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Attractant-mediated behaviour of mobile stages of *Heterodera schachtii*

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Summary – The responses of second-stage juveniles (J2) and males of *Heterodera schachtii* to root exudates and female sex pheromonal substances, respectively, at different distances from the attractant source, were studied by analysing orientation tracks left on agarose surfaces. Two classes of J2 were tested : stimulated J2, previously stimulated by root exudates, and unstimulated J2. Track lengths were quantified to differentiate between oriented and unoriented searching behaviour. The quantification of forward movements towards and away from the gradient, subsequent to each backward movement, enabled the differentiation of attractant and repellent effects. Males showed no repellent responses. The comparison of the sensory abilities between stimulated and unstimulated J2 points to the possible occurrence of " learning " processes.

Résumé – ***Influence de produits attractifs sur le comportement des stades mobiles d'*Heterodera schachtii** – La réaction des juvéniles de deuxième stade (J2) d'*Heterodera schachtii* aux exsudats radiculaires et des mâles aux phéromones produites par les femelles à des distances variées de la source d'attraction, est étudiée en analysant l'orientation des traces laissées à la surface de l'agar. Deux types de J2 ont été testés : des J2 stimulés – précédemment soumis à des exsudats radiculaires – et des J2 non stimulés. Les longueurs des traces ont été quantifiées de façon à différencier les comportements de recherche orientés et non orientés. La quantification des mouvements dirigés vers le gradient, ou à l'opposé de celui-ci, conséquence de chaque mouvement de recul, permet de différencier les effets attractifs et répulsifs. Aucun effet répulsif n'a été observé en ce qui concerne le comportement des mâles. La comparaison des capacités sensorielles entre J2 stimulés et non stimulés pourrait laisser supposer l'existence d'un processus d'apprentissage.

Key-words : Movement, searching, learning, root exudates, pheromonal substances, nematodes.

Males of amphimictic nematode species are thought to find their females by orientating themselves along sex pheromone gradients. Compared with insects, however, little is known about pheromone structures, pheromone-producing cells, pheromone receptors, and pheromone-mediated behaviour of nematodes (Haseeb & Fried, 1988).

Having hatched from eggs outside plant roots, juveniles of most plant-parasitic species search suitable roots by orientating themselves along root exudate gradients. Likewise, little is known about the structure of these attractants, nematode receptors, or root exudate-mediated behaviour (Robertson & Forrest, 1989).

The responses of nematodes to exogenous stimuli can be observed microscopically on agarose surfaces (Ward, 1973). We describe here the responses of mobile stages of *Heterodera schachtii* to root exudates and female sex pheromonal substances. Oriented and unoriented searching behaviour is characterized by the track lengths. Furthermore, the number of forward movements (after backward movements) towards and away from the attractant gradient can be used to differentiate quantitatively between attractant and repellent effects. Finally, learning processes can be studied by comparing the sensory abilities of second-stage juveniles (J2) that were previously stimulated or not stimulated by root exudates.

Materials and methods

NEMATODE CULTURES

Freshly emerged males of *H. schachtii* were obtained from monoxenic root cultures of oilradish (*Raphanus sativus* var. *oleiformis*) cv. Pegletta. In this resistant cultivar nearly all nematodes develop into males. J2 of *H. schachtii* were obtained from cysts reared under monoxenic conditions on root cultures of mustard (*Sinapis alba*) cv. Albatros (Grundler *et al.*, 1991). Excised roots of both plants were grown in the dark at 25 ± 2° C in a nutrient agar medium according to Sijmons *et al.* (1991) in 9 cm (oilradish) or 14.5 cm diameter (mustard) plastic Petri dishes.

NEMATODE ATTRACTANTS

Female substances with sex pheromone activity were obtained by transferring 100 white, living *H. schachtii* females into a microcentrifuge tube containing 1 ml of ultrapure water. They were incubated at 4-6° C and the supernatant was used 8-9 days later. Root exudates of *S. alba* cv. Albatros were produced in 1000 ml Erlenmeyer flasks under sterile conditions. The bottom of the flask

Treatment and fiction condition	Class of chain		
Stimulating fiction, 5 min	P5+	[illegible]	(9.27)
	P5−	[illegible]	12.77 (9.98)
Tears, 5 min	P5+	[illegible]	15.26 (10.83)
	P5−	[illegible]	25.77 (12.18)
Stimulating fiction, 10 min	P10+	[illegible]	16.23 (8.53)
	P10−	[illegible]	21.33 (14.72)
Tears, 10 min	P10+	[illegible]	15.41 (8.84)
	P10−	[illegible]	19.51 (11.08)
Contrasts: [illegible]	[illegible]	[illegible]	[illegible]

P10+ = P10−, ns

P5+ = P10−, ns

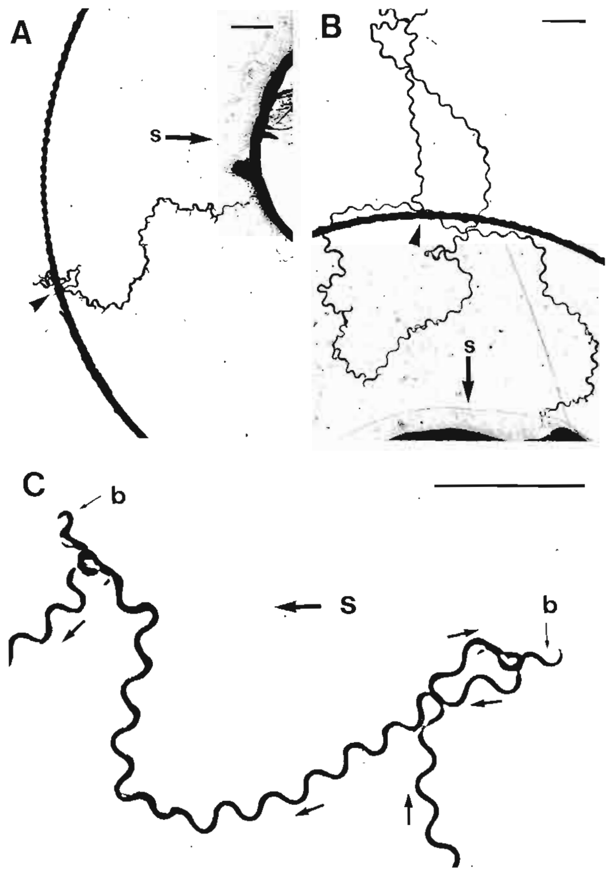

Fig. 1. Tracks left by Heterodera schachtii *J2 on agarose surfaces in a 60 min bioassay in the presence of root exudate (RE). A : Stimulated J2 with RE disk contact; B : Unstimulated J2 with RE disk contact; C : Typical backward movements (b), followed by forward movements. (Arrows indicate direction of movement; S with arrow = direction towards RE disk; dark rings in A and B = circles marked on the bottom of the plates.* Scale bars = 1 mm).

able to sense the exudate gradient. This remarkable behavioural change was evident from the evaluation of nematode tracks on the agarose surface.

Our results support the occurrence of two behavioural searching states in stimulated and unstimulated nematodes as previously described by Samoiloff *et al.* (1974) for *Panagrellus silusiae* males. Croll (1970) postulated that stimulation of nematodes may coincide with the release of energy quanta, followed by movement and orientation. The transition from unstimulated to stimulated behavior may be caused by external (e.g. chemicals, nutrient medium composition, temperature, light, etc.) or internal (e.g. physiological state, age, hunger, etc.) factors. The induction of chemotactic behaviour by an external factor was described by Albert and Riddle (1983) for the dauer juveniles of *Caenorhabditis elegans*. After pre-incubation with an appropriate food source, the immobile dauer juveniles became mobile and

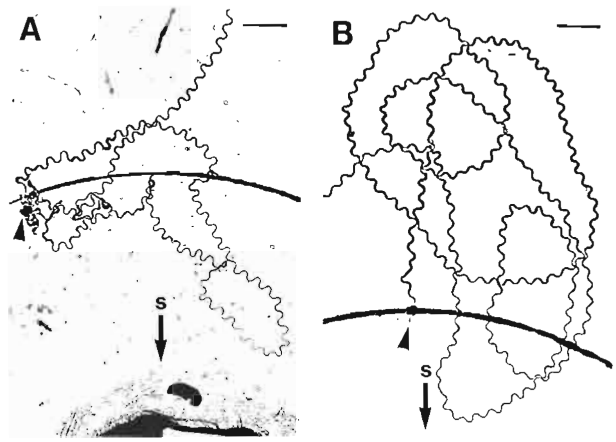

Fig. 2. Sections of tracks left by Heterodera schachtii *J2 on agarose surfaces in a 60 min bioassay in the presence of nutrient solution (NS). A : Stimulated J2 that moved towards and then away from the NS disk; B : Unstimulated J2 that showed no response to NS. (Arrowhead = point of nematode application; S with arrow = direction towards NS disks.* Scale bars = 1 mm*)*.

showed chemotactic behaviour and pharyngeal pumping. In the presence of the nutrient solution used to obtain root exudates, no direct orientation or exploratory behaviour could be observed for both behavioural states of *H. schachtii* J2 (Table 2). Furthermore, in contrast to unstimulated J2, stimulated J2 appeared to be repelled by the nutrient solution, supporting the hypothesis of nematode activation by specific stimuli.

This activation may be explained by " learning " processes. Rankin *et al.* (1990) showed for the first time that a nematode is capable of learning. They demonstrated for *C. elegans* several forms of non-associative learning, like short-term habituation, dishabituation and sensitization as well as long-term retention of habituation. Habituation was defined by them " as a decrease in response amplitude due to repeated stimulation, while dishabituation is the facilitation of that decremented response by a novel or noxious stimulus. Sensitization is defined as the facilitation of non-decremented or baseline responses by a strong stimulus ". Our observations on the orientation capacity of stimulated and unstimulated *H. schachtii* J2 indicate that these nematodes might be capable of the " learning " process of sensitization as defined by Rankin *et al.* (1990).

In conclusion, our results show that relatively simple bioassays can be applied for a quantitative analysis of nematode responses to attractants and repellents. Similar results have been obtained previously with free-living (Ward, 1973; Samoiloff *et al.*, 1974) and plant-parasitic nematodes (Riddle & Bird, 1985; Aumann *et al.*, 1990). Recent experiments showed that the bioassay described here can be used for the identification of attractive components of mustard root exudates (C. D. Clemens, unpubl.).

Table 2. *Movement patterns of stimulated and non-stimulated* Heterodera schachtii *J2 with and without contact with agarose disks wetted with root exudate or nutrient solution. Data from a 60 min bioassay.*

Treatment and distance from source	Class of J2	n (%)	Mean no. of forward after backward movements towards/away from the gradient (Std. dev.)	Mean track lengths in mm (Std. dev.)
Root exudate, 5 mm, stimulated	R5/1+	63 (73.3)	5.62/2.14 (2.92/2.00)	16.82 (10.85)
	R5/1–	23 (26.7)	3.13/4.43 (2.05/2.45)	42.04 (16.41)
Root exudate, 5 mm, unstimulated	R5/2+	28 (33.3)	2.64/1.79 (2.71/2.38)	25.42 (18.15)
	R5/2–	56 (66.7)	2.14/2.98 (1.75/2.20)	56.87 (23.16)
Nutrient solution, 5 mm, stimulated	N5/1+	35 (22.9)	1.80/1.54 (1.69/1.62)	55.24 (31.64)
	N5/1–	118 (77.1)	2.08/4.84 (1.94/3.48)	67.79 (24.58)
Nutrient solution, 5 mm, unstimulated	N5/2+	31 (39.7)	2.19/1.35 (2.33/1.75)	75.06 (41.41)
	N5/2–	46 (60.3)	1.70/2.00 (1.6/1.86)	84.36 (31.62)
Root exudate, 7.5 mm, stimulated	R7.5/1+	48 (45.1)	5.15/1.96 (3.36/1.80)	35.96 (19.45)
	R7.5/1–	56 (54.9)	2.16/2.88 (2.34/2.72)	49.71 (21.18)
Root exudate, 7.5 mm, unstimulated	R7.5/2+	30 (27.0)	2.03/1.33 (1.81/1.32)	47.00 (23.35)
	R7.5/2–	81 (73.0)	1.58/2.69 (1.40/2.20)	74.60 (25.70)
Statistical intergroup comparisons		R5/1+ > R5/1–; P < 0.001 R5/2+ < R5/2–; P < 0.001 N5/1+ < N5/1–; P < 0.001 N5/2+ < N5/2–; P < 0.05 R7.5/1+ = R7.5/1–; ns R7.5/2+ < R7.5/2–; P < 0.001	R5/1+; P < 0.001 R5/1–, P < 0.01 R5/2+; ns R5/2–; ns N5/1+; ns N5 / 1–; P < 0.001 N5/2+; ns N5/2–; ns R7.5/1+; P < 0.001 R7.5/1–; ns R7.5/2+; ns R7.5/2–; ns	R5/1+ < R5/1-; P < 0.001 R5/2+ < R5/2–; P < 0.001 N5/1+ = N5/1–; ns N5/2+ = N5/2–; ns R7.5/1+ > R7.5/1–; P < 0.001 R7.5/2+ < R7.5/2–; P < 0.001

Abbreviations : R5/1+, R5/2+, N5/1+, N5/2+, R7.5/1+, R7.5/2+, with agarose disk contact; R5/1–, R5/2–, N5/1–, N5/2–, R7.5/1–, R7.5/2–, without agarose disk contact; ns, not significant; Std. dev., standard deviation.

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