J. J. SCHUURMAN AND M. A. J. GOEDEWAAGEN

METHODS FOR THE EXAMINATION OF ROOT SYSTEMS AND ROOTS

Methods in use at the Institute for Soil Fertility for eco-morphological root investigations

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He died in 1970.

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Preface

In 1911 Maschhaupt initiated studies on root morphology at the Institute for Soil Fertility at Groningen. Zijlstra worked briefly in this field but discontinued these studies. Goedewaagen started work at the Institute in 1921 and spent 41 years there working on root morphology, publishing most of his results in Dutch. The experience over these years led to considerable development of morphological and ecological methods of research on roots, which were compiled into two small publications by Goedewaagen in 1948, 1949.

A fuller account, the first edition of this book, was published in 1965 but has sold out and the publisher has asked me to prepare a second revised edition. This second edition includes new findings, in particular new techniques since 1965. For auger soil cores, a new routine partly automatic method of cleaning roots is described. The schemes for description of roots have been transferred to appendices and the bibliography has been brought up to date. There are more figures and plates. However, as modern materials are not always at hand, old methods have usually been kept in the book. Root ecology may stress physiology or morphology. This book is confined to methods of morphological research.

I realize that my account is sometimes incomplete and will be happy to supply readers with further information. The methods described are as used in our institute. Many details are adaptable to circumstances elsewhere and to materials available. I am fully aware of the existence of other methods. Since description of these would have unduly enlarged the size of this book, an extensive literature list is given with a key that can be consulted by those who want more information.

Once again I thank Mr. J. J. H. de Boer and Mr. L. Knot for helping to prepare this edition. Mr. G. Mesker has produced many original ideas in the development of the new method of cleaning roots in soil cores. Miss A. C. G. Holle is largely responsible for the revision of the bibliography. Mr. J. Floris has helped to develop methods for examining finer details of roots. Thanks are also due to Mrs. E. Brouns and Mr. J. C. Rigg of Pudoc for English correction.

Haren-Groningen, March 1971

J. J. Schuurman

1. Introduction

Plant growth is governed by different processes: synthesis of organic material by green aerial parts, and uptake of nutrients and moisture by roots. These processes interact in that carbon dioxide assimilation is necessary for root growth and uptake by the roots for shoot growth. If fixation of carbon dioxide is poor, roots grow badly. Reciprocally if roots absorb only small amounts of nutrients and moisture, aerial growth is impaired even when aerial conditions are favourable. Symptoms observed above the ground may be caused by soil factors that affect the development of the root system and consequently the absorptive capacity of the root system.

Besides the content of nutrients and moisture in the soil and the vigour of the root system, absorptive capacity is determined by the distribution and extent of the root system. Agricultural crops usually need a well developed root system in order to exploit deeper layers more fully. They are then more resistant to periods of stress that often occur during growth and are more likely to yield well. Farmers should know what factors promote and impede root growth. With this knowledge, they can purposefully encourage root growth. Such help to the farmer is the ultimate aim of ecological research on roots. In root ecology, certain morphological characteristics are important (Section 1.1). They can be studied in the field or in containers. In either case there are problems in making roots accessible for study without damaging them. Methods will be described in later chapters in the same order as enumerated below (Section 1.2).

1.1. Morphological characteristics

1. Total amount of root. This indicates absorptive capacity. But two plants are comparable only if they have similar types of root, e.g. plants of the same species of perhaps the same family. The total amount of root is usually expressed in grams dry root but can also be expressed as total length of root or as total surface area. The total surface area is an important characteristic, but it is extremely difficult to calculate. Anyway, not every part of the surface is functionally identical. It is therefore ignored. Total weight of root or root weight in the surface soil can be expressed per hectare as a parameter of supply of organic matter to the soil.

2. Number of seminal and nodal roots. Particularly in monocotyledons. The number of nodal roots depends on soil properties.

3. Number of branch roots and diameter. Absorptive capacity depends on branching and diameter of the roots. Root diameter can help indicate the comparability of two root systems.
4. Vertical quantitative distribution in the profile. The amount of root in different layers is an indication of the nutrient and moisture status of these layers, and the crop's resistance to drought.
5. Lateral quantitative distribution in the profile. Lateral distribution has a bearing on such tillage operations as mechanical hoeing and on plant spacing.
6. Maximum depth and extent. Maximum depth indicates the limit of the plant's activity, in taking up nutrients and moisture. The significance of extent (maximum lateral spread) depends on the layer in which it occurs.
7. Course of development. Observations at different growth stages indicate rate of growth. Drought or frost are withstood better if plants have established a good root system early. Rapid root growth may also counteract leaching of nutrients.

1.2. Types of technique

1.2.1 Methods in field trials

Monoliths (Chap. 2). The pinboard method gives a fairly complete picture of the structure and shape of the root system and of the total amount of root. It supplies data on the distribution in the profile and maximum depth. Course of growth and branching can be assessed from pinboard specimens taken at intervals.
Soil cores (Chap. 3). Fragments of root in small soil samples are taken by augers. This method gives accurate data on the amount of root in each layer. This is also a fairly simple way of obtaining information on vertical distribution and maximum depth. Samples at various distances from the plant show lateral spread and differences between samples taken at intervals show growth rate.
Field assessment (Section 3.5) is a simplified process developed from the auger method.
Excavations (Chap. 4).
Profile walls (Chap. 5). This method includes quadrat mapping and root counting. It is a very suitable method of determining root distribution in the profile and maximum depth. The total amount of root in any quadrat can be counted. The method can easily express root branching and growth. Besides quadrat mapping, roots on the wall can be cleared of soil and then drawn or photographed (Weaver, 1926).

1.2.2 Methods in container trials (Chap. 6)
Plants can be grown under controlled conditions in containers or combinations of containers. Experimental design and analysis of such trials is often simpler than of field trials.
The container may be earthenware or asbestos pipes, concrete containers or glass-panelled boxes for soil cultures and glass cylinders for water cultures.

2. Monoliths: the pinboard method

Rotmistroff (1908) used this method for plants grown in boxes. Maschhaupt (1915) was the first to use it for a field crop. Later Goedewaagen used it for plants grown in boxes and in the field.

2.1. Equipment

To construct a pinboard two similar-sized pieces of plywood may be used 1.5 cm and 1 cm thick. In the board 1.5 cm thick small holes are drilled in vertical and horizontal rows 5 cm apart to hold pins made from cut-off knitting needles or stainless steel wire bent into a U with a 5 cm base and uprights whose length is the sum of the required length of the pins plus the thickness of the board (de Roo, 1957) (Fig. 1). The piece of plywood 1 cm thick is screwed on as backing. This shape of the pins prevents them from pushing through the back of the plywood when they are driven into the soil. If, as formerly, the pins are driven directly into the board it is advised to use a small tube whose length is equal to the required pin length. This tube

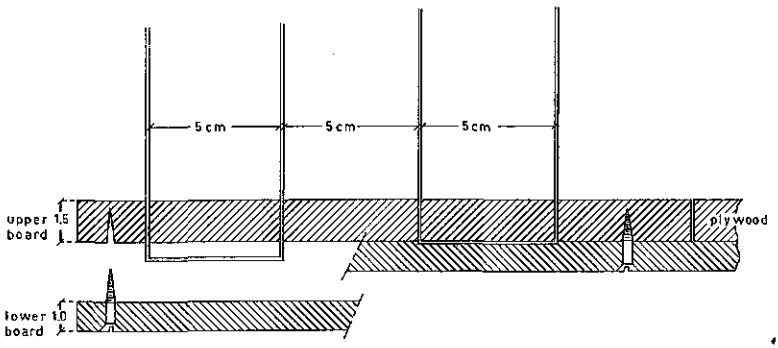

Fig. 1 ***Construction of pinboards***

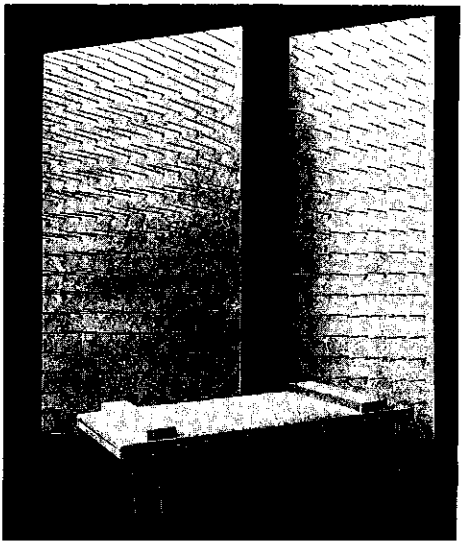

Fig. 2 Pinboards of various sizes

has a flat base. The tube prevents the pins from going crooked.
The dimensions of the pinboards and length of the pins differ according to the plants to be sampled (Fig. 2). The two most common sizes of boards are 1 m x 40 cm with pins 9.5 cm long, and 1 m x 60 cm with pins 15 cm long. The first type is for plants with restricted root systems, e.g. pasture plants and cereals, and the second for crops with extensive root systems, e.g. potato, beet, rape. Before sampling black polyethylene sheeting is stretched over the board and pressed with a lath down into the pins until it is against the board. The advantage of polyethylene sheeting is that the root system can be removed from the board intact after washing off the soil and draining.

2.2. Obtaining pinboard samples

When the pinboard is ready the plants are inspected. The crop stand and development, and any weed growth are noted. Alongside a representative site, a pit is dug about a metre square and the vertical wall against the plant or plants is smoothed off with a blade sliding up and down two supporting vertical laths (Fig. 3).
When plants are in rows, the pit is dug either parallel to the row or transversely to it. The specimen taken parallel to the row will contain part of a single row as wide as the pinboard and the specimen taken transversely

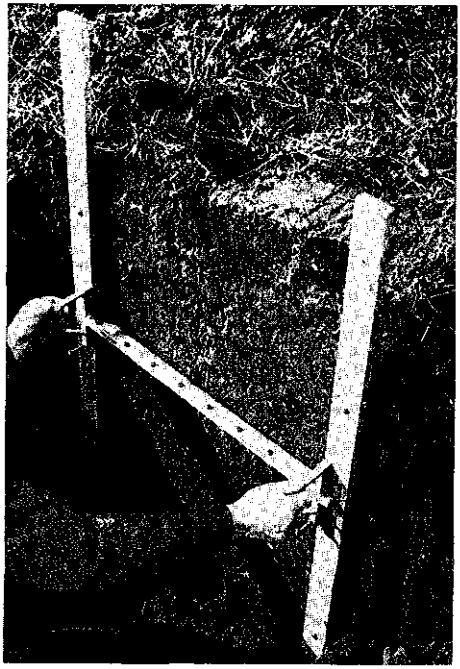

Fig. 3 Blade for smoothing a profile face

will contain plants from two or more rows depending on the width of the board. With a section across two or more rows differences in root distribution in and between rows, and the lateral extent of roots can be examined. If the wall is parallel to the row, the distance of the plants from the wall should be a few centimetres, so that the plants come to lie halfway up the pins or slightly beyond the middle of the soil slice after the monolith has been cut out. The soil profile is described and all relevant data are noted, e.g. type of soil, watertable or height of the site above the ditch level, and thickness and characteristics of the surface soil and subsoil.

The force required to drive a conical object into the various soil layers with a penetrometer is measured. At constant bulk density this force increases as moisture content decreases, and at constant moisture content it increases with bulk density (Schuurman, unpublished). Observations are noted on a standard form. If necessary, soil samples are taken in rings 5 cm diameter and height to the laboratory to determine bulk density. Roots from the profile face are teased out with a probe or the face is scraped with a three-tined hand cultivator to gain an impression of depth and extent of rooting.

Following this the board is held vertical with the pins against the profile face so that the top row of pins is at ground level. If the surface is uneven, the highest point is selected. The pins are driven into the soil with a jack (Fig. 4) or by hammering the back of the board with a wooden or rubber mallet. These operations over, large plants on the pinboard are cut and stored in plastic bags to avoid damage and loss of water.
After pressing the pins home, a few centimetres of soil underneath the pinboard is cleared, to a distance of a few centimetres beyond the tips of the pins (Fig. 4). The pinboard is then supported by the jack. Soil from the profile face is cut away on either side of the board, also a few centimetres further than the tips of the pins. A steel cable, up to 2 mm diameter with a handle at each end, is passed, down one side, along the bottom and up the other side of the pinboard, and drawn up and down in a sawing movement so that the monolith is cut away from the soil mass. When it is free, the pinboard is lowered, still resting on the jack, until it lies on the pit bottom. The soil is now held on the pinboard by the pins.
Two or four men can hoist smaller specimens out of the pit, if necessary with ropes. For heavy specimens, use of rope, block and tackle is advisable. The tackle should be fastened to the back of the pinboard before sawing off the monolith. Two smooth beams, resting in the pit bottom under the pinboard are leaned against the top edge of the opposite wall of the pit. The monolith can then easily be lifted along these beams, for instance, by towing with a car.
If horizontal pinboard specimens are needed (Fig. 5), the aerial parts of the

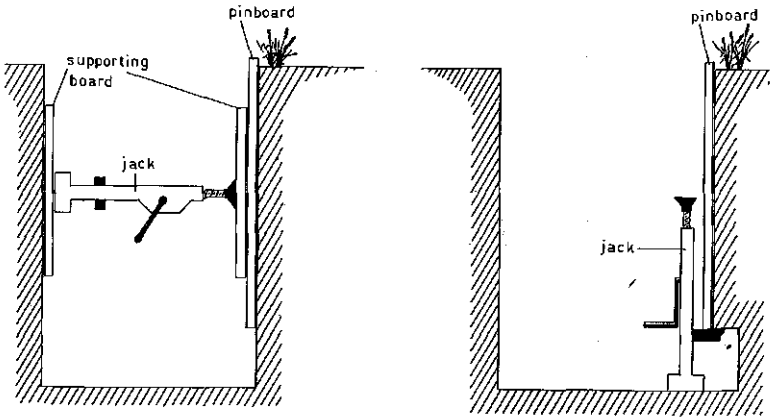

Fig. 4 Use of a jack during pinboard sampling

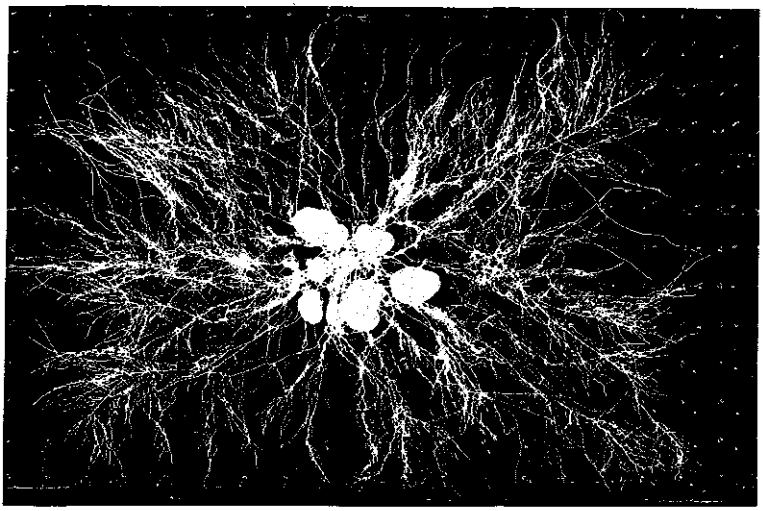

Fig. 5 Horizontal picture of a root system on a pinboard

plant are first cut off. The surface is levelled, if necessary. The pins are pressed into the soil from above. The soil is dug away round the board and a wooden frame is fitted round the pinboard and specimen. There is a groove in the frame below the level of the pins. With a jack, a steel plate with a triangular cutting edge is forced under the specimen. To prevent the specimen from being dislodged, the other side should be held in place. Once the specimen has been cut loose, it rests on the metal plate and can be lifted out. All the specimens are wrapped in sacking to prevent them from breaking during transport to the laboratory.
Fig. 5 illustrates the extent of a root system of potatoes in the topsoil.

2.3. Removing soil from the root systems

2.3.1. Sandy or loamy soils

If the monolith consists of sandy or loamy soil, it is directly placed in a slightly inclined bath of internal dimensions 150 x 95 x 35 cm (Fig. 6). The lower end of the bottom has 4 plugholes for quick drainage and is placed in a drain. The lower vertical wall of the bath has a series of 4 plugholes too, for maintenance of water levels of 3, 10, 17 or 24 cm (Fig. 7). The specimen is put in the bath, then the bath is filled with water and the specimen is kept immersed until the soil seems saturated, usually 12 to 24 h. Clods are then

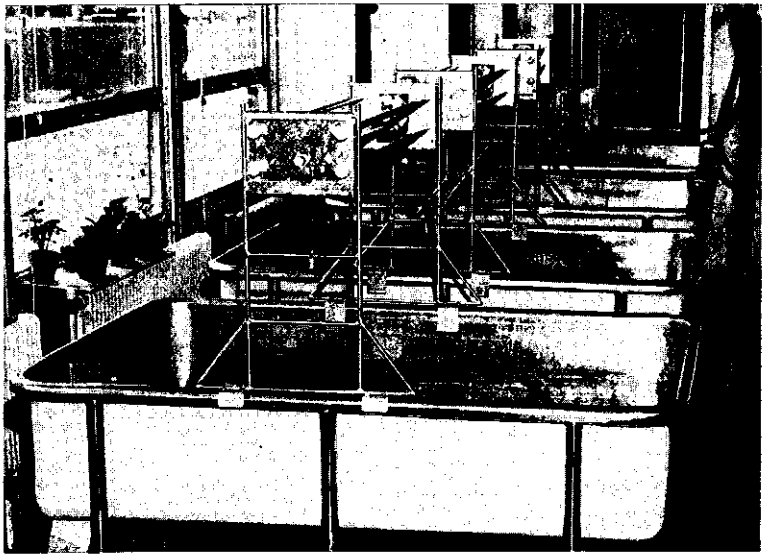

Fig. 6 View of washing baths

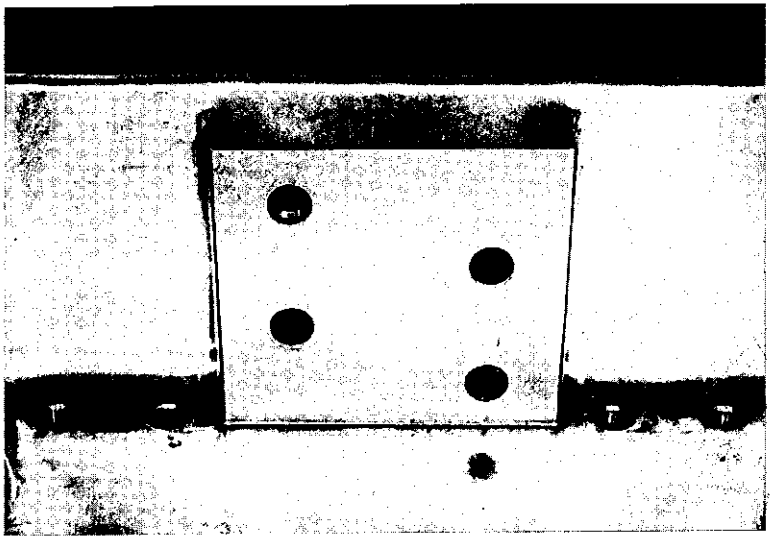

Fig. 7 Detail of washing bath

less likely to break off and roots to be lost. Washing can then begin. Damage during washing is avoided by keeping the water level in the bath just below the top of the pinboard specimen. This precaution is not essential. Sandy or loamy specimens can be washed with a hand-sprinkler or various types of automatic sprinkler.

Hand-sprinkler. One person can wash the specimen with a hand-sprinkler, but few technicians keep the pressure constant, particularly if there are harder and softer layers in the profile.

Rotary sprinkler. The specimen can be washed with a rotary sprinkler with three arms 20 or 33 cm long and perforated underneath slightly off-centre with a row of small holes (Fig. 8). The number of arms can be reduced to one by plugging the other ones, so that water pressure can be regulated. The slightly oblique position of these holes causes the sprinkler to rotate with the pressure of the water. The drops scatter evenly over the entire trajectory.

This method has several advantages. All material is washed with the same force. One technician can simultaneously supervise the washing of several profiles. Such debris as pieces of straw, stalk or leaves should be removed during washing with tweezers before they become entangled in the root mass. If washing is properly supervised, the amount of water need not

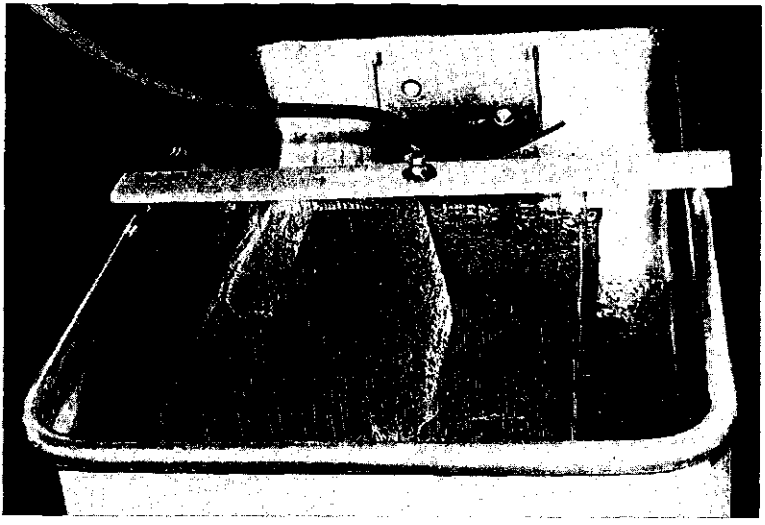

Fig. 8 Rotary sprinkler

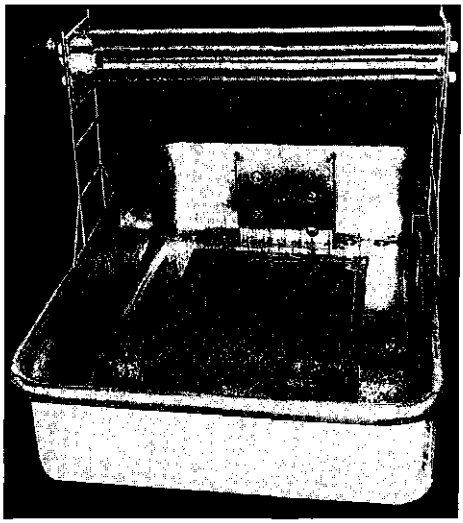

Fig. 9 Oscillating sprinkler

exceed that used with a hand-sprayer.
It is general rule that washing should not be too vigorous since a high pressure may damage roots, and wash away cut roots from plants outside the specimen.
Oscillating sprinkler. A rectangular area of the specimen may be automatically washed with a modified garden sprinkler placed on a stand. The nozzle moves to and fro with the pressure of the water (Fig. 9). This area depends primarily on the distance from the nozzle to the specimen and the length of the nozzle. Therefore the construction of the stand allows the sprinkler to be placed at three heights. In each position the spray can be switched to broad, narrow or to one side. A pinboard specimen size 60 x 100 cm can sometimes be washed in a single operation. The apparatus has the advantages of the rotary sprinkler but is superior since it cleans a rectangular area. It can also be used to compare the hardness of layers in two profiles, since a part of both profiles can be washed simultaneously and hence with the same force. During washing debris also should be removed by tweezers.

2.3.2. Heavy clays
Pinboard samples of heavy clay are extremely difficult to wash, sometimes it is even impossible without special preparation of the sample. Attempts have been made to use a motor pump for washing with aqueous solution of sodium pyrophosphate ($Na_4P_2O_7$). The solution was pumped from a tank,

sprayed on the specimen, drained to the tank and recirculated several times. These experiments were unsuccessful. Fairly good results have sometimes been obtained by drying the entire specimen at 100°C and then soaking it in an anhydrous sodium pyrophosphate solution, 270 gram per 100 litre. The drying allows the solution to penetrate quickly and evenly into the soil. Drying should be gentle to avoid severe cracking, which will break roots. After about 12 hours in the solution an attempt can be made to wash the specimen in the usual way. If the sodium pyrophosphate has not penetrated enough the process is repeated. Recently a method has been developed for processing soil cores (Section 3.3.2) by freezing and action of sodium pyrophosphate under vacuum. It has given also favourable results with small pinboard samples, but the latter must be kept longer than the auger samples in the vacuum chamber (Fig. 32).

2.4. Final steps

After the specimen has been washed the pinboard and roots are transferred to a level bath of water. Here the roots are profiled and cleaned. During profiling, dislodged and loose roots twisted round needles are restored to their original position. In the cleaning process debris, e.g. straws, leaves and roots of adjoining plants, etc., are removed. The specimen may be at least partially cleaned, by filling the basin with water and then passing through it a thin stream of water which runs over the edge.

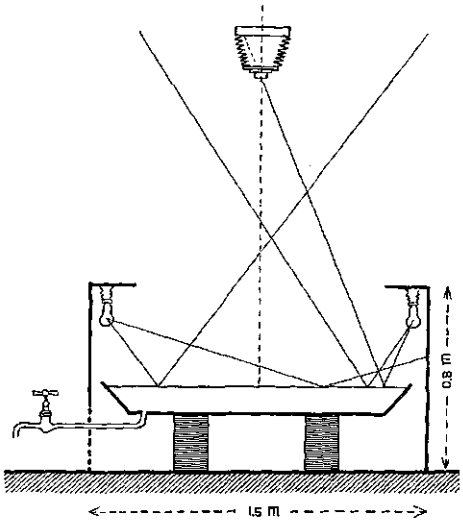

Fig. 10 Wooden case with light bulbs used for photography of a root system

2.5. Photography

2.5.1. Root systems

After the specimen has been washed and arranged the root system can be photographed under water as it lies on the pinboard. Under water the natural position of the finer roots is also preserved. The black polyethylene sheeting makes a good background. Roots can be evenly illuminated in a box in which are arranged a number of bulbs or fluorescent tubes (Fig. 10). The bulbs or tubes are placed sufficiently obliquely to avoid reflexion within the area of the sample. Since the photograph is taken vertically the front of the camera should be blackened in order to prevent it from being reflected in the water. Ceiling reflexion may be avoided in the same way. Alternatively four powerful 'Fotomirenta' bulbs can be arranged round the bath to

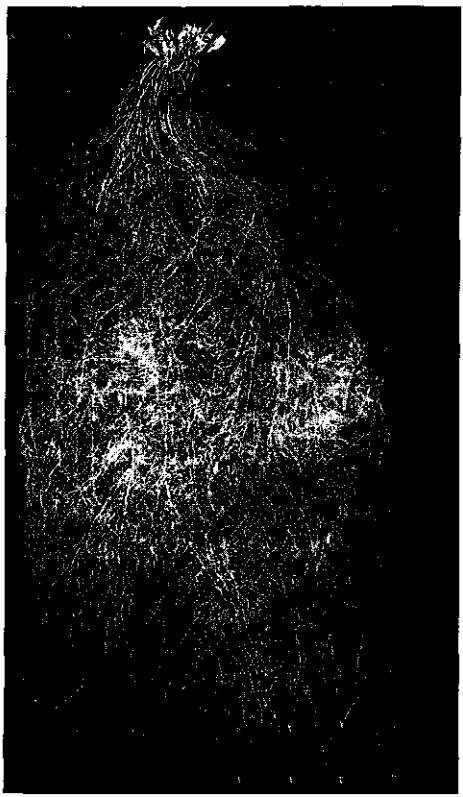

Fig. 11 Submerged root system on a pinboard

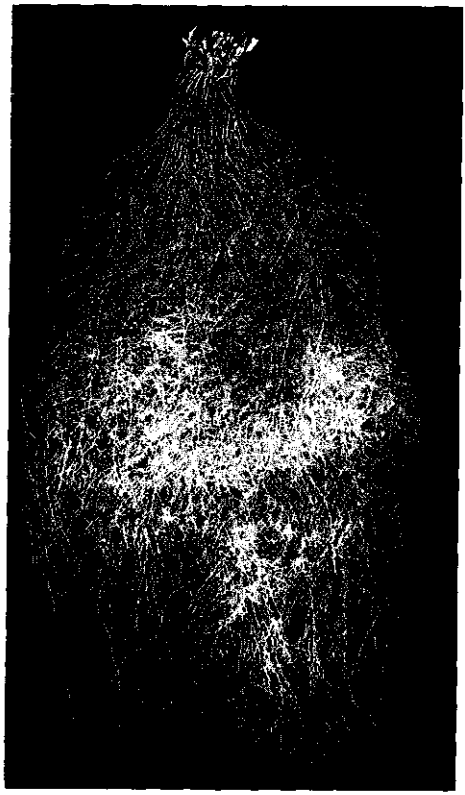

Fig. 12 Submerged root system of Fig. 11 removed from the pinboard

give a good distribution of the light and to avoid their reflexion within the area of the root system. Figure 11 shows a root system photographed on a pinboard under water. This figure illustrates the drawback of pins on a picture. To prevent this the root system on the polyethylene backing can be transferred to another bath and then photographed (Fig. 12). See also Section 2.8.3.

A third possibility is to photograph the dry root system after it has been arranged. The water is carefully siphoned out of the bath until the roots are free of the water. The root system is dried with a fan until the outside is practically air-dry. The root system and the polyethylene sheeting are carefully lifted off the pinboard and the root system is transferred onto black velvet or black-painted hardboard, for photography. The finer roots are not so clear as when photographed under water (Fig. 13). Moreover,

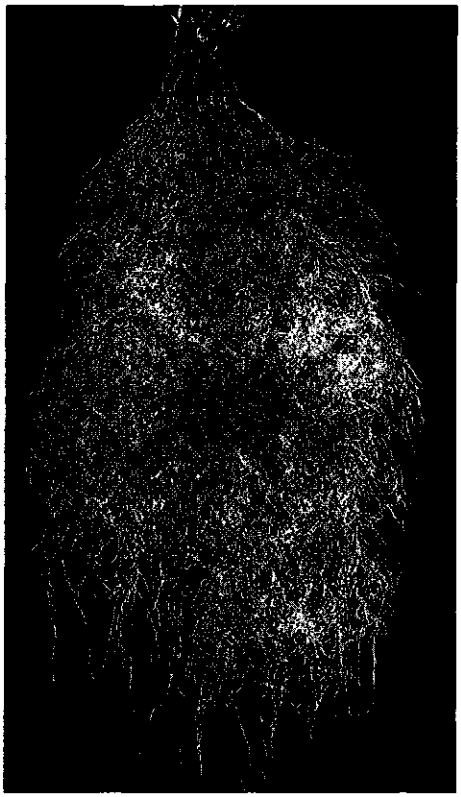

Fig. 13 Root system of Fig. 11 photographed in dried state

later description of roots is practically excluded. The best results are usually obtained by the second method.
It is clear that pinboard samples of larger plants do not include an entire root system but only a sort of vertical cross-section of it. Roots towards the front and the rear of the specimen are cut off.
However, a pinboard specimen gives a good impression of the root system as a whole. Hudig (1939) more or less circumvented this limitation by taking a cube of soil on two pinboards size 30 x 30 cm at right angles to each other.

2.5.2. Shoots
The aerial parts of those plants that are not cut off are photographed with the roots. Large plants are photographed separately, if possible from the same distance as for the roots so that photographs are on the same scale.

2.6. Calculation of root weights

After photographing the root system, the roots can be collected either as a whole or in layers to estimate the weights. Layers may correspond with soil horizons or be chosen arbitrarily. Roots are dried at 75°C before weighing.

2.7. Description of root systems

A root system must be considered as a whole before describing individual roots in detail.
A scheme for description is given in Appendix 1.

2.8. Description of individual roots

2.8.1. Selection of roots

For detailed description of roots, e.g. their branching two or more fully grown roots are selected and worked loose without breaking off branch roots. This is a difficult task that requires skill. Experience has shown that the method succeeds even with dense root systems if no force is used. The

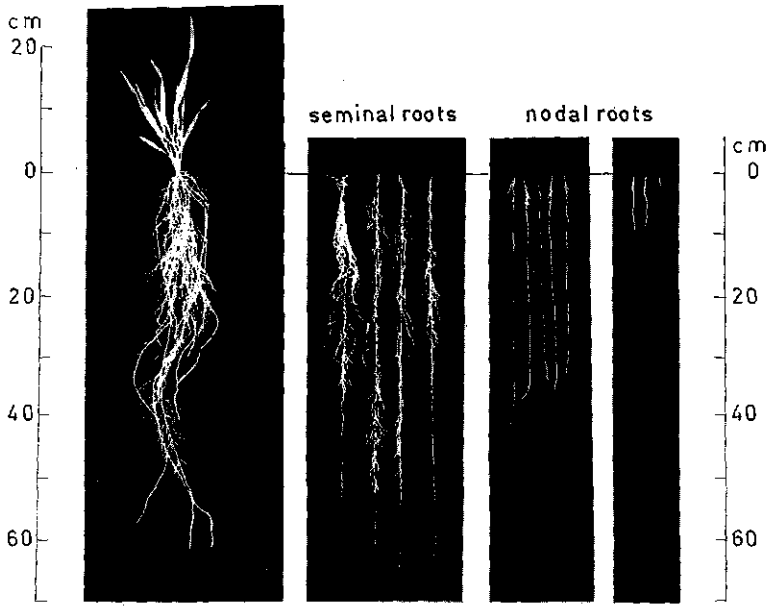

Fig. 14 Roots of one oat plant

number of roots required depends on the type of investigation and the kind of root system. Some plants such as potato have roots, differing markedly in growth habit, one part being confined to the top soil and another growing in a distinctly vertical direction. Roots have to be taken from both groups for detailed investigation. In the case of cereals either the nodal or seminal roots, or both can be taken. To analyse the effect of soil factors it is useful to choose fully grown roots. The oldest roots are better avoided as they may be partially decayed. This method also affords a good idea of the growth of the roots of different ages of a particular plant by working loose roots from young to old (Fig. 14).

For selecting roots the best procedure is to place the root system in a long, fairly broad and shallow bath with 4 to 5 cm water. The bottom of this bath is preferably dark for pale roots and light for dark ones. Since the bath is wide, any roots not used can be put aside without being cut off at once. However, the best procedure is to start with cutting away young unbranched roots since this makes it easier to free fully grown, densely branched roots. Roots selected for description and photography are cut when they are freed.

2.8.2. Preparing roots for photography

If the root is not needed for weight determination it is transferred to a long, comparatively narrow and shallow bath the bottom of which is lined with a sheet of fairly firm transparent polyethylene sheeting without folds or creases as it may later be used for photographing or photocopying. The

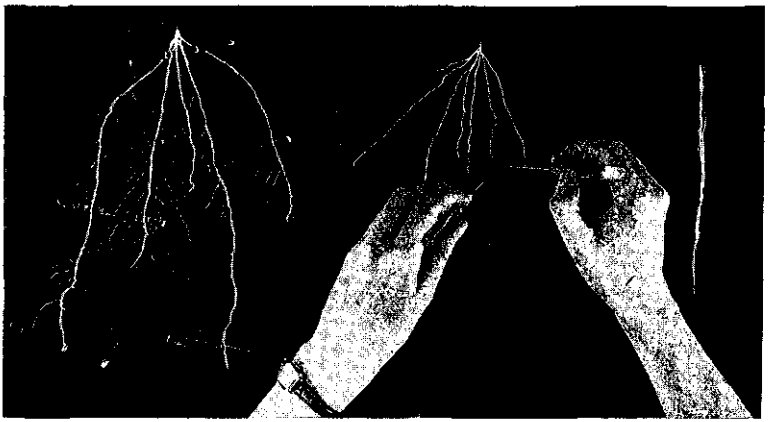

Fig. 15 Arrangement of roots by means of pins

margins of this plastic sheet are weighted to prevent it from drifting when the vessel is filled with a thin layer of water. If the water is too deep, fumbling may cause water currents that displace the roots. Fortunately when roots have only a few side-roots the water adhering to them is sufficient. The branch roots are arranged with pins (Fig. 15) from the base of the main root up to the tip. When the whole root has been arranged, the water is carefully removed with a water-jet vacuum pump. To prevent the roots from being displaced by the water they are weighted with glass rods. The roots are then left to dry until they begin to adhere loosely to the polyethylene sheet. To prevent roots coming away from the sheeting, as happens with uneven drying a vaporiser is used for spraying the roots with a clear glue: Saba 810 E, dissolved in water in a ratio of 1:5. Excess glue can be removed with a water-jet vacuum pump. The glue takes about 30 minutes to dry, or less with a fan. The roots then stick to the polyethylene sheet. Afterwards the dry specimen is covered with a second sheet of thin polyethylene to protect it (Fig. 16).

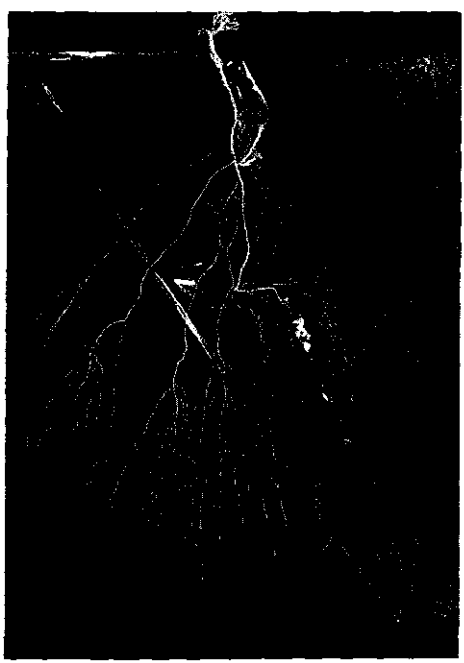

Fig. 16 Roots partly between polyethylene sheets

2.8.3. Photography

Before photography of the roots the thin polyethylene cover which could create reflexions should be removed. Roots can be photographed by either transmitted light or with a black background by reflected light (Fig. 17). The roots should again be covered as quickly as possible with a thin sheet of polyethylene.

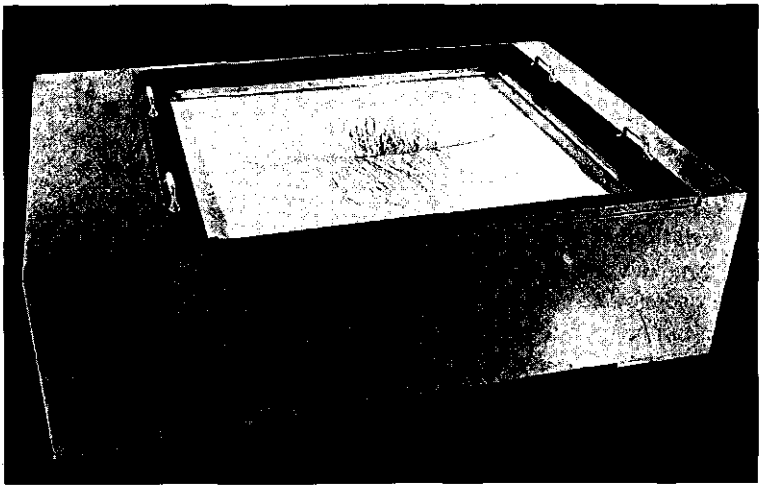

Fig. 17 Wooden case suitable for photography with transmitted light

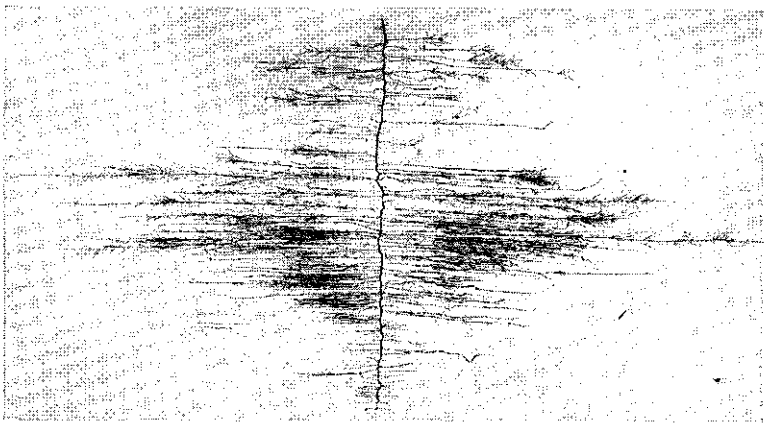

Fig. 18 Seminal root of oats (photographed with transmitted light)

The thin polyethylene sheet is also removed when photocopies are made. The light-sensitive paper comes into direct contact with the roots and a very sharp photocopy is obtained of the thin roots. Roots which vary extensively in thickness make poor photocopies and are better photographed. Photocopies or photographs, which give an accurate picture of the roots, can be used afterwards for measuring and counting.
The arranging of roots could also be carried out between glass sheets, instead of polyethylene, but then only photographs can be taken since these cannot be used in a rotating photocopier.
Roots needed also for weight determination are arranged in a shallow water layer in a glass bath (Fig. 17). They are photographed in this bath with either transmitted or reflected light (Fig. 18). In the latter case a black background of polyethylene is used.

2.8.4. Description
For measuring and counting, the root or a photocopy is laid under a binocular microscope with a calibrated background (Fig. 19). The binocular microscope can be moved from side to side and backwards and forwards independently. Alternatively branch roots are counted by projection on a squared screen. The description schemes used are given in Appendices 2 and 3.

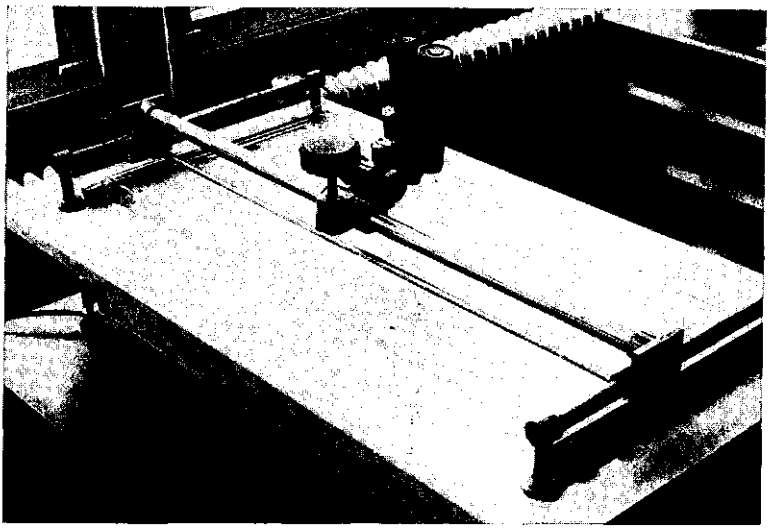

Fig. 19 Binocular microscope

2.9. Special applications and difficulties

If the pinboard is too short for the root system to be sampled, two pinboard specimens can sometimes be driven close together one below the other into the profile face. The specimens cannot be cut away until both pinboards are in position and the soil between them has been cut carefully with a knife to avoid tearing of the roots.

It is possible to supplement the data with boring specimens taken from the

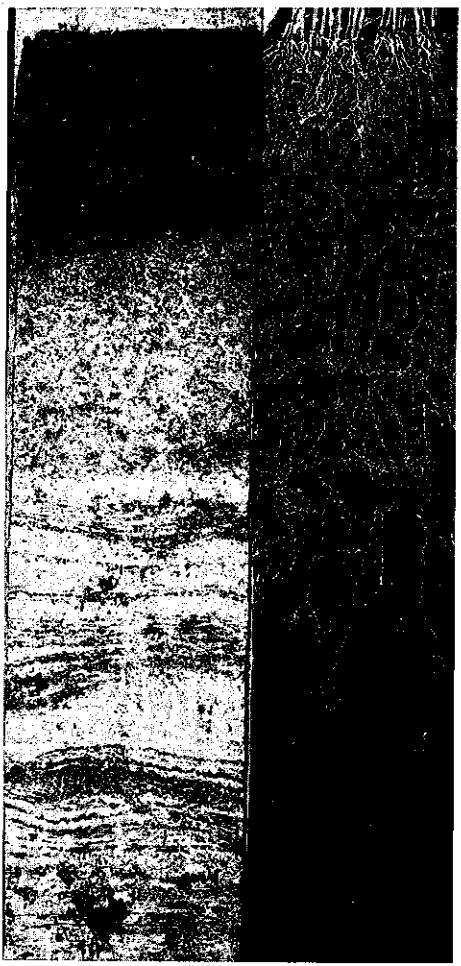

Fig. 20 Root system of oats and corresponding profile

soil of the hole or its immediate vicinity.
To allow comparison of the root system with the soil profile, both the profile and the root system may be preserved. In these cases the sample taken is slightly thicker than the length of the pins. The soil outside the pins is used for making a soil film. This method has been described (Schuurman, 1955) but has since been simplified. After drying the root system is now transferred from the polyethylene sheet to a blackened hardboard to which roots and monolith are stuck with a clear glue (Fig. 20).
Summarizing it will be seen from the above that the pinboard method has many possibilities. These specimens can be used to obtain information on all the points listed in Section 1.1. It is also extremely important that it gives an idea of the habit of the root system of a particular plant. Moreover the method is usually fairly easy to carry out and is not very laborious. One drawback is that quite a big pit is needed for taking a pinboard specimen. In experimental plots, unless the site adjoins a border, the excavated soil is difficult to dispose of without damaging the crop. The soil is usually deposited on a large sturdy canvas sheet that is spread near the pit. Top and subsoil and if necessary special horizons are kept apart. Moreover use of this sheet restricts damage since the soil can be tipped back easier. Despite this the crop is often damaged over an area of about 4 x 3 sq.m. Usually this upsets yield estimates from small plots. In such cases it is generally inadvisable to take more than one sample per plot. Even though the soil layers are kept separate during excavation the profile will never be entirely the same as before, upsetting results of trials in later years.
A restriction is also that the method cannot be used in stony soils.

3. Soil specimens having a slight volume: the auger method

3.1. Equipment

The auger method is used both in field trials and certain container experiments. Two types of augers are used. One, the heavy auger, is specially designed for sampling heavy soils and hardpans. This is driven into the soil with a mallet. (Fig. 21d). The light auger (Fig. 21a) was originally developed for sampling light soils, but after the cutting edge had been serrated it also proved suitable for heavy soils where it is now even preferred to the heavy one (Fig. 22). In stony soils the heavy auger must be used. Both types were developed by Goedewaagen from augers used by Visser, 1943 (cited by Goedewaagen, 1948).

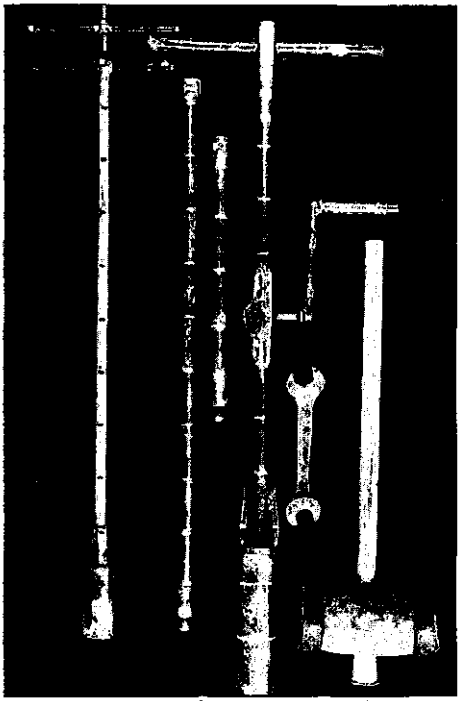

Fig. 21 Auger types with tools

Both augers consist of a cylindrical tube with an inside diameter 7 cm. The height of the tube of the light auger is 15 cm and of the heavy one 25 cm. Formerly augers 4 cm in diameter were used but the frictional resistance of the inner wall was so much greater on some soil types than in the 7 cm auger, that soil was partly forced away. With the 7 cm auger this occurs so rarely that it can be discounted. This has been proved by measuring the bulk density. Nevertheless soil cores drilled with the 7 cm auger vary in length, either caused by compression or expansion of the sample in the auger tube. Therefore the amount of root present is still that from the original volume of soil despite compression or expansion during drilling. A hollow shaft is fixed above the tube. There are marks on both the tube and the shaft at 10 cm intervals. The light auger has a handle fixed to the top of the shaft to enable it to be driven in the soil and pulled out again. The heavy auger is provided with a striking head in which can be inserted a sturdy crosspiece that can be used as a handle to lift the auger out of the soil. The auger may be lengthened by unscrewing the striking head and

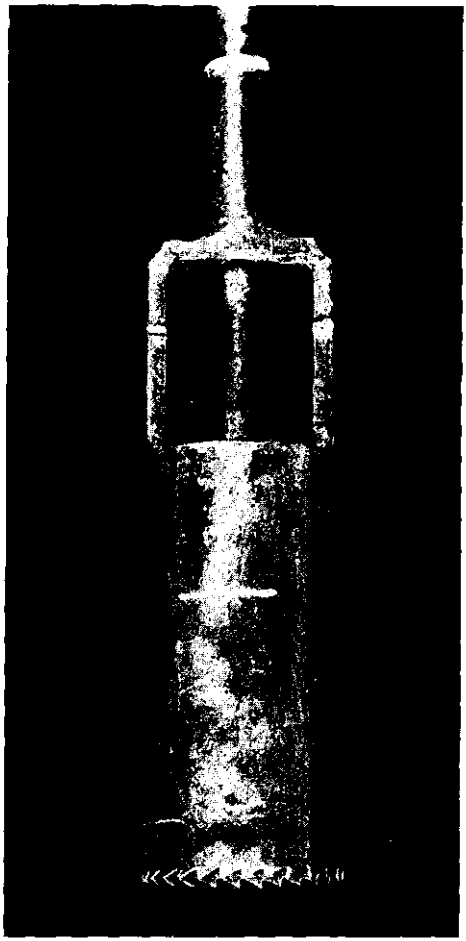

Fig. 22 Auger with serrated cutting edge

inserting an extension shaft (Fig. 21b, c).
The shaft of the light auger is normally 70 or 110 cm long so that they can be used for drilling to a depth of 80 or 120 cm respectively. The short auger is used for shallower sampling as in this case the operator's weight is put to better use. The long auger is used for deeper drilling.
The shaft of both auger types contains a rod with a disc at the bottom acting as a plunger. The light auger is provided with a handle at the top of the rod. The rod and stamp are used for forcing the soil specimens out of

the tube. In the shaft of the heavy auger a short rod is fixed at the bottom end to a plunger. The other end of the rod is a rack that engages a pinion in a housing about 40 cm above the tube mounted on the shaft. (Fig. 21d).

3.2. Sampling

3.2.1. The light auger

By slowly twisting the auger to and fro in short turns it is pressed vertically into the soil up to the first mark (Fig. 23). During drilling the plunger is forced upward by the sample. When the auger has reached the required depth it is rotated several times to free the sample and then pulled out. The hole is slightly widened by rocking the auger. This prevents soil and roots from being sliced off the wall when inserting the auger for the next sample. The sample is forced out of the tube by turning the auger upside down and pressing the handle of the plunger with one foot down. It is collected in a

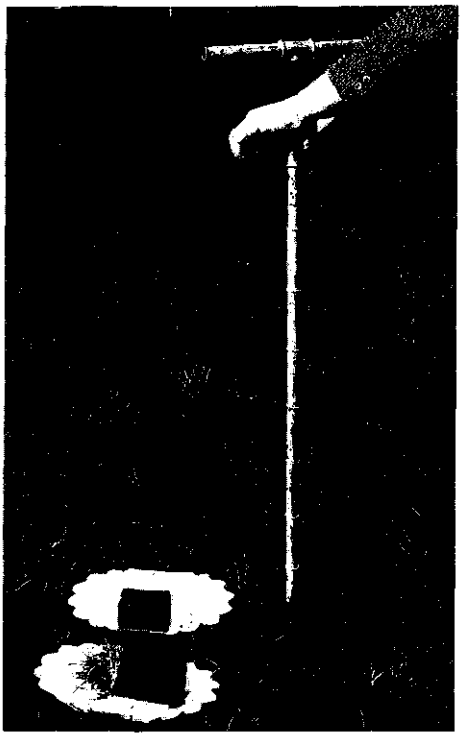

Fig. 23 Drilling a sample with a light auger

cardboard dish where it can be inspected. By arranging the samples in order of depth a rough description of the profile can be made (Fig. 24).

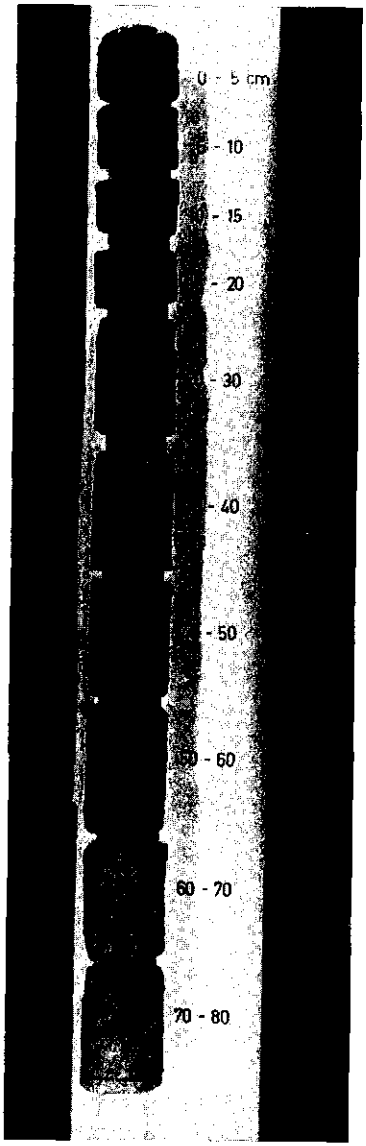

Fig. 24 Soil cores arranged according to depth

Afterwards each sample is transferred to a numbered paper or plastic bag. The auger is then replaced in the borehole and drilling is continued up to the next mark. The work proceeds until no further roots are encountered in the sample but, to be on the safe side, one more sample is taken. If the build-up of the profile is such that a distinct difference exists between two layers in one 10 cm sample, this may be subdivided into two parts at the interface between these layers. This subdivision is usually done. The 0 - 10 cm layer of grassland is usually subdivided in this way into layers of 0 - 5 and 5 - 10 cm.

3.2.2. The heavy auger

This auger should be of a much heavier design, since it is hammered into the soil with a wooden or rubber mallet. To prevent damage to the shaft the auger should be hammered gently. In heavy soils it is often impossible to push the sample out of the auger by hand. This is therefore done by rotating the pinion in the housing with a crank (Fig. 25). After that the same procedure can be followed as for the light auger. In clay soils the work, with either the light or the heavy auger, may be greatly facilitated by briefly dipping the auger into a pail of water before each drilling operation.

Fig. 25 Removing the sample from a heavy auger

3.3. Calculation of root weights

3.3.1. Hand washing

Drilling samples can be used for calculation of the root weights, after the specimens have been washed in the laboratory. Sometimes, however, they have to be prepared for washing. The procedure depends on whether the samples can be processed immediately, whether the moisture content has to be estimated and whether the samples are sandy or clay. Sand samples processed immediately on arrival and requiring no estimation of moisture can be washed at once. If the samples, irrespective of the soil of which they consist, cannot be processed immediately, they are dried at about 100°C and stored to prevent the roots from rotting. If the moisture content has to be estimated the samples are packed in the field in heat-proof plastic bags and are weighed in these bags, before being dried. After drying they are weighed again. Clay samples cannot be washed until they have been first dried and then the clay dispersed in a sodium pyrophosphate solution. Special care should be taken when washing dried samples. If dry fine roots were washed immediately they would be reduced to powder and lost. To prevent this, dry samples of sandy soil with the field data on a label are soaked in large bottles of water for about eight hours. About 5 ml detergent in 300 ml of water assists soaking. During this period the roots also take up moisture and become so flexible as to enable them to be washed. Clay samples should be soaked in an aqueous sodium pyrophosphate solu-

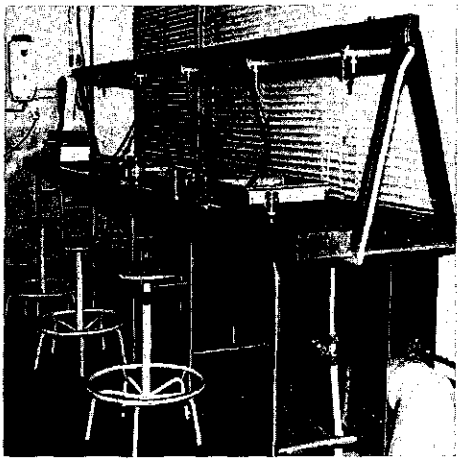

Fig. 26 View of washingtable

tion (270 g $Na_4P_2O_7$ in 100 litre), to disperse the clay. As a result the sample often disintegrates altogether. (Fig. 31a).
Although the roots are fairly shrivelled after the samples have been dried, they swell again when the samples are again contacted with water. The roots then substantially regain their normal habit, and even the root hairs do not seem to be greatly affected. This means that samples first weighed moist and then dried and again weighed to estimate bulk density, pore volume and the soil-water-air ratio, can easily be wetted again and washed for a qualitative and quantitative evaluation of the root fragments in the sample. This method can therefore be profitably used in studies on the influence of chemical and physical properties of soil on root development. The sample is washed by pouring it out through copper gauze about 0.3 mm gauge on a specially designed table (Fig. 26). These meshes are so fine that hardly any roots are lost. The gauze usually lets through most of the soil, except for clods and coarser soil components. During washing free roots are picked off from the gauze with tweezers and transferred to small bottles of water ready to hand (Fig. 27). Unfortunately not all technicians maintain

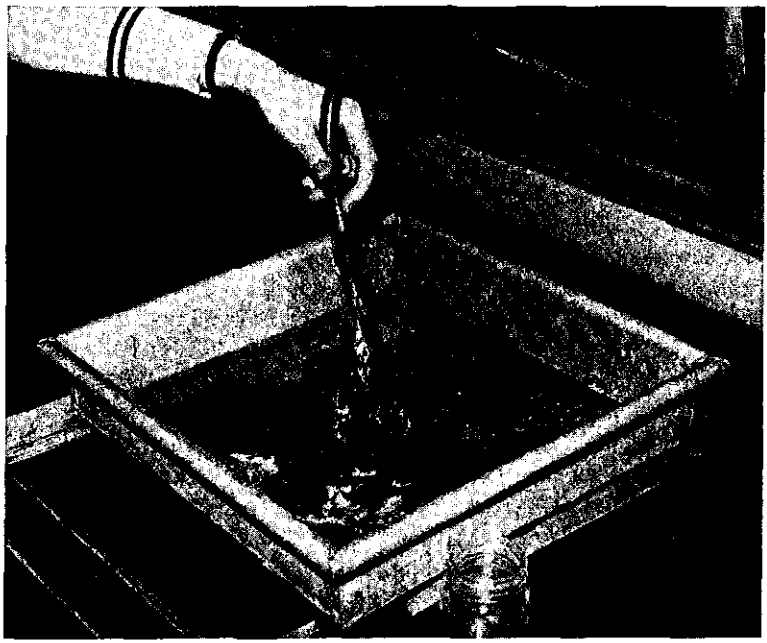

Fig. 27 Hand washing of a soil sample on a screen

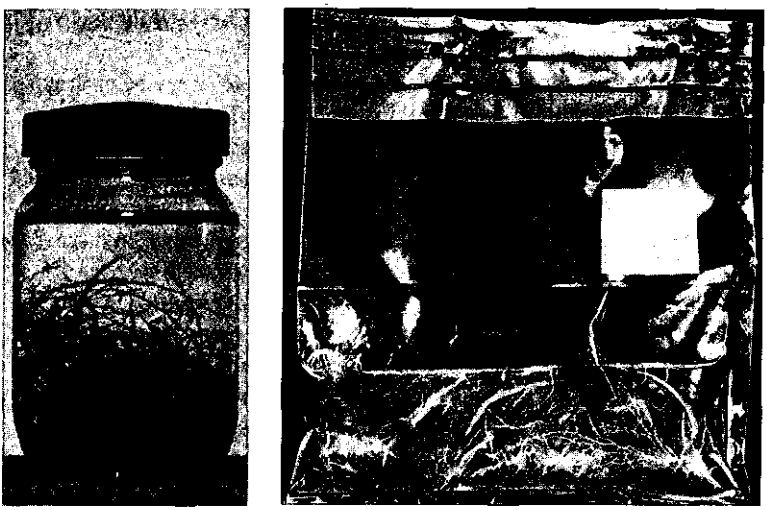

Fig. 28 Roots stored in a bottle and in a polyethylene bag

a constant water jet, and results may vary between workers.
When no more soil passes through the screen and only very fine roots are left, everything on the screen - rootlets, humus components, plant debris and soil particles - is washed into a glass cylinder in which the soil particles settle out and roots and other organic material continue to float a while. By decanting once or more and topping up the roots can be separated from the soil, but any plant debris and humus components remain among the roots, as they cannot be separated from them by decanting. Finally the mixture is poured onto a fine nylon or muslin gauze measuring 10 x 10 cm whence the mass remaining on the gauze is added to the other roots that were already separated.
If the samples cannot immediately be dealt with, formalin or ethanol 60% or 1.5 gram finely divided thymol per litre is added. Since glass bottles are breakable and take up space, roots have recently been packed in plastic bags with some water, heat-sealed and frozen (-20°C) or with some preservative solution (thymol) and then heat-sealed (Fig. 28). Afterwards the organic impurities are also removed. This is done at a specially designed table (Fig. 29) by pouring the sample into a shallow enamel bath measuring 26 x 20 x 4 cm, after which the impurities are removed with tweezers (Fig. 30). If there are many impurities removal of the roots from the mass is better.

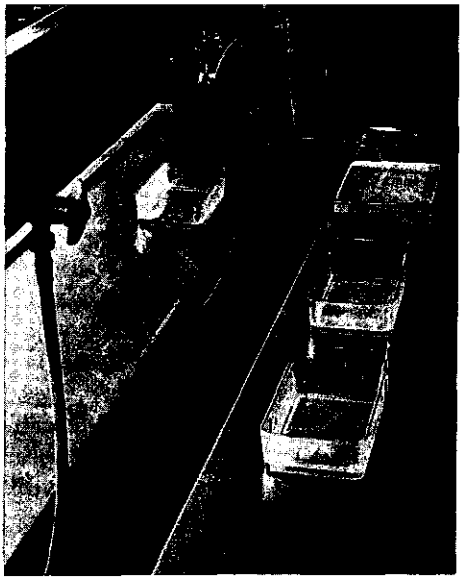

Fig. 29 View of table for separating roots and debris

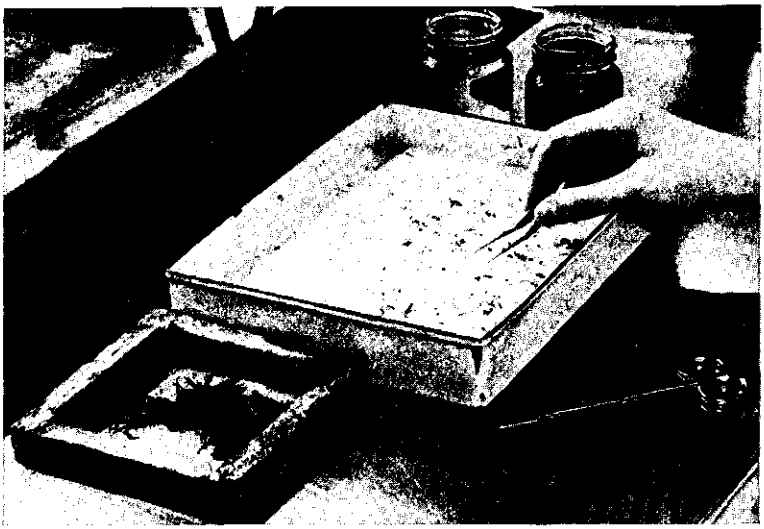

Fig. 30 Separation of roots and debris

It is difficult to distinguish living and dead roots, i.e. roots which were alive or dead when sampled. Four features are noted for drawing this distinction: the elasticity of the root, its colour, and the presence of cortex and lateral roots. A dead root is far less elastic, is often grayer, the cortex is often ravelled or it has no cortex and lateral roots have often broken off, leaving stumps with frayed ends. The *combined* assessment of these four characteristics determines whether a root should be regarded as alive or dead. If dead it is removed. Another test, although unsuitable for routine is to contact the roots with a tetrazolium chloride solution. Live roots stain red, whereas the dead ones remain colourless (Goedewaagen, 1954; Butijn, 1955 and 1961). After removing all impurities the roots are again tipped out on to the fine gauze, moisture is gently pressed out and then the roots are transferred with tweezers to a small paper bag on which all data are noted. The original label is also placed in the bag. Finally the roots are dried in the bags at 75°C. It was found, that the results hardly differed from those obtained at 105°C. A further advantage of this lower temperature is that it prevents the roots from being pulverised. After drying for about 48 hours, the bags of roots are placed in a desiccator to cool. The roots are then weighed.

The intention of washing is to remove all *soil particles*. They can, however, never all be removed as some particles adhere firmly to the roots, especially if the roots have an abundance of root hairs. The presence of soil particles biasses the root weights obtained. This inaccuracy was checked periodically by occasionally ashing washed and dried root samples. The percentage inaccuracy usually increases the deeper the layers from which the roots were taken. The maximum inaccuracy found was 12%. Fortunately only a small proportion of the roots is found below a depth of about 20 cm. It can be stated as a general conclusion that with carefully washing the impurities need not exceed 4%, for the entire depth of sampling. In some cases even lower values have been found. If a vibrator is used after washing this percentage can even be further reduced. As a rule we did not estimate organic matter contents of the roots, although it can be done with the auger method.

3.3.2. Routine method

In the previous pages a simple method for processing soil cores obtained by augers has been described. Even though the method may be considered as a good one it is clear that the operation contains personal elements, for instance when not everybody works with a uniform water yet. Moreover the method is very time consuming. Finally drying of soil samples, however,

necessary to preserve them, may be destructive to the roots.
We have developed a routine automatic and standard method that complies with reasonable requirements of being objective, not laborious, gentle so that the soil particles are loosened from the roots without damage to the latter, and simple provided the necessary equipment is present.
Firstly, soil samples, having normally a volume of 385 cm^3 are thoroughly frozen in the laboratory at a temperature of -20° C. While they are thawing they are submerged in about 800 ml solution of sodium pyrophosphate. The favourable concentration of sodium pyrophosphate after the freezing is 2 to 4 grams per litre (Fig. 31a). In contrast with this the action of sodium pyrophosphate without freezing and vacuum is small (Fig. 31b). No distinction is made between clayey and sandy soils. The samples are then placed in a vacuum chamber. When a small number of samples are to be processed an undamaged and high quality desiccator can be used. For many samples proper equipment is required (Fig. 32). The first step is then to evacuate the chamber to a pressure of about 45 cm Hg. Following this, air is admitted above the solution surface to restore normal pressure. Then again air is evacuated, now until the pressure is about 25 cm Hg. Air bubbles show that air is escaping from the sample. Again normal air pressure is re-established. Finally air is pumped out until a pressure of about 6 cm Hg is attained and this is maintained for about 15 minutes. Following this, air pressure is restored again to normal. By this alternating from a vacuum to normal air

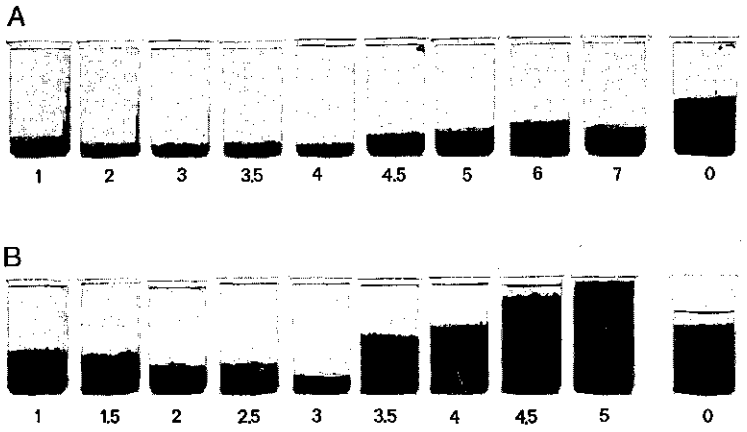

Fig. 31 Effect of various sodium pyrophosphate concentrations upon the soil. A. under vacuum after freezing; B. without vacuum and freezing

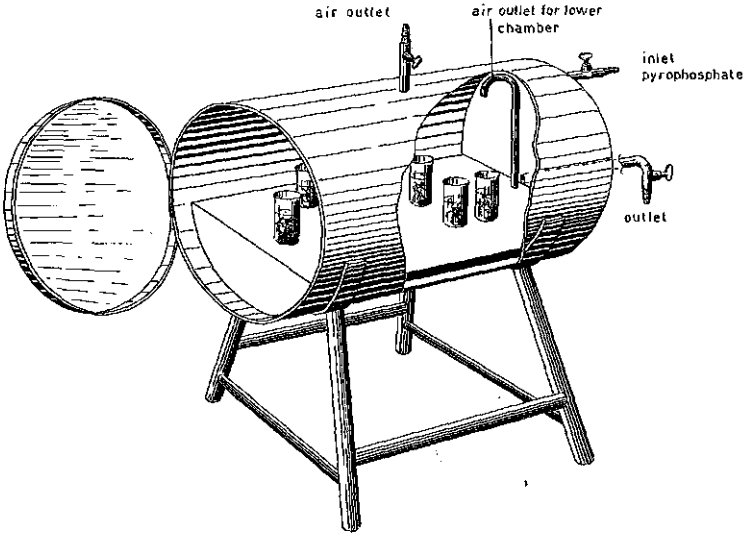

Fig. 32 Drum for treatment of samples with sodium pyrophosphate under vacuum

pressure the sodium pyrophosphate solution penetrates deeply in the soil sample and causes a nearly complete disintegration of the soil. This means that the roots are practically completely separated from soil particles although they are still mixed with these. The sample is next transferred to the top compartment of a plastic gutter, with a total length of about $^1/_2$ metre, divided into 3 compartments, separated by removable partitions (Fig. 33). The roots are washed down towards the final compartment by a stream of water to separate them from the soil particles. During passage they are picked up with tweezers by a supervisor and transferred to a gauze. Roots that pass the gutter unseen are caught in a gauze filter at the end of the gutter. These roots are mostly mixed with plant debris. By dipping the gauze in about 3 cm of water usually the roots and debris can easily be separated. The washing operation takes 5-15 minutes. One technician can supervise at least 8 gutters simultaneously. Treatment with sodium pyrophosphate and washing of about 25 samples takes about 60 minutes. It is calculated that one person can process between 100 and 150 samples per day.

If soil particles remain sticking to the roots after processing, the roots can be put in small bottles of water that are placed one by one on a vibrating apparatus for up to 3 minutes. This almost completely separates roots from

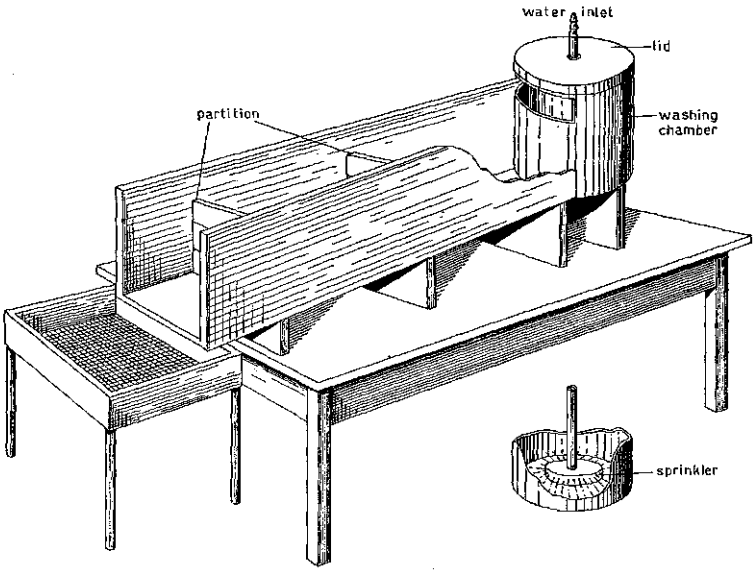

Fig. 33 Gutter for separation of roots and soil particles

sand particles.
The major part of the roots is recovered practically undamaged (Mesker and Schuurman, in press).

3.4. Results

The auger method supplies only data on fragments of the root system and gives therefore little idea of the entire root system as a whole. Nor is it possible to study the branching because many lateral roots are wholly or partly cut off by the auger. On the other hand, samples can be taken for weight determination without damage to the experimental field. Moreover, periodic auger samplings can indicate the development of the root system at particular sites. Due to the variation in root weight in the various soil layers an accurate idea of the roots weights can only be obtained if a large number of samples is taken from which the average values per layer can be calculated. The statistical reliability of these figures can then be determined. An example is given in table 1.
In our studies the minimum number of borings per plot of mixed cultures as grassland was set at 25; at least 24 borings are taken of such monocultures as arable crops. Where such crops are cultivated in rows, e.g.

Table 1. Reliability of root weights from auger samples of a pasture

Depth (cm)	*Weight of 25 root samples*	*Weight of root per sample*	ó	m	m%
0-5	3673	147	65	13	8.8
5-10	1112	44	14	3	6.8
10-15	917	37	14	3	8.1
15-20	483	19	9	2	10.5
20-30	544	22	9	2	9.1
30-40	327	13	7	1	7.7
40-50	201	8	5	1	12.5
50-60	158	6	4	1	16.6
60-70	66	3	2	0.4	13.3
70-80	73	3	3	0.6	20.0
80-90	3	sp	0.4	0.09	
Total	*7557*	*302*			

cereals, 12 borings are taken in the rows and 12 between. For crops not sown in rows other sites may be selected. The holes are drilled over the field in a selected pattern. Weedy patches are avoided. Since in most crops the weight of roots in the topsoil is 70% to 90% of the total, an accurate estimation of the weights of the surface roots is more important than of the subsoil roots. For this purpose, it is possible to adopt a system of taking a smaller number of complete borings with an additional number of samples from the topsoil. Although accuracy in the root-deficient subsoil is reduced as a result, there is a slightly greater accuracy in the root-rich topsoil. Since each boring is divided in layers of 10 cm thick or less, some 250 samples are obtained from 25 complete borings per plot at a drilling depth of 1 metre. These samples have to be processed in the laboratory. This indicates that this method is very laborious, and this is its great drawback. Given the diameter of the auger the root production per hectare can be calculated from the root weights per boring. This value is a parameter of the supply of organic matter to the soil.

3.5. Field assessment of root density in cores

Preparation of auger samples for weighing is a time-consuming operation. For this reason a quicker method has been developed in which an assess-

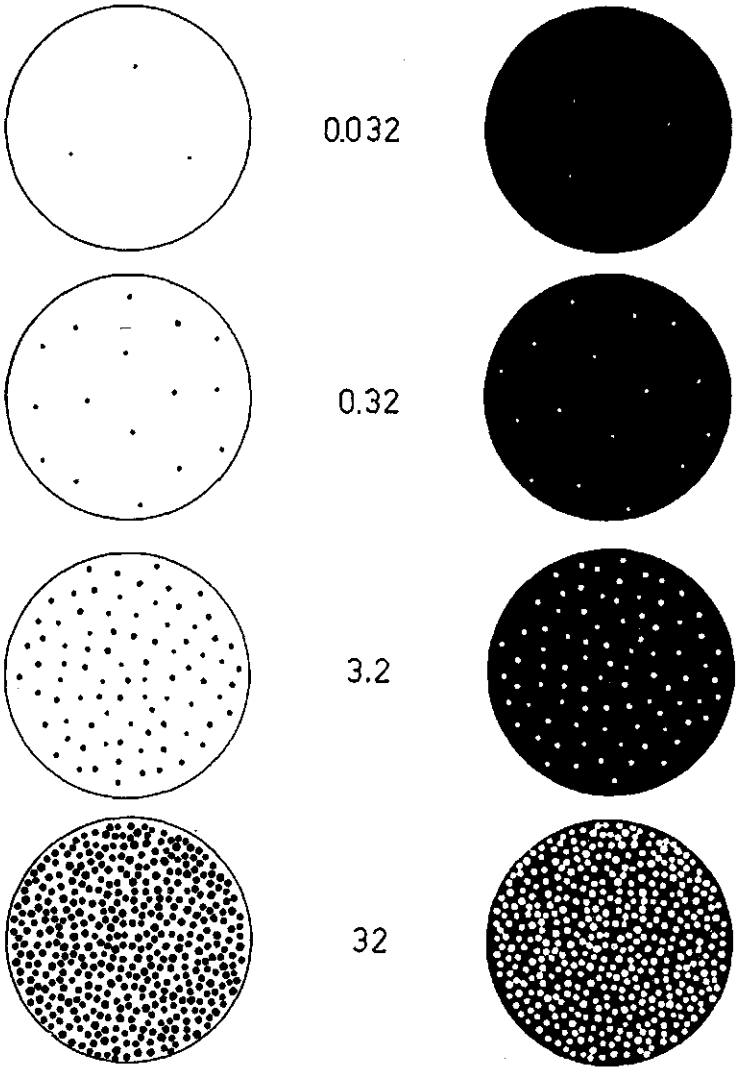

Fig. 34 Some standard figures for use in field assessment of root densities

ment is made in the field of the amount of roots in soil samples. Although slightly less accurate, this method is adequate for field work and requires little or no laboratory work. This method has been fully described in an earlier publication (Schuurman and Knot, 1957). Here we will only give a brief description of its principle. Hitherto the method has been elaborated only for crops like grasses and cereals whose roots have no secondary thickening. It is based on a comparison of root quantities with standard data.

The samples for the estimate are taken with the auger. After the sample has been pushed out of the auger it is broken horizontally in the middle. The

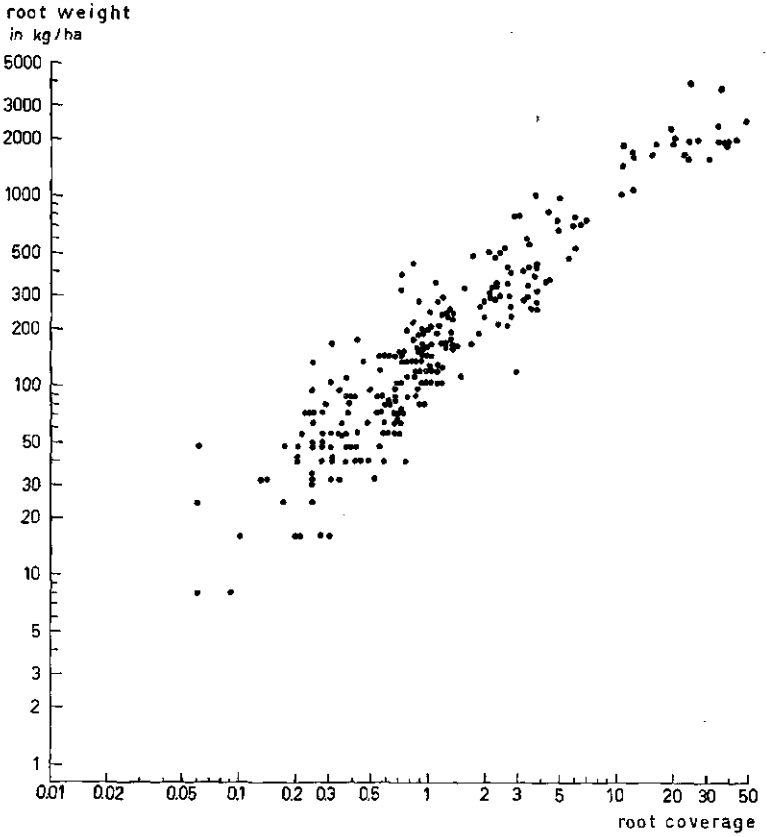

Fig. 35 Correlation between estimated values and ovendry weights of roots

roots growing across this plane of fracture do not usually break in this plane but near to it. They can then be seen and they can be compared with specially designed standard figures. These consist of a series of circles of the same diameter as the auger with an increasing number of light dots on a dark background (Fig. 34). Each circle has a known dotted area. The total coverage of the roots is calculated by adding together assessments for both planes of fracture. The accuracy can be enhanced by breaking the core at several points and assessment in all planes. This is especially important for samples in which there is a sharp decrease of the amount of roots in a downward direction. It is then possible to calculate the mean coverage of these planes of fracture. In order to eliminate the 'dissimilar height' factor of samples, the coverage figure is multiplied by the height of the samples expressed in centimetres. It has been found that root coverage is correlated with root weight of the same sample (Fig. 35) (Schuurman and Knot, 1957).

3.6. Special applications and difficulties

Samples taken with the auger in wet soils may not come up when the auger is lifted out. This can be overcome by providing a light auger with a leather or rubber piston above the plunger (Fig. 36). Since the piston fits tightly it has to be gradually raised during drilling. When the auger has reached the required depth the piston is raised a bit further to reduce the air pressure above the sample in the auger; as a result the sample remains in the auger while this is lifted out.

Good results have also been obtained with an auger round which is fitted another drilling tube about 2 mm wider than the usual one (Fig. 37). When the auger is lifted air can pass down the outside of the inner tube, so that no vacuum is formed below the sample.

When samples are taken in very soft soil the borehole does not remain entirely open. A similar difficulty occurs when many borings have to be made close together and the remaining soil has to be supported. In such cases use is made of thin-walled iron pipes with a detachable top-piece secured to the pipe by means of a bayonet closure. The pipe and the top piece are over 1 metre long and have a diameter enabling the drilling tube to fit in it with some play. The auger is placed in the pipe so that the handle is inserted in the corresponding cavities of the re-inforced upper edge of the detachable top-piece (Fig. 38). The saw-teeth of the auger then project some 2 mm below the bottom of the pipe. The auger and pipe are both pressed into the soil to the first mark on the pipe which is made 9.8 cm from the bottom. The auger is then withdrawn, the pipe remains in position and descends 10 cm more with each subsequent boring until the

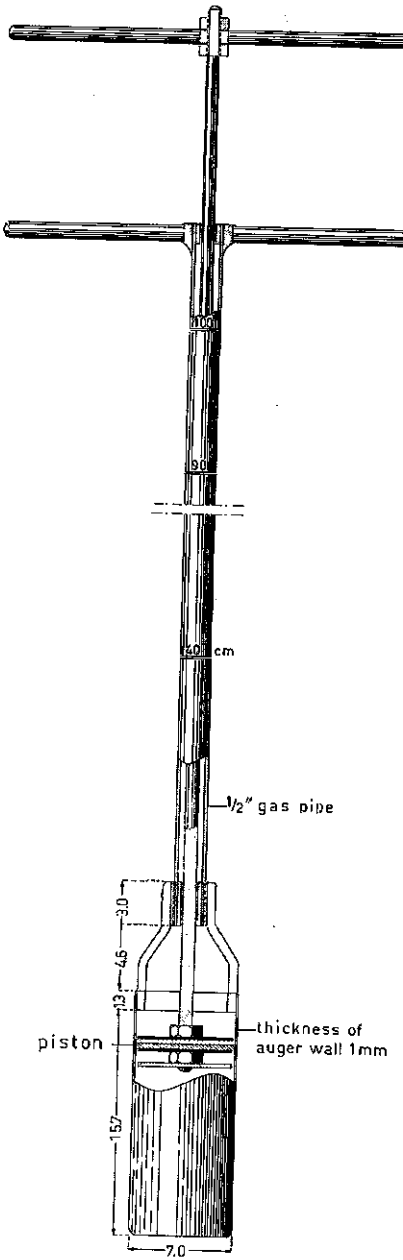

Fig. 36 Auger provided with a rubber piston

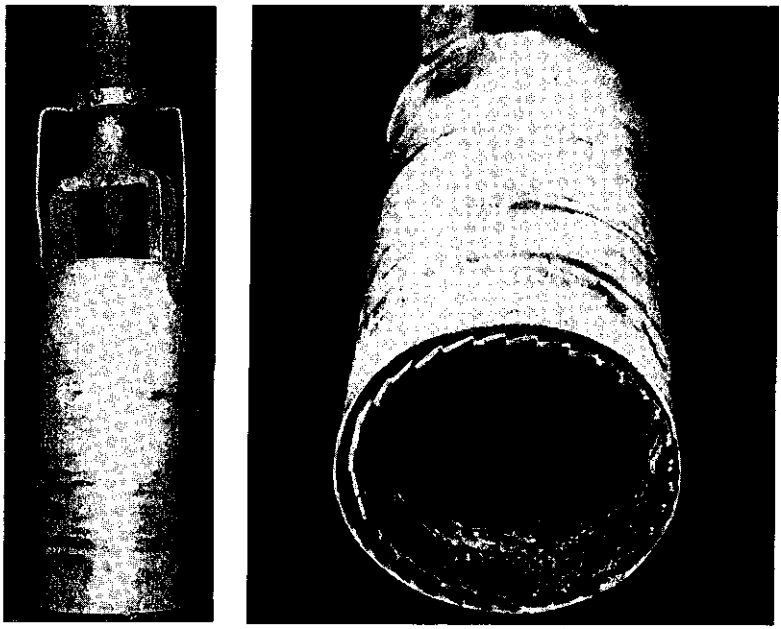

Fig. 37 Auger with extra tube

full depth has been reached. The top-piece is then taken off and used for the next boring. The pipe itself remains in the borehole either until all borings have been made, or indefinitely if more borings are to be taken periodically from a profile of a cylinder experiment. Moreover, the pipes seal off surrounding soil, thus preventing changes in aeration and moisture content in this soil (Fig. 39). This method can also be employed in humid soils.

Difficulties may also be encountered in the sampling of peat soils. In the first place the peat may be too loose or so stratified that the auger does not penetrate. Nor is it possible without preparatory measures to wash out all the peat from a sample without roots being lost. However, there are indications that a pre-treatment with a 5% hydrogen peroxide solution or a solution of sodium pyrophosphate has a good effect. Peat is also difficult to wash off from pinboard samples, although the drawbacks in this case are less considerable as an idea can be formed of the structure of the root system from a pinboard which is still partly covered with peat. Moreover, with careful handling the peat can be removed with tweezers.

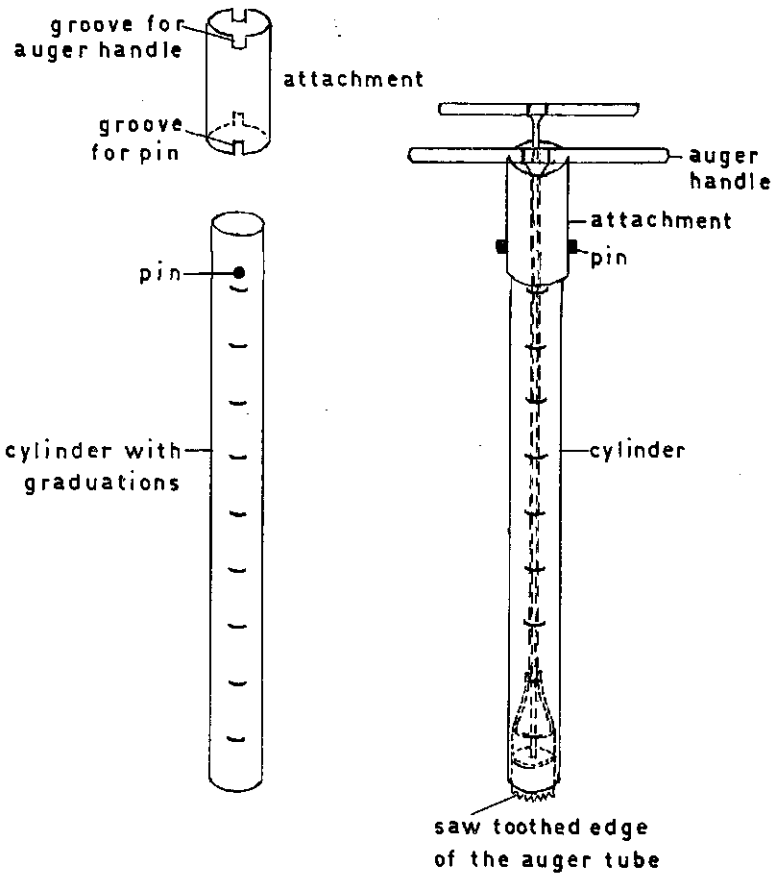

Fig. 38 Auger with accessory cylinder

When taking auger samples a further difficulty may arise which is equally applicable to the pinboard method. Auger and pinboard methods are impracticable in very stony soils. In these cases the method developed by Weaver (1926) in which the root development is studied in a profile wall is used.

Summarizing it may be stated that both the pinboard and auger method have their own particular advantages and disadvantages and that the data not supplied by one method can be supplemented by the other. Consequently it is advisable to employ both methods at the same time.

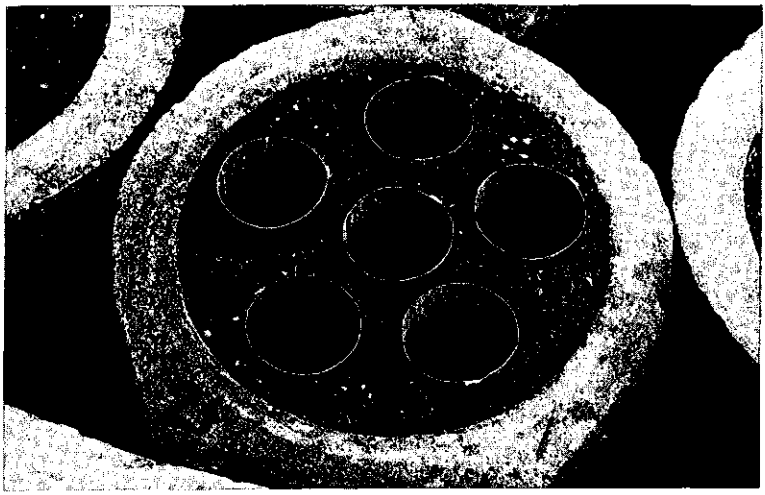

Fig. 39 Cylinders in the soil

4. Excavations

Goedewaagen already used rectangular iron frames to determine root masses in the topsoil as a parameter of supply of organic matter to the soil. These frames had a length of 24 cm, a width of 20 and a height of 30 cm or 30 x 27 x 40 cm. The bottom edge was sharpened. He pushed or hammered the frames vertically into the soil, excavated them and washed them on a screen with a spray nozzle. After drying he weighed the roots.
Recently Dilz used similar iron frames that are pushed into a profile wall. Samples are taken of the topsoil with a height of 25 to 30 cm and horizontal dimensions of 20 x 10 cm. The sample is first saturated with water and then weighed. By sprinkling the sample with a standard apparatus during a fixed time part of the soil is washed away. The remaining soil is again conditioned to the same moisture content and then weighed. This weight is seen as a parameter of the soil-binding capacity of root systems. This is particularly important for crops in relation with the sensitivity of the soil to erosion. Since washing of clay soils is difficult these samples are frozen on arrival in the laboratory and then after thawing treated like sandy samples.

5. Profile walls

5.1. Mapping, followed by counting

Oskamp and Batjer (1932) developed this method for trees. Afterwards we slightly modified and supplemented it. In this method either tangential or radial trenches are dug at a certain distance from a tree and the tips of the cut roots are mapped in one of the walls of the trench. This is done by covering the face with a system of squares of, say, 20 x 20 cm (Fig. 40). Nails are therefore driven into the profile wall at 20 cm intervals round the sampling area. Pieces of string weighted at one end are suspended from the upper horizontal row of nails, so that they hang vertically along the wall. Other pieces of string weighted at both ends are suspended over the corresponding nails of the two vertical rows thus forming horizontal lines. The wall is then carefully scraped with a long needle (sack needle with a handle) to reveal the tips of the cut roots. The point of this needle should not be sharp, to avoid cutting the roots. These are mapped, different categories being distinguished according to thickness. A different symbol is used for each category (Fig. 41). The profile may be sketched in on the map at the same time. It may be asked how the trench should be located with respect

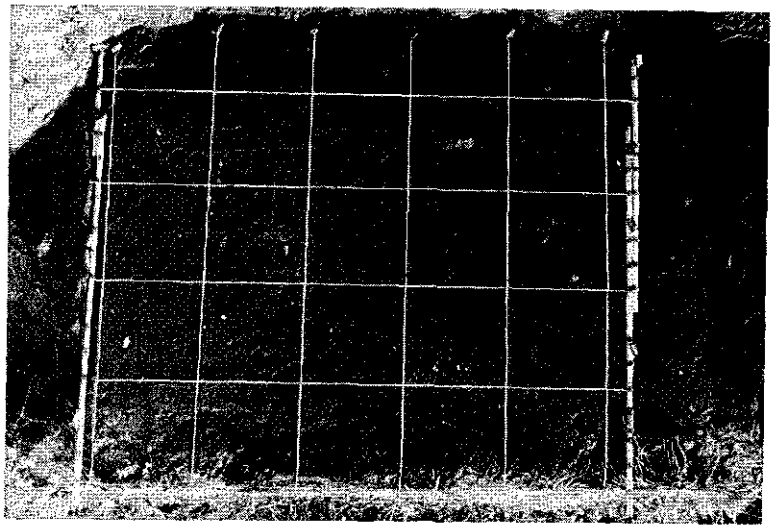

Fig. 40 Squares on a profile wall

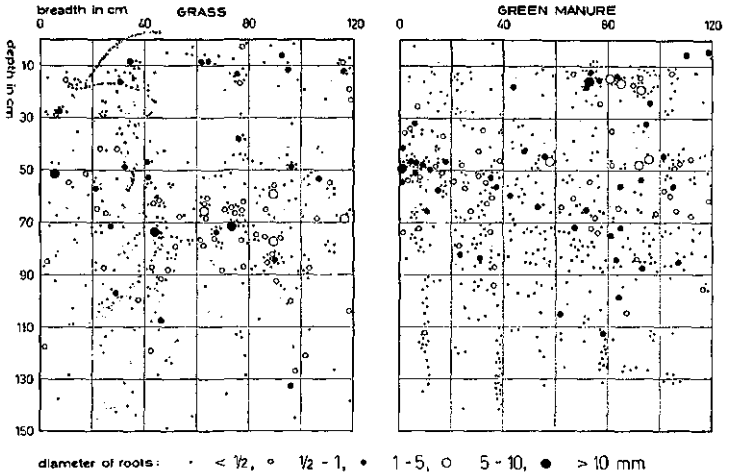

Fig. 41 Examples of root maps of a profile wall

Table 2. Comparison of the numbers of roots of apple trees on a grass plot in tangential trenches at equal distances from the tree

	Distance from the tree 200 cm Width of the profile 120 cm (= 1/12 circumference)										Distance from the tree 150 cm Width of the profile 80 cm (= 1/12 circumference)					
	Numbers of roots per trench					Percentages per trench					Numbers of roots per trench			Percentages per trench		
Layer	1	2	3	4	Av.	1	2	3	4	Av.	1	2	Av.	1	2	Av.
cm	*Diameter of the roots* $<½$ mm															
0- 10	0	13	4	0	4	0	10	2	0	3	8	14	11	6	6	6
10- 40	22	29	27	42	30	17	22	15	34	22	33	75	54	25	33	29
40- 70	52	27	75	47	50	39	20	40	38	35	34	82	58	25	36	32
70-100	43	39	52	16	38	32	30	28	13	26	37	51	44	28	23	24
100-150	16	24	25	19	21	10	18	13	15	14	22	5	14	16	2	9
Total	*133*	*132*	*183*	*124*	*143*						*134*	*227*	*181*			
	Diameter of the roots ½-1 mm															
0- 10	1	3	3	0	2	4	13	10	0	7	4	2	3	12	4	8
10- 40	9	12	3	13	9	37	54	10	30	30	8	10	9	24	21	22
40- 70	8	4	15	18	11	33	18	48	42	37	10	15	12	29	31	30
70-100	3	2	3	7	4	12	9	10	16	13	11	18	14	31	37	35
100-150	3	1	7	5	4	12	5	22	12	13	1	3	2	3	6	5
Total	*24*	*22*	*31*	*43*	*30*						*34*	*48*	*40*			

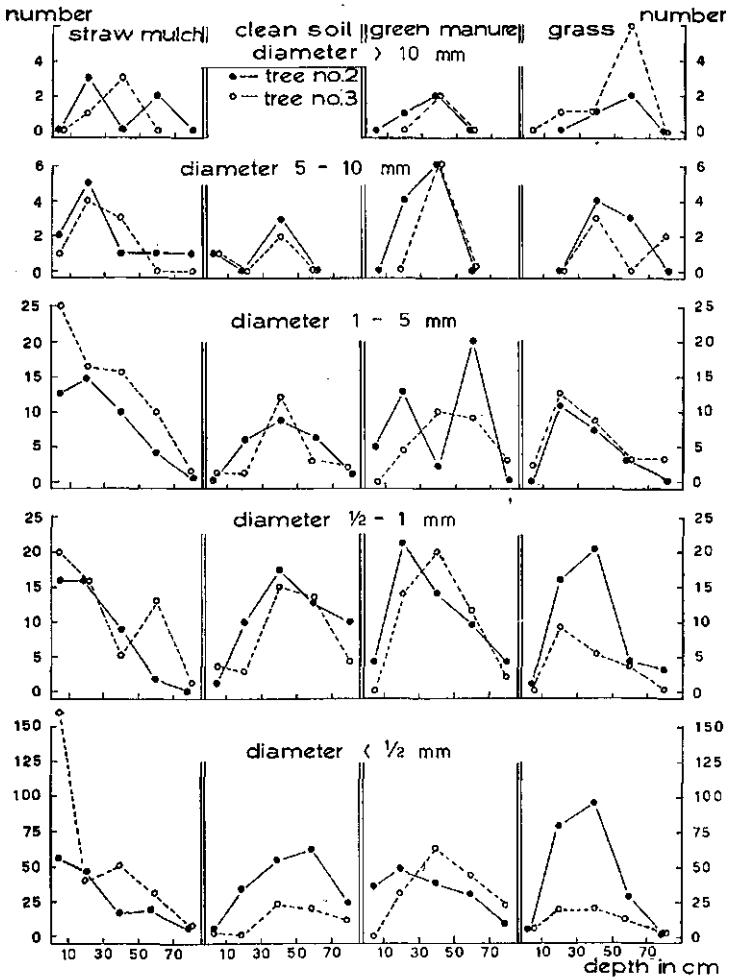

Fig. 42 Comparison of the root numbers of two trees subjected to the same treatment

to the tree. Some variations has been found in tangential trenches, but it is more or less immaterial on which side of the tree the trench is dug, provided conditions round the tree e.g. the structure of the profile and the hydrological regime are uniform (tables 2 and 3). A tangential wall can be divided into a number of parts of equal size which may be taken as replicates. Actually this division has already been made by the system of squares

during the sampling process. This is important as it means that the root zones can be compared at different times by moving to other places on the same circle in successive samplings. The radial trench indicates root development at different distances from a tree. Since in such a map each root has its own distance from the tree there can be no question of replicates and this means that the data provided by a radial trench are less reliable. The reliability can, however, be enhanced by sampling a greater number of radial walls per tree, or both walls of a radial trench.

Tangential trenches also afford an impression of the root development at different distances from a tree when they are dug at varying distances from the trunk (table 4).

The comparability of trees treated the same way has been shown by a trial with four groups of two apple trees in tangential trenches. Data on root density in various soil layers, given in figure 42 show a reasonably good agreement.

Table 3. Comparison of the numbers of roots of apple trees on a straw mulch plot in tangential trenches at equal distances from the tree

	Distance from the tree 200 cm Width of the profile 120 cm (= 1/12 circumference)										Distance from the tree 150 cm Width of the profile 80 cm (= 1/12 circumference)					
	Numbers of roots per trench					Percentages per trench					Numbers of roots per trench			Percentaces per trench		
Layer	1	2	3	4	Av.	1	2	3	4	Av.	1	2	Av.	1	2	Av.
cm	*Diameter of the roots* <½ mm															
0- 10	130	161	97	98	122	36	45	30	32	36	104	78	91	36	28	32
10- 40	135	80	118	120	113	37	22	37	39	34	87	98	92	30	35	33
40- 70	21	31	54	40	37	6	9	17	13	11	29	41	35	10	15	12
70-100	27	28	24	25	26	8	8	8	8	8	31	29	30	11	10	11
100-150	49	56	25	23	38	13	16	8	8	11	37	31	34	13	11	12
Total	*362*	*356*	*318*	*306*	*336*						*288*	*277*	*282*			
	Diameter of the roots ½-1 mm															
0- 10	10	29	16	16	18	16	32	26	20	24	17	8	12	22	16	19
10- 40	36	28	32	35	33	58	31	52	43	44	36	24	30	46	47	47
40- 70	7	19	10	15	13	11	21	16	19	17	13	6	10	17	12	15
70-100	8	10	4	9	8	13	11	6	11	11	10	7	8	13	14	13
100-150	1	4	0	6	3	2	4	0	7	4	2	6	4	2	12	6
Total	*62*	*90*	*62*	*81*	*75*						*78*	*51*	*64*			

Table 4. Comparison of the numbers of roots at three different distances from the tree

Diameter of the roots in mm	Width of the trench 1/12 circumference Distance from the tree		
	2 metres	1.50 metres	1.00 metre
	Straw mulch plot		
<½	336	282	272
½-1	75	64	54
1-5	32	36	28
5-10	7	6	6
>10	2	5	4
	Grass covered plot		
<½	143	181	150
½-1	30	40	36
1-5	14	22	16
5-10	4	2	3
>10	1	1	0

From the root map the number of root of each size category is counted per square. From these data the total number of roots in each layer can be calculated. The average number of roots per unit of length of a layer and the statistical reliability may be calculated.

The mapping method can provide important information on root development of woody plants. It has, however, a number of limitations. In the first place it is very time-consuming so that it is impractical to sample more than one tree in a plot. Secondly, as all work has to be done outdoors, good weather is essential unless a tent and artificial light are used. Finally, it should be pointed out that the sampling depth is governed by the water-table. This method can also be employed for agricultural and horticultural crops. Trenches are then usually dug parallel to a row or across one or more rows.

A variant of this method used in sandy soil, is that the soil is flushed out with water instead of being worked loose with a needle. A knapsack sprayer of vaporizer can be used for this purpose. After a wall is sprayed with the finest atomizer the cut roots show up well and can be mapped (Fig. 43). Only a small amount of water is required. This variant is also used by other scientists.

Fig. 43 **Spraying a profile face**

5.2. Weaver's method

The method as introduced by Weaver (1926), referred to on page 42 is also used for the investigation of profile walls, especially in stony soils where pinboards and augers are impractical.

6. Experiments in containers

In these experiments plants are grown under specific, pre-determined conditions. In some experiments several of the methods mentioned above may be used, but the procedure in many container experiments is often so adapted to root studies that the processing has its own particular character. The following will be discussed in succession: experiments with *cylinders, cases, boxes and pots* filled with soil, and cylinders filled with nutrient solutions. A general and favourable feature of container experiments is that complete root systems are available for investigations.

6.1. Cylinders

6.1.1. Material

Cylinders of different material and dimensions are used. Concrete cylinders were first used by Goedewaagen (Frankena and Goedewaagen, 1942). The original, laborious method has since been improved and simplified and new facilities created. Until recently only large cylinders, made of concrete with a 30 cm internal diameter and a height of 100 or 125 cm, and small cylinders, made of asbestos with a 15 cm internal diameter and a height of 75 cm were used.

The large cylinders are arranged in 2 groups in large, concrete vessels, that are divded in two sections by a concrete wall (Fig. 44). Each section can hold up to nine large cylinders. As concrete cylinders have a weight of about 100 kg each, they are difficult to handle. Therefore recently large asbestos tubes have been introduced with the same external dimensions. Besides being lighter these tubes have the advantage of a larger internal diameter of 36 cm instead of 30 cm and they can easily be cut longitudinally thus facilitating washing.

For both kinds of tubes care is taken that the diameter is correct and the tube is circular, since this is essential for uniform filling with soil. The upper edge of the cylinder is about level with the surrounding surface. A given soil watertable can be maintained in the concrete containers. The watertable required does not necessarily have to be constant - a varying

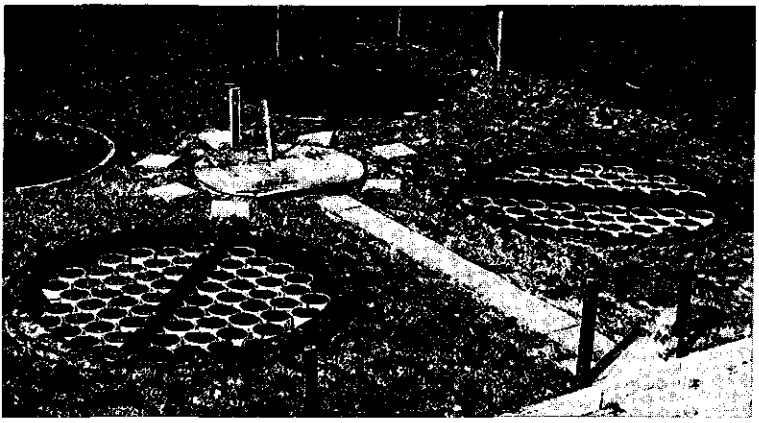

Fig. 44 Lay-out of an experiment with tubes in a concrete container

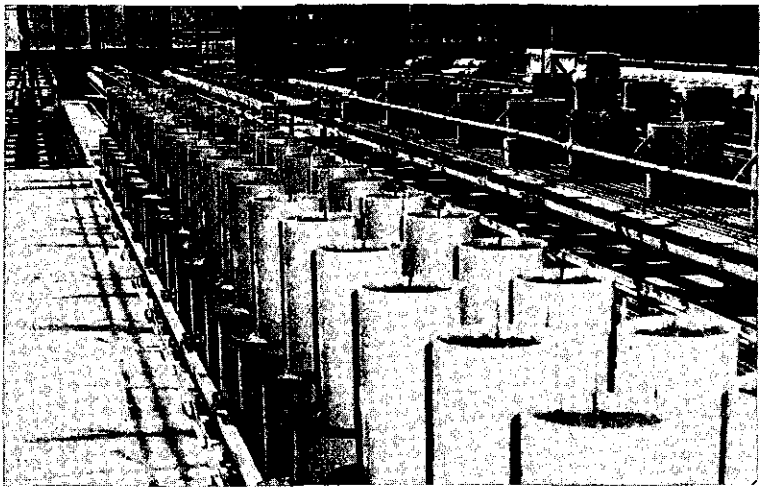

Fig. 45 View of an experiment with PVC cylinders

level may also be used.
The asbestos cylinders 15 cm in diameter, have recently been replaced by PVC cylinders with a 20 cm external diameter and a height of 90 cm. This material has the advantage of being chemically inert. The cylinders are usually placed on trolleys so that the experiment can be either out of doors or under glass, depending on the weather and the purpose of the experiment. Recently they are placed in a movable greenhouse (Fig. 45).

6.2. Large cylinders

6.2.1. Filling

The soil with which the cylinders are to be filled is first screened. The screened soil is mixed with fertilizers as uniformly as possible and stored in plastic bags to prevent loss of moisture. The moisture content is determined so as to enable a calculation to be made of the amount of soil to be introduced into the cylinder in order to obtain a predetermined bulk density. This amount of soil is weighed out and divided into portions which are again stored in plastic bags. Each portion is exactly enough to fill the cylinder with a 5 cm layer. Before filling the tube is closed at the bottom with porous nylon cloth (Fig. 46) and secured upon a concrete base of equal diameter provided with two wire loops by means of a bar and screw (see

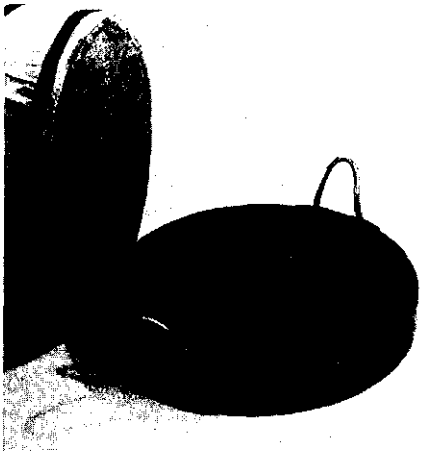

Fig. 46 Part of cylinder closed with nylon cloth, and base with loops

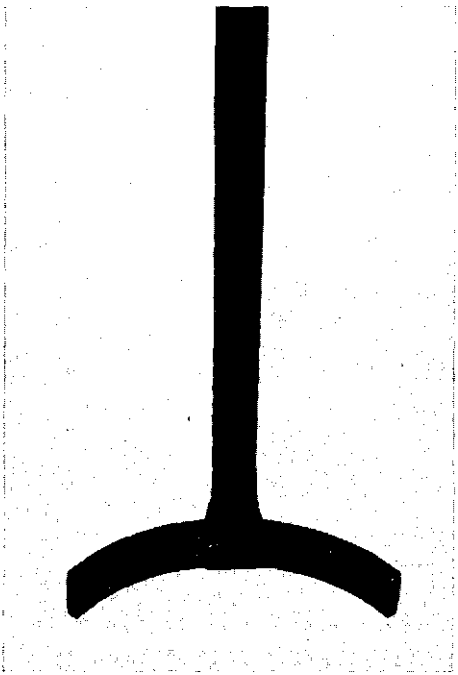

Fig. 47 Apparatus for compressing the soil along the wall of a cylinder

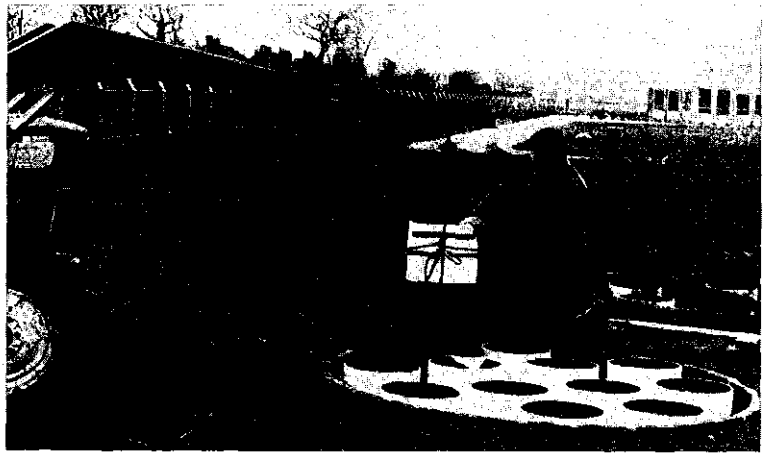

Fig. 48 Lowering filled cylinders into cement container

also fig. 56). During each filling operation half a bag is emptied into the cylinder and then tamped down. A regular check is made to ensure that the proper level is reached after tamping. If this is the case the soil along the edge of the tube is pressed down with a narrow curved iron bar (Fig. 47), so as to prevent growth conditions for the roots being more favourable in this part than in the rest of the profile. The topmost layer of from 2 to 5 mm is then carefully scraped loose over the entire surface to prevent the formation of layers (Goedewaagen, 1932 p. 182). The second half of the soil is then poured into the cylinder and treated in the same way. The whole procedure is repeated with each portion of soil until the cylinder is full. In this manner it is possible to build up a great diversity of profiles with variations in soil type and density. The filled tubes, placed upon the concrete bottom are transported to and lowered into the container by means of hooks and rope, attached to the wire loops (Fig. 48). After placing, the hooks are freed from the loops. Since the soil is capable of absorbing a great deal of moisture in the initial stages, the containers are regularly replenished with water until an equilibrium has been reached. The crop can then be sown.

Recently promising results have been obtained in an experimental tamping with a slatted iron ring with a handle. Tamping is done by one operator while the tube turns round and the soil is tipped in regularly by a second person.

Fig. 49 Regulation of soil watertable with syphons

6.2.2. Soil watertable

The watertable in each section of the containers used in the large cylinder experiments can be set to various heights with siphons (Fig. 49) in a central vessel that are connected with these sections.

The water level in the containers is checked several times a week and hence the corresponding soil watertable in the profiles also. During and after rainfall excess water is removed by the siphon offering the possibility of measuring these amounts. If the soil watertable has dropped it is made up. The amounts added and of precipitation are a measure of the absorption of water by the plants. It is advisable to cover the top of the profile with a layer of fine gravel of about 2 cm thick to prevent evaporation.

6.2.3. Sampling

There are various ways of studying the root development of a crop. It has already been shown in Figure 39 how a profile in a large cylinder can be drilled. Experience has shown that at least 6 borings can be obtained from cylinders of 30 cm diameter.

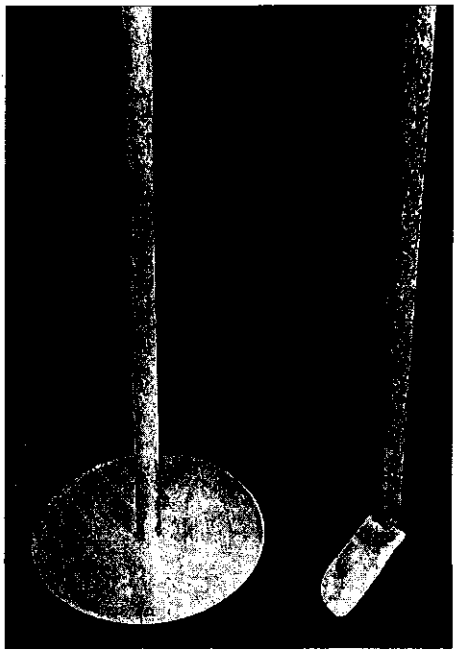

Fig. 50 Blades for cutting away soil horizontally in cylinder experiments

A second method is to remove all soil from the cylinder layer by layer. This is done by first removing some samples with an auger up to the required depth and then cutting loose the rest of the soil to the same depth by means of specially designed blades (Fig. 50) and taking it out of the tube. The drilling cores may be kept separate, e.g. for moisture determination, and added to the remainder afterwards. The resultant samples may be washed as discussed in Section 3.3.

A third method is to lift the tube out of the container together with the profile with a rope and hooks attached to the wire loops.

To prevent the soil from falling out of the tube this is lifted out whilst it stands on the bottom. It is transferred to the laboratory, where it can be laid on top of a steel basin (Fig. 51) or on a steel stand. On this stand the profile can be turned round horizontally and set at various slopes and thus be adjusted to the available light and most convenient height (Fig. 52). The crop may or may not be cut before transport. On the basin or stand the top of the profile should preferably be lower than the bottom, so that some water always remains in the tube during washing. This prevents the soil and

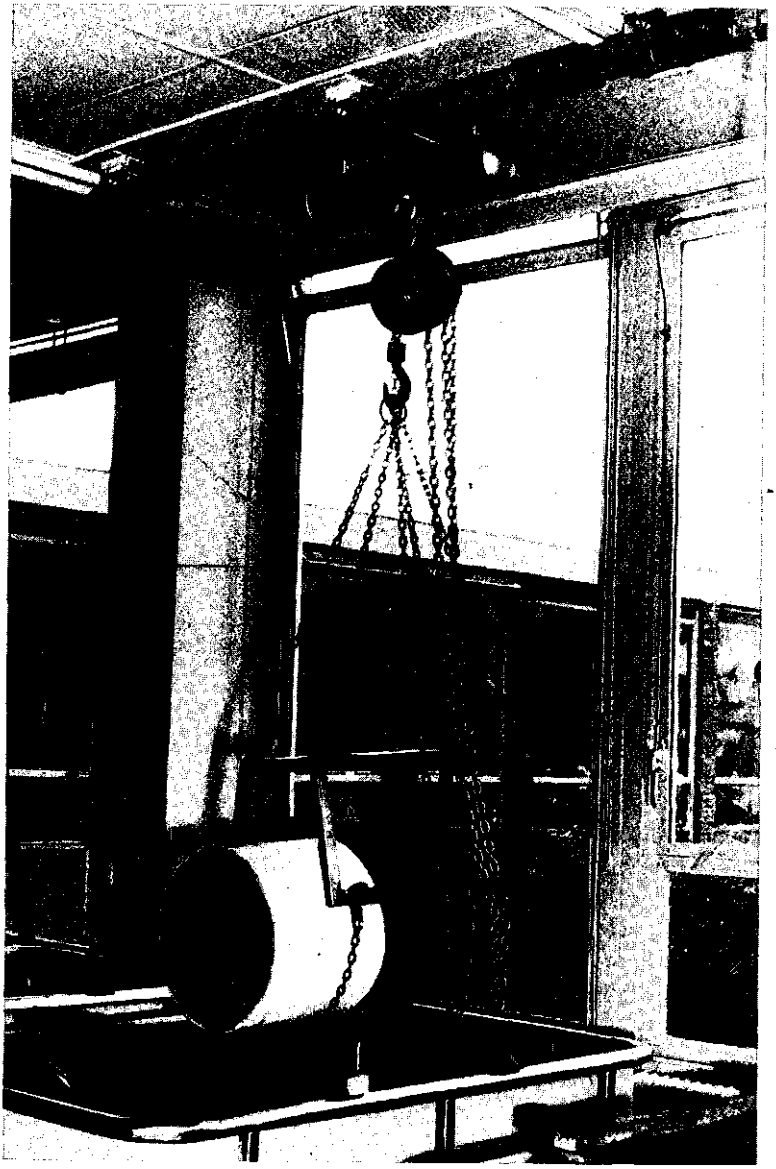

Fig. 51 Cylinder above a washing bath

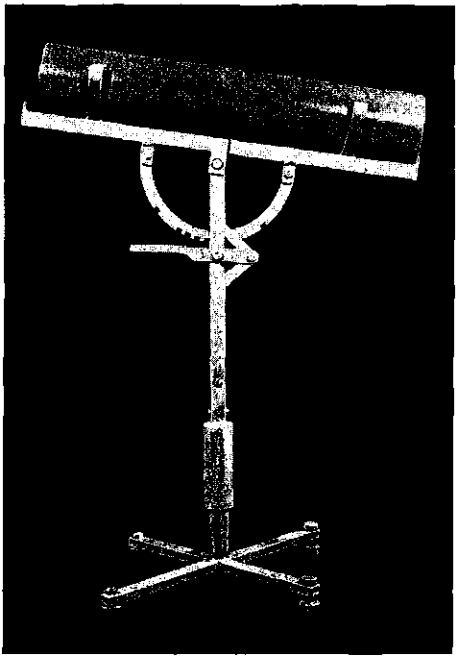

Fig. 52 Adjustable stand to hold PVC tube during washing

Fig. 53 Washing apparatus

the roots from breaking. However, the profile may slide out of the tube if the tube has a slope of more than 30°. The nylon cloth is removed and the soil is washed out of the tube. This can be done with a sprinkler about 80 cm long that allows either sprinkling frontally or sideways, regulated by a two-way switch (Fig. 53). Usually it is best to start washing from the bottom of the tube. The presence of many roots in the top layer makes it difficult to wash the soil from the top except at the last stage, after the top layer has been pierced. Moreover when the profile is being washed from the

Fig. 54 Removing debris from a root system after washing

bottom one can immediately note the depth to which the roots have penetrated the soil. As washing proceeds the slope can be reduced. In the last stage of washing the tube is so placed that the top of the profile is highest. Turning the tube during washing must be avoided, since this may damage the roots and twist them. When the entire root system has been washed free of soil it is carafully slid out of the tube into a shallow bath with the use of plenty of water. The size of the bath must be adjusted to that of the root system. Any extraneous matter is removed in this dish (Fig. 54). The root system can then be photographed. Finally the root system and the individual roots can be described in the manner indicated in Section 2.7 and 2.8.
One disadvantage of the third method is that no data is obtained on the moisture content of the soil. This may be overcome by drilling samples from the profile with a narrow drill with a diameter of 14 mm before the soil is washed away (Fig. 55). Owing to the fineness of the drill it is not possible to make a hole deeper than about 60 cm. This drilling also results in slight damage to the root system.
A fourth method of studying root development is employed by cutting the tubes longitudinally in two slightly unequal parts. The smallest part is removed, the larger piece of tube is laid with the profile in a bath. The soil can then be washed with an oscillating sprinkler (Fig. 9). This method saves much work and provides the least damaged root systems. Water content of the soil should be determined as described in the third method before the tube is cut.

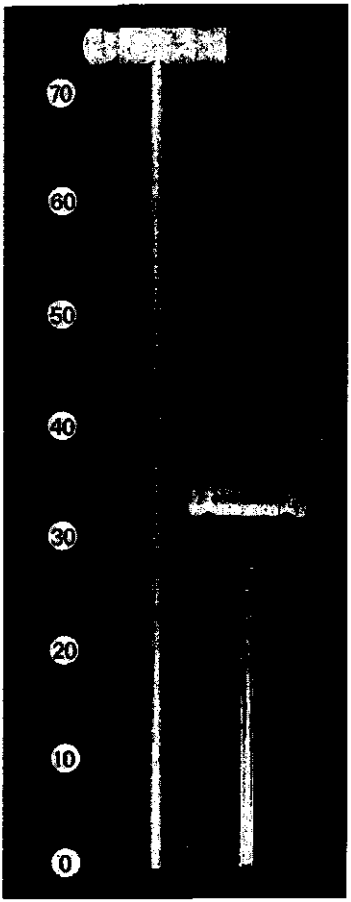

Fig. 55 Drills used to obtain small soil samples for moisture determination

6.3. Small cylinders

6.3.1. Filling

The small cylinders are usually above ground. They are filled with soil in exactly the same manner as the larger cylinders. (Fig. 56). It is also advisable to give the bottom of these profiles a permanent support by securing moisture-permeable nylon cloth to the bottom of the cylinders. In general the same kind of experiments can be carried out with these cylinders as with the large ones. Since they are lighter they are easier to handle, but the cylinders with a diameter of 15 or 20 cm do not take as many plants.

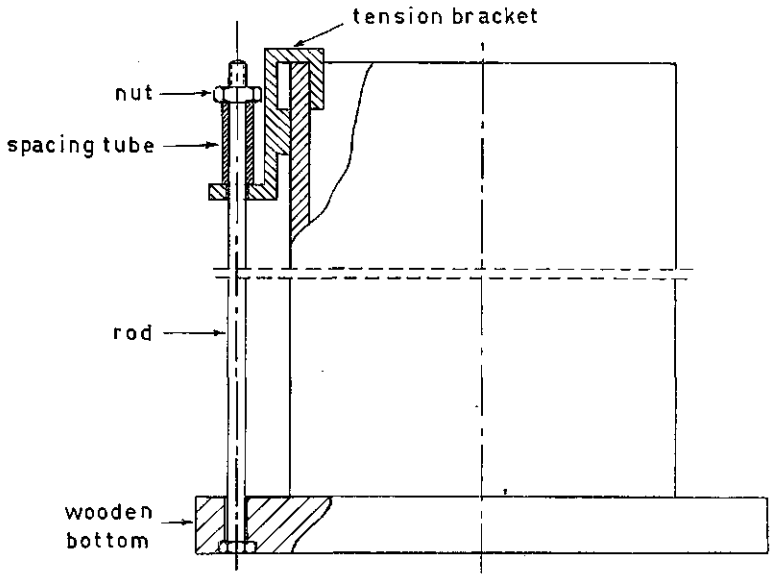

Fig. 56 Wooden board with tension bracket

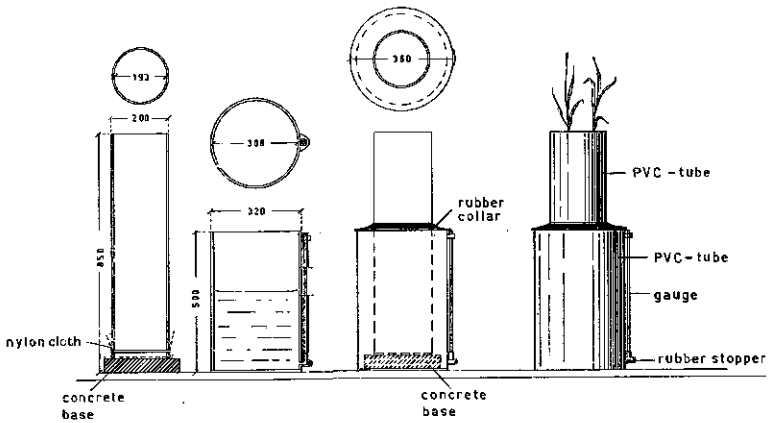

Fig. 57 PVC-tube with accessories

6.3.2. Soil watertable

The cylinders are placed in larger ones in which the watertable can be maintained at various levels (Fig. 57). In experiments where only deep soil

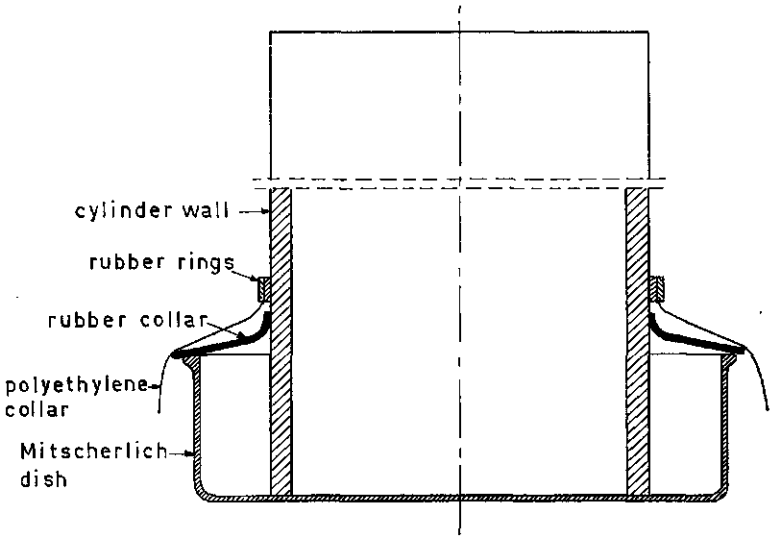

Fig. 58 Tube in a dish closed by rubber and polyethylene collars to prevent evaporation

watertables are wanted shallow dishes may be used. To prevent evaporation from these vessels they are covered with a rubber collar. This collar can also prevent rain from seeping in the vessel when the experiment is in the open, but as an extra precaution a second collar of flexible polyethylene material is then placed above the rubber one. This polyethylene collar is fastened between two strong rubber rings (Fig. 58). Evaporation from the profile is prevented by covering it with a layer of fine gravel or plastic granules as is done for large cylinders. The water used for replenishing the vessels is measured, so that the moisture uptake of the plant in each cylinder can be accurately determined.

6.3.3. Sampling

The roots are examined by washing away the soil, as discussed in connection with the large cylinders. Moisture samples are taken with the small drill (Fig. 55).

6.4. Special applications

Hitherto we have only discussed cylindrical tubes, but it is obvious that both the shape and material may be adapted to circumstances.
Cylinder experiments can be carried out with both natural and artificial

profiles. In their studies Frankena and Goedewaagen (1942) forced a steel tube with a cutting edge into the soil. This tube had a diameter of 30 cm equal to the internal diameter of the concrete cylinders. After being excavated the profile was transferred to a concrete cylinder.
Later experiments showed that it was possible, and probably even better, to excavate a soil column with a 30 cm diameter on which the cylinder stands. As the soil is dug more deeply and cut away, the cylinder gradually falls round the soil column by its own weight. A metal collar with a 30 cm diameter and provided with a cutting edge may be used. In this case the cylinder rests on the metal collar which it forces downward. This method can only be used in soils with a firm profile structure, i.e. the soil should neither be too wet nor too dry. Should it be too dry, one can attempt to make things easier by wetting the soil. Till now better results have been obtained with glazed earthenware cylinders than with the fairly rough concrete cylinders.
Undisturbed soil samples have likewise been obtained in cylindrical tins 20 cm high and with a diameter of 25 cm.

6.5. Cases and boxes

Cases of various design are used for root examinations. Wooden cases were first used by Goedewaagen (1932, 1933), following Rotmistroff (1908) and Maschhaupt (1915). The dimensions are 60 x 20 x 100 cm (Fig. 59). One wall measuring 100 x 60 cm is fastened by screws. The cases are filled with soil and then buried with their surfaces level with the surrounding soil. A crop can be grown on the 60 x 20 cm top surface. When the crop is to be exami-

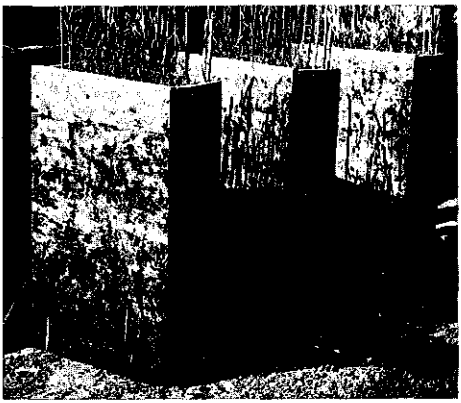

Fig. 59 Wooden cases used for root studies

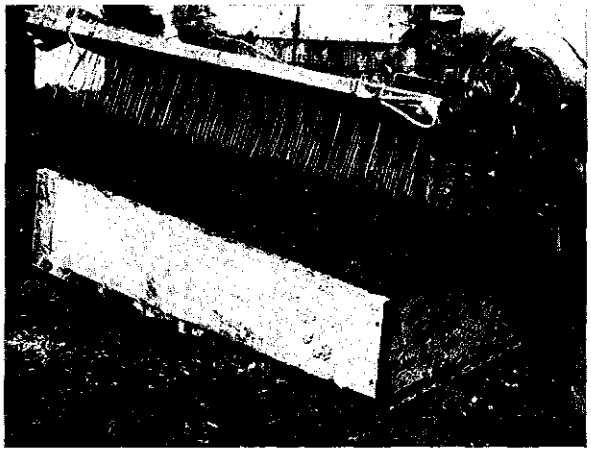

Fig. 60 Replacing one wall of a wooden case by a pinboard

ned, the wall fastened with screws is unscrewed and replaced by a pinboard of the same dimensions. The pins are driven into the profile (Fig. 60). The whole is then turned upside down with the pinboard underneath and the case is removed. The result is a pinboard sample that can be washed and further processed by the method described in Section 2.3.

There are two types of concrete vessel in use. One type is a ready-made vessel with dimensions 50 x 50 x 50 cm, the walls being fastened together. The only way to study root development in such boxes is by borings, as it is hardly possible to obtain pinboard samples.

The second type is made of loose rectangular concrete slabs fitted together to form a bottomless container measuring 1 x 1 x 1 metre. It is advisable to bury the slabs. After that the box can be filled with experimental soil. For sampling a hole can be dug on one side of the box, after which the adjoining side-plate can be removed. After partly slicing off the soil to avoid marginal effects, a pinboard sample can be taken. Eventually a second pinboard sample can be taken or the data can be completed by auger cores.

A particular method of root study is to observe root growth behind a window-pane. Goedewaagen (1955) carried out experiments in small wooden boxes with one vertical wall replaced by a glass panel. The inside area of these boxes is 10 x 10 cm and the height 25 cm. A network of squares was arranged on the glass panel to facilitate observation. The boxes were slightly tilted so that the glass wall leans forward. The glass wall should be

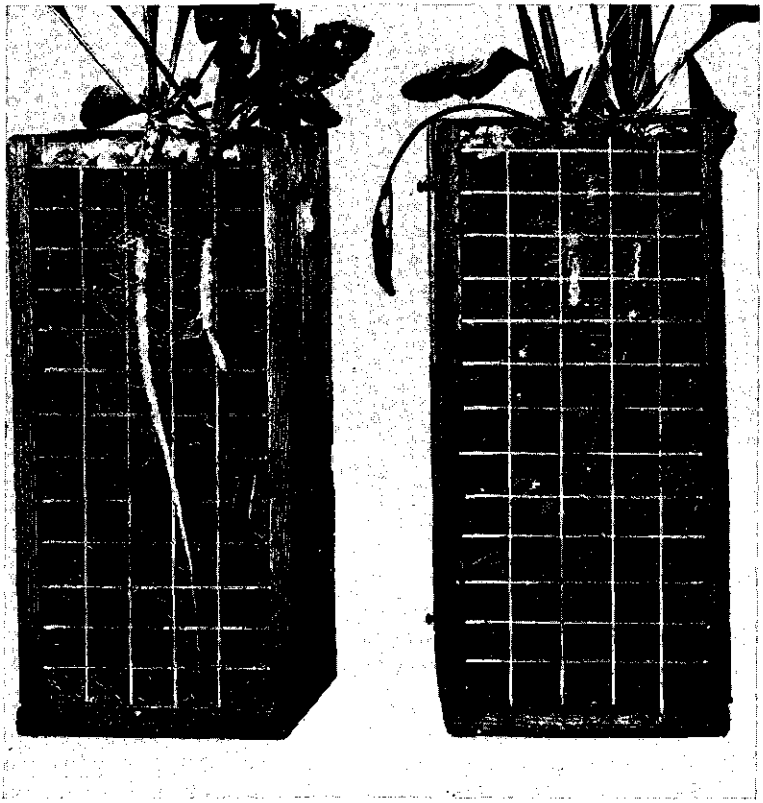

Fig. 61 Small wooden cases with one glass panel, provided with squares

covered during the experiment and the cover only removed when observations are made. These boxes are suitable for short term experiments in which the root growth can be observed through the glass (Fig. 61). At the end of the experiment the roots may be washed free by removing the glass panel and replacing it with a pinboard of suitable size.

6.6. Pot cultures

Different variations are possible. Use is made of ordinary flower pots, Mitscherlich pots, and combinations of pots and water cultures. The glazed earthenware vessels may also be mentioned under this heading as owing to their size and shape they belong here rather than to the boxes. The pots can

be filled in the same way as the cylinders. In the case of flower pots it should be remembered that the diameter is not the same at all points. Pot culture experiments are particularly useful when the plants are to be examined at an early stage of growth and fullygrown root systems are not required.

The disadvantages of pots are usually the shallow depth and slight volume, that limit the root depth. Consequently the root systems of plants grown in pots are often divergent from those grown under natural conditions. This is further exaggerated by the extremely intense root growth along the walls, as is often seen in porous flower pots. Pot culture experiments are therefore of limited use for root studies. This drawback is less applicable to the glazed earthenware vessels which are 25 cm long, 25 cm wide and 50 cm deep.

Roots of plants grown in pots can only be properly examined if the entire root system is washed free. The soil should be washed away in a downward direction. Sometimes it is also possible to remove the entire contents from the pot and then wash them.

Maschhaupt (1911) used a combination of flower pot and glass cylinder, widening as far as possible the opening in the bottom of ordinary flower pots so as to give the roots a better chance of growing out of the pot. This opening was covered with a thin film of cottonwool to prevent soil from falling into the beaker of nutrient solution underneath the pot. In the middle of the pot a tapering wooden stick was placed with a diameter about equal to that of the opening in the bottom. The soil was pressed in round the stick. After the stick had been removed the hole was filled up fairly loosely with earth. A cavity was made in the middle in which a germinated seed was placed. The pots were placed in round holes made in cases with detachable side walls, so that the beakers with nutrient solution were in the dark. The root growth could be observed at regular intervals by removing one of the side walls of the case.

Goedewaagen (1955) used a method which is substantially the same as Maschhaupt's. Asbestos pots were used of which the bottom consisted of wire netting with a 2 mm mesh. This wire netting was covered with a thin layer of glass wool. The pots were filled with soil and placed on glass cylinders filled with water or nutrient solution. In this way the subsoil was imitated. Glass wool prevents soil particles from falling through the gauze but permits free passage of the roots. The soil and water were separated by a thin layer of air.

At the transition from pot to cylinder a metal collar was used to prevent aeration and evaporation of the water in the cylinder, and to create a moist

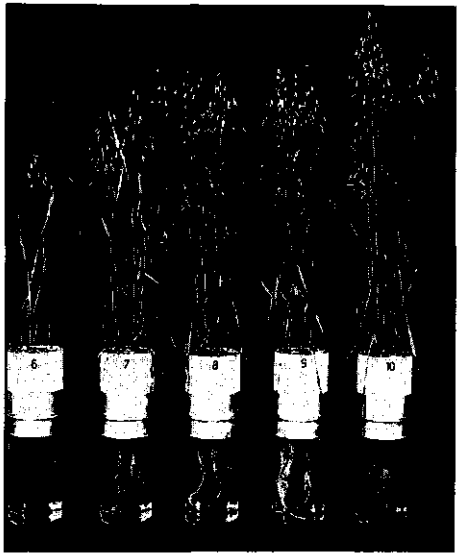

Fig. 62 View of a pot-cylinder experiment

medium in the layer of air between the soil and the water. A double layer of white polyethylene material was wrapped round the asbestos pots to prevent evaporation through the cylinder wall (Fig. 62). An experimental plan of this kind is very suitable for studying the importance of subsoil roots for the plant's supply of moisture. It is also possible to study fertilization problems in the topsoil and subsoil.
The soil in the pots was covered with a layer of gravel. The amount of water consumed by the plants was calculated by weighing the pots and cylinders separately at regular intervals.
The roots in the glass cylinders may be studied without making any further provisions, and it is even possible to measure the longitudinal growth of the roots during the experiment. The topsoil roots should be washed free.

6.7. Nutrient solutions

Zijlstra (1922) germinated seeds on hollow glass rings having a diameter of 13 cm on which was stretched 1 mm gauge gauze. The ring floated in a jar of water so that the gauze just touched the surface and the seeds placed on it came into contact with the water. After germination the roots grew through the meshes of the gauze and were freely suspended in the water. The main root was immediately marked. When it had attained a length of

some cm the cultures were transferred to nutrient solutions in 2½ litre glass cylinders 24 cm high.
Water cultures are comparatively rarely used for root studies as conditions differ so extensively from field conditions that it is difficult to compare the conclusions. In general they may be used for fertilizer and aeration problems. Cylinders or glass vessels of different sizes may be used for the purpose. The growth of algae in the water or nutrient solution may cause undesirable changes. This can be prevented by covering the vessel with black paper, black polyethylene sheeting or corrugated paper. It may be necessary to replenish and aerate the water regularly. The root system can be easily studied by temporarily removing the cover.

7. Tracers

Wiersum has carried out some work with tracers and tracer-techniques. In his publication (1967) he describes tracer techniques. He distinguishes between application of tracers to the soil and to the plant and gives an account of the amount of information obtained in both cases.

Appendices

Appendix 1 Scheme for description of root systems

1. General picture
a. shape (e.g. square, oblong, etc.)
b. maximum depth in cm
c. extent in cm
d. colour
e. number of zones distinguished according to change in the shape of the root system or in the density of the root zone.

2. Description by zones
a. depths in cm
b. extent in cm
c. density of rooting (in terms of sparse to abundant), e.g. $< 2\%$ sparse; 2-20% common; 20-70% many; 70-100% abundant
d. horizontal distribution of roots over the width of the board in the soil (in terms of regular or patchy)
e. colour

Appendix 2 Nomenclature of roots

1. Monocotylodons
1.1 Annuals
1.1.1 From seed (cereals)

Main seminal root	branch roots and root hairs
Secondary seminal root	
Nodal roots	

1.1.2 From bulbs or tubers (tulip, gladiolus, iris)
Adventitious roots
- Branch roots
- Root hairs

1.2 Perennials (pasture grasses)
Adventitious roots
- Branch roots
- Root hairs

(seedling axis, rhizome)

2. Dicotyledons
2.1 From seed (sugar beet, clover, lucerne)
Main root
- Branch roots
- Root hairs
2.2 From tubers (potatoes)
Adventitious roots
- Branch roots
- Root hairs
Adventitious roots at the nodes
- Branch roots
- Root hairs
(Rhizomes, stolons are stems not roots)

Appendix 3 Scheme for description of individual roots

I. Dicotyledons

1. Main root
a. shape (e.g. tap root, filiform, etc.)
b. length in cm
c. thickness in mm (where necessary at different depths or distances from the base)
d. colour (colour may vary according to soil composition)
e. number of zones, according to number of primary branch roots
f. number of primary branch roots in each zone per unit length of the main root.
g. length of the root-hair zone

2. Primary branch roots (per zone)
a. shape (e.g. elongated, twisted, etc.)
b. length in cm (average + extremes)
c. thickness in mm (average + extremes, where necessary at the base and at various distances from the base)
d. colour
e. number of zones, according to number of secondary branch roots
f. number of secondary branch roots, if necessary per zone, in terms of few to abundant per unit length
g. length of the root-hair zone

3. Secondary branch roots
a. estimated length (average + extremes)
b. presence of tertiary branch roots and of higher orders and their length
c. length of the root-hair zone

4. Adventitious roots (other roots)
details as under I.1, I.2, I.3

5. Root nodules
a. habit (single or multiple)
b. number
c. place

II. Monocotyledons (grasses and cereals)

1. Seminal roots
a. shape (e.g. elongated, twisted, etc.)
b. length in cm (average + extremes)
c. thickness in mm (average + extremes)
d. colour
e. number of zones, according to number of primary branch roots
f. number of primary branch roots per unit length in each zone

2. Primary branch roots
a. shape (elongated, twisted etc.)
b. length in cm (average + extremes)
c. thickness in mm (average + extremes)
d. colour
e. number of zones, according to number of secondary branch roots
f. number of secondary branch roots if necessary per zone in terms of few to abundant per unit length

3. Secondary branch roots
a. estimated length of 2nd order branch roots
b. presence of tertiary branch roots and of higher orders and their length
c. length of the root-hair zone

4. Nodal roots
as II.1, II.2 and II.3

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