Self-interactions of Meloidogyne hapla and of Heterodera schachtii on Beta vulgaris[1]

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Abstract: Double inoculations of sugar beet with larvae of *Meloidogyne hapla* resulted in a higher galling incidence in only one treatment than did a single inoculation using the same number of larvae. Double inoculations with larvae of *Heterodera schachtii*, however, resulted in three- to five-fold more cysts in most cases than did single inoculations using the same number of larvae. In general, plants died more quickly after double inoculations than after single inoculations of the same total number of either nematode. Ratios of total soluble carbohydrates to reducing carbohydrates were lower in multiple inoculated treatments than in other treatments. Plants infected with *M. hapla* had lower quantities of B, K, and P in leaf tissue than noninoculated plants, but no differences were correlated with type of inoculation. Plants inoculated with *H. schachtii* had lower quantities of B, K, and Mg than noninoculated plants. Also, quantities of Mn, Cu, and Zn were much lower in plants inoculated twice with *H. schachtii* larvae than in plants inoculated with the same total number of larvae in a single dose. *Key Words:* Cyst nematode, root-knot nematode, mineral elements, carbohydrates, double inoculations, parasitism, sugar beet.

Predisposition effects of environment on behavior of different pathogens and their hosts are important factors in the etiology of disease. Plant parasitic nematodes, for example, are found in various locales and are constantly associated with other organisms in specific habitats. As the environment of a particular locality changes, the pathogenicity of the nematodes and the physiological actions of the host may change. These changes may enhance or suppress the development of a parasite or the growth of a host at different temperature regimes prior to infection by the parasite. Studies of this nature have been conducted for many hosts as well as pathogens. Investigations regarding predisposition of a host to a particular nematode prior to reinoculation with the same nematode have not gained much attention.

The emphasis of this study was to investigate the importance of predisposition of plants to *Meloidogyne hapla* and *Heterodera schachtii* prior to reinoculation with the same parasite. An abstract has been published (3).

MATERIALS AND METHODS

Seedlings of *Beta vulgaris* L. hybrid var. 'USH9-A' were grown for 3 weeks in steam-sterilized sand. The sand was washed off the roots, and seedlings of similar size were transplanted into 350-ml (12 oz.) styrofoam cups containing pasteurized 3:1 soil-sand mixture. Three, 5-cm sections of plastic drinking straws were spaced equally around each seedling with one end in contact with the roots and the other projecting above the soil level. Plants were inoculated through the straws with 250, 500, or 1000 second stage larvae of *Meloidogyne hapla* Chitwood, 1949, or *Heterodera schachtii* Schmidt, 1871, in 6-ml of water. After 10 days, half of the plants were again inoculated with 250, 500, or 1000 additional larvae of the same nematode species, and the other half were left with only the first inoculation. Table 1 summarizes these treatments and their respective codes.

Two inoculated-control series were used. One series was inoculated only at day 1 with 250, 500, 750, 1000, or 1250 larvae of either species. The second series was inoculated only at day 10 with the same number of larvae used at day 1. Since no significant differences occurred between the two treatments, data are presented as the average of the two series.

Thirty days after the original inoculation, all roots were collected by soaking the pots in water and removing the soil. Although a few cysts remained in the soil, no attempts were made to recover them. Galls and cysts were examined and counted, and fresh weights of plant top and root were recorded. Samples from leaves and roots were taken for tissue and carbohydrate analyses, respectively.

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Leaf tissues were analyzed, by using an emission spectrograph with A. C. arc source, to determine the amount of 10 different elements (K, P, Ca, Mg, Mn, Fe, Cu, B, Zn, Al). Standard curves were determined by using different weights of a composite plant standard. Concentrations of the standards were determined by the official analysis methods of the Association of Official Analytical Chemists (6). Total soluble and reducing carbohydrates were obtained by using the method of Umbreit and Burris (12).

In a second experiment using the same treatments, the plants were allowed to grow up to 150 days. At that time, the only plants surviving were the noninoculated control series. There were five replications per treatment, and each experiment was repeated twice. Since there was no statistical difference between the two experiments, data are presented as the average of the two experiments.

The two-factor analysis of variance was used in statistical analyses for all experiments. The LSD values for 0.05 and 0.01 levels for all treatments are listed on the respective figures and in the tables.

RESULTS

Numbers of galls and cysts 30 days after inoculation increased with an increase in inoculum in the single-inoculated CM and CH series (Fig. 1, 2; see Table 1 for explanation of codes). More galls developed on roots when 500 larvae were applied in two doses than when they were applied in a single dose (M1, CM2). However, 750 and 1250 larvae applied in single doses produced more galls than when they were applied in two doses (CM3 M2; CM5 and M3 and M5). Also, when 750 larvae were applied in two doses, more galls developed when 500 were followed by 250 than when 250 were followed by 500 (M4 M2).

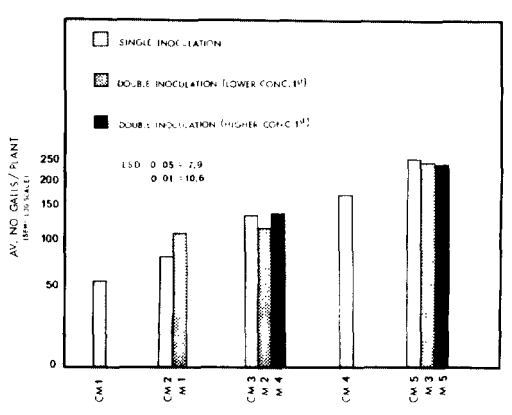

FIG. 1. Effects of single and double-inoculations of *Meloidogyne hapla* on galling of roots of *Beta vulgaris*. Roots were examined 30 days after the initial inoculation.

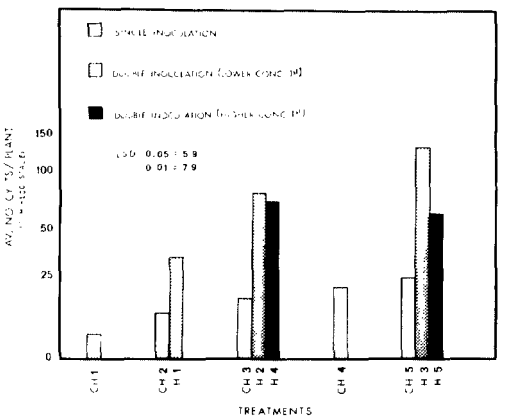

FIG. 2. Influence of single and double-inoculations on development of *Heterodera schachtii* on *Beta vulgaris*. Roots were examined 30 days after the initial inoculation.

Numbers of cysts were higher in all double-inoculation series (H1-H5) than in single inoculation series (CH1-CH5) (Fig. 2). The only significant differences observed were in treatments comparing H2 with H4 and H3 with H5.

In the *M. hapla* experiment, root weights were greater ($P = 0.01$) in CM1 (250), CM2 (500), and CM4 (1000) than in C, the control (Fig. 3). There were no differences in root weights between C and M treatments. There were significant increases in root weights in CM2 (500) compared to M1 (250 + 250), and in CM5 (1250) compared to M5 (1000 + 250). Top weights in CM1, CM2, CM4 and CM5 were greater than in C. Top weights in the M5 treatment were lower than in C. When top weights in the CM and M series were compared, CM2 was greater than M1, and CM5 and M3 were greater than M5.

In the *H. schachtii* experiment, there were significant increases in fresh top weights of CH1 (250 larvae), CH2 (500), CH4 (1000), and CH5 (1250) over C (noninoculated). Fresh top weights of plants in the double-inoculation treatments were lower than in either control (C) or single-

TABLE 1. Descriptive key to the population, time, and sequence of inoculation of *Beta vulgaris* roots with *Meloidogyne hapla* (M) and *Heterodera schachtii* (H).

Treatment designation	No. of *M. hapla* larvae inoculated		Treatment designation	No. of *H. schachtii* larvae inoculated	
	Original	10 days later		Original	10 days later
M1	250	250	H1	250	250
M2	250	500	H2	250	500
M3	250	1000	H3	250	1000
M4	500	250	H4	500	250
M5	1000	250	H5	1000	250
*Inoculated Control 1**			*Inoculated Control 1**		
C1 M1	250		C1 H1	250	
C1 M2	500		C1 H2	500	
C1 M3	750		C1 H3	750	
C1 M4	1000		C1 H4	1000	
C1 M5	1250		C1 H5	1250	
*Inoculated Control 2**			*Inoculated Control 2**		
C2 Mi		250	C2 H1		250
C2 M2		500	C2 H2		500
C2 M3		750	C2 H3		750
C2 M4		1000	C2 H4		1000
C2 M5		1250	C2 H5		1250
C-noninoculated Control			*C-noninoculated Control*		
	0	0		0	0

*Since no statistical differences were noted between two inoculated control series for each nematode, data throughout the text and on figures will be presented as the average of the two series, coded as CM1-5 or CH1-5.

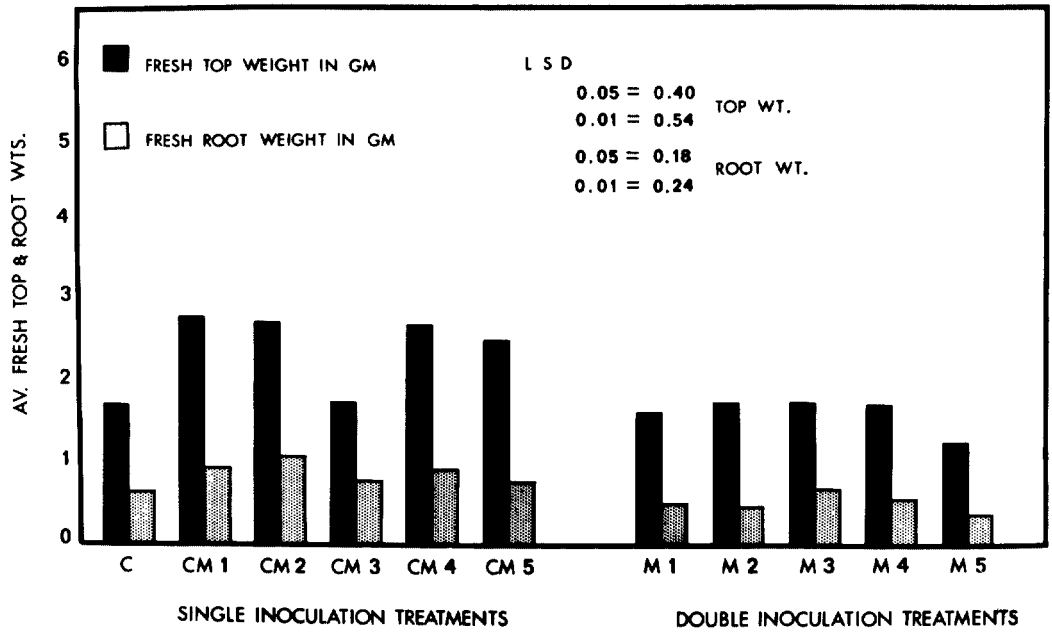

FIG. 3. Effects of single and double-inoculations of *Meloidogyne hapla* on the growth of *Beta vulgaris* 30 days after the initial inoculation.

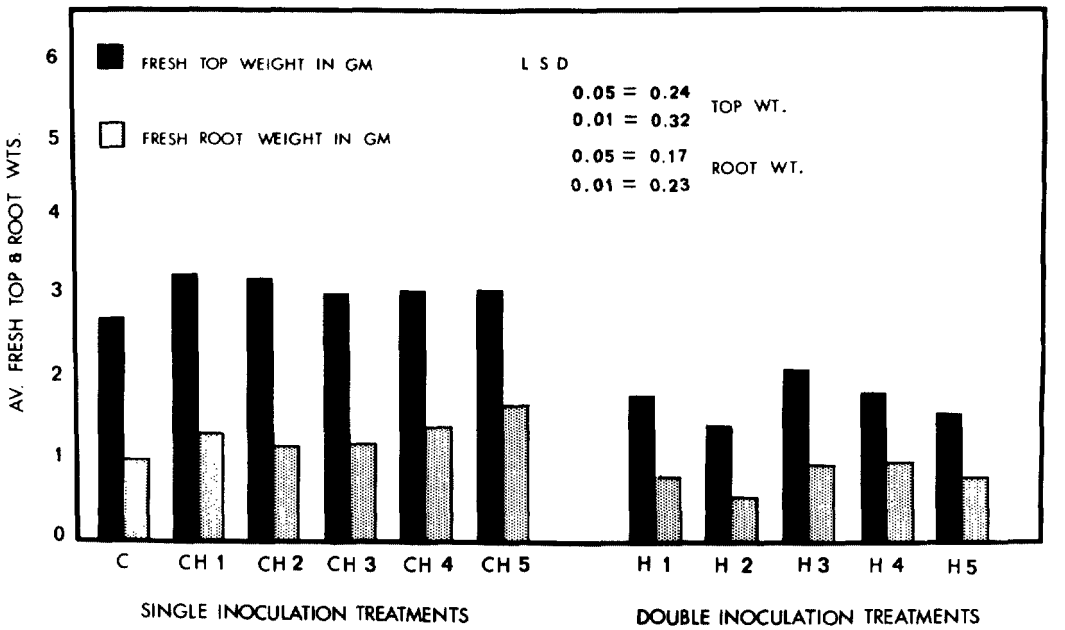

FIG. 4. Effects of single and double-inoculations of *Heterodera schachtii* on the growth of *Beta vulgaris* 30 days after the initial inoculation.

inoculation treatments (CH series). Fresh root weight of plants in the noninoculated treatment was lower than all single-inoculation treatments. The only notable difference in root weight between C and H series was between the C and H2 treatments. Root weights were lower in H1 (250 + 250 larvae), H2 (250 + 500), and H3 (250 + 1000) when compared with CH2, CH3 and CH5, respectively. Root weight in the H2 treatment was lower than H4, but there was no significant difference between H3 and H5 treatments.

Durations of plant longevity are summarized in Table 2. The only significant differences between the M and H series were between M1 and H1 and between M5 and H5 treatments. The longevity of plants in treatment C was greater than in any other treatment. Longevity of plants in M5 was lower than in other treatments of the M series and all treatments in the CM series. There were no significant differences among M2, M4, and CM3. Longevity of the plants was lower in M1 and M5 than in CM2 and CM5, respectively. Conversely, longevity of plants in treatments H1, CH5, CH3, and C was greater than in other treatments in the H and CH series.

In the *M. hapla* experiment, the quantities of B, K, and P were lower in all inoculated treatments then in the noninoculated control. Treatments M4 and M5 had greater amounts of Fe and Al than all other treatments except CM5, which contained a high amount of Al. Treatment M5 had a greater amount of Ca and Zn than all other treatments. In general, all

TABLE 2. Self-interactions of *Meloidogyne hapla* and of *Heterodera schachtii* on *Beta vulgaris*. Effects on plant longevity.[a]

Treatment designation for *M. hapla*	Number of days plant survived	Treatment designation for *H. schachtii*	Number of days plant survived
M 1	94.6	H 1	109.2
M 2	96.2	H 2	99.6
M 3	96.6	H 3	99.4
M 4	97.2	H 4	101.6
M 5	86.8	H 5	99.6
CM 1	110.4	CH 1	113.4
CM 2	106.4	CH 2	103.6
CM 3	102.0	CH 3	109.6
CM 4	105.0	CH 4	102.8
CM 5	103.0	CH 5	105.0
C	150.0	C	150.0
LSD: 0.05	7.74		7.30
0.01	10.35		9.76

[a]Refer to Table 1 for explanation of code.

inoculated plants contained more Ca than noninoculated plants. The quantities of Mg were lower in all inoculated plants (except treatment M1) than in noninoculated plants.

Quantities of different elements also varied in the *H. schachtii* experiment. A general depression in the quantities of B, K, and Mg was apparent in all inoculated plants when compared with noninoculated controls series. The quantity of Al was higher in H2, H3, H4, and H5 than in all other treatments. Similarly, the amount of Fe in H2, H3, and H5 was higher than in all other treatments.

In general, ratios of total soluble/reducing carbohydrates of the plants in all double-inoculation treatments in the *M. hapla* or *H. schachtii* experiments were lower than in the single-inoculation treatments. Similarly, plants in all the single-inoculation treatments had lower ratios of total soluble/reducing carbohydrates in comparison to noninoculated plants.

DISCUSSION

Self-interaction studies with plant pathogens have been mostly limited to viruses. Recovery from infection and immunity to reinfection are common in virus diseases in animals. Baron (1) and Isaacs (2) attributed this type of reaction to antibodies and interferon. Price (8) compared the apparent recovery of infected plants to acquired immunity in animals. Other workers reported a similar reaction in plants infected with viruses (4, 9, 10). Self-interactions of nematodes, at least with *M. hapla* and *H. schachtii,* appear to be completely different and contrary to those of viruses. Our results indicate that plants do not recover to become immune to these nematodes but support reinfection by the same parasite. Apparently, no interfering agent is produced by the infecting parasites or the host. The increased population in plants inoculated twice (over that in plants inoculated once) with the same total number of nematodes varied with different levels of inocula and species of nematode. With *M. hapla,* the only combination showing a significant increase in double-inoculations was 250 + 250 larvae (M1) inoculated at 10-day intervals. In contrast, all double-inoculation combinations with *H. schachtii* produced significantly (0.01) higher populations of cysts than single-inoculations with the same number of nematodes.

Increased populations in dual-inoculations cannot be attributed to the age of the host plant or the avenues for entry. This statement is supported by the performance of the plants inoculated once at various ages and growth times in soil.

Inhibition of root and top growth and reduced plant longevity by the greater population in the double-inoculation series were more pronounced with *M. hapla* than with *H. schachtii.*

The differences in mineral content that occurred in leaf tissue are not in agreement with the results of Owens and Novotny (7). They reported no changes in mineral content of tomato and cucumber plants infected with *M. incognita,* but their results were based on root analyses.

Lower starch content in potato tubers inoculated with *Ditylenchus destructor* has been reported by some investigators (11, 13). Our results indicate a general reduction in ratios of total soluble/reducing sugars in double-inoculated treatments. Muge (5) attributed the reduction of starch in tubers to amylase activities of nematode secretions and conversion of starch to soluble sugars for utilization by the parasite. Correlation of increased nematode population and decreased ratios of total soluble/reducing sugars in our double-inoculation treatments supports Muge's explanation.

The mechanism of increasing populations of *M. hapla* and, particularly, *H. schachtii* in double-inoculation treatments is probably due to predisposition. Correlation of increased population with reduction of total carbohydrates compared to reducing carbohydrates and the changes in mineral content indicate the importance of physiological and biochemical changes in double-inoculation treatments. Results of this research emphasize the need for studying self-interactions more fully in order to understand the complex reactions of nematode-nematode-host combinations.

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