Influence of Meloidogyne hapla Chitwood, 1949 on Development and Establishment of Heterodera schachtii Schmidt, 1871 on Beta vulgaris L.[1]

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Abstract: Influence of *Meloidogyne hapla* on establishment and maturity of *Heterodera schachtii* in sugarbeet was studied. Results indicated that when the majority of *M. hapla* were in second, third, or fourth larval stages within plants prior to *H. schachtii* inoculation, growth and development of the latter was retarded. However, when *M. hapla* reached the young female stage prior to inoculation of *H. schachtii*, establishment and development of the latter was greatly enhanced. As *M. hapla* reached maturity before and after egg production prior to *H. schachtii* inoculation, establishment and growth of the latter was progressively decreased. In each instance, *M. hapla* developed independently and matured at the same rate as in plants inoculated with only *M. hapla*. Usually ratios of total soluble carbohydrates to reducing carbohydrates were lower, but not significantly different, in plants receiving both nematodes as compared to other treatments. *Key words:* northern root-knot nematodes, sugarbeet nematode, sugarbeet, interrelationships, carbohydrates, predisposition.

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Studies concerning interrelationships of *Meloidogyne hapla* and *Heterodera schachtii* in sugarbeet, *Beta vulgaris* L., have shown each parasite could exert influence upon the other and upon the host when population densities, inoculation combinations, and inoculation timing were manipulated (2,3,4,5,6,7). Although several population combinations were used, the most interactive level was 250 *M. hapla* and 1,000 *H. schachtii* larvae. The effects of such interaction affect the ability of parasites to mature and to change host physiology. Thus, under certain conditions, a plant could become less or more suitable as a host for a second invading parasite. We report here the influence of various developmental stages of *M. hapla* on establishment and maturity of *H. schachtii* in sugarbeet.

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MATERIALS AND METHODS

Several sugarbeet seedlings cv. USH 9A were collectively grown in 10-cm plastic pots of sandy loam soil. Three weeks after germination, the seedlings were divided into four equal groups and inoculated as follows: each plant in two groups received 1,000 *M. hapla* juveniles (tomato cv. Rutgers source); another group 1,000 *H. schachtii* juveniles (commerical beet source); and the remainder were left as control for carbohydrate analysis. Six days after inoculation, seedlings were uprooted, washed to remove larvae that had not penetrated, and replanted in 350-ml styrafoam cups containing sterilized soil. Three inoculation tubes (7-cm sections of drinking straws), spaced equidistant from the plant, were inserted in each cup with one end in contact with roots and the other extending about 3 cm above the soil line. Subsequent inoculations were made through these tubes.

Treatments were as follows: 1) *H. schachtii* (Hs) alone, 2) *M. hapla* (Mh) alone, 3) Mh + Hs, and 4) noninoculated controls. At 0, 1, 2, 3, and 4 weeks after

transplanting, all treatments except the controls were inoculated a second time with 1,000 *H. schachtii* juveniles. Each treatment was replicated five times and the entire experiment was repeated. At weekly intervals after inoculation, entire root systems of five plants from treatment 2 (*M. hapla* alone) were washed free of soil and stained in acid fuschin-lactophenol (8) to determine *M. hapla* development.

Thirty days after the second inoculation treatments, roots of remaining plants were washed free of soil and numbers of cysts and galls recorded. Stained roots were examined with a stereo-microscope for various developmental stages of *M. hapla* and classified as follows: A = larvae from initial growth to conical tail; B = hemispherical posterior with terminal spike to onset of final molt; C = final molt to nearly mature; D = fully mature without eggs; and E = females with eggs in matrix (1). A composite root sample from each treatment (approximately 5 g green weight) was obtained for carbohydrate analysis. Total soluble and reducing carbohydrates were determined by standard methods (9). A one/two factor analysis of variance was used in statistical analyses of data obtained in this investigation.

RESULTS AND DISCUSSION

There were no significant differences in cyst numbers when *H. schachtii* was inoculated at increasing time intervals on plants previously infected with sugarbeet nematodes (Hs alone) (Table 1). However, when *M. hapla* and *H. schachtii* were present in plants prior to additional *H. schachtii* inoculations, there were significant differences in cyst numbers.

Cyst production in Mh + Hs treatments approximates a bell-shaped curve with the peak occurring at the third inoculation (2 weeks after transplanting). At this time, the majority of *M. hapla* had developed to stage C. As *M. hapla* developed beyond stage C, subsequent inoculations of *H. schachtii* resulted in a significant reduction in the number of cysts. Similar reductions in cyst numbers occurred when *H. schachtii* was inoculated before *M. hapla* reached staged C.

Significant increases in the number of *M. hapla* galls occurred in Mh + Hs treatments with some subsequent inoculations of *H. schachtii.* In treatment Mh, the last inoculation of *H. schachtii* resulted in the most significant numbers of galls. Numbers of cysts in Hs treatments were not significantly different. However, the numbers of cysts were significantly different from each other in each of the Mh + Hs treatments.

Extraction results of soluble and reducing carbohydrates and their ratios were not significantly different.

Invasion of sugarbeet roots by *M. hapla* prior to invasions of *H. schachtii* affected establishment, development, and population levels of *H. schachtii.* Variation in

Table 1. Effects of various *Meloidogyne hapla* (Mh) developmental stages on *Heterodera schachtii* (Hs) infection and cyst production on *Beta vulgaris.*

Weeks after inoculation with *H. schachtii**	Percent nematodes in each developmental stage of *M. hapla*†					Average no. cysts per plant after 30 days from *H. schachtii* inoculation		Average no *M. hapla* galls per plant 30 days after inoculation	
	A	B	C	D	E	Mh + Hs treatment	Hs treatment	Mh + Hs treatment	Mh treatment
0	95	5	0	0	0	4.8 a‡	24.0 a	48.0 a	51.2 b
1	24	76	0	0	0	17.6 c	24.0 a	47.8 a	47.2 a
2	6.4	1.3	63.2	17.6	0	52.8 e	24.0 a	48.2 a	47.0 a
3	1.4	2.1	9.1	63.3	24.1	35.2 d	23.6 a	55.0 b	56.8 b
4	0	1	3	32.3	63.7	10.8 b	23.6 a	64.2 c	63.8 c

*Five replications in each of two identical experiments (10 replications).

†A = larvae from initial growth to conical tail; B = hemispherical posterior with terminal spike to onset of final molt; C = final molt to nearly mature; D = fully mature without eggs; E = females with eggs in matrix.

‡Means followed by a common letter are not significantly different at the $P = 0.5$ level according to Duncan's multiple-range test.

H. schachtii cyst numbers in Mh + Hs treatments may have been caused by several factors, including availability of feeding sites, resulting from inoculation density or from competition between species, and changes in host physiology induced by the first pathogen that repress the second pathogen. Availability and competition for feeding sites appears to be a valid consideration, particularly when the plants are young or when second-generation *M. hapla* juveniles appear in the system. *M. hapla* may have been the more aggressive feeder, rapidly converting available feeding sites into malformed and galled tissue. However, in recent similar studies Griffin and Waite found *H. schachtii* to be more aggressive than *M. hapla* in competing for feeding sites on tomato (2). Such effects on host physiology appeared to be antagonistic to *H. schachtii* during the beginning and waning portions of the *M. hapla* life cycle. During *M. hapla* stage C, such effects were not apparent, as indicated by highest cyst numbers. Apparently such alterations did not effect total soluble and reducing carbohydrates or their ratios. Although most explanations given for increases or decreases in *H. schachtii* cyst numbers are hypothetical, additional biochemical studies of host physiology during interaction studies should be informative.

Gall numbers remained more-or-less constant during the first three *H. schachtii* inoculation periods. An increase in galls during the last two inoculation periods apparently occurred as second-generation *M. hapla* began feeding.

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