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Temperature and the Life Cycle of *Heterodera zeae*[1]

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Abstract: Development of the corn cyst nematode, *Heterodera zeae,* was studied in growth chambers at 20, 25, 29, 33, and 36 ± 1 C on *Zea mays* cv. Pioneer 3184. The optimum temperature for reproduction appeared to be 33 C, at which the life cycle, from second-stage juvenile (J2) to J2, was completed in 15–18 days; at 36 C, 19–20 days were required. Juveniles emerged from eggs within 28 days at 29 C and after 42 days at 25 C. Although J2 were present within eggs after 63 days at 20 C, emergence was not observed up to 99 days after inoculation. Female nematodes produced fewer eggs at 20 C than at higher temperatures.

Key words: corn cyst nematode, *Heterodera zeae,* life cycle, temperature.

The corn cyst nematode, *Heterodera zeae* Koshy, Swarup & Sethi, was first described from corn, *Zea mays* L., in India in 1970, where it is widespread in most corn-growing areas and is considered to pose an economic threat to corn production (4,5). *Heterodera zeae* was subsequently detected in several agricultural regions of Egypt (Oteifa, unpubl.) and in Pakistan (8).

The corn cyst nematode was first reported in the Western Hemisphere in 1981 when *H. zeae* was recovered from soil samples collected from corn fields in Kent County, Maryland (10). Subsequent surveys conducted cooperatively by the University of Maryland, Maryland Department of Agriculture, and the United States Department of Agriculture resulted in the detection of *H. zeae* cysts in soil from fields comprising a total of 1,332 ha in four contiguous Maryland counties (Roth, unpubl.). Studies to determine the pathogenicity of *H. zeae* on corn are in progress.

Limited information regarding the biology and temperature requirements of *H. zeae* is available, and that pertains primarily to the Indian population. The objective of this study was to investigate certain aspects of the biology of a Maryland population of *H. zeae,* including the influence of temperature on its life cycle.

Materials and Methods

Cultures of *H. zeae* were initially established on *Zea mays* cv. Pioneer 3184 in the greenhouse as previously described (3). Cysts from these cultures were the source of second-stage juveniles (J2) which were used as inoculum in these studies. Cysts collected from cultures were blended in tap water in a Waring blender for 90 seconds. The blended suspension was poured through a 45-μm-pore sieve, and the residue was incubated on modified Baermann funnels for 4–6 days at 22 C. Juveniles were collected daily from the funnels and stored in tap water at 7 C until used as inoculum. Prior to inoculation, aliquots of the suspension were examined microscopically to ensure that the J2 were active. The nematode suspension was adjusted to a density of 400 J2/ml water.

Corn seed were germinated in flats of acid-washed sand at 25 C. Seedlings 12–15 days old were inoculated by pipeting an aqueous suspension containing 2,000 active J2 onto roots supported on sterile sand in 60-cm³ plastic cups. Roots were covered with moist sand and seedlings were placed in growth chambers at 20, 25, 29, 33, or 36 ± 1 C, with a 12-hour photoperiod, for 48 hours. Following the infection period the seedlings were removed from the sand, the roots were rinsed thoroughly to remove any J2 that had not penetrated, and the seedlings were planted in sterile sand in 4-cm-d pots and returned to the growth chambers. Three plants were sampled

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TABLE 1. Development time and egg production of *Heterodera zeae* at five temperatures.

Developmental parameters	Days from inoculation to first observation				
	36 C	33 C	29 C	25 C	20 C
Second molt	5–7	4–5	6–7	10	12–13
Third molt	7–9	7–8	9–10	14–15	17–18
Fourth molt	9–11	10–11	11–14	19–21	27–28
Egg production	11–13	12–13	15–19	24–28	37–38
First molt (in eggs)	17–19	14–17	26–27	26–37	61–62
Hatch	19–20	15–18	28	42–43	†
Tan cysts	26	30	30	53–57	95
Adult males	14	12–15	15	†	64
Average no. eggs/cyst	190	191	209	235	129

† Not observed.

every 24 hours at 36, 33, 29, and 25 C and every 48 hours at 20 C until the nematode life cycle was completed, and then at intervals of 3–7 days until the experiments were terminated. Roots of infected plants were prepared for examination using the method of Byrd et al. (2). Nematodes were dissected from roots and observed microscopically to determine the stage of development. Washings from sand surrounding the roots of test plants were passed through a 45-μm-pore sieve and incubated on modified Baermann funnels in order to extract J2 and males. Residue from a 250-μm-pore sieve was examined for white females and cysts. The timing of the developmental stages of the nematode life cycle were delineated on the basis of the first observance of each molt and the presence of nematodes in the succeeding developmental stage. The life cycle was considered to be completed when the first emerged J2 was observed. Experiments at each temperature were conducted twice. The data presented in Table 1 are the range of days observed for all plants in the two experiments.

RESULTS

Nematodes penetrated corn seedling roots and developed to maturity at all temperatures (20–36 C) used. The optimum temperature for development appears to be 33 C at which the life cycle was completed in 15–18 days (Table 1). Development was also rapid at 36 C, at which the life cycle was completed in 19–20 days. Emergence of J2 occurred after 28 days at 29 C and after 42–43 days at 25 C. Although J2 in eggs were present after 61 days at 20 C, hatch did not occur even up to 99 days after inoculation.

Development of juveniles was uniform and rapid at 29, 33, and 36 C (Table 1). Nematodes remained as J2 for 4–7 days, third and fourth juvenile stages were completed within an additional 2–4 days for each stage, and mature females were observed 10–12 days following inoculation. Variation occurred in the time required for development to egg production, embryonation, and emergence of J2 from eggs. Juveniles emerged from eggs 1–2 days following the first molt within eggs, and reinfection of roots occurred almost immediately.

Nematode penetration of roots within the 48-hour infection period seemed to occur less readily and nematodes developed more slowly at 20 and 25 C than at higher temperatures. Despite uniform inoculum densities among experiments, roots consistently contained more nematodes at 29, 33, and 36 C than at 20 or 25 C. At 20 C fewer eggs were contained in cysts than at higher temperatures.

A thin subcrystalline layer was present on the body wall of newly developed female nematodes, and gelatinous egg sacs were produced at all temperatures. The maximum number of eggs observed within any single egg sac was 15, most eggs being retained within the female body.

Males were found infrequently at 29, 33,

and 36 C. A single male was found after 64 days at 20 C and none were found at 25 C. On several occasions large numbers of males were recovered from stock cultures maintained on Pioneer 3184 corn at 29 C in plant growth chambers. These cultures supported high nematode population densities, with single plants grown in 6-cm-d pots often yielding more than 20,000 cysts within 2 months of seeding. Males were never observed to mate with females.

Discussion

The development and reproduction of *H. zeae* are favored by relatively high soil temperatures. Under the conditions of these experiments, infection of corn roots was heaviest and nematode development most rapid at 33 and 36 C. At 20 C few J2 penetrated roots within 48 hours, development was slow, and adult females produced fewer eggs than at higher temperatures. A few J2 developed within eggs in females at 20 C, but no hatch was observed during the course of the experiments.

The results of our investigations are similar to those of Srivastava and Sethi (11) who reported that *H. zeae* completed its life cycle in 15–17 days following inoculation at greenhouse temperatures ranging from 27 to 38 C. The length of the life cycle varied on the different corn cultivars tested in their studies. Verma and Yadav (12) reported that development of *H. zeae* J2 to mature egg-filled females occurred in 20 days in the field at temperatures of 24–29 C.

Bajaj et al. (1) reported that adult females of CCN developed on corn within 12 days of inoculation at 23 C and within 6 days at 32 C. At 15 C, J2 penetrated roots but failed to develop further. Nematode development in their experiments occurred much more rapidly than in our experiments. This variation in nematodes of the two populations can be explored only by comparing their development at a single location under identical environmental conditions.

Lauritis et al. (7) studied the development of a Maryland population of *H. zeae* in root explant cultures of *Z. mays* cv. Kenworthy and reported a 22-day life cycle under those conditions at 29.5 C. An average of 229 eggs were deposited within the egg sacs of female nematodes in explant cultures. In contrast, Srivastava and Sethi (11) observed only 4–15 eggs within egg sacs of female nematodes recovered from greenhouse cultures, and they suggested that the Maryland and Indian populations of corn cyst nematode might differ in the number of eggs deposited within the female egg sacs. However, since only 0–15 were found in egg sacs of females during our study, it seems more likely that variation in numbers of eggs extruded results from differences in cultural conditions or differences in host cultivars rather than from inherent differences in nematodes of the Maryland and Indian populations.

Temperature has been reported to influence the susceptibility of relatively poor host plants to infection by *H. zeae.* Bajaj et al. (1) reported that under identical conditions, J2 penetrated maize roots but failed to penetrate roots of wheat at 15 and 23 C; however, nematode penetration and development were similar at 32 C in both corn and wheat roots. Similar behavior was observed during studies on the host range of *H. zeae* (9). Four cultivars of oats and three of wheat, which failed to support detectable reproduction of CCN when grown under fluctuating greenhouse conditions where average low temperatures were 11–22 C, supported slight nematode reproduction when grown in growth chambers at 33 C. These two observations should be considered when selecting experimental conditions for any future tests with *H. zeae.*

Soil temperatures during much of the growing season in Maryland are generally lower than those determined in these studies to be optimum for the development of CCN (6). Soil temperatures 15 cm deep at 7–9 A.M. during 1985 and 1986 ranged from 17 to 22 C during the spring and early summer and were usually 20–26 C during July through September. Soil temperatures of 26–28 C were recorded at 3–5 P.M. during July and August 1984.

The apparently reduced ability of CCN J2 to infect roots, their slow development at or below 25 C, and the resulting low reproduction of CCN may largely account for the low population densities (< 1–20 cysts/250 cm^3 soil) detected in 93% of the infested acreage found in Maryland (Krusberg, unpubl.). The existence of conditions suboptimal for infection of corn roots and development of nematodes, resulting in low soil population densities of *H. zeae* present in most infested soils, may be responsible for the restricted distribution of this nematode in Maryland. However, the high reproductive potential of *H. zeae* at soil temperatures of 33–36 C suggests that in warmer corn-growing regions of the United States CCN could develop high soil population densities and be an economically important pathogen of corn.

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