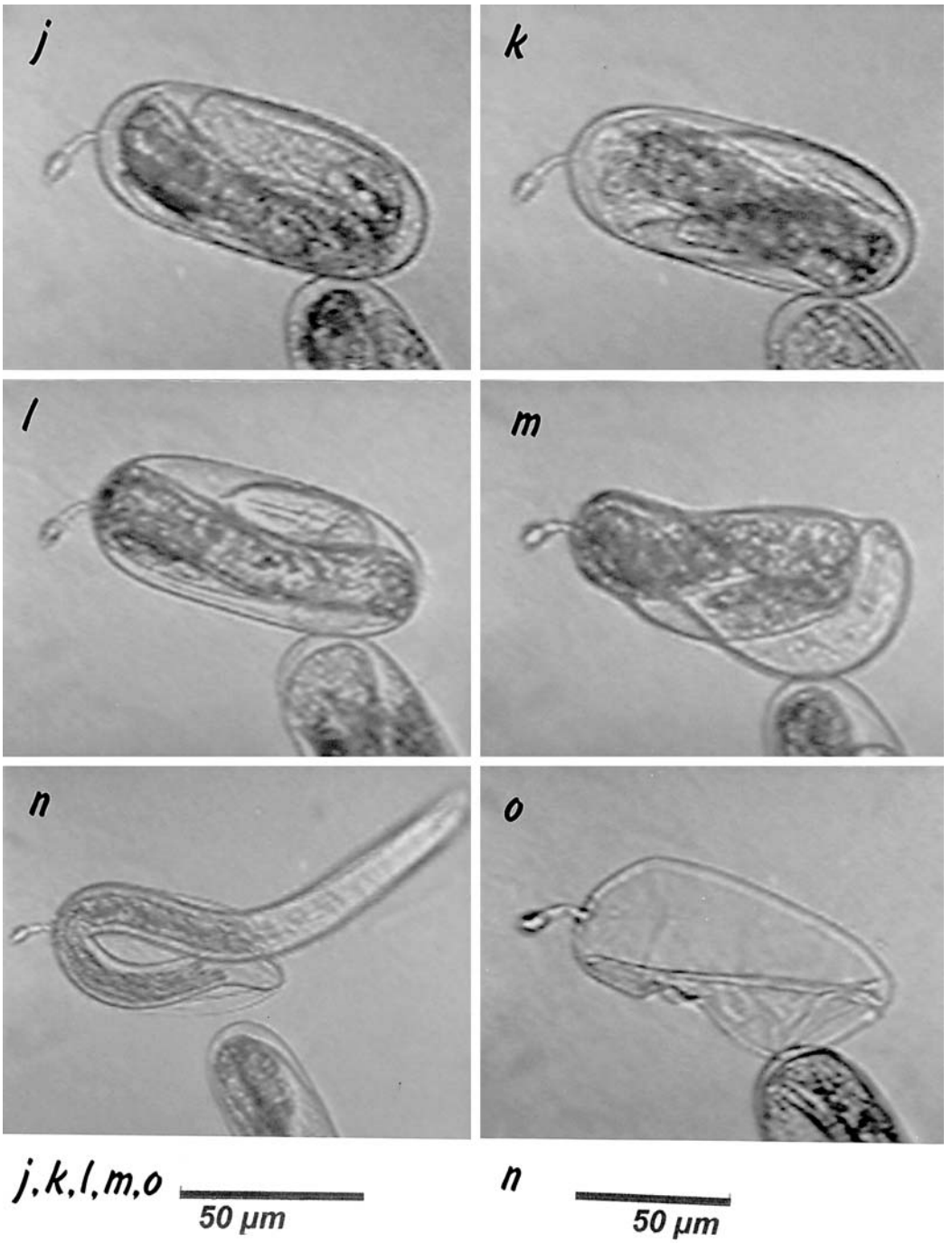

Fig. 1. Egg of *Hoplolaimus columbus* in various stages of embryonic development; (j) first-stage juvenile, (k, l) molting-first-stage juveniles, (m) second-stage juvenile, (n) hatching of second-stage juvenile, and (o) eggshell.

Table 1. Duration of embryonic developmental stages of an individual *Hoplolaimus columbus* on 'Braxton' soybean excised root culture at 30°C.

	Time (hours)	
Embryonic stag	Each stage	Cumulative
1 cell	0	0
2 cell	8	8
3 cell	2	10
4 cell	3	13
5 cell	4	17
7 cell	3	20
Multiple cell	3	23
Gastrula	52	75
Early 'tadpole'	14	89
First-stage juvenile	22	111
First molt	39	150
Second-stage juvenile	31	181
Hatching	36	217

fed ectoparasitically and endoparasitically and initiated necrosis in the root cortex. The nematode laid eggs in agar, on the root surface, and in the root cortex. After emergence from the eggshell the second-stage juvenile migrated toward the root surface. The nematode fed anywhere in the root but preferred the maturation zone of the lateral root. The nematode fed ectoparasitically on subepidermal cells. It was difficult to observe the endoparasitically feeding nematode by direct observation using stereoscopic or compound microscopes. The juvenile ceased all activity and lay outstretched close to the roots when it was ready to molt. Similar patterns of feeding and molting also occurred in the third and fourth-stage juveniles. The cuticle of the previous stage separated in the anterior region carrying with it the prorhabdion and the nematode emerged from the old cuticle by vigorous movements coupled with stylet piercing. Observation of the molting fourth-stage juvenile showed that all regions of the body became darker and granular. Two days later clear regions appeared around head, tail, and vulva. The head was separated from the old cuticle at the third day and the separation developed backward along the body. The nematode rotated, moved backward and forward, pressing the head and stylet to break the old cuticle, and emerged as an adult female. The molt took approximately 9 days to complete at 30°C.

DISCUSSION

Feeding behavior of *H. columbus* was similar to other tylenchid species (Doncaster and Seymour, 1973) and included wide and local explorations, stylet thrusting, and feeding. The nematode always migrated toward roots, particularly to the

maturation zone of roots. On older cultures, however, the nematodes did not show any feeding preferences and could be found any place along the roots. The infection site of one nematode often attracted other nematodes. These results suggest that roots chemically attract the nematode. Most juveniles and adults fed ectoparasitically and endoparasitically. Observation on the ectoparasitic nematode showed that feeding behavior was similar to the basic pattern described previously (Fassuliotis, 1975).

The female reproduced readily without the presence of males. At 30°C, the developing oöcytes were evident just 38 hours after feeding and a newly molted female began laying eggs 6 days after feeding began. These results demonstrate more rapid egg development and ovipositing than described previously (Fassuliotis, 1975), but incubation temperature may have been higher under our conditions. It is possible now to estimate the degree-days (DD) required for embryonic development of this species (Pedigo, 1999). Assuming a hypothetical developmental threshold of 15°C and a sustained developmental temperature of 30°C, 135.6 DD were required for embryonic development. Future investigations should focus on postembryonic developmental time so that knowledge of degree-days required for completion of the life cycle can be obtained. This will permit prediction of number of generations, the resulting population growth, and damage potential at a given plant developmental stage.

Continuous observation of the embryonic development of a single egg has not been conducted previously with *H. columbus*. Video recorded observation with time-lapse camera resulted in more precise observations. For example, the fourth cell division resulted in the formation of a five-celled embryo and occurred transversely on the most anterior cell (end possessing the stalk). This differs from previous information on *H. indicus* (Dasgupta *et al.*, 1970; Gupta and Atwal, 1971) that described the cleavage as longitudinal. It is not clear whether this divergence occurred due to species differences. Later egg development showed that at the gastrula and 'early tadpole' stages, the end possessing the stalk developed into the dark zone while the other end possessing a discernible knot developed into the light zone. This pattern occurred consistently, suggesting the polarity of the embryo is related to the stalk position, in contrast to previous opinion (Fassuliotis, 1975). The stylet developed within the first-stage juvenile and by the first molt, the developing stylet shaft and knobs were evident. A developed stylet was observed in the second-stage juvenile. Juvenile movements became much more intense when hatch approached. The juvenile moved around the egg and occasionally pressed its head against the eggshell. The eggshell looked more elastic right before hatching and the juvenile emerged by forcing its head to break the eggshell. There was little evidence indicating the involvement of the stylet in hatching. The eggshell was ruptured at the terminal end. The information on hatching has not been previously described for *H. columbus* and differs from that described for *H. indicus* (Dasgupta *et al.*, 1970; Gupta and Atwal, 1971).

By using a time lapse video recorder, it was possible to follow some, but not all, of the life history of a single nematode. The nematode's migratory behavior made observations difficult. Postembryonic development of *H. columbus* consisted of three juvenile stages and adult. Since feeding was essential for all stages outside the egg, the life sequences of feeding-molting occurred in the second-, third-, and fourth-stage juveniles, and feeding-egg oviposition occurred in the adult. This feeding-molt-

ing part of the life history was similar to that described in *H. indicus* (Dasgupta *et al.*, 1970). *Hoplolaimus columbus* completes its life cycle outside the root during root exploration, feeding as an ectoparasite, and molting. The nematode uses its vigorous movements to emerge from the egg-shell and old cuticle during hatching and molting, respectively. The system used here for culturing and observing the behavior of *H. columbus* can be adapted to experiments concerning resistance, host suitability, degree-hour development studies, natural enemies, and nematistatic compounds.

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