

# THE DISTRIBUTION OF *PRATYLENCHUS LOOSI* LOOF AMONG TEA ESTATES IN CEYLON, WITH PARTICULAR REFERENCE TO ALTITUDE

M. T. Hutchinson and M. K. Vythilingam

## Summary

A survey for the root-lesion or "meadow" nematode, *Pratylenchus loosi* Loof, has been continuing since 1939, and has included over 9,000 soil samples from a total of 526 estates. This nematode has been found to be distributed throughout the tea-growing regions of Ceylon; being present in all Planting Districts and at all elevations. It is found most abundantly, however, at elevations above 4,000 ft in the zone receiving the southwest monsoon. Obvious injury attributable to this nematode is most frequently found within these same geographic and climatic limits. Temperature appears to be the primary factor influencing the concentration of *P. loosi* in soil, with average soil temperatures greater than 80 to 85 degrees F markedly depressing population growth.

## Introduction

Tea, *Camellia sinensis* L. is a host in Ceylon, for several species of nematodes. The most important of which is the root-lesion or "meadow" nematode, now known as *Pratylenchus loosi* Loof. Since its discovery as a pest (Gadd 1939), this nematode has been found in soil samples received from or collected at more than two hundred estates; the total number of tea estates in Ceylon being approximately twelve hundred. When different concentrations of *P. loosi* recovered from soil were marked on a map by means of coloured pins, the highest levels of infection were seen to occur at elevations above 4,000 ft and only low levels were noted below 2,000 ft (Hutchinson, 1960).

Observations of nematode populations in relation to altitude have been made elsewhere in the world. Jensen et al. (1959), in surveying for plant parasitic nematodes associated with sugarcane in Hawaii, found that as altitude increased, numbers of the burrowing nematode, *Radopholus similis* (Cobb) Thorne, decreased, and that this nematode was rarely recovered above 2,000 ft elevation. However, the root-knot nematode, *Meloidogyne incognita acrita* Chitwood and Oteifa, increased in numbers with increasing altitude. Whitehead and de Grisse (1960) made a similar observation in Tanganyika, where *Meloidogyne* spp. were not found infecting coffee on Mt Kilimanjaro at an elevation of 3,000 ft but infected 80 per cent of the trees at 5,000 feet.

## Materials and Methods

*Survey for Pratylenchus loosi.* Sampling of tea estates for *P. loosi* was started before 1942, and the number of samples processed by the Institute since that time until the end of 1962 totals 6,281. Since June 1960, 2,762 additional samples, for which we have data, have been processed by commercial firms in Colombo. The number of samples upon which this study is based is therefore 9,043 and these were obtained from a total of 526 estates. The number of samples taken from an individual estate varies from a minimum of one to a maximum of more than two hundred.

These samples were either received from Estate Superintendents, or were collected by members of the Institute staff. While obtained primarily from areas of declining mature seedling tea, samples are included from nurseries and new clearings. These samples have been taken in a great variety of ways. However, all have been processed in a similar manner; a modification of the Baermann method for recovering nematodes from soil, that was very briefly described by Gadd and Loos (1941). One hundred grams of soil, representative of the sample, are placed in a circular copper container measuring  $3\frac{1}{2}$  inches in diameter and  $1\frac{1}{2}$  inches in height, covered on the bottom with 60 mesh copper gauze. Prior to the addition of soil, a thin layer of sand is sprinkled over the gauze. The container, with the soil, is then lowered into a glass funnel, 6 inches in diameter, filled with sufficient water so that the water level is just above that of the soil. A rubber tube, clamped shut by a pinchcock, prevents the water from leaving the funnel tip. Nematodes migrating through the soil sink to the bottom of the funnel. After 24 hours, approximately 5 ml of water is drawn off into a glass block with a circular concavity. The nematodes are allowed to settle for approximately  $1\frac{1}{2}$  hours, at which time approximately 1 ml of water from the lowest part of the concavity is drawn up into a pipette. After a further 3 hours, four drops, or approximately 0.05 ml is then pipetted onto a microscope slide. A cover slip is added, and the slide is flamed to kill the nematodes, which are counted under a compound microscope.

*Survey for injury to tea attributable to Pratylenchus loosi.*:—Injury caused by the root-lesion nematode was initially described by Gadd (1939) and subsequently by Loos (1953). The most commonly encountered symptom of injury consists of fairly large patches of unthrifty tea, through which are scattered bushes that do not appear to be affected. The affected bushes put out little new growth, the leaves tend to become smaller, and the internodes are reduced in length. Some affected bushes show considerable loss of maintenance foliage, with many of the remaining leaves being yellowed. Leaves at the level of the plucking table remain as tufts (earlier suggesting the name "Witches' Broom"), with few buds giving rise to pluckable shoots. A pronounced tendency toward flowering is often evident. The feeder roots of more severely affected bushes are dried or decorticated; a general absence of living feeder roots being typical of the condition. Black lesions of varying extent are found on the storage roots, and can be revealed by scraping the epidermis to expose the root cortex. These lesions frequently contain large numbers of the nematodes. Bushes become progressively less thrifty, and the more susceptible finally die during periods of drought or as a result of pruning.

Areas containing severely injured tea are as small as 2 acres on some estates, and as large as 500 acres on others. A total of perhaps 3,000 acres can be considered as being thus affected, and the crop loss, estimated by Visser (1959b) is from 200-300 lbs made tea per annum; a reduction of at least 25 per cent. A much larger acreage is affected to a lesser degree.

Both casual and planned surveys of estates having tea infected by *P. loosi* were undertaken during this study, on the basis of the symptoms noted above. These symptoms can be approximated by several unrelated conditions (see DISCUSSION) and therefore do not always denote injury attributable to the root-lesion nematode.

Estimation of the total number of estates in each elevation range, as required for the comparison noted in Table 5, was made from the acreage listed in Fergusons, Ceylon Directory 1962 edition, for low, mid, and high country tea. One hundred estates were selected from representative Planting Districts in each elevational range and the average acreage per estate was calculated. These averages were then divided into the total acreages and the approximate number of estates for each elevational range was obtained.

*Experimental research*.—The experiment reported herein was conducted as follows. Clonal tea plants (T.R.I. 2024), 5 months old, were placed in polythene bags 6 inches in diameter and 9 inches long, provided with drainage holes, and containing mixed soil from 25 tea estates. Soil from each estate was infested with *P. loosi*. After 2 months, 200 of these bagged plants were transferred to each of 5 locations (Table 1), and were set into the ground approximately 18 inches apart. All plants were manured and sprayed at regular intervals.

TABLE 1.—Locations at which tea plants, infected with *Pratylenchus loosi*, were placed for experimental purposes

Location	Elevation above sea level	Nature of original site
T.R.I. Substation, Kottawa, Talgampola	200	Light brush
Ekkeralle Estate, Opanaika	700	Guatemala grass after jungle
Balangoda Group, Balangoda	4,000	Jungle
T.R.I., St Coombs, Talawakele	4,300	Fallow area of tea nursery
Oliphant Estate, Nuwara Eliya	7,000	Jungle

The elevation of each location was determined by use of matched aneroid barometers, using known elevations as points of reference. The elevation of the site at Oliphant Estate, being above the upper range of the barometers, was estimated from the knowledge of the Superintendent. Soil temperature measurements were made at 6 inch depth with dial thermometers (1), except at St Coombs where a mercury soil thermometer was used. All thermometers used were calibrated against a standard thermometer. Temperature records were maintained at St Coombs and Kottawa on an almost daily basis. Records were taken once monthly at all other locations.

Sampling began one month after the plants were set out, and continued at monthly intervals. At each occasion, 20 plants were selected at random from each location, and the entire root system of each plant was removed for individual processing. The method used for recovering nematodes from the roots has been described earlier (Hutchinson, 1962b).

*Nomenclature.* The scientific names for the nematodes noted in this paper, and their synonyms, are from Baker (1962).

*Statistical.*—The methods used to evaluate significance in the results are those of the Chi-square test, and the analysis of variance.

(1) Weston Electrical Instrument Corporation, New York, N.J., U.S.A. The method used for recovering nematodes from the roots has been described earlier (Hutchinson, 1962b).

## Results

1. *Pratylenchus loosi* is distributed throughout the tea-growing areas of Ceylon:—When the known occurrences of *P. loosi* are located on a map showing the tea estates of Ceylon, the distribution is seen to be general, with occurrences in all Planting Districts. Approximately one half of these occurrences (133 of 268) are marked on Figure 1.

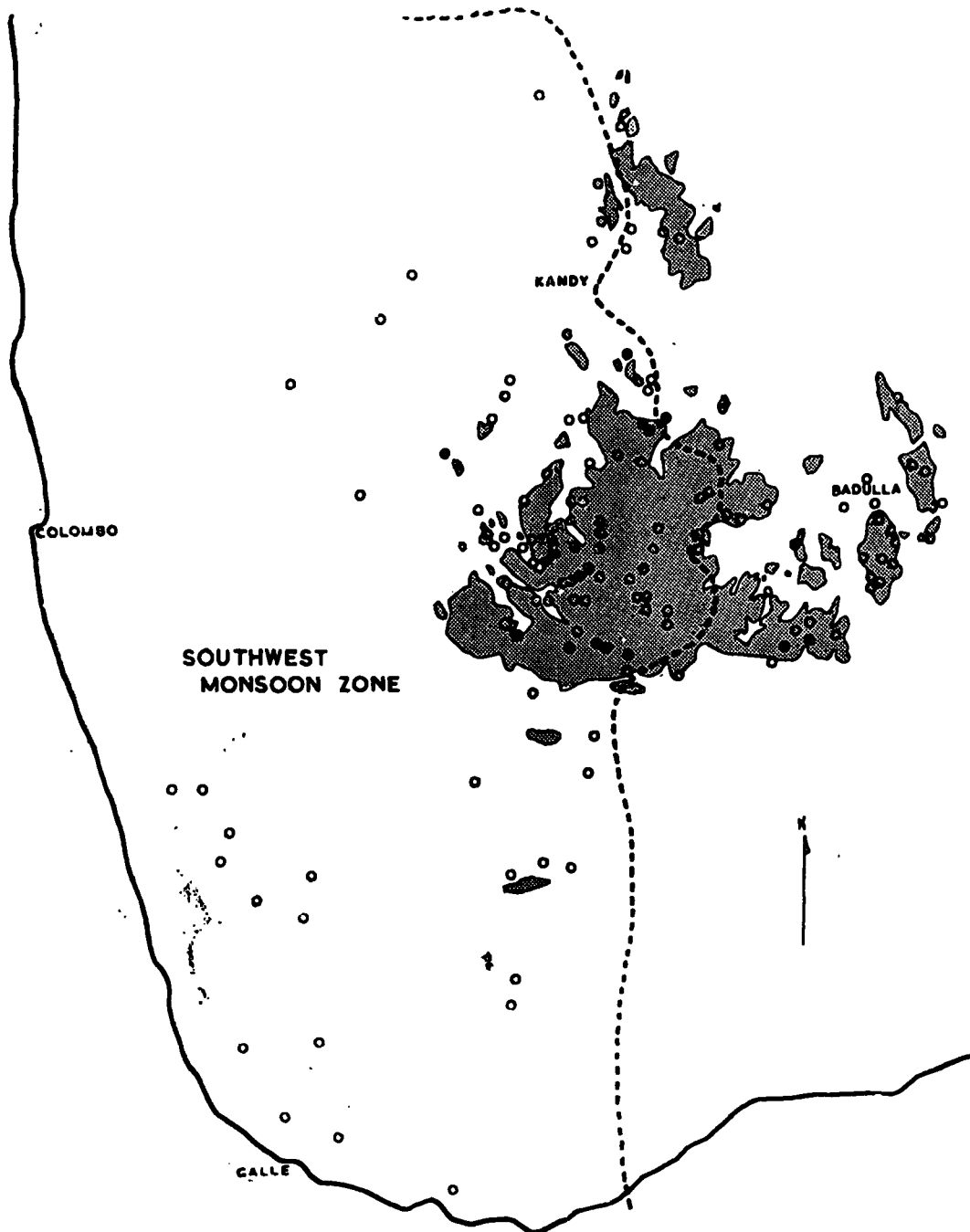


Figure 1. Distribution of *Pratylenchus loosi* Loof in Ceylon tea. Open circles indicate estates from which trace numbers have been recovered (less than 5 nematodes per 100 gm soil). Dark circles indicate estates from which very large numbers have been recovered (more than 125 nematodes per 100 gm soil). The shaded area is above 4,000 ft elevation, of which approximately half is in tea and half is jungle.

2. *Pratylenchus loosi* does not often occur in high concentrations below 4,000 feet elevation:—A preliminary summary of the results of soil sampling for *P. loosi* has already been given (Hutchinson, 1962a). The completed summary is now presented as Table 2, wherein each estate is represented by the highest number of *P. loosi* ever recovered from its soil. A part of this summary has been mapped to show the distribution of estates from which the lowest numbers of *P. loosi* (1 to 5 per 100 g soil) have been recovered, in comparison with those estates from which the highest numbers (more than 125 per 100 g soil) have been recovered. This is shown in Figure 1, whereon elevations of 4,000 feet or above are shaded in gray. Analysis of the data presented in Figure 1 shows that there is a very high association of elevation and concentration of *P. loosi* in the soil (Table 3.).

TABLE 2.—Summary of the distribution of *Pratylenchus loosi* among 526 tea estates in Ceylon

Numbers of <i>P. loosi</i> (1)	Numbers of tea estates from which <i>P. loosi</i> has been recovered according to monsoon zone and altitude (2)					
	South-west monsoon zone			North-east monsoon zone		
	0-2000	2-4000	4000+	0-2000	2-4000	4000+
125 and above	0	3	20	0	1	2
26-124	0	7	35	0	0	3
6-25	12	16	36	0	18	8
1-5	25	25	25	0	13	19
None	50	65	90	2	30	31

(1) Recovered per 100 gm soil

(2) Each estate is represented by the highest number of *P. loosi* ever recovered from its soil.

TABLE 3.—Relationship of *Pratylenchus loosi* to elevation when extremes are considered\*

Numbers of <i>P. loosi</i>	Elevation and number of tea estates	
	4,000 feet and above	below 4,000 feet
125 and above	22	4
1-5	44	63

\*data taken from Table 2

Chi-square = 14.13\*\*\* (P < 0.001) for 1 d.f.

Results of the experiment, wherein *P. loosi* in the roots of young plants was exposed to environmental conditions at several elevations show (Figures 2 and 3) that populations reached high levels at elevations of 4,000 feet and above, and rapidly declined at elevations below 1,000 feet. Population growth was intermediate at 7,000 feet.

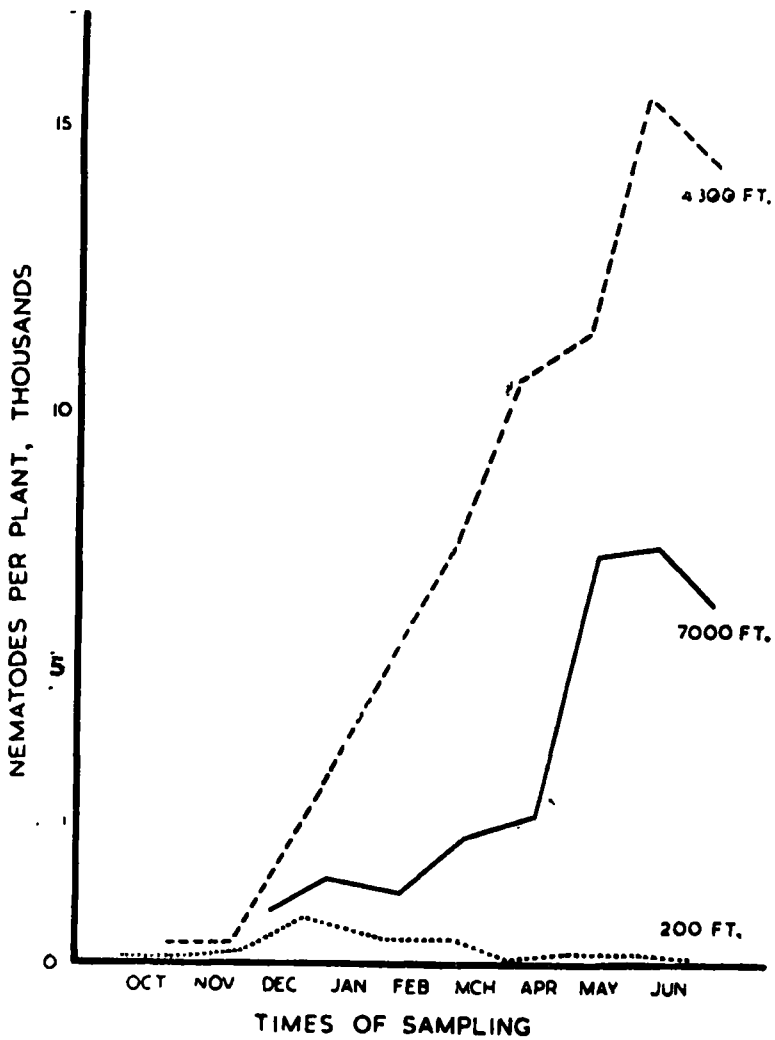


Figure 2. Population trends of *Pratylenchus loosi* during 1961-1962 in roots of tea plants set out at the T.R.1. Substation, Kottawa (200 ft) St Coombs Estate (4,300 ft) and Oliphant Estate (7,000 ft). Each point shows the average number of nematodes per 20 plants.

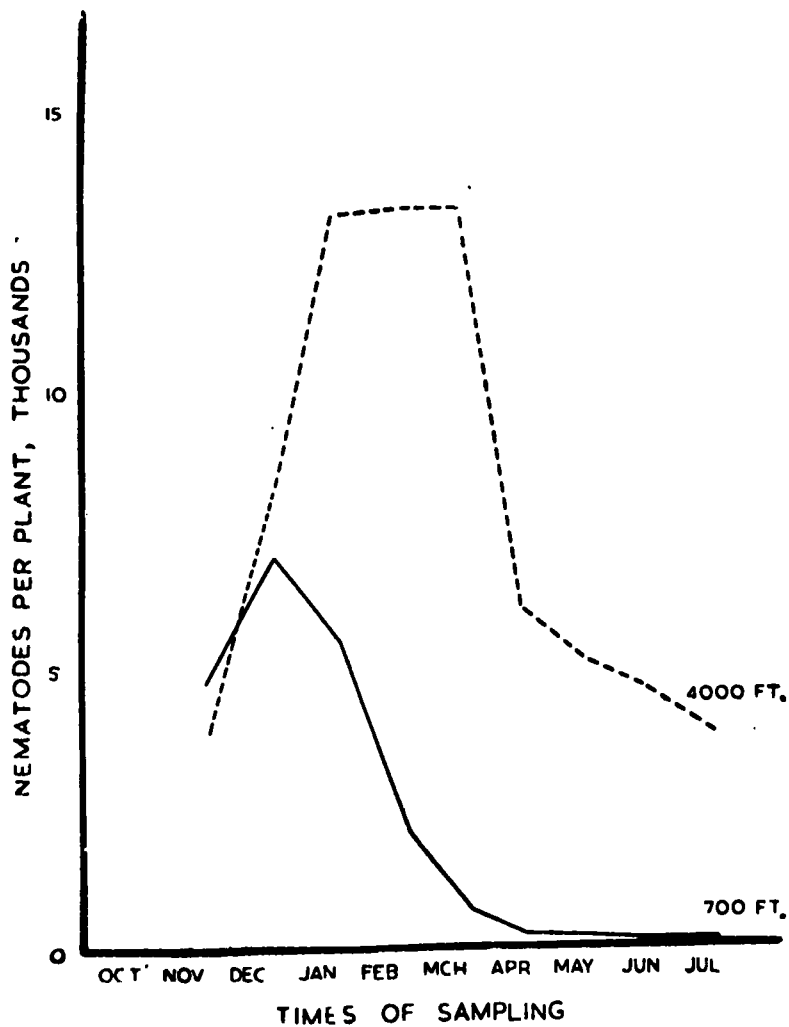


Figure 3. Population trends of *Pratylenchus loosi* during 1961-1962 in roots of tea plants set out at Ekkeralle Estate (700 ft) and Balangoda Group (4,000 ft). Each point shows the average number of nematodes per 20 plants.

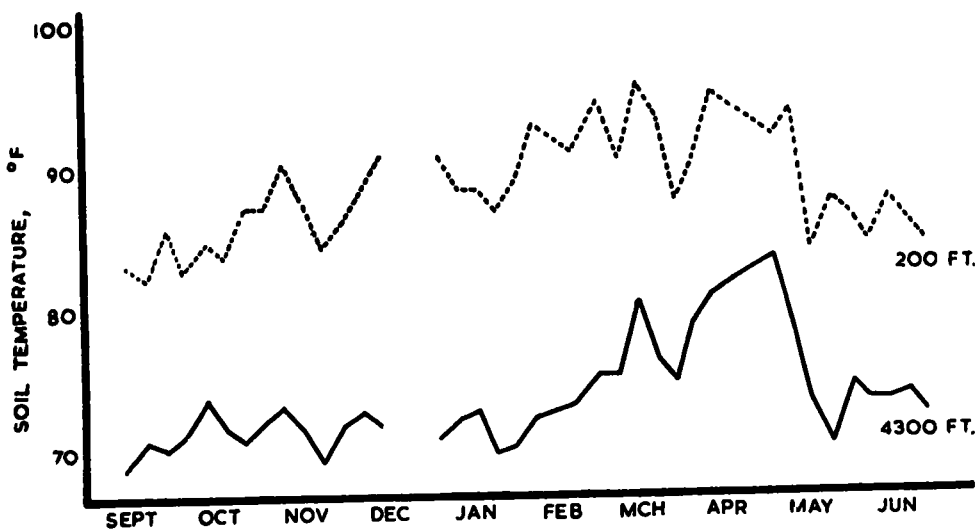


Figure 4. Soil temperatures at 6 inch depth for the experimental areas at Kottawa and St Coombs during 1961-1962. Each point represents the mean temperature for approximately 6 days.

3. *Pratylenchus loosi* occurs in higher concentrations at elevations of 4,000 feet and above in the zone receiving the southwest Monsoon than in the zone receiving the Northeast monsoon:—As shown in Table 4, there is good evidence that the concentration of *P. loosi* in soil is influenced by the monsoon zone. The largest nematode populations appear more often on estates in the south-west monsoon zone, while the very low populations tend to be associated with estates in the north-east monsoon zone. The demarcation between the two monsoon zones is sharply defined in most areas (see Figure 1), and is produced by ranges of low mountains oriented in a north-south direction.

TABLE 4.—*Relationship of monsoonal zone to numbers of Pratylenchus loosi recovered from tea soils\**

Number of <i>P. loosi</i>	Monsoon zone, and number of estates 4,000 ft and above in elevation		Chi-square value
	South-west monsoon zone	North-east monsoon zone	
above 25	55	5	7.61**
6-25	36	8	0.67
0-5	115	50	4.36**

\*data taken from table 2,  
Chi-square (total) = 12.64\*\* (P < 0.01) for 3d.f.

4. *Injury attributable to Pratylenchus loosi is most frequently found at elevations of 4,000 feet and above.*—Data presented in Table 5 show that there is an extremely high association of elevation and injury attributable to *P. loosi*. A different way of presenting this data shows that more than 90 per cent of the injured tea areas (40 of 43 estates) occur above 3,800 feet and that no injury attributable to *P. loosi* is known to occur below 2,700 feet.

TABLE 5.—*Relationship of obvious injury to seedling tea, attributable to Pratylenchus loosi, and elevation.*

Injury	Elevation and numbers of tea estates	
	4,000 feet and above	below 4,000 feet
Present	34	9
Absent	271	881

Chi-square = 64.4\*\*\* (P < 0.001) for 1d.f.

5. *Injury attributable to Pratylenchus loosi is associated with the number of nematodes recovered from soil.* Table 6 indicates that injured tea on 35 of 43 estates is associated with numbers of *P. loosi* recovered in excess of 70 per 100 gm soil.

TABLE 6.—*Relationship of obvious injury to seedling tea, attributable to Pratylenchus loosi, and numbers of nematodes recovered (1)*

Numbers of <i>P. loosi</i>	Numbers of estates having obvious injury attributable to <i>P. loosi</i>
125 and above	21
70-125	14
less than 70	8

(1) Estates are represented by the highest number of nematodes recovered at any time from 100 gms soil.

## Discussion

### METHODS

*Survey for Pratylenchus loosi*.—While the samples of tea soils on which this survey is based have been taken in many different ways, they have most often been taken from those areas where tea has shown declining growth. This fact, and the large number of samples, is believed to over-balance any lack of uniformity in sampling method.

The method described for the recovery of *P. loosi* from tea soils has the advantage that all nematodes recovered are contained on a single microscope slide, and that little or no soil debris is present. The method has the disadvantage that only a small proportion of the nematodes originally present in the soil are recovered. Visser (1959a), in studying this question, found that approximately nine times as many *Pratylenchus loosi* and larvae of *Meloidogyne* sp. were recovered from 100 g soil when it was processed in 5 g lots. Therefore, recovery of *P. loosi*, using 100 g samples, probably does not exceed 10 per cent. However, Visser decided to continue using 100 g samples because the results were sufficiently reproducible, and because a 100 g sub-sample is much more likely to be representative of a field-collected sample than is a 5 g sub-sample.

Feeder roots of tea yield much larger numbers of *P. loosi* than do soil samples of similar weight, and for this reason examination of roots is used in the research work of the Nematology Division. However, since all previous surveying has been carried out using soil samples, this method was continued for purposes of comparison.

*Survey for injury to tea attributable to Pratylenchus loosi*.—While above-ground injury to tea by the root-knot nematode, *Meloidogyne brevicauda* Loos, can easily be confused with that caused by *P. loosi*, the former nematode is very localized in its distribution, being known to occur on only three estates, all of which border on the same area of jungle. This, and the characteristic swelling and pitting of the roots caused by *M. brevicauda* is sufficient to distinguish the two conditions.

Rather, it is several unrelated conditions that are most commonly confused with injury induced by the root-lesion nematode. Of these, the most striking is the condition induced by the Shot-hole Borer beetle, *Xyleborus fornicatus* Eichh., the adults of which tunnel the living tissue of the matured shoots. Injured plants, however, tend to be more uniformly distributed than those injured by the nematode, and the presence of the borer can be readily detected by the ease with which shoots

snap off when one walks through a stand of affected bushes, and by the galleries in the broken shoots and old pruning stubs. On several estates, tea at elevations between 3,000 and 4,300 ft is attacked by both the nematode and the borer, and assigning to each pest its proper share in the resultant injury to tea is a very difficult, if not impossible, task.

Infestation by Scarlet Mite, *Brevipalpus* spp., can result in severe loss of maintenance foliage at a time when the mites have disappeared, and such injury has at times undoubtedly been confused with that caused by *P. loosi*. However, during the time when the mites are present, the leaves show brown necrotic areas along the midrib that are typical of the condition (Cranham, 1962).

Root disease caused by the fungus *Poria hypolateritia* Berk. produces approximately circular patches of dead bushes surrounded by declining bushes. The concentration of dead bushes usually serves to distinguish the injury from that caused by *P. loosi*, although when the bushes are found singly the reason for their decline is not so evident. However, the external mycelium of the fungus at the crown of the bush or along the roots positively distinguishes the condition from that caused by root-lesion nematodes. While the *Poria* fungus and the nematode cause serious injury to tea within the same range of elevation, instances of their occurring together are not common.

While waterlogged soil, prolonged drought, and extreme nitrogen deficiency can cause symptoms in tea very similar to those induced by root-lesion nematodes, distinguishing these conditions is not likely to be difficult. There remain, however, areas of declining tea for which no obvious reason can be assigned. Two such areas, both at an elevation of approximately 5,000 feet were carefully examined for *Pratylenchus loosi*, but none of these nematodes could be found. The possibility that poor soil structure or mineral imbalance was involved cannot be overlooked.

It is thus apparent that above-ground symptoms are only indicative of injury by the root-lesion nematode. Even the presence of dark lesions on the storage roots is not conclusive. Only recovery of the nematodes themselves, in the roots or adjacent soil, can be taken as proof that the observed injury is in some degree attributable to this nematode.

*Experimental methods*:—The method used exposed *Pratylenchus loosi* obtained from 25 locations to the environmental conditions at the several elevations, so that the result may be considered to be typical of the species.

Soil conditions at the five locations were similar, since the soil in the polythene bags came from the same source. Roots escaping into the soil would, however, encounter different conditions.

The use of polythene bags also enabled most of the root system to be recovered for examination. The processing of the entire root system of each plant, rather than only a part, precluded error due to the different rates of root growth at the different elevations.

This experiment exposed *Pratylenchus loosi* from up-country estates to low-country conditions. The reverse experiment has now been initiated, wherein *P. loosi* from several low-country estates are being exposed to conditions at St Coombs, to determine whether or not they will produce high populations where soil temperatures are lower.

## Results

*Reasons for the distribution of Pratylenchus loosi in relation to elevation:*—Seedling tea planted at different elevations is not of uniform *jat*; up-country tea tending to be low *jat* and low-country tea to be high *jat*. However, there is no evidence that *jat* in tea is correlated either with susceptibility or resistance to the root-lesion nematode, so that the observed differences in distribution of the nematode must be explained by some environmental factor or factors.

The fact that soil temperature is rather closely correlated with elevation, and that the warmest soils contain the fewest *P. loosi*, is strongly indicative (2). For example, the temperature differences between the experimental sites at Kottawa and St Coombs averaged 14.6 degrees F. (87.9 and 73.3 degrees, respectively) when soil temperatures were taken at 2-00 p.m. (Plate 4). The average of 7 temperature readings at Ekkeralle and Balangoda, respectively, was 81.9 and 70.6 degrees F., at 2-00 p.m., and the average of 8 readings at Oliphant was 61.0 degrees F. Maximum soil temperatures at the St Coombs and Kottawa sites, however, occurred later in the day, and were as much as 6.5 degrees F, higher than the temperatures recorded at 2-00 p.m.

This study clearly establishes the differences in numbers of root-lesion nematodes found at sea level with those found at 4,000 feet. Whether or not soil concentrations of *P. loosi* again decline at higher elevations, as suggested by the population curve obtained at 7,000 feet, cannot be positively determined. Large numbers of these nematodes have been obtained from elevations of 5,000 feet. However, not more than twenty-four estates are situated above 6,000 feet elevation, and although none of these estates are known to harbor numbers of *P. loosi* in excess of 15 per 100 g soil, the data available are insufficient for a conclusion to be reached.

Climatic variables other than temperature are not likely to affect the root-lesion nematode to the extent recorded in the survey and in the experimental trials. Rainfall, in the zone receiving the south-west monsoon, is fully adequate for good growth of tea at all elevations, and is therefore not likely to be limiting so far as the nematodes are concerned. Barometric pressure differences between 2,000 and 4,000 feet elevation are unlikely to have significant effect on growth of either plant or nematodes. Ultra-violet radiation is presumably more intense above 4,000 feet than below 2,000 feet. However, this could have no direct effect on nematodes below the soil surface, and its indirect effects, exercised through plant growth, are unlikely to provide such a marked stimulus for reproduction. If the region receiving the north-east monsoon is for the moment removed from consideration, soil types are sufficiently similar throughout the tea-growing areas (Tolhurst, 1962) that their effect on nematode populations should not be appreciable.

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(2) During the course of this study, two noteworthy exceptions to these general rules were encountered. At Hantane Estate (2,600 ft), where high and increasing numbers of *P. loosi* were maintained in the roots of experimental plants over a period of 10 months, the soil temperature was found to be much cooler than expected. A comparison with temperatures at St Coombs (4,300 ft) during September 1962, showed an average difference of only 2.2 degrees F, whereas a difference of at least 6 degrees was expected. An explanation may be that the particular slope at Hantane is exposed to cooler winds, or that heavy shade of secondary forest growth and the heavy shade maintained over the tea may cool the general areas, even though the experimental site itself was unshaded. At Kirimetiya Estate, a survey of 10 locations was made on a slope ranging between 2,685 and 4,165 ft. The soil temperatures at these two extremes were 80.0 and 68.0 degrees F, respectively on 15th June, 1961, three separate samples, each comprising 10 subsamples were taken at each location. Numbers of *P. loosi* recovered ranged up to 97 per 100 grams soil. Analysis of the data showed no significant difference in numbers of *P. loosi* in the different elevations tested. Because of the continued possibility of such variations, it would seem wise to consider all tea above 3,000 ft elevation as being potentially infectible by the root-lesion nematode in numbers sufficient to cause severe injury.

While temperature appears to be the most important factor governing the distribution of *Pratylenchus loosi* in Ceylon tea soils, experiments wherein infected plants were held at a single elevation and under conditions wherein temperature was the only variable, could give a more definite indication of its role. Such experiments with other *Pratylenchus* species have been performed by several investigators, using water-filled tanks or heating coils buried in soil, to provide relatively constant temperatures.

Graham (1951) found that when *Pratylenchus zeae* Graham and/or *P. leiocephalus* Steiner, now *P. brachyurus* (Godfrey) Filipjev and Schuurmans Stekhoven, were reared on corn, temperatures of 80–90 degrees F were more favourable for development than were temperatures of 60–70 degrees. Mountain (1954) noted that the optimum temperature for the development of *Pratylenchus minyus* Sher and Allen, now *P. neglectus* (Rensch) Filipjev and Schuurmans Stekhoven, on tobacco was higher than 100 degrees F. Riggs et al (1956) reported that population growth of *Pratylenchus coffeae* (Zimmerman) Filipjev and Schuurmans Stekhoven in roots of strawberry plants increased with each increase of temperature from 75 degrees F to 92 degrees. Mai (1960) conducted temperature tank studies using *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans Stekhoven in roots of apple seedlings, and found (Mai, 1962) that those nematodes exposed to soil temperatures of 65 degrees F produced the largest populations. Previous research with nematodes of the genus *Pratylenchus* therefore indicates that temperatures apparently lethal to *Pratylenchus loosi* are well tolerated by other species. The distribution of *P. loosi*, and the result of experiment, indicate an optimum temperature within the range 65 to 75 degrees F, so that of those *Pratylenchus* species so far tested, only *P. penetrans* seems to have a similar optimum temperature.

Why populations of *P. loosi* do not die out completely at low elevations is a question that is difficult to answer. Possible reasons include the occurrence of soil pockets at lower temperature than usual for the elevation, or that some of the nematodes can withstand the higher temperatures. In support of the second hypothesis, the preliminary test of ability of *P. loosi* to survive in the roots of clonal plants (TRI 1526) at Kottawa showed that all the nematodes were dead within 8 months (Hutchinson, 1962a). Nematodes from a single source were used in this test. In the final test, where nematodes from 25 sources were used, some nematodes survived over a period of 9 months (Fig. 2). Conceivably, 25 populations of the nematodes could contain more individuals adaptable to the climatic conditions at Kottawa than could one population. If heat resistance is accepted as the more probable explanation, the genetic character or characters involved must be recessive since otherwise the progeny of these heat-resistant individuals would soon create large populations.

*Reasons for the distribution of Pratylenchus loosi in relation to monsoon zones:*—Total annual rainfall in the tea-growing regions of Ceylon is highly variable, being as low as 60 inches in one area and over 200 inches in other areas. Rainfall patterns are also highly variable; some areas receiving a well-distributed rainfall, and others experiencing long droughts. The zone receiving the south-west monsoon has a total rainfall very significantly greater than the zone receiving the northeast monsoon, as indicated in Table 7. Even at locations in the two zones where the total annual rainfall is approximately equal, the number of rainy days is significantly less for the location in the north-east monsoon zone (Table 8). This latter location, at the Passara Sub-station of the Institute on Gonakelle Estate, is considered to have a climate more favourable for tea than most other areas of the north-east monsoon zone because of its location on the slope of a prominent mountain. No records of the successive number of days drought is immediately available for this location as compared with others in this monsoon zone. However, droughts of considerable

duration, to the point of killing out established tea, are not uncommon in the Úva Province, and may be an explanation of the lower numbers of nematodes recovered from these soils. During these droughts, the temperature of the upper layers of soil could rise to a point at which growth and reproduction of the nematodes was inhibited.

TABLE 7.—Average annual rainfall at locations in the South-west and North-east Monsoon Zones (1)

South-west Monsoon Zone		North-east Monsoon Zone	
Location (2)	Rainfall, ins.	Location (3)	Rainfall, ins.
Bogawantalawa	97.13	Badulia	72.00
Dickoya	111.51	Diyatalawa	65.62
Dimbula	117.51	Haputale	85.72
Hewaheta	111.70	Hanguranketa	83.10
Lindula	91.71	Passara	85.79
Maskeliya	119.02	Udapussellawa	103.78
Watagoda	121.16	Welimada	60.28
Mean annual rainfall, ins.	109.96		79.47

$$t = 4.34*** (P < 0.001)$$

- (1) Records from each location over a period of at least 28 years, as given in Furguson's Ceylon Directory for 1962, Page 97-98.
- (2) Locations in the vicinity of which *Pratylenchus loosi* occurs in high concentration in soil.
- (3) Locations throughout the North-east Monsoon Zone.

TABLE 8.—Comparison of climate between Gonakelle Estate, Passara (N.E. Monsoon Zone, 3,300 ft) and St Coombs Estate, Talawakele (S.W. Monsoon Zone 4,500 ft)

MONTH	Rainfall, ins (1)		Rainy days, no (1)		Sunshine, hours (2)	
	Passara	Talawakele	Passara	Talawakele	Passara	Talawakele
January	11.78	3.74	16	12	130	170
February	6.06	3.28	10	8	135	143
March	6.28	4.21	11	11	195	228
April	11.05	6.65	17	17	171	151
May	6.04	9.52	13	19	159	109
June	2.09	15.39	5	26	198	176
July	3.87	10.08	9	24	163	82
August	4.41	8.46	9	24	192	116
September	5.30	8.58	11	21	139	89
October	9.92	10.11	17	21	154	158
November	11.65	8.26	19	18	133	128
December	10.95	5.04	18	14	136	184
Total	89.40	93.32	155	215	1905	1734
Mean	7.45	7.78	13.91	17.92	158.75	144.50
t	t = 0.235		t = 1.926*		t = 0.213	

- (1) 11 year averages (January 1, 1950 to December 31, 1961).
- (2) 3 year averages (August 1, 1959 to July 31, 1962).

*Percentage of up-country tea believed to be infected:*—The number of tea estates known to be infected with *Pratylenchus loosi* has steadily increased since the nematode was first discovered in 1939. By 1943, 17 estates were reported as infected in the Annual Report of the Pathologist published for that year. A decade later, according to a note by the Pathologist, dated 12 January, 1953, "47 estates are known to be infected. The distribution is almost through the Ceylon tea districts". At the end of the succeeding decade, primarily due to a larger number of samples processed but in part doubtless due to spread of the nematode, a total of 268 estates were recorded as being infected.

Considering now the region most likely to suffer from infection by the nematode, there are approximately 224 estates at 4,000 ft elevation or above in the zone receiving the south-west monsoon. Of these, only 116 estates, or approximately half, are known to harbour the nematode, and many of these are apparently only lightly infected (Table 2). While 4 estates are known to be infected in every field, on most estates the infection is much more limited; at times being confined to a few acres. On average, it is most unlikely that more than 10 per cent of any given estate is actually infected.

This would mean that only 5 per cent of the most potentially infectible acreage is at present infected. However, even if we double this figure, to account for estates on which infection occurs without our being aware of the fact, and because of lack of intensive sampling on all but a very few estates, it is evident that most of the up-country tea acreage remains to be infected, and that the primary task is to prevent spread of the nematode from occurring.

Prevention of spread between estates is primarily a matter of refusal to buy or sell plants, since these can harbour the nematodes in their roots. We have positive evidence that 6 transfers of *P. loosi* have occurred in this way, from infected to previously non-infected estates, and circumstantial evidence of several additional transfers. In recognition of the serious nature of this problem, regulations currently in force (Mahadeva 1960) deny subsidy payment for plantings made with plants other than those originated on the same estate.

Prevention of spread within an estate is primarily a question of nursery management. Fumigation of rooting beds and of basketing soil is currently recommended for all estates above 3,500 ft elevation, but this practice cannot be relied upon to guarantee plants free of root-lesion nematodes. Nematodes present in the subsoil of the nursery can move upward into rooting beds, or even into the roots of plants contained in polythene bags that are placed on the soil. The nematodes can also be introduced into nurseries in water that is obtained from infected areas of mature tea. These difficulties might be lessened by fumigating the subsoil, by striking cuttings directly into polythene bags, and by keeping the bagged plants on racks above the soil. However, the approach being taken at the Institute is to select a pesticide that can be drenched onto nursery plants, to eliminate any nematodes present in the roots before the plants are transferred to the field.

*Relation of injury to seedling tea to numbers of nematodes recovered:*—Where estates showing serious injury to seedling tea have been sampled in many locations and at different times of the year, numbers of root-lesion nematodes as large as 300 per 100 g soil have eventually been recovered (2 estates), and numbers approaching or exceeding 200 are not uncommon (8 additional estates).

There is, however, great variability in the numbers of *P. loosi* recovered, depending upon the precise location sampled, the time of sampling, the method of sampling, and the condition of the tea. Not infrequently, one location may yield 50 or more nematodes per 100 g soil, while an adjacent location may yield none. Likewise,

the soil population of *P. loosi* varies with the season, and sampling of the same location will yield widely different numbers at different times of the year (Hutchinson, 1963b) Sampling variation is also considerable, even where the same number of samples is taken at random from a marked area by different teams of investigators (Hutchinson and Foster-Barham, 1962). On estates where extensive destruction of feeder roots has occurred throughout many previous years, together with extensive soil erosion, only low numbers of nematodes may be recovered. For example, from two such estates, known previously to be heavily infected in almost all fields, no numbers greater than 30 per 100 g soil could be recovered when the object was to recover the maximum number possible.

Hoestra and Oostenbrink (1962) estimate that 130 *Pratylenchus penetrans* per 300 ml soil is the level at which damage to apple trees always occurs under field conditions in Holland, and they believe that considerable damage probably occurs at levels below 100 per 300 ml soil. A similar correlation for tea and *Pratylenchus loosi* (Table 6) would be 125 per 100 g and 70 per 100 g, respectively, with 100 g loamy tea soil having a volume very close to 100 ml. The level of 70 per 100 g is chosen because most of the 8 estates from which lesser numbers have been obtained have not been intensively sampled, and because several of the estates are severely affected by the shot-hole borer.

Since the method used by Oostenbrink for recovery of *P. penetrans* from soil (Oostenbrink, 1960) is estimated to be 85–90 per cent efficient, and the method used by the present authors is estimated to be 10 per cent efficient, the correlation for tea, corrected for purposes of comparison with the work of Hoestra and Oostenbrink, would be approximately 3,000 per 3000 ml and 1,850 per 300 ml, respectively.

No obvious injury to tea, attributable to *P. loosi*, has ever been found to be associated with 5 or less nematodes recovered per 100 g soil. Since this number is comparable to the level at which Hoestra and Oostenbrink report invariable injury to apple, a reasonable conclusion is that *Pratylenchus penetrans* is more pathogenic to apple trees in Holland than is *P. loosi* to tea bushes in Ceylon.

The pathogenicity of *Pratylenchus loosi* to tea has been clearly established in tests wherein 12 clones have been grown in beds of infected and fumigated soil, respectively. Growth of young plants of the most susceptible clones is reduced by 80 per cent when they are grown in infected soil over a period of 15 months (Hutchinson 1963a).

*Distribution of injured tea*.—As indicated in Table 5, obvious injury to seedling tea, attributable to *Pratylenchus loosi*, is most often noted above 4,000 ft elevation, and the relationship of injury to elevation is very highly significant. The significance of this association, however, is probably much greater even than calculated, since of the 9 estates below 4,000 ft elevation where severe injury to seedling tea has been found, 4 are known to be heavily infested with Shot-hole Borer and 2 are known to be moderately infested. Thus, injury in some of these areas may be attributable primarily to Shot-hole Borer rather than to the nematode.

Above 4,000 ft elevation, in the zone receiving the south-west monsoon, severe injury attributable to *P. loosi* is not uniformly distributed, but is concentrated in six principal areas. Of these, the most notable is perhaps the area surrounding the town of Kotagala in the Dimbula District. The other areas include those centering around the towns of Talawakele, Bogawantalawa, Hewaheta, Lindula, and Maskeliya, respectively.

The distribution of injury in new clearings planted with clones may, however, prove to be a different matter. While a field of seedling tea presents the nematodes

with a wide range of plants, some of which are susceptible, and others of which are resistant or tolerant, a field of clonal tea can be highly and uniformly susceptible. Three dramatic instances of this possibility have occurred during the past several months, on estates not previously known to harbour the root-lesion nematode. All clearings were planted with clone TRI 2024, and all have sustained severe injury, associated with very high numbers of nematodes, ranging from 100 to more than 300 per 100 g soil. Two of these estates are above 4,000 ft elevation, but one clearing is located at 3,000 feet. The implication of these occurrences is that the geographic and elevational range of high concentrations of this nematode may become considerably extended as a result of planting susceptible clones. One remedy, to avoid excessive injury in new clearings, would be the planting of clones that are tolerant of infection by the nematode, and several such clones are now being recommended by the Institute.

### Conclusions

1. *Pratylenchus loosi* becomes increasingly numerous with increasing elevation, to at least 5,000 feet.
2. Injury attributable to *P. loosi* has not occurred below 2,500 feet elevation, and is most evident above 4,000 feet.
3. The north-east monsoon zone is less likely to experience injury from this nematode than is the south-west monsoon zone.
4. Numbers of *P. loosi* recovered from soil in excess of 125 per 100 grams are always associated with severe injury to tea. Numbers recovered in excess of 70 per 100 grams are very probably associated with severe injury to tea.
5. The optimum soil temperature for the development and reproduction of *P. loosi* appears to lie between 65 and 75 degrees F.
6. The most important consideration in control of this pest is to prevent its further dissemination in areas above 2,500 ft elevation.

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