DAMAGE OF ROOT-KNOT NEMATODE (*MELOIDOGYNE GRAMINICOLA*) TO RICE IN FIELDS WITH DIFFERENT SOIL TYPES

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Summary. The rice root-knot nematode (*Meloidogyne graminicola*) is an important pathogen of rice in Nepal. Surveys were conducted in 65 rice fields in the Rupandehi, Chitwan and Parsa districts of Nepal by collecting rice soil and root samples from 25, 20 and 20 grower fields, respectively, in 1999-2001. Soil bioassays using these field soils were done in 1999 and 2000 to understand the factors affecting variability in population densities of the rice root-knot nematode, severity of symptoms and crop yields. Rice fields with light soils and low yields had larger nematode population densities. Similarly, larger nematode population densities were recovered from symptomatic fields/symptomatic plants (chlorotic plants in patches with reduced growth and reduced tiller numbers) as compared to asymptomatic plants/fields. Higher nematode populations were observed with severe symptomatic plants. In the surveys and the screen-house tests, all rice cultivars tested were susceptible to the nematode but acted differentially. Soil physical and chemical properties from these fields were analyzed and correlated with nematode population densities using different models. A positive relationship with sand and phosphorus, a negative relationship with nitrogen, no relationship with silt and pH and a variable relationship with clay (negative to positive relationship) to nematode population densities were observed in the absence of other predictors and interaction terms in the models. However, when these predictor variables were considered in the presence of other predictor variables, with or without interactions, the relationships were different. In addition, volunteer rice and seedling rice were also sampled from different parts in 1999 to understand their role in nematode survival and dispersal, respectively. Volunteer plants and rice seedlings supported nematode survival, growth, and multiplication in the absence of a rice crop in the field, so helping to increase the severity of the nematode problem. The effect of the rice root-knot nematode populations on rice yield reduction was studied under the influence of nitrogen plus phosphorus and compost in micro-plots. The results indicated that nematode-induced rice yield reduction was low when plots were supplied with nitrogen and phosphorus as compared to control plots (no fertilizer or compost).

Keywords: Rice root-knot nematode, soil physical and chemical properties, correlation, yield loss.

Soilborne diseases are becoming increasingly important in the rice-wheat production systems of the Indo-Gangetic plains. Increasing evidence suggests that manipulating soil biological processes can benefit yield, and this area needs much more attention (Duxbury, 2002). One of the most important soilborne pests of rice (*Oryza sativa* L.) in rice-based cropping systems is the rice root-knot nematode, *Meloidogyne graminicola* Golden *et* Birchfield. This nematode reduced the rice yield by more than 17% in a greenhouse experiment and the yield losses might go as high as 80% (Tandingan *et al*., 1996; Soriano *et al*., 2000). Limited field surveys and management studies have clearly documented the importance of this nematode in rice production in Nepal (Pokharel, 1993). Unlike other root-knot nematode species, *M. graminicola* is adapted to survive and infect rice under upland, non-flooded (irrigated) and flooded soil conditions (Prot *et al*., 1994). Soriano *et al*. (2000), however, reported that raising rice seedlings in flooded soils or flooding early minimizes yield loss.

Manipulating soil biological processes can increase yield. For example, soil solarization in 25 randomly selected farmers fields in Chitwan district in Nepal increased grain yield of rice by 1-2 tons/ha (Duxbury, 2001). Rice seedlings in solarized plots had longer roots and fewer root-knot galls than seedlings in non-solarized plots. Nematodes survive and multiply in soil, and their numbers and the damage that they cause can be affected by soil chemical and physical factors. These factors, especially in rice, are important for nematode management.

Rice growers in Nepal have frequently reported that their plants are stunted and that the overall growth in the field is uneven (unpublished observations). They often attribute such symptoms to micronutrient deficiencies and ignore the possibility of nematode involvement. Examination of some of the fields, however, resulted in finding galls on the roots of symptomatic plants and high numbers of root-knot nematodes in the soil in a preliminary study. Therefore, the involvement of rice root-knot nematode in such symptom expression (chlorotic plants in patches with reduced growth and height) was suspected but actual information for these rice fields was not available.

In the present study, surveys in farmer fields were carried out to determine whether *M. graminicola* was involved in producing such symptomatic plants. In addition, the roles of rice varieties, soil types and rice yield

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were determined. The relationship of soil physical properties with the nematode population densities was studied using different models. Since the nematode survives in soil having the properties measured, knowledge of their interrelationships is important in understanding the biology of the nematode. These soil properties were never considered in different combinations. Although data already exist indicating that the nematode can be a problem in many fields other than those planted to rice, more field data are needed to determine whether symptoms caused by this nematode are being misdiagnosed. Moreover, no such study had been conducted in rice grown in flooded conditions. Thus, this study examined how soil, crop health, crop genotype, volunteers, sprouted plants and seedlings affect nematode infection, plant growth and/or damage and also studied the relationship of soil nutrients with the nematode population density. Finally, the study also determined whether nitrogen plus phosphorus and compost reduced yield losses caused by *M. graminicola*.

MATERIAL AND METHODS

Field surveys

Altogether, 650 rice samples (consisting of soil and roots) from 65 grower fields were collected, processed and nematode population densities assessed. The samples were collected from Rupandehi, Chitwan, and Bara/Parsa districts in 1999 and 2000 with a shovel. An additional twenty samples were collected in the same fields of Rupandehi district in 1998 by pulling a hill of rice and they are not included in the comparison except for comparing sampling methods. In all surveys, ten representative hills of rice (one hill representing one sample) in each field were dug out in a zig-zag "W" pattern, from each 0.1-0.5 hectare with a shovel or a spade, along with a big ball of soil around the plants. Excess soil (leaving about 4 to 5 kg soil around roots) were removed by hand to protect roots from breaking. The collected samples were labeled and brought to the Institute of Agriculture and Animal Science (IAAS) laboratory for processing. Roots and soil from each sample and field were processed separately to assess population densities of the nematode. Therefore, the soil of each sample was carefully separated from the roots, mixed thoroughly and the nematodes were extracted from 200-cm^3 sub-samples; an additional 100-cm^3 sub-sample was processed for physical and chemical analysis. The remaining soil from each sample was saved for the bioassay test. The roots were washed and assessed for root-galling severity (RGS) using a 1 to 9 scale in which 1 = no galls (healthy roots), 2 = ≤ 5% roots galled, 3 = 6-10%, 4 = 11-18%, 5 = 19-25%, 6 = 26-50%, 7 = 51-65%, 8 = 66-75%, and 9 = >76 -100% of roots galled (Mullin *et al*., 1991). Nematodes were also extracted from 5 g roots using the blender method (Prot *et al*., 1993) combined with modified Baermann's funnel method (Whitehead and Hemming, 1965), and from the soil by combining sieving with modified Baermann trays method. The nematodes were identified to species level, counted and presented per gram of roots or 100 cm^3 of soil.

Nematode population and rice cultivars in the field. During the field surveys, rice cultivars grown in each grower field were noted. Averages of the root-knot nematode data per cultivar in each field were calculated to evaluate the response of the cultivar to this nematode. The soil samples collected in each field were saved for soil bioassay.

Nematode population, soil types and rice yield. In Rupandehi, twenty irrigated (mostly with deep-well water) rice fields with known yield and soil types were selected. High and low yielding fields with different soil textures were previously identified by the Rice-Wheat Long-term Monitoring Project (Dr. Peter Hobbs, personal communication). Twenty fields (five fields in each category) were selected based on the following characteristics: *a*) high yield, light textured soil; *b*) high yield, heavy textured soil; *c*) low yield, light textured soil; and *d*) low yield, heavy textured soil. In the same district, an additional five fields with symptomatic (chlorotic plants in patches with reduced height and tiller numbers) rice plants and five normal looking fields were also sampled in 2000.

Nematode population, crop health and soil types. Similarly, twenty fields that were mostly rain-fed or channel-irrigated were sampled in Chitwan district. The four following field categories were selected as before; *a*) asymptomatic fields with light textured soil; *b*) asymptomatic fields with heavy soil; *c*) symptomatic plants/fields with light soil; and *d*) symptomatic plants/fields with heavy soil. Five fields in each category were sampled with ten random samples in each field. The samples were collected and processed following the protocol described before.

Nematode population and severity of symptoms. In Bara, all the 20 fields sampled were symptomatic (mild to severe) based on visual observations such as stunted growth, lower tiller numbers and chlorotic appearance in patches (Fig. 1). The fields having more than two such patches and that had a history (more than 2 years) of such patches as reported by growers were considered severely symptomatic fields, whereas those having only one or two such patches and no long history of occurrence were considered as less severely symptomatic fields. Ten of the fields were considered severely symptomatic, and the remainder less severely symptomatic in history and/or appearance. Rice root and soil samples were collected and processed as described above.

Determination of potentially economically important

Fig. 1. A rice field showing patches of chlorotic plants associated with large population densities of *Meloidogyne graminicola*.

fields. Since other nematode genera were observed only in low densities and frequencies, only the frequencies and abundances of *M. graminicola* were calculated and used to determine which fields out of the 65 grower fields in the survey would potentially suffer economically important damage. A nematode genus was considered potentially economically important when the abundance index was greater than or equal to 2.3 in the soil and 1.3 in the roots and with frequency greater than 30% (Fortuner and Merny, 1973). The abundance index was the logarithm of the average of the evaluated population in all the fields where the species was observed and the frequency index was the percentage of the fields where the species was observed (Fortuner and Merny, 1973).

Relationship of M. graminicola with soil physical and chemical properties. Physical and chemical soil analyses of all the samples collected from 65 grower fields were performed by the Soil Science Division, National Agriculture Research Council Khumaltar, Katmandu, Nepal. The hydrometric method was used for textural analysis and the Walkerley-Black method, Bray no. 2, Kjeldahl, and Flame Photometric methods were used for organic matter, phosphorus, and nitrogen contents, respectively. Soil pH was determined using a pH meter in 1:1 soil to water. Multi-variate regression analysis (Proc reg), a computer based SAS application (SAS Enterprise, SAS Institute) was performed to correlate the soil texture (sand, silt and clay percentage) and chemical content (nitrogen, phosphorus, pH and organic matter content) with the populations of root-knot nematode in rice soils and roots. Soil factors were considered as independent (predictor) variables and nematode populations in soils and roots as dependent variables. Three different sets of models were tested: 1) single predictor (assuming the absence of other variables), 2) predictor in presence of other variables but no interactions, and 3) predictor variables with interactions. Due to the high number of predictor variables in the model, all the interaction terms (with different levels) could not be adjusted in one analysis. Thus, a separate model was run for each level of interactions.

Influence of volunteer rice plants and seedlings on nematode populations. Volunteer rice plants and rice seedlings growing in nurseries were collected from grower fields. Five samples in each field, randomly taken in a zigzag manner, of volunteer rice plants along with soil, were collected in March-April of 1999. A total of 80 samples (roots and soil) were collected from grower fields throughout Nepal in 1999 and a bioassay was done in 2000. Rice hills were uprooted, washed, and root-knot nematode galling severity (RGS, scale of 1-9) assessed. The plants with any visible root galls were considered infected for the purpose of calculating the percentage of infection by root-knot nematode. Fields with only one sample infected out of five samples in each field (either root or soil) were still considered infected.

Screen-house tests

Bioassay and nematode severity. Bioassays of soil samples collected from Rupandehi, Chitwan and Bara were conducted at IAAS, Rampur. The soil samples collected in the 1999 surveys were used for the 2000 bioassays. Fifteen hundred cm^3 of soil of each sample was mixed with an equal volume of the autoclaved field soil. The resultant soils were placed in clay pots and planted to wheat CV RR 21. After wheat harvest, the same cultivar as grown in the relevant field during the 1999 survey was planted. The pots were maintained outdoors in a screen-house and watered regularly. After 75 days, the rice plants were uprooted, the roots were washed free of soil and rated for RGS.

Evaluation of grower cultivars. Twelve rice cultivars most commonly grown in the three districts were obtained from the National Research Council, Nepal, and evaluated in a screen-house for their reaction to the root-knot nematode. The experiment was conducted in sterile soil inoculated with 5,000 eggs per pot (500 cm^3 soil) of a population of *M. graminicola* reared on rice cv. Mansuli in a greenhouse at IAAS. The nematode eggs were obtained by blending rice roots. The pots were sown with ten seeds of a cultivar of rice per pot, replicated four times, and maintained in a screen-house with regular watering and monthly fertilization with a 20:20:20 NPK fertilizer. After 75 days, the plants were uprooted and the roots washed and rated for root-galling severity (RGS) rating.

Bioassay of additional fields. The root-knot nematode associated with the rice was studied by collecting soil samples from different farmer fields at Jhapa, Illam, Morang, Bara, Parsa, Rupandehi, Dhading, Gorkha and Lamjung districts in 1999. The soils from the 1999 sur-

vey were used for the experiment in 2000. Ten samples collected from each field were mixed together, diluted with sterile (1:1 v/v) soil and placed in three plastic pots (5-litre capacity). Ten seeds of rice cv. Mansuli were grown in each clay pot and regularly watered. These pots were maintained at the IAAS screen-house and arranged in a completely randomized design with four replicates. In this test, four pots filled with soil containing known population densities of the root-knot nematode were also included as a check. After 75 days, the plants were uprooted and the roots washed and rated for root galling caused by the root-knot nematode.

Micro-plot experiment

An experiment to assess the effect of *M. graminicola* on rice yield, using micro-plots (1 m × 1 m × 1 m), was conducted according to a completely randomized design with three replicates. The micro-plots were filled with uninfested soil or soil infested (15 juveniles per cm^3) with the nematode, obtained from production fields. The following treatments were evaluated in the micro-plots with and without nematodes: control, fertilizer applications (nitrogen, 10 g/plot and phosphorus 6 g/plot) and compost application (10 kg/pot). Thirty-day-old rice seedlings free of nematodes were transplanted at a spacing of 25 cm × 5 cm and maintained according to recommended practices for flooded rice production. At maturity (about 150 days after transplanting), plants were harvested and grain yields were recorded. The experiment was repeated in the same micro-plots the following year. The nematode population densities at harvest of the first crop of rice had increased 1-2 fold, but dropped after harvest and was again around 15 juvenile/cm^3 at the start of the next rice crop season (November-March)

Laboratory assays

Extraction of nematodes. For the inoculation of rice in the screen-house, root segments of rice cv. Mansuli, grown in an infected soil, were obtained and nematode eggs extracted by blending galled root segments in 1% sodium hypochlorite solution for 3 min and rinsing sev-

Table I. Number (per 100 cm^3 of soils or gram of roots) of *Meloidogyne graminicola* recovered from different rice cultivars in Bara, Chitwan and Rupandehi in 1999 field surveys and root galling severity in a trapping bio-assay in 2000 using the same field soil and same cultivar.

Cultivar	Field population 1999		Bioassay 2000
	Soils[a]	Roots[b]	RGS[1]
Bara/Parsa			
Makawanpur 1	1,158	1,785	6 ab
Philips	483	1,030	5 b
Sabitri	375	977	5 b
Radha 11	27	39	5 b
K. Masuli	27	2,281	5 b
Seto Maculi	231	650	5 b
Maculi	130	195	7 a
Average	**347**	**993.9**	**5.4**
Chitwan			
Amjhute	0	0	4 ab
Anadi	21	73	3 ab
Bam Morcha	0	11	4 b
Baspate	5	4	4 b
Bijaya 2	314	5,516	5 a
CW 2	9	33	3 b
Jhapali	5	0	3 b
Masuli	8	57	5 a
Radha	11	20	4 ab
Sabitri	5	35	4ab
Average	**37**	**574**	**3.9**
Rupandehi			
Mala	70	34	5 a
R. Maculi	17	327	5 a
Radha	6	164	6 a
Sabtri	0	158	6 a
Sarju-52	0	69	5 a
Mala	70	34	5 a
Average	**19**	**151**	**5.4**

[1]RGS = Root Galling Severity.

Means in the same column sharing a common letter are not significantly different within each site according to LSDT test (P = 0.05). [a] juveniles and [b] mostly eggs and some juveniles.

eral times with tap water. They were kept refrigerated (4 °C) until being inoculated near the root zone with a pipette tip. The protocol for processing soil and root samples for nematode extraction was as described below. Each sample consisted of roots and soil. Therefore, in the laboratory, roots were separated from the soil and processed separately. Before processing soil samples, clods were broken and the samples were mixed thoroughly. A 200 cm^3 sub-sample of soil was taken, water added to a final volume of five litres, stirred thoroughly, and allowed to settle for about one minute. The soil water suspension was then passed through a 60-mesh sieve nested over a 500-mesh sieve. The process was repeated twice for complete recovery of nematodes. The contents of the 500-mesh sieve were gathered into a beaker in about 50-100 ml of water. Roots were washed thoroughly, cut into 3-5 cm long pieces and mixed. Five grams of roots were blended for 3 minutes in a commercial blender. Then the root and soil suspensions were poured into a small sieve lined with tissue paper and placed in a Petri dish submerged in water for 36 hours (Hooper *et al*., 2005). The water suspension in the Petri dish was collected, the volume adjusted to 20 cm^3 and the nematodes were identified and counted. For the soil bioassay experiments, roots were washed and rated for root galling severity (RGS) according to the above-mentioned 1-9 scale.

Identification of nematodes. The nematodes were identified and enumerated using a dissecting microscope, as described by Zuckerman *et al*. (1971). All counts were adjusted to a value per 100 cm^3 of soil or per gram of roots.

Statistical analysis. As needed, log + 1 transformation of data was performed to obtain normality in all tests. Simple means and ranges were calculated. Analysis of variance was performed to test differences in means using Proc GLM computer based SAS (SAS Enterprise, SAS Institute). Student's t test for survey data and Tukey's test for screen-house and micro-plot data were used to separate the means.

RESULTS

Field surveys

In the rice fields, *M. graminicola* and mixed populations of *Hirschmanniella oryzae* Van Breeda de Haan and *H. mucronata* Das were observed. The first nematode was observed in high frequencies and comparatively low densities, whereas the latter two were observed in higher frequencies and lower densities. Similarly, species of *Heterodera, Hemicycliophora, Criconemoides, Tylenchchorhynchus* and *Helicotylenchus* were also observed. However, their frequencies and densities were low and they were identified to genus level only. Frequencies and densities (52 nematodes per litre of soils and eight nematodes per gram of roots) of the root-knot nematode recovered from rice fields surveyed in 1998 were low compared to 1999 and 2000 (Table I). In addition, only a few root-galls of root-knot nematode were observed in rice roots in 1998, as opposed to numerous and distinct root galls observed in the 1999 field surveys and 2000 bioassay. The largest densities of root-knot nematodes were observed in the soil and root samples from Bara followed by those from Chitwan and Rupandehi districts (Table I).

Nematode population and rice cultivars. In the field surveys, all of the grower rice cultivars supported development and multiplication of the nematode to a different extent and no resistant reaction was observed (Table I). In Bara district, root-knot nematode populations were largest in the roots of rice cv. K. Masuli followed by cv. Makawanpur 1, while soil populations were largest with cv. Makawanpur 1. In Chitwan district, the largest soil and root populations were observed in cv. Bijaya, whereas in Rupandehi district the greatest nematode populations were in the cv. R. Mansuli. The cvs Radha and K. Mansuli in Bara district and Bam Morcha in Chitwan district had the lowest nematode populations. Similarly, cvs Radha 11 in Bara, Jhapali in Chitwan and Mala in Rupandehi supported lower root populations. However, the lowest RGS was observed in many cultivars in Bara district, in CW and Jhapali in Chitwan district and in three cultivars in Rupandehi district. The cvs S. Mansuli in Bara, Amjhute and others in Chitwan, and Mala and Sarju in Rupandehi were among the cultivars supporting the lowest nematode populations (Table I). The same cultivar in different locations could support different levels of nematode population, such as with Sabitri, an improved cultivar commonly grown (Table I).

Nematode population, soil types and rice yield. Significantly larger root-knot nematode populations were recovered from rice roots in low yielding plots as compared to high yielding plots, but not from soils (Fig. 2A). Similarly, significantly larger root-knot nematode populations were observed in both roots and soils in the fields with light soils as compared to heavy soils, regardless of the rice yield categories (Fig. 2B). However, significantly larger soil and root population densities of the nematode were observed in light-textured soils as compared to heavy textured soils within low yielding plots or high yielding plots (Table II), but no nematodes were recovered from high yielding fields with heavier soil types during the 1999 surveys. In addition, significantly higher free-living nematode populations were observed in the fields with light soils as compared to heavy soils.

Nematode population density, crop health and soil type. Significantly larger nematode populations were observed in symptomatic fields as compared to asymptomatic fields irrespective of soil type (Fig. 3A). Similar-

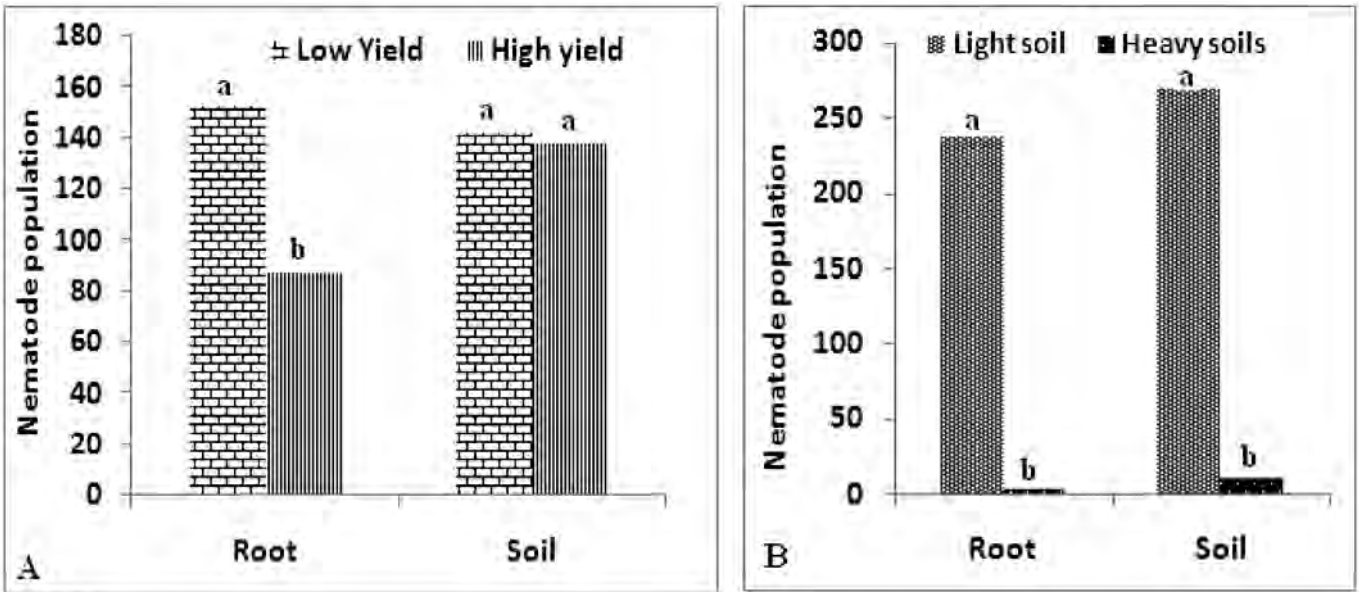

Fig. 2. Relationship of root (mostly eggs with some juveniles) and soil (juveniles) population densities of *M. graminicola* to yield (A; light soils *vs* heavy soils) and to soil texture (B; light soil *vs* heavy soils). Paired column means with the same lower case letter do not differ from each other according to Student's t test (P = 0.05). Population densities are per gram of roots and 100 cm^3 soil.

ly, significantly larger root and soil nematode populations were observed in light soils than in heavy soils irrespective of field symptoms (Fig. 3B). However, significantly higher root-knot nematodes populations were observed in plots with lighter soils as compared to the plots with heavier soil types within asymptomatic fields. In symptomatic fields, larger nematode populations were observed in light soil as compared to heavy soil, but not in the roots (Table III).

Nematode population densities and plant symptom severity. In the Bara district, more nematodes were observed in fields with severely symptomatic plants than in fields with mildly symptomatic plants (Fig. 4). Similar results were observed in soil bio-assays and repeated in the survey in 2000. However, the severity of the symptoms in soil bio-assays was less than the severity of symptoms in the field. Also, larger root-knot nematode populations were recovered from fields with severely

Table II. Effect of the interaction between rice yield (low *vs* high) and soil texture (light *vs* heavy) and of the presence of symptoms of infection by *M. graminicola* on the population densities of the nematode per 100 cm^3 soil and gram of roots.

Rice yield category	Soil Types	1999*		2000**	
		Soil[a]	Root[b]	Soil[a]	Root[b]
Low	Light soil	3,000 a	275 b	121 b	3,250 a
Low	Heavy soil	6 d	10 c	99 b	12 c
High	Light soil	175 c	264 b	59 c	230 b
High	Heavy soil	0 e	11 c	199 a	15 c
Symptomatic fields		288 b	414 a		513 b

In each column, the means sharing a common letter are not significantly different according to LSD test (P = 0.05). * = average of 10 samples, ** = average of 3 samples. [a] juveniles [b] mostly eggs and juveniles.

Table III. Population densities of *M. graminicola* in farmers' rice fields of Chitwan, in 1999, in different soil types collected from sick looking and healthy looking fields and their bio-assay in pots in 2000.

Rice yield category	Soil types	1999		2000	
		Soils[1]	Roots[2]	Soils[1]	Roots[2]
Normal looking fields	Heavy	4 c	6 c	5 b	8 c
Normal looking fields	Light	14 b	36 b	5 b	25b
Symptomatic fields	Heavy	4 c	201 a	7 a	27b
Symptomatic fields	Light	70 a	355 a	7 a	102 a

[1]Per 100 cm^3 of soil; [2]per gram of root (mostly eggs and some juveniles).
In each column, the means sharing a common letter are not significantly different according to LSD test (P = 0.05).

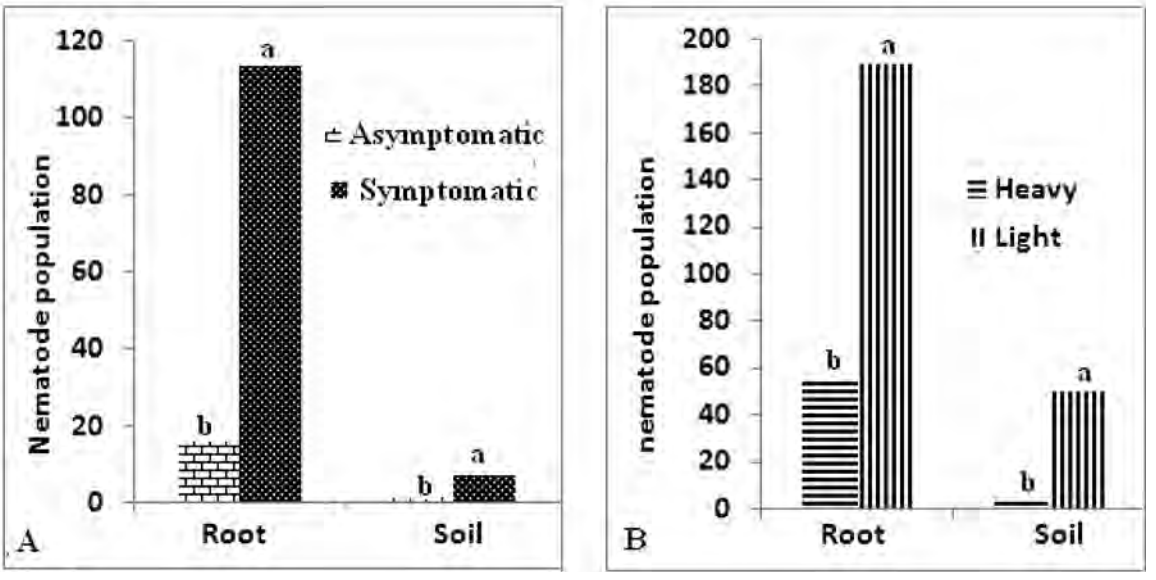

Fig. 3. Relationships of symptom expression (A; non-symptomatic *vs* symptomatic) and soil texture (B; heavy *vs* light) to population densities of *M. graminicola*. Paired column means with the same lower case letters do not differ according to Student's t test (P = 0.05). Population densities are per gram root (mostly eggs with some juveniles) and 100 cm^3 soil (juveniles).

symptomatic plants in the additional five fields in Rupandehi district, regardless of other factors, than in fields with less symptomatic or asymptomatic plants.

Potentially economically important fields. In the soil, the root-knot nematode population densities were potentially economically important in 5, 24 and 35% of the fields in Rupandehi (Fig. 5A), Chitwan (Fig. 6A) and Bara/Parsa/Rauthat (Fig. 7A), respectively. If the nematode population in the roots is considered, then 40, 60 and 100% of the fields in Rupandehi (Fig. 6B), Chitwan (Fig. 6B) Bara/Parsa/Rauthat (Fig. 7B), respectively, had nematode populations of potentially economic importance. Overall, 100, 55 and 40% of the fields were potentially economically important for *M. graminicola* infection in Bara/Parsa/Rautahat, Rupandehi, and Chitwan districts, respectively.

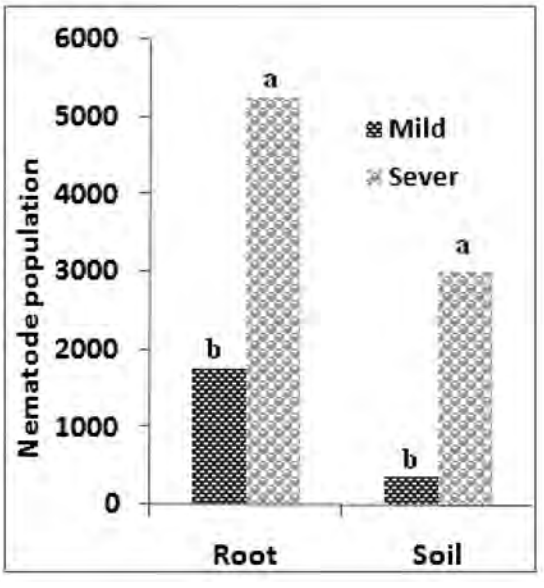

Fig. 4. Relationships between symptom expression on rice plants (mild *vs* severe) and population densities of *M. graminicola* in rice and soils in Bara. Paired column with the same lower case letters do not differ according to Student's t test (P = 0.05). Populations densities reported are per gram of root material (mostly eggs and some juveniles) and 100 cm^3 soil (juveniles).

Relationship of M. graminicola with soil physical and chemical properties. The physical attributes of the soil samples collected during the surveys were characterized in five classes (Table IV). The greatest number of fields had loam soil, and this soil type supported the lowest soil and root populations of the nematode. The fields

Table IV. Population densities of *M. graminicola* observed in each soil category during the survey of rice fields in different districts of Nepal in 1999.

Soil type	*M. graminicola*	
	Soil*	Root**
Clay (9)	20	2697
Loam (20)	2	109
Sandy (12)	299	3915
Silt (12)	14	146
Sandy clay (2)	1158	8924
Sandy Loam (10)	93	1498

Numbers in parentheses indicate the number of fields in each soil physical category

* per dm^3 of soil; ** per gram of roots.

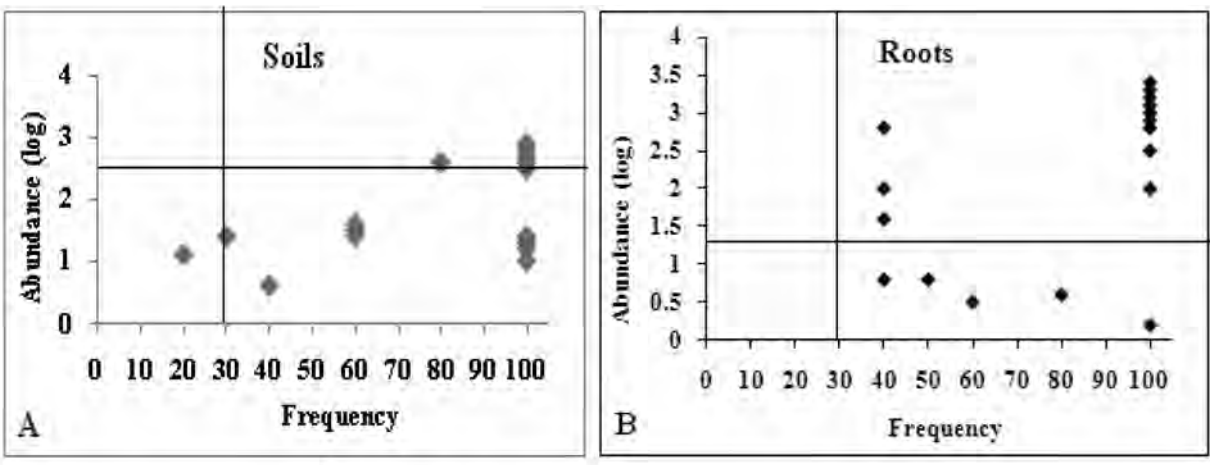

Fig. 5. Frequency and abundance *of M. graminicola* in soils (A) and roots (B) in Chitwan district. A vertical line and a horizontal line at a value of 30 and 2,3 or 1.3, respectively indicate the threshold level of the nematode in a particular field.

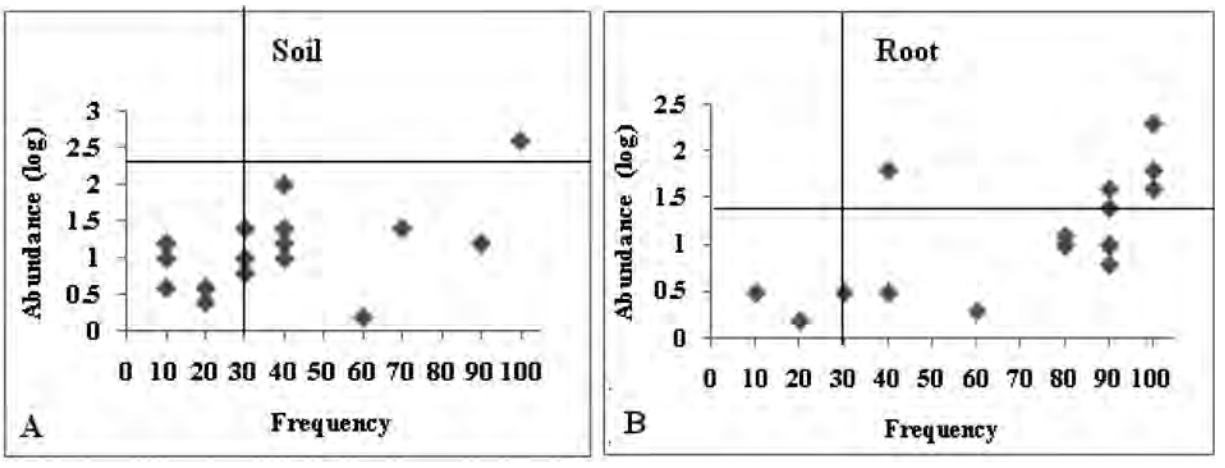

Fig. 6. Frequency and abundance *of M. graminicola* in soil (A) and roots (B) in Rupandehi district. A vertical line and a horizontal line at a value of 30 and 2,3 or 1.3, respectively indicate the threshold level of the nematode in a particular field.

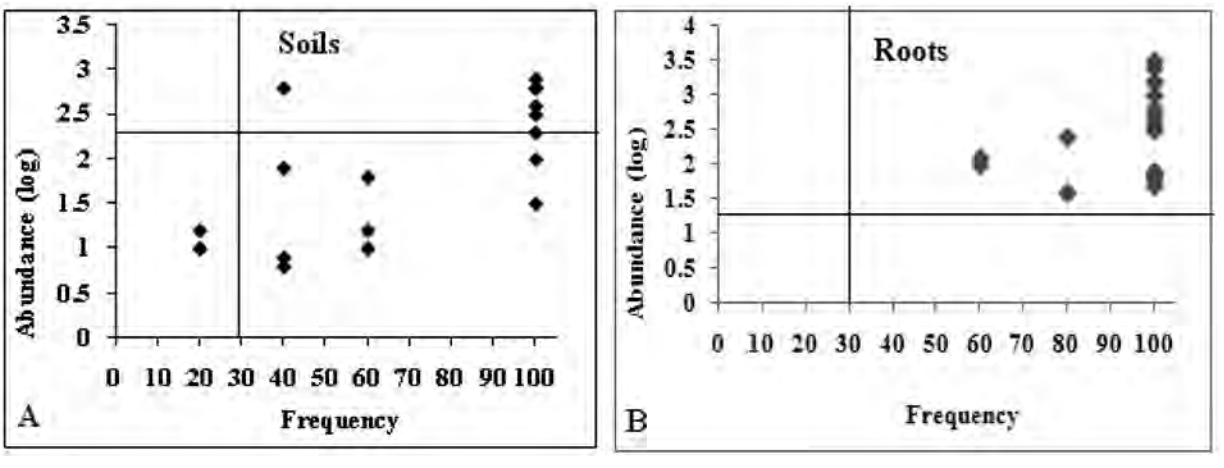

Fig. 7. Frequency and *abundance M. graminicola* in soil (A) and roots (B) in Bara/Parsa/ Rauthat district. A vertical line and a horizontal line at a value of 30 and 2,3 or 1.3, respectively indicate the threshold level of the nematode in a particular field.

Table V. Correlations between population densities of *M. graminicola* and various soil components (predictor variables) in soil and roots, in the absence of interaction but in the presence of other predictor variable.

Variable		In roots				In soils			
		P value	R^2	A value	B value	P value	R^2	A value	B value
C	-	0.003	0.221	3.399	0.829	0.004	0.2949	2.939	0.934
C	PH	0.036	0.211	2.955	0.949	0.004	0.2049	3.332	0.850
C	Om	0.007	0.121	2.741	0.973	0.008	0.2949	3.209	0.879
C	N	0.020	0.213	2.397	0.985	0.004	0.2949	2.7385	0.874
C	P	-	-	-	-	0.037	0.2949	2.158	0.982
C	S	0.021	0.213	3.165	1.311	0.006	0.2949	3.471	1.772
C	Si	-	-	-	-	0.001	0.2949	3.225	0.883
C	pH & Om	0.010	0.126	2.742	0.999	0.001	0.2949	3.267	0.900
C	pH & P	-	-	-	-	0.040	0.2949	2.137	0.997
C	pH & Si	0.006	0.241	2.940	0.998	0.001	0.2949	3.231	0.891
C	pH & S	0.026	0.263	3.134	1.347	0.009	0.2949	3.362	1.206
C	S & Si	0.038	0.211	3.588	1.661	0.046	0.2949	2.120	1.025
C	N & S	0.050	0.175	2.699	1.324	-	-	-	-
C	pH, om & N	-	-	-	-	0.007	0.2949	2.729	0.938
C	pH, S & Si	0.045	0.211	3.585	1.709	0.009	0.2949	4.211	1.516
C	Om & Si	0.001	0.241	2.719	1.031	0.002	0.2949	3.116	0.929
C	Om & N	0.031	0.211	2.276	1.019	0.006	0.2949	2.705	0.905
N	-	0.021	0.137	-1.654	0.683	0.008	0.1751	-1.772	0.634
N	Om	-	-	-	-	0.015	0.175	-1.678	0.653
N	pH	0.020	0.131	-1.699	0.700	0.009	0.1751	-1.804	0.651
N	pH & S	0.040	0.016	-1.557	0.708	-	-	-	-
N	S	-	-	-	-	0.031	0.175	-1.463	0.875
N	Si	0.028	0.137	-1.604	0.695	0.013	0.175	-1.692	0.645
N	pH & Om	0.035	0.137	-1.557	0.708	0.015	0.1751	-1.710	0.664
N	S & Si	-	-	-	-	0.039	0.1751	-1.409	0.656
N	P & Si	-	-	-	-	0.045	0.1751	-1.242	0.897
N	pH, Om & Si	-	-	-	-	0.025	0.1751	-1.604	0.693
N	pH & Si	-	-	-	-	0.002	0.281	-3.117	0.929
N	Om & Si	-	-	-	-	0.023	0.175	-1.581	0.665
N	Om & P	-	-	-	-	0.041	0.175	-1.272	0.600
P	-	0.001	0.175	2.781	0.767	0.005	0.175	2.735	0.720
P	pH	0.001	0.263	2.804	0.786	0.001	0.281	2.842	0.733
P	Om	0.002	0.263	2.662	0.816	0.001	0.281	2.684	0.768
P	S	-	-	-	-	0.004	0.281	1.080	0.851
P	Si	0.001	0.263	2.767	0.798	0.001	0.281	2.645	0.743
P	C	0.037	0.263	2.020	0.930	-	-	-	-
P	pH & Om	0.003	0.263	2.768	0.890	0.001	0.281	2.868	0.804
P	N & pH	0.003	0.263	2.399	0.740	-	-	-	-
P	pH & S	0.004	0.257	0.834		0.281	2.458	2.458	0.774
P	pH & Si	0.002	0.263	2.842	0.809	0.001	0.281	2.740	0.749
P	pH & C	0.034	0.263	2.103	0.051	0.045	0.281	1.777	0.857
P	Si & C	0.039	0.263	2.028	0.945	-	-	-	-
P	S & C	0.041	0.263	2.013	0.946	-	-	-	-
P	S & Si	0.006	0.263	2.468	0.838	0.005	0.289	2.322	0.779
P	pH, om & N	0.001	0.281	2.459	0.800	-	-	-	-
P	pH, N & S	0.009	0.263	2.354	0.841	0.007	0.264	2.197	0.764
P	pH, N & Si	0.005	0.263	2.468	0.823	0.004	0.281	2.352	0.675
P	pH, S & Si	0.005	0.276	2.523	0.847	0.004	0.281	2.399	0.778
P	pH, S and C	0.046	0.295	1.801	0.876	-	-	-	-
P	pH, Om, N & S	-	-	-	-	0.012	0.281	2.225	0.833
P	pH, Om, N, P & Si	0.011	0.263	2.405		-	-	-	-
P	pH, Om, N & Si	-	-	-	-	0.006	0.281	2.412	0.815
P	pH, N & S	0.009	0.263	2.354	0.841	-	-	-	-
P	pH, N and Si	-	-	-	-	0.007	0.281	2.197	0.764
P	pH, S, Si & C	0.044	0.263	2.147	1.027	-	-	-	-
P	pH, Om, N, S & Si	0.016	0.281	2.174		-	-	-	-
P	N & S	0.004	0.263	2.433	0.798	0.008	0.281	2.122	0.758
P	N & Si	0.008	0.263	2.364	0.823	-	-	-	-
P	Om & pH	0.010	0.281	2.254	0.828	-	-	-	-
P	N & S	0.008	0.281	2.317	0.823	-	-	-	-
P	pH & Si	0.004	0.263	2.432	0.798	-	-	-	-
P	Om & S	-	-	-	-	0.011	0.281	2.254	0.828
P	Om & N	0.007	0.263	2.364	0.816	0.004	0.281	2.332	0.752
Si		-0.030	0.103	-0.003	0.987	-	-	-	-
S	-	0.001	0.310	3.140	0.876	0.001	0.328	2.358	1.453
S	pH					0.020	0.126	-2.249	0.921
S	Om	-	-	-	-	0.022	0.126	-2.090	0.877
S	Si	-	-	-	-	0.038	0.126	-3.066	1.424
S	pH & Om	-	-	-	-	0.021	0.126	-2.214	0.916
S	pH & Si	0.031	0.126	-3.156	1.409	0.032	0.126	-3.229	1.444
S	Om & Si	0.044	0.125	-3.124	1.498	0.005	0.215	-3.124	1.408

Only significant values (P = 0.05) are given in the table. Negative values indicate negative correlations, otherwise correlations are positive. * C = Clay; Si = Silt; S = sand; N = nitrogen; P = phosphorus; . pH = soil pH; Om = soil organic matter.

that had sandy clay soil supported the highest soil and root populations of this nematode (Table IV). Soil analysis revealed ranges of soil pH (3.4-7.1), organic matter (0.39-0.78%), soil nitrogen (0.012-0.165 ppm) and phosphorus (80-181.12 ppm) content. Soil physical analysis showed sand (S) contents of 10-67%, silt (Si) contents of 8-67%, and clay (C) contents of 2.1-81.3%. The population densities of RKN ranged from 0 to 6,360 per g of roots and up to 5,790 per dm^3 of soil. Significantly positive correlations of the nematode population densities in roots and soil with that of soil sand content were observed when interactions terms were excluded in the model. This relationship changed to negative when the other predictor variables and interaction terms were included in the model (Table VI).

However, a negative correlation of the nematode population densities only in soils was observed with that of soil silt content. With the interactions included in the model, in the presence of sand, soil nematode population densities had a negative correlation with nitrogen

Table VI. Correlations between population densities of *M. graminicola* in rice roots and soil with soil chemical and physical factors in the presence of other predictors and interactions in the model with data from Bhairahawa, Chitwan and Bara, in 2000.

Variable levels	Soil properties*	In roots				In soil			
		P value	R^2	A value	B value	P value	R^2	A value	B value
2nd a	S	0.017	0.083	-336.1	121.2	-0.023	0.082	-328.0	127.2
	Si	0.006	0.056	-420.5	123.8	-0.005	0.076	-442.7	129.9
	C	0.004	0.061	-442.9	124.4	-0.014	0.034	-373.9	130.5
	N and Si	0.047	0.034	-27.7	12.5	-0.036	0.012	-31.0	13.1
	S and Si	0.026	0.032	58.9	23.1	0.032	0.219	59.0	24.3
	S and C	0.011	0.034	34.6	11.4	0.050	0.044	25.2	12.0
	Si and C	0.010	0.030	58.5	19.3	0.035	0.023	48.1	20.2
	pH and P	-	-	-	-	0.013	0.031	-25.6	8.7
3rd b	S	0.011	0.083	-184.5	51.3	-	-	-	-
	Si	0.016	0.057	-225.4	67.7	-	-	-	-
	C	0.005	0.061	-235.7	54.9	-	-	-	-
	PH	0.001	0.034	262.6	59.9	-	-	-	-
3rdc	N	0.002	0.054	-250.5	47.4	0.003	0.023	-79.5	32.1
	P	0.008	0.065	92.8	23.7	0.008	0.008	62.8	16.0
	PH	0.001	0.034	470.4	82.3	0.002	0.013	457.7	55.5
	C	0.006	0.034	-214.4	31.8	-	-	-	-
	S	0.007	0.012	180.4	45.4	0.005	0.045	133.8	30.6
	Si	0.041	0.018	138.8	53.6	0.001	0.051	208.9	36.1
	pH, N, P, S, & Si	0.007	0.007	15.3	3.7	0.002	0.006	-8.4	1.0
	pH, N, P, S & C	-	-	-	-	0.006	0.007	7.7	2.2
	pH, P, S & C	0.001	0.006	3.6	0.6	<.001	0.091	4.1	0.4
	pH, N, S, Si & C	0.001	0.003	-6.0	1.4	0.004	0.081	-6.9	0.9
	pH, N, S &,Si	0.007	0.005	15.3	3.7	0.001	0.003	22.1	2.5
	pH, N, S & C	0.025	0.011	11.4	3.8	-	-	-	-
	pH, N, Si & C	0.009	0.006	5.6	1.5	0.001	0.002	6.1	1.0
	pH, N, P & Si	-	-	-	-	0.001	0.006	9.4	1.4
	pH, N, P & C	0.013	0.005	-15.4	4.4	.0001	0.006	-28.2	3.0
	N, P, S & Si	-	-	-	-	0.006	0.066	3.9	0.5
	N, P, S & C	0.010	0.002	4.2	1.1	-	-	-	-
	P, S, Si & C	0.019	0.003	2.8	0.5	0.002	0.011	2.9	0.3
	pH, P, S, & C	0.048	0.034	-5.1	1.5	-	-	-	-
	pH, P, S & Si	0.015	0.007	-5.5	2.2	0.001	0.008	-9.2	1.0
	Om, N, P & S	0.006	0.003	-15.8	3.3	0.004	0.005	-11.2	2.5
	Om, N, P & Si	0.028	0.001	-9.5	3.3	0.005	0.001	-9.9	2.2
	Om, N, P & C	0.015	0.001	11.1	3.2	0.001	0.002	8.4	2.2
	Om, P, S & C	0.013	0.002	-11.8	3.4	0.007	0.001	-9.2	2.3
	Om, S, Si & C	-	-	-	-	0.038	0.001	-0.2	0.1
	Om, pH, N, P, & S	-	-	-	0.1	0.023	-1.820	0.5	1,1
	Om, pH, N, P, & S	-	-	-	0.0	0.033	3.733	1.0	1.3
	Om, pH, N, P & C	0.046	0.009	-3.0	1.2	0.026	0.045	-2.3	0.8
	Om, N, P, S & Si	0.012	0.006	5.4	1.5	0.003	0.056	4.9	1.0
	Om, N, P, S & C -		-	-	-	0.018	0.632	-1.5	0.4
	Om, P,S, Si & C	0.010	0.004	3.3	0.8	0.009	0.090	2.2	0.5

Only significance values (at P = 0.05) are given in the table. Negative values indicate negative correlations, otherwise correlations are positive. [a] Two variables were included at a time ; [b] Three variables were included in an analysis. [c] Four variables were included in an analysis. The abbreviations * C = Clay; Si = Silt; S = sand; N = nitrogen; P = phosphorus; S = sulfur; pH = soil pH; Om = soil organic matter;

and clay, and a positive correlation with that of the combinations of sand, nitrogen, pH, silt, phosphorus, and silt and clay (Table VI). Also, a negative correlation of the soil nematode population densities in the presence of sand and clay combination, and positive correlations of the same populations with that of clay and sand, clay and silt, and clay, phosphorus and sand were observed. However, when interactions were included in the model, in the presence of nitrogen, the only significant correlation observed was a positive correlation of sand with the nematode population densities in both root and soil. A positive correlation of the nematode population density with sand in the presence of nitrogen, but a negative correlation in presence of clay were observed (Table VI). Also, in the presence of nitrogen the relationship of sand with that of nematode populations changed from positive to negative.

Nematode population in volunteer and seedling nursery. Root infection and galling were observed in both volunteer and seedling rice in all locations. In volunteer rice, root populations (25-6,880 eggs and juveniles/g) and RGS (2 to 6) were higher than in seedling rice. However, significant differences in root populations and RGS were observed in both volunteer rice and seedling rice among localities (Table VII). Infested roots were found in 25-80% of the volunteer rice samples and 15-78% of the seedling rice samples.

Screen-house tests

Bioassay and nematode severity. The results of soil bioassays conducted in 2000, with field soils of the 1999 survey, confirmed the observations under field conditions, but higher RGS ratings were observed in soil bioassays than in the surveys. However, the bioassays of field soils in the screen-house showed RGS varying from 1 to 7 (Table I), whereas the bioassays of soil from the five farmer fields in Rupandehi district having severely symptomatic plants and large population densities produced RGS ratings of 8-9.

Evaluation of grower cultivars. Twelve rice cultivars collected from growers' fields and tested against *M. graminicola* exhibited susceptible reactions to the nematode in the screen-house as revealed by the large numbers of eggs and juveniles and high RGS ratings, al-

Table VII. Root galling severity (RGS) and reproduction of *M. graminicola* in volunteer rice and rice seedlings in different parts of Nepal in 1999.

Locality	Volunteer rice infestation %	RGS	Eggs+juveniles/g of root	Seedling infestation %	RGS	Eggs+Juveniles/g of root
Mangalpur	80a	5ab	6980a	78a	3b	300b
Rampur	78a	4b	562b	23bc	2c	214b
Parbatipur	60a	6a	695b	69a	3b	690a
Shivanagar	69a	3bc	251b	52ab	4a	236b
Budhbare	25ab	2c	63c	15c	3b	25c
Urlabari	36ab	3bc	98c	23bc	2c	36c
Belwa	23ab	4b	106bc	69a	4a	360b
Parwanipur	15b	3bc	108bc	36b	3b	251b
Average	48.25	4 b	1108	46	3	264

In a column, figures sharing a common letter are not significantly different according to Student's t test (P = 0.05 level).

Table VIII. Number of eggs and juveniles and root galling severity (RGS) index in 2000 pot experiment in screen-house with the 1999 survey soils.

Rice cultivar	1999 Surveys			2000 Bioassays		
	Juveniles	Eggs	RGS	Juveniles	Eggs	RGS
Bindeshowri	1,376 bc	2,780 e	6 b	13 bc	3,156 e	5 cd
Chaite-2	1,104 c	3,134 d	4 c	12 bc	4,138 de	4 d
Chaite-4	189 d	3,905 d	4 c	8 cd	3,989 e	4 d
Ghaiya	1,106 c	3,160 d	4 c	15 bc	4,356 de	5 cd
Janaki	1,304 c	8,680 c	5 bc	3 d	9,923 cd	6 bc
Makawanpur-1	1,352 c	1,664 e	4 c	4 d	2,546 e	4 d
Masuli	1,668 bc	1,8610 b	8 a	8 cd	2,3456 b	7 ab
R. Masuli	2,616 a	1,4540 bc	6 b	10 c	1,7256 bc	5 cd
Radha-7	3,183 a	5,190 cd	4 c	13 bc	8,373 cd	4 d
Radha-7	2,439 a	7,279 c	4 c	23 a	9,719 cd	4 d
Radha-9	1,967bc	6,5058 a	8 a	12 bc	6,7026 a	8 a
Sabitri	2,545 a	2,996 de	4 c	12 bc	5,541 d	4.d

Within each column, the means sharing a common letter are not significantly different according to LSD test (P = 0.05). * per gram of root.

Table IX. Root-galling severity (RGS) rating by *M. graminicola* in rice cv. Mansuli in a bioassay with soil collected from different grower's fields in different districts in 2000.

District	Number of fields	Average RGS	Percentage of fields infested	Root population
Bara	4	4.8	100	5,133
Chitwan	9	3.8	89	423
Lamjung	9	2.6	78	223
Parsa	5	3.4	100	566
Illam	2	3.5	100	654
Morang	4	3.5	100	567
Jhapa	2	3.0	100	456
Dhankuta	6	2.0	83	324
Rupandehi	7	4.6	100	4,532

though significant variation was observed among the different cultivars. All cultivars of the Radha and Janaki series had generally high numbers of eggs and juveniles in the screen-house test. The fewest eggs plus juveniles were observed in Makawanpur 1 but, although the fewest eggs were observed with Makawanpur 1, the fewest juveniles were observed with Chaite. Many cultivars exhibited a low RGS in both the surveys and the screen-house bioassay (Table VIII). However, the cultivars having the highest RGS did not necessarily have the largest nematode root populations.

Bioassay of additional fields. A very large percentage of the fields (83-100) had large nematode populations (223 to 5,133 eggs + juveniles) per gram of roots, with RGS ranging from 2.0 to 4.8. The greatest root population and RGS were observed in Bara, followed by Rupandehi districts and with the least in Lamjung and Dhankuta, both hill districts (Table IX). Bioassay of soil samples from growers' fields indicated that all fields, except in Gorkha and Dhanging in upland soils, had RGS greater than 1. A total of 18% of the fields had an RGS rating of 4-7, 42% had RGS of 2-3, and 44% had RGS 1 ratings. However, bioassayed plants did not show similar shoot symptoms to those observed in the field surveys irrespective of the root galling severity. All five bioassayed soils with known larger nematode population densities included in second year produced high RGS ratings.

Micro-plot experiment

The greatest rice yields were obtained in plots to which compost was applied in the absence of nematodes, followed by compost with nematodes, while the lowest yields were obtained when no nutrients (chemical or organic source) were added to the plots. These treatments were significantly different from the others. The lowest yield was observed in positive control plots (where nematodes were present without NP) (Fig. 8).

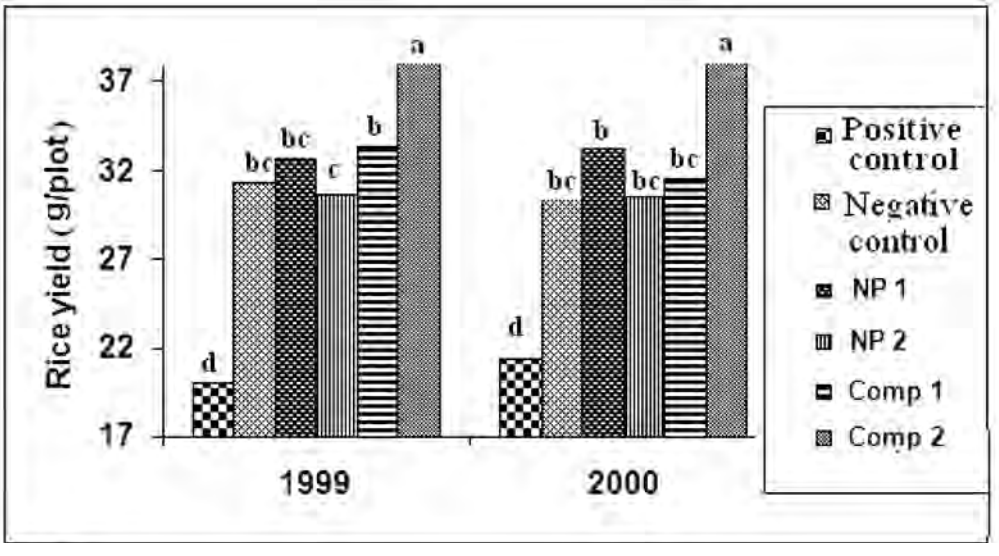

Fig. 8. Interaction effect of *M. graminicola* with nitrogen (N) and phosphorus (P) or compost (com) on yield (g/m^2) of rice cv. Mansuli in micro-plots during 1999 and 2000. Column means within the same year that have the same lower case letter do not differ according to LSD (P = 0.05 level). Positive control (with N and P and nematode); Negative control (without N, P and nematode); NP 1 = N and P without nematodes; NP 2 = N and P with nematode; Comp1 = Compost with nematodes; Comp 2 = Compost without nematodes.

DISCUSSION

Meloidogyne graminicola was the only species of root-knot nematode observed in lowland rice fields, thus confirming previous reports for the rice-wheat fields in Nepal by Pokharel *et al.* (2007). However, these authors also observed variability among and within fields. Lower frequencies and lower densities of the root-knot nematode, and the absence of or fewer root-galls due to root-knot nematode were observed in 1998 than in 1999 and 2000 in the same field. This may be due to difference in sampling techniques. In 1998, one hill of rice was removed by pulling, but in 1999 and 2000 all samples were dug out carefully by shovel. Sampling in rice is difficult as the crop is grown under constant flooding conditions; the easiest way of sampling was uprooting by pulling a hill of rice, as was done in 1998. But forceful uprooting could leave most of the roots and root-galls behind in the soil, and thereby lead to the lack of observed root galls and lower recoveries of nematodes in 1998 as compared to 1999 and 2000 in the same field – most of the nematodes are inside feeding roots and root tips, the very parts of the root system likely to be left behind by pulling. In other observations, root galls were detached from roots during washing just by water force and rubbing roots to get rid of soil particles, especially in heavy and clay soils. This clearly demonstrates the importance of using an appropriate sampling technique to estimate nematode populations.

Growing resistant cultivars is a cheap and sustainable solution to manage the nematodes. In the current study, none of the cultivars tested, under either field or screen-house conditions, exhibited resistance to the nematode. RGS ratings were more severe in the bioassay pots than in the field surveys with known population densities of the nematode. This was probably due to higher RGS ratings being associated with greater proximity of the inoculum in pots to roots as compared to fields, or perhaps to the virulence of the nematode isolates used.

The largest nematode population densities were observed in Bara/Parsa/Rauthat, followed by Chitwan and with the least in Rupandehi, where 100, 60 and 40%, respectively, of the fields had infestation levels that were economically important. One hundred percent of the fields in Bara/Parsa/Rauthat, 60% fields in Chitwan and 40% fields in Rupandehi were symptomatic and larger nematode populations were observed in symptomatic fields than in asymptomatic fields. Further, pot bioassays in the screen-house with the same soils confirmed the results of the surveys. However, the severity of plant symptoms was less in the bioassay pots than in the fields from which the soils had been taken for bioassay. This could be due to differences in plant age during sampling; in the screen-house the plants were uprooted 75 days after transplanting, at late maximum tillering stage, while in the fields surveyed the plants were collected at flowering to maturity stage (about 100 days old). Hussey (1985) reported that the severity of the symptoms caused by root-knot nematodes increase with the age of the plants. However, production of such symptoms in the fields caused by soil physical and chemical disorders (high or low) and other soil related biological constraints (Hussey, 1985) should not be ignored. In addition, association with other nematodes, especially rice root nematodes such as *Hirschmanniella* spp., should not be overlooked as their associations with and high incidences in rice have already been reported in Nepal (Pokharel, 2001; Sharma *et al.*, 2001; Bridge *et al.*, 2005).

Because root-knot nematodes dwell in soil, soil physical and chemical factors might affect survival, growth, and reproduction of the nematodes. The significantly larger nematode population densities observed in the fields with light soil as compared to heavy soil may be the result of better growth and multiplication of the nematodes favored by light soil (Israel and Rao, 1972; Prot *et al.*, 1994). A positive correlation of *M. graminicola* was observed with sand percentage in the soil with the exclusion of other independent variables. Nevertheless, the relationship of soil and root populations of the nematode with that of sand changed from positive, without interaction terms included in the model, to a negative relationship with the inclusion of other dependent variables and the interaction terms in the model. However, in the past, studies of such relationships did not consider the presence of other factors that co-exist under field conditions and affect nematode infection and reproduction. Soriano *et al.* (2000) found greater damage to rice varieties in sandy soils than in clay soil. Similarly, soil population densities of *M. incognita, M. javanica*, *M. arenaria* and *M. hapla* were positively correlated with that of the sand content of the soil (Taylor *et al.*, 1982). Limited information is available on the role of soil texture on *M. graminicola* in rice, especially in flooded conditions. This nematode has greater survival and infectivity in flooded soil than in non-flooded soils, in contrast to most other *Meloidogyne* species (Padgham *et al.*, 2004). The lack of correlation between silt content of the soil and nematode populations, when the other predictor variables were excluded from the model, and a negative to positive relationship when the other predictor variables were included, might be due to the fact that the effect of silt on nematode populations was highly variable.

Nematode populations were negatively correlated with N alone but positively correlated with N in combination with other soil components. Probably, N alone was not enough to support plant growth adequately, but it did when combined with P (Regmi, 1996). Rao and Israel (1971) and Swain and Prasad (1991) found an increase in nematode population density with increasing levels of N up to 40 kg (with 40 kg P) and a drastic decrease at 120 kg/ha even with 40 kg P. Similarly, the positive relationship of soil pH with nematode populations, only observed with the inclusion of more than four predictor variables, indicated that the nematode

populations increased with the increase in P and pH together. On the other hand the effect of P did not change with the inclusion of other predictors and their interaction, while the effect of pH did. Long-term use of N plus P increased *Hirschmanniella* populations in rice (Pokharel *et al*., 2004). The addition of P also increased the severity of root-knot nematode damage in the field (Pokharel *et al*., 2004), confirming findings by Rao and Israel (1972) who reported that P application alone or in combination with N was found to favour this nematode. Potassium content was not analyzed due to lack of laboratory facilities.

Severe infection of root-knot nematodes in the nursery leads to the possibility of widespread nematode dispersal in the field as rice seedlings are grown in nurseries and then transplanted in the fields. Also, the use of infected seedlings may result in greater infection and damage to rice plants in the field (Duxbury, 2001). Dense populations of infected volunteer rice plants leads to a high risk of severe infection and damage in such fields as self grown, sprouting rice maintains nematode population densities at damaging levels from one season to the next. However, weeds growing in rice fields, especially within self-sprouted rice fields, may also harbour this nematode and allow the nematodes to survive in the absence of a rice crop (Medina *et al*., 2009). Removal of such infected self-grown and sprouting rice stubbles and weeds and treating rice nurseries may help to reduce the severity of nematode infestation in the fields (Padgham *et al*., 2004). In Nepal, rice harvest is followed by winter crops. Generally rice stubbles start to sprout only when soil temperature and soil moisture conditions favourable to rice growth return in March, assuming they have not been killed by tilling and, therefore, especially under no-till or zero tilling of wheat production.

The yield loss of rice caused by the root-knot nematode in screen-house tests ranged from 31 to 97% with different initial inoculum levels (Sharma-Poudyal *et al*., 2005). However, nitrogen application resulted in increased rice growth and yield irrespective of root-knot nematode infestation in rice (Prot *et al*., 1994). In our micro-plot test, yield loss of rice was greatest in the control (without fertilizer or compost) and least in N+P fertlized plots. This suggests that a balanced dose of N+P might have provided a degree of tolerance to the rice plants. However, nitrogen application alone did not reduce the relative nematode damage in rice (Prot *et al*., 1994), which may be because other factors contributed to yield reduction in rice by this nematode. Soriano *et al*. (2000) reported reduced root development and grain yield in rice by *M. graminicola* in sandy soils and under all water regimes, including continuous flooding.

In conclusion, several factors were found to contribute to the infection and reproduction of the nematodes in fields. However, once the nematode is established in a field, nematode population densities could be directly related to the severity of the symptoms. Such factors need to be considered while studying the nematode, especially their role in rice production. The relationship of the nematode with various soil physical and chemical factors is important since they co-exist in soil. The relationship of the nematode with these factors changed with the inclusion of additional predictor variables and their interaction terms, so while predicting their relationship several factors and their interaction need to be considered together in a model to avoid misleading conclusions. The role of seedling rice in nematode dispersal and volunteer plants in increasing the severity of any damage needs to be considered while planning any nematode management strategy. Many rice fields showed severely symptomatic plants and large nematode population densities and, therefore, require the immediate application of appropriate management procedures in order to achieve the potential rice yield.

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