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Damage potential of *Heterodera zeae* to *Zea mays* as affected by edaphic factors (1)

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Summary – During 1986-1990, the effects of the corn cyst nematode, *Heterodera zeae*, on growth and grain yield of maize, *Zea mays*, were studied in field microplots. These experiments were conducted in microplots containing a coarse-textured or a fine-textured soil, with and without added mineral fertilizer, and with and without *H. zeae*. Maize growth (dry weight) and yield were suppressed by 13 to 73 % in 4 of the 5 years in the presence of *H. zeae*. These plant responses to *H. zeae* were greater in coarse-textured than in fine-textured soil. Fertilizer amendments did not alleviate suppression of plant growth by *H. zeae*. The nematode caused more damage to maize plants in hot, dry rather than cool, wet seasons.

Résumé – ***Effets de facteurs édaphiques sur le potentiel de dégats causés par* Heterodera zeae *au maïs*** – On a étudié les effets du nématode à kyste du maïs, *Heterodera zeae*, sur la croissance et la récolte en grain du maïs, *Zea mays*, en microparcelles pendant les années 1986-1990. Ces expériences ont été conduites en microparcelles contenant du sol à texture fine ou grossière, avec ou sans engrais minéraux, et avec ou sans *H. zeae*. La croissance du maïs (poids sec) et la récolte de grain ont décru de 13 à 73 % pendant 4 ans sur 5, en présence de *H. zeae*. L'effet de *H. zeae* sur les plantes a été plus important dans le sol de texture grossière que dans le sol à texture fine. Les engrais n'ont pas diminué les effets de *H. zeae* sur la croissance des plantes. Le nématode a plus endommagé les plants de maïs pendant les périodes chaudes et sèches que pendant les périodes fraîches et humides.

Key-words : cyst nematodes, *Heterodera*, maize, microplots, pathogenicity, soil type, weather.

The corn cyst nematode, *Heterodera zeae*, was first detected in the Western hemisphere in Kent County, Maryland in 1981 (Sardanelli *et al.*, 1981). In October 1992, an infestation of *H. zeae* associated with poorly growing maize was found in central Virginia about 200 miles (320 km) southwest of the Maryland infestation (Eisenback *et al.*, 1993). Many species of cyst nematodes are widely recognized as destructive to many economically important host plants, including, *Heterodera glycines* on soybean, *H. schachtii* on sugar beet and crucifers, and *Globodera rostochiensis* and *G. pallida* on potato (Miller, 1986). Because maize (*Zea mays*) is a major crop in the United States, discovery of the corn cyst nematode was cause for concern. *H. zeae* was placed under a joint federal and state quarantine in 1986 (Cawley, 1986).

Surveys for *H. zeae* and experiments using nematicides were conducted in naturally infested fields in Maryland in the early 1980s. During 1982 to 1984, several granular nematicides and one fumigant were applied in experimental field plots where soil population densities of cysts ranged from 50 to 300/250 cm^3 soil in Kent and Harford counties, Maryland (Krusberg *et al.*, unpubl.). Fumigation greatly lowered soil population densities of the corn cyst nematode based on soil analysis, but no increases in yield of maize were observed compared to unfumigated soil. Hence, it was decided to determine the ability of the corn cyst nematode to suppress yield of maize in Maryland using field microplots where treatments could be better controlled than in large field plots. A preliminary report on the results of these microplot studies has been published (Krusberg & Sardanelli, 1991).

The objectives of this study were to determine over several years the influence of *H. zeae* on yield of maize grown in a coarse or a fine-textured soil, with and without the application of fertilizer.

Materials and methods

This study was conducted in an isolated field in Prince George's County, Maryland outside the quarantine area with the approval of the Maryland Department of Agriculture. The study area was first cultivated to remove all plants. Microplots were located on a 3 × 3 m grid with a 3 m border kept devoid of plants around the outermost microplots. The soil around the microplots was kept fallow by rototilling

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when maize was growing in the microplots, and by applications of glyphosate during other times of the year.

Cylindrical microplot collars were constructed from sheet fiberglass, and were 45 cm in diameter by 60 cm long. These collars were inserted into holes dug with a tractor-mounted auger to a depth of 45 cm. Methyl bromide-fumigated silt loam field soil (Matapeake silt loam, Typic Hapludult, fine-silty, mixed, mesic soil consisting of 14 % sand, 62 % silt, 24 % clay and 1.8 % organic matter) or loamy sand field soil (Norfolk loamy sand, Typic Paleudult, fine-loamy, siliceous, thermic soil consisting of 90 % sand, 5 % silt, 5 % clay and 0.5 % organic matter) was used to fill the microplot collars.

A population of *H. zeae* from Kent County was propagated on *Zea mays* Pioneer brand 3184 dent corn growing in 17-liter plastic pots filled with washed builder's sand. The pots were placed on plant propagation mats on benches in the greenhouse to maintain the sand at about 30 °C. The sand was collected from 10-week-old cultures, mixed in a cement mixer to distribute the nematodes evenly, and was then thoroughly mixed into the soil by shovel in designated microplots at the rate of 13 l of sand inoculum per microplot. Samples collected from the microplots 10 days after infestation contained an average of 110 cysts full of eggs, *ca.* 209 eggs/cyst (Hutzell & Krusberg, 1990), plus 1900 second-stage juveniles (J2) per 250 cm^3 soil. Microplot soil was infested with *H. zeae* a single time, on 6 May 1986.

All microplots were planted with Pioneer brand 3184 dent corn with five seeds per microplot. When the seedlings were about 20 cm tall, they were thinned to the two most vigorous plants per microplot. Designated microplots received fertilizer at levels optimum for maize production, based on soil analyses. Fertilizer was applied when plants were 30 to 60 cm tall. During dry periods microplots were irrigated weekly at a rate of 20 mm/ha.

Planting and harvesting dates were as follows: 1986: planted on May 16 and harvested on September 9; 1987: planted on May 22 and harvested on September 14; 1988: planted on May 31 and harvested on September 12; 1989: planted on June 5 and harvested on September 18; 1990: planted on May 23 and harvested on September 20.

Soil samples were collected from microplots for assay of *H. zeae* population densities within 2 weeks before planting (*Pi*) and within 1 month after harvest (*Pf*). Soil samples of 250 cm^3, a composite of six cores per microplot, taken from each microplot were processed by washing and decanting. The suspension was passed through a 250-µm pore sieve (to retain the cysts) and over a 45-µm pore sieve (to retain the J2). The residue from the 250 µm sieve was processed, and the cysts and females counted using the filter paper/Büchner funnel technique (Krusberg *et al.*, 1994). The residue from the 45 µm sieve was placed in a modified Baermann funnel for recovery of vermiform nematodes.

All treatments were arranged in a randomized complete block design with ten replications. Plant dry weight and grain yield data were analyzed as a randomized complete block three-factor factorial combined over years (McIntosh, 1983). The main treatment effects were soil type, fertilizer and *H. zeae* inoculation. The initial and final nematode population density data that were collected from the infested plots were analyzed as a randomized complete block two-factor factorial combined over years. The main treatment effects were soil type and fertilizer. Soil processed from uninfested plots revealed no cross-contamination and therefore had no nematode populations to respond to treatments. Uninfested plots were thus not included in the analysis of nematode population levels.

Table 1. *ANOVA for plant dry weight and grain weight.*

Source	df	P*	
		Plant Weight	Grain Weight
Year	4	<.01	<.01
Block	9	<.01	<.01
Soil Type	1	<.01	<.01
Year × Soil	4	.02	.02
CCN Inoculation	1	<.01	<.01
Year × CCN	4	<.01	.05
Soil × CCN	1	<.01	<.01
Year × Soil × CCN	4	<.01	<.01
Fertilizer	1	<.01	<.01
Year × Fertilizer	4	<.01	<.01
Soil × Fertilizer	1	<.01	.02
Year × Soil × Fertilizer	4	.20	.13
CCN × Fertilizer	1	.42	.92
Year × CCN × Fertilizer	4	.98	.68
Soil × CCN × Fertilizer	1	.58	.67
Year × Soil × CCN × Fertilizer	4	.83	.89
error	350		

*Probability (0-1) that differences between source means are due to chance, based on F-Test.

Data were collected on microplot soil temperature at 15 cm depth at 8:00-9:00 a.m. and on rainfall once per week throughout each growing season.

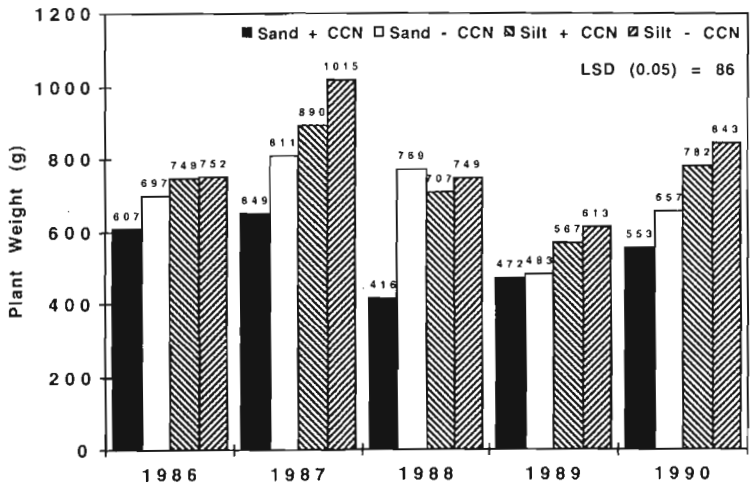

Fig. 1. *Effects of soil texture and presence or absence of* Heterodera zeae *on the dry weight of* Zea mays *plants grown in field microplots during 1986-1990.*

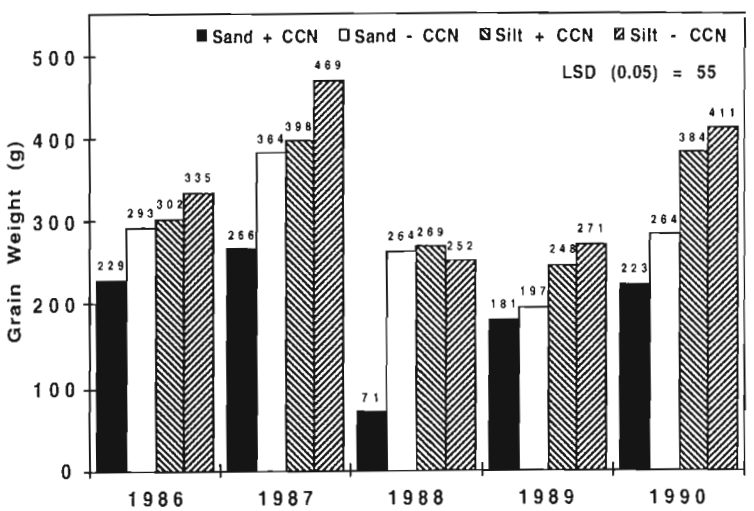

Fig. 2. *Effects of soil texture and presence or absence of* Heterodera zeae *on the yield of grain from* Zea mays *plants grown in field microplots during 1986-1990.*

Results

Maize biomass (Fig. 1) and grain yield (Fig. 2) were suppressed by *H. zeae* in 4 of the 5 years of this study. Biomass was suppressed less than grain yield in those 4 years. Average plant dry weight in the presence of *H. zeae* in sandy soil was decreased by 13 % in 1986, 20 % in 1987, 66 % in 1988, and 16 % in 1990. Grain weights were decreased by 22 % in 1986, 31 % in 1987, 73 % in 1988, and 21 % in 1990. In silty soil *H. zeae* suppressed maize biomass only in 1987, plant dry weight by 12 % (Fig. 1) and grain yield by 15 % (Fig. 2). In 1989 the nematode failed to suppress plant biomass or grain yield in either sandy or silty soil. Surprisingly, application of fertilizer had no effect on the suppression by *H. zeae* of plant biomass or grain yield (Table 1).

Both soil type (Fig. 3) and application of fertilizer (Fig. 4) affected the numbers of *H. zeae* cysts full of eggs extracted from soil samples during the 5 years of this study. In general, larger numbers of cysts were detected in sandy soil than silty soil (Fig. 3) and in soil from microplots receiving fertilizer annually, as compared to unfertilized microplot soil (Fig. 4). During

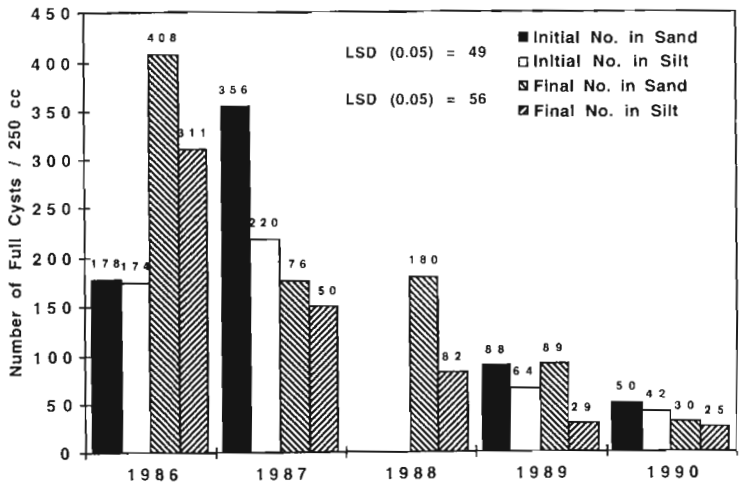

Fig. 3. Initial (Pi) *and final* (Pf) *annual soil population densities of* Heterodera zeae *cysts full of eggs in sandy or silty field microplot soil planted with* Zea mays *during 1986-1990 (per 250 cm³ soil).*

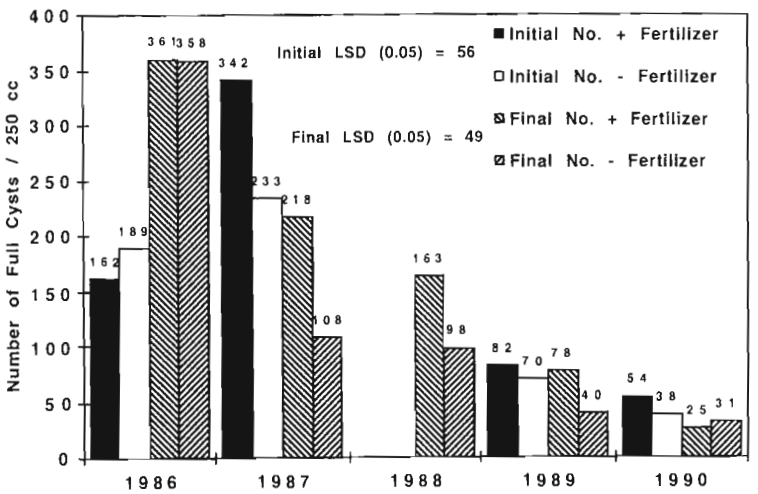

Fig. 4. Initial (Pi) *and final* (Pf) *annual soil population densities of* Heterodera zeae *cysts full of eggs in soil from field microplots with and without added fertilizer and planted to* Zea mays *during 1986-1990 (per 250 cm³ soil).*

the first growing season, 1986, average numbers of cysts increased in all microplots 3- to 4-fold from the time of infestation of microplot soil on 6 May 1986 until after-harvest soil samples were collected in late September 1986. In the following years, the numbers of cysts obtained from microplot soil decreased continuously during each growing season and from 1 year to the next; in fall 1990 soil from infested microplots contained 15-40 cysts full of eggs/250 cm^3. Numbers of total cysts extracted from soil samples during the 5 years of this study followed a pattern similar to that for cysts full of eggs, but at 2- to 10-fold greater numbers (data not shown).

Data on soil temperature and rainfall in the microplots for the years 1986-1990 are given in Fig. 5. In every year except 1989 morning soil temperatures reached 25 °C or above at some time during July and/or August. Rainfall was rather low during the 1986, 1987, and 1988 growing seasons, being close to a total of 38 cm for each year. Rainfall was abundant and rather evenly distributed during the 1989 growing season with a total of 80 cm, and the highest morning

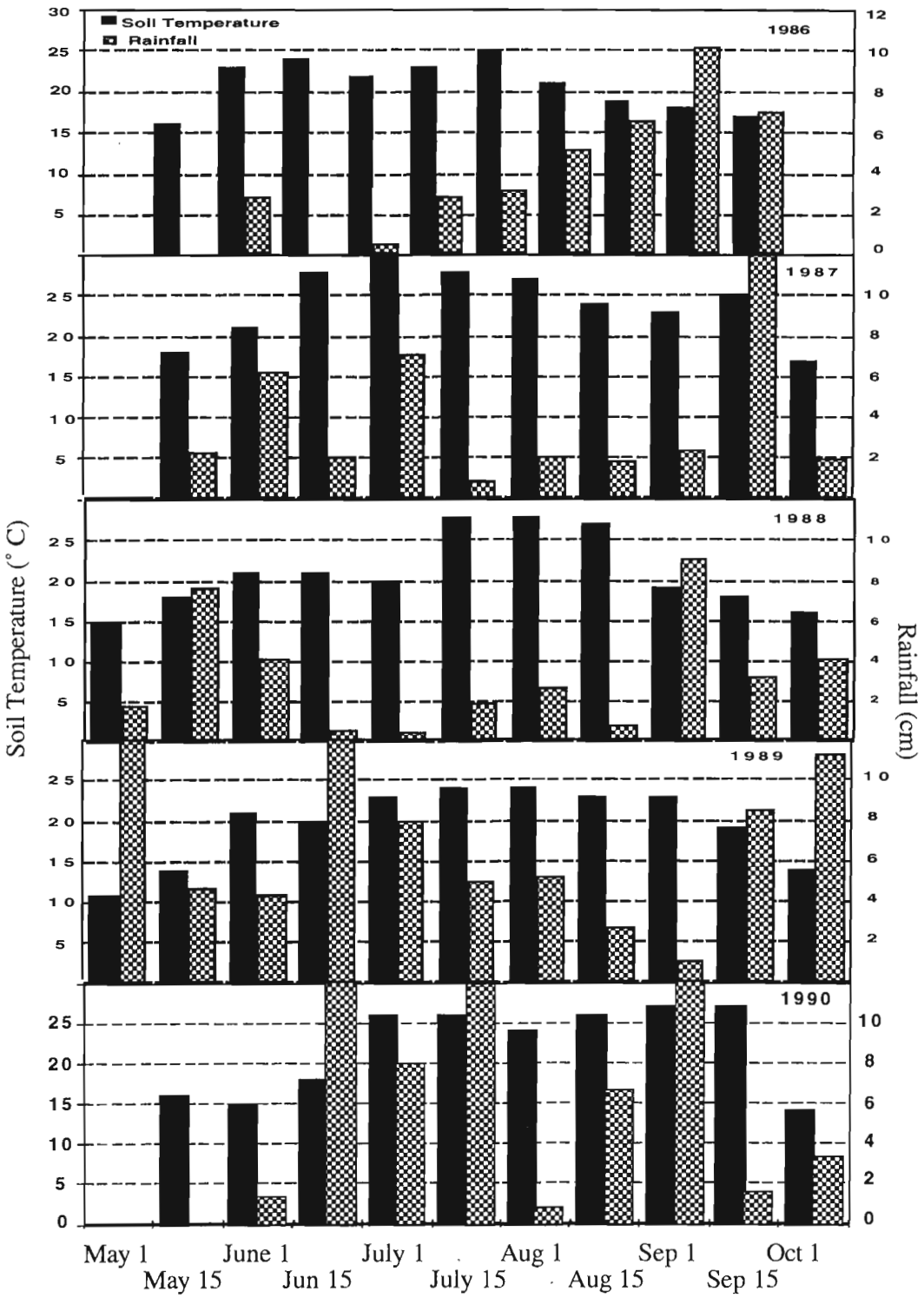

Fig. 5. *Mean morning (8 to 9 a.m.) soil temperature in microplots and total rainfall at 2-week intervals during the growing seasons of 1986-1990.*

soil temperature reached was 24 °C. The 1990 season experienced both high soil temperatures at 8 to 9 AM, above 25 °C in July-September, and ample rainfall, 620 mm total for the season.

Discussion

Suppression of grain yield in the presence of *H. zeae* was demonstrated in the field microplot studies, but not in nematicide evaluations previously conducted in naturally infested field plots. Several factors may have contributed to the differences in test results. Soils for the microplots were fumigated with the biocide, methyl bromide, and then added to the collars. Tests in natural field infestations would have less altered soil microbial populations and therefore would be more likely to contain antagonists and competitive organisms (Freckman & Ettema, 1993). However, examination of numerous cysts extracted from soil samples did not reveal the presence of fungal mycelium, discoloration or deterioration in either study. Microplot soils were artificially infested at calibrated nematode densities within a limited rooting area. In a naturally infested field, nematode distribution as to presence and density is clustered. This distribution contributes to variability in population determinations resulting from soil sampling in a field with moderate to low nematode densities (Noe & Campbell, 1985). Also, the field plot tests were all conducted in silty soil. In the microplot tests, *H. zeae* suppressed maize yields to a greater degree and more consistantly in sandy soil than in silty soil. Microplots were spaced on 3 m centers and seedlings were thinned to the two most vigorous. This spatial arrangement allowed for much greater exposure of microplot soil to the warming effects of sunlight than in a field soil planted with production equipment. Soil temperatures were not measured in the field plot trials, but shading effects on the soil surface due to close plant spacing may have resulted in cooler temperatures. Previous studies demonstrated this nematode to be favored by high soil temperatures. It completes its life cycle most rapidly, in 15-18 days, at 33 °C, requires 42 days at 25 °C, and will develop completely but eggs will not hatch at 20 °C (Hutzell & Krusberg, 1990). Reproduction of *H. zeae* on maize plants growing in soil is most rapid at 36 °C and the greatest juvenile emergence from cysts was at 30 °C (Hashmi *et al.*, 1993). Studies on an Indian population of *H. zeae* also found 30 °C as the most favorable temperature for emergence of juveniles from cysts (Srivastava & Sethi, 1984). Thus soil texture and temperature as well as inoculum distribution within the root rhizosphere appear to have been more conducive for CCN infestation and pathogenicity in the microplot studies than in the previously conducted field trials.

Table 2. *Population densities per 250 cm³ soil of* Heterodera zeae *in two adjacent fields in Kent County, Maryland from 1985 through 1994.*

Date soil collected	Field 1		Field 2	
	Total cysts	J2 *	Total cysts	J2
9/13/85	3	79	1	51
9/25/86	5	257	5	761
9/24/87	8	166	3	533
10/17/88	50	1485	28	2,805
11/15/89	13	16	48	501
10/11/90	3	23	8	170
10/24/91	58	503	53	413
11/2/94	25	198	23	403

* Second-stage juveniles.

With the exception of an increase after the initial infestation in 1986, cyst numbers declined each succeeding year in the field microplots to final low levels in 1989 and 1990. Cyst nematode population decline in microplots has been previously documented (Kerry, 1975; Hartwig, 1981). The decline is generally attributed to changes in the soil microbial population that eventually can include pathogens, antagonists and competitors to the cyst nematode population. Most of the infested fields detected in surveys in Maryland contained very low populations of this nematode (Krusberg & Sardanelli, 1989). The population density of *H. zeae* was followed in two adjacent naturally infested fields on a farm in Kent County, Maryland from 1985 to 1991 and in 1994 (Table 2). Four soil samples were collected from each field. Each sample was a composite of 50 soil cores collected along *ca.* 100 m of row. Sampling was done at the same location in each field between mid-September and mid-November each year. These fields were planted continuously to maize during this observation, the only exception being a planting of Field 1 in 1989 with soybeans. The soil was the same silty loam as used in the microplots. A natural rise and fall in the populations was observed but the population remains relatively low.

H. zeae can cause serious losses in maize yields, especially under conditions meeting its high optimum temperature requirements. We believe that *H. zeae* reduced maize biomass and grain yield in microplots but not in previous field trials because conditions were more conducive in the microplots. However, it appears unlikely for *H. zeae* to reduce yields in production fields of maize in Maryland except perhaps in very sandy fields with a low plant population under an

extended period of high ambient temperatures. It would be more likely to find production conditions conducive to the development of yield losses from the corn cyst nematode in the southeastern U.S. rather than the Mid-Atlantic or Corn belt regions.

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