BIODIVERSITY OF PLANT PARASITIC NEMATODES OF CASHEW PLANTATIONS IN TRIPURA, INDIA

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Re-submitted: 23 September 2012; Accepted: 27 October 2012.

Summary. Thirteen species of plant parasitic nematodes within nine genera were isolated and identified from roots and soil around cashew nut shrubs. They were: *Helicotylenchus astriatus, H. dihystera, H. elegans, Hemicriconemoides mangiferae, Hoplolaimus indicus, Meloidogyne incognita, Pratylenchus coffeae, P. brachyurus, Rotylenchulus reniformis, Scutellonema brachyurus, Tylenchorhynchus leviterminalis, Xiphinema brevicolle and X. neoamericaum.* Among them, *Helicotylenchus* spp. (95%), *Rotylenchulus reniformis* (83%), *Pratylenchus* spp. (70%), *Hoplolaimus indicus* (60%), *Tylenchorhynchus leviterminalis* (46%) and *Xiphinema* spp. (28%) were the most predominant species associated with cashew nut in Tripura. All these species of phytonematodes, except *Hemicriconemoides mangiferae*, are new records of association with cashew nut from Tripura state as well as from India. The plantation at Belonia (10 years old) was more diverse than either the younger or older plantations when diversity was based on the numbers of nematodes. The three-years-old plantation at Nalkata was the least diverse in this regard. The twenty-years-old plantation located at Nagichherra was the most diverse when diversity was based on nematode biomass.

Key words: *Anacardium occidentale*, community analysis, phytonematodes, nematode biomass.

Cashew (*Anacardium occidentale* L.) is one of the most delicious, nutritious and important nut crops in the world. It is one of the most important export crops earning foreign exchanges in India. It grows in areas with annual rainfall ranging from 1,000 mm to 4,000 mm up to an elevation of 1500 masl (Singh, 1992). In India, cashew producing states include Kerala and mostly coastal areas of Andhra Pradesh, Tamil Nadu, Karnataka, Orissa, West Bengal, Goa, Damon and Diu. Recently, its cultivation has also started in north-east India, for instance in Assam and Tripura states. In Tripura (22°51' - 24°32' N and 90°10' - 92°21'E), it is cultivated in an area of 3,220 ha and the productivity is 1,200 t/ha/yr (Anonymous, 2002). Information on the infestation of plant parasitic nematodes on this plant is scanty.

High populations of *Criconemoides* sp., *Xiphinema* sp. and *Scutellonema* sp. were found in the rhizosphere of unthrifty trees in Brazil (El-Borai and Duncan, 2005). In addition, *Xiphinema index* Thorne *et* Allen 1950 causes "Xiphinematose" disease, common in cashew in north-eastern Brazil. Other plant parasitic nematodes associated with cashew include *X. ifaculum* in Liberia (Lamberti *et al.*, 1992) and *R. reniformis* in Costa Rica (Lopez and Azofeifa, 1985; Lopez and Salazar, 1987). *Caloosia longicaudata* (Loos, 1948) Siddiqi *et* Goodey, 1963, *Hemicriconemoides mangifera* Siddiqi, 1961 and *Tylenchorhynchus mashhoodi* Siddiqi *et* Basir, 1959 have all been reported from Orissa, India (Ray and Das, 1980), and *Hemicycliophora attapadii* Rahaman, Ahmad *et* Jairajpuri, 1996 has also been reported from India (Rahaman *et al.*, 1996). However, no information about plant parasitic nematodes associated with cashew nut is available from Tripura. Hence, an intensive survey was conducted for qualitative and quantitative analysis of plant parasitic nematodes affecting cashew nut in the state.

MATERIALS AND METHODS

During April-September 2001, a survey of plant parasitic nematodes was conducted in the main cashew nut producing areas of Tripura. A total of 60 composite soil and associated root samples was collected from six widely distributed cashewnut plantations (viz. Nalkata in North Tripura, Bishalgarh, Jumerdepa, Nagichherra and Salbagan in West Tripura, and Belonia in South Tripura) of different age groups (three to 30 years old).

Soil moisture content was determined from representative soil samples that were collected from within each of the six plantations and placed in aluminium boxes with aluminium covers. The weight of the moist soil plus box was determined with the help of a digital, pocket-type electronic balance. The aluminium boxes were then uncovered and placed in an electric oven, where the soil was dried at 110 °C for 24 hours. The

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Table I. Physicochemical properties of cashewnut soils in Tripura[1].

Location	Sand (%)	Silt (%)	Clay (%)	Organic matter	Organic carbon (%)	Nitrogen (kg/ha)	Phosphorus (kg/ha)	Potassium (kg/ha)	pH
Nalkata	50	14	34	1.052	0.612	0.06	0.7	13	5.4
Bishalgarh	52	14	35	0.910	0.529	0.08	0.7	24	5.7
Belonia	47	14	38	1.299	0.755	0.08	0.9	16	5.5
Jumerdepa	47	16	37	1.204	0.700	0.06	0.6	13	5.0
Nagichherra	46	16	38	0.872	0.500	0.06	0.2	12	4.5
Salbagan	51	15	35	1.053	0.612	0.04	0.6	21	6.3

[1]Soil type: sandy clay in all locations.

aluminium boxes were then covered and cooled in a desiccator before weighing the soil plus boxes and then the boxes alone so that the moist and dry soil weights could be derived by difference. The soil moisture content was expressed as percentage of water in soil on oven-dry weight basis.

Sand, silt and clay contents (in percentages) of soil samples were determined according to the International pipette method (Piper, 1966) and organic matter was determined by the Walkley and Black rapid titration method (Piper, 1996).

Determinations of available nitrogen (alkaline permanganate method of Subbiah and Asija, 1956), extractable phosphorus (Bray and Kurtz, 1945) and extractable potassium (flame photometric method according to Toth and Prince, 1949) were carried out and expressed as kg/ha.

At each location, ten sites were selected at random and a sample was taken at each site. Each sample consisted of ten sub-samples of rhizosphere soil and a portion of the root system, covering over one hectare of plantation and taken to a depth of 40 cm with the help of a galvanized iron pipe (diameter 2.5 cm) sampling tool. The age of the plantations was recorded as reported by the government officials and planters.

An aliquot of 250 cm^3 of each composite soil sample was processed for nematode assay by decanting and sieving, followed by the modified Baermann funnel technique (Schindler, 1961). The roots from the main samples were thoroughly washed in running tap water, finely chopped and thoroughly mixed. Five grams of these composite root masses were stained with the acid fuchsin-lactophenol method, and the nematode population in roots was estimated by extraction through maceration in a blender (Marks and McKenna, 1981) followed by counting their numbers in a sub-sample.

Nematodes collected from soil samples were heat-relaxed, killed in a hot water bath, fixed in TAF, dehydrated by the slow glycerol method and mounted on permanent slides. The nematode population was estimated from a 10% aliquot of the suspension obtained from each sample. Plant parasitic nematodes were identified to genus and/or species level. The species of root-knot nematodes were identified by their perineal pattern, male and juvenile characters (Siddiqi, 1986).

Nematode population density (mean ± S.E.) with range and frequency of occurrence of each nematode genus was determined at each location and for all of the samples collected from each area of the survey. Nematode communities were analysed by Prominence value (= absolute density × √ absolute frequency) and Importance value (the sum of relative density, relative frequency and relative biomass) for each nematode species following the method of Norton (1978) and Norton and Schmitt (1978). Biomass was determined by morphometric measurement of ten mature females collected from each location.

The Shannon-Weiner diversity index was also calculated, to determine the diversity of nematode community, according to the equation:

$$H' = \Sigma^{s} Pi \log Pi$$

where 's' is the number of species at each location and 'Pi' is the relative abundance of the 'i'th species.

RESULTS

Soil characteristics. The physico-chemical properties of the cashew nut soils showed that the soils were of a sandy clay type in all locations, acidic (pH 4.5-6.3) with

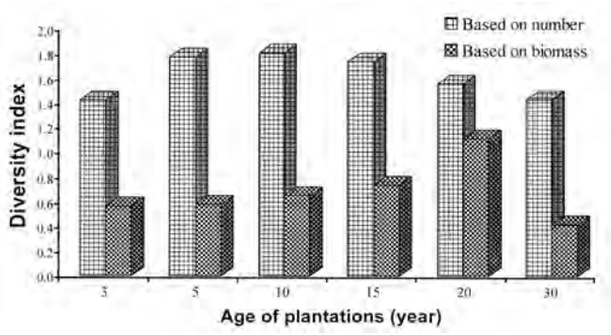

Fig. 1. Nematode community diversity in relation to age of cashew nut plantations in Tripura, India.

high sand (46-52%), high clay (34-38%) and low silt (14-16%) contents. Organic carbon varied from 0.50 to 0.75% and there was wide variation in NPK status (Table I). The diversity of the nematode community based on the numbers of nematodes (H'n) was greatest in the plantation located at Belonia (10 years old), than in younger or older plantations (Fig. 1). Hence, it was probably related to the age of plant communities rather than to the physico-chemical properties of the soils.

Nematodes encountered. Thirteen species of plant parasitic nematodes within nine genera were isolated and identified from roots and the soil around cashew nut. The species encountered were *Helicotylenchus astriatus* Khan *et* Nanjappa 1972, *H. dihystera* (Cobb, 1893) Sher 1961, *H. elegans* Roman 1965, *Hemicriconemoides mangiferae* Siddiqi 1961, *Hoplolaimus indicus* Sher 1963, *Meloidogyne incognita* (Kofoid *et* White 1919) Chitw. 1949, *Pratylenchus coffeae* (Zimmermann 1898) Flipjev *et* Schuurmans Stekhoven 1941, *P. brachyurus* (Godfrey 1929) Filipjev *et* Schuurmans Stekhoven 1941, *Rotylenchulus reniformis* Linford *et* Oliveira 1940, *Scutellonema brachyurus* (Steiner, 1938) Andrassy 1958, *Tylenchorhynchus leviterminalis* Siddiqi, Mukherjee *et* Dasgupta 1982, *Xiphinema brevicolle* Lordello *et* da Costa 1961, and *X. neoamericanum* Saxena, Chhabra *et* Joshi 1963 (Table II). Among these, *Helicotylenchus* spp. (95%), *R. reniformis* (83%), *Pratylenchus* spp. (70%), *Hoplolaimus indicus* (60%), *T. leviterminalis* (46%) and *Xiphinema* spp. (28%) were the most predominant nematode species associated with cashewnut (Table II).

Nematode abundance. The spiral nematodes *Helicotylenchus* spp. were represented by three different species, viz. *H. astriatus, H. dihystera* and *H. elegans,* which formed the bulk of the nematode load in soil samples. The mean density of *Helicotylenchus* spp. was 156 nematodes/250 cm^3 soil and the population ranged from 16 to 478 nematodes/250 cm^3 soil.

The lesion nematodes, *Pratylenchus* spp., were represented by two species, namely *P. coffeae* and *P. brachyurus*, with *P. coffeae* being the dominant species. The mean density of the lesion nematodes was 203 nematodes/250 cm^3 soil and the population ranged from 8 to 480 nematodes/250cm^3 soil.

Table II. Population densities (Mean ± SE), with ranges (in brackets) and frequency of occurrence (as % below Mean ± SE in Mean column on the right), of plant parasitic nematodes in cashewnut plantations of Tripura.

Nematode species	Locality						
	Nalkata (NT)[1] (3yr)[2]	Bishalgar (WT) (5yr)	Belonia (ST) (10yr)	Jumerdepa (WT) (15yr)	Nagicherra (WT) (20yr)	Salbagan (WT) (30yr)	Mean
Helicotylenchus spp. *(H. astriatus, H. dihystera, H. elegans)*	62±16 (25-188)	105±20 (36-220)	88±20 (16-176)	210±41.2 (48-368)	334±57 (66-478)	178±33 (54-310)	156±17 95[3]
Hemicriconemoides mangiferae	–	56±13.3 (22-114)	–	26±11.1 (12-48)	18±8 (10-26)	32±12 (20-44)	39.5±8 21
Hoplolaimus indicus	44±10.2 (18-76)	27±11.3 (10-105)	228±57 (46-372)	65±11.3 (24-96)	82±22.6 (34-145)	16±6.6 (8-36)	77.1±15.3 60
Meloidogyne incognita	128±95 (33-223)	70±38 (28-184)	142±53 (18-262)	88±32 (36-172)	56±36 (20-92)	30±8 (22-38)	90.4±19 30
Pratylenchus spp. *(P. coffeae, P. brachyurus)*	26±7.3 (8-46)	148±44 (16-287)	355±33 (160-480)	212±63 (35-360)	225±39.4 (44-356)	115±43.2 (35-250)	203.3±23 70
Rotylenchulus reniformis	37±6 (12-58)	86±21 (10-146)	270±28.4 (28-325)	266±48 (30-386)	152±25 (43-225)	108±30 (24-230)	157±17 83
Scutellonema brachyurus	64±17.3 (40-115)	–	272±77 (58-466)	144±38 (25-210)	205±52.6 (58-286)	–	180.4±32.2 31
Tylenchorhynchus leviterminalis	–	258±65.5 (36-430)	216±61 (25-340)	14±3.1 (5-28)	–	20±4 (6-34)	95.3±25.1 46
Xiphinema spp. *(X. brevicolle, X. neoamericanum)*	–	10±7 (2-38)	–	18±9 (8-45)	25±5 (16-38)	33±9 (24-60)	21±4 28

[1] Districts (NT = North Tripura, WT = West Tripura, ST = South Tripura);
[2] Age of plantations;
[3] Frequency of occurrence (%)

A mixed infestation of the species *P. coffeae* and *P. brachyurus* was found in 45% of the samples examined. The mean abundance of *P. coffeae* was highest (225 nematodes/5 g root) in roots at Nagichherra and the population ranged from 44 to 356 nematodes/5 g of root samples (Table II).

The mean density of the yam nematode, *Scutellonema brachyurus*, was 180 specimens/250 cm³ soil and the population ranged from 25 to 466 specimens/250 cm³ soil. This density was much greater in comparison to the other prevalent species but the frequency of this species (31%) was much lower than others.

The mean density of the reniform nematode, *R. reniformis*, was 157/250 cm³ soil and the population ranged from 10 to 386 nematodes per 250 cm³ soil. This polyphagous pest was uniformly present in all plantations of different age groups.

Second stage juveniles (J_2) of the root-knot nematode *M. incognita* were prevalent in all plantations and their populations ranged from 18 to 262 J_2/ 250 cm³ soil.

The mean density of the stunt nematode, *T. leviterminalis*, was 95 specimens/250 cm³ soil and the population ranged from 5 to 430 nematodes/250 cm³ soil.

The two species of the dagger nematodes, *Xiphinema* spp., were prevalent in four locations. Of the two, *X. brevicolle* was the predominant species. *Xiphinema brevicolle* is a major problem and caused decline of litchi and other fruit plants in Africa (McSorley, 1992).

The six cashew nut plantations sampled varied greatly in age (three to 30 years) and, as a result, in the population densities of the nematode species encountered. *Rotylenchulus reniformis, P. coffeae, P. brachyurus* and *S. brachyurus* are serious polyphagous/polytrophic nematode pathogens. The concomitant occurrence of these

Table III. Community analysis of plant parasitic nematodes associated with cashewnut plantations of Tripura.

Nematode species[1]	Relative density (%)	Relative frequency (%)	Relative biomass (%)	Prominence value	Importance value	Plantations code[2]	Pathogenic significance ranking[3]
Meloidogyne incognita	6.55	5.13	76.61	465.5	88.29	1-6 [4]	–
Rotylenchulus reniformis	11.78	14.18	4.98	1393.8	30.94	1-6 [4]	1
Helicotylenchus dihystera	12.48	16.24	0.50	1578.9	29.22	1-6 [4]	–
Pratylenchus coffeae	13.86	11.96	0.27	1505.9	26.09	1-6 [4]	2
Scutellonema brachyurus	13.17	5.29	7.39	952.0	25.85	1,3	3
Hoplolaimus indicus	5.93	10.25	2.20	596.4	18.38	1-6 [4]	–
Tylenchorhynchus leviterminalis	9.78	7.86	0.22	861.3	17.86	2-4,6 [5]	–
Helicotylenchus elegans	8.70	5.13	0.32	618.9	14.15	1,4,6	–
Helicotylenchus astriatus	7.32	6.49	0.32	585.6	14.13	2,3,5 [5]	–
Pratylenchus brachyurus	4.93	7.69	0.45	429.3	13.07	1-6 [4]	4
Xiphinema brevicolli	1.62	4.78	2.91	111.1	9.31	2,4-6 [5]	–
Hemicriconemoides magniferae	2.54	3.59	0.40	151.12	6.53	2,4-6	–
Xiphinema neoamericanum	1.31	1.36	3.40	48.0	6.07	5	–

[1] Nematodes are arranged in the descending order of their importance values.
[2] Plantations code: (i) Nalkata, (ii) Bishalgar, (iii) Begonia, (iv) Jumerdepa, (v) Nagicherra, (vi) Salbagan.
[3] Based on biological understanding.
[4] Most widely distributed.
[5] Least widely distributed.

Table IV. Nematode community diversity indices based on the numbers of nematodes and biomass in cashewnut plantations of Tripura.

Index based on number of nematodes		Index based on nematode biomass	
Age of plantations[1] (Years)	Index (H′n)	Age of plantations[1] (Years)	Index (H′b)
10	1.810	20	1.116
05	1.775	15	0.739
15	1.741	10	0.652
20	1.563	05	0.582
30	1.432	03	0.570
03	1.422	30	0.426

[1]Age of plantations arranged by descending diversity of nematode community.

pathogenic species in the cashew nut plantations of Tripura means that multiple species infections occurred in the investigated area.

Nematode community structure. Analysis of the nematode communities showed that *M. incognita* ranked first in importance value (88.2), which is three to five times more than for other nematode species, such as *R. reniformis* (30.9), *H. dihystera* (29.2), *P. coffeae* (26.1), *S. brachyurus* (25.8) and *H. indicus* (18.4), as shown in Table III. However, *H. dihystera* ranked first in prominence value (1578.9), followed by *P. coffeae* (1505.9), *R. reniformis* (1393.8) and *S. brachyurus* (952.0). The relative density of *P. coffeae* was greatest (13.9), followed by those of *S. brachyurus* (13.2), *H. dihystera* (12.5) and *R. reniformis* (11.8). As the relative biomass of *M. incoginta* was highest (76.6), having obese females, it out-ranked all other dominant species in the community in terms of importance value (Table III). From a plant pathological point of view it appears that *M. incognita* may establish as a key parasite in the future, although cashew is not its favoured host wherever nematode associations have been surveyed. Nevertheless, community analysis, as used in ecosystem studies, shows the high importance value of *M. incognita* in cashew.

Nematode community diversity. The diversity of plant parasitic nematode communities, measured by numbers of nematodes and biomass of individual species in different cashew nut plantations, was compared with indices of diversity based on number (H'n) and biomass (H'b) and correlated with the age of cashew nut plants (Fig. 1). H'n ranged from 1.422 to 1.810, while H'b ranged from 0.426 to 1.116 (Table IV). H'b values were less than H'n. This agrees with Norton and Edwards (1988) with maize in Iowa, USA. Nematode community diversity (H'n) was greater in the plantation located at Belonia (ten years old) than in either the younger or older plantations and least diverse at Nalkata (three years old) (Fig. 1). H'b was most diverse at Nagicherra (20 years old), showing its linear increase with age. With the increase in age of a plantation, plant parasitic species probably become more abundant and established to that monoculture plantation. Beyond a certain age of the monoculture plantation, the soil seems to be unsupportive to a very diverse pathogenic community of phytonematodes except the nematode specific for that plantation. These findings agree with those of Mukherjee and Dasgupta (1983) on banana in West Bengal, India, dogwood nurseries in Tennesse, USA (Niblack and Bernard, 1985), and with jackfruit in Tripura, India (Mukherjee *et al*., 2001).

CONCLUSIONS

In cashew nut plantations, five nematode pests that are of major importance on a world basis have been encountered in Tripura. Infestations by *P. coffeae, P. brachyurus, R. reniformis, M. incognita* and *S. brachyurus* are recorded for the first time from Tripura as well as from northeastern India. Although the extent of damage nematodes may cause to cashew nut is not known (El-Borai and Duncan, 2005), our findings may partially explain the low productivity of cashew in the state and warrant further investigations to assess the impact of these nematodes on the cashew nut crop. Biodiversity of plant parasitic nematode communities in cashew nut plantations in relation to the age of the plants showed that diversity of the nematode communities increased up to certain age in relation to nematode density and then gradually declined. However, the diversity of the nematode communities gradually increases in relation to biomass and becomes stabilised at the age of 15-25 years in the long life of this economically important crop.

Biodiversity, measured both by number and biomass, is age-related with a peak at a certain age. The nature of the relation along with the peak is dependent on highest pathogenic ranking (HPR), particularly life span, and the productive life of a tree under the soil climatic conditions and given level of management.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. S. Ganguly, Division of Nematology, IARI, New Delhi, and Dr. H.K. Bajaj, Department of Nematology, HAU, Hissar, Haryana, for confirmation of the identity of nematode species, the Indian Council of Agricultural Research, New Delhi, for financing the research scheme, and the Head, Department of Zoology, M.B.B. College, Agartala, for laboratory facilities. C. Bhattacharjee is grateful to her late co-supervisor Dr. B. Mukherjee for her Ph.D degree from Visva Bharati University.

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