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Relationship Between *Heterodera schachtii*, *Meloidogyne hapla*, and *Nacobbus aberrans* on Sugarbeet[1]

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Abstract: Heterodera schachtii, Meloidogyne hapla, and *Nacobbus aberrans* either alone, or in various combinations with each other, can, when inoculated at a concentration of 12 second-stage juveniles/cm³ of soil, cause a significant ($P = 0.01$) suppression of growth of sugarbeet (cv. Tasco AH14) seedlings. *M. hapla* and *H. schachtii* decreased growth of sugarbeet more than *N. aberrans* over a 60-day period. The adverse effect of *N. aberrans* on the final population/initial population (Pf/Pi) ratio for either *M. hapla* or *H. schachtii* was dependent on time, and was more accentuated on that of *M. hapla* than on that of *H. schachtii.* Neither *M. hapla* nor *H. schachtii* had an adverse effect on the Pf/Pi ratio of *N. aberrans. N. aberrans* is considered to be less aggressive on sugarbeet than either *H. schachtii* or *M. hapla.* Sections of sugarbeet roots infected simultaneously with *H. schachtii* and *N. aberrans* showed scattered vascular elements between the *N. aberrans* syncytium located in the central part of the root and that of *H. schachtii* in the peripheral position.

Key words: Beta vulgaris, false root-knot nematode, histopathology, northern root-knot nematode, Pf/Pi, sugarbeet cyst nematode, yield suppression.

Anatomical changes caused by *Nacobbus aberrans* Thorne and Allen on tomato (*Lycopersicon esculentum* Mill.) and sugarbeet (*Beta vulgaris* L.) roots are well documented (3,6,9,10). There is no information, however, on host yield suppression by this nematode (12). In the western United States *Heterodera schachtii* Schmidt and *Meloidogyne hapla* Chitwood concomitantly attack sugarbeet (4); in Nebraska *H. schachtii* occurs in association with *N. aberrans* in sugarbeet fields, however, it is not known to what extent these nematode species compete. This study compares competitiveness, pathogenicity, and population increase of *H. schachtii, M. hapla,* and *N. aberrans* on sugarbeet.

MATERIALS AND METHODS

Nacobbus aberrans was obtained from *Kochia scoparia* (L.) Schrad. collected in Nebraska, *H. schachtii* was obtained from sugarbeet collected in Utah, both nematodes were maintained on sugarbeet; *Meloidogyne hapla,* obtained from lettuce (*Lactuca sativa* L.) collected in Utah, was maintained on tomato. Inoculum for experiments was collected by incubating egg masses at 25 C on 75-μm microsieves and enclosed in petri dishes partially filled with distilled water.

Pre-germinated sugarbeet seed cv. Tasco AH14 were planted in individual 6-cm-d plastic pots containing 400 cm³ of methyl bromide treated sandy loam (72% sand, 18% silt, 10% clay). Three days later the soil was infested with *H. schachtii* or *M. hapla,* alone and in combination with *N. aberrans* or with only *N. aberrans.* The initial inoculum density was 5,000 second-stage juveniles (J2) of each nematode per pot (about 12 J2/cm³ of soil). The nematodes were introduced into the soil by pouring an aqueous suspension of inoculum into 10 holes, each 5 cm deep, around the base of the seedling. Treatments, including non-inoculated plants, were randomized in 14 replicates on a greenhouse bench. Greenhouse temperature was maintained at 25 ± 3 C with 19 hours light supplemented with high-output fluorescent lamps. Seven replications of each treatment were harvested 30 and 60 days after inoculation. At each harvest, total fresh weight, fresh root weight, and dry top weights were recorded. Dry tap root weights were taken only after 60 days when the seedlings had developed a storage root. Dry weights of total root system at 30 days or lateral roots at

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TABLE 1. The effect of *Heterodera schachtii, Meloidogyne hapla,* and *Nacobbus aberrans* alone or combined on the growth of sugarbeet seedlings cv. Tasco AH14, 30 and 60 days after inoculation.*

Nematode	Fresh plant weight (top & root) (g)		Fresh root weight (g)		Dry top weight (g)		Dry tap root weight (g)
	Days after inoculation						
	30	60	30	60	30	60	60
H. schachtii	0.28	5.80	0.046	0.92	0.017	0.50	0.06
M. hapla	0.21	2.00	0.056	0.50	0.016	0.10	0.02
N. aberrans	0.28	9.20	0.059	2.00	0.030	0.80	0.13
H. schachtii and *N. aberrans*	0.09	4.60	0.029	0.8	0.013	0.30	0.05
M. hapla and *N. aberrans*	0.19	4.20	0.070	0.94	0.013	0.30	0.03
Noninoculated	3.61	12.60	0.27	2.70	0.25	1.20	0.60
LSD ($P = 0.01$)	0.30	2.81	0.055	0.72	0.023	0.24	0.14

* Values are mean of seven replicates.

60 days were not measured because the roots were processed for nematode extraction.

To determine the reproductive rate of *H. schachtii, M. hapla,* and *N. aberrans,* under conditions of single nematode inoculations, eggs were recovered from roots by using the NaOCl method (2), and J2 in the soil were extracted by the Cobb sieve and decant method (1). Numbers of eggs from root, and J2 from soil were added together. White females and cysts of *H. schachtii* were removed from the roots soon after egg extraction and counted. The roots were then stained in hot lactoglycerol (1:1:1 lactic acid : glycerol : distilled water) and acid fuchsin and the remaining nematode stages in the roots were counted. Cysts in the soil were extracted with an elutriator and separated from the debris by an alcohol flotation method (11). Cysts were then crushed in water in a glass tube and the eggs counted. Eggs from the cysts were added to those obtained from the gelatinous matrix of the white females.

Root systems of the seedlings inoculated with the combination of *H. schachtii* or *M. hapla* plus *N. aberrans* were examined with a stereomicroscope, and the *N. aberrans* egg masses were removed from the characteristic large galls. Egg masses were crushed in water in a glass tube, and eggs counted. After the *N. aberrans* egg removal, the roots infected with *M. hapla* or *H. schachtii* were processed as described for the single nematode inoculation. Developmental stages of two combined nematodes in soil and stained roots were counted separately to compare their numbers with those of single inoculations (no adult male counts were made). Data were analyzed by a split plot analysis of variance.

Histological observations were made on sugarbeet root galls harboring *H. schachtii* cysts and *N. aberrans* females. Galls were fixed in FAA (23% formalin, 3% acetic acid, 30% ethyl alcohol, 44% distilled water), dehydrated in tertiary butyl alcohol series, and embedded in paraffin. Sections 10–15 μm thick were stained with safranin and fast-green, mounted in Dammar xylene and observed under a compound microscope (5). Sugarbeet roots infected with *M. hapla* and *N. aberrans* were too deteriorated for histological study.

RESULTS AND DISCUSSION

Total fresh (top and root) and dry top weights of inoculated plants were less ($P = 0.01$) than noninoculated plants 30 and 60 days after inoculation (Table 1). At 30 days after inoculation, total fresh weights of noninoculated plants were more ($P = 0.01$) than 75% greater than plants inoculated with either nematode alone, or concomitantly inoculated. At 60 days after inoculation, plants inoculated with *H. schachtii,* or *N. aberrans* alone, or the combination of the two nematodes, weighed 54, 27, and 64% less than noninoculated controls; plants inoculated with only *M. hapla* weighed 84% less than noninoculated controls.

Dry top and fresh top plus root weights of plants inoculated with the individual nematodes or their combination did not

TABLE 2. Numbers and ratios between final (Pf) and initial (Pi) population densities (Pf/Pi) of *Heterodera schachtii, Meloidogyne hapla,* and *Nacobbus aberrans* alone or combined 30 and 60 days after inoculation.

Nematode	Mean no. of juveniles in roots/plant*		Mean no. of immature and mature ♀/plant†		Mean no. of eggs + J2 per plant		(Pf/Pi)	
	Days after inoculation							
	30	60	30	60	30	60	30	60
H. schachtii	18 a	491 a	41 a	612 (91) a	3,562 a	24,231 a	0.71 a	4.84 a
M. hapla	42 a	1,779 b	19 b	99 b	9 b	27,074 b	0.002 c	5.41 a
N. aberrans	296 b	378 a	9 (1) bc	133 (111) b	0 b	1,763 e	0 c	0.35 b
H. schachtii + *N. aberrans*	11 a	541 a	34 a	438 (50) a	487 b	30,341 c	0.10 b	6.06 a
N. aberrans + *H. schachtii*	63 a	153 a	0.5 c	50 (44) b	0 b	402 e	0 c	0.08 b
M. hapla + *N. aberrans*	39 a	292 a	13 bc	20 b	5 b	5,167 d	0.001 c	1.03 b
N. aberrans + *M. hapla*	59 a	437 a	2.8 c	40 (32) b	0 b	1,151 e	0 c	0.23 b

* Values are mean of seven replicates. Column means followed by the same letters are not statistically different according to split plot analysis of variance ($P = 0.01$).

† The values in parentheses indicate only the number of *H. schachtii* females which reached the cyst stage and of those of *N. aberrans* that became swollen.

differ 30 days after inoculation, but at 60 days after inoculation plants inoculated with only *N. aberrans* were heavier ($P = 0.01$) than plants inoculated with *H. schachtii* and *M. hapla* alone or the combination of nematodes (Table 1). No additional effect in growth suppression was observed when *N. aberrans* was added to *H. schachtii* or *M. hapla* compared to *H. schachtii* and *M. hapla* alone 30 and 60 days after inoculation (Table 1).

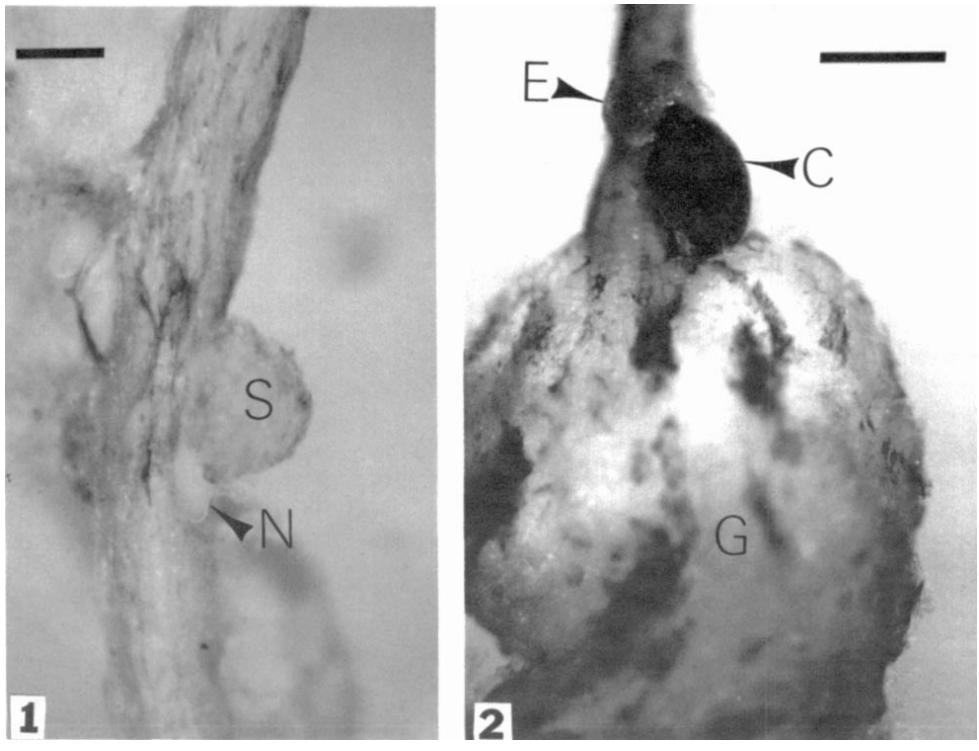
There were no differences in the dry weights of storage roots from plants inoculated with the individual nematodes or their combination. The fresh weight of lateral root from plants inoculated with *N. aberrans* alone was not different from that of the noninoculated controls and was higher ($P = 0.01$) than that of the plants infected with *H. schachtii* and *M. hapla* alone or in combination with *N. aberrans* 60 days after inoculation. The large galls on the *N. aberrans* infected roots may account for the increase in weight compared to plants infected with *H. schachtii* or *M. hapla.*

No seedling mortality was observed in plants inoculated with *N. aberrans* alone. However, 29% of the seedlings inoculated with either *H. schachtii* or *M. hapla* alone, or the *M. hapla* plus *N. aberrans* combination, and 43% of the plants inoculated with *H. schachtii* plus *N. aberrans* died 30 days after the inoculation. No additional seedling mortality occurred by 60 days after inoculation.

The numbers of parasitic juveniles recovered after 30 days from plants singly inoculated with *N. aberrans* were greater ($P = 0.01$) than those of *H. schachtii* or *M. hapla* in single or combined inoculations (Table 2). The greater numbers of *N. aberrans* juveniles were due to the longer period required by these juveniles to reach sexual maturity.

More ($P = 0.01$) juveniles were recovered from plants singly inoculated with *M. hapla* than from plants inoculated with *H. schachtii* and *N. aberrans* alone or in combination, after 60 days (Table 2). The smaller number of *M. hapla* juveniles recovered from plants concomitantly inoculated with *N. aberrans* and *M. hapla,* compared to the number from plants inoculated with only *M. hapla,* indicates an adverse effect of *N. aberrans* on *M. hapla* development. At 60 days after inoculation the number of juveniles obtained from plants inoculated with only *H. schachtii* or *N. aberrans* did not differ from that obtained from plants concomitantly inoculated with both nematodes (Table 2).

The numbers of *H. schachtii* adult females were greater ($P = 0.01$) than *M. hapla* or *N. aberrans* with either single or combined inoculations at 30 and 60 days after inoculation (Table 2). The numbers of *M.*



FIGS. 1, 2. Sugarbeet cv. Tasco AH14 roots infected simultaneously with *Heterodera schachtii* and *Nacobbus aberrans*. 1) *H. schachtii* white female (N) inserted at the base of a lateral root tip swelling (S) induced by *N. aberrans* juveniles. 2) *H. schachtii* cyst (C) with egg mass (E) in a root gall (G) induced by *N. aberrans* female. Scale bars = 586 μm.

hapla and *N. aberrans* adult females in either single or combined inoculations did not differ from each other after 30 and 60 days.

No *N. aberrans* eggs were found 30 days after inoculation. The greatest number of eggs and J2 were obtained from plants 30 days after inoculation with *H. schachtii* alone (Table 2); the number was greater (P = 0.01) than that from plants concomitantly inoculated with *H. schachtii* and *N. aberrans* (Table 2). This indicates that by 30 days after inoculation the *N. aberrans* root invasion and development had an adverse effect on *H. schachtii* reproduction. However, by 60 days after inoculation, numbers of *H. schachtii* eggs from the combined inoculation were the greatest and the adverse effects did not persist (Table 2). At 30 days after inoculation *H. schachtii* cysts had not yet developed and all the eggs were obtained from the egg masses deposited by white females. Fewer (P = 0.01) eggs and J2 were obtained from *M. hapla* than from *H. schachtii* indicating that *M. hapla* oviposition was initiated about 30 days after inoculation (Table 2).

The ratio between final and initial nematode population densities (Pf/Pi) at 30 days after inoculation was higher (P = 0.01) for *H. schachtii* than for *M. hapla* and *N. aberrans*. However the Pf/Pi of *H. schachtii* and *M. hapla* did not differ at 60 days after inoculation and were higher than that of *N. aberrans* (Table 2). At 60 days after inoculation the Pf/Pi ratio from plants inoculated with *M. hapla* alone was higher (P = 0.01) than that from plants inoculated with *N. aberrans* and *M. hapla* together, suggesting an adverse effect of *N. aberrans* on *M. hapla* population increase (Table 2). The *N. aberrans* final population was lower than the initial population in single or combined inoculations 60 days after inoculation (Table 2). The lack of *N. aberrans* reproduction in all pots at 30 days after inoculation confirms the long life cycle (about 48 days) reported in the literature (3,7,8). Very few swollen females, from 0

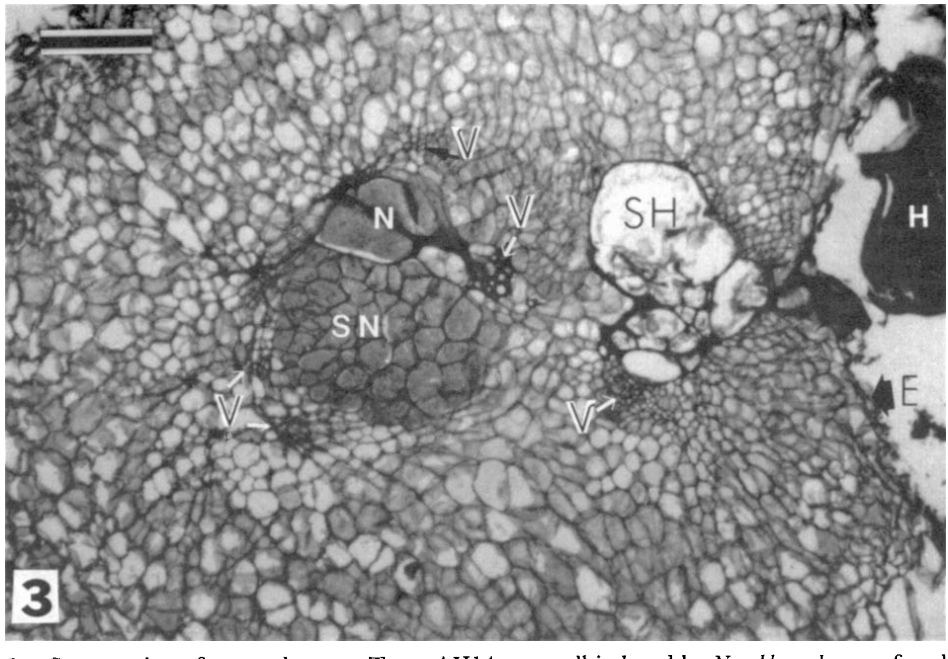


FIG. 3. Cross section of a sugarbeet cv. Tasco AH14 root gall induced by *Nacobbus aberrans* female (N) and infected simultaneously with *Heterodera schachtii* cyst (H). The *N. aberrans* syncytium (SN), occupying the center of the root stelar area and adjacent to the *N. aberrans* female body (N), had granular and dense cytoplasm. The syncytium of *H. schachtii* (SH), occupying the periphery of the root section, had empty cells indicating senescence. The vascular tissue (V) is fragmented and scattered between the two syncytia, E = epidermis. Scale bar = 140 μm.

to 7 per root system, were found in the galls 30 days after inoculation. The majority of the nematodes detected were immature female and juvenile stages in the slightly swollen roots or in the soil. By 30 days after inoculation *H. schachtii* and *M. hapla* developed to adult stages, but *N. aberrans* was still in an immature stage.

Because of its long life cycle and the limited fecundity of individual females, *N. aberrans* population increase was much slower than that of *H. schachtii* or *M. hapla*; by 60 days after inoculation *N. aberrans* Pf/Pi was 13 and 15 times less ($P = 0.01$) than that of *H. schachtii* and *M. hapla*, respectively. The slow development and low reproductive rate of *N. aberrans* enabled better sugarbeet seedling growth compared to seedlings infected by *H. schachtii* and *M. hapla*. Therefore, by 60 days after inoculation *N. aberrans* appeared to be less aggressive than *H. schachtii* and *M. hapla*. However there was no difference in the dry weight suppression of storage roots from plants inoculated with the three nematode species either alone or in combination.

Juveniles of *H. schachtii* and *M. hapla* were observed mixed with those of *N. aberrans* in the same root tip swelling. In some cases *M. hapla* females were detected in *N. aberrans* galls and were located in the external tissue layers of the galls. Females and cysts of *H. schachtii* were also observed at the base of *N. aberrans* induced swellings (Fig. 1) or galls (Fig. 2). Cross sections of galls with the two nematode species showed the *H. schachtii* induced syncytium at the periphery of the gall and the *N. aberrans* induced syncytium in the central part of the root and adjacent to *N. aberrans* body (Fig. 3). All the stelar area appeared obliterated and fragmented by the syncytium of *N. aberrans*, and the vascular elements were scattered between the *N. aberrans* and *H. schachtii* syncytial cells (Fig. 3). The *H. schachtii* syncytium extended from the subepidermal region towards the central portion of the root and in some cases was adjacent to the *N. aberrans* syncytium.

The results of these experiments indicate that at initial density of 12 J2/cm^3 of soil, *H. schachtii*, *M. hapla*, or *N. aberrans*

induced the same degree of damage to sugarbeet seedlings during the first 30 days after the inoculation. However by 60 days after inoculation *N. aberrans* damage was less pronounced than the damage caused by *H. schachtii* or *M. hapla.* Therefore, we consider *N. aberrans* to be less aggressive to sugarbeet than either *H. schachtii* or *M. hapla.* However, damage by *N. aberrans* was considered serious because the large galls induced by this nematode are capable of distorting and deforming the sugarbeet storage root.

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