A Comparison of the Hatching of Juveniles from Cysts of Heterodera schachtii and H. trifolii[1]

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Abstract: The effects of root diffusates of selected plants within the families Chenopodiaceae and Cruciferae and the hatching agent zinc chloride were tested for their effects on hatching and emergence of juveniles from cysts of *Heterodera schachtii* and a race of *H. trifolii* parasitic on Chenopodaceae and Cruciferae in The Netherlands. Although all diffusates strongly stimulated hatching of juveniles of *H. schachtii,* their effects on *H. trifolii* were less evident. *Key words:* sugarbeet diffusate, zinc chloride, *Beta* spp, Chenopodiaceae, Cruciferae, sugarbeet nematode, clover cyst nematode.
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In 1975 high populations of the clover cyst nematode, *Heterodera trifolii* Goffart, 1932, were discovered in sandy soils in the southeastern area of The Netherlands where sugarbeet had been grown (4). By 1977 the nematode was widespread in this area producing severe damage to sugarbeet. Subsequent investigations by Maas and Heijbroek (4) showed that this pathotype (host-race) of *H. trifolii* reproduced on, and was highly pathogenic to, sugarbeet and produced symptoms of injury similar to those produced by *H. schachtii* in sandy soils. They were able to distinguish this nematode from other pathotypes of *H. trifolii* by investigations of their host ranges. Although the females of this race have a distinctive yellow color immediately prior to formation of cysts and can be easily distinguished from *H. schachtii,* Maas et al. (3) could find no morphological basis to separate the "yellow beet cyst nematode" from other cyst-forming nematodes of the *H. trifolii* complex.

In comparing the hatching of *H. schachtii* and the pathotype of *H. trifolii* on sugarbeet, Maas and Heijbroek (4) found that at 15 C only a few *H. trifolii* juveniles hatched in 0.3 m*M* picric acid while about

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37% of *H. schachtii* juveniles hatched when treated similarly. Maximum hatches of *H. trifolii* and *H. schachtii* occurred at 25 C and 30 C, respectively.

Stimulation of hatching of second-stage infective juveniles by secretions of host-plant roots plays an important role in the host-parasite relationships and population dynamics of certain *Heterodera* spp. (9). This paper reports additional investigations to compare and to contrast the hatch of *H. schachtii* and the aforementioned race of *H. trifolii*. Root secretions leached from soil (root diffusates) of selected resistant and susceptible plants within the families Cruciferae and Chenopodiaceae were used.

MATERIALS AND METHODS

Two tests were designed to compare the effects of sugarbeet root diffusate and zinc chloride on hatching and emergence of juveniles from cysts of *Heterodera schachtii* Schmidt, 1871 from the Salinas Valley of California and a population of *H. trifolii* Goffart, 1937 from a field of infected sugarbeet in the southeast area of The Netherlands. The populations were increased on sugarbeet growing in nematode infested soil for 90 days. Newly formed cysts together with plant root debris were isolated by washing and screening infested potting soil. Cysts which appeared full of eggs and juveniles were removed from plant root debris and stored at 8 C to delay hatching until the tests were initiated (about 1 week).

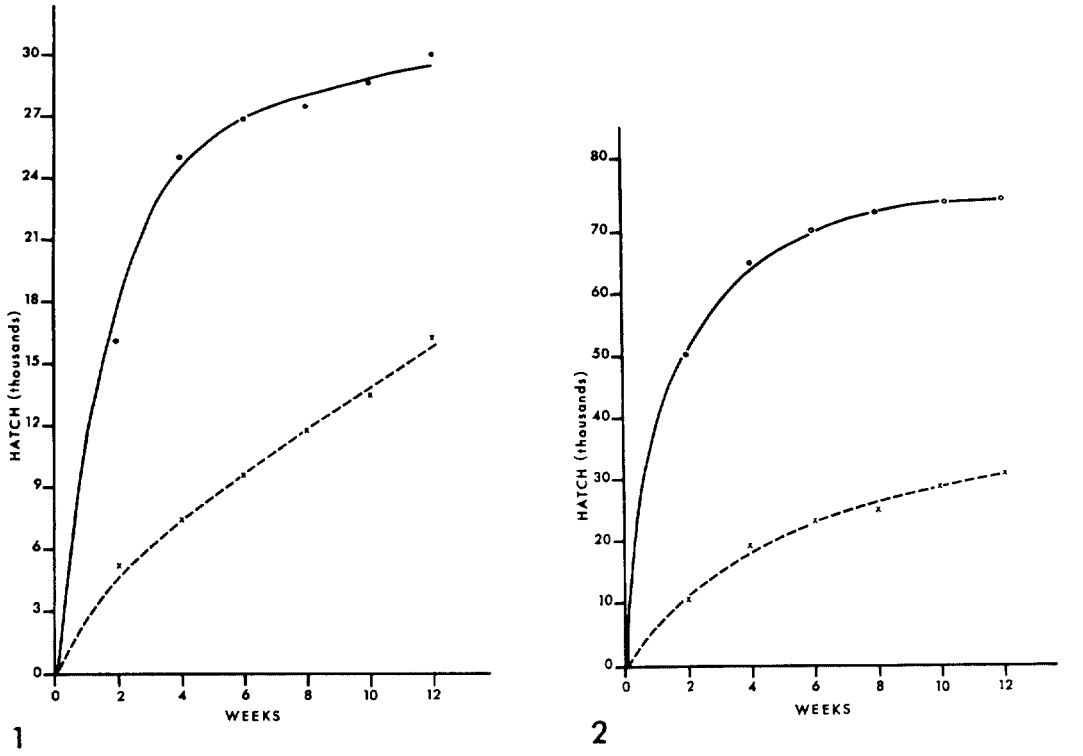
Three 10-day-old sugarbeet (*Beta vulgaris* L), cv. USH 11, were transplanted to nine 15-cm clay pots containing steam sterilized sand-clay soil (1:3) mixture and placed in a greenhouse. After 6 wk, 200 ml of sugarbeet root diffusate was leached from each pot by adding tap water to the soil at the base of the plants during a 24-h period. Aqueous solutions of 3 m*M* zinc chloride were prepared, and all solutions were stored at 8 C until needed.

Seven replicate groups of 40 randomly selected cysts of each *Heterodera* species were placed in individual dishes containing 5 ml of diffusate, zinc chloride, or tap water (8). The dishes were stored in an environmental chamber at 24 C for 12 wk. Cysts were changed to fresh solutions weekly. Juveniles emerging during consecutive 2-wk intervals were recorded as cumulative hatches. Treatments were replicated seven times.

The two additional tests evaluated root diffusates of various plant species resistant or susceptible to *H. schachtii* within the families Cruciferae and Chenopodiaceae on *H. schachtii* and *H. trifolii* parasites of sugarbeet in The Netherlands. In one experiment, root diffusates tested were *Brassica napus* L. (winter type oil seed rape, cv. Yet Neuf), *Sinapsis alba* L. (white mustard), susceptible cv. Hohenheimer and an unnamed resistant selection, *Raphanus sativa* L. (oil radish, unnamed susceptible and resistant populations), and *Beta vulgaris* L. (cv. Monohil). In a second experiment, root diffusates from *Beta vulgaris* L. (cv. USH 11), *B. vulgaris-B. procumbens* (resistant interspecific hybrids developed by Savitsky [6,7]), *Hesperis matronalis* L. (dames violet), *B. procumbens* Chrys. Sm., *B. patellaris* Moq., and *B. maritima* L. (breeding line 104 W) were tested. The latter three *Beta* species are wild beet, and all are resistant to *H. schachtii* as is *H. matronalis*. Treatments of 3 m*M* zinc chloride and tap water were also included. Each test consisted of four replicate groups of 25 cysts. The treatment solutions were changed weekly, and the study was terminated after 4 wk. Counts of emerged juveniles were analyzed for statistical significance among treatment means.

RESULTS AND CONCLUSIONS

Cumulative hatches of *H. schachtii* and *H. trifolii* are illustrated in Fig. 1 (sugarbeet root diffusate) and Fig. 2 (3 m*M* zinc chloride). Hatching of *H. schachtii* during the first 2 wk was significantly greater than for *H. trifolii* in both sugarbeet root diffusate and zinc chloride. Hatch of *H. schachtii* during the first 4 wk in diffusate and zinc chloride amounted to 83 and 87%, respectively, of the cumulative hatch for the entire 12-wk period. After 12 wk the cumulative hatch for *H. trifolii* in diffusate or zinc chloride was only 55 and 41%, respectively, of *H. schachtii*. From the 2nd through the 12th wk the rate of hatch of *H. trifolii* juveniles in diffusate or zinc



Figs. 1, 2. Influence of sugarbeet root diffusate (Fig. 1) and 3m*M* zinc chloride (Fig. 2) on cumulative hatch of *Heterodera schachtii* (solid line) and *H. trifolii* (broken line). Each point represents the total hatch from seven replicates of 40 cysts each.

chloride remained uniform; the cumulative hatch of *H. trifolii* in diffusate or zinc chloride was, respectively, 67 and 132 times greater than in water. Hatches of *H. schachtii* in diffusate or zinc chloride were, respectively, 19 and 78 times those in tap water.

Oostenbrink (5) demonstrated clear and distinct diapause from August to February in populations of *H. trifolii* from The Netherlands, whereas Winslow (11) reported a slight diapause from November through April for Canadian populations of this species. Maas and Heijbroek (4) reported that the seasonal effect of diapause in *H. trifolii* parasitic on sugarbeet is eliminated by the hatch stimulant picric acid. It is possible that cold (8 C) storage of cysts before initiation of this test induced dormancy (diapause) to a greater extent in *H. trifolii* than in *H. schachtii*. Although root diffusate of nonsusceptible mangold (*B. vulgaris* L.) does not stimulate hatching of a population of *H. trifolii* (2), the present study demonstrates that a race parasitic on sugarbeet is moderately stimulated by sugarbeet root diffusate or zinc chloride.

Cysts of *H. schachtii* employed in this experiment were heavily contaminated with fungi (probably *fusarium* sp.). This required repeated selection to obtain cysts with the least fungal contamination which resulted in selecting cysts with reduced numbers of viable eggs and juveniles. One or more species of fungi are nearly always associated with cysts of *H. schachtii* (10) and are invariably present as contaminants in hatching tests. The extent to which fungal contaminants differentially affected hatching of *H. schachtii* and *H. trifolii* populations in these experiments is not known. Therefore, valid comparisons of the total hatches of these nematodes cannot be made. However, since there were no significant interactions ($P = 0.05$) between diffusate treatments and nematode species in either of the two experiments, it is unlikely that differences in hatch potential

precluded the bioassay of diffusate hatching activities.

Root diffusates of all crucifers, Monohil sugarbeet, and zinc chloride significantly ($P = 0.05$) increased hatching of *H. schachtii* and *H. trifolii* compared with tap water (Table 1). Diffusate of oil seed rape appeared to be the most active for *H. schachtii* but not significantly greater than the susceptible population of oil radish. Hatch in diffusates of plants susceptible or resistant to *H. schachtii* were not significantly different indicating that the factor(s) affecting nematode hatch is not linked to plant resistance. Significantly greater numbers of juveniles emerged from cysts of *H. trifolii* than *H. schachtii* (Population mean, Table 1). When analyzed separately, none of the diffusates exhibited significant differences in activity for hatching of *H. trifolii*.

All diffusates except that of the interspecific hybrid (*B. vulgaris* L.-*B. procumbens*) stimulated hatching of *H. schachtii* while none showed activity for *H. trifolii* (Table 2). The cyst population of *H. trifolii* employed in the test was greatly infested with fungi (probably *Fusarium* spp.) and thus could account for the apparent lack of strong hatching responses. Although there was no interaction between the nematode populations and treatments in this

Table 2. Cumulative numbers of *Heterodera schachtii* or *H. trifolii* larvae emerging from cysts treated with plant-root diffusates, zinc chloride, or tap water over a 4-wk period.

Treatment	*Heterodera schachtii*	*Heterodera trifolii*
Beta vulgaris L.	6,192* cd†	4,126‡
B. vulgaris L.-*B. procumbens*	5,566 ab	4,305
B. procumbens	6,620 cd	4,217
B. patellaris	6,034 bc	4,095
B. maritima	6,081 c	4,627
Hesperis matronalis	7,425 d	4,524
Zinc chloride 3 m*M*	4,739 a	3,768
Tap water	4,777 ab	4,042
Population mean	5,929§	4,212§

*Mean number emerged per four replicates, each of 25 cysts.

†Values in a column not followed by the same letter differ significantly according to Duncan's multiple-range test ($P = 0.05$).

‡Means in this column not significantly different.

§Means significantly different at $P = .01$.

Table 1. Cumulative numbers of *Heterodera schachtii* and *H. Trifolii* emerging from cysts treated with various plant root diffusates, zinc chloride, or tap water over a 4-wk period.

Treatment plant	Host status*	*Heterodera schachtii*	*Heterodera trifolii*
Brassica napus L. cv. Yet Neuf	S	4,205† d‡	5,400† b‡
Sinapsis alba L.	R	3,177 bc	5,526 b
S. alba L. cv. Hohenheimer	S	3,096 bc	5,330 b
Raphanus sativa L.	R	3,245 c	4,781 b
R. sativa L.	S	3,647 cd	5,246 b
Beta vulgaris L. cv. Monohil	S	2,745 bc	5,505 b
Zinc chloride 3 m*M*	...	2,337 b	5,155 b
Tap water	...	1,117 a	3,426 a
Population mean		2,946§	5.046§

*R = resistant; S = susceptible to *H. schachtii*.

†Mean number emerged per four replicates, each of 25 cysts.

‡Values in a column not followed by the same letter differ significantly according to Duncan's multiple-range test ($P = 0.05$).

§Population means significantly different at $P = .01$.

test, within each test the diffusates showed a rather wide range of activities for *H. schachtii* but not for *H. trifolii*. The uniform response of *H. trifolii* may have resulted from triggering of diapause during storage at 8 C prior to initiation of these experiments. Other research (8) has shown that *H. trifolii* does not respond to diffusates of red clover (*Trifolium pratense*) which is a good host, but diffusates of pea (*Pisum sativum*), a non-host, stimulates hatching of this nematode.

In this test, root diffusates of *Hesperis matronalis, B. patellaris,* and *B. procumbens,* all highly resistant to *H. schachtii,* greatly increased hatching and emergence of juveniles from cysts of this nematode, but pronounced activity for *H. trifolii* was not evident. Golden (1) previously reported that root diffusates of *B. procumbens, B. patellaris,* and *B. webbiana* greatly increased hatching of *H. schachtii* populations from the Salinas Valley of California.

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