Parasitism of Beta vulgaris by Meloidogyne hapla and Heterodera schachtii Alone and in Combination[1]

PARVIZ JATALA and H. J. JENSEN[2]

Abstract: Interrelationships of *Meloidogyne hapla* and *Heterodera schachtii* in combinations of several population levels and different inoculation periods were studied. Results indicated suppression of gall development of *M. hapla* in any treatment in which inoculations of *H. schachtii* preceded those of *M. hapla* by 10 days. This interrelationship was characterized by amensalism with *M. hapla* serving as an amensal and *H. schachtii* serving as an inhibitor. Conversely, when inoculations of *M. hapla* preceded *H. schachtii* inoculations by 10 days, there were increases in cyst development. This relationship was characterized by commensalism with *H. schachtii* serving as a commensal. In both interactions, the preinvading parasites acted independently and established populations equal to treatments receiving either parasite alone. When both nematodes were inoculated simultaneously, there were no effects on populations of either. Relationships of this nature were characterized by neutralism. Ratios of total soluble/reducing carbohydrates were lower in treatments when *M. hapla* inoculations preceded those of *H. schachtii*. Plants inoculated with both nematodes died earlier than those inoculated with either parasite alone. High concentrations of Al and Fe occurred in treatments wherein *M. hapla* or *H. schachtii* inoculations preceded each other by 10 days. Generally, noninoculated control plants exhibited higher concentrations of K, P, Mg, and B than other treatments. *Key Words:* Interactions, carbohydrates, mineral elements, amensalism, commensalism, neutralism.

Occurrence of several genera of plant-parasitic nematodes in or near the rhizosphere of a given host plant is common. Associations of more than one kind of plant-parasitic nematode in a given rhizosphere may have a greater impact on the physiology of a host than either pathogen alone. Each pathogen has its own requirements for growth and multiplication. Therefore, when more than one kind of plant-parasitic nematode is found in the rhizosphere of a particular host, the plant may become a target for infection by these parasites at various times during the growing period. Physiology and response of a host to infection by a second pathogen may be altered by the presence of an established pathogen. This change could favor or hinder the second pathogen.

There are several reports on interactions among various nematodes (1, 2, 3, 4, 5, 6, 7, 8, 9, 12). Bird et al. (1), who studied dynamics of field populations of *Hoplolaimus columbus* and *Meloidogyne incognita* in a cotton field, reported that the presence of either species significantly inhibited the occurrence of a concomitant species. They reported that *H. columbus* was predominant in one part of the field, whereas *M. incognita* was predominant in the other part. Both species were recovered only in the center of the field. Gay and Bird (4) found that populations of *Pratylenchus brachyurus* on cotton were increased significantly in the presence of *M. incognita* either when plants were inoculated simultaneously with both pathogens or when the plants were previously inoculated with the latter. Simultaneous inoculation with or previous invasion by *P. brachyurus,* however, inhibited root penetration by *M. incognita.* Chapman and Turner (2, 3) reported that *M. incognita* repressed reproduction by *P. penetrans* in red clover and alfalfa. Oviposition by *P. penetrans* decreased as the number of nematodes, the ratio of entrant *M. incognita* to

Received for publication 23 October 1973.

[1] Oregon Experiment Station Technical Paper No. 3689. Portion of a Ph.D. dissertation submitted by senior author to Oregon State University, Corvallis 97331.

[2] Nematologist, The International Potato Center, Apartado 5969, Lima, Peru, and Professor of Nematology, Oregon State University, Corvallis 97331, respectively.

P. penetrans, and the priority of root invasion by *M. incognita* increased.

Interaction studies between sedentary parasites have received limited attention (5, 6, 7, 8, 12). The emphasis of this study was to determine the interrelationships of *Meloidogyne hapla* Chitwood and *Heterodera schachtii* Schmidt on a common host *Beta vulgaris* L. Preliminary results of this study have been reported (6, 8).

MATERIALS AND METHODS

Seedlings of *Beta vulgaris* 'USH9A' were grown in sterile sand for 3 weeks, after which the sand was washed off the roots and seedlings of the same size were selected and transplanted into 350-ml styrofoam cups containing pasteurized 3:1 soil-sand mixture. At transplanting time, three 5-cm sections of plastic drinking straws were spaced equally around each seedling with one end in the root zone and the other projecting above the soil. Plants were inoculated with newly hatched larvae through the straws. Inocula combinations included: both nematodes simultaneously, *M. hapla* (Mh) preceding *H. schachtii* (Hs), or Hs preceding Mh by 10 days. Inoculated-control plants received either Hs or Mh, and noninoculated plants served as control. Treatments are summarized in Table 1.

Two inoculated-control series were used in this experiment: one series consisted of initial inoculations of 250, 500, 750, 1,000, and 1,250 larvae of either species; a second series received the same treatments 10 days later. There were five replications/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.

Thirty days after the initial inoculation, roots were carefully removed from soil by being soaked in water. Although very few cysts remained in the soil, no attempts were made to recover them. Roots were examined, galls and cysts were counted (with the aid of a stereoscopic microscope), and fresh plant top and root weights were recorded. Dried leaf and fresh root samples were collected for tissue and carbohydrate analysis, respectively.

Using an emission spectrograph with A. C. arc source, we analyzed leaf tissues 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 A.O.A.C. methods (11). Total soluble and reducing carbohydrates were obtained by using the method of Umbreit and Burris (13). In the second experiment, which involved the same treatments, longevity of plants was recorded to 150 days, at which time the only surviving plants were those of noninoculated series.

A two-factor analysis of variance was used for statistical analysis of all experiments.

RESULTS

MELOIDOGYNE HAPLA (Mh) INOCULUM CONSTANT (250 LARVAE) AND HETERODERA SCHACHTII (Hs) INOCULUM VARIABLE: *Gall and cyst development.*—Gall counts in simultaneous-inoculation treatments or in treatments when Mh preceded Hs inoculations by 10 days were not different and were within the range obtained by inoculation with 250 Mh alone (Table 1). However, when Hs (250, 500, or 1,000 larvae) inoculations preceded Mh by 10 days, there was suppression in total gall formation in each treatment. Gall formation resulting from inoculation with 250 Mh was lower when it followed the inoculation with 250 Hs than when it followed inoculations of 500 or 1,000 Hs.

Except for one treatment, cyst production was not different among treatments in which Hs preceded Mh inoculations, Hs inoculation alone, or in simultaneous inoculations with Mh (Table 1). Cyst formation was higher in plants inoculated with 1,000 Hs alone than in plants in which inoculations of 1,000 Hs were followed, by simultaneous inoculations with 250 Mh. However, in all treatments in which Mh preceded Hs inoculations, cyst formation was greater than in any other treatment. This increase was directly correlated with the increase in Hs inoculum. There was an increase of up to eight-fold in cyst formation when inoculation with 250 Mh was followed by inoculation with 1,000 Hs as compared to adding 1,000 Hs alone, preceded by or simultaneously inoculated with 250 Mh.

TABLE 1. Influence of varying inoculum levels and sequences on the concomitant development of *Meloidogyne hapla* (Mh) and *Heterodera schachtii* (Hs) on *Beta vulgaris*.

Inoculum level and sequence	No. galls	No. cysts	No. plants with cysts and galls[a]	Fresh weights (gm)		No. days plants survived
				Shoots	Roots	
Single Species						
Mh 250	56.4	—	—	2.80	0.94	110.4
Mh 500	82.0	—	—	2.74	1.10	106.4
Mh 750	137.4	—	—	2.37	0.86	102.0
Mh 1000	177.2	—	—	2.72	0.97	105.0
Mh 1250	257.4	—	—	2.54	0.80	103.0
Hs 250	—	5.6	—	2.10	0.87	113.4
Hs 500	—	12.4	—	2.06	0.76	103.6
Hs 750	—	16.4	—	1.97	0.79	109.6
Hs 1000	—	21.4	—	1.97	0.92	102.8
Hs 1250	—	25.4	—	1.99	1.10	105.0
Simultaneous						
250 Mh + 250 Hs	53.6	5.4	0.2	2.59	0.90	98.8
250 Mh + 500 Hs	54.2	9.0	0.4	2.54	0.95	91.0
250 Mh + 1000 Hs	58.6	14.0	0.8	2.38	1.00	91.4
500 Mh + 250 Hs	85.6	5.2	0.2	2.47	1.04	95.0
1000 Mh + 250 Hs	209.0	5.0	0.6	2.40	0.93	88.2
Mh 1st by 10 days						
250 Mh — 250 Hs	47.2	20.8	1.6	1.70	0.47	87.4
250 Mh — 500 Hs	59.8	46.0	1.4	1.39	0.50	81.8
250 Mh — 1000 Hs	56.6	113.6	2.2	1.75	0.70	86.6
500 Mh — 250 Hs	87.8	41.0	3.6	1.38	0.41	81.0
1000 Mh — 250 Hs	96.6	12.8	1.6	1.17	0.58	72.6
Hs 1st by 10 days						
250 Hs — 250 Mh	22.8	8.1	0	1.13	0.41	105.2
250 Hs — 500 Mh	51.2	3.4	0	1.31	0.55	102.2
250 Hs — 1000 Mh	74.0	4.8	0.2	1.62	0.82	95.4
500 Hs — 250 Mh	34.0	7.4	0.6	0.81	0.28	101.0
1000 Hs — 250 Mh	34.6	15.2	0.4	1.42	0.48	101.0
Noninoculated control	0	0	0	1.77	0.55	>150.0
LSD: ($P = 0.05$)	7.84	4.57	1.1	0.32	0.18	9.3
($P = 0.01$)	10.40	6.06	1.5	0.43	0.24	12.3

[a]Refers to mean number of plants on which cysts and galls occur together (in same site).

Plant Growth.—When separate inoculations of Mh and Hs were made, fresh top weights of plants were variable but greater than those in the noninoculated control series (Table 1). The fresh top weights in all combinations of simultaneous inoculation treatments were higher than those in other treatments when both nematodes were present. Whenever Hs preceded Mh inoculations or vice versa, top weights were lower than when both nematodes were inoculated simultaneously. Top weights were higher when inoculation with 250 Mh was followed by inoculation with 250, 500, or 1,000 Hs than when a reciprocal inoculation sequence was used.

There were no differences in root weights among plants which were inoculated with: 250 Mh followed by 1,000 Hs; those inoculated with 1,250 Hs; or those in the noninoculated control series (Table 1). Plants inoculated with 250 Mh and 1,000 Hs together or with 1,250 Mh had greater root weights than plants inoculated with 1,250 Hs. Fresh root weights of all other treatments were a reflection of their top weights in most cases.

HETERODERA SCHACHTII (HS) INOCULUM CONSTANT (250 LARVAE) AND MELOIDOGYNE HAPLA (Mh) INOCULUM VARIABLE: *Gall and cyst development.*—Gall formation was less

in all three treatments in which Hs preceded Mh inoculations than it was in any other treatment (Table 1). There were no differences in gall formation among plants inoculated with 250 Mh followed by 250 Hs, or plants inoculated with both species together; or plants inoculated with 500 Mh followed by 250 Hs, 500 Mh and 250 Hs together, and by 500 Mh alone. Plants inoculated with 1,000 Mh, then with 250 Hs exhibited fewer galls than plants inoculated with 1,000 Mh and 250 Hs together and 1,000 Mh alone. Gall count in plants inoculated with 1,000 Mh followed by 250 Hs was higher than in those inoculated with 250 Hs and then with 1,000 Mh.

There were no differences in total cyst formation when plants were inoculated with 250 Hs alone, with both nematodes simultaneously, or with Hs preceding Mh (Table 1). There were increases in cyst formation when inoculation with Mh (all levels) preceded inoculation with Hs by 10 days. However, the increase in cyst formation in plants inoculated with 1,000 Mh and then with 250 Hs was less than in those inoculated with 250 Mh and then with 250 Hs or 500 Mh and then with 250 Hs. The optimum number of Mh as a predisposing factor for 250 larvae of Hs was 500 larvae.

Plant Growth.—Fresh top weights of plants inoculated with Mh or Hs alone were variable, but in most cases they were greater than weights of noninoculated plants (Table 1). They were greater for plants inoculated simultaneously than they were for plants inoculated sequentially with both nematodes, regardless of order. Fresh top weights of plants inoculated with 1,000 Mh followed by 250 Hs were lower than those inoculated with the reciprocal sequence and those of plants inoculated with 250 Hs followed by 250 Mh were lower than those inoculated with the reciprocal sequence.

When both nematodes were present, root weights reflected top weights with the exception of the plants inoculated with 250 Hs and then with 500 Mh, or with 1,250 Hs alone. In these instances, increases in root weights had no correlation with top weights (Table 1). Similarly, in the inoculated-control series, treatment showed an increase in root weight which was not correlated to top weight.

OCCURRENCE OF CYSTS AND GALLS TOGETHER: The frequency with which cysts and galls occurred together was higher in most of the treatments when inoculation with Mh preceded inoculation with Hs. Plants inoculated with 500 Mh and then with 250 Hs exhibited a higher rate of cyst and gall (together) formation than any other treatment. Rates of cyst and gall (together) formation in plants inoculated with 250 Mh and then with 1,000, 250, or 500 Hs, respectively, and in plants inoculated with 1,000 Mh followed by 250 Hs were lower. There was no cyst and gall formation in plants when 250 or 500 Mh inoculations followed inoculation with 250 Hs.

EFFECTS OF INTERACTIONS ON PLANT LONGEVITY: When Mh preceded Hs by 10 days in all inoculation levels, the plants, in most cases, had lower longevity rates (Table 1) than in other treatments. In general, plants receiving inoculations of either 250 to 1,250 Mh or 250 to 1,250 Hs alone survived longer than plants receiving both nematodes species. However, the statistical significance of this behavior was shown in only a few cases. Longevity rates of plants in all treatments receiving both nematodes simultaneously were generally lower than in treatments in which Hs preceded Mh.

Longevity rates in plants inoculated simultaneously with 250 Mh and 500 Hs or 1,000 Mh with 250 Hs were lower than in those inoculated with 500 and 250 Hs followed by 250 and 1,000 Mh, respectively. Longevity rates in plants inoculated with 1,000 Mh and then with 250 Hs were lower than in any other treatment except for those inoculated with 250 or 500 Mh followed by 500 and 250 Hs, respectively.

Plants in the noninoculated-control series survived for 150 days, which was the termination period of the experiment. Their longevity rate was greater than that of any other treatment.

INFLUENCE OF INTERACTIONS ON MINERAL ELEMENTS AND CARBOHYDRATES: Results of elemental analyses for all treatments are presented as the concentration of 10 elements/gm of dried leaf tissue. Although there were variations in concentrations of different elements in all treatments, for most cases, noninoculated control plants had higher concentrations of K, P, Mg, and

B than those of other treatments. Treatments with high concentrations of Fe included: plants on which Mh preceded Hs inoculations; those on which 250 or 1,000 Mh followed inoculation with 250 or 250 Hs, respectively; those on which 1,000 Mh were added with 250 Hs together; and those which received 750 or 1,250 Hs alone. Similarly, treatments with high concentrations of Al included: plants inoculated with 250 Mh followed by 250, 500 or 1,000 Hs; plants inoculated with 250, 500 or 1,000 Hs followed by 250 Mh; plants inoculated with 250 Mh and 500 Hs together; and plants inoculated with 1,250 Mh alone. Plants inoculated with 500 Hs followed by 250 Mh also had higher concentrations of K, P, and Zn than those of other treatments. Plants inoculated with 1,000 Mh followed by 250 Hs had the lowest concentrations of K and P and the highest concentration of Ca. The highest concentration of Mn was found in plants inoculated with 250 Hs followed by 1,000 Mh. Plants inoculated with 250 Hs followed by 500 Mh had the lowest concentrations of Ca, Mg, and Al.

The lowest ratios of total soluble/reducing carbohydrates in all treatments (compared with ratios in noninoculated control plants) were those in which Mh preceded Hs inoculations. Treatments with lower ratios of total soluble/reducing sugars than those in noninoculated plants included: plants inoculated with 250, 500, or 1,000 Mh after being inoculated with 1,000, 250, and 250 Hs, respectively; those inoculated simultaneously with 500 or 1,000 Hs and 250 or 250 Mh, respectively; those which received 750 and 1,000 Mh alone; and those with 750 or 1,000 Hs alone. The highest ratios of total soluble/reducing carbohydrates were found: in plants inoculated with 250 or 500 Hs followed by 250 and 250 Mh; in plants inoculated simultaneously with 250 or 1,000 Mh and 250 Hs, respectively; and in those inoculated with 1,250 Mh alone.

DISCUSSION

Interactions between Mh and Hs on *B. vulgaris* resulted in a definite shift in the development of both species if either nematode preceded the other in inoculation.

Suppression of *M. hapla*-induced galls occurred when inoculation of Hs preceded that of Mh. This fact indicates an amensalism type relationship, with Mh serving as the amensal and Hs serving as the inhibitor. Conversely, there were increases in cyst formation by Hs when Mh preceded Hs by 10 days in inoculation. This type of relationship indicates commensalism, with Hs serving as a commensal. In both cases, however, the preinvading species acted independently and had a population growth equal to that when introduced alone. In simultaneous inoculations, there were no changes in population of either parasite. Relationships of this nature are termed neutralism. These three types of interactions fit the description of terms used by Odum (10).

Stimulatory effects of Mh on Hs development and the inhibitory effects of the latter on development and growth of Mh are certainly important factors in etiology of the disease caused by either pathogen. *Meloidogyne hapla* is a polyphagous nematode and probably has more complex enzyme systems than Hs which has a limited host range. Results of this experiment suggest that Mh modifies and/or alters the physiology of the host plant to the advantage of the incoming Hs larvae. Subsequently, Hs establishes itself and continues to grow vigorously. Conversely, preinvasion by Hs results in moderation and/or alteration of host physiology to the disadvantage of Mh which was expressed as suppression of gall formation. The information reported here agrees with the findings of Ross (12).

When the plants were pre-exposed to 250 Mh larvae for 10 days prior to inoculation with 1,000 Hs larvae, there was an almost six-fold increase in cyst production. This ratio seemed to be the most effective combination for Hs cyst production over other treatments. Reversal of this combination was effective in decreasing Mh-induced galls.

When Mh preceded Hs in inoculations, decrease in total soluble carbohydrates resulted, probably because the rate of photosynthesis by the host was suppressed. Ratios of total soluble/reducing carbohydrates were lower when inoculations of Mh preceded those of Hs. These results were reflected in plant survival. When inoculation with Mh preceded inoculation with Hs,

survival of plants was, in most cases, significantly less than with other treatments. Although there were variations in longevity rates of plants in all treatments, the plants inoculated with both nematodes had shorter longevity rates than those inoculated with either parasite alone.

Occurrence of both organisms in one feeding site is of great interest. This type of association was most common when Mh served as the preinvading parasite. Histopathological and biochemical studies of this nature and synergistic, additive, or deterrent effects of these nematodes on disease of the host warrant attention. It would be of interest to determine at which stage of growth a certain nematode has either stimulatory or inhibitory effect on growth and development of the other parasite.

Since these nematodes occur together frequently in the same field or planting area, this study provides some basis for interest in breeding sugar beets for resistance to these two parasites.

LITERATURE CITED

1. BIRD, G. W., O. L. BROOKS, and C. E. PERRY. 1974. Dynamics of concomitant field populations of Hoplolaimus columbus and Meloidogyne incognita. J. Nematol. 6:190-194.
2. CHAPMAN, R. A., and D. R. TURNER. 1972. Effect of entrant Meloidogyne incognita on reproduction of concomitant Pratylenchus penetrans in red clover. J. Nematol. 4:221 (Abstr.).
3. CHAPMAN, R. A., and D. R. TURNER. 1975. Effect of Meloidogyne incognita on reproduction of Pratylenchus penetrans in red clover and alfalfa. J. Nematol. 7:6-10.
4. GAY, C. M., and G. W. BIRD. 1973. Influence of concomitant Pratylenchus brachyurus and Meloidogyne spp. on root penetration and population dynamics. J. Nematol. 5:212-217.
5. JATALA, P. 1972. Interrelationships of Meloidogyne hapla Chitwood, 1949 with Heterodera schachtii Schmidt, 1871 on Beta vulgaris L. Ph.D. Dissertation, Oregon State University, Corvallis, Oregon. 91 p.
6. JATALA, P., and H. J. JENSEN. 1972. Interrelationships of Meloidogyne hapla and Heterodera schachtii populations on Beta vulgaris. J. Nematol. 4:226 (Abstr.).
7. JATALA, P., and H. J. JENSEN. 1972. Histopathological interrelationships of Meloidogyne hapla and Heterodera schachtii on Beta vulgaris. Phytopathology 62:1103-1104 (Abstr.).
8. JATALA, P., and H. J. JENSEN. 1973. Effects of Meloidogyne hapla and Heterodera schachtii interrelationships on carbohydrates and mineral content of Beta vulgaris. 2nd Int. Congr. Plant Pathology, Minneapolis, Minn., September 5-12, 1973 (Abstr.).
9. JOHNSON, A. W., and C. J. NUSBAUM. 1970. Interactions between Meloidogyne incognita, M. hapla and Pratylenchus brachyurus in tobacco. J. Nematol. 2:334-340.
10. ODUM, E. P. 1959. Fundamentals of ecology. Second edition. W. B. Saunders Company, Philadelphia. 546 p.
11. Official and Tentative Methods of Analysis of the A.O.A.C. 1955. Eighth Ed., Published by Association of Official Agricultural Chemists, P. O. Box 540. Benjamin Franklin Station, Washington 4, D. C., U.S.A.
12. ROSS, J. P. 1964. Interaction of Heterodera glycines and Meloidogyne incognita on soybeans. Phytopathology 54:304-307.
13. UMBREIT, W. W., and R. H. BURRIS. 1964. Chemical Analyses. Pages 205-225 *in* W. W. Umbreit, R. H. Burris and J. F. Stauffer, eds. Manometric techniques, Burgess Publishing Co., Minneapolis, Minn.