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Survival of *Heterodera zeae* in Soil in the Field and in the Laboratory[1]

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Abstract: Eggs and (or) second-stage juveniles (J2) inside cysts of *Heterodera zeae* survived over winter in the field with no detectable mortality at all six depths to 30 cm from which soil samples were collected between corn stubble in the row at 4–8-week intervals. Few or no free J2 were recovered from soil collected in January–April from the top 5 cm, but some were recovered at all samplings from soil collected at greater depths. Emergence of J2 from cysts and numbers of females developing on corn roots in bioassays of cysts increased substantially between January and April. Cyst numbers in a fallow area of the corn field did not decline at any depth to 30 cm during 20 months. Free soil J2, J2 emerged from cysts, and females from the bioassay of cysts were highest at the first soil sampling in July after 10 months of fallow; numbers of nematodes in all three categories declined thereafter, but a few were still detectable after 20 months of fallow. Some cysts were still being recovered after 51 months from naturally infested field soil stored moist in the laboratory at 2 C and 24 C. Females were produced in the bioassays of cysts recovered from soil stored for 38 months at 24 C and for 32 months at 2 C. No free J2 were recovered from soil after 1 month of storage at −18 C, but even after 7 months storage J2 emerged from cysts that were recovered and many females developed in bioassays of those cysts.

Key words: corn cyst nematode, *Heterodera zeae,* survival.

The corn cyst nematode, *Heterodera zeae,* was discovered for the first time in the western hemisphere in corn fields in Kent County, Maryland, in early 1981 (4). The economic pest status of this nematode is being evaluated, and studies are being conducted on various aspects of its biology. The survival ability of the corn cyst nematode in the field in Maryland was considered an important facet of its biology, since the other areas of the world where it is known to occur—India, Pakistan, and Egypt—all have much warmer climates than Maryland. Also, most of the infested fields detected in surveys in Maryland contained very low soil population densities of this nematode.

Our objectives were to determine survival of *H. zeae* in a naturally infested corn field and in infested soil from that field stored at constant temperatures.

Materials and Methods

The field study was conducted in Kent County, Maryland, in a grower's field naturally infested with *H. zeae* where corn cultivars and nematicide trials were conducted in 1981 and 1982. The specific sites in this field were selected on the basis of high cyst population densities in 1982. The soil type was a Matapeake silt loam, Typic Hapludults, fine-silty, mixed, mesic consisting of 14% sand, 62% silt, 24% clay, 1.8% organic matter.

Air and soil temperatures were measured with an Electro Therm HT 680 digital thermometer (Electromedics, Inc., Denver, CO) at each soil sampling. Air temperatures were measured in the shade at 15 cm above the ground and at the soil surface. Soil temperatures were measured at 5-cm intervals to 30 cm. A 3-cm-d glass tube with a rubber stopper in the bottom was inserted vertically into the soil to 30 cm deep and left in place for the duration of the experiment. To measure temperature, the thermometer probe was inserted into the glass tube with the tip at the desired depth. At each depth, the probe was allowed to equilibrate for at least 2 minutes and the temperature was measured for 30 seconds. Table 1 presents the soil temperatures at the six depths on each of the 13 days soil samples were collected.

Soil samples for nematode analysis were collected in the row and in an alley between plots at least 2 m from any corn plants.

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Layers of soil ca. 20 × 20 cm square and ca. 5 cm thick were removed with a spade to 30 cm deep. Approximately 1,000 cm^3 soil from each depth was placed in a plastic bag and transported to the laboratory for processing. Soil samples and temperature data were collected similarly from three in-row sites each about 10 meters apart in this field on 3 November and 8 December 1982 and 12 January, 9 February, and 14 March 1983; and from a second set of three sites on 8 December 1983 and 6 January, 13 February, and 11 April 1984. The entire field was plowed and harrowed in April 1983, mixing the topsoil to 30 cm deep before planting. Soil samples were collected from three sites in the alley away from corn plants on 6 July, 8 August, 7 September, 11 October, and 8 December 1983 and 6 January, 13 February, and 11 April 1984. This area (fallow) had supported no corn plants since September 1982.

A 75,000-cm^3 soil sample was collected on 3 November 1982 from areas heavily infested with *H. zeae* and transported to the laboratory for storage at constant temperatures. This soil was passed through a 1.27-cm-pore sieve and mixed thoroughly. Two subsamples of soil were immediately processed for nematode analysis (see following paragraph). The remainder of the soil was divided among six 12-liter polypropylene buckets loosely covered with plastic bags to retard water loss. Two buckets were placed at each of three temperatures: 24, 2, and −18 C. The portion of this study at −18 C had to be abandoned after 7 months because of freezer malfunction. Two subsamples from the bulk soil held at each temperature were processed at various intervals to recover nematodes.

All samples and subsamples of soil processed for nematode analysis throughout this study were 250 cm^3. Cysts were collected on a 850-μm-pore sieve and vermiform nematodes on a 45-μm-pore sieve. The residue from the 45-μm-pore sieve was placed in a modified Baermann funnel and left for 2 days. Numbers of full (containing eggs) and empty cysts were counted and then one-half of the cyst-containing residue was placed in modified Baermann funnels for 2 weeks to allow emergence of second-stage juveniles (J2) from cysts. The other half of the residue was placed in autoclaved sand in 10-cm-square plastic pots into which four seeds of corn (*Zea mays* L. cv. Pioneer 3184) were planted. The corn was grown in the pots for 8 weeks in climatoria at 25–30 C to assess infectivity.

Results

Overwinter: The data for the overwinter survival and distribution in the soil profile for *H. zeae* in the field were variable (Figs. 1, 2). In both winters the highest soil population densities of full and total cysts were generally between 5 and 20 cm deep; these population densities varied little over each 4-month winter sampling period. In both winters free soil J2 were not recovered from the top 5 cm of soil (December 1982, January 1984). In the winter of 1982–83, free soil J2 were recovered at all depths sampled below 5 cm through the February sampling. The lowest soil temperature of 2 C was reached on the 12 January 1983 sampling date (Table 1). In the winter of 1983–84, free soil J2 were not recovered from soil samples taken to 10 cm deep on the 6 January 1984 sampling; no J2 were recovered thereafter at 0–5 cm, but a few were recovered at 5–10 cm deep in April 1984. On 6 January 1984, the soil was frozen to 30 cm deep, varying from +0.5 C at 5 cm deep to −0.1 C at 30 cm deep. Below 10 cm deep, low numbers of free J2 were recovered throughout both winters.

Few J2 emerged from cysts recovered from soil collected at 0–5 cm deep and few bioassay females developed on corn plants inoculated with these cysts either winter. In the winter of 1982–83, J2 emergence from cysts collected at 5–10 cm was steady at an intermediate level through January 1983, then increased dramatically in February and March (Fig. 1). Few females formed on corn roots inoculated with cysts recovered from the November and December 1982 soil samples. The numbers of bioassay females increased through January, February, and March 1983 when corn

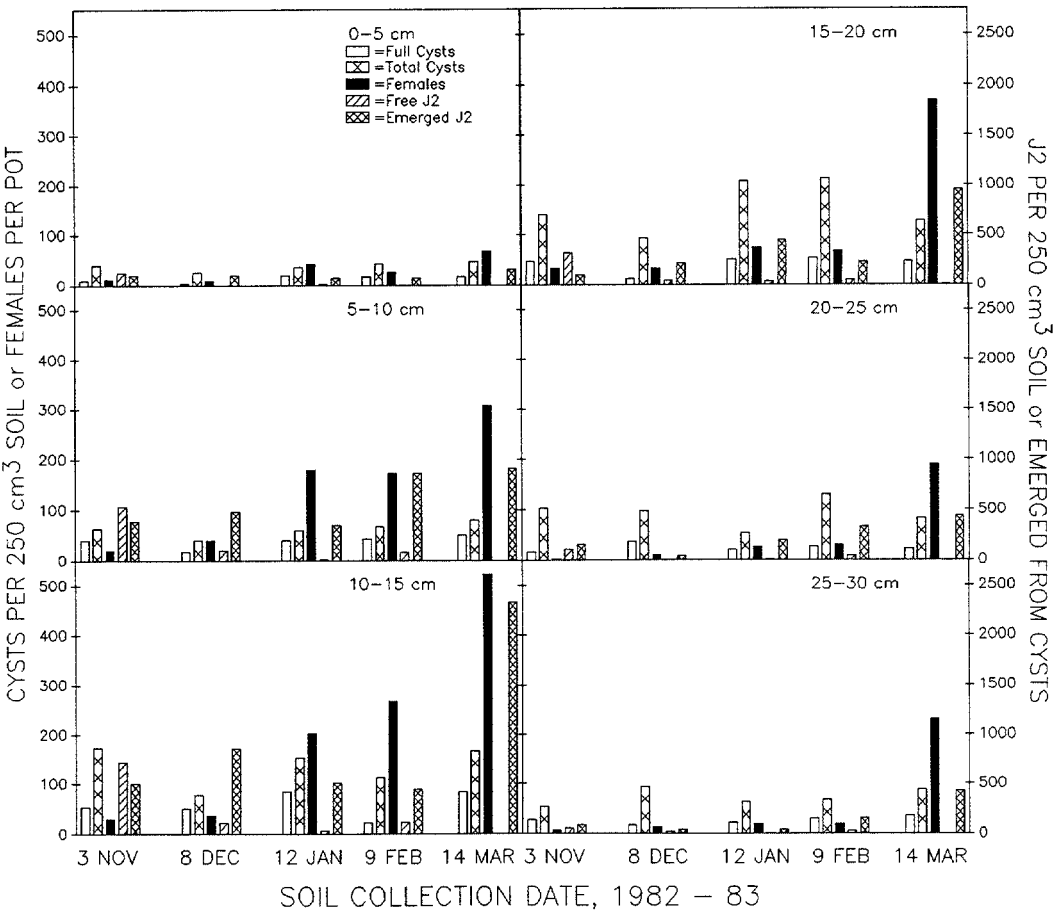

FIG. 1. Population densities of different stages of *Heterodera zeae* at soil depths of 0–30 cm in a naturally infested field from November 1982 to March 1983.

plants were inoculated with cysts recovered at 5–15 cm deep, but the increase in females did not occur until March 1983 when the inoculum was cysts recovered at 15–30 cm deep.

Many J2 emerged from cysts recovered from soil collected on 8 December 1983; however, very few J2 emerged from cysts recovered from frozen soil collected on 6 January 1984 (Fig. 2). Emergence of J2 from cysts from 15 to 30 cm deep was low in December 1983, dropped even lower in January 1984, then gradually increased through April. The fewest females developed from cysts collected in December 1983, and the numbers gradually increased through April 1984. The largest numbers of females developed from cysts collected at 5–15 cm deep.

Fallow: The numbers of cysts recovered remained relatively constant at all sampling depths over the entire 10-month sampling period (Figs. 3, 4). Population densities of cysts with eggs were low (average 20/250 cm^3 soil) throughout the sampling period; however, slightly fewer cysts with eggs were recovered in April 1984 than in July 1983.

Many J2 emerged from cysts recovered at all depths from soil samples collected on 6 July 1983 (average 727/250 cm^3 soil), and this number increased in the 8 August samples (average 1,053). Numbers of emerging J2 plummeted in September (average 134), declined gradually through October (average 17), increased at all depths in December (average 408), and then dropped to a low level for the re-

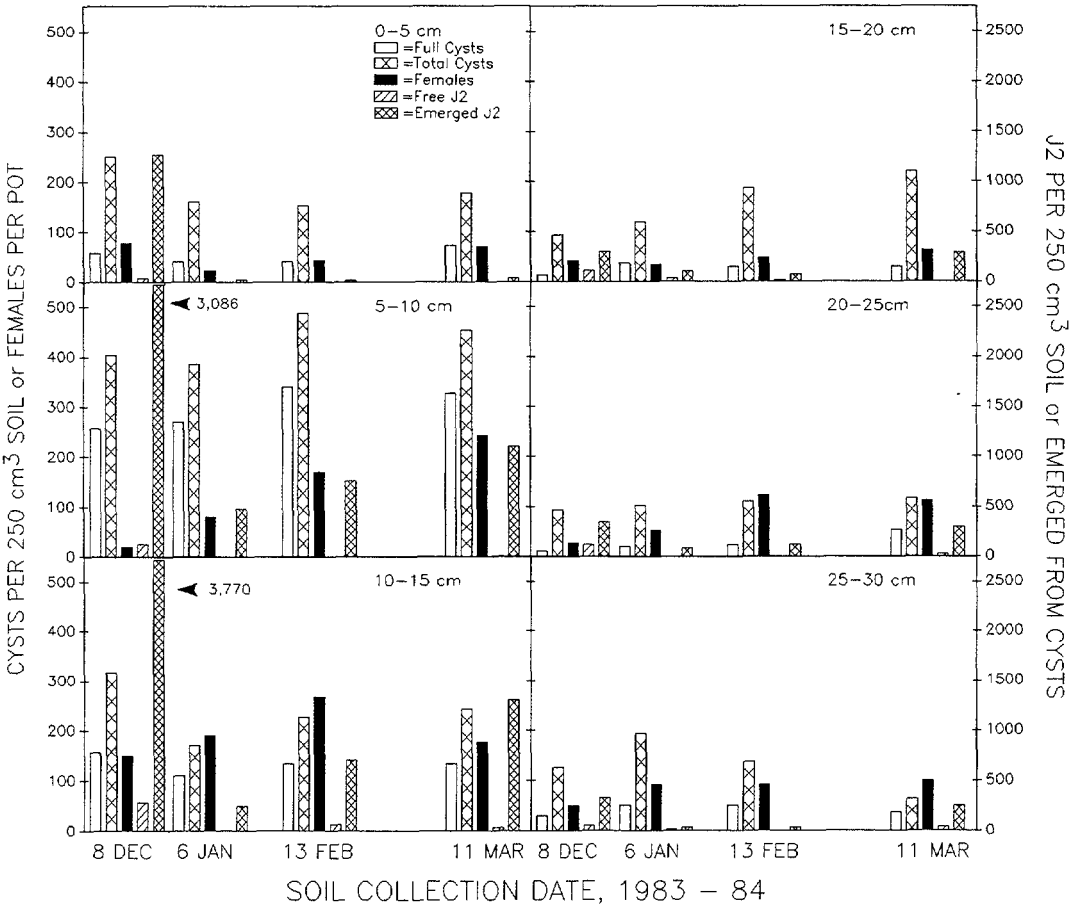

FIG. 2. Population densities of different stages of *Heterodera zeae* at soil depths of 0–30 cm in a naturally infested field from December 1983 to April 1984.

mainder of the samplings through 11 April 1984 (average 31).

During this 10-month sampling period, the greatest numbers of bioassay females resulted when corn plants were inoculated with cysts recovered from soil samples collected at five depths on 6 July 1983 and at 10–15 cm deep on 8 August 1983 (Figs. 3,

TABLE 1. Average field soil temperatures (°C) at 5–30 cm deep on soil sampling dates.

	5 cm	10 cm	15 cm	20 cm	25 cm	30 cm
03 Nov 82	20.3	17.4	16.1	15.3	14.7	14.4
08 Dec 82	11.6	10.4	9.3	9.3	9.5	9.8
12 Jan 83	4.8	4.9	5.2	5.5	5.8	6.1
09 Feb 83	1.9	1.7	1.8	1.9	2.0	2.2
14 Mar 83	10.9	8.9	6.8	5.8	5.3	5.2
06 Jul 83	27.8	28.2	27.2	26.3	25.6	24.7
08 Aug 83	27.2	25.9	25.3	25.2	25.2	25.2
07 Sep 83	27.6	26.7	25.9	25.8	25.9	25.9
11 Oct 83	15.6	15.3	15.3	15.8	16.2	16.9
08 Dec 83	1.5	1.8	2.8	3.7	4.4	5.0
06 Jan 84	0.5	0.0	−0.3	−0.3	−0.3	−0.1
13 Feb 84	8.2	6.8	5.5	4.3	3.9	3.5
11 Apr 84	8.7	7.8	6.9	7.0	7.3	7.8

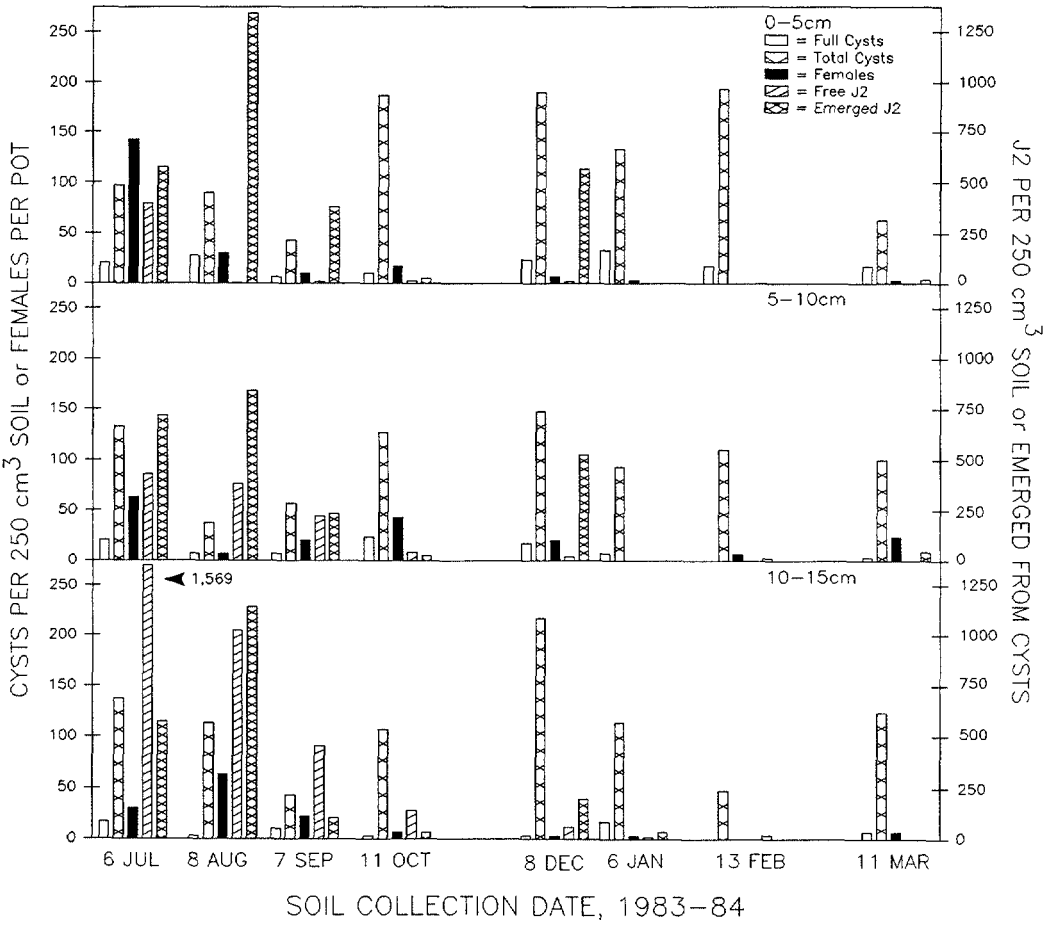

FIG. 3. Population densities of different stages of *Heterodera zeae* at soil depths of 0–15 cm in a naturally infested fallow field from July 1983 to April 1984.

4). Few (average < 10) bioassay females resulted from subsequent soil samples.

Laboratory: The total numbers of cysts recovered from soil stored at 24 C remained relatively constant for 28 months, then decreased somewhat (Table 2). Cysts with eggs were abundant for 4 months, then declined to a relatively constant number through 22 months after which they virtually disappeared. A few cysts with eggs were recovered at 38 months, but none thereafter.

Free soil J2 were relatively abundant through 2 months storage of soil at 24 C; the numbers decreased by ca. 50% at months 3 and 4, gradually increased to peak at 9 months (August 1983), and declined erratically to very few at 25 months (Table 2). A few free soil J2 were recovered at 35 months, but none thereafter. Juveniles emerged from cysts in large numbers through 22 months of soil storage, then declined with few recovered at 38 months and none thereafter.

Few (20) bioassay females resulted from cysts separated from freshly collected soil (Table 2). The numbers of bioassay females increased to a peak of 770 at 4 months storage at 24 C (March 1983), declined to a low at 9 and 10 months (August and September 1983), increased somewhat through 32 months, and declined until none were detected at 44 or 51 months.

The total numbers of cysts recovered from soil stored at 2 C remained relatively constant over the 51 months of this study (Table 2). Numbers of cysts with eggs declined after 31 months, but variation was

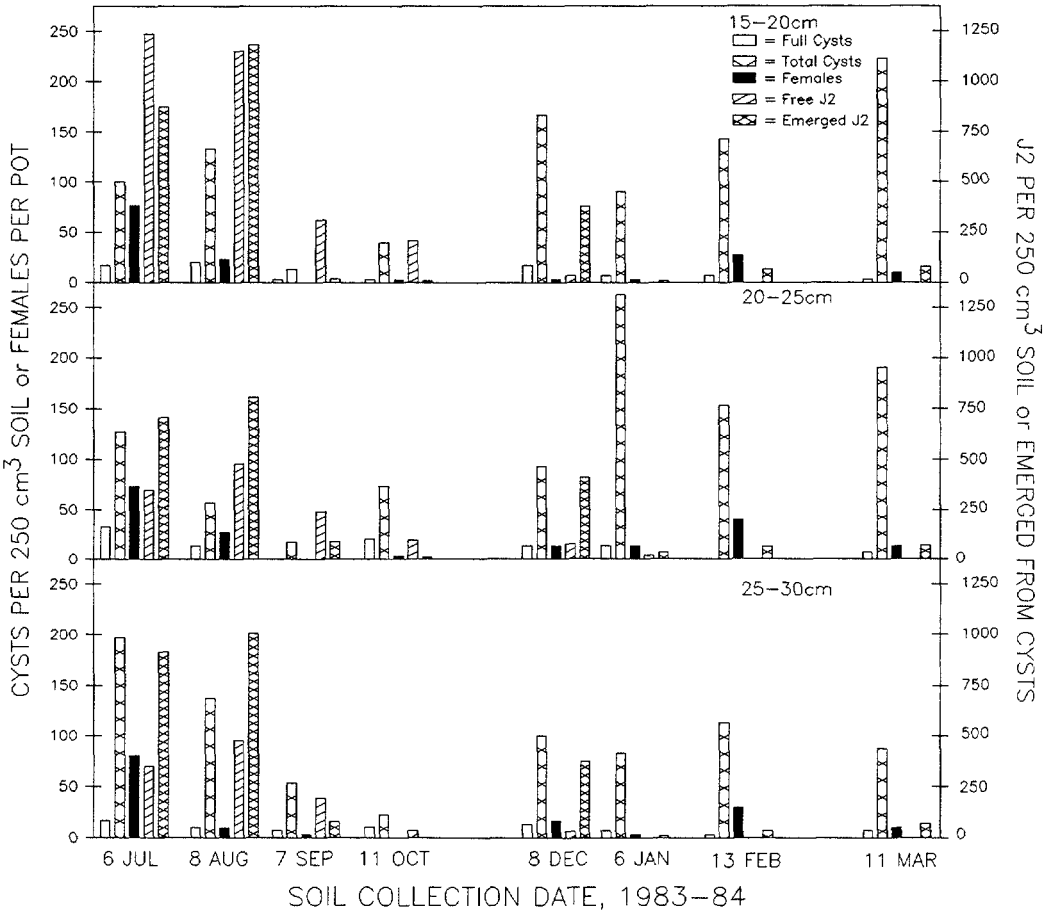

FIG. 4. Population densities of different stages of *Heterodera zeae* at soil depths of 15–30 cm in a naturally infested fallow field from July 1983 to April 1984.

great. Numbers of free soil J2 were variable for 3 months, none were recovered at 4–7 months, large numbers were recovered at 8 months, and few or none were recovered thereafter. Emergence of J2 from cysts was high for the first 9 months, generally light for the next 10 months, and nonexistent after 19 months. Numbers of bioassay females rose from 65 after 1 month of storage to 910 after 6 months (May 1983), then declined and was erratic for 13 months until June 1984 (19 months) after which few females appeared; no females were detected after 35 months.

No free J2 were recovered from soil held at −18 C (Table 2). In contrast, many J2 emerged from cysts and many bioassay females resulted from cysts recovered from soil held at −18 C at all samplings through 7 months.

DISCUSSION

Soil population densities of full cysts (cysts with eggs) were expected to decline with time rather than remain relatively constant, especially in the fallow location where the first soil samples were collected 10 months after the last planting of corn in the area. In the laboratory the numbers of total cysts and full cysts decreased earlier at 24 C than at 2 C. It might have been interesting to determine the numbers of eggs inside the various groups of cysts recovered, but the proportion of those eggs that were viable and would give rise to infective J2 could not be ascertained. The best measure of nematode survival is the bioassay.

Heterodera zeae cysts in the field are reasonably durable, persisting for 51 months

TABLE 2. Population densities of different developmental stages of *Heterodera zeae* per 250 cm³ infested field soil stored moist in the laboratory at 24, 2, and −18 C.

Date soil processed	Months stored	Full cysts	Total cysts	Free soil J2	J2 emerged from cysts	Bioassay females
			24 C			
03 Nov 82	0	55	170	579	1,255	20
14 Dec 82	1	55	150	827	1,872	45
12 Jan 83	2	65	150	657	761	185
09 Feb 83	3	60	190	319	1,398	415
14 Mar 83	4	80	120	325	2,587	770
16 May 83	6	25	145	845	2,860	425
15 Jun 83	7	30	80	1,177	1,950	95
15 Jul 83	8	15	60	1,001	533	35
18 Aug 83	9	35	90	2,275	4,095	5
14 Sep 83	10	20	35	1,547	1,287	0
17 Oct 83	11	65	185	403	403	40
14 Nov 83	12	20	75	111	1,300	100
15 Dec 83	13	25	80	1,060	2,015	15
14 Feb 84	15	40	175	267	260	100
16 Apr 84	17	10	40	13	644	25
18 Jun 84	19	20	105	247	1,047	75
17 Sep 84	22	25	90	26	967	20
17 Dec 84	25	5	135	7	663	50
25 Mar 85	29	0	65	0	442	30
02 Jul 85	32	0	15	0	182	120
02 Oct 85	35	0	50	7	117	5
07 Jan 86	38	10	45	0	26	5
01 Jul 86	44	0	25	0	0	0
04 Feb 87	51	0	25	0	0	0
			2 C			
14 Dec 82	1	50	105	247	1,209	65
12 Jan 83	2	115	245	91	273	105
09 Feb 83	3	65	190	130	1,183	630
14 Mar 83	4	115	185	0	754	455
16 May 83	6	10	80	0	403	910
15 Jun 83	7	15	50	0	397	295
15 Jul 83	8	25	85	3,380	377	40
18 Aug 83	9	25	50	0	501	30
14 Sep 83	10	50	140	13	65	0
17 Oct 83	11	60	195	0	26	65
14 Nov 83	12	60	250	7	130	20
15 Dec 83	13	40	175	0	306	0
14 Feb 84	15	65	195	0	3	85
16 Apr 84	17	25	75	0	33	15
18 Jun 84	19	80	210	0	13	205
17 Sep 84	22	30	100	0	0	5
17 Dec 84	25	5	170	0	0	5
25 Mar 85	29	0	50	0	0	0
02 Jul 85	32	0	20	0	0	5
02 Oct 85	35	5	30	0	0	5
07 Jan 86	38	25	125	0	0	0
01 Jul 86	44	40	155	0	0	0
04 Feb 87	51	0	55	0	0	0
			−18 C			
14 Dec 82	1	45	85	0	260	45
12 Jan 83	2	60	225	0	72	130
09 Feb 83	3	80	240	0	13	100
14 Mar 83	4	145	215	0	72	310
16 May 83	6	70	155	0	163	390
15 Jun 83	7	40	50	0	39	185

in field soil stored moist in the laboratory at 2 or 24 C, even though they are thin-walled and appear fragile compared, for example, with cysts of *H. glycines*. In this field, however, a single year (1984) in red clover resulted in a sharp decline in recoverable cysts, to 10–20/250 cm^3 soil, and this soil population density of cysts remains low for some unknown reason despite 3 years of continuous corn (1985–88). *Heterodera glycines* cysts survived up to 1.5 years in flooded soil, more than 3 years in air dry soil, and nearly 8 years in soil held near field capacity (6).

Heterodera zeae appears to have a biological clock for hatch. Population densities of free J2 in soil in the field and in field soil stored in the laboratory followed a similar trend. They were most numerous during May through July and decreased to low numbers by midwinter. Free J2 in soil, emergence of J2 from cysts, and bioassay females followed this seasonal pattern whether from freshly collected field soil or soil stored at 24 C or 2 C. Eggs of *Globodera rostochiensis* hatched seasonally from February to May in England (5), and in Australia a similar seasonal hatch of *H. avenae* eggs occurred with the onset of cool soil temperatures in April–June (1). Information until 1977 on the effects of season on egg hatch, diapause, and dormancy in cyst-forming nematodes has been summarized (2).

Our field study lasted 18 months, and the differences between the winters of 1982–83 and 1983–84 emphasize the need to conduct such studies over several years to determine the variation in nematode activity associated with variations in environmental conditions. Projections based on too small a data base can lead to erroneous conclusions. Unfortunately the grower no longer wished to cooperate with us, so our field study had to be abandoned prematurely. Okada (3) similarly observed large differences in hatch of *H. glycines* eggs obtained from cysts from soil samples collected in two consecutive Octobers.

The top 5 cm of exposed field soil would appear to be an inhospitable soil environment for nematodes because of its rapid changes in moisture content and temperature. The low nematode population densities obtained from the surface soil stratum in the winter of 1982–83 supported this conclusion, but then much higher numbers of nematodes were found in the surface stratum the following winter of 1983–84. Only free J2 seemed consistently less able to survive in the top 5 cm of soil in the field than at deeper strata. *Heterodera schachtii* survived in cysts over summer in the top 2.5 cm of fallow clay-loam soil where the soil temperature frequently exceeded 40 C in the daytime (7). It also thrives in cooler areas like Michigan and New York where the soil freezes in winter. Thus *H. schachtii* probably can survive a wider range of environmental conditions than can *H. zeae*.

The laboratory study of soil stored at three temperatures demonstrated that although *H. zeae* J2 free in soil died quickly at −18 C, J2 and (or) eggs inside cysts survived well at −18 C and were infective after 7 months. *H. zeae* survived and was infective after almost 4 years in soil at 24 C and almost 3 years in soil at 2 C.

Heterodera zeae does not appear to survive as long as certain other cyst nematodes, such as *H. glycines* and *G. rostochiensis*; however, infective *H. zeae* can survive at least 19 months in a fallow field with fine silty-clay soil and more than twice as long in the laboratory. Studies are needed to determine how long *H. zeae* can survive in the field with different soil types, temperatures, moisture levels, and plant covers from the ones we studied. Such information is needed to develop the best methods for managing this nematode in infested fields.

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