Population Development of *Pratylenchus hexincisus* in Eight Corn Inbreds[1]

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Abstract: Of eight corn inbreds tested in the greenhouse and field, three (H60, H95, and H84) supported lower populations of *Pratylenchus hexincisus* than other inbreds included in this study. No apparent differences existed among inbreds in nematode invasion or development in the roots, or in population structure. Differences in population were therefore attributed to differential reproduction. *Key words:* lesion nematode, corn nematode, screening.

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Pratylenchus hexincisus Taylor and Jenkins is probably the most important species of *Pratylenchus* on corn in the Midwest (4, 7,11). The possibility of variation in the ability of corn breeding material to serve as hosts for *P. hexincisus* has not been investigated previously, although Thomas (8) reported variable response of a mixed population of *Pratylenchus* spp. to corn hybrids in Iowa. It has been reported frequently that other nematode species invade resistant and susceptible plants in comparable numbers but fail to establish, develop, or reproduce in the resistant plants (1,2,3,9,10). The present investigation was undertaken to determine whether eight corn inbreds differed in ability to support population increase of *P. hexincisus* and, if so, to determine the nature of the differences.

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MATERIAL AND METHODS

The following corn inbreds were selected for study. W64A, B73, Mo17, H60, H84, and H95. These inbreds (suggested by Dr. L. F. Bauman of the Agronomy Department, Purdue University) are commonly used in corn breeding in the Midwest. In addition, Mo17bm3, a low-lignin (brown midrib) inbred, and Mo17o2, a high-lysine (opaque-2) inbred, were included.

Greenhouse experiments: Greenhouse experiments were carried out to establish whether differences existed among the eight inbreds in their ability to support population increases of *P. hexincisus* (exp. 1) and to investigate invasion and early population development in four of these inbreds (exp. 2, 3). For initial screening, five plants of each corn inbred were grown in randomized complete blocks for 10 wk. Early population development was determined by harvesting (1, 2, 3, and 5 wk after planting) two blocks chosen at random from eight randomized complete blocks, each containing two plants of each of four inbreds (H60, H95, W64A, and Mo17). Data were taken on number and size distribution of nematodes recovered from roots (exp. 2). Population development in seedling (seminal) and adventitious (nodal) roots of these same inbreds was examined separately (exp. 3). In this experiment, number of blocks was increased to 18 and internal replication was eliminated. Six randomly chosen blocks were harvested at 3-wk intervals.

Naturally infested Chalmers silty-clay loam (pH 6.6) from a field in continuous corn production in Tippecanoe County, Indiana, was planted with corn (Dekalb XL45A) in the greenhouse to build up populations of *P. hexincisus*. Prior to initiation of each greenhouse test, all soil for the experiment was thoroughly mixed by coning. Initial populations per pot of plant parasitic nematode species present in the soil were determined by decanting and sieving, followed by Baermann funnel extraction of four 500-cm^3 soil samples (Table 1). Two to three seeds were planted per pot and later thinned to a single plant (exp. 1, 3), or a single pregerminated seed was planted per pot (exp. 2). All plants were grown in 20-cm pots under fluorescent lights on a 15-h photoperiod and were fertilized regularly with a solution of complete (NPK) fertilizer. The ambient temperature fluctuated around a 24 C average.

In all experiments, fresh weights of roots were obtained for each plant. A maximum of 5 g of roots per sample was incubated at room temperature in 250-ml beakers containing 200 ml water through which air was bubbled for 1 (exp. 2) or 2 (exp. 1, 3) wk to extract nematodes. If the root system of a plant weighed 5 g or less, the entire root system was incubated. Measurements of a maximum of 20 (exp. 2) or 10 (exp. 3) randomly chosen *P. hexincisus* per root sample incubated were made after the first week of incubation using a compound microscope and ocular micrometer.

Table 1. Initial soil populations of plant-parasitic nematodes (number per 500 cm^3) for greenhouse and field tests.

Species	Greenhouse*			Field**	
	Exp. 1	Exp. 2	Exp. 3	Plot 1	Plot 2
Pratylenchus hexincisus	422 ± 95	1,098 ± 285	588 ± 146	23 ± 14	31 ± 12
Helicotylenchus pseudorobustus	134 ± 20	174 ± 45	818 ± 218	30 ± 14	93 ± 42
Hoplolaimus galeatus	18 ± 6	6 ± 5	28 ± 17	4 ± 6	10 ± 6
Xiphinema americanum	4 ± 2	–	5 ± 6	20 ± 5	7 ± 3
Tylenchorhynchus spp.	–	–	–	24 ± 4	–

*Mean ± standard deviation of four observations.
**Mean ± standard deviation of five observations.

When fewer *P. hexincisus* were extracted, all were measured.

Data were anlyzed using ANOVA and Duncan's multiple-range test or Newman-Keuls sequential range tests. The Tukey test of nonadditivity was used as needed to produce separate blocks by inbreds interaction and error estimates. Since only one representative of each inbred was present per block (exp. 1), no proper homogeneity of variance tests could be performed on these data; however, when blocks were treated like simple repeat observations, variation in root nematode counts increased as mean count increased, whether expressed as nematodes per gram root or total. Since the relationship seemed to approximate standard deviation proportional to mean, a log transformation was used in analyzing root nematode data in this experiment. Homogeneity of variance tests were performed on data from the remaining experiments, and log transformation was applied where indicated to stabilize variance. In addition, the ANOVA assumption of normal distribution was tested (exp. 3). As long as variances were reasonably uniform, departures from normality of fewer than a majority of distributions involved in the analysis were not viewed as cause for concern.

Field test: The farm from which soil for greenhouse experiments was obtained served as the site for field tests of the eight inbreds. Two plots in different areas of this farm were each laid out in a randomized complete block design of five blocks. Rows were spaced 76 cm apart with a spacing within rows of about 30 cm. Butylate plus R-25788, atrazine, and Cyanazine herbicides were used. Rows were marked and fertilized with a tractor-drawn planter and were planted by hand on 9 May 1980. Cultivation throughout the season was performed mechanically.

Initial soil populations of plant parasitic nematode species present were established for each plot by samples taken between rows on 20 May (Table 1). Root samples were taken twice from each plot during the growing season, on 4 and 30 June for plot 1 and 12 June and 20 August for plot 2. Roots from 4 June were incubated as described above for 1 wk; roots from the remaining samples were incubated for 2 wk. ANOVA, Tukey test of nonadditivity, and Newman-Keuls sequential range tests were performed on log-transformed data.

RESULTS AND DISCUSSION

Greenhouse experiments: Differences were found in both *P. hexincisus* per gram root and total root populations of *P. hexincisus* among inbreds after 10 wk (Table 2). Inbreds H60, H95, and H84 supported the lowest total root and per gram root populations. Inbred Mo17 and its converted versions did not differ significantly from one another (Duncan's multiple-range test, $\alpha = 0.05$).

Most invading *P. hexincisus* were early-stage juveniles (Fig. 1). Through 5 wk after planting, median length of *P. hexincisus* individuals recovered increased with time. This suggests first, that the effect of growth of nematodes already in the root outweighed the effect of continued influx of predominantly small invaders; and second, that egg hatch was also relatively unimportant, although the slight bulge in numbers of small nematodes at week 5 (Fig. 1d) may represent the vanguard of the next cohort. The cumulative frequency distributions of nematode lengths in the four inbreds were similar in both shape and position at weeks 1, 2, and 5. At week 3, nematode development in W64A seemed to lag behind, and in H 95 was slightly more advanced than in the other inbreds. Inbred W64A had a lower root weight (data not shown); this may be a factor in the tardi-

Table 2. Numbers of *Pratylenchus hexincisus* per gram root and total root populations after 10 wk in greenhouse on various corn inbreds.

Corn inbred	*P. hexincisus*	
	Per gram*	Total*
H60	510 a	6,440 a
H95	742 a	13,653 ab
H84	1,898 b	19,479 abc
B73	2,882 b	34,219 cd
Mo17	4,352 b	76,494 d
Mo17o2	4,382 b	64,282 cd
Mo17bm3	2,694 b	44,449 cd
W64A	5,550 b	51,016 bcd

*Column numbers followed by the same letter do not differ significantly at the .05 level in Duncan's multiple-range tests on log-transformed data.

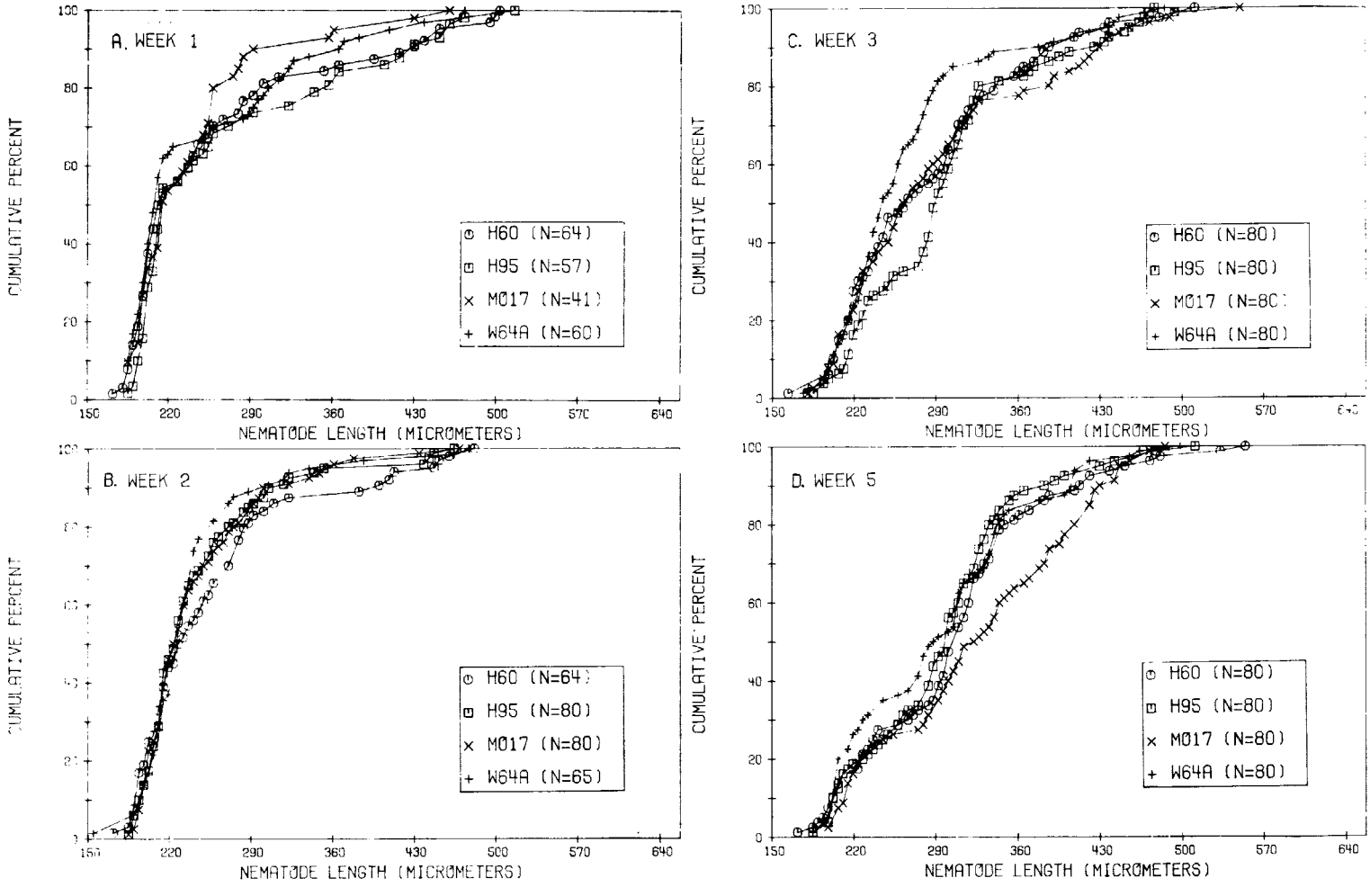

Fig. 1. Cumulative frequency distributions of total lengths of *Pratylenchus hexincisus* specimens from roots of four corn inbreds. A) week 1. B) week 2. C) week 3. D) week 5.

ness of nematode development, but no similar explanation can be invoked to explain the behavior of the populations in H95.

There were no differences (α = 0.05, ANOVA F test) in *P. hexincisus* numbers among inbreds at any sampling time through week 5 (Table 3). This, in fact, is what one would predict, based on the hypothesis that all inbreds are invaded equally and that differences among inbreds later are the result of differences in development and reproduction of the nematode. The nodal root system (the only system for most of the life of the plant) appears several days to a week after germination, and invading nematodes are mostly juveniles. If one assumes a life cycle of about 30 days for this species, differences due to differential reproduction could not be expected to show up until after week 5.

Additional results further support the above hypothesis. There were no differences in *P. hexincisus* populations among inbreds in either the seminal or the nodal root systems at week 3 (Table 4). Significant differences emerged by week 6 in total seminal root population and by week 9 in total nodal root population and per-gram root system population for both seminal and nodal root systems.

Seminal root populations were developmentally ahead of nodal root populations at week 3 (Fig. 2) At week 3, length distributions of the seminal root nematodes resembled those obtained at week 3 for combined seminal and nodal root systems (Fig. 1c), but the distributions of the nodal root nematodes, with a larger component of small nematodes, more nearly resembled curves previously obtained for week 2 (Fig.

Table 3. Numbers of *Pratylenchus hexincisus* per gram root and total root populations among four corn inbreds representing lines which support low (H60 and H95) and high (Mo17 and W64A) buildup.

Inbred	Week*			
	1	2	3	5
H60	217 (129)	621 (425)	353 (492)	99 (185)
H95	122 (54)	426 (297)	332 (365)	361 (837)
Mo17	131 (73)	387 (261)	702 (1,077)	281 (802)
W64A	186 (89)	567 (354)	857 (500)	388 (597)

*Numbers per gram of root (and total per root system).

Table 4. Partitioning, at various sampling intervals following planting, of total root populations and per gram root populations of *Pratylenchus hexincisus* between seminal and nodal roots of corn plants (exp. 3).

Inbred	Week 3*		Week 6*		Week 9*	
	Seminal	Nodal	Seminal	Nodal	Seminal	Nodal
			Total root populations			
H60	203 a	52 a	1301 b	5188 a	280 b	48934 b
H95	425 a	42 a	1840 ab	6248 a	430 ab	28006 b
Mo17	1460 a	76 a	21650 a	12907 a	3333 a	99066 ab
W64A	328 a	55 a	2793 ab	9359 a	795 ab	124140 a
			Numbers per gram root			
H60	842 a	343 a	15498 a	975 a	2800 b	1394 b
H95	1233 a	124 a	12560 a	1258 a	2931 ab	787 b
Mo17	2545 a	250 a	39771 a	2042 a	10525 a	2276 b
W64A	1153 a	303 a	15604 a	1506 a	3620 ab	4428 a

*Column numbers followed by the same letter do not differ significantly at the .05 level in Newman-Keuls sequential range tests. Seminal and nodal root data (total root populations) and seminal root data (numbers per gram root) were subjected to log transformation.

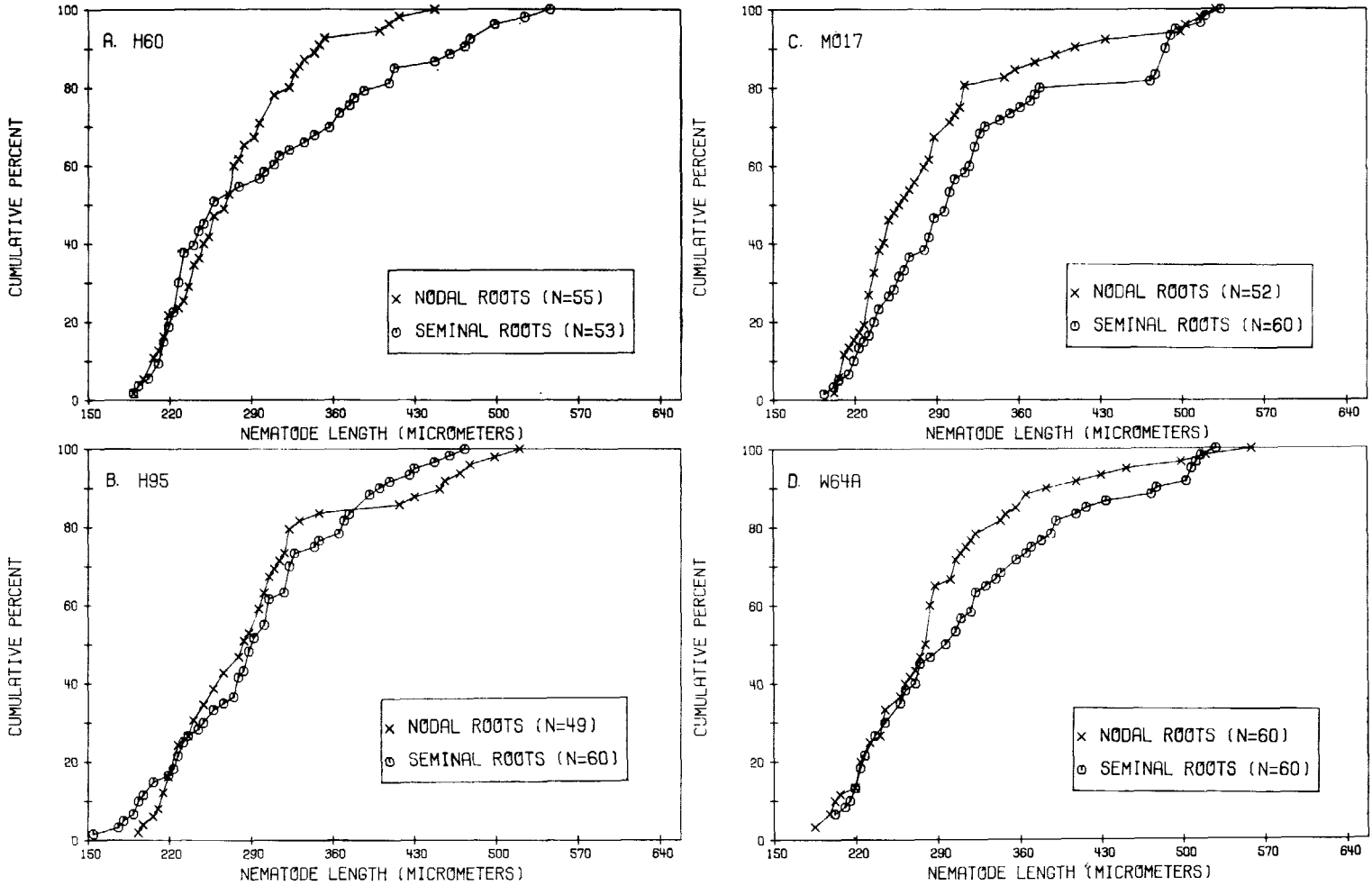

Fig. 2. Cumulative frequency distributions of total lengths of *Pratylenchus hexincisus* specimens from seminal and nodal roots, week 3. A) H60. B) H95. C) Mo17. D) W64A.

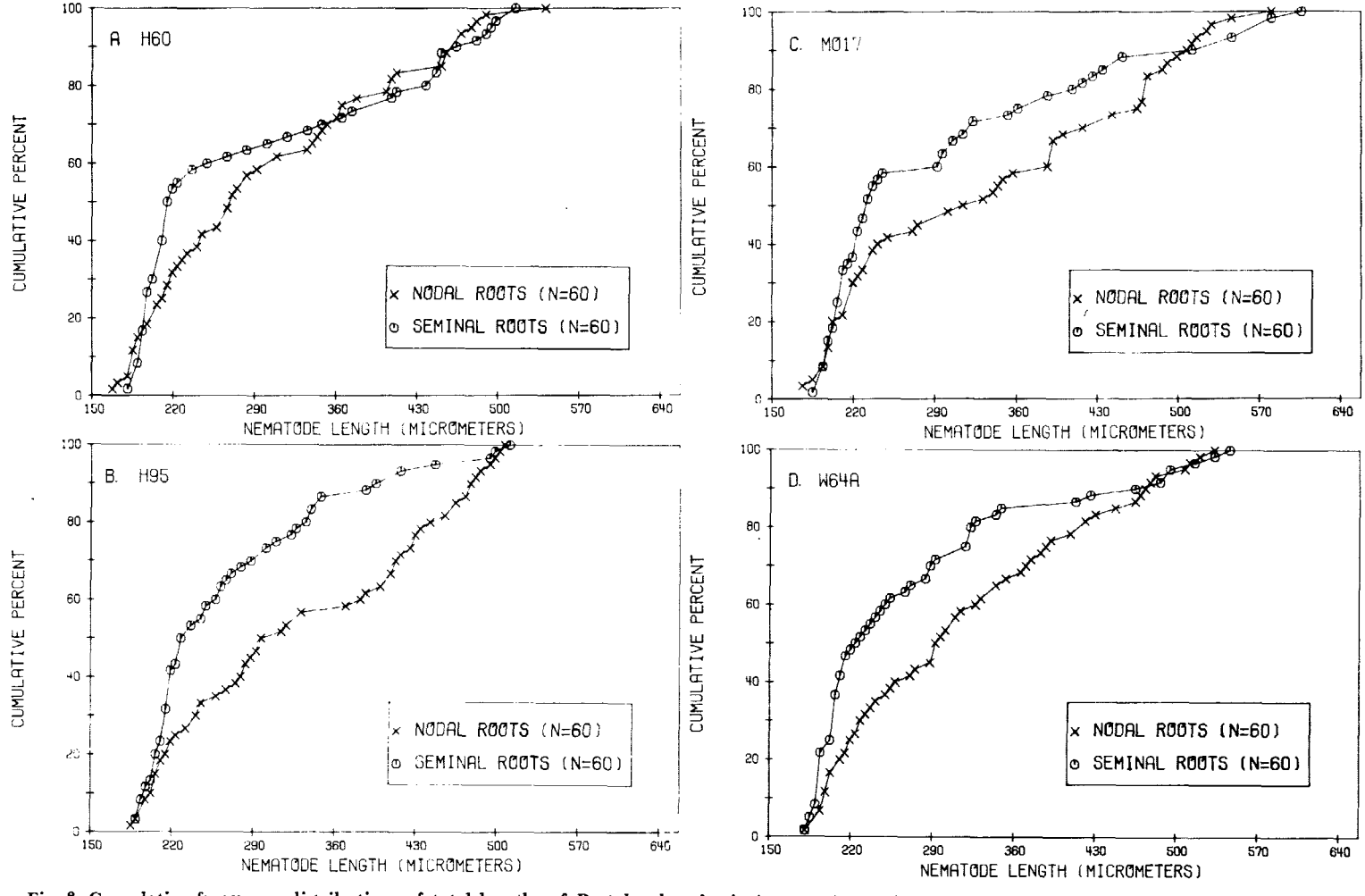

Fig. 3. Cumulative frequency distributions of total lengths of *Pratylenchus hexincisus* specimens from seminal and nodal roots, week 6. A) H60. B) H95. C) Mo17. D) W64A.

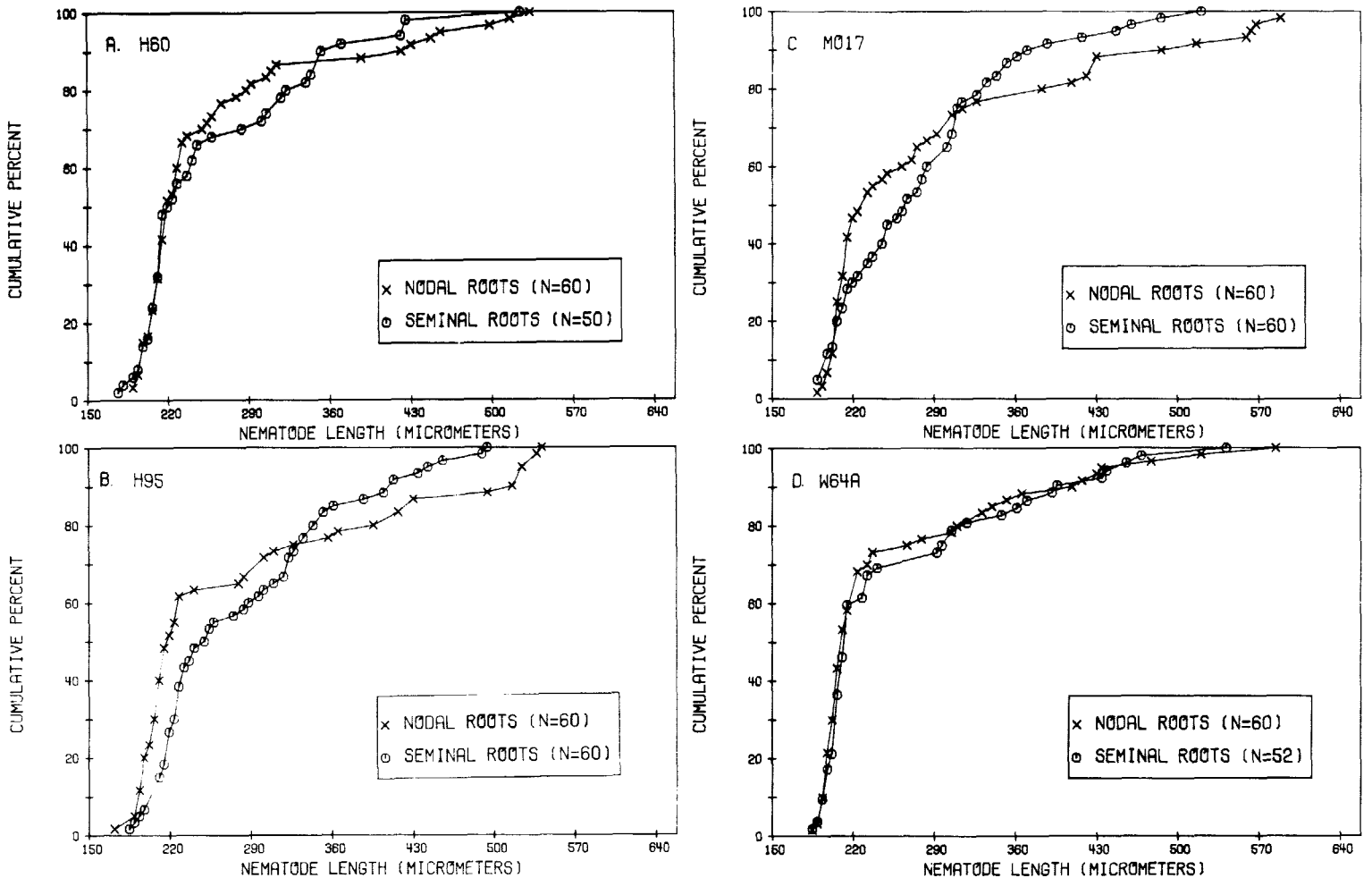

Fig. 4. Cumulative frequency distributions of total lengths of *Pratylenchus hexincisus* specimens from seminal and nodal roots, week 9. A) H60. B) H95. C) Mo17. D) W64A.

1b). This indicates the early predominance (and availability to nematodes) of the seminal roots. Curves of cumulative frequency distribution at week 6 (Fig. 3) reflect continued population development, with seminal root populations still developmentally ahead of nodal root populations, although seminal and nodal curves have reversed their relative positions from week 3. Distributions at week 6 indicate egg hatch in the seminal roots; nodal root nematodes continued to grow but did not reproduce. At week 9 (Fig. 4) length distributions of seminal and nodal root populations were basically indistinguishable. Except for Mo17, seminal root population distributions remained unchanged from week 6 or shifted toward smaller nematodes. By week 9 the second generation had hatched in nodal roots.

At week 3 population distributions were essentially identical for nematodes in roots of the four inbreds (Fig. 2). Moreover, nothing in the distributions at weeks 6 and 9 seemed to correspond to differences among inbreds in numbers of *P. hexincisus* recovered.

Field test: Separate analyses of variance on log-transformed data from the two samples from plot 1 yielded very different error estimates, so separate analyses were chosen in preference to a single analysis of combined data (Table 5). No differences existed among inbreds at the time of the first sampling ($\alpha = 0.05$, ANOVA F test). In the second sample, differences emerged ($P < .005$ for Friedman's Q, a nonparametric test statistic), but Tukey's test of additivity revealed apparent interactions between blocks and corn inbreds complicating interpretation of results. The interaction appeared to be due in large part to the erratic behavior of W64A, which ranged from the highest to the lowest ranking inbred in a block in terms of *P. hexincisus* population per gram root. This inbred had also shown the greatest inconsistency in the greenhouse screening. The interaction disappeared when W64A was dropped from the analysis. As in the greenhouse, H60, H95, and H84 yielded fairly low numbers of nematodes per gram fresh root. In a notable departure from previous results, however, B73 did not differ significantly from these three (Newman-Keuls sequential range test, $\alpha = .05$). This test did not detect differences between Mo17 and its converted versions.

Data from plot 2 (Table 5) were analyzed in a single overall ANOVO model. Differences in mean number of *P. hexincisus* per gram fresh root appeared among inbreeds in both samples (Newman-Keuls sequential range test, $\alpha = .05$). In the second sample, H 60 differed from all other inbreds. Of the remaining inbreds in the sample, only B73 and W64A differed from each other. Again, Mo17 and its converted versions did not differ.

The absence of differences among inbreds 1 month after planting (plot 1) and the presence of differences in all subsequent samples supports the hypothesis advanced earlier that the nematode invades all inbreds equally well, and that the dif-

Table 5. Numbers of *Pratylenchus hexincisus* per gram of root at various time intervals following planting of eight corn inbreds in two field plots.

Inbred	Plot 1*		Plot 2*	
	4 June	30 June	12 June	20 August
H60	9.0	21.1 a	27.4 ab	227 a
H95	16.0	33.4 ab	24.2 ab	2336 bc
H84	15.0	72.8 bc	76.2 b	7666 bc
B73	9.4	36.2 ab	22.2 a	2160 b
Mo17	11.4	121.8 c	89.4 b	6484 bc
Mo17o2	17.3	116.0 c	97.7 ab	5389 bc
Mo17bm3	25.0	148.0 c	82.8 b	7837 bc
W64A	19.8	210.4 **	96.2 b	9879 c

*Column numbers followed by the same letter do not differ significantly at the .05 level in Newman-Keuls sequential range tests on log-transformed data

**Not included in analysis. See text for explanation.

ferences among inbred populations later in the season are the result of post-invasional phenomena.

CONCLUSIONS

The corn inbreds tested differed in susceptibility to *P. hexincisus* as measured by population buildup. No differences were detected among Mo17 and its converted versions. Apparently, the biochemical differences generated by the opaque-2 (high lysine) and brown midrib genes do not affect *P. hexincisus*. Since the opaque-2 gene is expressed chiefly (or exclusively) in the endosperm, it is not surprising that its presence evokes no response from a root parasite. The lowered lignin content of cell walls in plants carrying the brown midrib gene does make these plants more susceptible to invasion by other pathogens, but invasion does not seem to be a problem for *P. hexincisus*.

Differences in *P. hexincisus* populations developed several weeks after invasion began. This situation was also observed for *Heterodera avenae* on wheat (6) and *Globodera rostochiensis* on potato (5). In both these cases, juveniles invaded the resistant host in numbers similar to those invading susceptible hosts but failed to establish and develop. In contrast, *P. hexincisus* developed at a similar rate in all corn inbreds examined in this manner, as shown by the size distributions over the first several weeks. Although reproduction clearly occurs on all inbreds, differences in reproduction appear to be more important than establishment and development in explaining the differences in *P. hexincisus* populations among corn inbreds.

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