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# DEVELOPMENTAL BIOLOGY AND FEEDING BEHAVIOR OF *TYLENCHORHYNCHUS AGRI* ON TWO HOSTS, *TRIFOLIUM PRATENSE* AND *POA PRATENSIS*

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## ABSTRACT

Coates-Beckford, Phyllis L. 1982. Developmental biology and feeding habits of *Tylenchorhynchus agri* on two hosts, *Trifolium pratense* and *Poa pratensis*. Nematropica 12: 1-5.

Eggs of *Tylenchorhynchus agri* Ferris were deposited as a single cell and the embryogeny was similar to that of other *Tylenchorhynchus* Cobb species. At 27C, hatching of eggs occurred 7 days after oviposition and the second-, third- and fourth-stage juveniles and adults were formed at 7, 13, 19 and 25 days, respectively, after oviposition. Nematodes fed ectoparasitically on red clover (*Trifolium pratense* L.) and bluegrass (*Poa pratensis* L.), with only the stylet tip inserted in epidermal cells of the root-hair zone. Duration of feeding was usually less than 5 min.
*Additional key words: temperature, interspecific comparison, stunt nematodes.*

## RESUMEN

Coates-Beckford, P.L. 1982. Biología del desarrollo y hábitos alimenticios de *Tylenchorhynchus agri* en dos hospederos, *Trifolium pratense* y *Poa pratensis*. Nematrópica 12: 1-5.

Huevos de *Tylenchorhynchus agri* Ferris fueron depositados como células individuales y la embriogénesis fué semejante a la de otras especies de *Tylenchorhynchus* Cobb. La eclosión de los huevos ocurrió a los siete días después del desove a 27C y la segunda, tercera y cuarta etapas de larvas y adultos se observaron a los siete, 13, 19 y 25 días respectivamente, después del desove. Los nematodos se alimentaron ectoparasiticamente en trébol rojo (*Trifolium pratense* L) y en poa (*Poa pratensis* L) insertando sólo la punta del estilete en las células epidermales de la zona pilífera de las raíces. La duración del período de alimentación fué tipicamente de menos de cinco minutos.
*Palabras claves adicionales: efecto de temperatura, comparaciones interespecificas, gramíneas, leguminosas.*

## INTRODUCTION

Studies on a few species of the stunt nematodes, *Tylenchorhynchus* Cobb (*sensu lato*) have shown variation in the duration of nematode development (6, 8, 11) and feeding behavior (2). *T. agri* Ferris, which is commonly found in soils of Illinois, has a wide host range including temperate and tropical plant species (5), and its pathogenicity has been demonstrated in greenhouse tests (4). Studies were conducted to investigate the duration of embryogeny, postembryonic development and the feeding behavior of this species.

## MATERIALS AND METHODS

*Developmental Biology.* Gravid females of *T. agri* were extracted from monospecific pot cultures on 'Kenland' red clover (*Trifolium pratense* L.) using the method of Christie and Perry (3) and transferred individually to distilled water in micro culture slides. Females were removed after 10 hr following oviposition. Eggs were observed at regular intervals throughout ontogeny at a magnification 375X.

For postembryonic development studies, two red clover seedlings were planted in each of 92 10-ml beakers containing 5 $cm^3$ autoclaved sand. Fifteen gravid females were transferred to each beaker. One day after inoculation, and at 2-day intervals thereafter, nematodes from four randomly-selected replicates were extracted by a modified Baermann funnel technique (1) in which nematodes were collected in a watch glass for 18 hr. Developmental stages of the recovered nematodes were noted. All studies were carried out at 27C.

*Feeding Behavior.* Aseptically grown 10-17 day old seedlings of 'Newport' Kentucky bluegrass (*Poa pratensis* L.) and 3-day old seedlings of red clover were placed at the bottom of Petri dishes containing freshly prepared 0.5% water agar. Nematodes were surface sterilized by a 5-min immersion in 0.5% hibitane diacetate before inoculation. Seedlings were inoculated with freshly extracted nematodes or with refrigerated distilled water suspensions of nematodes starved for 1 and 2 mo. The Petri dishes were incubated at 25-27C under natural light. Nematodes feeding on roots growing along the bottom of the containers were observed at magnifications of 150X and 375X.

## RESULTS AND DISCUSSION

*Developmental Biology.* Gravid females, usually with a single egg in the uterus, often began ovipositing in distilled water within 10 hr of extraction from soil, but sometimes retained eggs for 2-3 days. Eggs were usually single-celled at oviposition. Subsequent ontogeny was similar to that described for *T. dubius* (Butschli) Filipjev (8) and *T. claytoni* Steiner (10). The two-, three-, four-, five-cell, blastula and gastrula stages were first formed at 16, 20, 24, 27, 63 and 83 hr, respectively, after oviposition (Table 1). The actively moving $J_1$ and $J_2$ stages were completely formed within the egg at 5 and 7 days, respectively. Hatching was first observed on the seventh day. The

Table 1. Development of *Tylenchorhynchus agri* from egg to adult at 27C.

| Stage of development | Minimum time from oviposition |
|---|---|
| Embryonic | |
| | Hours |
| One-cell (oviposited) | - |
| Two-cell | 16 |
| Three-cell | 20 |
| Four-cell | 24 |
| Five-cell | 27 |
| Blastula | 63 |
| Gastrula | 83 |
| $J_1$ within egg | 111 |
| $J_2$ within egg | 135 |
| $J_2$ hatched | 145 |
| Postembryonic | |
| | Days |
| $J_2$ | 7 |
| $J_3$ | 13 |
| $J_4$ | 19 |
| Adult | 25 |

durations of the stages were shorter than those noted for *T. dubius* at 20C (8) but time from oviposition to hatching was similar to that noted for *T. claytoni* at 22-25C (6, 10).

The life cycle of *T. agri* from egg to adult was completed in 25 days. Time taken from eclosion to development of the adult was much shorter than that reported for *T. dubius* at 20C (8) and *T. claytoni* at 24C (6), but longer than that of an undescribed species (11). The second-, third- and fourth-stage juveniles and adults of *T. agri* first appeared 7, 13, 19 and 25 days, respectively, after inoculation. However, the apparent interspecific variation noted may be partly due to suboptimal test temperatures since work by Malek (7) showed that optimum temperatures for development of *T. dubius* and *T. agri* were 25C and 30C, respectively.

Eggs of *T. agri* were seen in the watch glass on the first and third days and numerous second-stage juveniles again were present at 23 days. This indicated two periods of oviposition by females used for inoculation, the first within 3 days of and the second about 16 days after inoculation.

*Feeding Behaviour.* Feeding behaviour was similar on both plant species examined. Penetration of the cell wall by the stylet occurred after 3-10 rapid, irregular probes. An opaque mass accumulated around the stylet tip in a bluegrass cell indicating salivation by the nematode. A feeding position typical of other species of stunt nematodes (2, 6, 8, 9, 12) was assumed during stylet penetration and ingestion, the head being positioned at a right angle to the longitudinal axis of the root surface whereas the posterior region lay against the surface. Only the stylet tip was inserted into the cell and the stylet angle was altered occasionally. Continuous rhythmic pulsations of the median bulb occurred during ingestion.

All juvenile stages as well as adults of *T. agri* fed. For the majority of nematodes, duration of feeding was short (15 sec - 4 min 30 sec). After feeding, the nematodes moved away from the feeding sites and continued moving around the root system. An exception to the short duration of feeding was noted on two occasions when a single nematode was seen feeding on each plant species for about 30 min, after which it migrated into the agar away from the feeding site and remained motionless for over 1 hr. Subsequently, the nematodes moved away from the vicinity of the root.

*T. agri* always fed singly and ectoparasitically, usually on epidermal cells in the root-hair zone of clover and at all regions, except the tips, of bluegrass roots which bore root hairs over the entire surface. Nematodes ocassionally probed at root hairs of bluegrass and root cap cells of clover. Individuals starved for 1 and 2 mo began probing cells within 10 min of arriving at the root surface, unlike those freshly extracted which wandered about most of the time. However, only a few starved nematodes were vigorous enough to migrate to the roots. The migratory ecotparasitic and single feeding behavior was also typical of other stunt nematodes (2, 6, 8, 12) although aggregate feeding has been observed in *T. maximus* Allen and *T. lamelliferus* de Man (2).

Although numerous cells were observed, response to parasitism was detectable only in a single bluegrass epidermal cell which was fed on for 30 min. The cytoplasm became granular and moved rapidly, suggesting disturbance of cyclosis, and remained so for more than 1 hr after the nematode had stopped feeding. When examined 10 hr later, the cell appeared normal and was not distinguishable from the surrounding ones. Wyss (12) noted cessation of cyclosis in cells parasitized by *T. dubius.*

Differences in duration, sites and effects of feeding noted by Bridge and Hague (2) indicate differences in histopathogenic effects by various species. Interference with cyclosis of numerous host cells, as could occur with high populations of *T. agri*, could be a cause of poor host growth noted by Coates (4).

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