

# Life Cycle of *Heterodera zae* Koshy, Swarup, and Sethi on *Zea mays* L. Axenic Root Explants<sup>1</sup>

J. A. LAURITIS,<sup>2</sup> R. V. REBOIS,<sup>2</sup> and L. S. GRANEY<sup>3</sup>

**Abstract:** Monoxenic cultures of *Heterodera zae*, the corn cyst nematode (CCN), were established on root explants of corn *Zea mays* L., cv. Kenworthy. The life cycle of *H. zae* was determined from light and scanning electron microscopic observations of the root explants grown in the dark at  $29.5 \pm .5$  C under gnotobiotic conditions. The life cycle, from the time the explants were inoculated with second-stage larvae (L2) to the first appearance of newly hatched second-generation L2, required 22 days. The occurrence of males was rare suggesting that reproduction in *H. zae* is parthenogenetic.

Journal of Nematology 15(1):115-119. 1983.

In 1978, a new cyst forming nematode species was first reported in India as *Heterodera zae* Koshy, Swarup, and Sethi, the corn cyst nematode (CCN), with *Zea mays* L. as the type host (3). Initial studies indicated that corn and barley were susceptible, but not wheat, oats, sorghum, pearl millet, finger millet, and rye (3). Srivastava and Swarup (9) reported different levels of susceptibility of corn cultivars to the CCN. Reports on the host status of economically important cereals such as barley, oats, and wheat are conflicting. Barley was reported as both a good (1,3) and a

poor (9) host, whereas oats and wheat were either nonhosts (1,3) or poor hosts (9).

The CCN is reported to infect weed plants (*Urachloa panicades* var. *panicades* P. Beauv., *Echinochloa colonum* [Lim.] Link, and *Digitaria longiflora* Pers.), which commonly occur in corn fields in Rajasthan State, India (12). Since some weeds can serve as alternate hosts during postharvest, the difficulty in controlling the pest is increased.

In 1978, cysts of the CCN were first found in soil samples from the Nile Valley, Egypt (Bakir A., Oteifa, unpublished rept.). The host range study showed that the CCN reproduced on all dent corn, sweet corn, milo, and sudan grass cultivars tested and that two out of eight cultivars of barley and five out of six cultivars of wheat were susceptible to the CCN. Two corn varieties, *Z. mays indurata* and *Z. mays tunicata*, were nonhosts of the CCN.

Maghbool (6) reported the first observations of the CCN in Pakistan in 1981. The

Received for publication 24 May 1982.

<sup>1</sup>This study was supported in part by the U. S. Department of Agriculture Competitive Grants Program, Agreement No. 5901-0410-9-0255-0 and the United States-Israel Agricultural Research and Development Fund-BARD, Grant No. 1-96-80.

The use of a trademark name of proprietary product does not constitute a guarantee or warranty of the product by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

<sup>2</sup>Respectively, Microbiologist and Nematologist, Nematology Laboratory Plant Protection Institute, USDA ARS NER, Beltsville, MD 20705.

<sup>3</sup>Agricultural Technician, Department of Botany, University of Maryland, College Park, MD 20742.

host range included not only the cereals, corn, and wheat, but extended also to citrus and pear.

In 1981, Sardanelia et al. (8) reported the initial observations of the CCN in the USA. The CCN was found in soil samples from corn fields located on the Eastern Shore region, Kent County, Maryland. Preliminary host range studies identified field corn, sweet corn, and barley as hosts, whereas wheat, oats, rye, and buckwheat were not susceptible.

In view of the potential threat of severe economic losses to corn production in the USA and other countries, laboratory studies of the CCN were undertaken. In this report, we describe the establishment of monoxenic cultures and the life cycle of the CCN under gnotobiotic conditions.

#### MATERIALS AND METHODS

*Specimens:* Seeds of hybrid field corn *Zea mays* L. cv. Kenworthy (Lot C 726) and sweet corn cv. I.O. Chief (Lot B197) were purchased from the Meyer Seed Company of Baltimore, Maryland. Cysts and egg masses of *Heterodera zae* were provided by Dr. L. R. Krusberg of the University of Maryland Department of Botany.

*Larval axenization:* Cysts and egg masses were placed on six layers of sterile laboratory tissue supported by a wire screen in a petri dish filled with an antibiotic solution of 50 ppm streptomycin sulfate and 20 ppm quinolinol sulfate (4). After 24 hours, the antibiotic solution containing second-stage larvae (L2) was poured into sterile centrifuge tubes (12 ml) fitted with cotton plugs. The tubes were centrifuged for 5 min at 1,000 rpm, the supernatant discarded, and the L2 resuspended with 5ml methoxyethylmercuric chloride (Aretan) solution (4 ppm). After 3 hours, the L2 which had settled into the conical portions of the centrifuge tubes were collected with a sterile glass pipet (1 ml) and used to inoculate axenic corn root explants.

*Axenic corn root explants:* Seeds of Kenworthy and I.O. Chief were surface sterilized and germinated in the manner as described for soybean seeds (4). After germination, the terminal 2–3-cm portion of each radicle was aseptically excised and transferred to petri dishes (one root/plate)

containing a holidic agar medium (2) and incubated for 3 days in a controlled environmental growth chamber in the dark at  $29.5 \pm .5$  C.

*Establishment of monoxenic cultures:* Three-day-old axenic Kenworthy root explants were inoculated with a 0.1-ml suspension of Aretan-treated L2. The larval suspension was placed at a point farthest from the root system. After 24 hours, roots from uncontaminated plates were transferred to petri dishes with fresh medium (2). The petri dishes were sealed with Parafilm and these and all subsequent cultures were incubated in the dark at 29.5 C. Monoxenic cultures were maintained by transferring gravid females to Kenworthy root explants.

*Determination of the life cycle:* Kenworthy axenic root explants were inoculated with a 0.1-ml sterile aqueous suspension containing  $(50 \pm 2)$  L2. Plates were left unsealed to reduce excess moisture and incubated overnight to allow L2 penetration of the corn roots. After 24 hours, the root explants were transferred intact to petri dishes with fresh medium to prevent further root invasion by L2. Petri plates were sealed with Parafilm<sup>R</sup> and incubated.

For light microscopy (LM), root systems were removed from the petri dishes daily and stained with acid fuchsin in lactophenol (7) for 5 min. Roots were cleared in lactophenol and stained nematodes were teased from the tissue. Specimens were mounted on slides and examined with a LM. The first occurrence of each molt and developmental stage was the criteria used to delineate the time periods of the life cycle sequence.

For scanning electron microscopy (SEM), axenic corn root explants were grown on cellophane discs. Discs were cut from cellophane sheets (DuPont #124PD) to fit the bottom of the petri dishes. The discs were boiled for 10 min in distilled water and then washed for 10 min in running distilled water to ensure removal of the plasticizer. The cellophane discs were placed in glass petri dishes (20/dish) containing distilled water and sterilized by autoclaving for 15 min at 15 psi. Each disc was aseptically overlaid on the surface of hardened medium in petri dishes. Corn root

explants were grown on the cellophane overlay, inoculated with L2, and incubated. The CCN infected root segments were excised and fixed by immersion in 5% glutaraldehyde (Biological grade) in 0.1 M phosphate buffer (pH 7.2) for 24 hours at 5 C. Specimens were washed (3X) with buffer for 15 min each and then post-fixed with 2% OsO<sub>4</sub> in the same buffer for 3 hours at 25 C. Infected roots were dehydrated with 2,2-dimethoxypropane (5) followed by absolute ethanol and critical point dried using CO<sub>2</sub>. Specimens were mounted on aluminum stubs, sputter coated with gold-palladium, and examined with a Hitachi SEM-430 at 10 or 15 KV.

**Maintenance of stock cultures:** At periodic intervals (3-4 wk), white females with egg masses were transferred to fresh I.O. Chief root cultures. Older cultures (ca. 6 wk) exhibited limited root growth possibly resulting from desiccation and "staling" of the medium (10). Continued root growth and nematode development was maintained by adding a thin overlay of fresh medium to the cultures. By using the nutrient agar overlay, stock cultures could be maintained over an extended period (ca. 6 months).

### RESULTS AND DISCUSSION

The CCN life cycle was derived from

observations of the developmental stages on Kenworthy excised root explants. Kenworthy was selected as the host, since the CCN was originally found in soil samples taken from an infested field planted with Kenworthy. Since the CCN can reproduce well on the prolific root cultures of I.O. Chief, this cultivar was used to propagate and maintain monoxenic stock cultures. In contrast, Kenworthy root explants had relatively poor growth in culture. A temperature of 29.5 C was selected for growth and development based on the report that L2 of the CCN hatched at 29-31 C under laboratory conditions (3). A schematic illustration of the CCN life cycle is shown in Fig. 1.

LM observations of CCN infected roots stained with acid fuchsin in lactophenol revealed that 20% (10/51) of the L2 penetrated the roots 1 day after inoculation (DAI). The L2 invaded both the main and lateral roots, including the junctions between the lateral and parent roots (Fig. 2). The second molt (M2) occurred between 3 and 4 DAI and female third-stage larvae (L3) protruded from the root tissues 6 DAI (Fig. 3). The third molt (M3) occurred between 6 and 7 DAI. Fourth-stage larvae (L4) were observed singly or in aggregates on the roots as shown in Fig. 4. The fourth

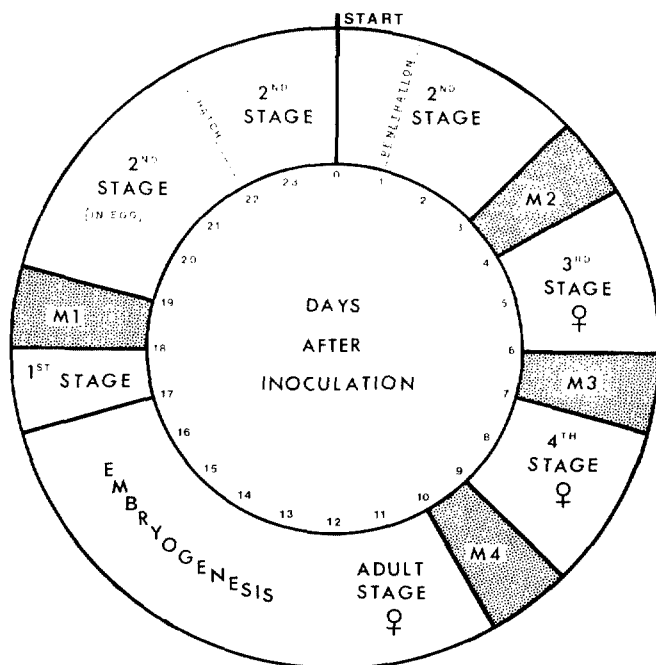
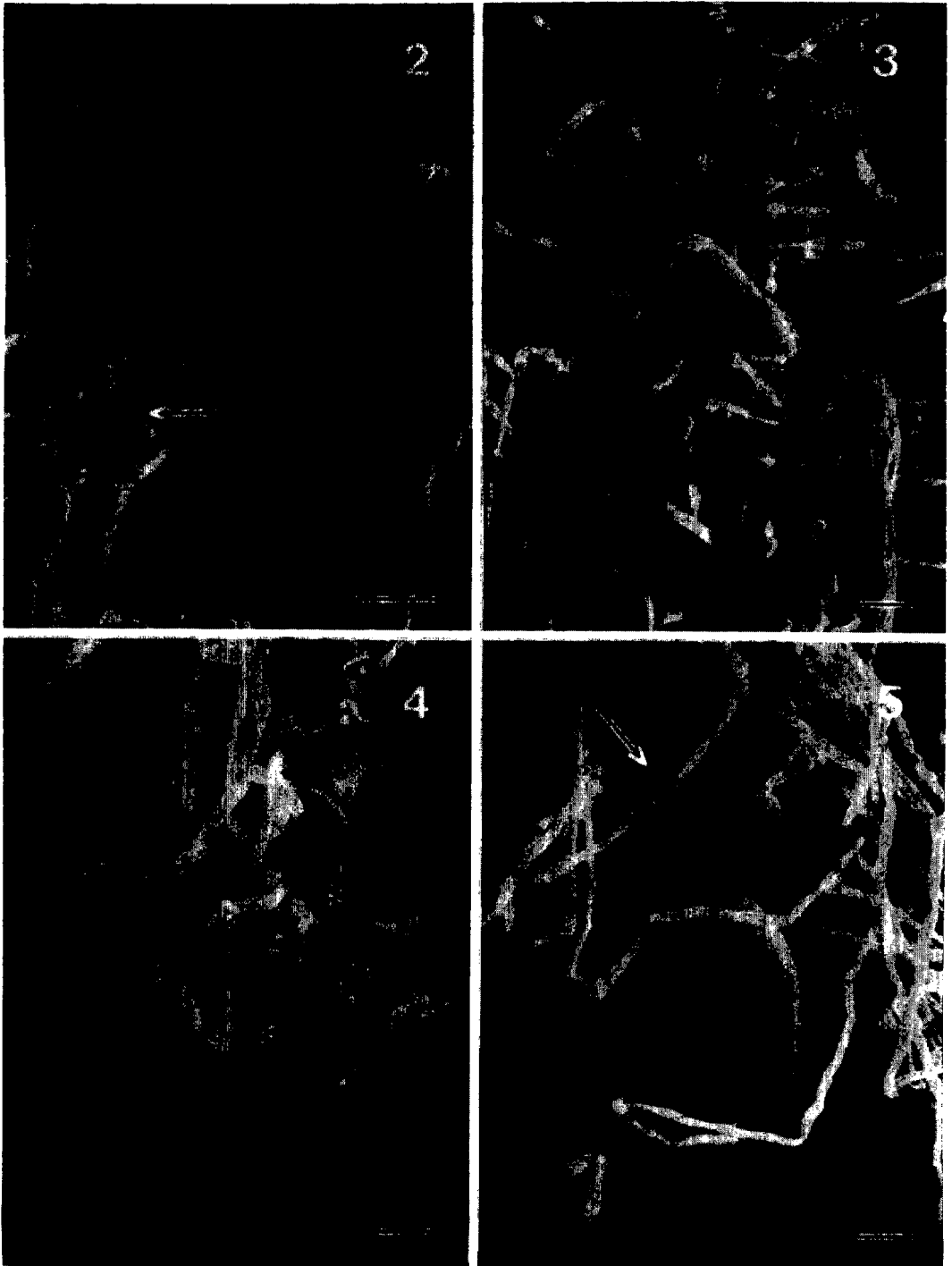


Fig. 1. Schematic representation of the life cycle of *Heterodera zae* on *Zea mays* cv. Kenworthy axenic root explants incubated in the dark at 29.5 ± .5 C.



Figs. 2-5. SEM micrographs of the developmental stages of *Heterodera zeae* on *Zea mays* cv. Kenworthy axenic root explants. 2) Second-stage larva (arrow) penetrating at the junction of the main and lateral root 1 day after inoculation (DAI). 3) Third-stage larva protruding from the root 6 DAI. 4) Aggregate of fourth-stage larvae (\*) 8 DAI. 5) Adult female with gelatinous sac (arrow) 10 DAI. Bar equals 50  $\mu\text{m}$ .

molt (M4) occurred between 9 and 10 DAI. Adult females with gelatinous egg sacs were observed 10 DAI (Fig. 5). A few unembryonated eggs were found within the female body 12 DAI. Thirteen DAI, numerous eggs were observed within the female; the first eggs appeared in the gelatinous sac at 14 DAI. First stage larvae (L1) were observed in eggs at 17 DAI. Molting (M1) of the L1 occurred between 18 and 19 DAI. Initial hatching of the L2 was observed 22 DAI, completing the L2-to-L2 life cycle. Hatching continued with numerous L2 on the agar surface 23 DAI. Since the occurrence of males was rare (1 male/10 cultures) and mating was not observed, it was assumed that reproduction of the CCN was by parthenogenesis. Within the cyst body and gelatinous egg sac, the average number of eggs was 237 and 229, respectively. Thus, eggs were rather evenly distributed between cyst and egg sac.

Verma and Yadav (11) reported that the life cycle (entry of L2 to presence of eggs in adult females, "white cyst") of the CCN on corn was 20 days under field conditions at temperatures of 24–30 C. This cycle appears shorter than the 22 DAI observed under gnotobiotic conditions. In the field study (11), the developmental stage at 20 days would probably correlate to observations of white females with eggs at 13 DAI under gnotobiotic conditions.

The life history of the CCN derived from controlled conditions can provide information on nematode behavior under natural environments. The utilization of monoxenic cultures should prove useful in studies of host-parasite interactions and ensures safe handling of this potentially serious pest of corn.

## LITERATURE CITED

1. Bhargava, S., and B. S. Yadav. 1978. Host range study and evaluation of certain barley varieties to the maize cyst nematode, *Heterodera zae*. Indian J. Mycol. and Pl. Pathol. 8:72.
2. Jacob, J. J. S., and J. van Bezooijen. 1977. A manual for practical work in nematology (revised edition). Intern. Postgraduate Nematology Course, Intern. Agric. Center, Wageningen, The Netherlands.
3. Koshy, P. K., G. Swarup, and C. L. Sethi. 1970. *Heterodera zae* n. sp. (Nematoda: Heteroderidae) a cyst-forming nematode on *Zea mays*. Nematologica 16:511-516.
4. Lauritis, J. A., R. V. Rebois, and L. S. Graney. 1982. Technique for gnotobiotic cultivation of *Heterodera glycines* Ichinohe on *Glycine max* (L.) Merr. J. Nematol. 14:422-424.
5. Lin, C. H., R. H. Folk, and C. R. Stocking. 1977. Rapid chemical dehydration of plant material for light and electron microscopy with 2,2-dimethoxypropane and 2,2-diethoxypropane. Amer. J. Bot. 64:602-605.
6. Maqbool, M. A. 1981. Occurrence of root-knot and cyst nematodes in Pakistan. Nematol. medit. 9:211-212.
7. McBeth, C. W., A. L. Taylor, and A. L. Smith. 1941. Note on staining nematodes in root tissue. Proc. Helminthol. Soc. Wash. D.C. 9:26.
8. Sardanelli, S., L. R. Krusberg, and A. M. Golden. 1981. Corn cyst nematode, *Heterodera zae*, in the United States. Plant Disease 65:622.
9. Srivastava, A. N., and G. Swarup. 1972. Preliminary studies on some graminaceous plants for their susceptibility to the maize cyst nematode, *Heterodera zae* Koshy et al. 1970. Indian J. Nematol. 5:257-259.
10. Street, H. E., M. P. McGonagle, and S. M. McGregor. 1952. Observations on the 'staling' of White's medium by excised tomato roots. II Iron availability. Physiol. Pl. 5:248-276.
11. Verma, A. C., and B. S. Yadav. 1975. Life history of *Heterodera zae* on maize under Udaipur conditions. Indian J. Mycol. and Pl. Pathol. 5:19.
12. Verma, A. C., and B. S. Yadav. 1978. New hosts of maize cyst nematode. Indian J. Mycol. and Pl. Pathol. 8:72.