Attraction of *Mononchoides longicaudatus* and *M. fortidens* (Nematoda : Diplogasterida) towards prey and factors influencing attraction

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SUMMARY

The attraction of *Mononchoides longicaudatus* and *M. fortidens* towards prey and factors influencing their attraction were studied on agar plates. These predators were attracted towards excised and non-excised (live) prey in response to prey secretions, attractants. Predators also responded positively towards agar containing unidentified bacteria. *M. longicaudatus* and *M. fortidens* showed preference for the prey nematodes which were tested either alive or by cutting into two pieces (excised). *Hirschmanniella oryzae, Tylenchorhynchus mashhoodi* and *Hoplolaimus indicus* attracted predators most while *Longidorus* sp., and *Helicotylenchus indicus* elicited least response from the predators. The rest of the prey nematodes attracted predators moderately. Both species of predators were also attracted towards their own individuals. Different factors viz., prey number, temperature, incubation period of prey, starvation of predators and distance of predators from prey affected attraction of the predators towards *Hirschmanniella oryzae* and the second stage juveniles of *Meloidogyne incognita.* Both predators were attracted in maximum numbers when 50 prey nematodes were incubated for 12 h in Petri-dishes, at 25-30°. Starvation of predators increased their attraction towards prey; increase in prey distance decreased attraction.

RÉSUMÉ

L'attraction de Mononchoides longicaudatus *et de* M. fortidens *(Nematoda : Diplogasterida) par les proies, et les facteurs qui l'influencent*

L'attraction de *Mononchoides longicaudatus* et de *M. fortidens* par les proies et les facteurs qui l'influencent ont été étudiés en boîtes de Petri. Ces prédateurs sont attirés par les proies, disséquées et non-disséquées (vivantes), en réponse à des substances attractives sécrétées par ces proies. Les prédateurs répondent également positivement à la présence sur l'agar d'une bactérie non identifiée. *M. longicaudatus* et *M. fortidens* montrent une préférence parmi les proies, que celles-ci aient été testées vivantes ou coupées en deux : *Hirschmanniella oryzae, Tylenchorhynchus mashhoodi* et *Hoplolaimus indicus* ont la meilleure attraction tandis que *Longidorus* sp. et *Helicotylenchus indicus* provoquent la réponse la plus faible. Les autres espèces testées n'attirent les prédateurs que modérément. L'une et l'autre espèces sont également attirées par des individus appartenant à leur propre espèce. L'attraction par *Hirschmanniella oryzae* et par les juvéniles de 2e stade de *Meloidogyne incognita* est influencée par différents facteurs : nombre des proies, température, période d'incubation des proies, jeûne des proies, distance entre proie et prédateur. Les deux espèces prédatrices sont attirés en nombre maximal lorsque 50 proies ont séjourné 12 heures sur l'agar de la boîte de Petri, à 25-30°. Le jeûne des prédateurs accroît l'attraction; enfin celle-ci est inversement proportionnelle à la distance entre proie et prédateur.

Predatory nematodes are unable to detect their prey and their predation depends on chance encounters (Yeates, 1969; Grootaert & Maertens, 1976; Small & Grootaert, 1983; Bilgrami, Ahmad & Jairajpuri, 1984). Esser (1963) speculated that dorylaim predators were attracted towards excised prey from short distances. Bilgrami, Ahmad and Jairajpuri (1985*a*), and Shafqat, Bilgrami and Jairajpuri (1987), however, did not find any positive attraction in *Aquatides thornei* (Schneider) and *Dorylaimus stagnalis* (Dujardin) respectively towards excised prey, but observed aggregation of more than one predator taking place around a prey injured by another predator. Yeates (1969) and Wyss and Grootaert (1977) also reported similar aggregation in *Diplenteron potohikus* (Yeates) and *Labronema vulvapapillatum* (Loof & Grootaert). Bilgrami and Jairajpuri (1988) observed the pre- and post-feeding aggregation of *Mononchoides longicaudatus* (Khera) and *M. fortidens* (Schuurmans Steckhoven) at feeding sites and attributed this phenomenon to lingering prey attractants in that area. The predatory mononchs, however, did not show such a response towards their prey (Bilgrami, Ahmad & Jairajpuri, 1984).

In the present work observations on *Mononchoides*

longicaudatus (Khera) and *M. fortidens* (Schuurmans Stekhoven) were made to study their attraction towards prey and factors influencing their attraction.

Materials and methods

General conditions

Mononchoides longicaudatus and *M. fortidens* were cultured separately in 1 % water-agar using *Rhabditis* sp., as prey. Five mg of infant milk powder (" Lactogen ") was spread on the surface of the agar to encourage bacterial growth which served as food for the prey nematodes. The predators, as and when their population declined, were transferred to fresh culture dishes containing prey.

In all experiments, attraction of *M. longicaudatus* and *M. fortidens* was tested in 0.5 cm thick layer of 1 % water-agar using ten prey nematodes at 28 ± 1°. The most suitable concentrations and layer of agar was 1 or 2 % and 0.5 cm respectively. It was therefore, these characteristics used for all experiments. The nematodes were incubated for eight hours in Petri-dishes containing autoclaved water-agar (without bacteria) prior to the inoculation of predators. The distribution of predators was recorded after four hours. Each experiment was replicated 20 times. All nematodes used as prey were extracted fresh from the soil and sterilized with 0.01 % mercuric chloride. Unless mentioned otherwise the conditions remained identical in all experiments.

All experiments were carried out in 5.5 cm diameter Petri-dishes, divided into three zones viz., inner, middle and outer by drawing two concentric circles 0.5 and 2.5 cm in diameter on the bottom. A plastic straw pipe 5 mm high, 5 mm in diameter, with a small piece of filter paper glued at one end was placed vertically in the inner zone of the Petri-dishes, with the filter paper in contact with the Petri-dish. Water-agar was then poured into the Petri-dishes and straw pipe to make a 0.5 cm thick agar layer. Prey nematodes were released in the straw pipe and left for incubation. Five predators were then released at the periphery of the middle zone. The distribution of predators was recorded and scores were obtained by summing up the product of the number of worms in each zone with their corresponding weighting factors. The weighting factors were obtained by dividing the area of outer zone by that of each of the three zones (Ahmad & Jairajpuri, 1980; Bilgrami, Ahmad & Jairajpuri, 1985 *a*). In the text MLS stands for " Mean Log Score ".

Attraction in *M. longicaudatus* and *M. fortidens*

Attraction was tested separately towards excised (cut in two pieces) and non-excised (live) *Rhabditis* sp., and agar containing unidentified bacteria (cultivated on agar by adding Lactogen milk powder). Petri-dishes without prey or bacteria served as control. Observations were made after 1, 2 , 3 and 4 h intervals.

Attraction towards different prey nematodes

Attraction of *M. fortidens* and *M. longicaudatus* was tested separately by Petri-dish experiments with excised and non-excised prey nematodes. Adult *Hirschmanniella oryzae, Hoplolaimus indicus, Helicotylenchus indicus, Hemicriconemoides mangiferae, Paralongidorus* sp., *Longidorus* sp., *Xiphinema americanum, Tylenchorhynchus mashhoodi, Mononchoides fortidens, Mononchoides longicaudatus* and the second stage juveniles of *Meloidogyne incognita* and *Anguina tritici* were used as prey.

Factors influencing attraction

To determine the effect of the following factors on the attraction, live adult *H. oryzae* and the second stage juveniles of *M. incognita* were used. *M. fortidens* and *M. longicaudatus* were tested separately towards individual prey nematodes.

Prey number : To observe the effect of prey numbers on the attraction of *M. fortidens* and *M. longicaudatus* 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 prey nematodes were placed in the straw pipe. They were incubated prior to the inoculation of predators.

Incubation of prey nematodes : Prey nematodes were incubated for 2, 4, 6, 8, 10 and 12 h by placing in the straw pipe of the separate Petri-dishes. After incubation *M. longicaudatus* and *M. fortidens* were released and their distribution recorded after the desired time.

Temperature : Prey nematodes were incubated at 5, 10, 15, 20, 25, 30, 35 and 40° to determine the effect of temperatures on the attraction of *M. longicaudatus* and *M. fortidens.* After incubation the predators were released and Petri-dishes containing prey and predators were again placed at the same temperatures at which they were kept earlier. Observations on the distribution of predators were made when required.

Starvation of predators : M. longicaudatus and *M. fortidens* were starved for 0, 2, 4, 6, 8, 10 and 12 days by placing them in cavity-blocks containing water (without prey). Predators were transferred to fresh water each day. Day 0 refers to fresh predators (well fed). Each group of starving predators was tested separately towards previously incubated prey nematodes.

Distance of predators from prey : The effect of the distance of predators from the prey on their attraction was tested in 7 cm diameter Petri-dishes containing water-agar. The dishes were marked at the bottom by straight lines into seven zones and numbered serially as 1, 2, 3... 7 (Bilgrami, Ahmad & Jairajpuri, 1985*b*). A plastic straw pipe with one end sealed with a piece of

filter paper was placed vertically in zone 1 so that the sealed end remained inside the agar. Prey nematodes *(H. oryzae)* were released in the straw pipe and the whole set was left for incubation. Twenty-five predators were then released at different points in zone 2. Similarly, predators were released in the zones 3, 4, 5, 6 and 7 of the separate Petri-dishes and observations were made after 4 h. Attraction of *M. longicaudatus* and *M. fortidens* was tested in separate Petri-dishes.

Results

ATTRACTION IN *M. FORTIDENS* AND *M. LONGICAUDATUS* (Fig. 1)

Both the predators responded positively towards live and excised *Rhabditis* sp., and agar containing bacteria. Attraction increased significantly from 1 h to 4 h ($p < 0.05$). The attraction of *M. longicaudatus* (Fig. 1 A) and *M. fortidens* (Fig. 1 B) (MLS 2.43 and 2.44 respectively) was more towards *Rhabditis* sp., than the live (MLS 2.26 and 2.28 respectively) or agar containing bacteria (MLS 1.90 and 2.13 respectively) after 4 h ($p < 0.05$). Minimum number of both the predators were attracted in the agar containing bacteria.

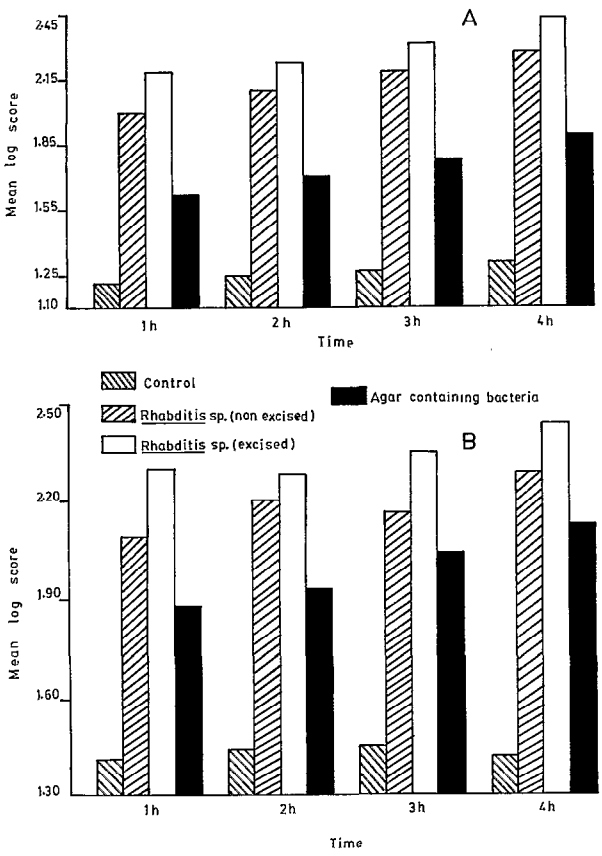

Fig. 1. Attraction of *M. longicaudatus* and *M. fortidens*. A : *M. longicaudatus;* B : *M. fortidens.*

ATTRACTION TOWARDS DIFFERENT PREY NEMATODES (Fig. 2)

M. longicaudatus and *M. fortidens* responded differently towards live and excised prey. When tested towards live prey, *H. oryzae* was most attractive to *M. longicaudatus* (Fig. 2 A). Prey nematodes viz., *Helicotylenchus indicus, H. mangiferae* and *Longidorus* sp., were least attractive. The attraction towards *H. oryzae* (MLS 2.06) was higher than towards *Helicotylenchus indicus, H. mangiferae* and *Longidorus* sp., (MLS 1.58, 1.58 and 1.59 respectively, $p < 0.05$). Excised prey attracted more *M. longicaudatus* than the non-excised ones (Fig. 2 B). Excised *T. mashhoodi* attracted most (MLS 2.34) while *Helicotylenchus indicus* the least (MLS 1.46) *M. longicaudatus.* There was no significant difference in the

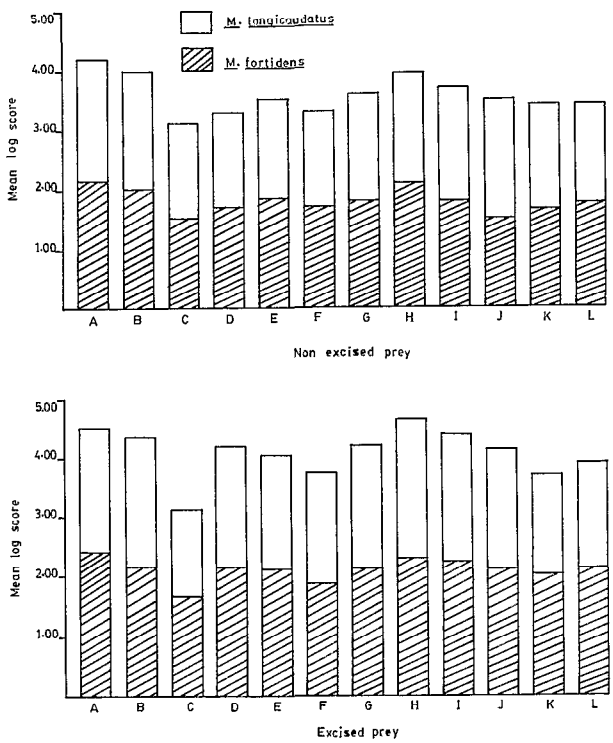

Fig. 2. Attraction of *M. longicaudatus* and *M. fortidens* towards non-excised and excised prey nematodes. Above : Non-excised. Below : Excised prey. A : *Hirschmanniella oryzae;* B : *Hoplolaimus indicus;* C : *Helicotylenchus indicus;* D : *Hemicriconemoides mangiferae;* E : *Paralongidorus* sp.; F : *Longidorus* sp.; G : *Xiphinema americanum;* H : *Tylenchorhynchus mashhoodi;* I : *Anguina tritici* juveniles; J : *Meloidogyne incognita* juveniles; K : *Mononchoides longicaudatus;* L : *Mononchoides fortidens.*

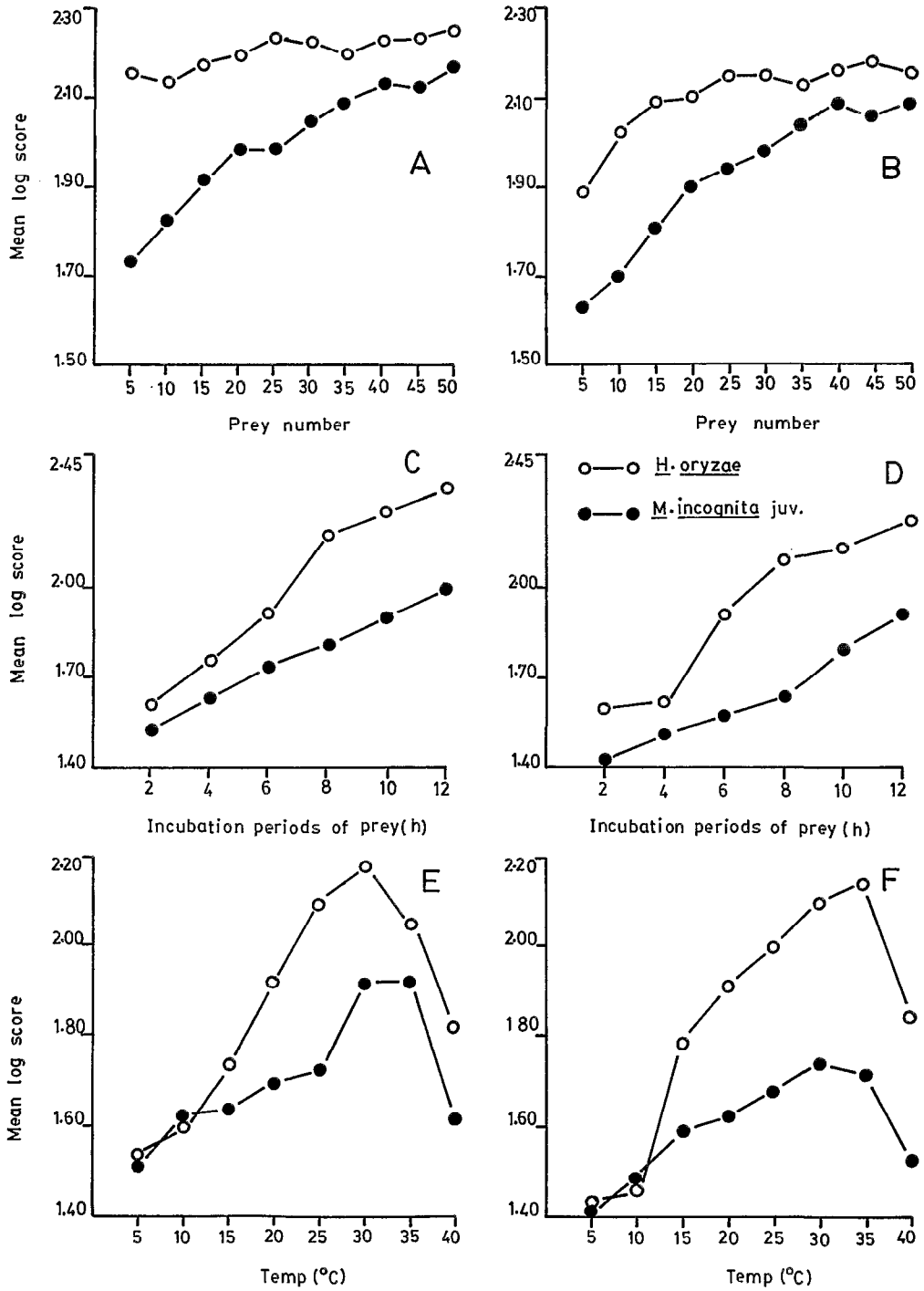

Fig. 3. Factors influencing attraction of *M. fortidens* and *M. longicaudatus*. A : Effect of prey numbers on the attraction of *M. fortidens;* B : Effect of prey numbers on the attraction of *M. longicaudatus;* C : Effect of incubation period of prey on the attraction of *M. fortidens;* D : Effect of incubation period of prey on the attraction of *M. longicaudatus;* E : Effect of temperature on the attraction of *M. fortidens;* F : Effect of temperature on the attraction of *M. longicaudatus*.

attraction of *M. longicaudatus* towards excised *H. oryzae, Hoplolaimus indicus* and *A. tritici* juveniles (MLS 2.20, 2.19 and 2.16 respectively); and *M. incognita* juveniles and *H. mangiferae* (MLS 2.04 and 2.02 respectively, $p > 0.05$). *M. longicaudatus* was also attracted towards its own individuals.

Among all the prey nematodes tested alive, *H. oryzae* attracted most *M. fortidens* (MLS 2.15) while *Helicotylenchus indicus* did the least (MLS 1.53, Fig. 2 B). There was little variation in the attraction towards non-excised *T. mashhoodi* (MLS 2.08) and *Hoplolaimus indicus* (MLS 2.02); and *A. tritici* juveniles (MLS 1.81) and *Paralongidorus* sp., (MLS 1.84). Excised *H. oryzae* attracted maximum predators and the attraction (MLS 2.38) was more than that towards other nematodes ($p < 0.05$). Secretions of excised *Helicotylenchus indicus* were least attractive to *M. fortidens* (MLS 1.65). These predators also responded positively towards their own individuals when tested alive (MLS 1.72) or excised (MLS 2.12).

FACTORS INFLUENCING ATTRACTION

Prey numbers (Fig. 3 A & B) : Attraction of *M. fortidens* increased with the increase in the number of prey (Fig. 3 A). The attraction towards five *M. incognita* juveniles and *H. oryzae* (MLS 1.74 and 2.14 respectively) increased significantly and was maximum when 50 individuals of the same prey nematodes were present (MLS 2.17 and 2.22 respectively, $p < 0.05$). *M. longicaudatus* also responded indentically (Fig. 3 B).

Period of incubation of prey (Fig. 3 C & D) : Attraction of both type of predators increased with the increase in the period of incubation of prey. The predators responded more towards *M. incognita* juveniles than *H. oryzae* which were incubated for the maximum duration (12 h) and least when incubated for only 2 h ($p < 0.05$).

Temperature (Fig. 3 E & F) : *M. fortidens* and *M. longicaudatus* were attracted more towards *M. incognita* juveniles and *H. oryzae* at 30 and 35°. Higher and lower temperatures showed declining attraction of both the predators ($p < 0.05$). Least attraction was recorded at 5°.

Starvation of predators (Fig. 4 A & B) : Twelve day starved *M. fortidens* (Fig. 4 A) and eight day starved *M. longicaudatus* (Fig. 4 B) exhibited maximum attraction towards *H. oryzae* and the second stage juveniles of *M. incognita.* Their attraction was more than the fresh predators ($p < 0.05$). *M. longicaudatus,* starved for more than eight days showed considerable decline in the attraction (Fig. 4 B).

Distance of predators from prey (Fig. 5) : Increase in the distance of predators from the prey affected their movement towards prey. *M. fortidens* and *M. longicaudatus* were attracted more towards *H. oryzae* (zone 1) when tested from the zones 2 and 3. The attraction was moderate when predators were tested from other zones of the Petri-dishes.

Discussion

Predatory nematodes are unable to detect their prey even from short distance (Nelmes, 1974). However, Esser (1963) and Yeates (1969) observed aggregation of predators around previously injured prey and attributed this phenomenon to prey secretions. Bilgrami and Jairajpuri (1988) in their studies on *Mononchoides longicaudatus* and *M. fortidens* have attributed the pre- and post-feeding aggregation of these predators at feeding sites to the lingering effect of prey secretions/attractants; they have also speculated on the role of predators

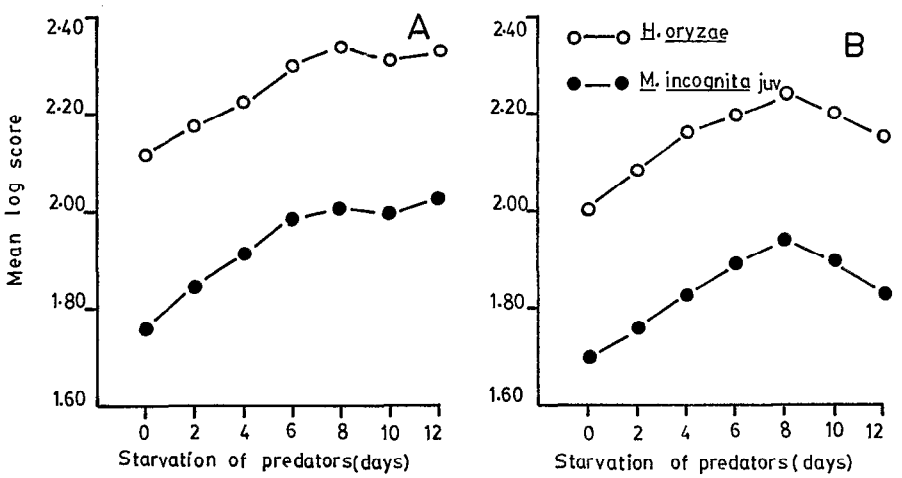

Fig. 4. Effect of starvation of predators on their attraction towards prey; A : *M. fortidens;* B : *M. longicaudatus.*

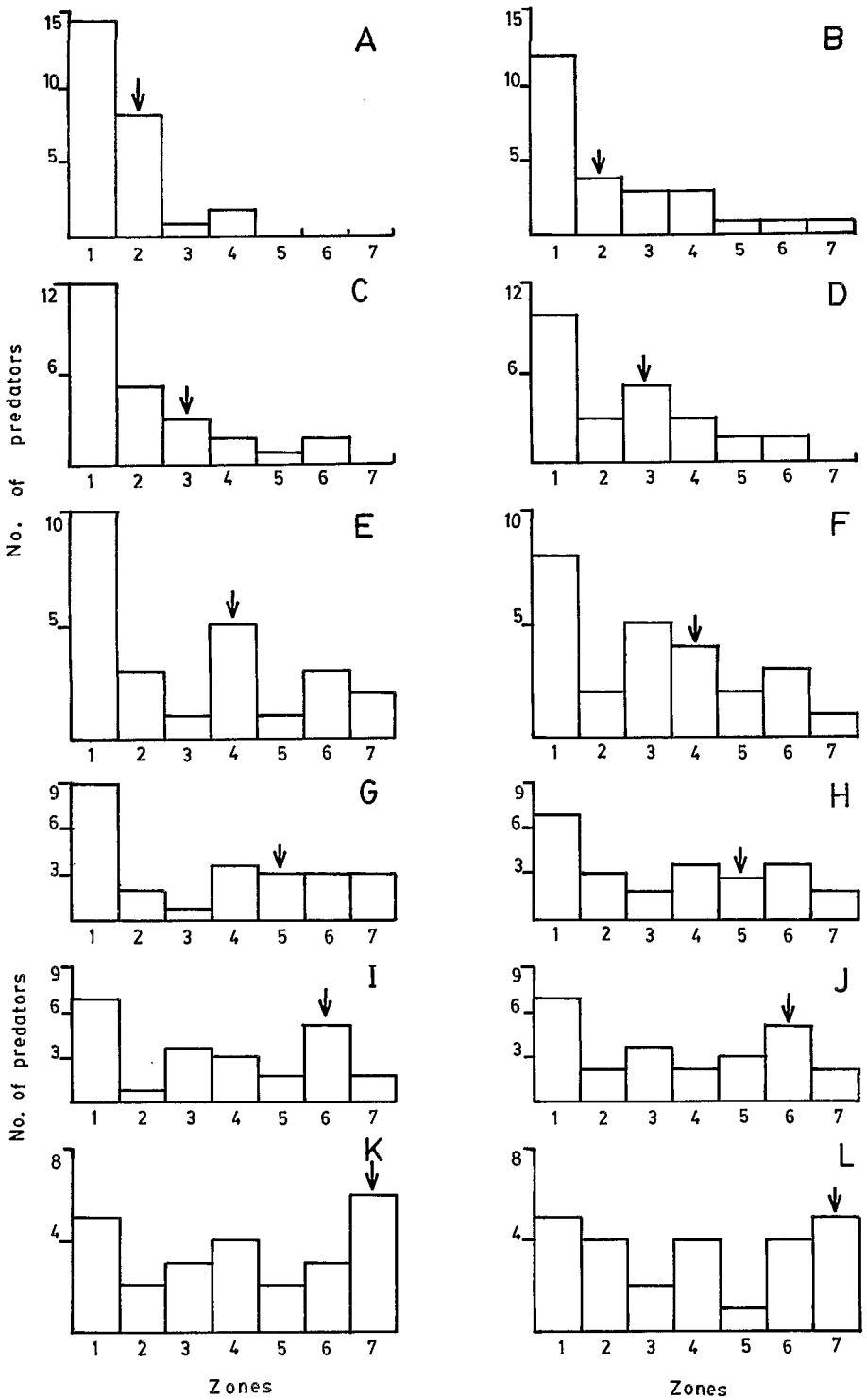

Fig. 5. Effect of distance of predators from prey on the attraction. A, C, E, G, I & K : *M. fortidens;* B, D, F, H, J & L : *M. longicaudatus.* Arrows indicate point of inoculation of predators.

secretions (released during extra-corporeal feeding and digestion) in the aggregation of other predators.

The present observations on the attraction of *M. longicaudatus* and *M. fortidens* reveal that these predators respond positively towards different prey in response to prey secretions/attractants. Nematode reach bacteria by chance or wilful movements (Poinar & Hansen, 1986), their orientation is determined by probing movements of the head (Ward, 1973; Seymour, Wright & Doncaster, 1983) and location of bacteria depends upon papillae and amphids (McLaren, 1976; Wright, 1983). The present study also indicates attraction of *M. longicaudatus* and *M. fortidens* towards bacteria.

The differential response of predators towards different prey suggests their preferential behaviour. It could also be possible that some prey nematodes secrete repellents which may be undesirable for the predators. Esser (1963) has suggested that species of *Helicotylenchus* secrete some toxic substances which make them resistant to predation. The fact that the two predators, *M. longicaudatus* and *M. fortidens* were less attracted towards *Helicotylenchus indicus, Longidorus* sp., and *X. americanum* indicates that these prey nematodes may secrete substances which are either toxic or unfavorable, leading to meager or moderate response of the predators. Besides, the composition, concentration, quantity and quality of prey secretions may also play a role in differential responses of *M. longicaudatus* and *M. fortidens* towards different prey since these characteristics may vary from species to species and individual to individuals.

Wallace (1963) found lower (5-10°) and higher (40° or more) unfavorable for the nematodes and Bilgrami, Ahmad and Jairajpuri (1983) found temperatures influencing activity of the predatory nematodes, *Mononchus aquaticus.* The decrease in the attraction of *M. longicaudatus* and *M. fortidens* at temperatures lower than 25 and higher than 35° may be attributed to their activity which might be inhibited by the lower and higher temperatures. Besides, temperatures may also influence the dispersion of prey attractants in the surrounding resulting in a differential predator responses.

The differential response of *M. longicaudatus* and *M. fortidens* towards prey nematodes, which were incubated for variable durations suggest that a minimum period is necessary for the development of the perceptible gradients of prey attractants. Doncaster and Seymour (1973) concluded that the minimum response threshold decreases in starving nematodes which could sense even the weaker stimuli. However, Bilgrami, Ahmad and Jairajpuri (1985*b*) had suggested an increase in the minimum response threshold level in starving *H. oryzae.* During present observations it seems that the minimum response threshold of *M. longicaudatus* and *M. fortidens* decreased with the increase in the period of starvation resulting in increased attraction from two to twelve day starved *M. fortidens* and two to eight day starved *M. longicaudatus.* In the latter predator the threshold level increased after eight of starvation and resulted in their lesser attraction towards prey.

Attraction of nematodes is influenced by the distance of nematodes from the source of attraction (Bilgrami, Ahmad & Jairajpuri, 1985*b*). The effect of distance of *M. longicaudatus* and *M. fortidens* from the prey on the attraction may be attributed to the time taken by the prey secretions/attractants to form an optimum threshold level in agar as well as the time taken by the attractants to disperse in the surroundings and to reach the predators. Stimuli of stronger intensities may require lesser time to develop a minimum response threshold and could easily be spread in a particular area faster than those of the weaker intensities.

Cannibalism seems to be a natural phenomenon occurring in most predators (Bilgrami, Ahmad & Jairajpuri, 1986) under conditions of non-availability of prey nematodes (Bilgrami & Jairajpuri, 1985). The ability of predators to recognize their own chemical secretions (Esser, 1963; Grootaert & Small, 1982; Bilgrami, Ahmad & Jairajpuri, 1985*a*; Shafqat, Bilgrami & Jairajpuri, 1987) may induce cannibalism. During present observations the attraction of *M. longicaudatus* and *M. fortidens* towards their own individuals suggests their ability to recognize their own chemical secretions which may also lead them to prey upon their own individuals (cannibalism).

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