

HATCHING ACTIVITY, INVASION RATE AND REPRODUCTION OF *HETERODERA SCHACHTII* ON OILSEED RAPE CULTIVARS

S. Fatemy and E. Abootorabi

*Nematology Department, Plant Pests and Diseases
Research Institute, P.O.Box 1454-19395, Tehran, Iran*

Summary. Effects of root diffusates of oilseed rape cultivars, Cobra, Global, K16 and Tower on hatching activity, rate of invasion and reproduction of *Heterodera schachtii* were determined. Root leachates were obtained by drenching 50 day old plants with 500 ml distilled water, 5 ml of which was added to Petri dishes containing 50 cysts and kept at 20 °C for four weeks. Rate of penetration of *H. schachtii* was determined by inoculating 40 day old seedlings with 1200 juveniles and roots were examined at three days intervals. To measure the susceptibility of cultivars, 40 day old plants were inoculated with ca. 3400 juveniles of *H. schachtii* and harvested after two months in the glasshouse. Nematodes hatched in root leachates were 24.5, 17.4, 9 and 8% for Cobra, Global, K16 and Tower respectively. Beet cyst nematodes penetrated the roots of all rape cvs, invasion rate being 18% for K16, 15% for Tower, 13% for Global and 12% for Cobra. Adult females were produced on the roots of all cvs, with K16 being the most susceptible compared with the others.

Cultivation of oilseed rape in rotation with sugar beet in soil infested with *Heterodera schachtii* may create problems for both crops. The Beet cyst nematode is widely distributed in Iran and in some area causes heavy yield losses on sugar beet (Omidvar, 1968; Damadzadeh *et al*, 1995; Sharafeh, 1996; Parvizy *et al*, 1998).

In Iran Oilseed rape is often used in rotation with sugar beet. *H. schachtii* hatched in root diffusates of Regents, Rafael, Cerez and Blinda cultivars and reproduced on them (Fatemy, 2000) developing from juvenile to adult in about 18 days (Abootorabi *et al*, 2000).

This paper reports the results of glasshouse experiments on the effects of root leachates on the hatching of *H. schachtii* *in vitro*, nematode penetration into the roots and reproduction.

MATERIALS AND METHODS

Heterodera schachtii Schmidt was reared on sugar beet, *Beta vulgaris* L., cv. IC1 for nearly 7 years in microplots located out doors at the Plant Pests and Diseases Research Institute, Tehran. The cysts were obtained by wet sieving and stored in 0.1% salt water at 5 °C until required. Freshly hatched juveniles were recovered by placing cysts on 200 µm sieves in a dish containing 100 ml distilled water which was substituted with 4mM ZnCl₂ (Southey, 1970) after 24 h. Hatched juveniles were kept at +5 °C and used within a week.

The Hatching test was carried out in the laboratory. Five seeds of each cultivar of oilseed rape, *Brassica napus* L., cvs. Tower, Cobra, Global and K16 and the sugar beet cv. IC1 were planted in 12.5 cm pots containing steam sterilized soil, and maintained in a glasshouse for

50 days. Soil without seeds was also included as control. Pots were not watered during the 24 hours prior to collection of leachates. Then each pot was drenched with 300 ml distilled water and three days later with 200 ml. The leachate from three pots of each cultivar was collected in a large beaker positioned beneath each pot and combined, filtered through No.42 Whatman filter paper and stored at 5 °C. Cysts were surface sterilized with 1% sodium hypochlorite solution for 5 min and rinsed in sterile distilled water. Each experimental unit consisted of 50 full cysts in a 5 cm diam. Petri dish. Cysts were incubated in 5 ml sterile distilled water. Treatments were replicated three times and arranged in a completely randomized design in the dark at 20 °C.

After one week, suspension of water and hatched juveniles were replaced with root diffusate of an appropriate treatment and the experiment continued for four weeks. At the end of each week, the root diffusates were renewed and the number of hatched juveniles were counted. When the experiment was terminated, the cysts were crushed, total J2 and eggs counted and percentage hatch was calculated.

To assess the rate of invasion of *H. schachtii*, 40 days old seedlings of the oilseed rape cultivars were planted in 12.5 cm pots containing 1000 g steam sterilized soil and were inoculated with 10 ml suspension of 1200 newly hatched juveniles. Nematodes were injected in three holes around each seedling. The pots were distributed in a completely randomized design in the glasshouse at 25.5 ±3 °C. Three days after inoculation three plants of each cultivar were uprooted and the number of invaded juveniles was determined. This procedure was repeated twice each week up to 21 days. Nematodes were extracted from the roots by a maceration and centrifugal flotation technique (Coolen and

D'Herde, 1972). Rate of penetration was calculated as proportion of the number of juveniles in the roots at each harvest to initial population density $\times 100$.

Susceptibility and host status of the four cultivars of oilseed rape were determined in a glasshouse experiment. Pots of 12.5 cm diam. containing 1000 g steam sterilized soil were planted with a seed of each cultivar. After 40 days 5 ml suspension of ca. 3400 freshly hatched juveniles were injected into three holes around each seedling. Uninoculated plants served as control. There were three replicates for each treatment and pots were arranged at random on a glasshouse bench. At harvest, two months after inoculation, tops were cut off, roots were washed, blotted dry and fresh weight of plants were taken. A 250 g sub sample of wet soil from well mixed soil of each treatment was processed, to extract cysts and females and to assess final populations.

RESULTS AND DISCUSSION

Most of the hatch took place in the second week of the experiment for all treatments and afterwards declined. Significantly more juveniles hatched in the root diffusates of Cobra and Global (24.5 and 17.4% respectively) than other rape cultivars ($p < 0.01$) (Fig. 1). Leachates from the roots of Tower and K16 stimulated ca. 8 and 9% of juveniles to hatch. In comparison, 23% of the eggs hatched in leachate from sugar beet, the susceptible host of *H. schachtii*. Only 2% of the eggs hatched in leachate from unplanted soil during a four week period, and distilled water stimulated 2 to 4% of the juveniles to emerge in the first week of the experiment.

Eggs of *H. schachtii* hatch over a wide range of temperature with an optimum of 20-25 °C. (Wallace, 1955;

Cooke, 1985). The rate of juvenile emergence at 20 °C was lower than recorded for other rape cultivars (25 to 38%) that have been tested (Fatemy, 2000). Cultivar differences, leachate concentrate, plant age and sources of nematodes may have been contributed to this variation. Differences in hatch of cyst nematodes have been observed with different cultivars. Leachates from different cultivars of potato had different effects on the hatch of *Globodera rostochiensis* and *G. pallida* (Evans and Perry, 1976). Also varying root weight and length has induced differences in hatch of soybean and potato cyst nematodes (Teft and Bone, 1985; Rawsthorne and Brodie, 1986; Sikora and Noel, 1996). Root diffusates from 2-6 week old plants stimulated greater hatch of *H. goettingiana* eggs than did diffusate from older or younger plants (Perry *et al.*, 1980).

Furthermore, in this experiment, the population of *H. schachtii* that had been reared on sugar beet for nearly 7 years responded well to leachates of rape, whereas Decker and Dowe (1990) reported that the population from sugar beet monoculture virtually did not respond to the run off from rape roots.

Juveniles of *H. schachtii* penetrated the roots of all cultivars and the highest numbers recorded were on the 6th day after inoculation (Fig. 2). Roots of K16 and Tower were invaded by more juveniles (18 and 15%) than Cobra and Global (12 and 13%). The rate of invasion declined after the first week but was more or less uniform up to 15 days and on the 21st day there were hardly any nematodes inside the roots.

Nematodes reproduced on the roots of all four cultivars (Table I). Multiplication rates (final/initial nematode population) were greater than one in all cultivars and were less on Tower and Cobra than on Global and K16 although the differences were not significant. Also,

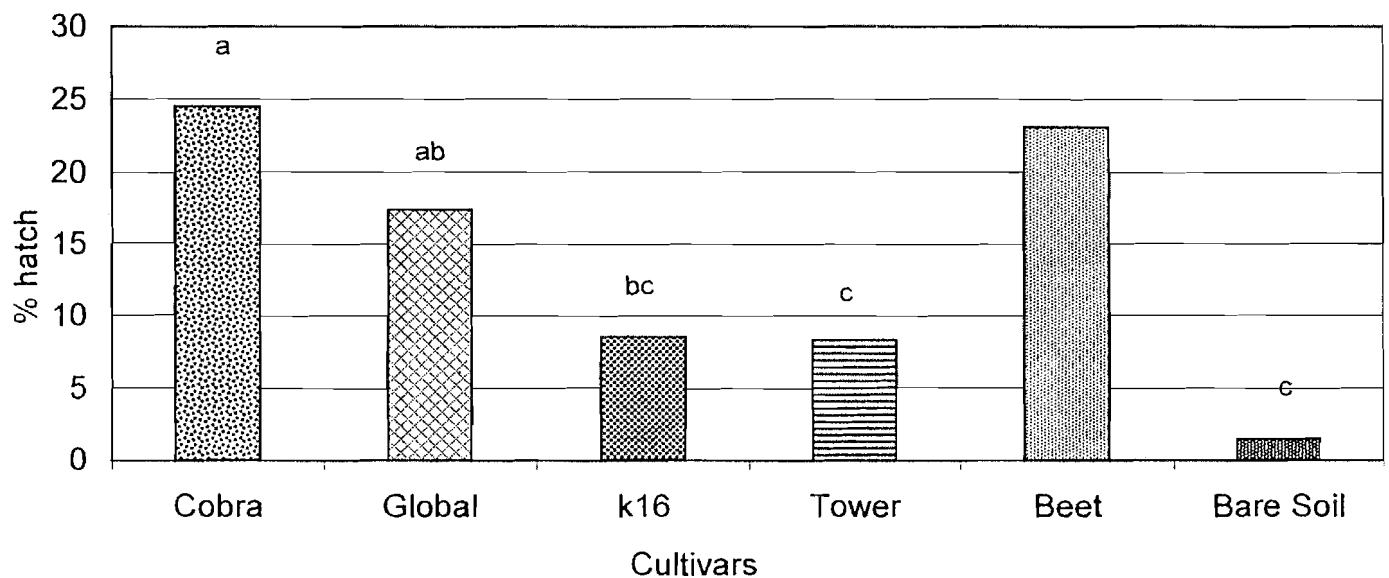


Fig. 1. Hatch of *Heterodera schachtii* in root leachates of four rape cultivars, beet and bare soil after four weeks at 20 °C. Columns with the same letter are not significantly different at 5% level.

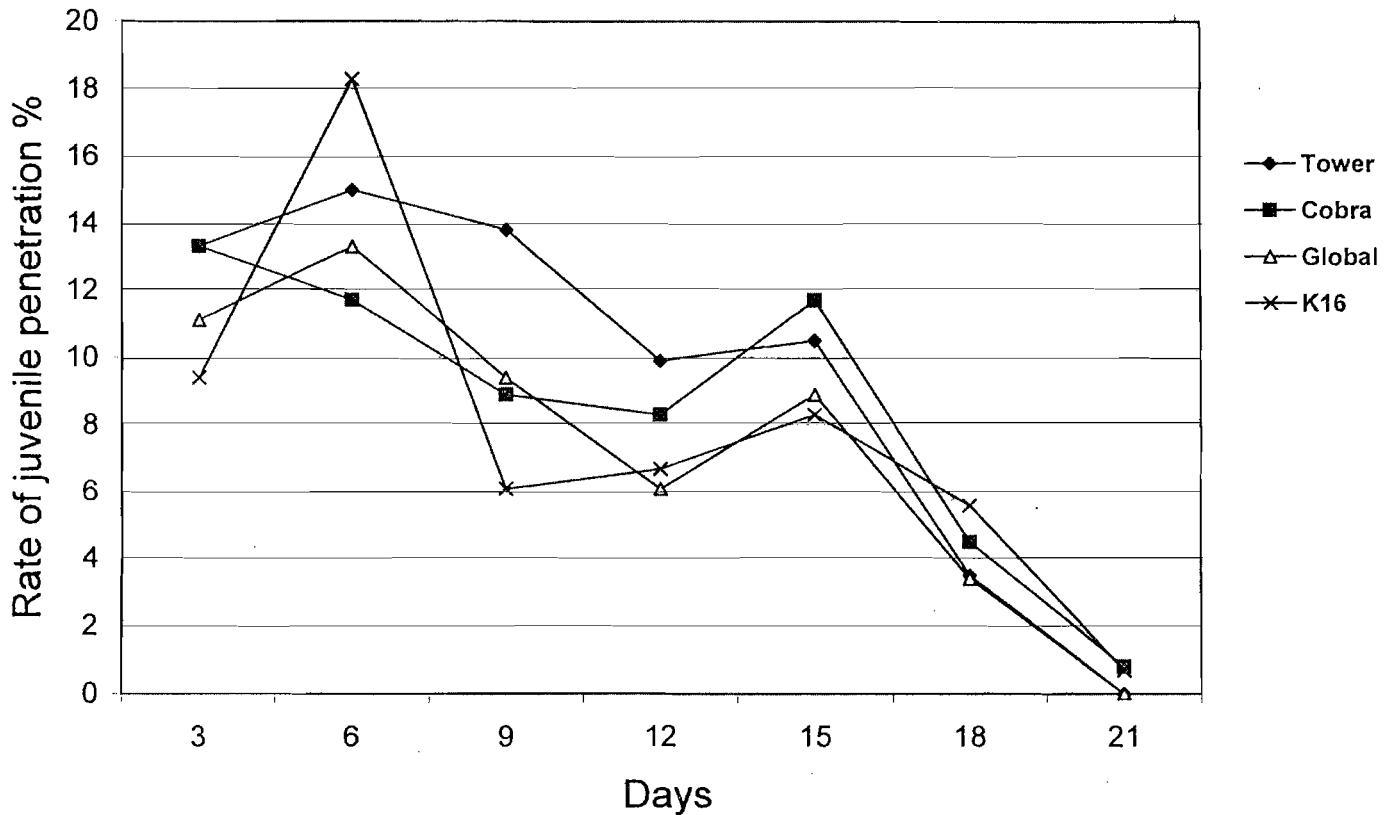


Fig. 2. Rate of penetration of *H. schachtii* in roots of four oilseed rape cultivars at three days interval.

nematodes were able to significantly produce more cysts ($P= 0.01$) on the roots of K16 and least on Tower and Cobra, Global being in between.

Weight of plants was affected differently by nematode attack; infested roots of cultivars were smaller than uninfested roots with the exception of Tower, whereas fresh weight of tops slightly increased when infested ($p < 0.01$). The root system of the autumn cultivar K16 was the largest of all when uninfested, but was reduced by almost 55% when infested and supported up to 213 females per gramme of root. On the other hand, the spring cultivar Tower, which had the smallest root system whether infested or not, was not affected and supported ca.125 cysts per gramme of root.

Cobra and Global, autumn and spring cultivars re-

spectively, showed 26 and 38 % reduction in root weight when nematodes were present and 71 and 98 cysts were counted on unit weight of root, respectively (Table I). The results of this experiment are in agreement with others who have reported that *H. schachtii* multiplied well on oilseed rape (Storey *et al.*, 1985; Bowen *et al.*, 1986). In this experiment population density of *H. schachtii* increased 3 to 6 fold, from an initial density of 3.8 eggs/g soil to 9 to 19 eggs/g soil in the four cultivars.

No relationships between percent hatch and rate of penetration was detected in these tests; similar results were also reported by others (Decker and Dowe, 1991; Fatemy, 2000).

Root diffusates of Cobra and Global stimulated more

Table I. Effect of *Heterodera schachtii* on growth of four cvs of oilseed rape and final nematode population after two months in the glasshouse.

Cultivars	Nematodes	Fresh weight root (g)	Fresh weight top (g)	Cysts/pot ₃	Eggs/g soil	Pf/Pi
Tower	with	2.0 d	19.7 cd	246.7 b	7.1	2.1
	Without	1.7 d	15.9 d	0.0 d	0.0	0.0
Cobra	with	6.8 bc	19.5 cd	483.3 b	7.5	2.2
	Without	9.2ab	18.2 cd	0.0	0.0	0.0
Global	with	6.1 c	24.1 bc	601.7 b	11.8	3.5
	Without	9.9a	21.3 cd	0.0	0.0	0.0
K16	with	4.9 c	29.6ab	1041.7a	19.0	5.6
	Without	10.9a	31.6a	0.0	0.0	0.0

Data with the same letters in each column are not significantly different at 5% level. Pf/Pi=final/initial nematode population density.

juveniles to emerge (Fig. 1) *in vitro* (24.5 and 17.4% respectively) whereas in the soil, roots of K16 and Tower attracted more juveniles (18 and 15%) (Fig. 2). However, the overall nematode populations at harvest were higher on K16 and then Global and Cobra. Although Tower attracted more juveniles, it supported fewer number of females, perhaps because of its small root system which could not provide enough space and food for all the developing nematodes. Sexual differentiation is influenced by environmental factors (Müller, 1986). In a similar experiment, equal numbers of males and females emerged from the roots of this cultivar after three weeks (Abootorabi *et al.*, 2000).

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