Population Dynamics of Heterodera schachtii on Tomato and Sugarbeet

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Abstract: Experiments showed that development of male and female *Heterodera schachtii* on tomato and sugarbeet are disproportionately influenced by the nematode inoculum level and root size, which together determine the density of invading larvae. Slight overcrowding favored development of males over females, whereas severe overcrowding equally affected development of males and females. Differential population changes of host-selected races on tested cultivars was attributable to selective development of male and female nematodes. *Key Words:* differential development, sugarbeet nematode, *Lycopersicon esculentum, Beta vulgaris.*

In 1920, Molz (8) first reported that the sex ratio of *Heterodera schachtii* Schmidt is strongly influenced by the physiological condition of the host plant. He suggested that the sex of *H. schachtii* is probably determined by the environment within host-plant roots. However, Sengbusch (11) noted that females have much greater nutritional requirements than males, and attributed shifts in sex ratio to differential death rates. Kerstan (7) determined that numbers of maturing female *H. schachtii* on roots of *Brassica rapa* L. var. *oleifera* (turnip) was always correlated with increases in degenerating and dead larvae in all stages of development, and concluded that density of infection and thickness of roots are the main factors influencing the sex ratio. However, Nemazi (9) reported that tomato varieties varied in the number of developing females that were embedded under the epidermis of roots and unable to break through to complete their life cycle. Larger secondary roots with a thicker epidermis trapped more females than did the smaller primary roots.

Steele (12) reported experiments in which two races of *H. schachtii* were cultured on tomato. Although female populations of the tomato race increased 195-fold, the sugarbeet race increased only 12-fold. Culture of the tomato race on tomato for several months did not result in its increased infectivity to tomato. The sex ratios of these biotypes were not recorded.

The apparent ease with which the population dynamics of *H. schachtii* is influenced by environmental factors is of considerable importance to studies of nematode resistance in sugarbeet. The experiments herein reported provide additional information on the influences of inoculum levels, nematode races, and plant cultivars on the population dynamics of *H. schachtii.*

MATERIALS AND METHODS

Nine tests conducted in this study are grouped under four headings that identify the test objectives. This grouping facilitates discussion of methods not common to all tests.

Influence of inoculum levels on rates of emergence of adult Heterodera schachtii males from roots of sugarbeet: Fifteen sugarbeet seedlings *(Beta vulgaris* L.) were transplanted individually to 20-cm clay pots containing steam-treated sand-soil mixture, and inoculated with 20 cysts. Another group of 15 plants were inoculated with five cysts/plant. All plants were grown in a greenhouse. Twenty-one days after inoculation, the roots were carefully washed free of soil, and plants were incubated in individual funnels for 22 days. Adult males that emerged from roots of each plant were counted.

Influence of inoculum levels on selective development of Heterodera schachtii on sugarbeet: Four tests were conducted. In one test (Table 1), plants were grown in a greenhouse and inoculum levels of 5, 20, and 40 cysts/plant were tested. In tests Nos. 2-4 (Table 2), inoculum levels of 20 and 60 cysts/plant were compared for plants grown in a growth chamber and adult males were counted on plants harvested 18 days after inoculation. Counts of adult females and weights of plants were obtained at harvest 30 days after inoculation.

The influence of host-selected biotypes on selective development of Heterodera schachtii

Received for publication March 28, 1974.

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on tomato: Two tests (5 and 6 in Table 3) were designed to measure the effects of two population levels of sugarbeet nematode maintained 12 years on tomato *(Lycopersicon esculentum* Mill.) by consecutive transfers (tomato race) on the final numbers of nematodes on tomato.

An additional test (7 in Table 3) compared the effects of a tomato race and a sugarbeet race of sugarbeet nematode on the relative increase of adult nematodes on tomato. Fifty cysts of each race were exposed 5 weeks to sugarbeet root diffusate to evaluate the inoculum potential of the two populations.

Influence of plant age on the development of Heterodera schachtii females on sugarbeet and tomato: Seedlings of cultivar US H9A germinate more rapidly and grow more vigorously in the seedling stage than does US H7A (John McFarlane, *personal communication).* The effects of plant age on development of *H. schachtii* on these cultivars were evaluated. Ten-day-old seedlings of each cultivar were started from seed in steam-treated sand and transplanted to 950-ml styrofoam cups filled with a steam-treated mixture of sand and soil. Seedling planting dates were adjusted so that half of the plants of each cultivar were inoculated with 50 cysts at transplanting or at 30 days after transplanting. The plants were grown 30 days in a growth chamber, after which the roots were washed and weighed, and adult female nematodes counted.

The last test of this study was designed to measure the effects of two population levels of the tomato race inoculated on Pearson A-1 tomato at 10, 20, or 30 days after germination. Each plant was exposed to 15 or to 30 cysts. Plants were grown in 1.9-liter waxed cartons kept in a growth chamber. Roots were examined for adult female nematodes 30 days after inoculation.

For all tests, newly formed cysts of *H. schachtii* were recovered from soil and plant debris in which tomato or sugarbeet had been grown for 90 to 120 days after inoculation with viable cysts by methods previously described (16). Cysts containing viable eggs and larvae were broken open in tap water, and the cysts and contents added to holes in the potting medium. The potting medium was a steam-treated one part sand, three parts clay loam soil mixture. Ten-day-old seedlings of 'Pearson A-1' tomato or 'US 75' sugarbeet were planted in holes in soil immediately after the nematodes were added. The inoculated plants were grown in 20-cm diameter clay pots in a greenhouse or in 1.9-liter waxed milk cartons (tomato) or 950-ml styrofoam cups (sugarbeet) in a growth chamber. All plants of a given test were grown in a greenhouse or a growth chamber. Ambient temperatures in the growth chamber were regulated to maintain soil temperatures at 24 C $\pm$ 0.5 during both 16 h of high-intensity illumination (about 55,974 lux.), and 8 h of darkness.

Adult males were counted from one of two paired plants and females from the other pair member. Plant pairs were replicated as indicated for the individual tests. Thirty days after inoculation, selected plants were removed from their containers and roots and soil washed and screened to recover adult females. Randomly selected plants used to recover adult males were removed from their containers 16 or 18 days after inoculation. Roots were carefully washed free of soil and placed in funnels on a laboratory table. The root systems were supported by wire screens below the tap-water surface for periods extending from the 16th to the 40th days after inoculation. Data on nematode counts for each test were analyzed for statistical significance by the t-test for paired characteristics or by the multiple-factor analysis of variance method.

RESULTS

Adult males of *H. schachtii* emerged from roots of sugarbeet at a relatively constant rate from 21-43 days after plants were inoculated with five cysts/plant. The total number of males emerging daily from roots of 15 plants varied from 42 to 102. In contrast, the mean daily rate of emergence of males from 15 plants inoculated with 20 cysts/plant varied from 40-550 (Fig. 1). The mean numbers of adult males emerging from 15 plants inoculated with 5 or 20 cysts/plant amounted to 46 and 250, respectively.

Increasing the inoculum level on sugarbeet produced dissimilar results in three tests. In one test (Table 1), the number of adult males recovered was proportional to the inoculum level. Although increasing the inoculum level from five to 20 cysts/plant did not increase the numbers of adult females, raising the inoculum from 20 to 40 cysts/plant caused a

5.9-fold increase in females.

In a second test (Table 2), increasing the inoculum level from 20 to 60 cysts/plant significantly increased males, but not females, on roots of sugarbeet. In a third test (Table 2), the higher inoculum level resulted in development of fewer males and females. However, there was a great disparity in plant weights of the second and third tests. Comparisons of tests 4, 5, and 6 (Tables 2 and 3) revealed that increasing the inoculum levels of the tomato race of *H. schachtii* on tomato and sugarbeet did not effect similar changes in the adult male populations. Increasing the inoculum level of the tomato-selected biotype did not significantly increase the numbers of adult males or females on sugarbeet. However, increasing the inoculum level from 20 to 60 cysts/plant on tomato significantly increased males (slightly less than threefold).

Development of females on tomato were not the same for two host-selected races. The

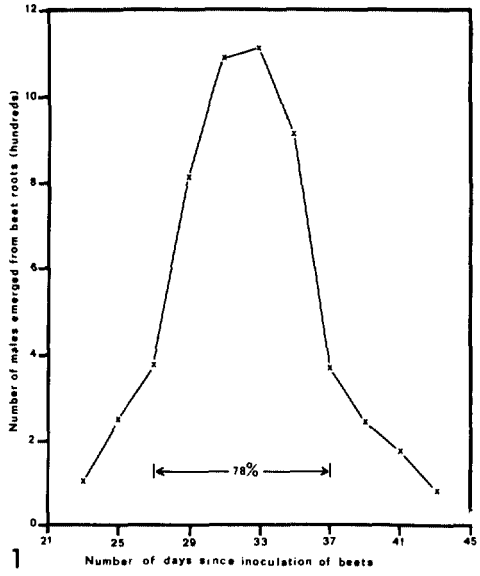

FIG. 1. Total numbers of adult male *Heterodera schachtii* emerged from roots of 15 infected sugarbeet plants 21-43 days after inoculation with 20 cysts.

TABLE 1. Influence of cyst inoculum level on numbers of adult *Heterodera schachtii* on sugarbeet.

Cyst inoculum level[a]	Mean plant weight (g)[b]	Mature males per plant	Mean plant weight (g)[c]	Mature females per plant[c]
5	9.9[d]	45.6	47.1	77.0
20	7.6	249.2	31.8	51.2
40	5.1	627.4	35.2	303.6
LSD ($P = 0.05$)	N.S.	297.6	N.S.	240.8

[a]Numbers of cysts inoculated on each of 15 sugarbeet seedlings transplanted to clay pots 10 days after germination.
[b]Plants harvested 21 days after inoculation and incubated 30 days in water to collect males.
[c]Plants harvested 35 days after inoculation.
[d]Mean of five plants.
[e]N.S. = differences not significant.

TABLE 2. Influence of cyst inoculum level on numbers of adult *Heterodera schachtii* on sugarbeet.

Test no. and cyst inoculum level[a]	Mean plant weight[b] (g)	Mature males per plant[b]	Mean plant weight[c] (g)	Mature females per plant[c]
Test No. 2				
20[e]	8.4[d]	168	24.0	315
60[e]	6.6	434	21.3	531
Significance	N.S.	*	N.S.	N.S.
Test No. 3				
20[e]	1.5	138	6.6	141
60[e]	0.7	98	4.8	84
Significance	N.S.	**	N.S.	N.S.
Test No. 4				
20[f]	6.6[d]	188	17.5	362
60[f]	2.8[d]	179	16.3	514
Significance	N.S.	N.S.	N.S.	N.S.

[a]Numbers of cysts inoculated on each of 15 transplanted sugarbeet seedlings 10 days after germination.
[b]Plants harvested 18 days after inoculation and incubated in water to collect males.
[c]Plants harvested 30 days after inoculation.
[d]Mean of 10 plants.
[e]Populations from sugarbeet.
[f]Tomato-selected biotype.

TABLE 3. Influence of cyst inoculum level and biotype on numbers of adult *Heterodera schachtii* on tomato.

Test no. and cyst inoculum level[a]	Mean plant weight[b] (g)	Mature males per plant[b]	Mean plant weight[c] (g)	Mature females per plant[c]
Test No. 5				
20[d]	4.8[e]	629	6.3	457
60[d]	3.1[e]	1,613	3.1	445
Significance	N.S.	**	N.S.	N.S.
Test No. 6				
20[d]	17.0[f]	210	44.1	340
60[d]	11.2[f]	612	37.1	364
Significance	**	**	N.S.	N.S.
Test No. 7				
Beet[f]	12.5	234	17.1	26
Tomato[d]	10.9	877	18.8	1,167
Significance	N.S.	**	N.S.	**

[a]Numbers of cysts inoculated on transplanted seedlings 10 days after germination.
[b]Plants harvested 18 days after inoculation and incubated in water to collect males.
[c]Plants harvested 30 days after inoculation.
[d]Tomato-selected biotype.
[e]Mean of 10 tomato plants. N.S. = difference not significant.
[f]Population from sugarbeet.

TABLE 4. Influences of plant age and sugarbeet cultivar on root weights and nematode counts.[a]

Sugarbeet cultivar (A)	Weights of plant roots (g)			Numbers of mature females		
	Age of plant at inoculation (B)		Mean by cultivar	Age of plant at inoculation (B)		Mean by cultivar
	10	40		10	40	
US H7A	0.2[b]	27.2	13.7	94.7	946.8	520.8
US H9A	0.2	31.8	16.0	32.8	378.2	205.5
Mean by plant age	0.2	29.5		63.8	662.5	
Significance[c]	**		N.S.	**		**

[a]Sugarbeets were inoculated with 50 cysts/plant at transplanting or 30 days after transplanting. All plants were examined 30 days after inoculation.
[b]Mean of six plants.
[c]Interaction (A × B) highly significant for nematodes, but not significant for root weights.

TABLE 5. Influences of age of tomato and inoculum level on development of *Heterodera schachtii*.[a]

Inoculum level (A)	Weights of plant roots (g)				Numbers of mature females			
	Age of plant at inoculation			Mean by inoculum level (B)	Age of plant at inoculation			Mean by inoculum level (B)
	10	20	30		10	20	30	
15	4.9[b]	13.6	19.7	12.7	11	99	201	104
30	4.1	11.9	16.7	10.9	13	275	537	275
Mean by plant age	4.5	12.7	18.2		12	187	369	
Significance		**		N.S.		**		**

[a]Tomato plants examined 30 days after inoculation.
[b]Mean of five replications.
[c]LSD ($P = 0.05$) for means by plant age was 22.9; by means of inoculum level, 2.7. There was a highly significant interaction (A × B) for nematodes, but not for root weights.

numbers of adult females recovered from tomato plants inoculated with the sugarbeet biotype amounted to only 2.2% of the adult females recoverable when similar plants were inoculated with the tomato race.

Ten times as many adult females of *H. schachtii* were recovered from sugarbeet cultivars inoculated 40 days after germination as from plants inoculated 10 days after germination (Table 4). Significantly fewer females developed on 'US H9A' than on 'US H7A.' Statistical analysis also revealed that the combined effects of plant age at inoculation and cultivar influenced development of adult females significantly more than the effects of either factor alone.

Increasing the inoculum level from 15 to 30 cysts/plant significantly increased the numbers of adult females developing on tomato inoculated 20 or 30 days after germination (Table 5). However, there was a disproportionate increase in females developing on plants inoculated with 30 cysts. For each inoculum level, the numbers of adult females on tomato roots 30 days after inoculation was proportional to plant weights at harvest (Fig. 2) and time lapsed between germination and inoculation. The data also revealed an interaction between inoculum level and plant age at inoculation, which significantly affected development of *H. schachtii* females. Mean plant weights and root weights were proportional to plant age, indicating that in this test, addition of 15 or 30 cysts per plant did not measurably affect plant growth.

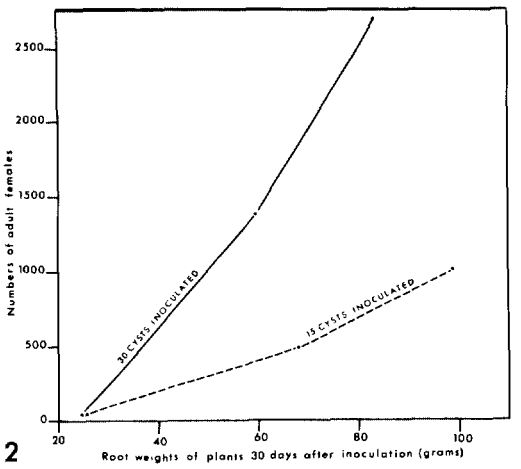

FIG. 2. Influences of inoculum levels of 15 and 30 cysts on root weights of tomato and numbers of adult females 30 days after inoculation.

DISCUSSION

Data illustrated in Fig. 1 suggest that, with inoculum levels of 20 cysts/plant, invasion of larvae is not constant during the 21-day period after inoculation (assuming rates of emergence of adults closely reflect rates of invasion of larvae). The initial increasing rate of invasion suggested by the emergence curve (Fig. 1) is probably in response to availability of new feeding sites in roots of rapidly growing seedlings. Increasing rates of diffusion of hatch-promoting substances from the newly formed roots would also contribute to increased larval invasion. The emergence curve also illustrates that, although the maximum rate of emergence occurred from about 30 to 33 days (the period when adult females break through the roots), males emerged during a period of about 20 days. This prolonged period of emergence precludes the collection of both males and females from the same plant without introducing factors which may affect the parameters of either or both males and females. Therefore, collection of data from paired plants seemed to offer the best opportunity for comparisons of the effects of various treatments on the dynamics of male and female development.

Data of these studies suggest that when only slight overcrowding of *H. schachtii* occurs in sugarbeet and tomato, males and females do not have an equal opportunity for development and proportionately fewer females develop. Minimal environmental changes that are sufficient to alter either plant root growth or numbers of invading larvae, will affect the density of larvae (numbers/unit of root). These changes in parasite density may result in large shifts in populations of adult females and smaller changes in the development of males. However, conditions of severe overcrowding in parasitized roots may equally affect development of males and females. In the present studies, severe overcrowding apparently restricted development of both males and females, effecting similar downward shifts of both sexes. Although there is a great decrease in the total population, the sex ratio remains relatively unchanged, as demonstrated by test No. 3. Chitwood and Feldmesser (1) reported that severe overcrowding by *H. rostochiensis*, may cause death and pruning of invaded roots, with resultant death of nematodes in the pruned tissue.

Results of test No. 7 suggest that differential development of male and female *H. schachtii* may be greatly influenced by the selective effects of the preceding host. In this study, females appear to be affected by host-selection to a greater degree than males. Root diffusates of tomato do not stimulate hatching of *H. schachtii* (6). Consequently, population differences observed in this study cannot be attributed to dissimilar hatch stimulation.

Triantaphyllou (17) noted that most of the recent work suggests that unbalanced sex ratios in the genus *Heterodera* are due to failures of female larvae to reach maturity under adverse conditions. Steele (15) found that *H. schachtii* males can develop on tomato or sugarbeet with only their head region embedded in the roots, whereas females developed only when they penetrated to the vascular parenchyma of roots. He suggested that factors that restrict deep penetration of female larvae to the central stele may tend to favor higher male to female ratios. In these contexts, initial parasitization of tomato by *H. schachtii* may have resulted in the selection of a race in which female larvae are more able to successfully invade and find their way to the vascular region of tomato roots. Similar tests with other host-species may clarify the nature of variations in host specificity between races of *H. schachtii*.

A great degree of variability in host-preference may occur within and between populations of *H. schachtii*. This variability is suggested by the wide host-range (13), the occasional development of this nematode on highly resistant plant species (14), and overlapping of host ranges of species within the genus *Heterodera*. The extended dormancy of eggs within cysts in soil may also contribute to heterogenous populations with regard to host preference. It is at least theoretically possible for first-generation larvae to exist simultaneously with larvae of subsequent generations up to and exceeding the 50th generation.

The development of females may be influenced by genotypic variation within a host-plant species, as demonstrated by the responses of 'US H7A' and 'US H9A' (Table 4). However, the plant age (which directly affects both linear root size and root diameters, and, hence, population density) influenced development of females to a greater extent than did varietal differences.

Plant age (size of root system) also influenced development of females to a greater extent than did inoculum level, although it is the interaction of these two factors that determines final densities of adult populations. Fedorko (4, 5) found, however, that the degree of parasitism of *H. schachtii* on rape decreased as the age of the plants at inoculation increased.

Lack of agreement on the occurrence of resistance in commercial cultivars of sugarbeet (2, 3, 10) is probably caused in part by plant-to-plant variability in early root growth. This affects development of females and precludes evaluation of resistance by visual inspection. The use of small vials for growing plants to be evaluated for resistance may be undesirable, because increasing the inoculum level on plants with small root systems will not measurably increase the production of females. Chitwood and Feldmesser (1) indicated that increasing the inoculum level on plants with restricted root growth may actually decrease production of females. Inoculation of plants only after they have grown adequate root systems may allow more efficient use of inocula and better control of density-dependent variables.

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