

Factors influencing attraction of adult *Hirschmanniella oryzae* towards cabbage seedlings

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SUMMARY

Observations on various factors influencing attraction of *Hirschmanniella oryzae* towards cabbage roots revealed that all factors except coloured lights and darkness affected attraction. Responses of *H. oryzae* declined as the age of the roots increased and also with an increase in the period of food deprivation of nematodes. Maximum attraction was recorded towards 4-6 day-old cabbage roots with fed nematodes as well as 2, 4 and 6 day unfed ones. Live roots attracted nematodes but roots killed by heating lost attractiveness. Maximum number of nematodes were attracted when roots were incubated for 10-12 h in 3-4 mm thick agar layer of 0.5 % concentration at 20-25°. The nematodes showed preference to the region of root tip and aggregated most around it. Attraction decreased with increase in the distance of inoculation of nematodes from the roots.

RÉSUMÉ

Facteurs influençant l'attraction des adultes d'*Hirschmanniella oryzae* par les plantules de chou

L'étude de l'influence de différents facteurs sur l'attraction des adultes d'*H. oryzae* par le chou révèle que tous ces facteurs, sauf les lumières colorées et l'obscurité, ont un effet. Les réponses d'*H. oryzae* diminuent en intensité lorsqu'augmentent soit l'âge des racines soit la durée du jeûne chez les nématodes. L'attraction maximale est observée avec des plantules de chou de 4-6 jours et des nématodes fraîchement nourris ou soumis au jeûne depuis seulement 2, 4 et 6 jours. Les racines vivantes sont attractives, mais perdent ce caractère lorsqu'elles sont tuées par la chaleur. Un nombre maximum de nématodes sont attirés lorsque les racines sont incubées pendant 10-12 heures dans une couche de 3-4 mm d'agar à 0,5 % maintenue à 20-25°. Les nématodes montrent une préférence pour l'extrémité apicale de la racine autour de laquelle la plupart d'entre eux se rassemblent. L'attraction décroît lorsqu'augmente la distance entre la racine et le lieu d'inoculation des nématodes.

Several workers have studied the attraction and feeding behaviour of plant-parasitic nematodes (Luc, Lespinat & Souchaud, 1969; Azmi & Jairajpuri, 1977a; Prot & Netscher, 1978; Prot, 1980; Wyss, 1981) but few have dealt with the factors influencing attraction of nematodes towards plant roots. Attractiveness was proportional to the rate of root growth (Weiser, 1955; Lavalley & Rohde, 1962) but if the roots were killed by heating they lost their attractiveness (Linford, 1939; Bird, 1962). Lownsbery & Viglierchio (1961) suggested that roots became unattractive when their growth ceased. Moussa, De Grisse & Gillard (1972) did not observe any significant differences in the attraction of *Globodera rostochiensis* juveniles when coloured lights were used. Davies & Fisher (1976) found temperature, initial inoculum density, duration of exposure and the distance at which the juveniles were placed from the root to be limiting factors in the invasion of barley seedlings by *Heterodera avenae* juveniles. Prot and Van Gundy (1981a; 1981b) concluded that the soil texture, clay components, photo-period and temperature acted as influencing factors on the migration of the juveniles of *Meloidogyne incognita* and *M. hapla* towards tomato roots. Recently, Prot and

Kornprobst (1983) observed decreased rate of penetration of tomato roots by *M. javanica* juveniles when an increased concentration of seed extract was used.

The present work was undertaken to find out the effect of various biotic and abiotic factors on the attraction of adult *Hirschmanniella oryzae* (Van Breda de Haan, 1920) Luc & Goodey, 1963 to cabbage seedlings (*Brassica oleracea* L. var. *capitata*) in agar plates. Bilgrami (1983) has shown that cabbage roots attract adult and juveniles *H. oryzae* though it is not as good a host as rice (*Oryza sativa*). The present observations were made on cabbage seedlings to study in detail the mode of attraction of adult *H. oryzae*.

Materials and methods

The soil samples containing *H. oryzae* were collected from paddy fields near the village Jamalpur, Aligarh and the nematodes were extracted by decantation and sieving and modified Baermann's funnel techniques.

Most experiments were carried out by Petri-dish techniques (cf. Ahmad & Jairajpuri, 1980). Excised root tips of 4-6 day old cabbage seedlings were placed in the

inner circle of the Petri-dishes containing 0.5 % water-agar and incubated for 12 h. Five adult *H. oryzae* were then introduced at different places on the reference circle. Observations on the distribution of nematodes were made after 4 h and were converted into scores and log scores. All experiments were carried out at $25 \pm 1^\circ$ and replicated fifteen times. Controls were run without host roots.

The above conditions were similar in all experiments unless mentioned otherwise.

EFFECT OF DIFFERENT AGE GROUPS OF ROOTS ON THE ATTRACTION OF FED AND UNFED NEMATODES

To determine the effect of different age groups of cabbage roots on the attraction of fed (kept on roots in agar for one day) and unfed (kept without roots in tap water) nematodes, six age groups of roots *viz.*, 2, 4, 6, 8, 10 and 12 day old were subjected to attraction of 0, 2, 4, 6, 8, 10 and 12 day unfed nematodes. The water in which they were placed was changed each day. Each group of unfed nematodes was tested towards each age group of the roots separately. The 0 day refers to fed nematodes.

EFFECT OF LIVE AND DEAD ROOTS

The influence of live and dead cabbage roots on the attraction of *H. oryzae* was observed by placing in the inner circle of separate Petri-dishes and incubating prior to the introduction of nematodes. Cabbage roots were killed by boiling in water for 5 min.

EFFECT OF LENGTH OF INCUBATION PERIODS OF ROOTS

To observe the influence of different periods of incubation of roots on the attraction of nematodes, roots were incubated separately for 2, 4, 6, 8, 10 and 12 h prior to the introduction of nematodes.

EFFECT OF REGIONS OF ROOT

To observe the effects of different regions of the root on the attraction two experiments were performed. In the first experiment attraction was tested towards intact non-excised roots. Water-agar (0.5 %) was poured on a glass slide and was allowed to cool. The root was placed in the agar parallel to the length of the slide and divided into three 1 cm regions by markings lines at the bottom of the slide (Fig. 1). The shoot remained outside the agar. After incubating roots for 12 h, 25 adult *H. oryzae* were introduced at the edges of the slide opposite the middle region of the root. Distribution of nematodes was observed after 4 h.

In the second experiment the Petri-dish technique was used. A 3 cm long excised root was cut into three equal parts *viz.*, root tip (0-1 cm); zone of elongation (1-2 cm) and zone of maturation (2-3 cm). These were incubated in separate Petri-dishes prior to nematode introduction.

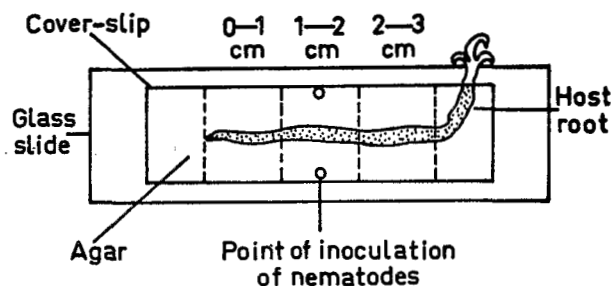


Fig. 1. Experimental design for testing the attraction of *H. oryzae* towards different regions of cabbage roots 0-1 cm: root tip; 1-2 cm: zone of elongation; 2-3 cm: zone of maturation.

EFFECT OF AGAR THICKNESS

To determine the effect of different thicknesses of agar on the attraction, nematodes were placed with roots in Petri-dishes containing 1, 2, 3, 4, 5 and 6 mm thick agar layers.

EFFECT OF AGAR CONCENTRATION

To observe whether agar concentrations influence attraction, roots were incubated in separate Petri-dishes containing 0.5, 1, 2, 3, 4, 5 and 6 % water-agar prior to the introduction of nematodes.

EFFECT OF LIGHT AND DARKNESS

Light from 60 Watt lamp kept at a distance of 65 cm from the Petri-dish was considered as normal light. Coloured effects were obtained by covering the lamp with red, yellow, green and blue cellophane papers. Roots were incubated in Petri-dishes under the different light conditions after which the nematodes were introduced and the dishes were replaced under similar lights.

EFFECT OF TEMPERATURE

To find the effect of temperature on the attraction of *H. oryzae* the roots were incubated in agar at 5, 10, 15, 20, 25, 30 and 35°. The nematodes were then introduced and Petri-dishes were again kept at the same temperature at which they were stored previously.

EFFECT OF DISTANCE OF ROOT ON ATTRACTION

The distance from which nematodes can be attracted towards roots was studied in 7 cm Petri-dishes. These were marked into 7 equal zones at the bottom similar to Azmi and Jairajpuri (1977a). A 1 cm long part of root from cabbage seedlings was placed in zone 1 and incubated for 12 h. After incubation 25 *H. oryzae* were introduced in zone 2. Similarly, the nematodes were introduced in zone 3, 4, 5, 6 and 7 of separate Petri-dish and their distribution was recorded after 4 h.

Results

The attraction of *H. oryzae* was influenced by all factors except coloured lights and darkness.

EFFECT OF DIFFERENT AGE GROUPS OF ROOTS ON THE ATTRACTION OF FED AND UNFED NEMATODES (Fig. 2 and 3)

All groups of roots attracted fed nematodes except 12 day old roots (mean log score 1.35; $p > 0.05$). Maximum attraction was recorded towards 4 and 6 day old cabbage roots (mean log score 2.59 and 2.58 respectively) (Fig. 2). This was higher than the attraction towards 2 day (mean log score 2.07) and 8 day old roots (mean log score 2.24) or 10 day old roots (mean log score 1.98) ($p < 0.05$). The attraction towards 2, 8 and 10 day old roots was not different from each other ($p > 0.05$).

The two day unfed nematodes were attracted most by 4 and 6 day old roots (mean log score 2.59 and 2.55 respectively) (Fig. 3, A). The roots of 2, 8 and 10 days attracted lesser number of nematodes compared to 4 and 6 day old roots ($p < 0.01$). No attraction was observed towards 12 day old roots (mean log score 1.45, $p > 0.05$).

Similarly, when 4, 6 and 8 day unfed nematodes showed maximum attraction towards 2, 4 and 6 day old roots (Fig. 3, B, C, D) which declined significantly in the presence of higher age groups and became unresponsive towards 12 day old roots ($p > 0.05$).

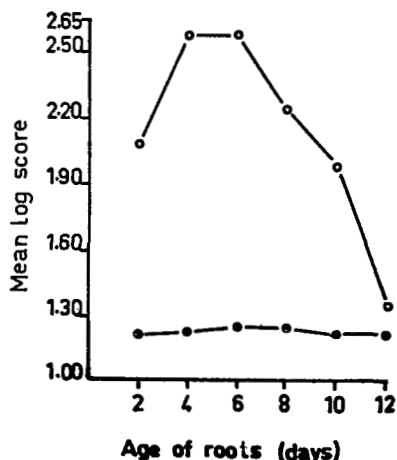


Fig. 2. Effect of different age groups of cabbage roots on the attraction of fed *H. oryzae* (Black circles : control; open circles : with roots).

H. oryzae which were deprived of food for 10 days showed positive response towards 2, 4, 6 and 8 day old cabbage roots (Fig. 3, E) and there was no difference in the attraction towards individual age groups ($p > 0.05$). The nematodes were unresponsive towards 10 and 12 day old roots (mean log score 1.45 and 1.42 respectively, $p > 0.05$).

Nematodes kept away from food for 12 days showed maximum attraction towards 4 day old roots (mean log score 1.76). This attraction was not different either from 2 day old (mean log score 1.75) or 6 day old roots (mean log score 1.70) ($p > 0.05$). However, roots 8, 10 and 12 day old did not attract nematodes significantly ($p > 0.05$) (Fig. 3, F).

EFFECT OF LIVE AND DEAD ROOTS (Fig. 4)

Live roots attracted nematodes (mean log score 2.55, $p < 0.05$) while dead roots failed to attract them (mean log score 1.38, $p > 0.05$).

EFFECT OF LENGTH OF INCUBATION PERIODS OF ROOTS (Fig. 5)

The period of incubation of roots influenced the degree of attraction. While 2 and 4 h incubation did not attract the nematodes (mean log score 1.18, $p > 0.05$), the attraction became positive only after 6 h (mean log score 1.63, $p < 0.05$). Further, it increased significantly up to 10 and 12 h incubation ($p < 0.05$). There was no difference in the attraction after 10 or 12 h incubation (mean log score 2.40 and 2.55 respectively, $p > 0.05$).

EFFECT OF REGIONS OF ROOT (Fig. 6 & 7)

In Petri-dish experiments when excised roots were used maximum attraction (mean log score 2.58) was observed towards 0-1 cm region of roots, while least (mean log score 1.80) towards 1-2 cm region (Fig. 6). Similar results were obtained in glass slide experiments also when whole roots were used (Fig. 7). However, in Petri-dish experiments the attraction towards 0-1 cm was more than towards the 2-3 cm region (mean log score 2.20, $p < 0.05$) while in glass slide experiment there was no difference ($p > 0.05$).

EFFECT OF AGAR THICKNESS (Fig. 8)

In various thicknesses of agar, the nematodes responded positively but their attraction was influenced by different agar layers. Attraction was maximum in 3 and 4 mm (mean log score 2.51 and 2.38 respectively) but declined in other layers, thicker or thinner than these two

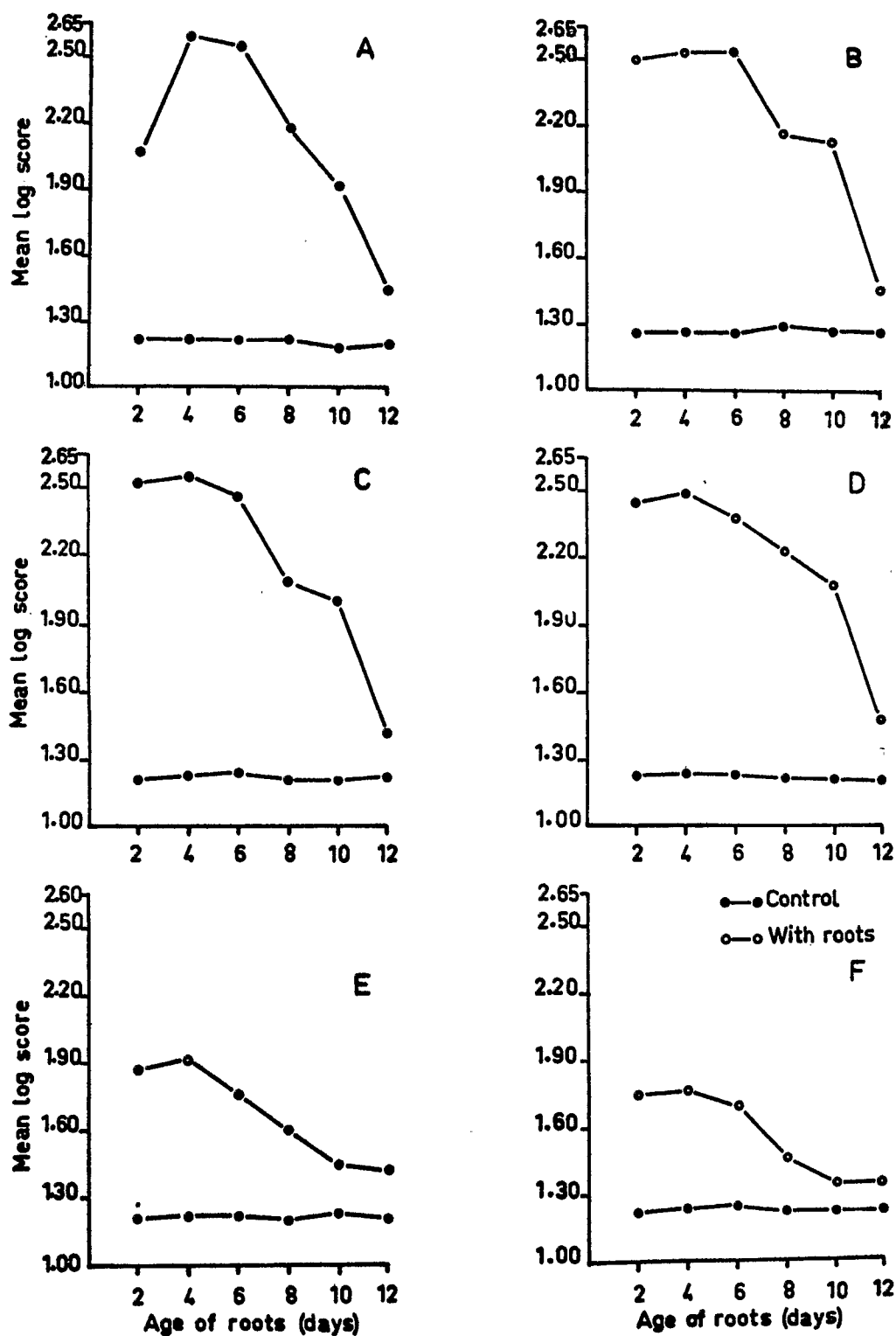


Fig. 3; Effect of different age groups of cabbage roots on the attraction of unfed *H. oryzae*. A : 2 day unfed adults; B : 4 day unfed adults; C : 6 day unfed adults; D : 8 day unfed adults; E : 10 day unfed adults; F : 12 day unfed adults.

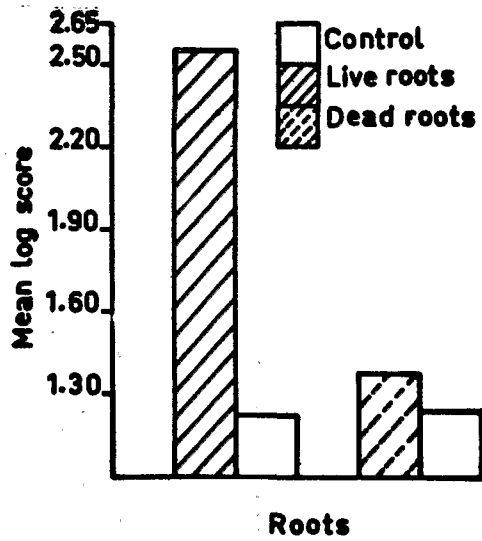


Fig. 4. Effect of live and dead cabbage roots on the attraction of *H. oryzae*.

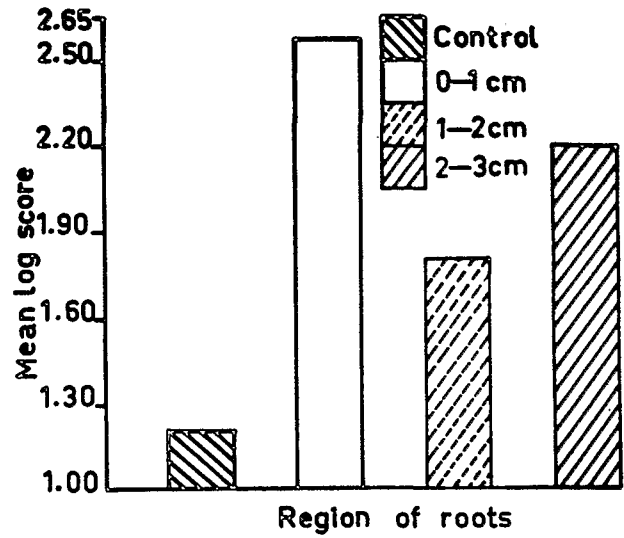


Fig. 6. Attraction of *H. oryzae* towards different regions of excised cabbage roots (Petri-dish experiments). 0-1 cm : root tip; 1-2 cm : zone of elongation; 2-3 cm : zone of maturation.

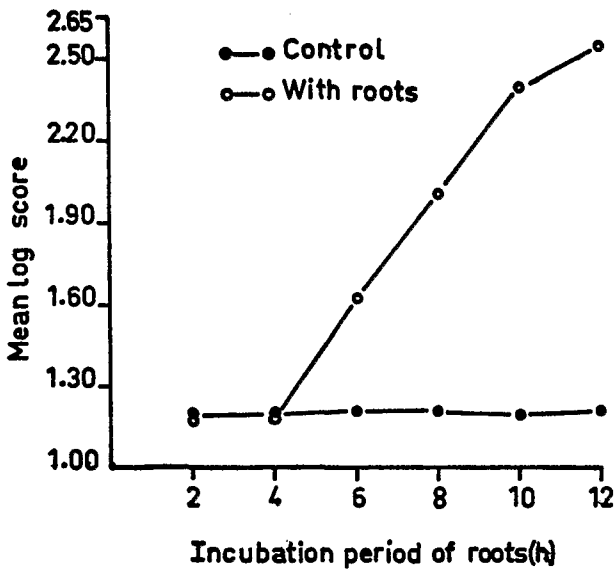


Fig. 5. Effect of length of incubation periods of cabbage roots on the attraction of *H. oryzae*.

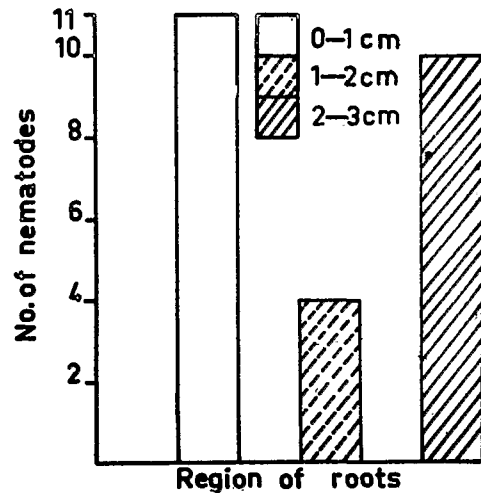


Fig. 7. Attraction of *H. oryzae* towards different regions of intact cabbage roots (Glass slide experiments). 0-1 cm : root tip; 1-2 cm : zone of elongation; 2-3 cm : zone of maturation.

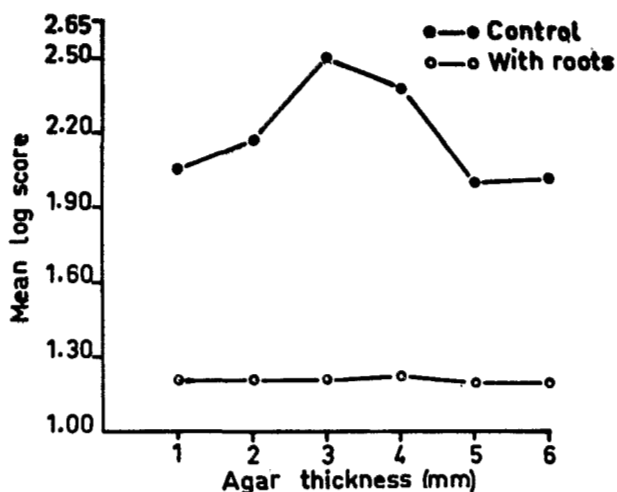


Fig. 8. Effect of agar thicknesses on the attraction of *H. oryzae* towards cabbage roots.

($p < 0.05$). The attraction was least in 6 mm (mean log score 2.01).

EFFECT OF AGAR CONCENTRATION (Fig. 9)

The attraction was greatly influenced by all concentrations of water-agar, except 0.5 and 1 % in which it was

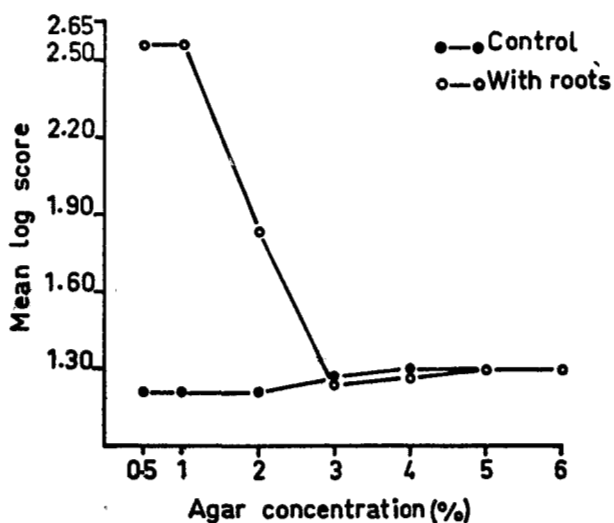


Fig. 9. Effect of agar concentrations on the attraction of *H. oryzae* towards cabbage roots.

maximum. Attraction in 2 % water-agar (mean log score 1.83) declined significantly ($p < 0.01$) and there was no attraction in 3, 4, 5 and 6 % agar dishes ($p > 0.05$).

EFFECT OF LIGHTS AND DARKNESS (Fig. 10)

Red, yellow, green, blue, normal lights and darkness did not significantly influence attraction of nematodes ($p > 0.05$).

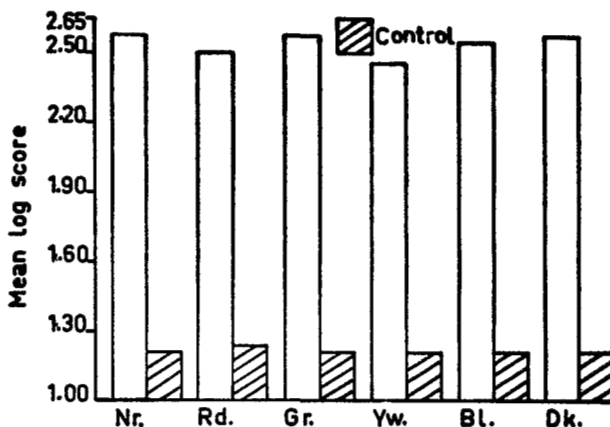


Fig. 10. Effect of lights and darkness on the attraction of *H. oryzae* towards cabbage roots (Nr : Normal; Rd : red; Gr : green; Yw : yellow; Bl : blue; Dk : darkness).

EFFECT OF TEMPERATURE (Fig. 11)

Attraction was influenced by different temperatures. Maximum attraction (mean log score 2.55) was at 20 and 25° and was higher than the attraction at 15 or 30° ($p < 0.01$). Nematodes were attracted least at a temperature of 35°. Lower temperatures of 5 and 10° proved

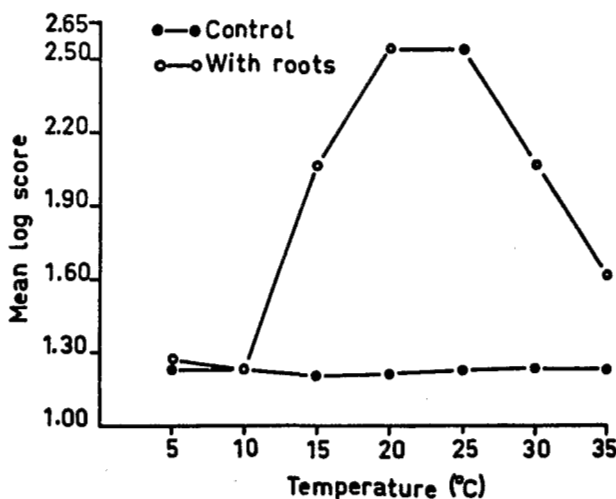


Fig. 11. Effect of temperatures on the attraction of *H. oryzae* towards cabbage roots.

unfavourable and no positive response was observed ($p > 0.05$).

EFFECT OF DISTANCE OF ROOT ON ATTRACTION (Fig. 12)

The number of nematodes reaching zone 1 increased as the distance of their introduction from the root decreased. Maximum numbers were recorded in zone 1 when the nematodes were introduced in zones 2 and 3 i.e., 1 cm and 2 cm from roots. They also showed positive

response when placed 3 cm and 4 cm away from the roots i.e., zone 4 and 5 but not when introduced in zones 6 and 7 i.e., 5 and 6 cm from the roots.

Discussion

From the above observations it is evident that as the cabbage roots grew older, their attractiveness decreased and failed to elicit a response from fed nematodes,

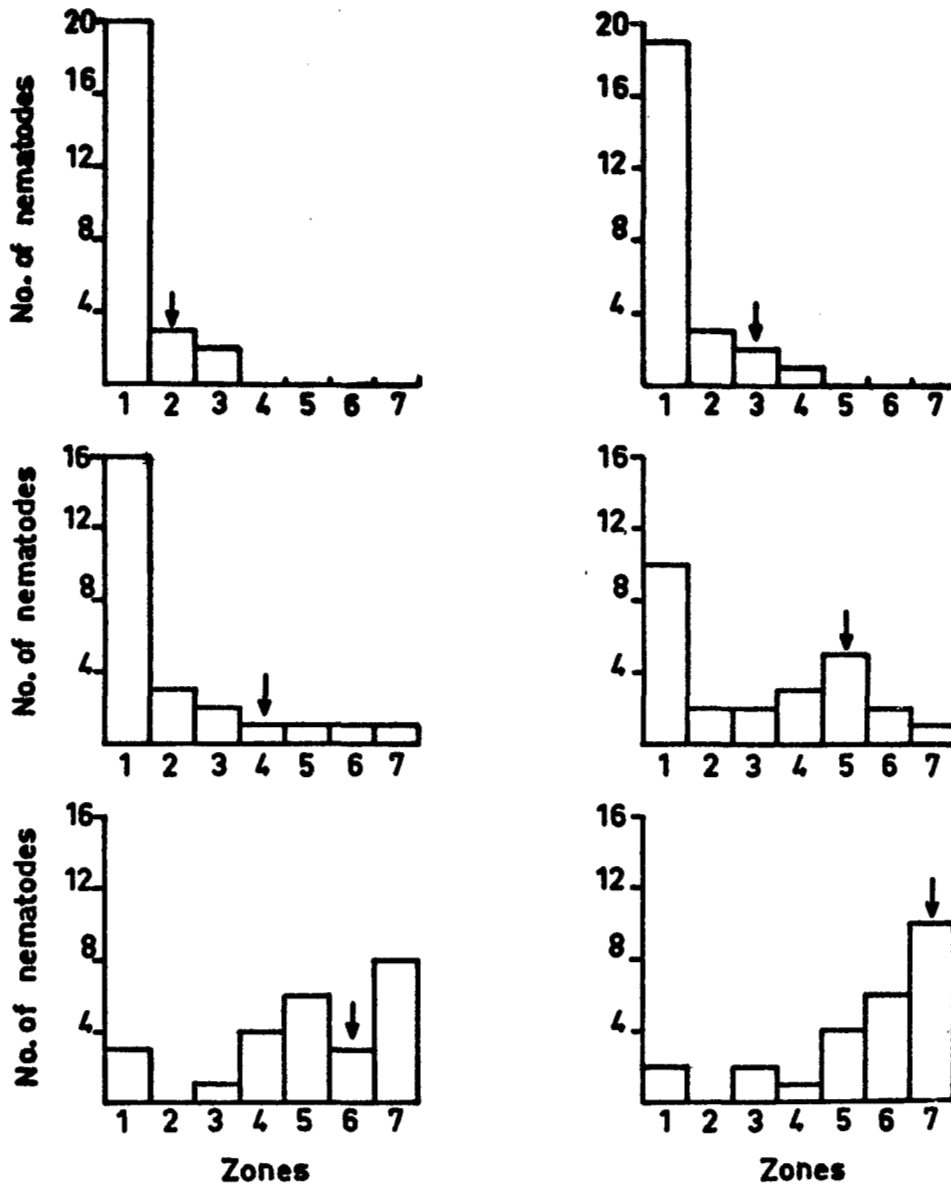


Fig. 12. Effect of distance of cabbage roots on the attraction of *H. oryzae*.

suggesting that they were no longer attractive. Similarly, as the period of food deprivation of nematodes increased their response towards increasing age groups of roots also decreased indicating that besides the unattractiveness of roots some other factor(s) within the nematodes was (were) also governing their responses. Doncaster and Seymour (1973) concluded that in starved nematodes the minimum response threshold decreased and unfed nematodes could perceive weaker stimuli. These observations show that in *H. oryzae*, perhaps, deprivation of food caused an increase in minimum response threshold as 12 day unfed nematodes failed to respond to some of those age groups of roots which attracted lesser unfed nematodes or perhaps, a loss in the degree of attraction with an increase in the period of food deprivation may have resulted from loss of energy, as starving nematodes utilize their reserve food material (Cooper & Van Gundy, 1970; Reversat, 1981a, 1981b). Hence, both factors, age of roots and period of food deprivation appear to independently influence attraction of *H. oryzae* towards host roots.

Weiser (1955), and Lavalley and Rohde (1962) found attractiveness of roots proportional to their growth and Viglierchio (1961) observed attraction to be independent of the age of the roots. The present observations are contrary to both. This could be due to different hosts and nematode species involved but it may also be possible that all nematodes are not equally sensitive to the same stimulus or all plants do not produce similar attractants (Prot, 1980). The possible inhibition of growth of roots in Petri-dishes containing only water-agar may have also influenced attraction of *H. oryzae* as was suggested by Viglierchio (1961). Moreover, roots killed by heating lost their attractiveness (Linford, 1939; Bird, 1962) which confirms the results on the responses of adult *H. oryzae* towards live and dead cabbage roots.

The observations on the effect of various coloured lights support the findings of Moussa, De Grisse and Gillard (1972) who also didn't find any difference in attraction of juveniles of *Globodera rostochiensis*.

The influence of different incubation periods of roots possibly suggest that a minimum period is necessary for the development of perceptible attractant gradients by the roots. The effect of agar concentrations and temperatures on the attraction may be related to the activity of nematodes. Wallace (1969), and Azmi and Jairajpuri (1977b) suggested that the activity of nematodes declined in increasing agar concentrations. Perhaps this reduced activity inhibited attraction of *H. oryzae* adults in more than 2 % water-agar. Similarly, it has been shown (Wallace, 1963) that nematodes became inactive at temperatures 5-10° and 30-40° and that activity of *Meloidogyne incognita* and *M. hapla* juveniles was inhibited by low temperatures (Prot & Van Gundy, 1981b). Thus no response in *H. oryzae* at 5-10° may be attributed to inhibited activity. Besides, the diffusion of root attractants in the agar may also have been influenced by

different temperatures and agar thicknesses which resulted in different nematode responses.

The maximum attraction of *H. oryzae* towards root tip, both excised as well as intact, suggests that perhaps this is the most attractive region as was also proposed by other workers (Pitcher & MacNamara, 1970; Schilt & Cohn, 1975; Stirling, 1976). However, excised roots may introduce into the medium additional unknown substances due to cell breakdown. But these substances apparently did not seem to influence behaviour as the attraction towards different regions of roots, excised and intact, showed a similar pattern.

As compared to *Globodera rostochiensis* juveniles which migrated upto 45 cm in soil (Rohde, 1962) and *Anguina tritici* juveniles which travelled 30 cm upwards to reach their host (Leukel, 1962), *H. oryzae* showed a comparatively weak response as it could reach its host only from a distance of 4 cm which is similar to *Ditylenchus dipsaci* (Blake, 1962). Prot (1977) has shown a 50 cm horizontal and vertical migration in soil of *Meloidogyne javanica* in 9 days towards a susceptible tomato variety. Similarly, the juveniles of *M. incognita* migrated upto 20 cm in soil to reach tomato roots (Prot & Van Gundy, 1981a).

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