

# DISTRIBUTION OF PLANT PARASITIC NEMATODES IN THE SOILS OF TEA ESTATES IN CEYLON

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## Summary

The spiral nematode, *Helicotylenchus dihystera* (Cobb) Sher is more widely distributed in tea soils than is the "meadow" or root-lesion nematode, *Pratylenchus loosi* Loof, despite the fact that tea, *Camellia sinensis* (L.) O. Kuntze, is either not a host or a very poor host plant for *H. dihystera* and is a very suitable host for *P. loosi*. Other plants, interplanted with tea, particularly the "dadap" tree, *Erythrina lithosperma* Blume, appear to be the primary hosts of *H. dihystera*. Of the several other nematodes that occur in tea soils, only the pin nematode, *Paratylenchus curvatus* van der Linde, and the root-knot nematode, *Meloidogyne brevicauda* Loos, seem to be parasites of tea.

## Introduction

The distribution of *Pratylenchus loosi* in tea soils has been summarised by Hutchinson and Vythilingam (1963), and the pathogenicity of this nematode to tea was demonstrated by Hutchinson (1963). Root-knot nematodes of the genus *Meloidogyne* have been found in roots of nursery tea plants and in the roots of *Tephrosia Vogelii* Hook. and of *Erythrina lithosperma* for many years, and the literature is summarised by Visser (1959a). Spiral nematodes are parasitic upon *E. lithosperma* and are therefore commonly found in tea soils, but are unable to thrive upon tea roots, according to Gadd (1943 and 1947). However, Gadd (1946) found pin nematodes of the genus *Paratylenchus* when examining tea roots for *P. loosi* and presumed that they were parasitic upon tea. Studies by Loos (1953) showed that *Meloidogyne brevicauda* could be a destructive pest of tea, and that nematodes of other genera could be recovered from jungle and patna soils (Loos, 1948).

TABLE 1.—*Plant parasitic nematodes recovered from tea soils in the zone receiving the south-west monsoon.*

Estates and elevation	Plant parasitic nematodes, species and number of occurrences				
	<i>P. loosi</i>	<i>Meloidogyne</i> spp.	<i>P. curvatus</i>	<i>H. dihystera</i>	<i>S. brachyurum</i>
<b>0-4,000 ft.</b>					
Estates, number	13	16	32	38	6
percent	28.9	35.6	71.1	84.5	13.3
Samples, number	55	47	153	194	8
percent	22.6	19.3	62.8	79.5	3.3
<b>4,000 ft. &amp; above</b>					
Estates, number	38	46	58	62	11
percent	55.1	66.6	84.0	90.0	16.0
Samples, number	183	186	313	333	27
percent	44.7	45.5	76.5	81.4	6.6

TABLE 2.—*Plant parasitic nematodes recovered from tea soils in the Zone receiving the north-east monsoon.*

Estates, all elevations	Plant parasitic nematodes, species and number of occurrences				
	<i>P. loosi</i>	<i>Meloidogyne</i> spp.	<i>P. curvitalus</i>	<i>H. dihystra</i>	<i>S. brachyurum</i>
Estates, number	9	15	19	29	3
percent	29.0	48.4	61.3	93.5	9.7
Samples, number	49	38	74	119	10
percent	32.2	25.0	48.6	78.3	6.6

TABLE 3.—*Concentration of plant parasitic nematodes in soil from mature stands of seedling tea in the zone receiving the south-west monsoon (elevation 4,000 ft and above).*

Nematode species	No. samples from which 100 or more nematodes were recovered per 100 g soil	Largest no. nematodes recovered per 100 g soil
<i>P. loosi</i>	29	472
<i>Meloidogyne</i> spp.	17	1297
<i>P. curvitalus</i>	42	1606
<i>H. dihystra</i>	62	480
<i>S. brachyurum</i>	12	440

TABLE 4.—*Plant parasitic nematodes occasionally recovered from tea soils or from jungle and patna soils.*

Nematode	Date	Location	Environment
<i>Boleodorus</i> sp. <sup>1</sup>	24- 5-60	Elladallua Estate, Badulla	mature tea
<i>Helicotylenchus</i> , n.sp. <sup>1</sup>	28-11-60	Blairlmond Estate, Udapussellawa	mature tea with ground cover of <i>Stylosanthes</i> and <i>Drymaria</i>
<i>Hemicylophora longicaudata</i> Loos <sup>2</sup>	6-12-59	Gikiyanakanda Estate, Neboda	nursery basket soil (jungle)
<i>Hemicylophora typica</i> de Man <sup>2</sup>	11- 6-60	Brownlow Estate, Maskeliya	proposed new clearing site (patna?)
<i>Hoplolaimus</i> (near <i>seinhorsti</i> Luc) <sup>1</sup>	1- 6-60	Lelwala Group, Wanduramba	mature tea?
<i>Scutellonema</i> , n.sp. <sup>1</sup>	8- 7-60	Kadiclenla Estate, Kotmale	mixed grasses (patna)
<i>Xiphinema americanum</i> Cobb <sup>2</sup>	25- 8-60	Kellicwatte Estate, Patana	mixed grasses
<i>Xiphinema radicola</i> Goodey <sup>2</sup>	25- 8-60	Kellicwatte Estate, Patana	mixed grasses

<sup>1</sup> Identification by S. A. Sher.

<sup>2</sup> Identification by A. C. Tarjan.

## Materials and Methods

A survey for all plant parasitic nematodes present in tea soils was conducted between September 1959 and March 1961, and encompassed all Planting Districts. A total of 805 samples was examined from 145 estates. The methods used have been described by Hutchinson and Vythilingam (1963). Briefly, 100 g samples of soil are processed, using a modification of the Baermann funnel technique.

Inoculations of several plant species, to determine whether or not they are hosts of *Helicotylenchus dihystra*, were made using the nematodes suspended in water, and with the plants growing in soil containing this species as the only plant parasitic nematode present. In each type of inoculation, the plants were contained in polythene bags filled with soil which were perforated to permit drainage of water. At the close of the tests, a total of three or four 10 g lots of soil were processed separately to recover the nematodes from each soil sample. In the second test, the entire root system of each plant was also processed. The roots were carefully washed, surface dried with a towel, weighed, and cut up prior to maceration in water in a mechanical blender. The resulting macerate was poured into a 60-mesh sieve covered with a special nylon-cottonwool filter (Oostenbrink, 1960). A spray of water was directed on to the filter to settle the froth produced by blending the roots, and the sieve was then placed in a shallow enamel pan containing sufficient water to flood the surface of the filter. Nematodes in all stages of post-embryonic development migrate through the filter, and on the following day the water in the pan was poured off into a tall 100 ml beaker. The nematodes were allowed to settle for 2 hours, and the supernatant liquid was carefully removed with a rubber-bulb pipette. The remaining 4-5 ml containing the nematodes was aspirated into a standard pipette with a short length of rubber tubing attached to the upper end. When aspiration was complete, the tube was clamped shut so that the column of water remains within the pipette. After 3 hours, 4 drops, or approximately 0.05 ml. was pipetted on to a microscope slide. A cover slip was added, and the slide was flamed to kill the nematodes, which were counted directly under a compound microscope.

The scientific names of the nematodes discussed in this paper have been obtained from Baker (1962) and Sher (1961). The scientific names of the plants have been obtained from Goodey, and Franklin (1958), Goodey, Franklin and Hooper (1959) and through the courtesy of Dr U. Pethiyagoda of the Tea Research Institute of Ceylon and of Mr K. L. D. Ameratunge of the Botany Department, University of Ceylon, Peradeniya.

## Results

The survey showed that *Helicotylenchus dihystra* is by far the most prevalent plant parasitic nematode, in each of the three major climatic regions in which tea is grown (Tables 1 and 2). This nematode also frequently occurs in high concentrations in tea soils (Table 3). Next in order of frequency, in each of the three regions, were *Paratylenchus curvatus*, second stage larvae of *Meloidogyne* spp., *Pratylenchus loosi*, and *Scutellonema brachyurum* (Steiner) Andrassy. Identification of *H. dihystra* was made from 25 locations, and identification of *S. brachyurum* was made from 11 locations by S. A. Sher (1961a, and in other correspondence). Identification of *P. curvatus* was made by Tarjan (1960) from specimens collected from mixed tea clones at Mooloya estate, Hewaheta. Examination of this population showed a sex ratio of approximately 9 per cent males (123 males and 1,217 females).

Other nematodes encountered in tea soils include *Meloidogyne brevicauda* and a species of *Rotylenchulus*. *M. brevicauda* has been recovered from the soils of only 3 estates: Mooloya (Hewaheta), Kabaragalla (Ellamulla) and Oliphant (Nuwara Eliya). The latter is the type location. Identification of this species, collected

from Mooloya estate, was confirmed by Tarjan (1960a). Larvae, males and immature females of *Rotylenchulus* sp, were found in large numbers in a tea nursery at Aighburth Estate, Rakwana (Tarjan, 1960). Subsequently, this nematode was recovered from 19 additional estates, predominantly located at low and mid-country elevations (below 4,000 ft), and confirmatory identifications were made by Sher (1961a). However, no mature females of this species have yet been found in tea roots. The largest number of these nematodes (1393 per 100 g soil) was recovered from around roots of clone T.R.I. 2025 in a clonal multiplication plot at Kirimetiya Estate, Galaha, in February, 1960. However, no nematodes of this genus could be obtained from the same location in April, 1962.

Nematodes less frequently encountered in tea soils, and those recovered from patna (grassland) or jungle soils adjacent to tea estates, are noted in Table 4. The fact that two of these are new species, found only in Ceylon, is a matter of some interest.

TABLE 5.—*Test of tea as a host of the spiral nematode, Helicotylenchus dihystrera* (Cobb) Sher, in comparison with several other plant species.<sup>(1)</sup>

Type of examination and replication	<i>Helicotylenchus dihystrera</i> associated with:						
	Tea, Clone			Marigold	Coffee	<i>Sesbania</i>	Dadap
	DT1	TRI 2024	KW 16/3				
Soil (100) <sup>(2)</sup> a	27	30	10	13	107	77	507
b	20	10	13	7	100	173	770
Root (1 g.) <sup>(3)</sup> a	0	0	0	0	55	24	558
b	0	0	0	0	17	167	244

<sup>(1)</sup> Inoculation 26-6-62. Examination 31-11-62.

<sup>(2)</sup> Estimated from numbers recovered from a total of three 10 g. samples, processed separately.

<sup>(3)</sup> Estimated from numbers recovered from the entire root system.

Results of the inoculations of *H. dihystrera* on tea roots show that tea is an unlikely host of this species, and confirm the results of inoculations made by Gadd (1943). Preliminary tests had shown that this species probably did not survive more than 7½ months when inoculated in water suspension on roots of tea, but multiplied nearly 30-fold on the roots of *E. lithosperma* within 6½ months. Some survival around the roots of Guatemala grass, *Tripsacum laxum* Nash, was also indicated. Results of growing several plant species in soil containing *H. dihystrera* are given in Table 5. The plants tested were tea (*Camellia sinensis*, 3 clones), marigold (*Tagetes erecta* L.), coffee (*Coffea arabica* L. x *C. robusta* Linden), *Sesbania cinerescens* Welw. and dadap, *Erythrina lithosperma*. There is an interesting apparent correlation between the numbers of nematodes found in soil and in roots, indicating that of the plants tested, only *Erythrina*, *Sesbania* and coffee are likely hosts of this nematode. However, *H. dihystrera* has rather frequently been recovered in small numbers from tea roots that were carefully washed prior to processing. Under certain conditions, therefore, tea may be a host of this nematode.

## Discussion

The percentage recovery of plant parasitic nematodes from tea soils, using the modified Baermann funnel technique, varies greatly with sample size, Visser

(1959) found that nine times as many *Pratylenchus loosi* and larvae of *Meloidogyne* spp. were recovered per unit weight of soil when 5 g samples were used rather than 100g samples. Six times as many nematodes were obtained using 10 g samples. No record was kept of the recovery of other plant parasitic species, but the effect of sample size would probably be similar. The numbers of nematodes recovered from 100 g soil (Table 3, and in the text) therefore represent only a fraction of the nematodes actually present. However, the same method was used throughout the survey period, so that results are comparable.

The fact that the "dadap," *Erythrina lithosperma* is a very good host for *Helicotylenchus dihystrera* explains the abundance of this nematode above 4,000 ft elevation in the south-west monsoon zone, since *Erythrina* is commonly planted for shade. Below 4,000 ft, however, *Gliricidia maculata* is the predominant shade tree, so that this species must also be a suitable host for *H. dihystrera*. Likewise, in the north-east monsoon zone *Albizzia moluccana* Miq. is commonly planted, and this also would be worth testing as a host plant. We seem to have here a very curious situation, in which the crop itself is possibly not a host of the nematode most frequently found in the soil.

A test of 8 species of plants grown among tea, for susceptibility to *Pratylenchus loosi* (Hutchinson, 1963), has incidentally revealed that several hosts of *H. dihystrera* in addition to *Erythrina*. Of the plants tested, *Erythrina* and *Crotalaria clarkei* harboured moderate numbers of the nematodes in their roots (avg. 25 and 14 per g respectively), and *Acacia decurrens* and *A. pruinosa* harboured small numbers (avg 4 and 1 per g respectively). Roots of *Crotalaria anagyroides* H.B. and K. contained none of the nematodes. However, roots of potatoes *Solanum tuberosum* L. have been found to harbour large numbers of *H. dihystrera* on several occasions. There is also good evidence from surveys that *H. dihystrera*, and possibly *S. brachyurum* are able to maintain themselves around the roots of Gutemala grass, whereas the other nematodes found in tea soils are apparently unable to do so.

Of interest is the fact that in 1898, Zimmerman discovered a very similar spiral nematode from *Erythrina lithosperma* in Java, which he described as *Tylenchus erythrinae*. This nematode is now known as *Helicotylenchus erythrinae* (Zimm.) Golden.

At this point a note on the synonymy of names given to the spiral nematodes recovered from tea fields in Ceylon may be useful. The previous name of the nematode primarily concerned in the present study was *Helicotylenchus nannus* Steiner, prior to the change of name to *H. dihystrera* by Sher (in 1961). The names of the spiral nematodes recovered from *Erythrina* in Ceylon by Gadd (1944) were given as *Anguillulina erythrinae* (Zimm.) Goodey, and *Hoplolaimus brachyurus*. The identifications were said to have been made by T. Goodey and G. Steiner, respectively. *A. erythrinae* has now been synonymised with *Helicotylenchus erythrinae* (Zimm.) Golden. *Helicotylenchus nannus* was not described by Steiner until 1945. Since that time, *H. erythrinae* has not been reported from tea soils in Ceylon, and in the present study, only *H. dihystrera* (of the described species of the genus) was recovered. It is therefore probable that the nematode now known as *Helicotylenchus erythrinae* has never been recovered from Ceylon tea soils, and it is possible that Zimmerman's spiral nematode either was the nematode now known as *Helicotylenchus dihystrera*, or included this species. A survey of Javanese coffee and tea soils for spiral nematodes might therefore be of taxonomic interest.

Regarding species of *Meloidogyne*, Visser (1959) noted that *M. javanica* (Treub) Chitwood was present in tea soils, and *M. arenaria* (Neal) Chitwood has been identified from roots of *Tephrosia vogelii* by Oostenbrink (1958). Confirmatory identifications of the latter species from roots of *T. vogelii* were made during the course of the present study. Careful tests have shown that *M. arenaria* from *T. vogelii* can invade

roots of nursery plants of both seedling and clonal tea, producing females containing apparently viable eggs (Hutchinson, 1963). However, roots of mature tea are apparently not colonized by *Meloidogyne* spp. other than *M. brevicauda* (Loos, 1951, 1953). *Erythrina lithosperma* is a superior host of root-knot nematodes, becoming heavily galled under field conditions. *Albizzia sumatrana* may also be a good host, as it harboured moderate numbers of second stage larvae (av. 27 per g) in the same pot tests (Hutchinson, 1963) in which roots of *E. lithosperma* harboured an average of 54 per gram. These results confirm the observations made by Visser (1959) from roots collected from these two species in the field. Thus, it would appear that roots of shade trees are also the primary source of the second stage larvae of *Meloidogyne* found in tea soils. Other plants that are hosts of root-knot nematodes, such as *Tephrosia vogelii* and *Mimosa invisa* Mart. (Visser, 1959) are not commonly interplanted with tea.

The distribution and occurrence of the remaining root-knot nematode, *Meloidogyne brevicauda*, is a most interesting subject from a scientific point of view, in addition to having at least two practical aspects. Examination of severely galled roots from four bushes at the type location showed the scarcity of living nematodes in the root galls, and the extreme rarity of mature females containing eggs. Small numbers of second stage larvae were, however, recovered from the soil. Examination of infected tea from Mooloya estate showed a much greater number of females in the roots, although females with eggs were uncommon. A few males were recovered, and small numbers of larvae were present in the soil. Larvae were present in moderate numbers (16 per 100 g) from soil around infected roots at Kabaragalla estate. Apparently, tea is a poor host for this nematode, but is hypersensitive to its presence in the roots. Roots of all ages are attacked. However, the nematodes seem to require several years to establish themselves in the roots of tea planted in infected soil. Figure 1 shows a healthy storage root of tea, compared to one that has been heavily galled by *M. brevicauda*. For comparison, a lesion produced by *Pratylenchus loosi* in the cambial and phloem tissues of a storage root is shown in Figure 2.

Of considerable interest is the fact that all three of the estates where *M. brevicauda* has been found border on the same area of up-country jungle (elevation range from 5,000-7,000 feet). Of the jungle hosts of this nematode, however, nothing is known. The practical aspects of our present knowledge are (1) that the nematode may in time be found on other estates bordering this same, rather extensive, jungle area and (2) that tea clones can possibly be selected for immunity to the nematode. Spread of the nematode does not appear to have occurred since Loos (1951) first mentioned root-knot disease of mature tea, as the number of estates known to be infected is still three, despite a greatly increased survey programme in the interim. A step in the direction of clonal selection has been taken with the establishment, in July 1962, of a half acre block of clones in an infected area at Oliphant estate.

Of the other nematodes recovered from tea soils, or from nearby patna or jungle soils, little is known. *Paratylenchus curvatus* is occasionally recovered in large numbers from the roots of tea, and has been demonstrated in the roots by staining. At the moment, it seems reasonable to consider *P. curvatus* as a probable parasite of tea, and *Scutellonema brachyurum* as a probable parasite of shade trees. Gadd (1943) noted that roots of *E. lithosperma* were readily colonized by the latter species, and that *T. vogelii* is also a host. This observation was confirmed by us, when sampling a mature stand of *T. vogelii* on St Coombs estate. The *Hemicycliophora typica* de Man, reported by Loos (1948) from jungle and patna soils, has since been considered to be a separate species *i.e.* *H. membranifer* (Micoletzky) Loos, and *Hemicycliophora longicaudata* Loos is considered to be a species belonging to an as yet undescribed genus (Thorne, 1955). Neither species has been found in tea soils. The *Rotylenchulus* sp. may be parasitic upon tea, but if so, it is unlikely to be of importance.

## Conclusions

Of the nematodes found in tea soils, only the "meadow" or root-lesion nematode, *Pratylenchus loosi* is of present importance. Of the remaining species, only *Meloidogyne brevicauda* appears to be of potential importance. However, further tests to determine the host range of *Helicotylenchus dihystrera*, *Scutellowema brachyurum*, and *Pratylenchus curvittatus* would be of interest.

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