

# STUDIES OF NEMATODE POPULATIONS IN SUGARCANE SOIL PROFILES

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## Abstract

The vertical distribution of nematode populations in soil profiles treated with nematicide and untreated, was investigated at frequent intervals during the plant and first ratoon crops in both a root laboratory and in the field. Nematodes were found to be associated with damaged roots and were not restricted to any particular level down to a depth of 2 m. There was evidence to suggest possible seasonal fluctuations in numbers. Treatment with nematicides at a depth of 23 cm affected nematodes to a depth of 2 m and resulted in dramatic growth responses of sugarcane. Sampling for and extraction of nematodes are discussed.

## Introduction

The experiments were undertaken for three reasons:

- (1) To confirm observations made in the Mount Edgecombe root laboratory that plant parasitic nematodes were a major cause of growth reduction in a sandy soil. (Glover<sup>5</sup>).
- (2) To substantiate the results of a number of field trials on sandy soils in which large growth responses had been obtained following the use of nematicides.
- (3) To obtain a better understanding of the occurrence and distribution of nematodes in the soil with a view to finding better control methods.

## Experiment 1

### Materials and Method

This experiment was conducted in two sections of the Mount Edgecombe root laboratory (Anon<sup>1</sup>) where a number of sugarcane crops had previously been grown in a nematode-infested soil of the Clanshal series. The soil is a structureless sand, 216 cm deep and with the following mechanical composition: clay 2%, sand 96% and silt 2% as determined by the Bouyoucos<sup>3</sup> method. In one of the two root laboratory sections ethylene dibromide in hydrocarbon diluent (E D B 4,5) at the rate of 225 l/ha was applied as a full cover treatment with a hand injector gun at a depth of 23 cm. The other section was left untreated. Twelve days after this nematicide treatment was applied, setts of the sugarcane variety NCo 376 were planted at a depth of 10 cm in rows 1,2 m apart. The first row was adjacent to the root laboratory window to facilitate sampling and observation.

Sampling was carried out 11 times during the plant crop and 9 times during the first ratoon crop. It was done by removing the light screens and windows of the root laboratory and removing on each occasion seventeen soil samples distributed vertically between 5 and 120 cm. Because the sampling was destructive only small samples (60 ml) were taken, and a different vertical axis was used each time. The Baermann funnel was used to extract nematodes from all samples.

## Results

### 1. Plant Growth

From the time of visible sprouting of the setts, the growth of both roots and tops of the sugarcane in the EDB-treated section was observed to be far more vigorous than that in the

untreated section. Sixteen days after planting, shoots were visible above ground only in the treated section and root development had already taken place down to 38 cm. In the untreated section no roots were observed at this depth until 34 days later. These results are similar to those recorded by Glover.<sup>5</sup>

Microscopic examination revealed that there were considerable morphological differences between the roots from the two treatments. Those from the untreated section were short, sparse, thickened, malformed and almost devoid of root hairs when compared with those growing in the EDB treated area. Root damage and malformations were present in the ratoon crop in both treatments. The root malformations were typical of those resulting from the presence of plant parasitic nematodes (Harris<sup>6</sup>).

Top growth was closely related to root development. Eleven weeks after planting, shoots in the treated area were twice as numerous as those in the untreated area and on average 65% taller. These growth differences persisted through to the harvest of the first ratoon crop as shown in Table 1.

TABLE 1  
Harvested crop characteristics. Plant crop harvested after 326 days and first ratoon after 345 days.

Item	Plant Crop		1st Ratoon	
	Treated	Untreated	Treated	Untreated
No. of stalks . . . .	134	90	122	105
Average length of stalk, cm . . . . .	163	111	125	109
Gross mass of fresh cane, kg . . . . .	104	47	80	59

### 2. Nematodes

Analyses of soil samples taken before treatment and planting showed that the following plant parasitic nematodes were present in the experimental area: *Meloidogyne* sp., *Pratylenchus* sp., *Trichodorus christiei* Allen, *Hemicycliophora labiata* Colbran, *Criconemoides* sp., *Tylenchorhynchus* sp., *Rotylenchulus* sp., *Xiphinema* sp., and *Hoplolaims* (nematodes in the sub-family *Hoplolaiminae*). *Tylenchorhynchus* sp., *Xiphinema* sp. and *Rotylenchulus* sp. were found too seldom to warrant discussion. The Baermann funnel technique does not effectively extract *Criconemoides* sp. and so this nematode is not discussed here but other sampling methods discussed later (Experiment 2) indicated that it was present, probably in large numbers.

The nematodes found and their distribution through the profile of untreated soil at the time of the first sampling, one week after planting, are given in Figure 1. Analyses of soil from the treated section indicated a moderately good control of nematodes down to the depth of nematicide application, but below this depth the same nematodes particularly *Meloidogyne* sp. larvae persisted. No *H. labiata* were detected and the

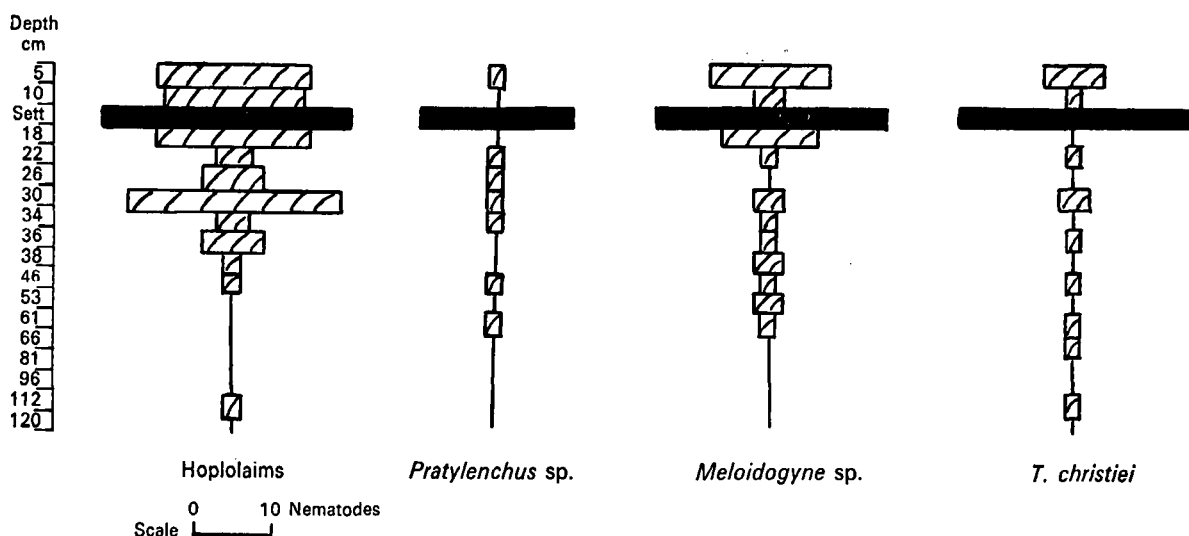


FIGURE 1 Profile distribution of plant parasitic nematodes 7 days after planting in the untreated soil.

analyses also indicated a large reduction in numbers of saprobiotic nematodes.

At the second sampling, 46 days later, little change was found in the nematode population of the untreated area and no nematodes were extracted from soil in the treated area at any sampled depth. This suggests that the nematicide treatment had not had time to be fully effective at the time of the first sampling.

The general conclusion was therefore that the control of nematodes by the nematicidal treatment had been good and that the reduction in their numbers was associated with greatly improved crop growth.

(a) *Meloidogyne* sp.

Larval counts of *Meloidogyne* sp. remained low and they were fairly evenly distributed down the profile to 112 cm in the untreated area, until the fifth sampling 143 days after planting, when a marked population increase was detected. Peak populations were recorded 43 days later at the next sampling. The highest numbers were found immediately above and below the sett with another apparent concentration between 34 and 44 cm deep. (Figure 2). An earlier flush of young developing roots may have been associated with the increased numbers of larvae at this depth.

Numbers of *Meloidogyne* remained high, but by early July (291 days after planting) the zones of highest concentration had moved further down the profile and were again associated with areas of more prolific and deeper root development. Samples taken later and until the end of the experiment indicated fewer nematodes evenly distributed through the profile. Good control of *Meloidogyne* sp. persisted in the treated soil. A total of only 6 larvae were extracted until the time of the eighth sampling, 247 days after planting, when increased numbers of larvae began to appear but only down to a depth of 26 cm. This indicated that reinfestation was probably taking place from the soil surface. By the middle of August, 326 days after planting, greater numbers of larvae were found to the deepest sampling depth. This situation continued until the termination of the experiment, with persistently lower total numbers of larvae being found in the treated area. In spite of being associated with a far larger volume of roots in the treated area than in the untreated, the population of this nematode had not recovered fully from the nematicide treatment by the end of the experiment. See Fig. 5.

(b) *Pratylenchus* sp.

Analyses of samples taken from the untreated soil 7 days after planting showed that the surviving population of *Pratylenchus* sp. occurred down to the deepest sampling depth of

120 cm. Samples taken during the subsequent 136 day period yielded no *Pratylenchus* sp. below 26 cm, presumably due to starvation resulting from lack of suitable host material below 26 cm. The flush of young roots which developed soon after planting, and was mainly confined to the upper 26 cm of soil, probably sustained this nematode and accounted for its survival in this zone. However from the time of the sixth sampling (186 days after planting) and until the final sampling, *Pratylenchus* sp. was found down to 112 cm, and presumably was associated with the deeper root penetration. No *Pratylenchus* sp. was found in the treated area until the sixth sampling, when it was detected down to a depth of 44 cm. Subsequent sampling 276 days after planting indicated that this nematode may have recovered to the extent that numbers in the treated area were almost equal to those in the untreated area. Their vertical distribution through the profile appeared to be fairly uniform. However, samples taken during the first ratoon crop indicated that only a partial recovery had taken place, as the numbers remained appreciably lower in the treated area until the end of the experiment 671 days after planting. This is indicated in Figure 5.

(c) *Trichodorus christiei*

Samples taken in the untreated area one week after planting showed that *T. christiei* were fairly evenly distributed throughout the profile down to 120 cm. At the second sampling 39 days later none was found below 36 cm but an increase in numbers was found immediately below the setts (Figure 2). Later samples indicated a drop in numbers throughout the profile with no particular zones of concentration. This situation appeared to continue until shortly before harvesting the ratoon crop when there was an increase in numbers down the profile. Suppression of this nematode appeared to be excellent in the treated area until the fourth sampling, 109 days after planting, when there was nothing less than a population explosion, as is often found with *Trichodorus* spp. following the use of halogenated hydrocarbon nematicides. (Dick and Harris<sup>4</sup>). The highest numbers were found in the 10 cm of soil immediately above the setts (Figure 2). This large increase in numbers in the treated area compared with the untreated area, persisted for nearly 16 months after planting (Figure 2). By the time the ratoon crop was harvested population differences between the two treatments had become appreciably smaller.

(d) *Hemicycliophora labiata*

Although pre-treatment sampling indicated the presence of *H. labiata* it was not found again until 187 days after planting.

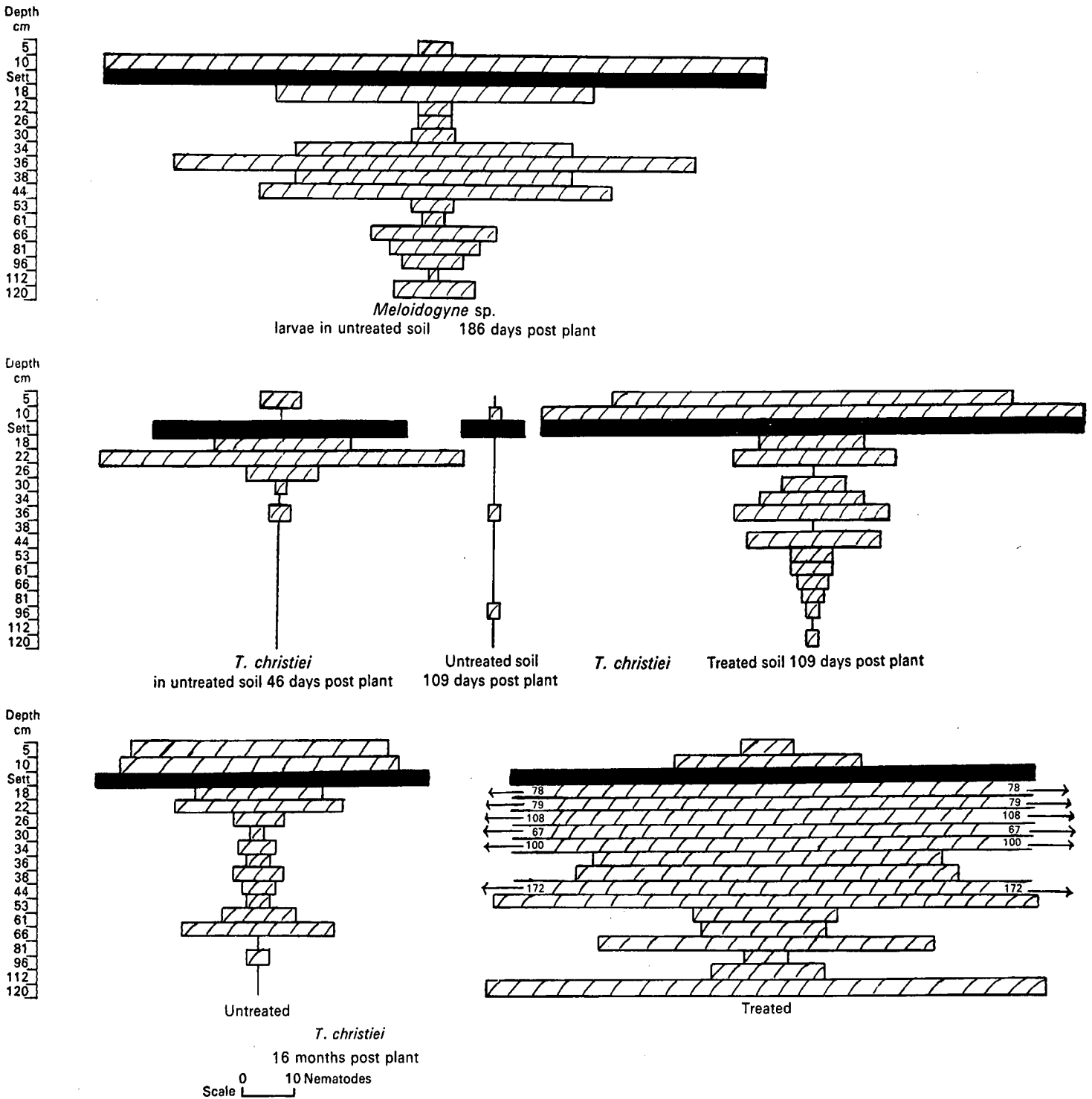


FIGURE 2 Profile distribution of *Meloidogyne* sp. larvae and *T. christiei*.

Then it was found only in the treated area and only below 44 cm depth. Numbers in the treated area increased during the plant crop. The results of analyses of the final samples taken during the plant and ratoon crops are shown in Figure 3.

No *H. labiata* were found in the untreated area until 8 months after planting, when only one was found at 120 cm depth. For the remaining period of the experiment a total of only 20 individuals were found sporadically in the untreated soil, and were restricted to the deeper levels.

Populations found in the treated area during the ratoon crop declined but remained far higher than those in the untreated area. Their rapid increase in numbers like those of *T. christiei*, may have been associated with EDB treatment, but unlike *T. christiei* they were restricted to levels deeper down the profile, as shown for the final sampling in Figure 3

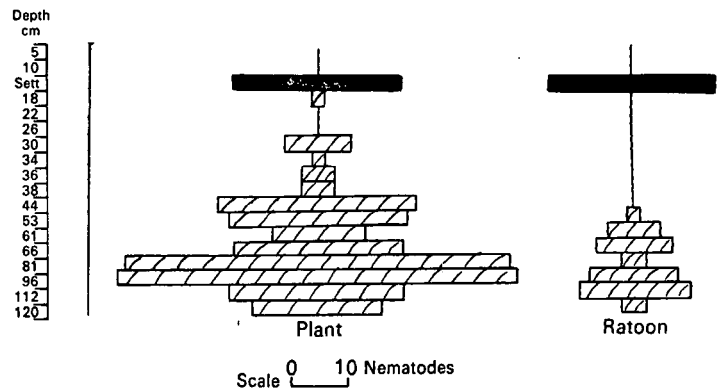


FIGURE 3 *H. labiata* profile distribution at the final samplings in the treated soil of the plant and ratoon crops.

and also in Figure 5, where mean numbers for each depth are shown.

(e) *Hoplolaims*

Hoplolaim nematodes were found in very low numbers. Nevertheless they were found down to the lowest depth of 120 cm. No Hoplolaims were detected in the treated area until 6 months after planting. Because of the low numbers extracted it was not possible to make valid comparisons between the treatments.

(f) *Mononch and Saprobiotic nematodes*

The predatory nematodes in the family Mononchidae were numerous throughout the soil profile of the untreated area

for the full duration of the experiment. A typical distribution of their numbers is shown in Figure 4.

Total numbers of saprobiotic nematodes (on some of which the mononchs may have been preying) had recovered in the treated area 46 days after planting, and were as numerous as those in the untreated soil. Despite this rapid recovery, mononchs were absent for 15 months after planting in the treated area. Later an increase in their numbers was seen at the deeper levels (Figure 4) where they remained more concentrated until the end of the experiment.

Experiment 2

When results from Experiment 1 had shown that appreciable numbers of plant parasitic nematodes may occur at depths in excess of 120 cm it was decided to make investigations further down the profile both at the root laboratory and in the field.

Method and Materials

Investigatory pits were dug in the treated and untreated areas of the root laboratory, used in Experiment 1, and also in the field. The findings from 4 such pits are discussed here. The two pits at the root laboratory were dug 33 months after planting, during the second ratoon crop, to a depth of 216 cm at which level there was an abrupt transition to a heavy clay soil.

The two excavations made in the field were in a structureless sandy soil of the Clansthal series with the following mechanical composition: clay 6%, sand 91% and silt 3%.

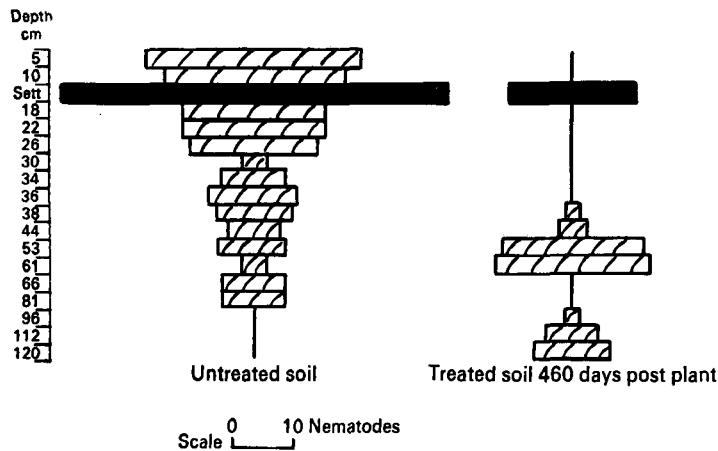


FIGURE 4 Profile distribution of Mononchidae.

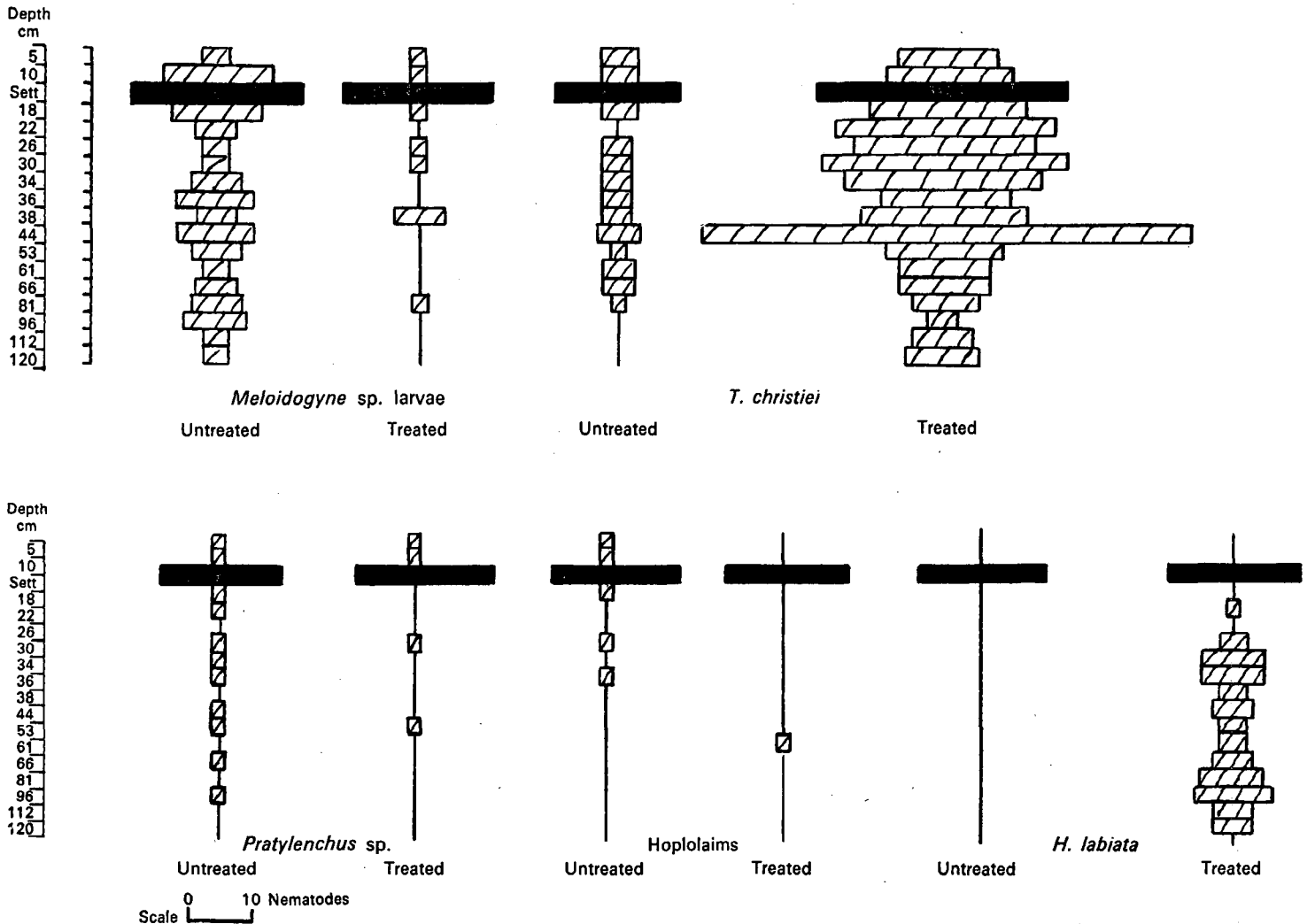


FIGURE 5 Mean number of nematodes for all sampling times at each level.

The two field pits were dug 4 months and 13 months after planting the crop in order to expose profiles 210 and 240 cm deep respectively, at which depths the contact between sand and clay was reached. The first of these pits, shown in Figure 6, exposed the root systems of 4 month old N55/805 sugarcane growing in a nematicide-treated area on the left, and an untreated area on the right. Three weeks before planting the treated area had been injected, at a depth of 23 cm, with 1,3-dichloropropene and 1,2-dichloropropane ("D-D"). At the time of planting, a 10% granular formulation of aldicarb (2-methyl-2-(methylthio) propionaldehyde-0 — (methylcarba-

moyl) oxime), was applied in the furrow at a rate of 56 kg/ha. The dramatic growth responses of both tops and roots to this nematicide treatment can be seen in Figure 6.

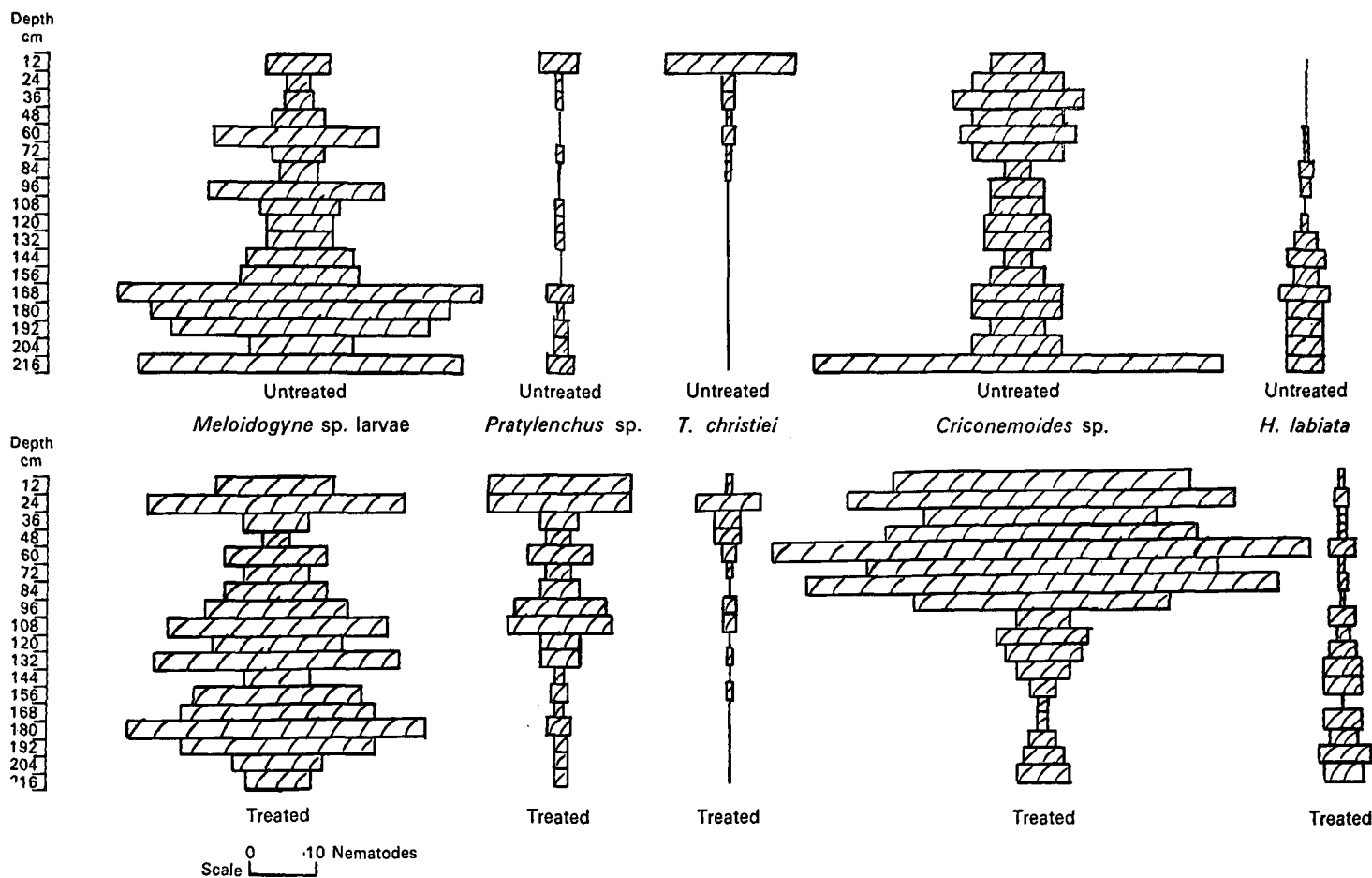
Four months after planting the cane, roots in the untreated area were sparse, malformed and had penetrated only to a depth of 120 cm, whereas roots in the treated area, which had penetrated the clay layer below 210 cm, were well developed and much healthier in appearance. The second field pit was dug adjacent to the first 9 months later, exposing a similar profile in the treated and untreated areas. Soil sampling was carried out at regular intervals down the profiles of all four pits with precautions being taken to avoid contamination of the samples. The numbers of nematodes in each extract given in Figures 7 and 8 are each the mean of six 100 ml sub-samples, and the extracts were made by the centrifugal flotation technique as modified by Jenkins.<sup>7</sup>

**Results**

Figures 7 and 8 show the distribution of the different plant parasitic nematodes occurring in the root laboratory and field pits respectively. It can be seen from Figure 7 that *Meloidogyne* sp. *Pratylenchus* sp. and *Criconemoides* sp. were noticeably more numerous in the treated area, in particular at depths less than 120 cm. *T. christiei* were not found right down the profile in either treatment and their number and distribution were not very different in the treatments. *H. labiata* was also similarly distributed in both treatments but as in Experiment I they were more numerous at the deeper levels. Total numbers of plant parasitic nematodes in the EDB-treated soil were higher presumably because of the greater volume of roots. The position indicated in Figure 7 shows that the large differences which existed between the treatments in Experiment I had become less obvious and that the effects of nematicide



**FIGURE 6** Field pit 4 months post plant. Left half is the treated area



**FIGURE 7** Profile distribution of nematodes from pit at root laboratory.

treatment could not be recognised except for an overall increase in population, presumably due to the presence of more roots.

In the field pit excellent control of *Meloidogyne* sp. *Cricone-moides* sp. and *Hoplolaims* was gained with nematicide. None of these nematodes was found at any depth in the profile. A resurgence of the *T. christiei* population in the treated soil appears to have taken place at this stage, mainly at depths below 150 cm as shown in Figure 8. The maximum depth of root development after 4 months in the treated area was more than 210 cm whereas rooting depth in the untreated area had reached only 120 cm. *Meloidogyne* sp. larvae, *Criconemoides* sp. and *T. christiei* were found below 120 cm suggesting the existence of a residual population presumably from the previous crop, whose roots at these depths were found to be completely decomposed.

The situation down the profile of an adjacent investigatory pit, in the treated area, dug 9 months later, showed considerable changes in comparison with the untreated area. *Meloidogyne* sp. larvae and *T. christiei* were more numerous, the latter in the upper levels, *Hoplolaims* had become distributed deeper down the profile, and *Criconemoides* sp. populations were still depressed. *Pratylenchus* sp. which were not found in the earlier samplings, were present in very low numbers in the upper 15 cm of soil only.

Although Figure 9 shows little difference in total numbers of plant parasitic nematodes in the two pits 13 months after planting, there were large population differences between the different genera. For example, *T. christiei* made up the bulk of the population in the treated soil. It is also apparent that populations of plant parasitic nematodes were higher in the shallower soil levels at this time.

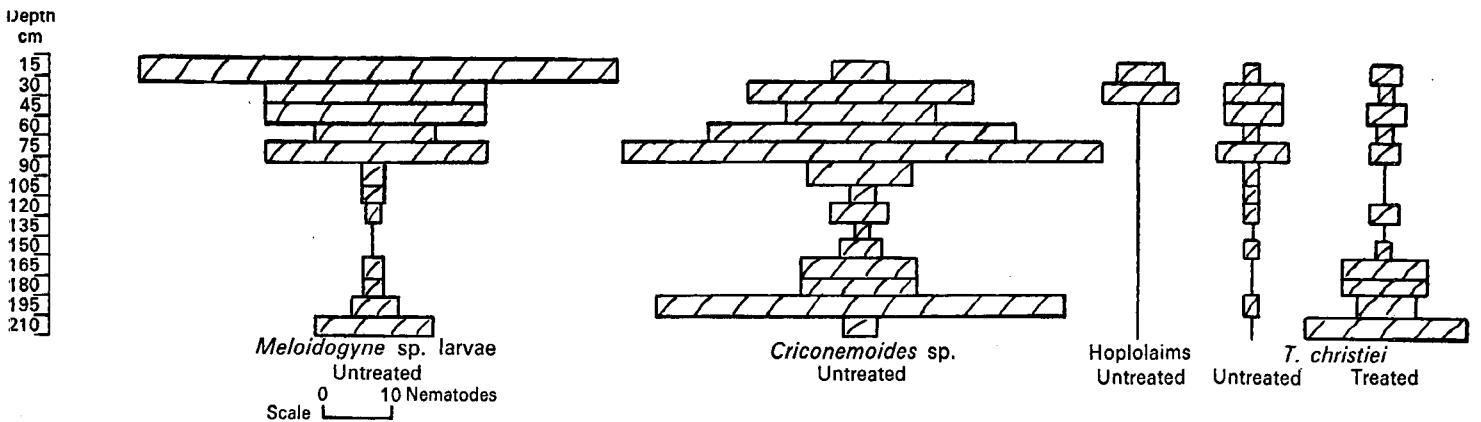
**Discussion**

Two dimensional evaluations and illustrations of the results of an experiment conducted in three dimensions are not always entirely satisfactory. However it is suggested that the association between areas of prolific root development on the one hand, and ultimately higher numbers of nematodes on the other, appeared to be close.

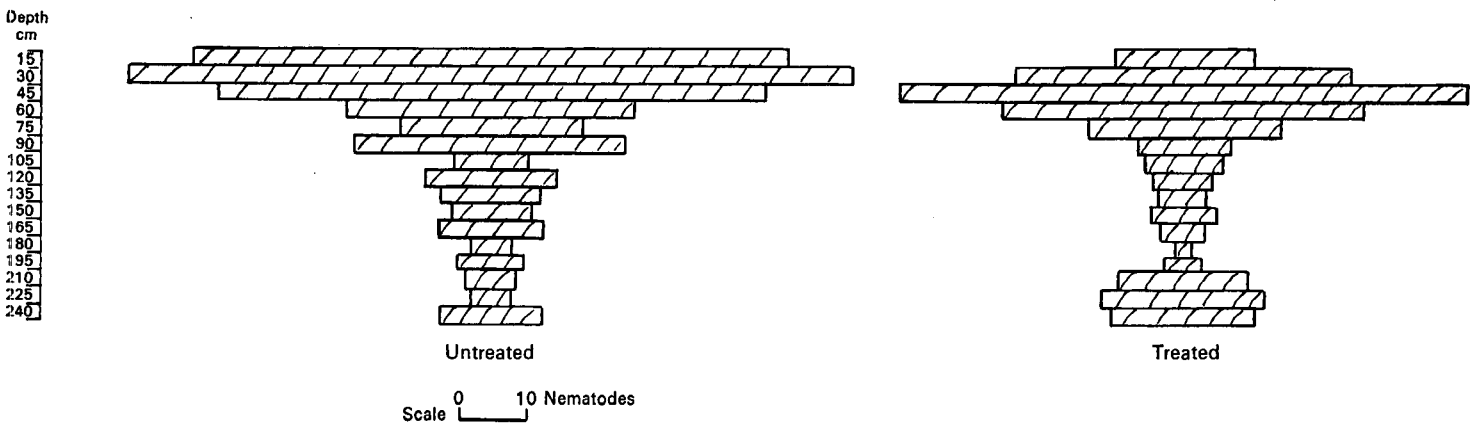
Methods of soil sampling and of separating nematodes from soil are known to give variable results and often account for quantitative as well as qualitative differences. Consequently only large and consistently occurring differences were assumed to be real. Soil and root sampling for nematodes often indicates the presence and relative abundance of only certain stages in the life cycles of nematodes, and therefore results should be interpreted with caution. Since nematodes seldom if ever damage roots without having a close interrelationship with other organisms, any factors influencing the development of the one are important to the other. In view of the complex nature of the soil environment, an accurate identification of cause and effect must always be difficult.

The period covered was probably too short to show whether the increased populations (which resulted from increased root development in the treated soil) would cause sufficient root damage to result in a period of lower nematode numbers in the treated than in the untreated soil. The results from comparatively superficial sampling in "good" and "poor" growth areas in the field suggest that such a cross-over point could occur, since lower populations of harmful nematodes are generally found to be associated with the most severely nematode-damaged sugarcane.

Sampling for adults of *T. christiei* and *H. labiata* shortly after treatment of the soil with nematicide gave no indication



**FIGURE 8** Profile distribution of nematodes from field pit, 4 months post plant.



**FIGURE 9** Profile distribution of numbers of all plant parasitic nematodes totalled from field pit, 13 months after planting.

of their being more tolerant of nematicides than the other plant parasitic nematodes found. The reasons for a subsequent increase in their numbers in the treated soil are not known, but it could be due to the suppression of a biological controlling agent, less competition from other organisms, or a more resistant egg stage. These results suggest that the increases in nematodes brought about by the use of halogenated hydrocarbons are not very persistent. (Figure 7).

The results from the root laboratory experiment indicated that EDB practically eliminated the predatory mononch nematodes. They occurred throughout the soil in the untreated area, but did not exert sufficient, if any, control on the harmful nematodes to decrease significantly the root damage caused there.

Barker *et al.*<sup>2</sup> found that Baermann funnel extractions may give different results depending on the time of year. Results obtained in these experiments using both Baermann funnel and centrifugal flotation techniques, showed that numbers of certain nematodes varied independently of crop cycle. Nematode numbers in soils sampled from field crops at different stages of growth support these findings (unpublished data). *Meloidogyne* sp. larvae were more numerous during the months March to July and *T. christiei* was extracted in higher numbers in September to November.

Findings of immediate practical significance from these investigations were (a) that nematodes were associated with

root damage at depths below 2 m and (b) that they were not necessarily concentrated nor restricted to any particular depth that was sampled. It was found that the nematicide treatments although made only at the 23 cm depth, could suppress and affect nematodes down to a depth of at least 2 m in these soils.

In sugarcane the generally accepted methods of sampling for nematodes can at best give only a limited indication of the whole situation, since in the field very little of the root system and soil profile can be sampled either horizontally or vertically.

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