Supplement to Journal of Nematology 25(4S):820–823. 1993.

Resistance in *Zea mays* to *Heterodera zeae*[1]

GHAZALA HASHMI,[2] SARWAR HASHMI,[2] LORIN R. KRUSBERG,[2] AND ROBIN N. HUETTEL[3]

Abstract: Twenty-three precommercial field corn lines (*Zea mays*) were screened in the greenhouse and in vitro for the ability to support reproduction of *Heterodera zeae*. Although *H. zeae* reproduced on all corn lines, reproduction was only 0.4 to 4.5% on the five least suitable corn lines in greenhouse tests compared with the susceptible check line Pioneer brand 3184. The least suitable experimental line supported an average of 30 cysts plus females after 8 weeks growth, whereas the susceptible check, Pioneer brand 3184, averaged 8,183 cysts plus females per pot. Reproduction of *H. zeae* in in vitro root cultures of the 23 lines and susceptible check cultivar, Iochief, was too low to be of any value in detecting resistance to this nematode under the conditions of these tests.

Key words: corn cyst nematode, corn, greenhouse, *Heterodera zeae*, in vitro, nematode, reproduction, resistance, *Zea mays*.

The corn cyst nematode, *Heterodera zeae*, was first described from corn, *Zea mays*, in Rajasthan State in India (8). This economically important nematode is widely distributed in most corn growing areas in India (7) and Egypt (1), and it also occurs in Pakistan (2). In 1981, *H. zeae* was reported for the first time in the Western Hemisphere in corn fields in Kent County, Maryland (13).

A Kent County, Maryland, population of *H. zeae* reproduced on 22 corn cultivars and certain cultivars of barley (*Hordeum vulgare*), oat (*Avena sativa*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), sugarcane (*Saccharum* interspecific hybrid), and wheat (*Triticum aestivum*) in greenhouse and growth chamber tests (12). Suppression of corn plant growth by the Maryland population of *H. zeae* was demonstrated in tests in microplots (9) and in plant growth chambers (6).

Because *H. zeae* can reproduce on and suppress growth of many corn cultivars, untested breeding lines needed to be tested against this nematode. In vitro screening techniques have been used to detect resistance to *H. glycines* in soybean lines (10) and for resistance to *Meloidogyne incognita* and *M. hapla* in tomato (3,4).

The objectives of this study were i) to compare greenhouse tests with plant tissue culture methods for screening corn lines for resistance to this nematode and ii) to determine whether resistance to *H. zeae* in corn breeding lines could be detected by in vitro studies.

MATERIALS AND METHODS

Greenhouse studies: An isolate of *H. zeae* was maintained on plants of *Zea mays* Pioneer 3184 growing in autoclaved washed builder's sand contained in 10-liter pots placed on plant propagation mats to maintain the sand at approximately 30 C in the greenhouse. Cysts were collected from the sand by washing in a bucket and passing the suspension through a 250-μm-pore sieve. Inoculum was prepared by placing the cysts on a 150-μm-pore sieve and breaking them open by gentle rubbing with a rubber stopper to release the eggs and second-stage juveniles (J2). The eggs and J2 were collected on a 25-μm-pore sieve and suspended in tap water. The aqueous suspension (20 ml) containing 5,000 ± 250 eggs and J2 was pipetted onto the surface of 800 cm^3 autoclaved washed builder's sand contained in each 15-cm-d plastic pot. Seeds of 23 precommercial corn lines were provided by Pioneer Hi-Bred International, Inc., Des Moines, Iowa. Iochief and Pioneer 3184 corn

Received for publication 16 February 1993.

[1] Scientific article number A 6434, contribution number 8628 from the Maryland Agricultural Experiment Station. Supported in part by Cooperative Agreement Number 58-319R-1-004 from OICD, USDA, and the Egypt NARP program.

[2] Graduate Research Assistants and Professor, Department of Botany, University of Maryland, College Park, MD 20742-5815.

[3] Microbiologist, USDA ARS, Nematology Laboratory, Beltsville, MD 20705-2350.

served as susceptible checks. Five seeds of a corn line were placed onto the sand in each pot and covered with another 200 cm^3 autoclaved sand. Pots were placed on plant propagation mats to maintain a sand temperature of approximately 30 C. The experiment was arranged in three randomized complete blocks. After 7 days, the plants were thinned to two per pot. Plants were fertilized once weekly and watered as required. Two tests, each of 8 weeks duration, were conducted: 5 June 1990 to 5 August 1990 and 25 May 1991 to 25 July 1991. Cysts were collected by washing all the sand in each pot in a bucket and passing the aqueous suspension through a 250-μm-pore sieve. Cysts were counted with a stereoscopic microscope at ×20 magnification.

Data from the two experiments were combined for statistical analysis as a randomized complete block design (11). Treatments means were separated by LSD ($P = 0.05$).

In vitro studies: An isolate of *H. zeae* from a Maryland population was established in vitro and maintained on sterile root explants of *Z. mays* cv. Iochief growing on Gamborg's B5 medium (Sigma Chemical Co., St. Louis, MO) at pH 5.6–5.8 at 30 C (5). Twenty seeds of each corn line, replicated three times, were surface sterilized with 1.0% sodium hypochlorite for 10 minutes followed by immersion in 95% ethanol for 3 minutes, and then germinated in petri dishes on 1.0% water agar. For growth rates, two roots per plate from each corn line were transferred to Gamborg's B5 medium, and fresh and dry weights of roots were obtained after 3 weeks of growth. For screening of corn lines in vitro, five petri plates, each containing two roots from each corn line, were inoculated with five mature cysts propagated in vitro, which were broken open aseptically. Visual observations were made with a dissecting microscope at ×45 magnification to observe nematode development at 10, 12, 15, and 35 days. Total counts of cysts and females were made on day 35. The corn cultivar Iochief was the susceptible check. This study was conducted three times.

Data were combined from all three experiments for statistical analysis as a randomized complete block design (11). Treatment means were separated by LSD ($P = 0.05$).

Results and Discussion

In both greenhouse and in vitro tests, the interaction effect between experiments and treatments was not significant, indicating that treatment performance was consistent among experiments. The combined results of the two greenhouse screening tests of precommercial corn lines demonstrated that resistance to *H. zeae* exists in *Z. mays* (Table 1). All of the precommercial corn lines screened in both tests supported less reproduction of *H. zeae* than the susceptible checks, Pioneer 3184 and Iochief. Two lines, numbers 4 and 5, were particularly resistant, supporting only 0.4 and 1.0%, respectively, of the reproduction obtained on Pioneer 3184. Visual observation

Table 1. Reproduction of *Heterodera zeae* on precommercial corn lines grown in pots of soil in the greenhouse.

Corn line	Total number of cysts + females	Corn line	Total number of cysts + females
Pioneer 3184 (check)	8,183	19	2,677
Iochief (check)	6,542	12	2,438
20	4,188	23	2,412
13	4,075	9	1,895
17	3,677	10	1,537
21	3,607	8	1,477
15	3,502	11	1,233
14	3,397	7	572
22	3,297	1	368
16	3,142	3	212
18	3,052	2	148
24	2,755	5	82
		4	30
LSD (0.05)	919		

Data are the mean numbers of cysts and females produced per pot after 8 weeks in two tests, with three replications in each test.

of the root systems showed no obvious differences in growth among corn lines.

In vitro reproduction of *H. zeae* on all corn lines 5 weeks after inoculation was lower ($P = 0.05$) than on the susceptible check Iochief. Root weight data indicated that corn lines 2, 3, and 15 grew better than Iochief, and corn lines 8, 12, 13, and 14 had less root growth than Iochief. Corn lines 1, 5, 13, 15, 17, 18, 19, 22, and 24 showed no nematode development before 35 days (Table 2). Late nematode development on these lines might indicate some tolerance towards the nematode. Comparison of the results on nematode development with root weights of corn lines 1, 2, 3, 5, and 15 indicates that lines showing low nematode development had good root growth. Therefore, we conclude that low nematode reproduction was not due to failure of root growth. Corn lines 14 and 19 did not support any nematode reproduction and also had less root growth than the other lines.

The logical sequel to this study would be to screen the parent material of the most resistant corn lines to determine the source(s) of the resistance. The resistant parent(s) could then be used to develop resistant cultivars for planting in *H. zeae*-infested land. Due to proprietary rights, it has not been possible to pursue such investigations. According to a Pioneer official (Bryan Anderson, pers. comm.), lines 1–5, which supported the lowest nematode reproduction, all have a common inbred parent. Lines 10, 11, 22, and 24 also have some of the same ancestry as lines 1–5. However, line 7, which supported only moderate nematode reproduction, has no common pedigree with the aforementioned lines. A search for *H. zeae* resistance in nonproprietary corn lines has been initiated.

TABLE 2. Dry weights of roots of corn lines and development of *Heterodera zeae* on root explants in vitro.

Corn line	Root dry weight (mg)		Cumulative number of nematodes developing†			
			Day 10	Day 12	Day 15	Day 35
3	11.4	a	0	0	2	4
2	8.6	ab	0	0	2	5
15	8.3	abc	0	0	0	2
5	7.7	bcd	0	0	0	5
1	7.7	bcd	0	0	0	2
23	7.1	bcde	0	1	5	11
7	6.9	bcde	2	3	4	13
Iochief (check)	6.6	bcde	7	12	20	32
18	6.5	bcde	0	0	0	2
16	6.4	bcdef	0	0	2	5
22	5.9	bcdefg	0	0	0	9
24	5.8	bcdefg	0	0	0	11
4	5.6	cdefg	0	0	1	2
9	5.4	cdefg	0	0	3	5
10	5.2	cdefg	0	1	2	12
21	5.0	defg	1	2	3	8
13	4.7	defg	0	0	0	7
17	4.6	efg	0	0	0	8
20	4.5	efg	0	2	5	10
11	4.2	efg	1	2	4	9
8	3.7	fg	1	2	4	11
12	3.6	fg	0	2	2	7
19	3.4	g	0	0	0	3
14	3.4	g	1	1	2	4
LSD (0.05)						7

Data are the means of three tests, with five replications in each test.
† Mean numbers of cysts and females produced per plate.

Literature Cited

1. Aboul-Eid, H. Z., and A. I. Ghorab. 1981. The occurrence of *Heterodera zeae* in maize fields in Egypt. Egyptian Journal of Phytopathology 13:51–61.

2. Ahmed, S. I., and M. Qasim. 1990. A survey of corn cyst nematode in North West Frontier Province and in northern areas in Pakistan. International Nematology Network Newsletter 7:26–27.

3. Dropkin, V. H., and R. E. Webb. 1967. Resistance of axenic tomato seedlings to *Meloidogyne incognita acrita* and to *M. hapla*. Phytopathology 57:584–587.

4. Dropkin, V. H., D. W. Davis, and R. E. Webb. 1967. Resistance of tomato to *Meloidogyne incognita acrita* and to *M. hapla* (root-knot nematode) as determined by a new technique. Journal of the American Society for Horticultural Science 90:316–323.

5. Gamborg, O. L., T. A. Thorpe, and I. K. Vasil. 1976. Plant tissue culture media. In Vitro 12:473–478.

6. Hashmi, S., L. R. Krusberg, and S. Sardanelli. 1993. Reproduction of *Heterodera zeae* and its suppression of corn plant growth as affected by temperature. Journal of Nematology 25:55–58.

7. Koshy, P. K., and G. Swarup. 1971. Distribution of *Heterodera avenae, H. zeae, H. cajani* and *Anguina tritici* in India. Indian Journal of Nematology 1:106–111.

8. Koshy, P. K., G. Swarup, and C. L. Sethi. 1971. *Heterodera zeae* n. sp. (Nematoda: Heteroderidae), a cyst-forming nematode on *Zea mays*. Nematologica 16:511–516.

9. Krusberg, L. R., and S. Sardanelli. 1991. Pathogenicity of *Heterodera zeae* to *Zea mays* in field microplots. Journal of Nematology 23:536–537 (Abstr.).

10. Lauritis, J. A., R. V. Rebois, and L. S. Graney. 1988. Screening of soybean for resistance to *Heterodera glycines* Ichinohe using monoxenic cultures. Journal of Nematology 14:593–594.

11. Macintosh, M. S. 1983. Analysis of combined experiments. Agronomy Journal 75:153–155.

12. Ringer, C. E., S. Sardanelli, and L. R. Krusberg. 1987. Investigations of the host range of the corn cyst nematode, *Heterodera zeae,* from Maryland. Supplement to the Journal of Nematology (Annals of Applied Nematology) 19:97–106.

13. Sardanelli, S., L. R. Krusberg, and A. M. Golden. 1981. Corn cyst nematode, *Heterodera zeae,* in the United States. Plant Disease 65:622.