

Feeding Behaviour and Histopathology of *Hirschmanniella oryzae*, *H. imamuri*, and *H. spinicaudata* on Rice¹

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Abstract: The feeding behaviour of *Hirschmanniella oryzae*, *H. imamuri*, and *H. spinicaudata* on excised rice roots was very similar to that of other tylenchid nematodes on other hosts. Juveniles and adults of all three species invaded rice roots, causing extensive mechanical damage. Feeding and burrowing activities predisposed roots to secondary infection in unsterile cultures. Surface-sterilized nematodes did not induce necrosis in axenic cultures. **Key Words:** Rice root nematodes, migratory endoparasites.

The genus *Hirschmanniella* contains some of the largest tylenchid nematodes. Eleven species, *H. spinicaudata*, *H. imamuri*, *H. mucronata*, *H. gracilis*, *H. thornei*, *H. belli*, *H. caudacrena*, *H. mangaloriensis*, *H. shamimi*, and *H. indica*, have been found in association with rice, *Oryza sativa* L. (1, 14, 16, 17).

H. oryzae was originally thought to be the causal agent of the 'Omomentek' disease complex of rice in Indonesia (21), which is now known to be a virus disease, 'cadang cadang,' transmitted by a leafhopper, *Nephotettix impicticeps* (14). Root-rot disease of rice has been linked with the feeding activities of *H. oryzae* (5, 20). The occurrence of 'Akiochi' disease, a physiological disorder, is associated with high infestations of *H. oryzae* (8). The browning of rice roots is suspected to be due to micro-organisms but can be aggravated by *H. oryzae* (10).

Stained roots of rice infected with *H. oryzae* show gregarious invasions through the same entry point, and large numbers of

the nematode have been found at the base of the coleoptiles (21). Sivakumar and Seshadri (18) sectioned rice roots infected with *H. oryzae* and observed mechanical damage in cortical parenchyma, necrosis around the feeding area, and dissolution of cell walls.

The apparent lack of information on the biology of *H. spinicaudata* and *H. imamuri* is probably because *H. oryzae* is usually considered the more important species in sympatric occurrences of species of *Hirschmanniella* in most rice-growing areas of the world.

Presented here are detailed observations on the comparative feeding behaviour and associated damage of *H. oryzae*, *H. spinicaudata*, and *H. imamuri* on rice.

MATERIAL AND METHODS

Feeding was observed on excised roots of rice cv IR8. The rice seeds were dehusked and surface-sterilized in 0.1% mercuric chloride for 10 min followed by three washes in sterile distilled water. Single seeds were germinated in 9-cm-diam plastic petri dishes containing 25 ml 1% ion agar made up with modified White's medium in a 25±1 C incubator (12). Roots were excised three days after germination and the seeds were discarded.

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H. oryzae was obtained from infested soil from New Delhi, India; *H. imamuri* came from Suweon, South Korea; and *H. spinicaudata* was from Badeggi, Nigeria. All species were cultured on rice in a heated greenhouse in large sealed pots, simulating flooded paddy conditions (3). The nematodes were extracted by soaking the soil samples in water, sedimenting the large particles and passing the supernatant through a bank of graded-mesh sieves. The contents of the sieves were washed onto a tissue on a plastic mesh in a tray of water, and nematodes were collected in suspension after 24 h. Infected roots were chopped into 0.5-cm pieces, and nematodes were extracted by the maceration-filtration technique (19, 22).

Active nematodes were surface-sterilised in 0.5% hibitane diacetate for 10 min, followed by three washes in sterile distilled water. About 20 to 30 nematodes were introduced around the excised roots and the petri dishes were sealed with plastic tape. The same numbers of unsterilised nematodes were introduced around excised roots in separate petri dishes. All operations were carried out in a sterile laminar-flow chamber, and all petri dishes were stored at 25 ± 1 C in an incubator.

Observations on feeding were begun 24 h after inoculation at magnifications of 100 and 400. Detailed observations were limited to ectoparasitic feeding on the roots because feeding within roots is difficult to observe. The amount of work done per stylet thrust during feeding was calculated by use of the formula $G \times d$, where G is the mass of the nematode and d is the distance travelled by the stylet. G was calculated with Andrassy's (2) formula: $G = (a^2b)/(16 \times 100,000)$, where a is the maximum width, and b is the length of the nematode; $16 \times 100,000$ is a constant.

Infected rice roots from pot cultures of each species were cut into 0.5-to-1.0-cm pieces and fixed overnight in boiling formalin acetic acid or in 3% gluteraldehyde in 0.025M phosphate buffer, pH 6.8, at 0 C (13). The root pieces were dehydrated in a graded alcohol series and embedded in Paraplast (MP 56-57 C). Ten-micrometer sections were cut and stained in modified Weigert's hematoxylin (7). Some infected roots were stained in 0.1% cotton blue or 0.5% acid fuchsin in lactophenol before observations were made.

RESULTS

The general pattern of feeding was the same for all three species (Table 1). Four distinct feeding phases were observed in all species: 1) probing or exploration; 2) stylet penetration; 3) salivation; and 4) ingestion.

Probing or exploration of roots was followed by stylet penetration, which was normally at right angles to the root surface. Rate of stylet thrust was initially low and irregular. The energy output involved in stylet penetration by *H. spinicaudata* was about four times that by *H. imamuri* and 53 times that by *H. oryzae* (Table 2). Often, two layers of cells were punctured by *H. spinicaudata* and *H. imamuri*. After stylet penetration, the nematodes remained motionless. During salivation, the ampulla of the dorsal oesophageal gland duct enlarged, followed by a continuous flow of secretions through the stylet into the punctured cell. The cytoplasm of the cell slightly darkened and the flow of secretion gradually stopped as the median oesophageal bulb began to twitch spasmodically. The twitching soon built up into a steady pulsation, and a reverse flow was observed of cell contents through the stylet into the nematode. The rate of pulsation was considerably lower in

TABLE 1. Times (in seconds) required for feeding functions of three *Hirschmanniella* species.*

	<i>H. imamuri</i>	<i>H. oryzae</i>	<i>H. spinicaudata</i>
Stylet penetration	160 (105-194)	186 (112-206)	148 (93-192)
Salivation	278 (165-401)	368 (183-520)	207 (131-310)
Ingestion	247 (228-317)	286 (102-378)	192 (154-321)
Median bulb pulsation per sec.	3.2 (2.9-3.4)	3.4 (3.0-3.5)	1.8 (1.4-2.2)

*n = 30.

TABLE 2. Forces applied per stylet thrust during feeding by *Hirschmanniella* species.*

	<i>H. imamuri</i>	<i>H. spinicaudata</i>	<i>H. oryzae</i>
Force applied	44.14 $\mu\text{mg}\mu$	169.09 $\mu\text{mg}\mu$	3.17 $\mu\text{mg}\mu$
Stylet length	29-32 μ	40-48 μ	16-19 μ

*Means of 15 observations.

H. spinicaudata than in *H. oryzae* and *H. imamuri*. When the cells collapsed, the nematodes withdrew completely from the root, or started to puncture adjacent cells.

Adults and juveniles of all stages invaded roots, often through the same opening. Juveniles and males moved in and out of the roots more frequently than did gravid females, which remained inside the roots and laid eggs along their burrowed channels. The three species fed at all points along the root but were found more often around root tips and along lateral roots. Both *H. spinicaudata* and *H. imamuri* became coiled during salivation and ingestion, while *H. oryzae* assumed an open C posture.

Damage to the tips of main roots was severe, resulting in cessation of growth and development of lateral roots around the tips. Necrosis was observed in two to four cells around the feeding and invasion sites in roots inoculated with unsterilised nematodes.

In stained roots there were lesions extending along the burrowed channels of the nematode for some distance from the entry point, until, eventually, the nematodes were surrounded by broken, although non-necrotic, cells. No necrosis was observed on or around nematode feeding and invasion sites in axenic cultures. Root growth stopped after 5 weeks, and thereafter general browning occurred around tips, lateral root primordia, root axils, and root hair bases in both axenic and unsterile cultures. Groups of adult nematodes were often observed within roots infected by either *H. imamuri* or *H. oryzae*, but only single individuals of *H. spinicaudata* were observed at any one site. Mechanical damage caused large cavities and collapse of cell walls, mainly within the cortex. The stele was damaged only in roots infected by *H. spinicaudata* (Fig. 1A). The burrowing activities of *H. imamuri* almost severed primordia of lateral roots (Fig. 1B), and

observations were similar in roots infected by the other two species. Such root primordia turned brown when killed, and could be mistaken for lesions caused during feeding. Cortical and lacunal cells were collapsed, and cell walls were broken by nematode feeding.

In infected roots from flooded soil cultures, roots invaded by any of the three species were predisposed to secondary infection by rot-inducing micro-organisms. The nematodes moved progressively from necrotic areas to healthy cortical tissues as necrosis spread. Necrosis, together with separation and breakdown of cell walls and collapse of cells, ultimately killed roots invaded by as few as 10 nematodes.

DISCUSSION

The feeding patterns of the three species are similar to those described for other tylenchid nematodes (6, 9, 23). In all three species the feeding and invasion of root tissues, both inter-cellularly and intra-cellularly, caused extensive mechanical damage. There was a complete lack of necrosis around feeding and invasion sites in axenic cultures of all three species 5 weeks after inoculation. Similar observations have been made on banana roots attacked by *Radopholus similis* (4). The occurrence of necrosis along burrowed channels within rice roots around feeding and invasion sites in contaminated cultures of all three species agrees with other observations (5, 11, 18, 21), and other studies have shown that necrosis is less in uninoculated roots than in roots inoculated with *H. oryzae* (10).

The continuous diminution of necrosis along burrowed cavities and its absence around the nematode for some distance within the root suggests that there may be a phoretic relationship between soil micro-organisms and the rice root nematodes.

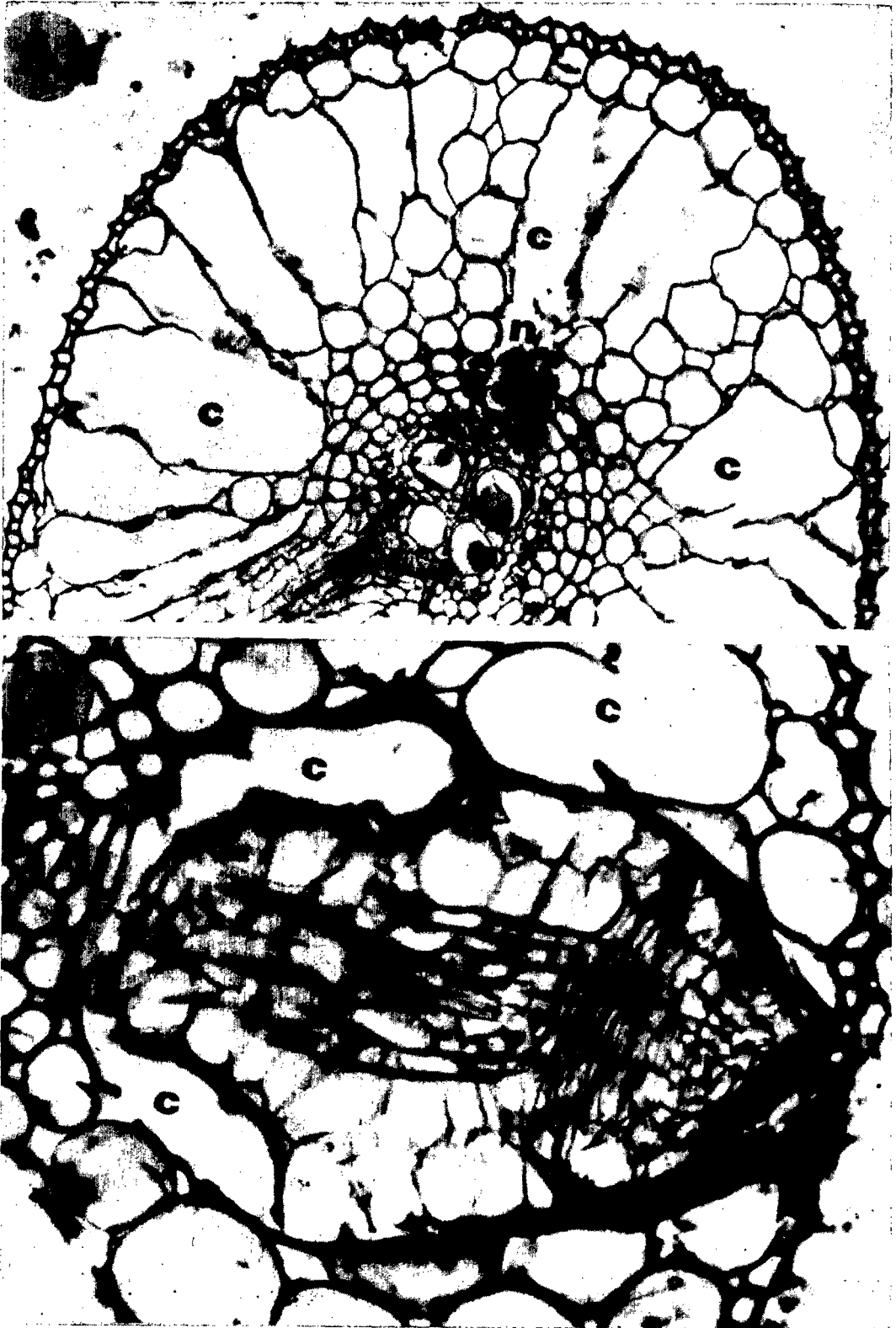


FIG. 1. A) Damage to the stele and cortex of rice root cv IR 8 caused by the feeding of *H. spinicaudata* (c = cavities, n = nematodes). B) Cavities, c, around the base and sides of a lateral root primordium caused by *H. imamuri*.

Feeding of the nematodes caused cessation of growth of the main roots and a proliferation of lateral roots close to the damaged root tips, apparently from breaking of the apical dominance of the roots. The creation of large cavities in the cortical parenchyma of the root, often accompanied by rot-inducing micro-organisms, caused disruption and eventual death of the roots. Because *H. spinicaudata* feeds on the stele, allowing secondary contamination of the vascular tissues, it appears to have a greater damage potential than the other two species. The collapse of cell walls also reduced the mechanical support that roots can offer the plant. Observations of Sivakumar and Seshadri (18) and Mathur and Prasad (11) showing a cell-wall thickening and dissolution in rice roots around sites infected with *H. oryzae* are similar to our observations made on young roots infected by all the three species of *Hirschmanniella*. This did not occur in the absence of secondary organisms, however. Nematodes were commonly found in the aerenchyma tissues and, since aerenchyma cells are adaptations to aquatic life, permitting the movement of oxygen from leaves to roots (15), the damage to these tissues is likely to restrict the passage of oxygen, thus placing the roots under oxygen stress.

The feeding activities of the three *Hirschmanniella* species thus appear to have deleterious effects on the rice plant in terms of mechanical damage to the stele and parenchyma tissues, disruption of growth, and predisposition of the rice root to secondary infection through the feeding and invasion sites. All of these would result in poor grain yield.

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