BIOLOGY OF *Heterodera longicolla*

by

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Introduction

The identification of Heterodera longicolla, Golden and Dickerson, as a parasite of buffalograss (Buchloë dactyloides (Nutt.) Englem.) in west central Kansas prompted queries about various aspects of its biology in relation to buffalograss as well as other economic grasses in the area.

Information concerning the life cycle, optimum temperature requirements, hatching requirements, histopathological effects, and host range was needed to assess its potential as a plant pathogen. A series of experiments were conducted to 1) gain an understanding of the parasite's biology, and 2) establish the nematode's economic potential.

Literature Review

Members of the genus Heterodera have caused great losses to economic crops in most agricultural areas of the world (22).

Life history studies reveal a complex association developed between Heterodera spp. and their hosts. Emergence of larvae from a cyst requires a series of processes involving the diffusion of a stimulus through the cyst and egg wall to cause hatching, stimulation of the larvae to break out of the egg, and movement of the larvae out of the cyst (9). The second process is biological in nature and probably is the limiting factor for emergence (9). Specific root diffusates are required for some species to stimulate hatching but others will hatch in water (21).

H. rostochiensis nematodes from free or encysted eggs hatched, infected, and reproduced equally well (18). Thus variability of cyst content could be eliminated and low density population experiments could be utilized (18). It was also shown that hatched larvae were as reliable as cysts for inoculum in screening tests (20). Hatched larvae developed

simultaneously, while cysts resulted in protracted invasion and development (20).

Infective larvae of most Heterodera spp. penetrate host roots relatively quickly. H. trifolii penetrated Ladino clover roots within 15 min after contact (16) and H. glycines entered soybean roots in 4 hrs (6). Intracellular penetration along the axis of the root is common (6), and entrance at wounds where secondary roots had ruptured cells has been observed (16).

Specialized feeding sites known as syncytia are an integral part of the host-parasite relationship for members of the genus Heterodera. Syncytia, induced by the nematodes, are usually elongate with ends merging with normal tissue, and usually only one syncytium is associated with each larva (8). Syncytial morphogenesis is a two-stage process involving introduction of specific compounds to induce the syncytial state and then a withdrawal of cytoplasm by the nematode (13). Cell wall breakdown seems to be localized at pitfields or at plasmodesmata with continued dissolution until only fragments remain. Thus breakdown is probably enzymatic in nature because cell wall fragments remain smooth (10,13,14). No cell wall dissolution was observed at infection sites of H. graminophila on Echinochloa colonum roots (2).

Syncytium development may occur in cortical parenchyma, endodermis, pericycle, or vascular tissue, depending on where initiation of the site occurs. Syncytium induced by H. avenae on oats was restricted by the endodermis and caused severe damage to vascular tissues (12). Translocation of minerals, food, and water by the root was seriously impaired from infections of H. leuceilyma on St. Augustinegrass (5). Development in the cortical parenchyma, however, appears to be less damaging to active xylem and phloem.

Wall thickenings adjacent to the nematode's head and at the periphery of the syncytial site were observed (1,6,12,13,16). Changes in cytoplasm involved in the syncytial site include hypertrophy of the nuclei and nucleoli, increase of cytoplasmic organelles, reduction and loss of the central vacuole, fusion of contiguous protoplasm through perforations in the wall, and an increase of endoplasmic reticulum (10,13,14).

Ultrastructural studies have shown wall modifications in syncytial sites that are associated with the contiguous wall adjacent to xylem and phloem vascular elements. These are membrane-bound wall outgrowths and probably serve as a solute transfer system for nematode feeding, utilizing the increased surface to volume ratio (10,13,14,15).

Materials and Methods

Heterodera longicolla used in experiments were collected from greenhouse cultures of a population collected in Ellis County, Kansas. Cultures were maintained in the greenhouse on buffalograss (Buchloë dactyloides (Nutt.) Englem.). The variety 'USDA-SCS-PMC Knox City, Texas #PM-T-1181' was used throughout experimentation as a susceptible host.

Cysts were obtained by washing infected roots and infested soil onto a 149 µm screen. Cysts were individually transferred by hand-held loops to 20 ml distilled water in watch glasses and stored at 4 C until needed.

Soil used for experiments was steam-sterilized at 250 C for 3 hours and stored at least 1 week prior to use.

Hatching of Encysted Eggs. - Fifteen H. longicolla cysts were placed in each of 15 BPI dishes containing 1 ml distilled water. BPI dishes were placed singly in moist chambers consisting of petri dishes lined with moistened filter paper. These moist chambers were placed into incubators, 5 each at 20, 25, and 30 C. Hatched larvae were counted and

removed daily. Water was added to BPI dish and filter paper as needed. The experiment was terminated when larval emergence ceased.

Hatching of Free Eggs. - H. longicolla cysts were ruptured in distilled water with a Thomas Model-A tissue homogenizer. Maceration continued until all cysts were disrupted. Eggs were suspended in 50 ml distilled water and 5 ml suspensions were poured into each of 10 sterile 9-cm petri dishes. An additional 20 ml of distilled water were added to each dish. Eggs were dispersed by swirling, and incubated at 27 C. Larvae were counted daily and the experiment was terminated when remaining eggs did not contain developed larvae.

Inoculum preparation for the remainder of the experiments followed the liberated-egg hatching method with a 6-day incubation at 27.5 C.

Optimum Temperature Experiments. - Buffalograss seeds were sprouted in vermiculite. Ten to 15-day-old seedlings were transplanted singly into sterile-steamed soil contained in 8-cm plastic pots. Five hundred L_2 larvae in 1 ml water were added about the roots of 90 plants at transplanting. Another 90 plants served as controls and received 1 ml distilled water about the roots at transplanting.

Pots were placed into 15-cm plastic containers and the space between the pot and container was packed with sand so the pot containing the seedling and the sand was level with the water level in the temperature tanks.

Assembled pots were randomized and placed in temperature tanks at 15, 20, 25, 30, and 35 C. Plant and nematode development were checked at 5-day intervals, starting at day 25 for the first series, day 15 for the second series, and day 35 for the third series.

Soil was gently washed from the roots over a 149 µm screen to catch

any dislodged cysts. Roots and screenings were microscopically examined for cysts and numbers were recorded. Plants were oven-dried and weights recorded.

Life Cycle. - Buffalograss seeds were washed in 80 C distilled water for 1 min, transferred to sterile petri dishes lined with moistened sterile filter paper, and incubated at 27 C. Seedlings with radicles 2 to 4 mm long were transferred to steam-sterilized loam with particle size of 149 µm or less. Fifty L_2 larvae in 1 ml water were placed about the roots of each seedling. Roots were covered with soil and incubated for 4 days at 27 C.

Seedlings were removed from the soil by gentle washing and examined microscopically for larval penetration. Infected seedlings were transferred to test tubes filled with vermiculite and placed back into the 27 C incubator. The vermiculite was gently removed from two test tubes daily and nematode development observed. Infected roots were subsequently processed for histological observations.

Histopathology. - Light Microscopy. H. longicolla-infected roots were fixed in FPA for at least 7 days, dehydrated through a TBA series (11), and embedded in Paraplast (M.P. 55-56 C). Blocks were sectioned on a Model 820 Spencer rotary microtome at 10 µm for cross sections and 15 µm for longitudinal sections. Sections were adhered to glass slides with Haupt's adhesive and allowed to dry for 24 hr. Sections were stained with 1% saffarin (aqueous) and counterstained with 1% fast green (95% ethanol), then coverslipped.

Electron Microscopy. Material to be examined was fixed in 2.5% gluteraldehyde in pH 7.4 2M phosphate buffer at 22 C. After 1 hr, fresh gluteraldehyde was substituted and fixation continued for an additional

2 hr. Tissues were rinsed in pH 7.4 2M phosphate buffer at 4 C with 3 changes and stored in buffer at 4 C.

Tissues were post fixed in 1% OsO_4 in pH 7.4 2M phosphate buffer at 4 C for 30 min. Fresh OsO_4 was added and fixation continued for an additional hr at 4 C. Tissues were rinsed with 3 changes of distilled water then dehydrated using a 50, 70, 80, 95 and 100% ethyl alcohol series. Two changes of 100% propylene oxide followed alcohol dehydration.

Tissues were embedded by adding small amounts of Mollenhauer's (17) mixture (Aralidite 506 and Epon 812) to the tissues in propylene oxide. Two changes of pure resin mixure followed, allowing tissues to remain in the second change for 24 hr. Tissues were transferred to flat forms containing fresh Mollenhauer's mixture and cured for 48 hr at 60-65 C.

Sections were cut using a Reichert Ultra Microtome to give ribbons in the gold interference region with thickness of 600-800 Å. Sections were supported on copper grids having carbon coated Form Var membranes. Sections were stained 30 min in uranyl acetate and counterstained in lead citrate for 12 min at 22 C. Material was observed on a Philips 201 Transmission Electron Microscope using a 60 KV accelerating voltage.

Host Range Experiments. - Seeds were germinated on moist filter paper and three seedlings were transplanted to a 13-cm pot of sterilized soil. Five pots of each host were included. One ml of water containing 500 larvae were pipetted around the roots of each plant. The plants were grown for 3-6 months, depending upon the maturity date of the plant, at 30 C in the greenhouse. At termination, plant roots and soil were washed to dislodge cysts. Cysts were collected on 149 µm screen and counted.

Survey. - Soil samples were taken in early Fall in Ellis and Rush counties in western Kansas. Samples were obtained with a 2.5 cm soil

probe which was thrust into the ground 10-15 cm deep. The resulting plugs were washed and cysts collected on a 149 μm screen. H. longicolla cysts were identified by their characteristic long neck and small size. Presence or absence of this nematode was recorded.

Results

Hatching. - Hatching of Encysted Eggs. A 21-day cumulative hatch of individual cysts was recorded, and proportional hatch values for predetermined intervals were computed (Table 1). Percentage of hatch for each interval was converted to probits (3) and plotted against log time in days for 25 and 30 C treatments (Fig. 1). Mean hatch-time was 6 days at 25 C and 12 days at 30 C. The log of time limits between which 95% of all larvae would emerge was 2-15 days at 25 C and 4-36 days at 30 C. The average hatch/cyst at 25 C was 74.6 and at 30 C was 120.2. At 20 C only 5-7 larvae hatched in the 3-week period.

Hatching Free Eggs. - H. longicolla eggs readily hatched in tap water. No significant difference was obtained with the use of whole roots in the hatching water or with distilled water. In 6 days at 27 C, 60% of free eggs and 50% of the encysted eggs hatched.

Optimum Temperature Experiments. - Two parameters for the establishment of the optimum temperature regime for life cycle development were 1) time of reinfection of host plant by L_2 larvae, and 2) peak build-up of maturing females and cysts. Average numbers of females and cysts were plotted against days after inoculation (Fig. 2). Four temperatures (20, 25, 30, and 35 C) were used for comparisons.

At 20 C there was no significant increase in female and cyst populations 50 days after inoculation (Fig. 2). At 30 C populations increased, indicating that one life cycle had been completed in 40 days. At 25 C

Table 1. Data for Plotting Probit Curve for Heterodera longicolla Larvae Emergence at 25 C.

Time (Days)	Number Larvae Hatching in Each Interval	Larvae Hatched at End of Each Interval			Log of Time
		Number	Percentage	Probit	
0					
2	1	1	1.6	2.86	.301
4	16	17	23.4	4.27	.602
6	23	40	54.1	5.10	.778
8	16	56	75.5	5.69	.903
9	5	61	82.4	5.93	.954
11	7	68	91.0	6.34	1.041
13	3	71	95.4	6.68	1.113
16	2	73	98.2	7.11	1.204
19	1	74	99.3	7.46	1.279
21	1	75	100.0	--	1.322

Fig. 1. A) Probit line plotted for the hatch from 5 individual Heterodera longicolla cysts in water at 25 C and B) at 30 C.

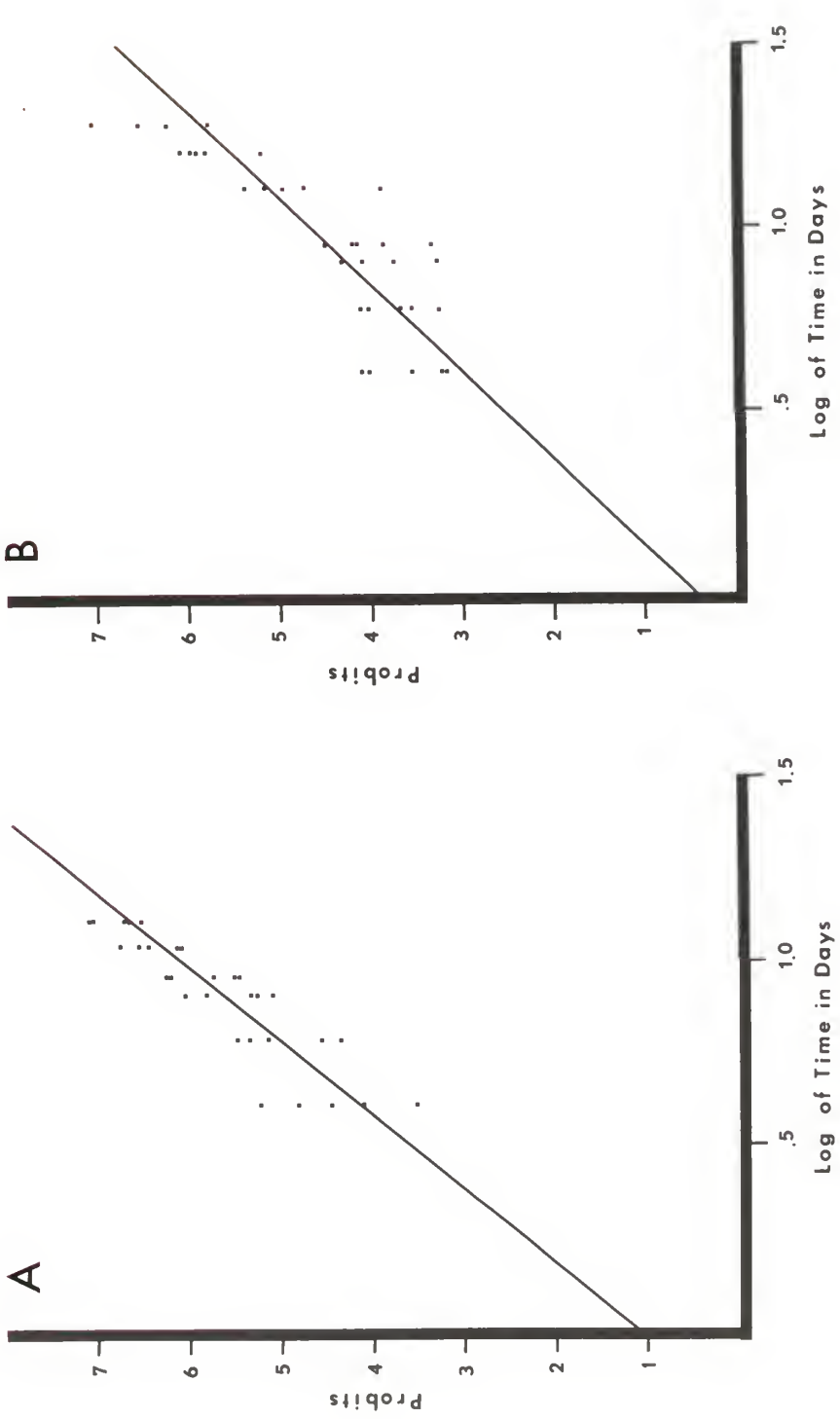
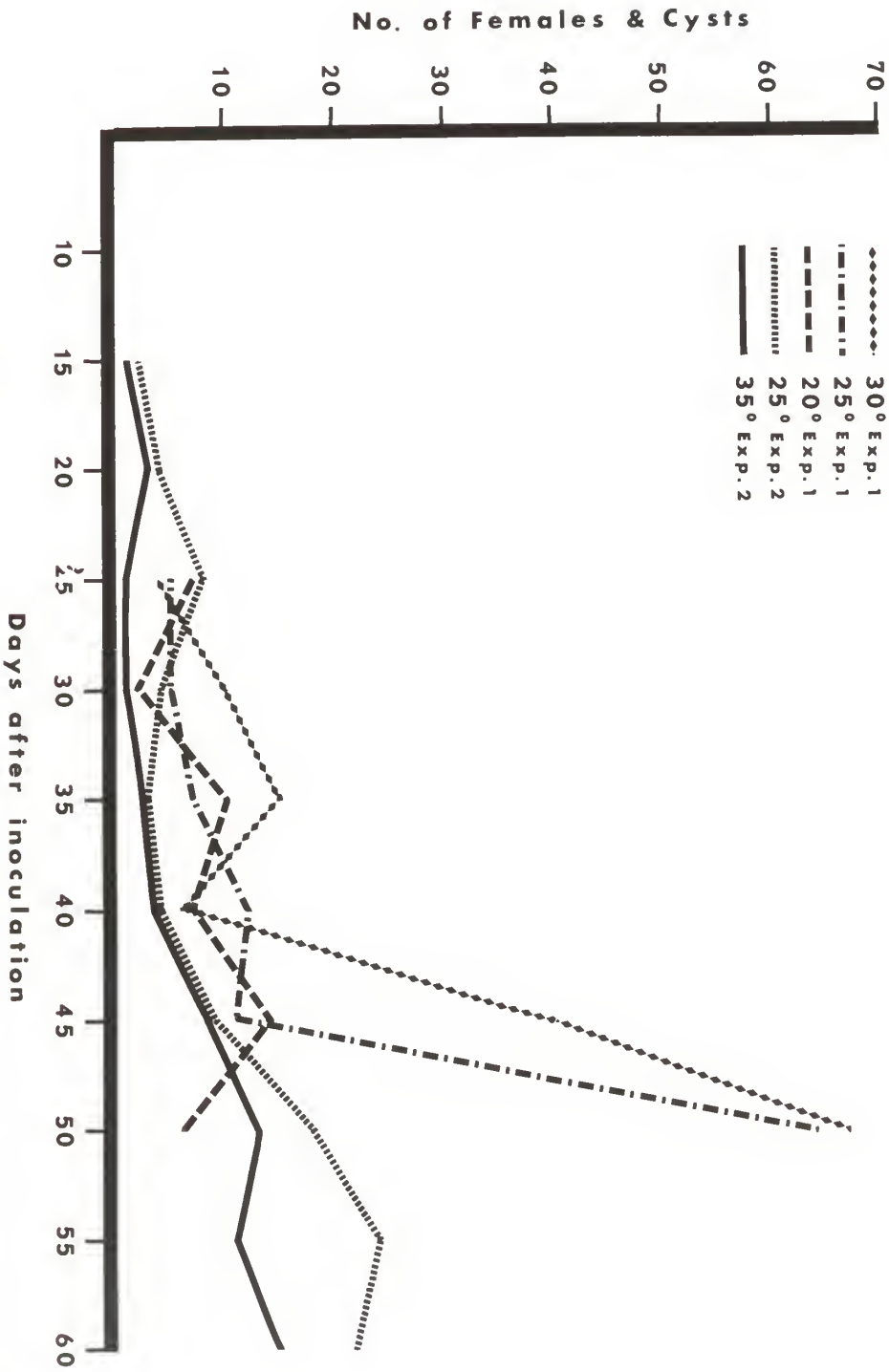

A
B
Probits
7
6
5
4
3
2
1
.5
1.0
1.5
Log of Time in Days

Fig. 2. Average No. of Heterodera longicolla per pot on dates indicated. Each plant was inoculated with 500 larvae.


No. of Females & Cysts
10
20
30
40
50
60
70
30° Exp. 1
25° Exp. 1
20° Exp. 1
25° Exp. 2
35° Exp. 2
10
15
20
25
30
35
40
45
50
55
60
Days after inoculation

development was slightly slower and took 45 days before significant increase in numbers was found. In a second experiment, the same trend was observed for development at 25 C. At 35 C the population increase was slowed but increases were found at 45 days after inoculation. The best temperature for development was about 30 C (Fig. 2).

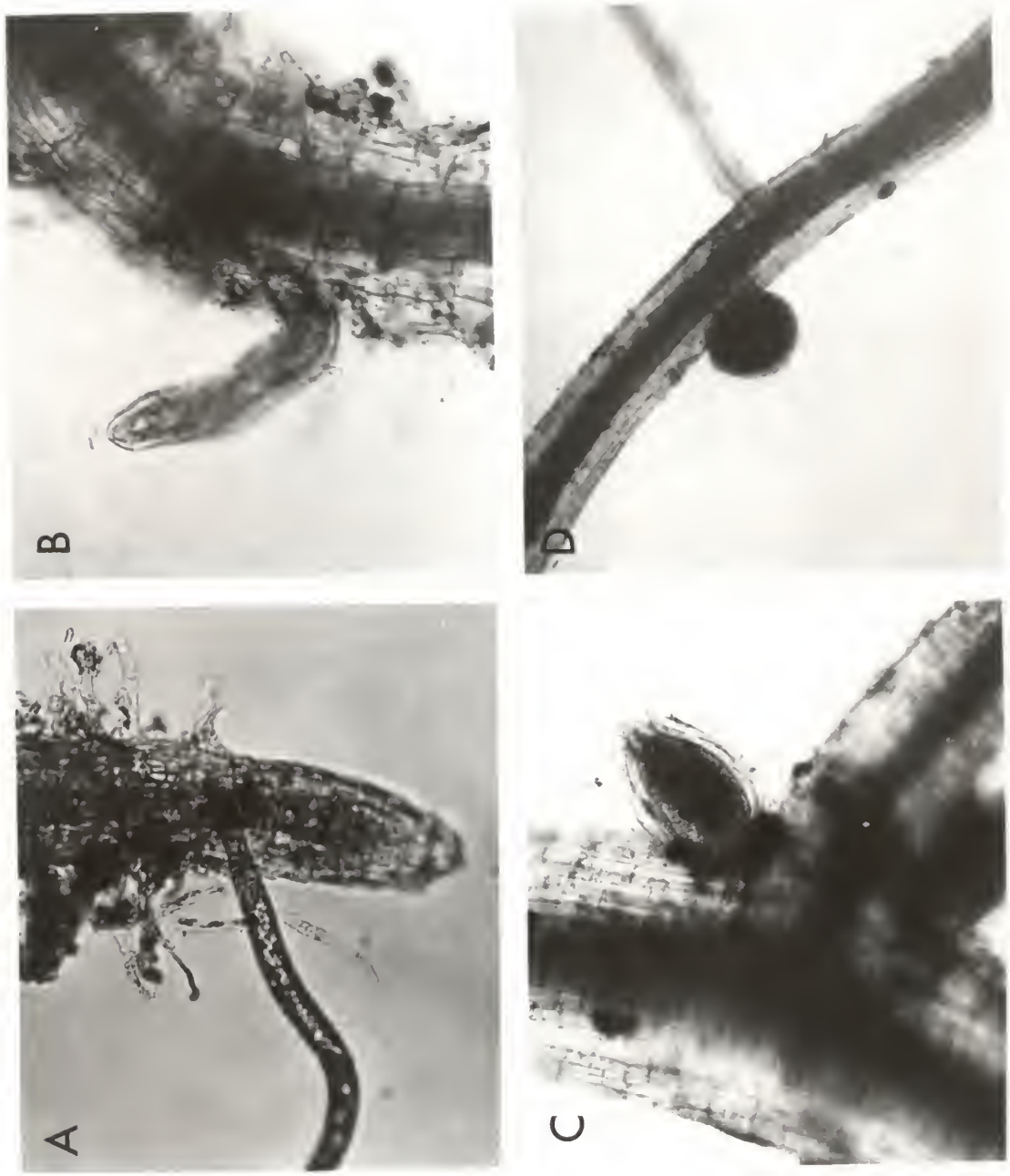
Weights of infected and non-infected plants were not significantly different. A regression analysis of the infected plant weights vs nematode numbers showed that the larger the plant, the more nematodes it could support. No plant stimulation due to nematode association could be detected. At the levels of inoculum used in the experiments, nematode development caused no serious symptoms.

Life Cycle Studies. - Hatching required 2 to 6 days at 27 C. The resultant L_2 larvae continued development after penetrating a suitable host's roots and establishing a permanent feeding site. Body movement ceased as the nematode established a permanent feeding site (Fig. 3-A).

Upon feeding, body contents of the nematode became granular. Five to 7 days after establishment, larvae underwent the second molt, and could be detected by the persistent remnants of the second cuticle. Two to 4 days later the third molt occurred and gave rise to the L_4 or cigar-shaped form (Fig. 3-B), which was never observed feeding. Two to 4 days later the fourth molt occurred to give rise to the adult (Fig. 3-C). If the feeding site was poor, a male developed. Developed males, coiled in their L_4 cuticles, hatched from 4 to 6 days after the fourth molt.

Female vulval-matrix deposition occurred by day 16 post-infection and enlarged white females were observed by day 20. Oviposition occurred as early as day 21 and usually the first eggs were deposited in the vulval matrix. Oviposition continued for 4-6 days during which time the female

Fig. 3. Stages of Heterodera longicolla development on buffalograss roots. A) L_2 larva penetrating at the root-hair zone (X 400), B) L_4 larva (X 360), C) Young female (X 800), and D) Adult female (X 220).


B
D
A
C

became full of eggs. Then the cuticle wall changed from white to yellow which marked the termination of the female (Fig. 3-D). The wall then hardened and turned brown as early as 35 days post-infection. Table 2 presents the stages of development at 27 C.

Histopathology. - Observations made from whole mount and sectioned roots indicated that the preferred larval entry site was in the young roothair zone, but entry was occasionally observed elsewhere. Larvae were observed at the base of a lateral rootlet where entry was gained by the separation of cortical cells (Fig. 3-C.)

Larvae penetrated the root intracellularly and perpendicular to the root axis. Entry of the entire larva or extensive burrowing was rarely observed. Usually only the anterior portion of the larva entered the root. Penetrating larvae ruptured the cells. The surrounding cortex contained the damaged cells and no serious injury was sustained by the root.

Larvae penetrated until their heads were at a suitable location for initiation of the syncytium complex which was typically either an endodermal cell or an adjacent cortical parenchyma cell (Fig. 4-E).

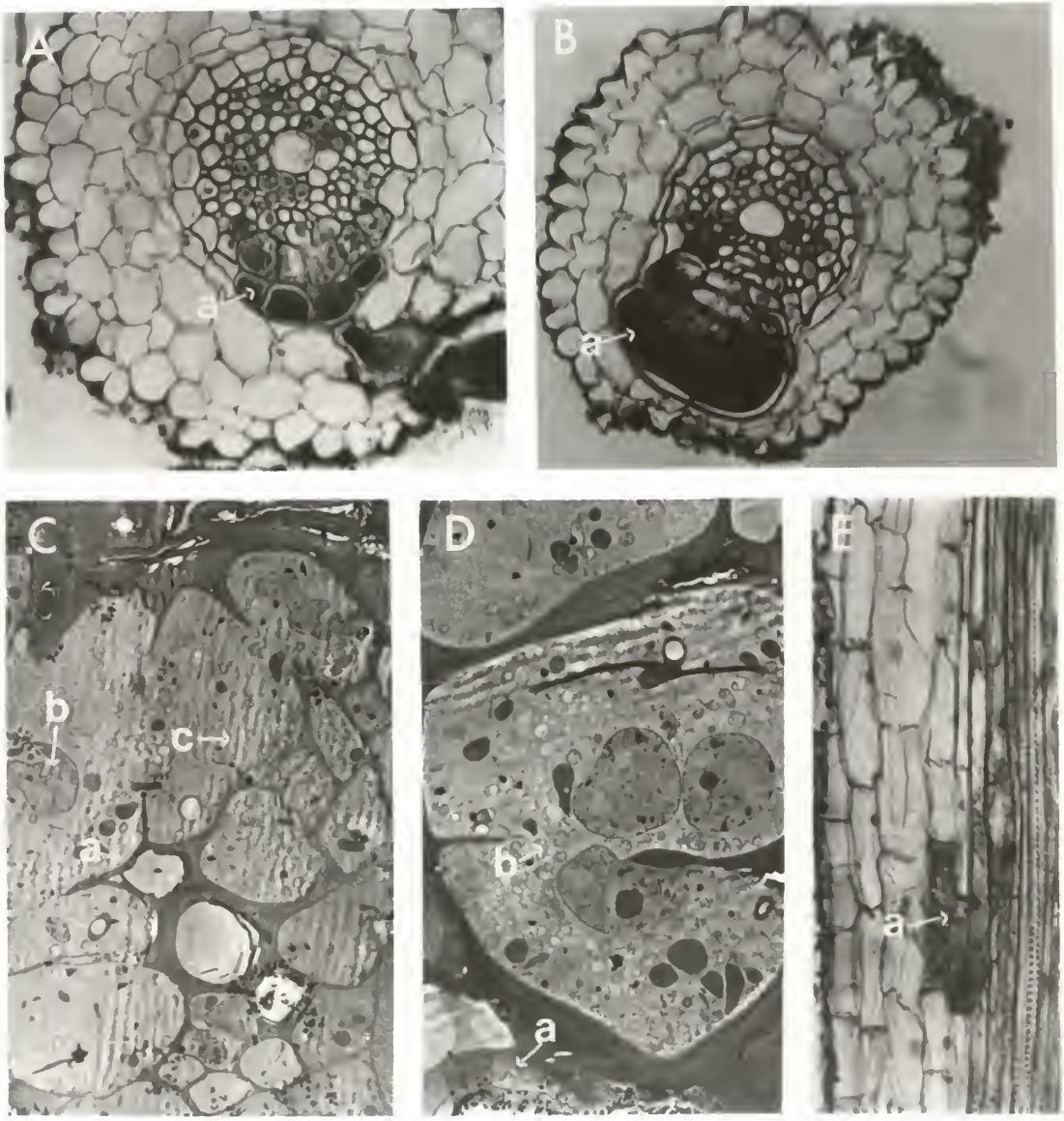
Syncytial Development. - Syncytial development depended upon the location of the nematode's head. It developed in the cortical parenchyma when the initial cell was a cortical parenchyma cell and the cortex, endodermis, pericycle and phloem parenchyma were involved in the syncytial complex (Fig. 4-B). When the initial cell was an endodermal cell, development was oriented centripetally and involved only endodermis, pericycle and phloem parenchyma (Fig. 4-A). Cortical cells hypertrophied 2 to 3 times their original size resulting in a larger syncytium but the nematode was not affected by the location of the syncytium.

The central vacuole became filled with dense and granular protoplasm.

Table 2. Development of Heterodera longicolla at 27 C.

Stages of Development	Days after Infection
Second Molt	5 - 7
L_3 Larvae	
Third Molt	9 - 11
L_4 Larvae	
Fourth Molt	11 - 14
Pre-adult Males & Females	
Matrix Deposition	16
White Females	20
Egg Formation	21 - 25
Hatching	25 - 28
Brown Cysts	35

Fig. 4. A) Syncytium site initiated by Heterodera longicolla in the endodermis of a buffalograss root (X720). B) Syncytium site initiated in the root cortex (X720). C) EM micrograph (X1960) of a portion of a syncytial site demonstrating (a) the lack of cell wall invaginations at the protoxylem, (b) irregularly lobed nuclei, and (c) dense cytoplasm. D) EM micrograph of a portion of a syncytial site with (a) thickened endodermal wall adjacent to a nematode head and (b) interior cell wall breakdown (X4650). E) Longitudinal section of an H. longicolla-incited syncytium initial in a buffalograss root (a) (X800).


A
a
B
a
C
b
c
a
D
b
a
E
a

Nuclei and nucleoli enlarged. Interior cell walls began dissolving while periphery walls of the complex thickened. Breakdown of cell walls appeared to occur outward from a centrally located plasmodesma, leaving fragments where secondary wall thickenings had occurred.

Pericyclic cells adjacent to the syncytium hypertrophied, cell walls dissolved, and contents of affected cells merged with that of the syncytium. Phloem cells incorporated in the syncytium underwent no hypertrophy although cell wall breakdown occurred. Protoxylem elements retained their integrity and were not incorporated into syncytia even when surrounded by syncytial cells. Metaxylem did not appear to be affected. Nuclear division was not observed. No vacuoles were observed at the peak of the nematode's feeding and cellular cytoplasm appeared densest at this time.

Transmission Electron Microscope Observations of Syncytium. - Characteristics of Active Syncytia. Material was observed between the nematode's head and host cell from which it was feeding (Fig. 4-D). This was thought to be used to prevent leakage from the cell after stylet penetration. Secondary thickening of the periphery cell wall of the syncytium was observed with maximum thickness adjacent to the nematode's head (Fig. 4-D).

Breakdown of cell walls appeared to start in interior cell wall plasmodesmata. Gradual dissolving of the walls progressed to the periphery, often leaving wall remnants extending into the syncytium. Syncytial wall invaginations were not observed (Fig. 4-C & D).

Cytoplasm of the active syncytium was characteristic of rapidly metabolizing cytoplasm. Abundant rough and smooth endoplasmic reticulum and ribosomes, both clumped and singular, were present throughout the cytoplasm. Vacuoles were small. Amyloplasts with starch grains and plastids containing crystalline matrices were present. Mitochondria were abundant.

Nuclei of the syncytium were irregularly lobed and in close proximity with one another, made possible by the cell wall breakdown. Often nuclei appeared to follow the contours of the walls, suggesting fluid movement in the cytoplasm (Fig. 4-D). Cells involved in the syncytium were noticeably enlarged and often forced the endodermal and adjacent cortical cell walls to be crushed. No compressive force to the interior vasculature could be observed. When a cortical syncytium site developed, the endodermal cells or cell between the syncytium and central stele were enlarged and intact.

Protoxylem elements were not involved in the syncytium but phloem elements and companion cells were active in the syncytium. Pericycle, endodermal, and cortical parenchyma cells were active in syncytium sites observed.

When the nematode matured, small vacuoles formed in cytoplasm and then coalesced. Rough and smooth endoplasmic reticulum became less obvious. Clumping of scattered ribosomes occurred. Numbers of plastids were reduced. Nuclei were lobed but reduced in size. The cytoplasm became thin and appeared less active.

Host Range. - The ability of H. longicolla to utilize alternate hosts to complete its life cycle was examined. No cysts developed on fifteen plant species inoculated (Table 3).

Preliminary Survey of Geographical Range. - Surveys were conducted in eastern and west central and western Kansas wehere buffalograss grows as a native cover. H. longicolla was found only in Rush and Ellis counties in west central Kansas.

Discussion and Conclusions

Hatching of H. longicolla was not dependent on root diffusates. The

Table 3. Plant Species Tested as Hosts for *H. longicolla*.

Plant	Common Name	Result
Agropyron cristatum	Fairway wheatgrass	-
Andropogon gerardi	Big bluestem	-
Andropogon scoparius	Little bluestem	-
Bouteloua curtipendula	Sideoats grama	-
Buchloë dactyloides	Buffalograss	+
Calamovilfa longifolia	Prairie sandreed	-
Cynodon dactylon	Bermudagrass	-
Echinochloa colonum	'Baldwin' jungle-rice	-
Echinochloa crusgalli var. *frumentacea*	'Chiwapa' Japanesemillet	-
Eragrostis trichodes	Sand lovegrass	-
Festuca sp.	Fescue	-
Panicum virgatum	Switchgrass	-
Poa sp.	Bluegrass	-
Poa pratensis	Kentucky bluegrass	-
Secale cereale	'Balboa' rye	-
Triticum aestivum	'Bison' wheat	-

best hatching temperature was 27 C where ca. 60% of the free eggs hatched. These characteristics of H. longicolla did not make it distinctive from several of the other cyst nematodes (19,21).

No pathogenicity was demonstrated on buffalograss at the inoculum levels used and environmental conditions tested. Reproduction was very slow at 20 C and under, best at ca. 30 C and apparently slowed at 35 C. Development from infective larvae to infective larvae occurred in 25-28 days at 27 C. Development at this rate was possible due to hatching of the eggs deposited in the vulvular matrix. Eggs in the cyst remained dormant. No data was generated to establish the length or conditions of dormancy.

Several aspects of the host-parasite relationship differed from that of most Heterodera spp. Larval penetration was only a few cells deep. Therefore, only a few cortical cells were damaged for each infection site. Both cortical and endodermal syncytial sites developed. In both cases, the syncytium developed from gradual cell wall breakdown and merging of cytoplasm of contiguous cells as has been described for Heterodera spp. (1,4,5,6,7,10,12,13,14,15,16). However, protoxylem and metaxylem elements retained their integrity even when syncytial development encircled these structures. There were no morphological aberations outside of the infected quadrant. Usually the quadrant was delimited by the protoxylem adjacent to the infected site. Normal root functions continued adjacent to syncytial sites with no apparent hindrance.

Syncytial wall modifications similar to those described for H. glycines, H. tabacum, and H. rostochiensis (10,13,14,15) were not found.

It appears that a massive invasion and infection rate would be necessary for H. longicolla to become pathogenic in nature to buffalograss.

Development of the nematode is as rapid as most Heterodera spp., but the effects to the host seem to be intermediate when compared with other members of the group which attack members of the Grass family. H. leuceilyma is a severe pathogen on St. Augustinegrass (5) while H. graminophila doesn't cause the typical syncytial site when parasitizing Echinochloa colonum (2).

Buffalograss with its common fibrous root system constantly replaces old roots with new. Since the nematode does not require root diffusate for hatching, it seems unlikely that a massive invasion of L_2 larvae would attack newer roots to cause serious damage.

H. longicolla appears to be restricted to buffalograss in its presumable native habitat, the short grass prairie of west central Kansas.

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VITA

Phillip Stephen Weberg was born December 7, 1950 in Salina, Kansas, to Earl A. and Mildred E. Weberg. He attended the Sacred Heart parochial school system and graduated from high school in 1969.

He entered Arizona State University in the fall of 1969 and majored in Biology. In 1971 he transferred to Kansas State University and obtained his B. S. in Biological Sciences in the spring of 1973.

In the fall of 1973, he accepted a Graduate Research Assistantship in the Department of Plant Pathology, Kansas State University and started a Master's Degree Program in Nematology.

In the summer of 1977, he started full time farming at Salina, Kansas, taking over for his retired parents.

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BIOLOGY OF *Heterodera longicolla*

BY

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Heterodera longicolla Golden and Dickerson was found parasitizing buffalograss in west central Kansas. Since some Heterodera spp. are economically important parasites of gramineous hosts, the objectives of this study were to 1) gain an understanding of its biology and 2) establish its economic potential.

H. longicolla eggs will readily hatch in distilled or tap water at the rate of 50-60% in a temperature range from 25-30 C. Eggs hatched best when free in tap water at 27 C. Live roots in the hatching medium did not enhance larvae emergence.

Optimum temperature for development was ca. 27 C and it took a minimum of 31 days to complete a life cycle at that temperature. Most eggs do not hatch that soon. The nematode developed very slowly at 15 C (well over 60 days for a life cycle) but did reproduce at 35 C.

H. longicolla did not reduce nor stimulate the rate of buffalograss growth. Nematode development was essentially as described for other Heterodera spp. The infective stage is the L_2 larva. Once feeding began the nematode enlarged and became sessile. The second and third molts came within 7 to 11 days during which time no feeding took place. Males developed during the fourth stage if the feeding site was poor. A few eggs were deposited in a vulvular matrix. The female changed from white to yellow. The cuticular wall hardened and turned brown.

The preferred entry site for L_2 larvae was in the young root-hair zone but occasionally entered openings left by lateral root formation. Penetration was intracellular. Usually only the anterior portion of the larvae entered the root. Surrounding cortex contained the damaged cells.

A syncytial complex developed from a cortical parenchyma cell initial and included cortical parenchyma, endodermis, pericycle and phloem

parenchyma. When the initial cell was endodermal, development was centripetally located and involved only stelar tissues. As the nematode began feeding, the cytoplasm became dense and granular and nuclei and nucleoli enlarged. Interior cell walls dissolved and periphery walls thickened. Cell wall breakdown occurred outward from a centrally located plasmodesma. Syncytial wall invaginations were not observed. At nematode maturity small vacuoles coalesced. Rough and smooth endoplasmic reticulum decreased, ribosomes were clumped and plastid numbers were reduced.

Buffalograss was the only host found for H. longicolla and populations were found only in west central Kansas.