Pathological Interaction of a Combination of Heterodera schachtii and Meloidogyne hapla on Tomato[1]

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Abstract: Increased culturing of a tomato population of *Heterodera schachtii* (UT1C) on tomato for 480 days (eight inoculation periods of 60 days each) significantly increased virulence to 'Stone Improved' tomato. A synergistic relationship existed between *Meloidogyne hapla* and *H. schachtii* on tomato. A combination of *H. schachtii* (UT1C) and *M. hapla* significantly reduced tomato root weights by 65, 64, and 61% below root weights of untreated controls, and single inoculations of *M. hapla* and *H. schachtii*, respectively. This corresponded to root reductions of 42, 44, and 46% from a combination of *H. schachtii* (UT1B) and *M. hapla*. Antagonism existed between *H. schachtii* and *M. hapla* with regard to infection courts and feeding sites. The root-knot galling index dropped from 6.0 with a single inoculation of *M. hapla* to 4.3 and 3.3 with combined inoculations of *M. hapla* plus UT1B and *M. hapla* plus UT1C cyst nematode populations. The pathological virulence of *H. schachtii* to sugarbeet was not lost by extended culturing on tomato; there were no differences in penetration, maturation, and reproduction between sugarbeet populations continually cultured on sugarbeet and the population continually cultured on tomato. *Key words:* sugarbeet cyst nematode, northern root-knot nematode, *Lycopersicon esculentum, Beta vulgaris,* physiological variability, races, synergism, antagonism.

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The northern root-knot nematode, *Meloidogyne hapla* Chitwood, and the sugarbeet cyst nematode, *Heterodera schachtii* Schmidt, commonly occur in the intermountain region of the western United States. Although they occasionally cohabit the same soil, they do not usually parasitize the same host. *H. schachtii* and *M. hapla* were found together in a tomato field in northern Utah. Plant roots were extensively galled and parasitized by *M. hapla,* but few sugarbeet cyst females were observed. Since *M. hapla* and *H. schachtii* have not been previously reported to occur concomitantly on tomato, a study was made to determine the host response of tomato to *H. schachtii* and the pathological interaction of *H. schachtii* and *M. hapla* on tomato.

MATERIALS AND METHODS

The following *H. schachtii* populations were used in this study: 1) UT1A—collected from a tomato (*Lycopersicon esculentum* Mill.) planted at Ogden, Utah, and cultured on sugarbeet (*Beta vulgaris* L.) 'TASCO AH14'; 2) UT1B—the UT1A population continually cultured over four inoculation periods of 60 days each on 'Stone Improved' tomato; 3) UT1C—obtained by culturing the UT1A population continually cultured over eight inoculation periods of 60 days each on 'Stone Improved' tomato; and 4) UT2, UT3, ID1, ID2, and OR1—collected from sugarbeet fields and cultured on 'TASCO AH14' sugarbeet. The *M. hapla*

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population used in this study was collected from a lettuce field and cultured on 'Stone Improved' tomato.

Cysts of *H. schachtii* and egg masses of *M. hapla* were collected from culture plants and surface sterilized in 0.5% sodium hypochlorite. *H. schachtii* eggs were hatched in a $ZnCl_2$ solution and *M. hapla* eggs were hatched in deionized water in an oxygenator and the larvae used for inoculum. Tomato 'Stone Improved' and sugarbeet 'TASCO AH14' were used as host plants.

Continual culturing of *H. schachtii* on tomato increased the virulence of the nematode to tomato (4). Therefore, an experiment was made to determine the possible variability in population virulence of *H. schachtii* on tomato, the relationship of *H. schachtii* and *M. hapla* on tomato, and the effect of possible differences in the virulence ratings of the *H. schachtii* populations (UT1A, UT1B, and UT1C) on the *H. schachtii-M. hapla* interactions. Fourteen-day-old tomato transplants were inoculated with 1,000 *M. hapla* and *H. schachtii* (population UT1A, UT1B, or UT1C) larvae, singly, or in combination, or left uninoculated. Plants were grown at a greenhouse temperature of 22 ± 4 C under a 16-h day. Each treatment was replicated 10 times. After 80 days growth, plants were harvested, and root and top growth weights and root-knot indices were determined. Root-knot indices were: 1 = no galling, 2 = 1–10% galling; 3 = 11–30% galling; 4 = 31–70% galling; 5 = 71–90% galling; 6 = 91–100% galling (1).

A similar study consisted of inoculating 14-day-old tomato transplants with 1,000 *M. hapla* and *H. schachtii* (population UT1A, UT1B, or UT1C) larvae, singly, or in combination, or left uninoculated; 30 days later the single species inoculated plants were inoculated with 1,000 larvae per plant of the other nematode species. Plants were harvested 50 days after the final inoculations and plant weights and galling indices determined as previously described.

To determine differences in the ability of UT1A, UT1B, UT1C, UT2, UT3, ID1, ID2, and OR1 *H. schachtii* populations to penetrate, mature, and reproduce on tomato, 14-day-old tomato transplants were inoculated with 100 *H. schachtii* larvae of each nematode population and each treatment replicated 40 times. After 14 days, 20 plants of each treatment were harvested and the roots stained with acid fuschin and nematode penetrations determined. After 42 days, 10 plants of each treatment were harvested, the soil carefully removed from the roots, and the number of females per plant were determined. The remaining 10 plants of each treatment were harvested after 60 days, and nematode reproduction (eggs/cyst) was determined by breaking 10 cysts, chosen at random from each treatment, in a grinding tube.

To determine what effects the culturing of *H. schachtii* on tomato have on the virulence of the nematode to sugarbeet, 14-day-old sugarbeet transplants were inoculated with 1,000 larvae of UT1A, UT1B, UT1C, UT2, or ID1 nematode populations with 10 replicates of each treatment. After 60 days growth at 22 ± 4 C, the plants were harvested and root and top weights were determined.

A final study compared the penetrations, maturations, and reproductive rates of the different *H. schachtii* populations on sugarbeets. Fourteen-day-old transplants (AH14) were inoculated with 100 larvae of UT1A, UT1B, UT1C, UT2, and ID1 *H. schachtii* populations. Each treatment was replicated 40 times. After 14 days, 20 plants of each treatment were harvested and nematode penetration was determined; after 42 days, 10 plants were harvested and nematode maturation (females per plant) were determined; and after 60 days, the final 10 plants per treatment were harvested, 10 cysts chosen at random from each replicate, and the number of eggs per cyst determined.

RESULTS

Neither single or combined inoculations of *H. schachtii* (UT1A) and *M. hapla* significantly decreased root growth of tomato plants. Tomato top weights were significantly ($P = 0.05$) reduced by M. hapla alone and in combination with *H. schachtii* (Table 1), but no synergism was detected in the combined inoculations. However, there was a direct relationship between the length of the culture period of *H. schachtii* on tomato and the damage caused by the nematode on tomato. The UT1C population

Table 1. The effect of a combination of *Heterodera schachtii* and *Meloidogyne hapla* on the growth and root galling of tomato, *Lycopersicon esculentum* Mill., (Stone Improved).*

Treatment	Root weight (g)	Top weight (g)	Root-knot indices†
H. schachtii‡	28.6	67.4	...
H. schachtii‡ +*M. hapla*	26.3	43.9	6.0
H. schachtii§	24.7	66.6	...
H. schachtii§ + *M. hapla*	21.3	37.4	4.3
H. schachtii‖	22.3	56.8	...
H. schachtii‖ + *M. hapla*	13.7	32.6	3.3
M. hapla	27.4	46.2	6.0
Control	30.1	75.8	...
LSD ($P = 0.05$)	6.9	10.8	0.6

*14-day-old transplants inoculated with 1,000 *H. schachtii* and/or 1,000 *M. hapla* larvae and grown for 80 days at 22 ± 4 C.

†1 = no galling; 6 = 71–100% galled root tissue.

‡UT1C population (inoculum obtained from tomato and maintained on sugarbeet).

§UT1B population (inoculum obtained by culturing UT1A population on tomato plants over four inoculation periods of 60 days each).

‖UT1C population (inoculum obtained by culturing UT1A population cultured on tomato plants over eight inoculation periods of 60 days each).

cultured on tomato for more than 480 days significantly ($P = 0.05$) reduced tomato root growth. A synergistic effect on root growth reduction existed between the *H. schachtii* UT1C population and *M. hapla*. However, only an additive effect between UT1B and *M. hapla* on root growth, and UT1B and *M. hapla* and UT1C and *M. hapla* on top growth was observed. Inoculation with *M. hapla* significantly ($P = 0.05$) reduced top weights below that of uninoculated control plants and UT1A and UT1B *H. schachtii* inoculated plants.

When *M. hapla* was inoculated in combination with *H. schachtii*, *M. hapla* gall indices on tomato roots were significantly ($P = 0.05$) reduced; the index dropped from 6.0 to 4.3 and 3.3 when UT1B and UT1C populations, respectively, were combined with *M. hapla*.

Neither combined nor sequential inoculations with *H. schachtii* (UT1A) and *M. hapla* significantly reduced tomato root growth compared to untreated controls, or top growth compared to *M. hapla* inoculations (Table 2). However, synergistic interactions were observed between *M. hapla* and the UT1B and UT1C *H. schachtii* populations when simultaneous inoculations were made (Tables 3, 4). A combined inoculation of *H. schachtii* (UT1B) and *M. hapla* synergistically reduced tomato root weigths by 42, 44, and 46% below untreated controls and *M. hapla* and *H. schachtii* inoculated plants, respectively. This corresponded to a synergistic reduction in root weight of 65, 64, and 61% below untreated controls and *M. hapla* and *H. schachtii* inoculated plants, respectively, induced by a combined inoculation of *H. schachtii*

Table 2. Pathogenicity of *Heterodera schachtii* (UT1A) and *Meloidogyne hapla* on tomato, *Lycopersicon esculentum* Mill., (Stone Improved) using combined and sequential inoculations.*

Treatment	Root weight (g)	Top weight (g)	Root-knot indices†
H. schachtii‡	26.7	69.8	...
M. hapla	24.4	43.7	6.0
H. schachtii§ + *M. hapla*	20.3	46.3	6.0
H. schachtii‖ + *M. hapla*	19.7	42.9	6.0
H. schachtii¶ + *M. hapla*	21.4	48.7	6.0
Control	21.7	70.3	...
LSD ($P = 0.05$)	6.7	12.3	

*Transplants inoculated with 1,000 *H. schachtii* larvae and/or 1,000 *M. hapla* larvae and grown at 22 ± 4 C. Plant harvested 50 days after final inoculation.

†1 = no galling; 6 = 71–100% galled root tissue.

‡UT1A population (inoculum obtained from soil collected from a tomato planting and cultured on sugarbeet.).

§14-day-old transplants inoculated with *H. schachtii* and *M. hapla* larvae.

‖14-day-old transplants inoculated with *M. hapla* larvae and 30 days later with *H. schachtii* larvae.

¶14-day-old transplants inoculated with *H. schachtii* larvae and 30 days later with *M. hapla* larvae.

Table 3. Pathogenicity of a combination of *Heterodera schachtii* (UT1B) and *Meloidogyne hapla* on tomato, *Lycopersicon esculentum* Mill., (Stone Improved) using combined and sequential inoculations.*

Treatment	Root weight (g)	Top weight (g)	Root-knot indices†
H. schachtii‡	23.2	72.1	...
M. hapla	22.5	44.7	6.0
H. schachtii§ + *M. hapla*	12.6	44.9	3.8
H. schachtii| + *M. hapla*	21.9	39.3	5.7
H. schachtii¶ + *M. hapla*	23.2	42.3	3.6
Control	21.7	75.8	...
LSD ($P = 0.05$)	6.3	11.7	0.9

*Transplants inoculated with 1,000 *H. schachtii* larvae and/or 1,000 *M. hapla* larvae and grown at 22 ± 4 C. Plant harvested 50 days after final inoculation.

†1 = no galling; 6 = 71–100% galled root tissue.

‡UT1B population (inoculum obtained by culturing UT1A population on tomato plants over four inoculation periods of 60 days each).

§14-day-old transplants inoculated with *H. schachtii* and *M. hapla* larvae.

|14-day-old transplants inoculated with *M. hapla* larvae and 30 days later with *H. schachtii* larvae.

¶14-day-old transplants inoculated with *H. schachtii* larvae and 30 days later with *M. hapla* larvae.

Table 4. Pathogenicity of a combination of *Heterodera schachtii* (UT1C) and *Meloidogyne hapla* on tomato, *Lycopersicon esculentum* Mill., (Stone Improved) using combined and sequential inoculations.*

Treatment	Root weight (g)	Top weight (g)	Root-knot indices†
H. schachtii‡	22.2	59.2	...
M. hapla	24.3	44.3	6.0
H. schachtii§ + *M. hapla*	8.7	39.9	3.6
H. schachtii|| + *M. hapla*	9.9	46.7	5.9
M. schachtii¶ + *M. hapla*	20.3	39.9	3.4
Control	24.9	69.1	...
LSD ($P = 0.05$)	7.4	12.4	1.2

*Transplants inoculated with 1,000 *H. schachtii* larvae and 1,000 *M. hapla* larvae and grown at 22 ± 4 C. Plant harvested 50 days after final inoculation.

†1 = no galling; 6 = 71–100% galled root tissue.

‡UT1C population (inoculum obtained by culturing UT1A population on tomato plants over eight inoculation periods of 60 days each).

§14-day-old transplants inoculated with *H. schachtii* and *M. hapla* larvae.

||14-day-old transplants inoculated with *M. hapla* larvae and 30 days later with *H. schachtii* larvae.

¶14-day-old transplants inoculated with *H. schachtii* larvae and 30 days later with *M. hapla* larvae.

(UT1C) and *M. hapla*. A sequential inoculation of *M. hapla* followed by *H. schachtii* (UT1C) also caused a synergistic reduction in root growth. This resulted in reductions in root weights of 60, 59, and 55% below untreated controls and *M. hapla* and *H. schachtii* inoculated plants. *M. hapla* reduced ($P = 0.05$) top growth of tomato plants, but there was no synergistic interaction when *M. hapla* was combined with *H. schachtii* populations. The synergistic effect of the UT1B cyst nematode inoculum was lost by sequential inoculations of *H. schachtii* and *M. hapla,* regardless of when inoculation sequence was followed. Inoculations of *H. schachtii* (UT1B and UT1C), however, reduced the galling index of *M. hapla* on tomato when simultaneously inoculated or when *M. hapla* followed either *H. schachtii* population in a sequential inoculation.

Continual culturing of *H. schachtii* on tomato increased ($P = 0.05$) penetration, maturation, and reproduction of *H. schachtii* on tomato plants (Table 5). Although UT1B and UT1C did not differ from one another, both were significantly different from UT1A. Larval penetration of tomato roots by the three cyst nematode populations obtained from tomato (UT1A, UT1B, and UT1C) was greater ($P = 0.05$) than that of the populations obtained from sugarbeet fields (UT2, UT3, ID1, ID2, and OR1). These sugarbeet populations not only penetrated tomato roots to a lesser degree, but also failed to mature to females.

Virulence of *H. schachtii* to sugarbeet was not lost during extended culturing on

Table 5. Physiological differences of six *Heterodera schachtii* populations on tomato, *Lycopersicon esculentum* Mill., (Stone Improved).*

Nematode populations	Nematode infection, maturation, and reproduction		
	Larvae/ seedling†	Females/ plant‡	Eggs/cyst§
Utah 1A‖	36	13	87
Utah 1B¶	55	39	104
Utah 1C#	58	43	108
Utah 2**	29	0	...
Utah 3**	32	0	...
Idaho 1**	26	0	...
Idaho 2**	32	0	...
Oregon 1**	27	0	...
LSD ($P = 0.05$)	9	7	24

*14-day-old transplants inoculated with 100 *H. schachtii* larvae and grown at 22 ± 4 C.

†14 days after inoculation.

‡42 days after inoculation.

§60 days after inoculation.

‖UT1A population (inoculum obtained from soil collected from a tomato planting and cultured on sugarbeet).

¶UT1B population (UT1A population cultured on tomato over four inoculation periods of 60 days each).

#UT1C population (UT1A population cultured on tomato over eight inoculation periods of 60 days each).

**Sugarbeet field collections cultured on sugarbeet.

tomato roots. Populations UT1B and UT1C cultured on tomato for 240 and 480 days, respectively, were as virulent on sugarbeets as populations cultured continually on sugarbeet (Table 6). There were no significant differences in root and top weights of sugarbeet parasitized by either UT1A, UT1B, UT1C, UT2, or ID1 populations; all reduced ($P = 0.05$) root and top weights compared to uninoculated controls. No differences in root penetration, nematode maturation, and reproduction were found among nematode populations cultured on tomato and sugarbeet (Table 7).

DISCUSSION

This study substantiates results obtained in other investigations on the host-parasite relationship of *H. schachtii* on tomato (2, 4,9) and confirms reports of *H. schachtii* populations with different host preferences (2,3,4,6,7,8,9).

Table 6. The effect of different *Heterodera schachtii* populations on the growth of AH14 of sugarbeet, *Beta vulgaris L.*, (AH14).*

H. schachtii populations	Root weight (g)	Top weight (g)
Utah 1A†	6.9	46.3
Utah 1B‡	7.3	42.7
Utah 1C§	6.7	40.9
Utah 2‖	7.2	43.6
Idaho 1‖	7.4	47.2
Control	11.7	54.7
LSD ($P = 0.05$)	3.2	8.2

*14-day-old sugarbeet seedlings inoculated with 1,000 *H. schachtii* larvae and grown at 22 ± 4 C for 60 days.

†Inoculum obtained from soil collected from a tomato planting and cultured on sugarbeet.

‡UT1A population cultured on tomato over four inoculation periods of 60 days each.

§UT1A population cultured on tomato over eight inoculation periods of 60 days each.

‖Sugarbeet field collections cultured on sugarbeet.

The inability to alter the virulence of *H. schachtii* to sugarbeet after extended exposure to tomato further indicates that the nematode population of the Utah tomato field included at least two races; one

Table 7. Comparative infection, maturation, and reproduction of *Heterodera schachtii* populations on sugarbeet previously cultured on tomato *Lycopersicon esculentum* Mill., (Stone Improved) and sugarbeet, *Beta vulgaris* L., (AH14).*

H. schachtii populations	Larvae/ seedling†	Females/ plant‡	Eggs/cyst§
Utah 1A‖	68	42	283
Utah 1B¶	66	39	265
Utah 1C#	62	46	274
Utah 2**	64	44	259
Idaho 1**	66	43	278
LSD ($P = 0.05$)	10	7	42

*14-day-old AH 14 sugarbeet seedlings inoculated with 100 *H. schachtii* larvae and grown at 22 ± 4 C.

†14 days after inoculation.

‡42 days after inoculation.

§60 days after inoculation.

‖Inoculum obtained from soil collected from tomato plantings and cultured on sugarbeet.

¶UT1A population cultured on tomato over four inoculation periods of 60 days each.

#UT1A population cultured on tomato over eight inoculation periods of 60 days each.

**Sugarbeet field collections cultured on AH 14 sugarbeet.

pathogenic on sugarbeet only, and one pathogenic on both sugarbeet and tomato.

Results of this study also indicate that a combination of a population of *H. schachtii* and *M. hapla* cultured on tomato can significantly reduce the growth of tomato plants below that of *M. hapla* alone. However, in only one experiment did *H. schachtii* (UT1C) cultivated on tomato for 480 days reduce ($P = 0.05$) plant growth below that of uninoculated controls. This plant growth reduction agrees with the findings of Lear and Miyagawa (4) who reported that after an extended culture period a tomato strain of *H. schachtii* reduced tomato top growth below that of the uninoculated controls. Competition existed between *H. schachtii* and *M. hapla* for infection courts and feeding sites, and *M. hapla* root galling was reduced when combined inoculations of *M. hapla* and *H. schachtii* were made. This agrees with the findings of Jatala and Jensen (5), who studied the relationship of *H. schachtii* and *M. hapla* on sugarbeet.

The adaptation of *H. schachtii* to mature and reproduce on tomato agrees with the findings of Lear and Miyagawa (4) and Steele (9) who obtained large numbers of cysts from tomato roots after extended periods of exposure. However, although *H. schachtii* can reproduce on tomato after extended periods of exposure, tomato should be considered only a fair to moderate host because the number of eggs per cyst is only 40% of the number produced on sugarbeet. Continual exposure to tomato failed to significantly increase the number of eggs per cyst above that of the field collection.

This study confirms the possibility of potential economic problems for tomato production (4). Adequate agronomic practices such as proper crop rotation, however, should sufficiently control this parasite even in the presence of another synergistic coinhabitant.

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