Threshold Populations of *Heterodera cruciferae* and *H. schachtii* Causing Damage to Cabbage Seedlings

J. McCANN, National Vegetable Research Station, Wellesbourne, Warwick CV 35 9 EF, England

ABSTRACT

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Cabbage cv. Firmhead seedlings were grown in a glasshouse in soil infested with either the cabbage cyst nematode (*Heterodera cruciferae*) or beet cyst nematode (*H. schachtii*). Nematode infestation retarded the growth of the plants, and their root systems were less extensive than those of plants in uninfested soil, but no specific aerial symptoms of attack occurred. Damage caused by the two species varied according to the soil population level: *H. schachtii* caused more damage than *H. cruciferae* at 80 cysts per 100 g of soil.

Additional key words: *Brassica oleracea* var. *capitata*

Cabbage (*Brassica oleracea* var. *capitata* L.) is a host to the cabbage cyst nematode *Heterodera cruciferae* Franklin and the beet cyst nematode *H. schachtii* Schmidt (5), but there is uncertainty about the nematode population density required to cause significant damage and the symptoms of damage caused by the two species. *Brassica* seedlings infested with *H. cruciferae* produce extra lateral roots and stunted shoots with either reddened leaves (7) or interveinal chlorosis (3). *H. schachtii* causes extreme stunting and some chlorosis in the absence of excessive lateral root development in cabbage grown in pots (12) but causes discoloration and reduction in size of cabbage roots under field conditions (1).

Under field conditions, 25 or more *H. cruciferae* eggs per gram of soil measurably decrease the yield of winter cauliflower (*B. oleracea* var. *botrytis* L.) (14). Similarly, approximately 34 *H. schachtii* eggs and larvae per gram of soil reduce the yield of fieldgrown cabbage (6), but loss in cabbage yield has recently been attributed to six to nine viable *H. schachtii* eggs and larvae per gram of soil (1). When grown in pots containing about 33 *H. schachtii* eggs and larvae per gram of soil, infested cabbage seedlings die (9).

This paper assesses the effect of a range of *H. cruciferae* and *H. schachtii* population levels on cabbage seedlings grown for 36 days. The objective was to determine the threshold population density of nematodes required to cause significant damage and to describe the symptoms of damage caused by the two nematode species.

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MATERIALS AND METHODS

In preliminary experiments, inocula of more than 200 *H. cruciferae* and *H. schachtii* eggs and larvae per gram of soil were required to cause significant damage to cabbage seedlings. Cyst inoculum levels similar to those found in the field were therefore used in the study. Inoculum levels of 80, 40, 20, 10, and 5 cysts (approximately 150, 80, 40, 20, and 10 eggs and larvae) per 100 g of soil were obtained from sandy loam soils in which pure nematode populations were increased on *Brassica* in the glasshouse and diluted with soil sterilized with methyl bromide (2 ml/0.08 m^3). Cyst-free soil was used as an uninfested control.

Each volume of soil was well mixed, and one half was distributed into 30 pots (10 cm diameter). The other half was further diluted to produce the next lower population level. Before being mixed with infested soil, soil treated with methyl bromide was allowed to equilibrate for 14 days to eliminate possible toxic effects caused by the methyl bromide, as verified by the "cress-test" (11,2). Liquid nutrient (Fisons NPK) solution was added to all pots during a 7-day period so that differences in nutrient levels between infested and uninfested soils were not apparent.

Two or three seeds of cabbage cv. Firmhead F_1 hybrid were sown per pot and thinned to one per pot 5 days later. All pots were arranged randomly and maintained in the glasshouse at 19–21 C and 65–85% RH. The experiments for the two species were done at different times.

Ten plants from each nematode population level were sampled 12, 24, and 36 days after germination. The tops of the plants were weighed fresh and reweighed after drying at 100 C for 24 hr. The roots were washed with water and stored in 10% formaldehyde. The appearance of tops and roots of uninfested plants and plants grown in soil infested with either *H. cruciferae* or *H. schachtii* at 80 cysts per 100 g of soil was recorded at each sample date.

The preserved roots were stained in

Table 1. Top dry weights of cabbage seedlings infested with *Heterodera cruciferae* or *H. schachtii*

Days after germination	Nematode	Inoculum (cysts/100 g of soil) 0	5	10	20	40	80
12	*H. cruciferae*	0.032[a]	0.031	0.032	0.033	0.032	0.031
	H. schachtii	0.033	0.032	0.029	0.030	0.027*	0.016*
24	*H. cruciferae*	0.30	0.31	0.28	0.25*	0.19*	0.16*
	H. schachtii	0.29	0.31	0.31	0.19*	0.15*	0.06*
36	*H. cruciferae*	0.63	0.59	0.56	0.50*	0.22*	0.22*
	H. schachtii	0.84	0.82	0.87	0.60*	0.41*	0.15*

[a] Mean weight in grams; n = 10. * = Significant difference from uninfested control at $P \leq 0.05$; Student's *t* test.

Table 2. Growth rates of cabbage seedlings infested by *Heterodera cruciferae* and *H. schachtii*, determined from log top dry weight

Days after germination	Nematode	Inoculum (cysts/100 g of soil) 0	5	10	20	40	80
12–24	*H. cruciferae*	0.209[a]	0.205	0.214	0.191	0.171	0.159*
	H. schachtii	0.297	0.220	0.232	0.197	0.187	0.156*
25–36	*H. cruciferae*	0.056	0.049	0.046	0.052	0.009*	0.018*
	H. schachtii	0.079	0.075	0.072	0.069	0.066	0.043*

[a] Mean log dry weight as grams per day; n = 10. * = Significant difference from uninfested control at $P \leq 0.05$; Student's *t* test.

boiling lactophenol-cotton blue for 3 min (4). The nematodes infesting the roots of five plants per treatment were counted for each sample period. Roots of 12-day-old seedlings were examined directly in water, but those of 24- and 36-day-old plants were macerated with a top-drive blender for 5–10 sec., and one-tenth aliquots were examined.

RESULTS

H. cruciferae infestation showed no effect on plant growth 12 days after germination (Table 1), but plants growing in soil infested with 40 or more *H. schachtii* cysts per 100 g of soil weighed significantly less than their uninfested controls ($P \leq 0.05$). Moreover, the mean dry weight of plants grown in soil infested with 80 *H. schachtii* cysts per 100 g was less than that of *H. cruciferae*-infested plants, but the uninfested plants from the two experimental runs were the same.

At days 24 and 36, plant dry weights were significantly lower ($P \leq 0.05$) when the population level of the two nematode species was 20 or more cysts per 100 g of soil (Table 1). The average growth rates between days 12 and 24 of plants grown in soil infested with 20 or more cysts per 100 g of soil of either nematode species were lower than growth rates of uninfested controls (Table 2). Between days 24 and 36, *H. cruciferae* populations caused a reduced growth rate at or above 40 cysts per 100 g of soil, whereas *H. schachtii* altered the growth rate, although not as drastically, when the inoculum level exceeded 20 cysts per 100 g of soil. The population range of both nematode species, expressed as log cysts per 100 g of soil, exhibited an inverse relationship with the dry weight of tops at day 36 (Fig. 1).

No visible difference was found between *H. cruciferae*-infested and uninfested plants at day 12, but plants grown in soil infested with 80 *H. schachtii* cysts per 100 g appeared to be smaller. *H. cruciferae*- and *H. schachtii*-infested plants were smaller than their respective controls at day 24 and had less extensive root systems when grown in soil infested with 40 or more cysts per 100 g of soil. Plants grown in soil infested with 40 or more *H. cruciferae* or *H. schachtii* cysts per 100 g were smaller and had fewer leaves than uninfested plants 36 days after germination (Fig. 2).

Leaves of infested plants differed slightly in color and lacked definite symptoms. The maximum extension of roots on uninfested plants was about four times that of plants infested with 80 cysts per 100 g of soil (approximately 200 mm compared with 50 mm), but lateral root development was not excessive in response to infestation (Fig. 3).

At day 12, no larvae of *H. cruciferae* were found in stained roots, but second- and third-stage larvae of *H. schachtii* were present within roots of infested plants (Table 3). The roots of all plants in inoculated soil were about equally infested by larvae and adults of the two nematode species at days 24 and 36.

DISCUSSION

Both nematode species caused reduction in plant weight within 36 days but not to the same degree. At day 24 more damage was caused by *H. schachtii* than by *H. cruciferae* at an inoculum level of 20 or more cysts per 100 g of soil (Table 1). The growth rates at these population levels were about the same (Table 2). Plants were damaged more by *H. cruciferae* than by *H. schachtii* at the higher inoculum levels 36 days after inoculation, as indicated by lower growth rates (Table 2).

H. cruciferae appeared to reach a population threshold for maximum damage at 40 cysts per 100 g of soil (Fig. 1). Conversely, *H. schachtii* had not reached a population threshold at this level, because further damage was observed at 80 cysts per 100 g of soil. A plant growth period longer than 36 days and an increased inoculum level would be

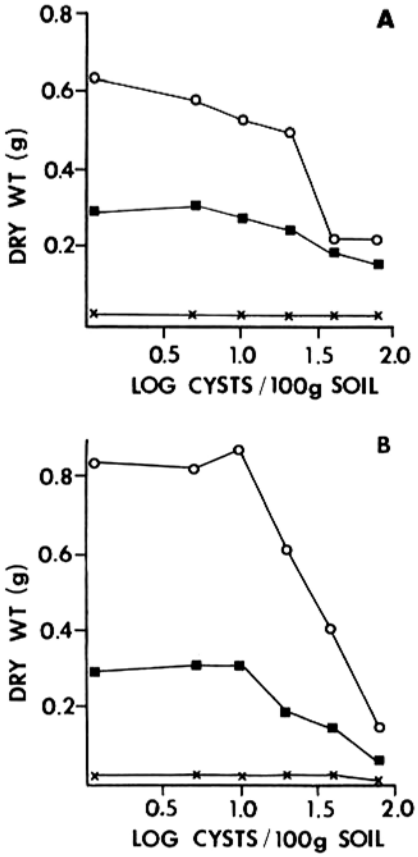

Fig. 1. Relationship of top dry weight of cabbage seedlings to different population levels of **(A)** *Heterodera cruciferae* and **(B)** *H. schachtii* sampled at 12 x———x, 24 ■———■, and 36 o———o days after germination.

Table 3. Number of nematodes per root system of cabbage seedlings.

Days after germination	Nematode	Inoculum (cysts/100 g of soil)					
		0	5	10	20	40	80
12	*H. cruciferae*	...	...	...	...	...	...
	H. schachtii	...	...	...	...	18[a]	8
24	*H. cruciferae*	...	6	10	18	30	26
	H. schachtii	...	4	12	20	52	38
36	*H. cruciferae*	...	6	20	40	48	42
	H. schachtii	...	16	22	36	44	58

[a] Mean of five plants.

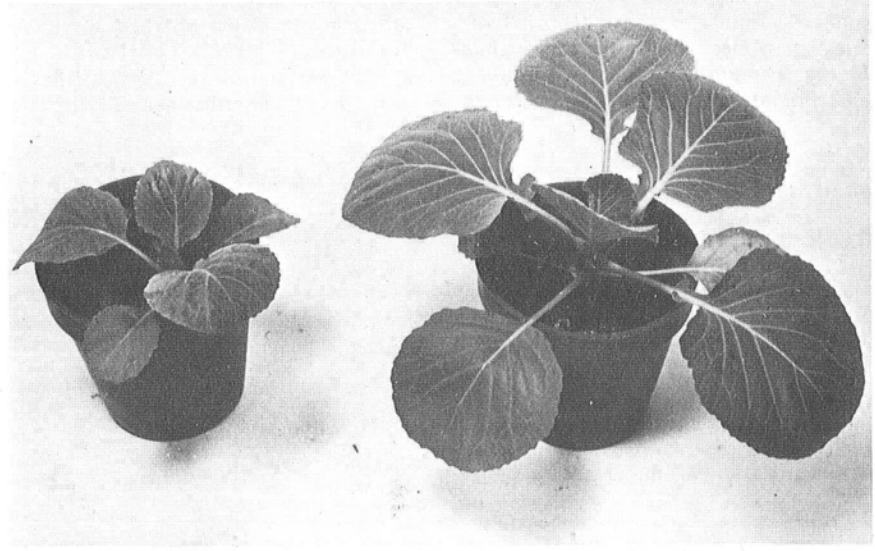

Fig. 2. Appearance of 36-day-old cabbage seedlings grown in soil infested with 80 *Heterodera cruciferae* cysts per 100 g (left) and in uninfested soil (right).

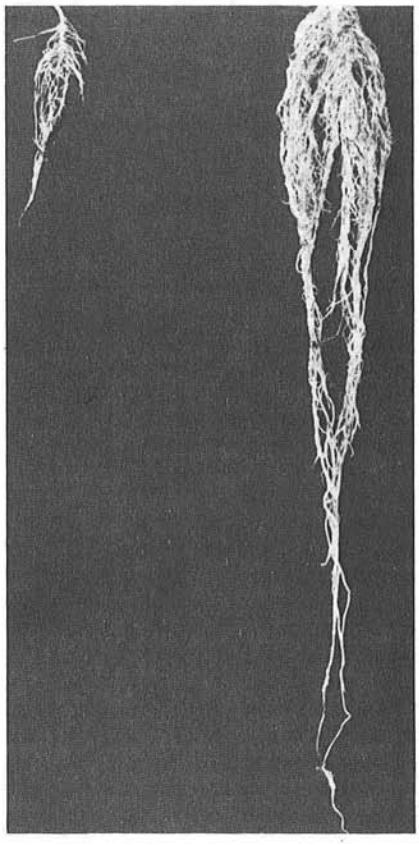

Fig. 3. Root systems of 36-day-old cabbage seedlings grown in soil infested with 80 *Heterodera cruciferae* cysts per 100 g (left) and in uninfested soil (right).

required to determine the population threshold of *H. schachtii*. However, assessed individually, a single *H. schachtii* caused more damage to a cabbage plant than did a single *H. cruciferae*.

The critical population density of 20 *H. cruciferae* and *H. schachtii* cysts per 100 g of soil (approximately 40 eggs and larvae per gram of soil) required to cause significant damage to cabbage in this study was similar to that of *H. cruciferae* infesting winter cauliflower (more than 20 eggs per gram of soil [14]) and *H. schachtii* infesting cabbage (more than 34 eggs and larvae per gram of soil [6]). The damage caused by *H. schachtii* did not, however, result in death of the cabbage seedlings as observed by others (9).

Plants grown in soil infested with 80 *H. cruciferae* and *H. schachtii* cysts per 100 g did not show extreme symptoms of nematode infestation 36 days after inoculation except that they grew slowly. The reduced growth rate of infested plants was manifested by fewer leaves, smaller plant size and less extensive root systems (Figs. 2 and 3). The results suggest that the less extensive root system of infested plants restricted water and nutrient uptake and thereby reduced the growth rate, even though the plants never became potbound and adequate nutrients and water were available. In contrast to earlier work (7,12), the specific symptoms of nematode attack, such as chlorosis or reddening of the leaves, were not observed. When such symptoms occur, they are probably due to nutrient deficiency in the soil that is exaggerated by the decreased root extension of infested plants. Differences in the results may also be due to different pathotypes or races of the two nematode species examined in other work.

The presence of second- and third-stage larvae of *H. schachtii* in roots 12 days after inoculation indicated that additional larval activity around the root could have been high enough to cause damage, resulting in reduced plant size. The increased activity of *H. schachtii* in the soil at this time may be due to water hatch (13), whereas hatch of *H. cruciferae* may depend more on the leaching of root exudates from the developing seedlings. As a result, *H. cruciferae* invaded later than *H. schachtii* but developed more quickly so that mature adults of both species were present 36 days after inoculation (Table 3).

Further experimentation under field conditions would be required to clarify the effect of *H. cruciferae*- and *H. schachtii*-infestation on the final yield of cabbage and other *Brassica* species as described by others (7,8,10,14). The present study, however, suggests that 20 or more cysts of either nematode species per 100 g of soil reduces the growth rate of cabbage seedlings. The production of *Brassica* transplants in seed beds containing such infested soil is therefore ill-advised.

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