POPULATION DENSITIES OF RICE ROOT NEMATODE (*HIRSCHMANNIELLA* SPP.) IN LONG-TERM FERTILITY EXPERIMENTS IN NEPAL

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Summary. Long-term soil fertility experiments at Bhairahawa (rice-wheat-rice), Tarahara (rice-wheat), and Rampur (rice-wheat-maize) were sampled to assess the densities of rice root nematodes *Hirschmanniella* spp. Nematode densities in roots and soil samples were determined by the blender-cum-modified Baermann trays and sieving-cum-modified Baermann trays techniques, respectively. The rice root nematodes, *Hirschmanniella oryzae* and *H. mucronata,* were common and observed in most of the rice samples collected, although the population varied greatly among the samples. In wheat crops, rice root nematodes were observed at low densities only in the soil samples, not in roots. Significantly higher numbers of nematodes were observed in the plots receiving N and P fertilizers as compared to those receiving N alone. Potassium fertilizer, up to 50 kg/ha, had no effect on rice root nematode populations, but significantly lower nematode populations were observed in plots fertilized with 100 kg/ha potassium as compared to the control. Also, significantly lower nematode populations and higher rice yields were observed in fields where farmyard manure was applied annually for 16-17 years. Incorporation of rice stubble and Dhaicha before flowering had no effect on nematodes.

Rice (*Oryza sativa* L.) is the most important food grain crop in Nepal, grown by most farmers in all agro-ecological zones, from the Terai (100-300 m above sea level), valleys and foot-hills (100-1000 m), to the high mountains (2600 m). Wheat (*Triticum aestivum* L.) is the third most important cereal crop in Nepal and is grown in the winter season in the same fields after rice. The rice-wheat system covers 30% of the rice area and 75% of the cultivated wheat area (Huke *et al.*, 1993). Despite an increasing trend to use more inputs and adopt new technologies, productivity of rice and wheat is not responding in terms of yield increase as it should be. Productivity per unit area is declining in some locations and production is less than that found in other developed countries in the region. Several factors are responsible for the low production. Of these, availability of irrigation water, soil nutrient status and outbreaks of insect pests and diseases are major constraints to higher productivity (Pimentel, 1983). Blast, bacterial blight, and sheath blight are among the important diseases on rice, whereas rust, leaf blight and loose smut are important for wheat (Dahal *et al.*, 1992). Nematodes that feed on roots and generally do not produce specific above-ground symptoms are also possible candidates for this decline.

Rice root nematodes (*Hirschmanniella* spp.) and root knot nematodes (RKN, *Meloidogyne* spp.) are the most important nematodes on lowland rice (Fortuner and Merny, 1979). These nematodes have been reported to cause as much as 50% yield reduction (Jairajpuri and Baquri, 1991), but generally they cause about 20% yield reduction on lowland rice (Prot, 1993). *Tylenchorhynchus, Pratylenchus, Hoplolaimus* and *Helicotylenchus* nematodes are reported to cause significant yield reduction in wheat in India (Prasad and Gaur, 1975).

Another major constraint in rice production is plant and soil nutrition. Fertilizers have to be used annually to maintain yield (Gami, 1996). Long-term fertility experiments have been conducted in Bhairahawa and Tarahara in rice-wheat systems for the past 16 years and have clearly shown that nitrogen alone cannot sustain the yield of rice or wheat. However, these experiments showed that addition of nitrogen together with phosphorus is essential to maintain productivity.

Plant nutrients not only play a vital role in crop yield, but also may impact the incidence and damage of insect pests and diseases. High use of fertilizers has often been considered as conducive to the outbreak of a number of insect pests and diseases of rice. Fortuner (1977) reported that fertilizers had no effect on *Hirschmanniella* spp.), but Mathur and Prasad (1972) noted that fertilizers improved plant growth and increased nematode populations. Data on the effect of long-term use of fertilizers on the rice root nematode are not available. This investigation was conducted to understand the role of long-term use of various fertilizers and manures on population dynamics of *Hirschmanniella* spp. in on-going research plots in Nepal.

MATERIALS AND METHODS

Long-term experiments on rice-rice-wheat at Bhairahawa and on rice-wheat-fallow in Tarahara have been maintained for the past 17-18 years with nine treatments

(Table I) in randomised block designs replicated three times.

Rice and wheat were grown according to commercial recommendations for each crop. These plots were sampled during the rice and wheat growing seasons and after the wheat crop. Another long-term rice-wheat fertility experiment at Bhairahawa, running for six years to assess the impact of inorganic and organic manures, especially Daicha incorporated as green manure, was also sampled. This experiment was a split plot randomised block design with three replications (plot size was 10 x 5 m) with the treatments indicated in Table II. Plots established in farmers' fields in Rupandehi and Chitwan were fertilized with 0, 50 or 100 kg/ha potassium in combination with 100 kg nitrogen and 100 kg phosphorus. Soil and root samples were collected by following a zigzag track within each plot. The samples were processed separately to assess the nematode populations

Table I. Number of *Hirschmanniella* in rice and wheat in different fertility regimes at Bhairahawa and Tarahara 1993-94.

	1993		1994			
	Rice		wheat[1]	wheat[2]	Rice	
Fertilizers	soil	root	soil	soil	root	soil
Rice-wheat-rice (Bhairahawa)						
$N^{0}P^{0}K^{0}$	55.0bc	3.3b	1.5b	25.0cd	3.7b	12.0d
$N^{100}P^{0}K^{0}$	8.0c	3.0b	1.5b	15.0d	3.4b	18.0d
$N^{100}P^{30}K^{0}$	53.0ab	22.6a	8.0a	366.5a	13.0a	36.5c
$N^{100}P^{0}K^{30}$	9.0c	15.0ab	1.5a	25.0cd	2.4b	13.0d
$N^{100}P^{30}K^{30}$	80.0ab	8.6ab	8.0a	98.0bc	13.0a	57.0b
$N^{100}P^{0}K^{0}+S$	5.0c	3.6b	1.5b	80.0bc	1.0b	12.0d
$N^{50}+S$	26.5c	12.5ab	1.5b	18.0d	1.7b	17.0d
$N^{50}P^{50}+S$	211.5a	23.6a	8.0a	283.0ab	15.0a	77.0a
FYM	50.0bc	8.0ab	1.5b	53.0cd	1.3b	20.0cd
Rice-wheat-fallow (Tarahara)						
$N^{0}P^{0}K^{0}$	4.6b	23.0ab	8.0ab	-	2.0b	20.0ab
$N^{100}P^{0}K^{0}$	3.0b	16.5bc	5.0b	-	1.7b	8.0b
$N^{100}P^{30}K^{0}$	10.0a	24.0ab	13.5a	-	5.3ab	39.0a
$N^{100}P^{0}K^{3}$	3.7b	16.5bc	8.5ab	-	1.0b	12.0ab
$N^{100}P^{30}K^{30}$	7.0ab	21.5ab	10.0ab	-	5.0ab	43.5a
$N^{100}P^{0}K^{0}+S$	3.4b	13.0bc	8.5ab	-	1.7b	10.0ab
$N^{50}+S$	3.0b	13.0bc	10.0ab	-	2.0b	13.5ab
$N^{50}P^{50}+S$	11.0a	50.0a	16.5a	-	7.0a	43.5a
FYM	2.7b	8.0c	10.0ab	-	1.7b	23.0ab

1 = at maximum crop growth period, 2 = after crop harvest.
All fertilizers rates are given as kg per hectare), S = stubbles and FYM = Farmyard Manure (10 tonnes per ha was applied).
Counts are per g of roots and per 200 cm^3 soils.
In each column, means with the same letter are not significantly different at P = 0.05 by DMRT test.

Table II. Rice root nematode populations as affected by fertilizers and green manuring, 1995-1996.

Treatment	Wheat/ Soil	Rice[1/] Soil	Rice[2]/ Soils	Rice/ Roots	Rice/ Roots
	1996	1995	1996	1995	1995
$N^{100}+P^{o}$	4.3b	6.17c	29.3bc	27.0bc	35.0a
$N^{100}+P^{50}$	4.3b	55.0a	138.7a	31.5b	32.0ab
N^{100} $+P^{100}$	4.3b	42.3ab	91.0abc	14.5c	26.6abc
$N^{100}+P^{60}$	6.0ab	36.7abc	68.0abc	42.5a	25.6abc
N+Daincha	7.3a	15.9bc	59.67abc	10.0c	17.6bcd
NP+Daincha	9.0a	14.0bc	124.7ab	23.0bc	16.0bcd
N+FYM	7.6a	15.0bc	40.3bc	27.5bc	12.3cd
NP+FYM	2.3b	46.0ab	73.6abc	27.0bc	11.0cd

N = Nitrogen, P = Phosphorus, FYM = Farmyard manure
Counts are per g of roots and per 200 cm^3 soils.
In each column, means with the same letter are not significantly different at P = 0.05 by DMRT test.

under different types and levels of fertilizers.

Since no nematodes were observed in the wheat roots, additional pot and field experiments were conducted in the National Agricultural Research Station (NARC) at Bhairahawa. The wheat was grown in pots in soils naturally infested with *Hirschmanniella spp*. The experiments included five fold replication in a completely randomised design. The nematode populations were assessed before wheat sowing, at the maximum tiller stage and at harvest. Similarly, the nematode populations were also assessed on rice at different growth stages.

Sampling of the field experiments was done according to the methods of Barker (1985). Five samples (each 250 cm^3) from each plot were taken, and consisted of roots and soil. A 200 g sub-sample of soil and a 1 g sub-sample of roots were taken for processing separately.

Before processing the soil sub-samples, clods were broken and the samples were mixed with about 5 litres of water, stirred thoroughly and allowed to settle for about one minute. The soil suspension was passed through a 60-mesh sieve nested over a 325-mesh sieve. The process was repeated twice for complete recovery of nematodes. The material over the 325-mesh sieve was transferred to a beaker and the solution was processed further. For processing root samples, roots were washed thoroughly, cut into 1-5 cm lengths and blended thoroughly. The root and soil suspensions were placed in small sieves lined with tissue paper and the sieves were placed in Petri plates filled with water for 36 hours. The water suspension was collected and the volume was adjusted to 10 ml. The number of nematodes was determined directly (Zuckerman *et al.*, 1971).

Nematode means and ranges were calculated and t-tests and analyses of variance were carried out to test differences. Duncan's multiple range test was used to compare the means.

RESULTS

In rice crop, *Hirschmanniella* spp. were observed in both roots and soils, whereas the nematodes were observed only in soils and not in roots of wheat grown in the same fields or pots. The nematode populations were low at the maximum growth stage of wheat but were higher immediately after wheat harvest, with significant differences between treatments (Table I). Data from pots are not presented.

In the rice-rice-wheat crop rotation at Bhairahawa, higher nematode populations were observed than in the rice-wheat crop rotation at Tarahara (Table I). Lower numbers of nematodes were observed in rice plots in Bhairahawa in 1994 than in the same rice plots in 1993, and also after the wheat crop. Similarly, root populations of the nematodes were lower in 1994 than in 1993 in the plots at Tarahara.

There was no significant difference between *Hirschmanniella* spp. populations in the plots supplied with 100 kg nitrogen (N) and those without N applications (Table I). Plots receiving N (50 and 100 kg per hectare) but no phosphorus (P) had generally low population levels of *Hirschmanniella* spp. in roots. The yield of rice was also low in the plots without P (Fig. 1).

However, nematode populations were significantly

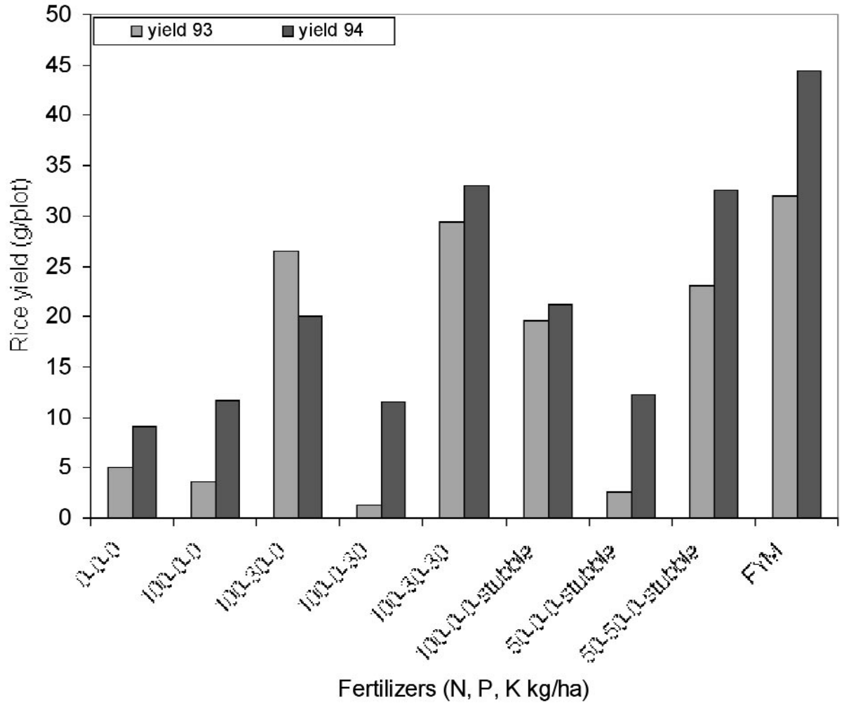

Fig. 1. Rice yield (g/plot) in the long-term fertility experiment at Bhairahawa, 1993-1994.

higher in the plots receiving P and N as compared to the plots without P (Table I), and these plots had higher yields than plots without P (Fig. 1). Also, the populations of *Hirschmanniella* spp. were not different in the plots fertilized with 30 kg/ha of P and various combinations of N and potassium (K) at 30 kg/ha (Table I).

When different levels of K were applied in combination with N 100 and P 50, no differences were observed between the nematode populations in the plots supplied with 0 and 50 kg K or those with 50 and 100 kg K. However, *Hirschmanniella* populations were higher in the plots supplied with 0 K than in plots receiving 100 kg/ha K. (Table III). Overall, there was a trend for a decreasing *Hirschmanniella* population with increasing rate of K application.

Table III. Effect of different levels of potassium applications to rice on *Hirschmanniella* populations.

Fertilisers	Chitwan		Bhairahawa	
	Root	Soil	Root	Soil
NPK_0	88.78a	111.80a	68.0a	125.0a
NPK_{50}	50.67ab	66.80ab	45.0ab	73.55ab
NPK_{100}	42.00b	46.8b	29.33b	23.55b

In each column, means with the same letter are not significantly different at (P = 0.05) by t test. Counts are per g of roots and per 200 cm^3 soils.

When rice stubble was incorporated into rice field soils with the addition of P, there were significantly higher populations of *Hirschmanniella* spp. than in plots receiving only the rice stubble. In addition, there were no significant differences between populations of *Hirschmanniella* spp. in the soils of plots receiving the rice stubble and those without the stubble, but receiving similar N and P rates (Table I).

Daicha incorporated before flowering as a green manure had no significant effect on populations of *Hirschmanniella* spp. in rice (Table II). Similarly, the incorporation of farmyard manure in combination with N or N plus P for 6 years had no significant effect on the populations of *Hirschmanniella* spp. Interestingly, plots receiving farmyard manure without N and P applications for 16 years had fewer *Hirschmanniella* spp. in rice roots and higher rice yields.

DISCUSSION

In rice, *Hirschmanniella* was commonly observed in all fertility experiments irrespective of location or crop rotation. Although *Meloidogyne* was observed at higher densities, it was found in few samples and locations; *Tylenchorhynchus* and *Hemicycliophora* were observed in low frequencies and densities in few samples only (data not presented). *Hirschmanniella* spp., endo-parasites, were observed in most of the rice root and soil samples processed, but only in rhizosphere soil samples from wheat. The failure to observe *Hirschmanniella* spp. in wheat roots suggests that the nematodes may be feeding ectoparasitically on wheat or that wheat is not a host. Rao (1978) also observed that wheat is not a host for this nematode. The presence of this nematode in wheat soil may be due to carryover from a previous rice crop. Pokharel (1993) observed the survival of this nematode for up to 6 months in soil in the absence of a rice crop. In wheat rhizosphere soil, the *Hirschmanniella* spp. population was very low with no significant differences between treatments at the maximum growth stage. This may be due the effect of temperature, which was low during maximum crop growth of wheat and increased at the time of wheat harvest. The optimum temperature for survival of *Hirschmanniella* spp. was reported to be 25-30 °C, with a minimum of 10 °C (Babatola, 1981). Thus, during the relatively cold wheat-growing season and in the absence of a suitable host, these nematodes may become inactive in order to survive and become active again with increasing temperatures. It has been reported that, under adverse conditions, this nematode survives through quiescence or anabiosis mechanisms (Thorne, 1961; Babatola and Bridge, 1979; Babatola, 1981). At favourable temperatures, reactivation of this nematode was obtained by providing sufficient moisture.

Higher nematode populations were found in a rice-wheat-rice rotation than in a rice-wheat-fallow rotation. Khuong (1987) obtained similar results in Vietnam in a general survey. The higher populations obtained in Bhairahawa and Tarahara in 1993 compared to those obtained in 1994 may be due to sampling time. In 1994, sampling was done at crop maturity, whereas sampling in 1993 was performed at an early crop growth stage.

Soil fertility management is an important factor in crop production. Fertilizer application may also directly or indirectly affect survival, multiplication and damage caused by the nematodes to host crops. The higher numbers of nematodes in the plots fertilized with both nitrogen and phosphorus may be due to the better growth of rice, which was reflected in the higher yield obtained. In this investigation, levels of N applications alone did not affect nematode populations and neither did they support acceptable crop yield.

These results were contradictory to those of Ishikawa as cited by Ichinohe (1971) and Rao (1970) who reported that the application of nitrogenous fertilizers increased nematode populations as well as plant growth. Rao (1978) also found a positive correlation between the level of nitrogenous fertilisers supplied and the number of *Hirscmanniella* spp. However, the soils where the present investigations were conducted were deficient in phosphorus, which resulted in reduced plant growth (Regmi, 1996). This suggests that nematodes are influenced more by plant growth rather than simply by the direct applications of nitrogen. Fortuner (1977) also found no effect of fertilizers on *Hirschmanniella* spp. Mathur and Prasad (1972) reported increased *Hirschmanniella* spp. populations and plant growth with fertilizer applications but did not mention the types of fertilizers used.

Significantly lower nematode populations were observed in plots in the field supplied with 100 kg/ha K as compared to those with 0 kg/ha K. Ishijawa, cited by Ichinohe (1971), reported that application of potassium fertilizers drastically reduced *Hirschmanniella* spp. populations, although there was no mention of the level of K used.

Dhaicha (*Sesbania* spp.) is a potential green manure crop as well as a nematode trap crop (Prot *et al*., 1992). No effect on *Hirschmanniella* spp. was observed when Dhaicha was incorporated into soils in combination with chemical fertilizers (N and NP). Similar results were observed when Dhaicha was incorporated before flowering (Pokharel, 1993). However, incorporation of Dhaicha after flowering reduced nematode populations by its trapping action (Prot *et al*., 1992). This could not be confirmed in the long-term experiments sampled in this investigation.

The data obtained in this investigation suggest that the main effect observed on the populations of *Hirschmanniella* spp. was due to P applications rather than the incorporation of rice stubble. This result is in contrast to that of Babatola (1984), who reported that the spreading and burning of stubble reduced *Hirschmanniella* spp. populations. The latter may have resulted from the addition of potassium in the ash (Babatola, 1984).

Farmyard manure application reduced the populations of *Hirschmanniella* spp. This result was in agreement with that of Ishijawa, cited by Ichinohe (1971), who reported that the application of compost decreased nematode populations throughout the rice-growing season. The low population densities of *Hirschmanniella* spp. in these plots may be either due to an antagonist present in farmyard manure or to chemicals released from it.

There is a need to develop an integrated approach to manage populations of *Hirschmanniella* spp. to assure high rice yield. The application of higher doses of potassium and continuous use of farmyard manure might help to reduce nematode populations and the damage they cause to rice.

ACKNOWLEDGEMENTS

We are thankful to CIMMYT Regional Office, Kathamndu for providing funds for this study and Prof. George Abawi for critical review of the manuscript.

LITERATURE CITED

Babatola J.O., 1981. Effect of oxygen and temperature on the activity and survival of *Hirschmanniella* sp. *Nematologica, 26*: 289-294.

Babatola, J.O., 1984. Rice nematode problem in Nigeria, their occurrence, distribution and pathogenesis. *Tropical Pest Management, 30*: 256-265 (Abstr.)

Babatola J.O. and Bridge J., 1979. Pathogenicity of *Hirschmanniella oryzae, Hirschmanniella imamuri* and *Hirschmanniella spinicaudata* on rice. *Journal of Nematology, 12: 48-53*

Barker K.R., 1985. Nematode extraction and bioassay. Pp. 19-35. *In*: An advanced Treatise on *Meloidogyne*, Volume II. Methodology (Barker K.R., Carter C.C. and Sasser J.N. eds). North Carolina State University, Raleigh, N.C., USA.

Dahal G., Amatya P. and Manandhar H.K., 1992. Plant Diseases in Nepal. *Review of Plant Pathology, 71*: 797-807.

Fortuner R., 1977. The fertilisation of rice and damage caused by *Hirschmanniella oryzae* (Van Breda de Haan 1902) Luc and Goody 1963. *Process Verbal de la seance du Juin 1977. Academie d'Agriculture de France, 624-630.*

Fortuner R. and Merny G., 1979. Plant parasitic nematodes of rice. *Revue de Nématologie, 2*: 79-102.

Gami S.K., 1996. Country paper on appropriate use of fertilisers. Pp. 331-352. *In*: Proceedings on appropriate use of fertilisers in Asia and the Pacific (Ahmed S. ed.). Asian Productivity Organisation, Food and Fertiliser Technology Centre for the Asian and Pacific Region, Taipei, Taiwan.

Huke R., Huke E. and Woodhead T., 1993. Rice-Wheat Atlas of Nepal. IRRI, CIMMYT and NARC, Kathmandu, Nepal, 33 pp.

Ichinohe M., 1971. Nematode diseases of rice. Pp. 127-143. *In*:_Economic Nematology (Webster J. M. ed.). Academic Press, New York, U.S.A.

Jairajpuri, M. S. and Baquri Q. H., 1991 *Nematode Pest of Rice*. New York, Oxford and IBH Publications Co, 66 pp.

Khuong N.B., 1987. *Hirschmanniella* spp. in rice fields of Vietnam. *Journal of Nematology, 19*: 82-84.

Mathur V.K. and Prasad S.K., 1972. Role of rice root nematode (*Hirschmanniella oryzae*). *Indian Journal of Nematology, 2*: 158-168.

Pimentel D., 1983. Environmental aspects of pest management, pp 185-201. *In*: Chemistry and World Food Supplies: The New Frontiers, Chemarawn II (Shemilt L.W. ed.). Pergamon Press, Oxford, U.K.

Pokharel R.R., 1993. Population dynamics and Management of rice root and other plant parasitic nematodes in lowland rice ecosystems in Nepal. *Journal of Institute of Agriculture and Animal Science, 14* : 69-76.

Prasad S.K. and Gaur H.S., 1978. Relation between nematode population and yield of wheat. *Indian Journal of Nematoloy, 4*: 152-159.

Prot J.C., 1993. Monetary estimates of nematode problems, research proposal and priorities: The example in south and Southeast Asia. *Fundamental and Applied Nematology, 16*: 385-388.

Prot J.C., Soriano I.R.S., Matias D.M. and Savary S., 1992. Use of green manuring crops in control *of Hirschmanniella mucronata* and *H. oryzae* in irrigated rice. *Journal of Nematology,* 24: 127-132.

Rao Y.S., 1970. Rice nematodes chapter 5, PANS Manual No. 3. RIE, London, U.K., 99-107 pp.

Rao Y.S., 1978. Nematode problem of rice and line of future research. Pp. 401-411. *In*: Increasing Yields of Rice in Kharif. Central Rice Research Institute Cuttack, India.

Regmi A.P., 1996. Long term effect of fertilisers and manures in a rice wheat system and sustainability of soil fertility under this system. Proceeding of Wheat Research Report September 7-10, 1995, N.W.R.P. Sidharthanagar, Nepal, pp. 363.

Thorne G., 1961. *Principles of Nematology*. McGraw-Hill Book Co., New York, U.S.A. XIV + 553 pp.

Zuckerman B.M., Mai W.F. and Rohde R.A. 1971. *Plant Parasitic Nematodes, Vol. 1. Morphology, Anatomy, Taxonomy and Ecology.* Academic Press, New York, U.S.A., 360 pp.

Accepted for publication on 3 July 2004.