# 5 METHODS

## 5.1 SAMPLING

At the four experimental plots, the samples were taken about two feet from the citrus tree trunk, as the chance of contacting big main roots would be smaller and also because the small profusely branched roots were more abundant at distances over two feet.

By means of a sampler, ten samples were taken at each plot per season. Each sample consisted of four subsample parts, thus constituting 40 subsamples per plot. Samples were taken in clusters. Subsequent samplings were taken close to previous samplings in the same basin.

### 5.12 The sampler

For sampling, a special sampling device was used. It consists of a steel tube section into which four aluminium cylinders fit, and a cap piece with a handle bar (plate 6). The tube section consists of two half cylinder parts (plate 7). Externally, the tube section has two small bolts, situated in a direct line with each other on the upper parts of the separate halves. These bolts correspond with two rectangular slots in the cap piece. The slots can be seen on plate 6. The tapering lower end of the sampling tube facilitates the sampling procedure. The inner side of the sampling tube "mouth" or

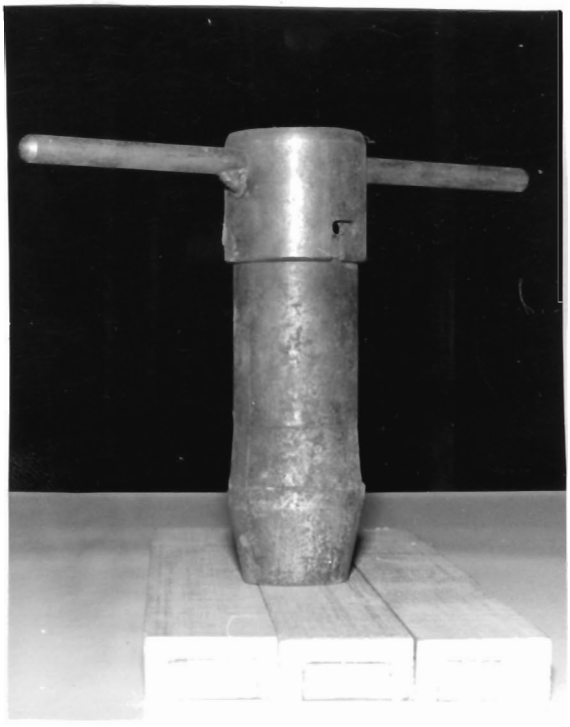

Plate 6 : The Sampler

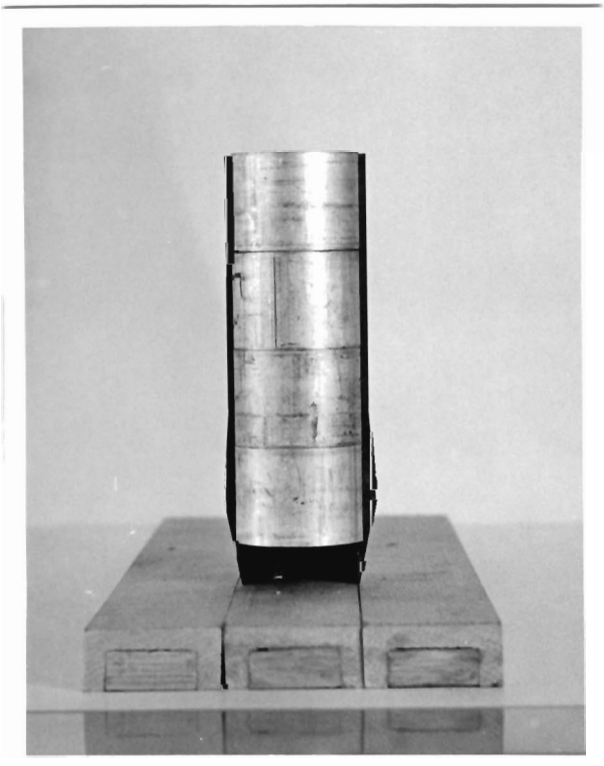

Plate 7 : Longitudinal section through the sampler showing the four subsample cylinders cased in a half cylinder section.

opening has a ridge on which the lower sampling ring rests. The ridge has a width of 2.5 mm and the aluminium cylinder wall is 2.2 mm thick, providing a flush fitting of ring and ridge, which leaves no obstruction to the soil thoroughfare during sampling. The cap piece consists of a cylindical section, which fits over the upper tube section, with a blocking ridge in the upper section, and a handle bar with which to force the sampler into the soil, as well as to close and open the sampling device itself.

Dimensions:

| | | |
|---|---|---|
| Overall length of sampler | : | 21 cm |
| Length of sampling core | : | 16 cm |
| Length of subsample cylinder | : | 4 cm |
| Inner diameter of subsample cylinder | : | 4.64 cm |
| Soil volume of subsample | : | 67.664 $cm^3$ |
| Surface area of subsample | : | 16.916 $cm^2$ |

## 5.13 Sampling procedures

To load the sampler, four subsample cylinders must be fitted into one half of the tubular section (plate 7), after which the other half section is located in the closed position with the first. Finally, to secure the halves, the cap piece must be slid into position, (with the bolts in the tubular section in their corresponding slots in the cap piece), and then twisted to the right, so as to slide the bolts to the end of the rectangular slots.

After the sampler is extracted from the soil, it is placed upright on a hardboard surface. The cap piece, as well as one half of the tubular section are then removed. Then, by thrusting a thin circular plate in between the first and second subsample cylinders, the first subsample core is divided from the rest and lifted free from the tubular section. To close and secure this subsample core, two hardboard discs, (half an inch thick, and with circular grooves corresponding to the diameter of the subsample cylinder) are fitted to both ends of the cylinder with its core (plate 8). The circular grooves in the discs are deep and the discs fit tightly enough to the cylinders to ensure safe transportation of the subsample; As a precautionary measure, however, the discs are secured with two crossed rubber rings (plate 9). Labels, prepared in advance, with the relevant information, indicating the specific plot numbers, sample and subsample numbers are attached to every subsample taken. Subsamples 2, 3 and 4 all receive the same treatment. Finally, the subsamples are carefully packed in cartons for transportation to the laboratory.

## 5.2 EXTRACTION

After much consideration it was decided to employ two different extraction apparatusses in the course of the present study namely the "heat extraction battery system" and the "grease filme extraction system" (Aucamp & Ryke, 1965). As no heat extraction system big enough to contain 200 subsamples simultaneously was available, the author decided to transform eight large

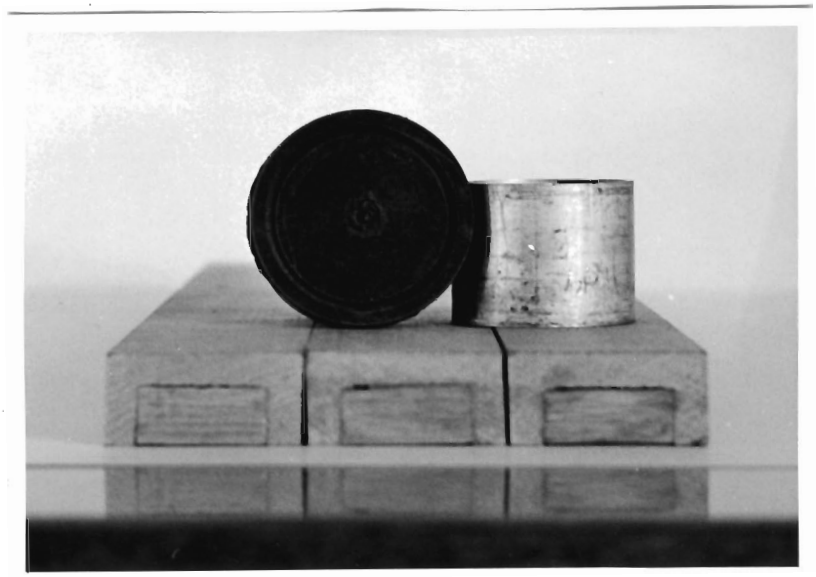

Plate 8 : Hardboard disc and subsample cylinder

Plate 9 : Subsamples prepared for transportation.

housed in square tin boxes, were attached to the inner side of the lid roof sections. A thermostat, with its indicating

Berlese funnel extractors for the purpose.

5.21 The heat extraction battery system (plate 10)

The system consisted of eight units (all identical in design), to hold 25 subsamples each. The overall length of a unit was 44 inches and its width 18 inches.

The framework, made from angle iron, supports on its upper deck an half inch thick hard board tray, 18 inches (46 cm) long and 18 inches wide. The hard board trays were perforated with 6 cm diameter holes, arranged in 5 rows of 5 holes each. Each hole was fitted with a small polythene funnel, 11 cm long and 8 cm wide at its rim. Inside each funnel, circular 1 mm mesh sieves, 6 cm in diameter, were placed (plate 11). During extraction the subsamples were placed on the sieves. The position of the cores on the sieves within the funnels produced no problems.

On top of the funnel - bearing trays, each unit had a square removeable lid, 18" by 18" by 9½" high. The lids were all provided with chimneys 11 inches high and 3½ inches in diameter, constructed in the centre of the roof section, to release excess moisture during extraction, thus preventing moisture condensation and accumulation on the inner walls of the lid.

The heat sources, which consisted of two 12 inch elements housed in square tin boxes, were attached to the inner side of the lid roof sections. A thermostat, with its indicating

Plate 10 : The battery extraction system

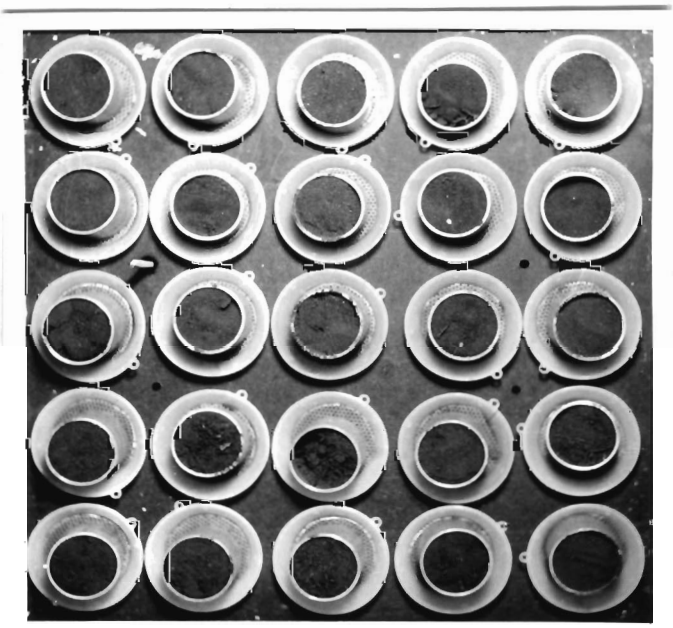

Plate 11 : Subsamples seen from above in funnel extraction unit.

pilot light on the front face section of the lid, together with a thermometer protruding downwards from the funnel tray made exact temperature control possible (plate 12). During operation, temperature was regulated in such a manner that an initially low temperature of 28°C was gradually increased over the entire extraction period of 5 days to reach a maximum of 40°C on the last day. The lids on top of the sample trays fitted neatly in angle iron seats, so that apart from the upper chimneys and the small funnel end openings, there were no other openings which might cause large temperature fluctuations.

Just below the funnels, scafoldings were constructed to carry the collecting containers.

### 5.211 Funnel extraction procedures

Extraction procedures always commenced the day after sampling, since the samples had to be transported 300 miles from Zebediela to the laboratory at Potchefstroom. Underneath the funnels, collecting bottles were placed. The four ounce collecting bottles were filled with 70% alcohol, to which were added a few crystals of phenol for the prevention of bacterial and fungal growth. The samples were then sorted out according to plot- and sample numbers, and placed in upright positions on the sieves. The lids of the collecting bottles were all numbered according to the subsamples on the sieves, and placed on the bench, directly beneath their corresponding sampling containers.

During extraction the fluid level in the collecting bottles was observed so that as evaporation occurred 70% alcohol was added when necessary.

After extraction, the collecting bottles were removed and their corresponding funnels were carefully washed out in their containers, as smaller organisms might adhere to the funnels. For subsequent extraction with the grease film extractor, the subsample cores were preserved in 70% alcohol in special wide mouth 8 ounce containers (plate 13).

## 5.22 Grease film extraction

### 5.221 Preliminary experiments

During the period allocated to preparatory word, experiments were made to ascertain whether different preservative fluids added to the soil cores might affect successful extraction by means of the grease film. In the course of the experiment, the following chemicals were tested:

1 Terpineol (diluted: 20 ml. constr. to 2½ litre)
2 Phenol " " " " " "
3 Creosote " " " " " "
4 Alcohol 50%
5 Alcohol 70%

During this experiment, 240 samples were examined. Forty samples were taken stratigraphically from each of the five

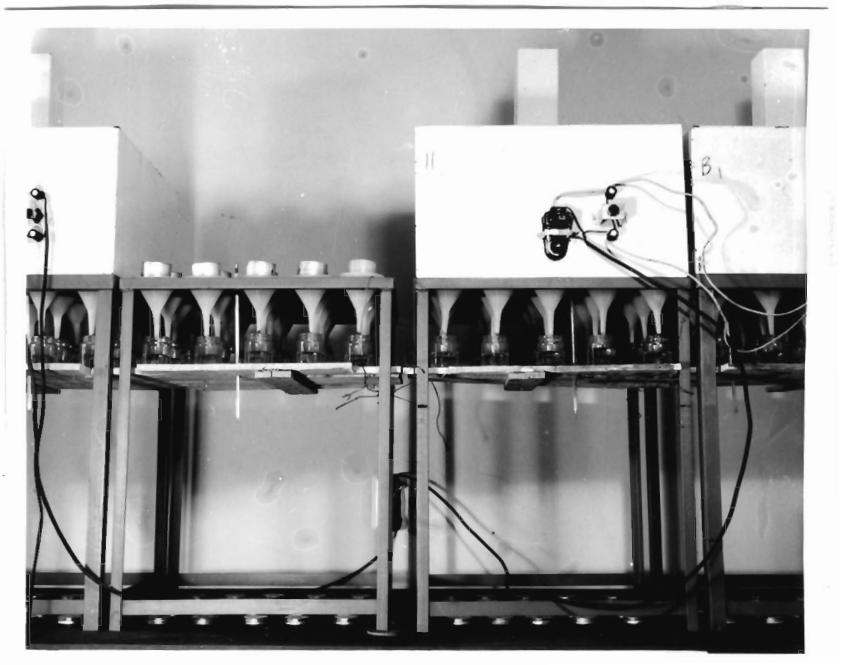

Plate 12 : Front view of extraction system.

Plate 13 : A group of mesofaunal collecting bottles and preserved soil cores from one sampling.

experimental plots. They were then divided in five groups of eight samples. Each of the five collections of eight samples were treated with a specific preservative from the abovementioned list and stored for two months to exclude the time factor. Forty samples, however, (eight samples from each plot) received no chemicals for control purposes, and were extracted directly after sampling. After storation, the treated soil samples were extracted by means of the grease film extractor and the recovered arthropods recorded.

From an evaluation of the results, the following conclusions were made:

(a) that no significant differences in the arthropod numbers between the chemically treated and the untreated soils existed, which means that the applied chemicals had not noticably disturbed the cuticle of the preserved arthropods, so as to effect the efficiency of the grease film method.

(b) that no significant difference occurred in the arthropod numbers in the differently treated soils, which means that not one of the chemicals used specifically, or the difference in concentration used had any "side effect" on the efficiency of the grease film and that thus, any of the chemicals experimented with could be used.

It was therefore decided to use 70% alcohol as it was in general use in the laboratory, and always available in that concentration.

### 5.222 The grease film extraction

The grease film extraction of the preserved soil cores was done after methods described by Aucamp & Ryke (1965), with the same extractor they described in their paper (plate 14). After extraction, the soil-water mixture in the plastic tanks was poured through two circular sieves, of 2 mm and 3 mm mesh respectively. This addition to the extraction process was made to capture some bigger arthropods, which, it was found, do not all adhere to the thin layer of the grease covered plates. A considerable number of Myriapods were recovered by this additional method. During colder months, luke warm water was used in the extraction process, so as to retain the adhesiveness of the grease film.

## 5.3 SORTING AND IDENTIFICATION

With the aid of a Wild stereoscope and microscope, both funnel and grease film extracted organisms were sorted and identified. To improve recognition of the organisms during sorting, the Petri-dishes (which were used during the sorting of the funnel extracted samples) were painted with a special black paint, registered as School Board Paint (Manufactured by United Painst Limited), for its non-reflective quality. The paint was specifically applied to the inner side of the Petri-dish, since the stereoscope was focused mainly there on submerged organisms. To facilitate sorting, a grid was drawn on the paint by means of an orange wax pencil.

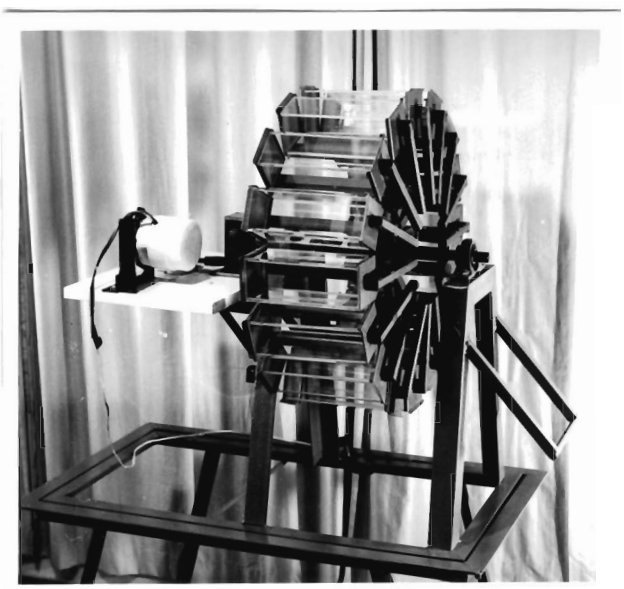

Plate 14 : The grease film extractor.

## 5.4 BIOMASS ESTIMATION

Biomass estimations were done after the methods described by Olivier & Ryke (1965). Alterations and additions (table 12), were made to the existing table 2 of Olivier & Ryke (1965).

TABLE 12 Estimates of biomass of mesofaunal components

| Organism | | Mean biomass per individual (mg) |
|---|---|---|
| ACARI | | |
| TROMBIDIFORMES | | |
| Tydeidae: | Tydaeolus sp. | 0.0015 |
| | Microtydeus sp. | 0.0020 |
| | Lorryia africanus | 0.0135 |
| | Lorryia sp. nov. | 0.0135 |
| | Paralorryia sp. | 0.0135 |
| Nanorchestidae: | Speleorchestes sp. | 0.0020 |
| | Nanorchestes sp. | 0.0020 |
| Cunaxidae: | Cunaxa sp. | 0.0140 |
| | Cunaxoides sp. | 0.0140 |
| Bdellidae: | Bdella sp. | 0.0140 |
| | Cyta sp. | 0.0140 |
| Raphignathidae: | Acheles aethiopica | 0.0180 |
| Stigmaeidae: | Ledermulleria sp. | 0.0160 |
| | Ledermulleriopsis sp. | 0.0160 |
| | Neophyllobius sp. nov. A. | 0.0200 |
| | Neophyllobius sp. nov. B. | 0.0200 |
| Pseudocheylidae: | Pseudocheylus sp. | 0.0150 |
| Cheyletidae: | Cheyletia sp. | 0.0020 |
| Erythraeidae: | Smaris biscutatus | 0.0400 |
| | Leptus sp. | 0.0400 |
| Pyemotidae: | Pygmephorus sp. | 0.0050 |
| Tarsonemidae: | Tarsonemus sp. | 0.0020 |
| Scutacaridae: | Scutacarus sp. | 0.0020 |
| Eupodidae: | Eupodes variegatus | 0.0140 |
| | Eupodes parafusifer | 0.0140 |
| Anystidae: | Anystes baccarum | 0.0200 |
| Cryptognathidae: | Cryptognathus cucurbita | 0.0150 |
| Pachygnathidae: | Bimichealia sp. | 0.0160 |
| | Pachygnathus sp. | 0.0160 |
| Paratydeidae: | Scolotydeus sp. | 0.0120 |
| Tetranychidae: | Brevihalpus obovatus | 0.0140 |
| Lordalychidae: | Lordalychus | 0.0020 |

| Organisms | Mean biomass per individual (mg) |
|---|---|
| MESOSTIGMATA | |
| Rhodacaridae: Rhodacarus sublapideus | 0.0020 |
| Digamasellidae: Digamasellus sp. | 0.0180 |
| Laelaptidae: Geolaelaps queenslandicus | 0.0200 |
| Hypoaspis quinquelongisetus | 0.0200 |
| Phytoseiidae: Amblyseius usitatus | 0.0200 |
| Ascidae: Gamasellodes sp. | 0.0180 |
| Protogamasellus primitivus similus | 0.0020 |
| Protogamasellus dispar | 0.0020 |
| Protogamasellus brevicornis | 0.0020 |
| Lasioseius sp. | 0.0020 |
| Pachylaelaptidae: Pachylaelaps sp. nov. | 0.0500 |
| ORIBATEI | |
| Oribatulidae: Scheloribates sp. A | 0.0180 |
| Scheloribates sp. B. | 0.0200 |
| Liodidae: Liodes sp. A. | 0.0150 |
| Liodes sp. B. | 0.0150 |
| Plateremaeidae: Plateremaeius sp. | 0.0500 |
| Pedrocorticella sp. | 0.0150 |
| Passalozetidae: Passalozetes sp. | 0.0050 |
| Euphthiracaridae: Rhyzotritia sp. | 0.1000 |
| Cosmochthoniidae: Cosmochthonius sp. | 0.0050 |
| Opiidae: Oppia nova | 0.0050 |
| Perlohmanniidae sp. | 0.0150 |
| Epilohmanniidae: Epilohmannia sp. | 0.0120 |
| Carabodidae sp. | 0.0150 |
| ACARIDIAE | |
| Acaridae: Tyrophagus sp. | 0.0140 |
| Hypopus | 0.0020 |
| INSECTA | |
| COLLEMBOLA | |
| Onychiuridae: Onychiurus camerunensis | 0.0200 |
| Isotomidae: Isotomina termophila | 0.0350 |
| Achorutidae: Brachystomella parvula | 0.0300 |
| Entomobryidae: Seira squamoornata | 0.0160 |
| Entomobrya sp. | 0.0160 |
| Sminthuridae: Sphaeridia pumilis | 0.0040 |
| DIPLURA | |
| Japygidae: Japyx sp. | 0.1000 |
| CORRODENTIA | |
| Liposcellidae sp. | 0.0200 |
| HEMIPTERA | |
| Reduviidae sp. | 0.4000 |
| Isometopidae: Letaba bedfordi | 1.5100 |
| HOMOPTERA | |
| Aphididae: Macrosiphum sp. | 0.3000 |
| Coccidae sp. | 0.0150 |

| Organism | Mean biomass per individual (mg) |
|---|---|
| THYSANOPTERA | |
| Phlaeothripidae: Urothrips minor | 0.4000 |
| Faureothrips reticulatus | 0.4000 |
| LEPIDOPTERA | |
| Noctuidae: Lapygma exempta | 0.1800 |
| DIPTERA | |
| Cecidomyiidae sp. | 0.2500 |
| Larva | 0.2000 |
| COLEOPTERA | |
| Tenebrionidae sp. | 0.2400 |
| Larva | 0.1800 |
| HYMENOPTERA | |
| Trichogrammatidae: Trichogramma sp. | 0.2500 |
| Formicidae: Pheidole megacephala | 0.6000 |
| ARACHNIDA | |
| ARANEIDA | |
| Salticidae sp. | 0.8000 |
| Eresidae sp. | 0.8000 |
| Linyphyiidae sp. | 0.8000 |
| DIPLOPODA | |
| SPIROSTREPTOMORPHA | |
| Spirostreptidae sp. | 0.6800 |
| CHILOPODA | |
| GEOPHILOMORPHA | |
| Geophilidae sp. | 1.9500 |
| PAUROPODA | |
| Pauropidae: Pauropus sp. | 0.0180 |
| TARDIGRADA | |
| EUTARDIGRADA | |
| Macrobiotidae sp. | 0.0020 |
| CRUSTACEA | |
| COPEPODA | |
| Cyclopidae: Mesocyclops sp. | 0.0150 |