POPULATION BEHAVIOUR OF *MELOIDOGYNE INCOGNITA* IN SOIL AND ROOTS OF TEA IN TRIPURA, INDIA

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Summary. Spatial and temporal variations in population density of *Meloidogyne incognita* in roots and rhizosphere soil of tea were studied for a period of two years (2000-2001) in a 3-year-old root-knot nematode infested and rain-fed tea plantation at Lakhilunga tea estate, West Tripura. Samples were taken at three depths (0-20, 21-40 and 41-60 cm) and at two horizontal distances (0-20 and 21-40 cm) away from the plant base. The number of second stage juveniles in the soil was low. The samples collected at 40 cm from the plant base and at 40 cm depth yielded significantly greater nematode populations and therefore can be considered as the optimal sampling zone for the development of the root-knot nematode in tea. The largest nematode population was observed in November when the soil was rather dry and rainfall was minimum in the post-monsoon season.

Key words: *Camellia sinensis*, population dynamics, root-knot nematode.

Tea (*Camellia sinensis* L.) is grown on the plain land and hillocks as a plantation and cash crop in Tripura, West Bengal and Assam in the North Eastern States of India. There are about 59 tea plantations distributed in different sub-divisions of Tripura but mostly confined in West Tripura and North Tripura districts (Anonymous, 2001). However, among the nematodes encountered in tea, root-knot nematodes and root lesion nematode *Pratylenchus brachyurus* (Godfrey) Filijev *et* Schuurmans Stekhoven are widespread and cause damage in nurseries and plantations in Tripura (Mukherjee, 2001). Information on nematode dynamics in soil and roots would be useful to design appropriate management strategies. Therefore, an investigation was undertaken to obtain insights on spatial and temporal variation of *Meloidogyne incognita* (Kofoid *et* White) Chitw. in roots and rhizosphere soil of tea.

MATERIALS AND METHODS

The investigation was conducted in a three-year-old root-knot nematode infested and rain-fed tea plantation at the Lakhilunga tea estate from January 2000 to December 2001. The mean annual rainfall is 2400 mm and the soil is sandy clay of acidic pH (5.7-6.5).

Fifteen randomly located root-knot infested tea bushes were selected for the experiment. Composite soil and root samples were collected from three blocks of bushes, each containing five bushes in a fixed sampling location. To represent each tea bush, three equidistant points were fixed and samples were collected at monthly intervals from three depths (0-20, 21-40 and 41-60 cm) and at two horizontal distances (0-20 and 21-40 cm) away from the plant base. The corresponding depth sub-samples collected at each distance from the plant base of each of the five tea bushes were mixed together thoroughly and aliquots of 250 cm^3 from the composite soil samples were processed for extraction of nematodes using wet sieving and modified Baermann funnel techniques (Whitehead and Hemming, 1965). Nematodes extracted from soil samples were heat relaxed, killed in a hot water bath, fixed in TAF or FAA (Fortuner, 1991) and counted.

Approximately 25 g of younger feeder roots (white in colour) were collected from the rhizosphere of every bush. Roots were thoroughly washed in tap water and cut into smaller pieces (2-3 cm in length). Five gram root sub-samples were then stained in acid-fuchsin-lactophenol. Stained roots were washed several times in tap water to remove the excess stain, and then macerated in an electric blender with 10-15 ml of water to dislodge secondary endoparasites. Three 1 ml sub-samples were removed from the suspension and the population counted. The roots containing galls were immersed in an aqueous solution of Phloxin B (0.15 g/l tap water) for 15 minutes to stain the egg masses in the roots (Dickson e*t al*., 1965), and excess stain was removed from the roots by rinsing in tap water. The stained egg masses were isolated with a fine needle under a dissecting binocular microscope, the eggs dispersed in water and counted. Thus the numbers of females and egg masses in the roots were counted and the numbers of eggs/egg mass were also assessed.

Freshly collected representative soil samples were taken in aluminium boxes with aluminium covers. The weight of the moist soil was determined with the help of a digital pocket-type electronic balance. The aluminium boxes were then uncovered and placed in an electric

Table I. Physicochemical properties of the soil in the tea root zone at Lakhilunga (Tripura).

Soil depths		Mechanical analysis				Chemical analysis			
Horizontal distance (cm)	Vertical depths	Sand (%)	Silt (%)	Clay (%)	Organic carbon (%)	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Potassium (kg/ha)	pH
0-20	0-20	50	14	36	0.853	0.05	0.2	13.6	5.7
	21-40	42	12	46	0.695	1.8	2.6	20.5	6.0
	41-60	37	11	52	0.478	1.4	2.4	14.9	6.2
21-40	0-20	52	16	32	0.930	0.7	1.4	9.8	5.5
	21-40	46	14	40	0.563	1.6	1.6	10.9	6.3
	41-60	38	10	52	0.521	0.9	0.9	8.7	6.5

Soil type at the site was sandy clay.

oven, where the soil was dried at 110 °C for 24 hours. The aluminium boxes were then covered and cooled in a desiccator before weighing the soil plus boxes and then the boxes alone so that the soil weights could be derived by difference. The soil moisture content was expressed as percentage of water in soil on oven-dry basis.

On each sampling date, the soil temperature was recorded by inserting a soil thermometer to a depth of 30-40 cm for 15 min twice a day at fixed times (9 am and 5 pm). The means of the two observations were recorded as the daily temperature.

The rainfall and number of rainy days per month were obtained from the Meteorological Observatory located at Agartala Airport, approximately 2 Km away on the north of the sampling site.

Sand, silt and clay contents (percentages) of soil samples were determined according to the International pipette method (Piper, 1966) and organic matter according to feel method (Khanna and Yadav, 1979).

Determinations of available nitrogen (alkaline permanganate method of Subbiah and Asija, 1956), extractable phosphorus (according to Bray and Kurtz, 1945) and extractable potassium (Flame photometric method according to Toth and Prince, 1949) were also made in January 2002 and expressed as kg/ha.

Correlations between nematode population density data and soil temperature (°C), soil moisture (%) and total rainfall per month (mm) and number of rainy days were calculated. The data of the nematode population of tea root and soil were $\log_{10}(x+1)$ transformed (where x = mean value of the population) before multiple ANOVA.

RESULTS

Climatic conditions. The climatic conditions at the study site were characterized by a high pre-monsoon (April) and monsoon (May-August) rainfall pattern with comparatively little rainfall recorded during post-monsoon (November) and winter months (December-February) during the period of study. The rainfall was negligible or nil during winter months (December-January). The rainfall pattern was grossly different in the two successive years of study. The highest rainfall were recorded in August, 2000 (667.3 mm) and in May, 2001 (1250.3 mm). The soil temperature started increasing from March (23.3 °C) onward to September (28.0 °C). The coldest month was January at 5.3 °C (Fig. 1).

Soil moisture content varied from 17.3% to 25.8% at different depths in successive months of study. From April to August soil moisture was at its highest in the uppermost depth (0-20 cm) and it gradually declined at

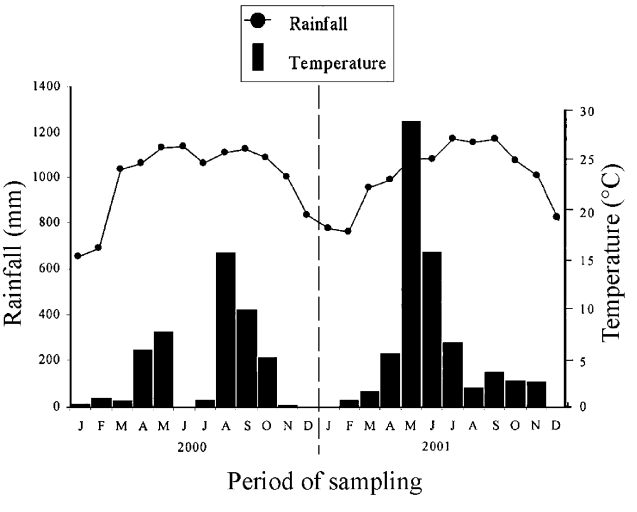

Fig. 1. Monthly rainfall and mean temperature during the sampling period (January 2000 – December 2001).

the lower depths (21-40 and 41-60 cm). In contrast, during the post monsoon months (September to December) and winter months soil moisture was at its lowest in the uppermost layer (0-20cm) and gradually increased with depth.

Soil properties. The soil was a sandy clay of acidic pH (5.7-6.5) (Table I). The pH gradually increased with depth. In the 0-60 cm soil profile, the inorganic nutrients were in the ranges 1.8-1.6 kg/ha, 2.6-1.6 kg/ha, and 20.5-10.9 kg/ha for N, P, and K, respectively, and highest at the middle depth (21-40 cm). Sand (52%) and silt (16%) contents were greatest in the upper soil layer (0-20 cm), while clay content was greatest (52%) in the deepest soil layer (41-60 cm) Thus, with increasing soil depth there was a decline in sand and silt contents and an increase in clay content.

Seasonal variation and vertical distribution. The density of the nematodes varied from sample to sample and season to season. A few root samples (>25 g) did not contain any nematode even in the most favourable season. Between the two horizontal distances (0-20 cm and 21-40 cm) and the three soil depths (0-20, 21-40, 41-60 cm) studied, the samples collected at 21-40 cm away from the plant base and at 21-40 cm soil depth yielded significantly greater *M. incognita* J2 populations (Table II). Therefore, this can be considered the optimal (active root) zone for root-knot nematode in tea at the Lakhilunga estate. The population levels differed significantly from each other (P = 5%) at the three depths and the two distances (P = 5%) and between months. The interaction effect between months and depths was also significant (Tables III and IV).

The number of *M. incognita* females in the roots was greatest at the 0-20 cm depth in February, May, July and September and least in April 2000 (Fig. 2A), and the number of eggs/egg mass had three annual peaks at the 21-40 cm depth – in March, June and October-November 2000 (Fig. 2B). So the increase in the adult population was followed by a marked increase in the egg population in subsequent months (Figs 2A and 2B). The population densities of juveniles in the soil increased gradually and reached three peaks – in March, July and November. The gradual increase of the population of the endoparasitic nematodes in roots during post-monsoon months continued up to November and declined in December.

Correlation between nematode population density and climatic factors. When the populations of the nematode in soil and roots was correlated with soil temperature (^{0}C), soil moisture (%), rainfall (mm) and number of rainy days, positive but poor correlations were observed only between root-knot nematode populations in the soil and soil temperature in the three depths at 20 cm distance from the plant base, viz 0-20 (r = 0.454), 21-40 (r = 0.424) and 41-60 (r = 0.459), and soil moisture at 21-40 cm depth and 21-40 cm distance from the base of the plant (r = 0.437). The cumulative effect cannot be ignored (Table V). The nematode population was lowest in August at both horizontal distances in the two successive years of study and following the highest rainfall of the year. The highest nematode populations were observed in November, when the soil was rather dry and rainfall was least in the post-monsoon months, and also in pre-monsoon months (March-April).

DISCUSSION

The dynamics of nematode females and eggs masses in the roots, eggs/egg mass and juveniles in the soil were greatly affected by environmental conditions through-

Table II. Second stage juveniles (J_2) of *Meloidogyne incognita* population in soil at three depths and two distances away from the tea plant base (2000-2001).

Distance from plant base (S)	Soil population per 250 cm^3 soil at three soil depths (D)				
	20 cm	40 cm	60 cm	Mean	LSD (S) at P = 0.05
20 cm	1.623 (41.99)	1.676 (47.49)	1.699 (50.06)	1.666 (46.51)	1.30
40 cm	1.695 (49.58)	1.736 (54.45)	1.700 (50.14)	1.710 (51.391)	4.57
Mean (D)	1.659 (45.78)	1.706 (50.97)	1.699 (50.10)		
LSD (D) at P = 0.05		1. 34			

Nematode data were log $_{10}$ (x+1) transformed. Figure in parentheses are weighted means after re-transformation.

out the two year study. The increase of the soil nematode population was at its highest in November when the soil was less moist and rainfall was its lowest in post-monsoon months, due to increased hatching of eggs during this period. Similar nematode population changes were reported by Souza *et al.* (2008). These authors observed that, in an upland coffee plantation in Brazil, the J2 population density of *M. exigua* Goeldi increased during the dry months. Seasonal fluctuations data suggest that *M. incognita* completes at least three generations per year, each taking 3-4 months under natural conditions.

In the present study, the occurrence of peaks of the population of *M. incognita* during March, June-July and October-November may have been caused by the pre-monsoon and post-monsoon rainfall pattern coupled with soil temperatures during these periods, which favoured the onset of root-flush of tea plants. The increase in suitable roots may have increased nematode abundance. Seasonal production of new root biomass in perennials affects seasonal dynamics of the nematode density (Pinochet *et al.*, 1990). A similar explanation was also given by Souza *et al.* (2008) for the J2 population dynamics of *M. exigua* in coffee. The population maxima of juveniles in soil during these months suggested increased hatching of eggs during this period (Fig. 2). The increase in root-knot infection depends on active root growth flushes of the host trees during the initial and heaviest monsoon periods, whilst the lack of fresh roots coupled with moisture stress during December-January reduced infestation. On the basis of such evidence, Mukherjee and Dasgupta (1993) and Rama and Dasgupta (1987) postulated that the production of more juveniles during the root flush of a host plant is a

Table III. Analysis of variance of *M. incognita* population densities in the rhizosphere soil of tea.

Source of variation	df	SS	MS	F
Month	23	07	0.30	7.5**
Depth	5	2.66	0.53	13.25**
Error	115	4.69	0.04	

** Significant at 1% level.

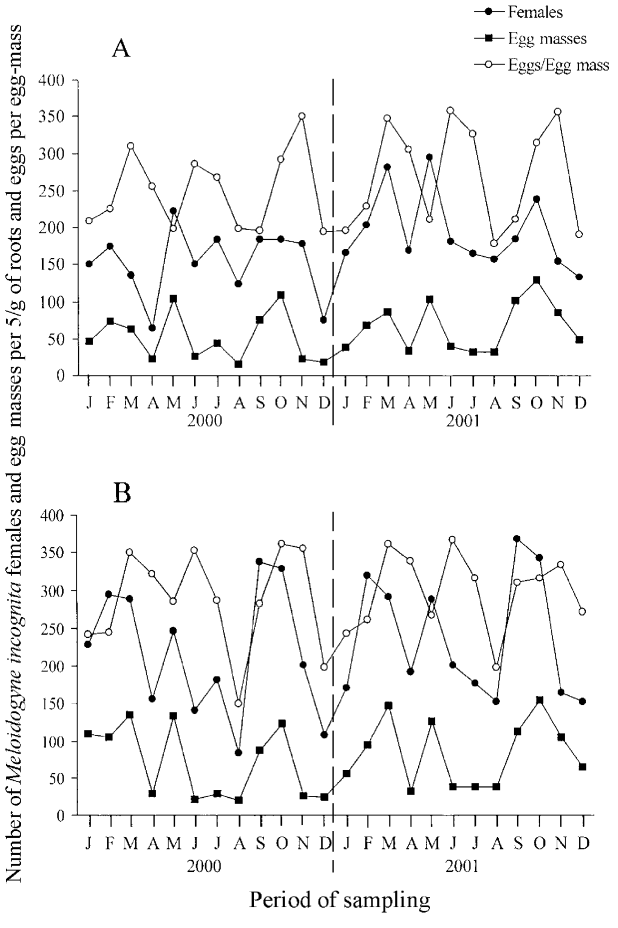

Fig. 2. Seasonal fluctuation (January 2000 – December 2001) of root populations of *Meloidogyne incognita* females, egg masses and eggs/egg mass on tea, at two horizontal distances from the plant base. A: 0-20 cm depth; B: 21-40 cm depth.

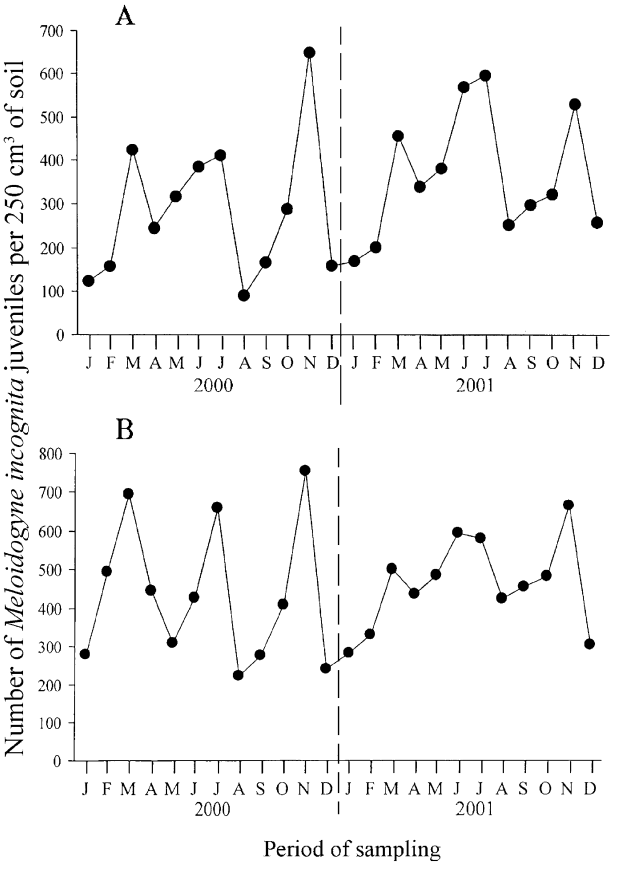

Fig. 3. Seasonal fluctuation (January 2000 – December 2001) of soil populations of *M. incognita* second stage juveniles on tea, at two horizontal distances from the plant base. A: 0-20 cm depth; B: 21-40 cm depth.

sign of parasitic adaptation to the host plant that enhances the survival capacity of the nematodes. This is a strong ecological adaptation as a successful parasite (Nath *et al*., 1998; Yeates, 1999). The optimum temperature for the development of *M. incognita* is 25-30 °C. Also, *M. incognita* is less sensitive to low temperature (10 °C) than *M. javanica*, which would account for its widespread distribution (Kamra and Sharma, 2000).

Soil texture affects plant growth and then nematode communities and densities (Yeates, 1999). The pre-monsoon and post-monsoon rainfall patterns of the two years may have had an indirect influence on the build-up of the nematode population by keeping the mean soil temperature (25.9-28.2 °C) at an optimum level and increasing the soil moisture level at the 21-40 cm and 41-60 cm depths (at post-monsoon). Nematodes are poikilothermic animals having a distinct thermal requirement for the hatching of their eggs, development, reproduction and survival. So, these two factors might have favoured reproduction, survival and hatching of eggs of the root-knot nematode and influenced nematode population build-up during these periods. Hence, soil temperature and soil moisture were limiting factors for the population size of *M. incognita* in tea. The present study showed that favourable conditions for growth and reproduction of *M. incognita* prevailed during pre-monsoon and post-monsoon months. The climatic conditions were optimal as there was moderate rainfall and favourable ranges of temperature during these periods. In addition, there was a consistent increase in the biomass of new feeder roots, which favoured the population build-up of parasitic nematodes. Thus, resource limitation may impact the survival of organisms, their reproductive potential and their

Table IV. Analysis of variance of *M. incognita* population densities in tea roots.

Source of variation	df	SS	MS	F
Year	1	2.46	2.46	14.47**
Month	23	24.66	1.07	6.29**
Depth	5	20.14	4.02	23.64**
Y × M	23	12.12	0.52	3.05**
Y × D	5	13.41	2.68	15.76**
M × D	115	54.30	0.47	2.76**
Error	115	20.16	0.17	

** Significant at 1% level

Table V. Correlation coefficients (r) between population densities of *M. incognita* in soil and root and mean monthly soil temperature, soil moisture, total rainfall and number of rainy days.

Population	Horizontal distance (cm)	Vertical depths (cm)	Soil Temperature (°C)	Soil moisture (%)	Rainfall total (mm)	Number of rainy days
Soil	0-20	0-20	0.454*	0.367	-0.058	0.079
		21-40	0.424*	0.305	0.092	0.051
		41-60	0.459*	-0.238	-0.182	-0.042
	21-40	0-20	0.245	0.076	-0.132	-0.073
		21-40	0.310	0.437*	-0.279	0.007
		41-60	0.306	-0.192	-0.232	0.025
Root	0-20	0-20	0.340	0.291	0.051	0.053
		21-40	0.357	0.209	0.010	0.039
		41-60	0.294	0.303	0.216	0.219
	21-40	0-20	0.320	0.219	-0.019	-0.035
		21-40	0.231	0.102	-0.179	-0.119
		41-60	0.252	0.189	-0.105	-0.054

* Significant at 5% level.

longevity. The crop phenology (Barker and Campbell, 1981; Eapen, 1993) as well as environmental conditions also appears to interact with the development, multiplication and seasonal behaviour of parasitic nematodes around perennial crops. For the development of an effective control schedule and for bioassay in tea, sampling should be done in March, June, July and October/November. Finally, any nematode management programme should be deployed preferably prior to March, June-July and October/November to prevent the occurrence of population peaks in tea plantations.

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