CHAPTER 6

Auger Sampling, Ingrowth Cores and Pinboard Methods

Maria do Rosário G. Oliveira[1], M. van Noordwijk[2], S.R. Gaze[3], G. Brouwer[4], S. Bona[5], G. Mosca[5], and K. Hairiah[6]

[1] University of Évora, Apartado 94, 7002-554 Évora, Portugal

[2] ICRAF, Jl CIFOR, PO Box 161, Situ Gede, Sindang Barang Bogor 16680, 16001Bogor, Indonesia

[3] University of Reading, PO Box 233, Reading, RG6 6DW, United Kingdom

[4] Plant Research International, Postbus 16, 6700 AA Wageningen, The Netherlands

[5] Università di Padova Instituto di Agronomica, Dipt. di Agronomia Ambientale e Produzioni Vegetali, Agripolis – Via Romea, 16, 35020 Legnaro (Padova), Italy

[6] University Brawijaya, Faculty of Agriculture, Department of Soil Science, Jl. Veteran, 65145 Malang, Indonesia

CONTENTS

6.1 Introduction 176

6.2 Methods of Root Sampling 177
6.2.1 Auger Sampling 177
6.2.1.1 Hand Sampling 179
6.2.1.2 Mechanised Techniques 179
6.2.1.3 Core Diameter 180
6.2.1.4 Sampling Strategy in the Field 180
Position of Sampling 180
Depth of Sampling 181
Number of Replications 181
6.2.1.5 Special Techniques for Tree Roots 182
6.2.1.6 Drawbacks 182
6.2.2 Ingrowth Cores 183
6.2.2.1 Concept 183
6.2.2.2 Drawbacks 183
6.2.2.3 Results and Perspectives 185
6.2.3 Pinboard Method 186
6.2.3.1 Concept 186
6.2.3.2 Procedure 187
6.2.3.3 Drawbacks 189
6.2.3.4 Results and Perspectives 190

6.3 Procedures for Root Washing 191
6.3.1 Hand Washing 191

A.L. Smit et al. (Eds.), Root Methods
© Springer-Verlag Berlin Heidelberg 2000

Soil Cores *191*
Washing Pinboard Samples *192*
6.3.2 Automatic Washing *192*
6.3.3 Chemical Dispersing Agents *194*
6.3.4 Errors *194*
6.3.4.1 Loss of Fine Roots *194*
6.3.4.2 Loss of Dry Weight *195*
6.3.4.3 Change in Chemical Composition *196*
6.3.4.4 Presence of Debris and Soil Particles *196*
6.3.4.5 Presence of Dead Roots *197*
6.3.4.6 Operator Differences *197*
6.3.5 Time Investment *198*

6.4 Methods of Storage *198*
6.4.1 Storage Before Washing *198*
6.4.2 Storage After Washing *199*
Cold Storage *199*
Freezing *199*
Drying *199*
Chemicals *200*
6.4.3 Effect of Storage Methods on Root Properties *200*

6.5 Root Quantification *200*
6.5.1 Root Weight *201*
6.5.2 Root Length *201*
6.5.2.1 Direct Measurement *201*
6.5.2.2 Line Intersect Method *202*
6.5.2.3 Visual Estimation Method *203*
6.5.3 Root Diameter and Surface Area *205*
6.5.4 Presentation of Root Data *205*

6.6 Conclusions and Perspectives *206*

References *206*

6.1 Introduction

This chapter outlines those methods for assessing root systems structure and function in the field which are based on washing roots free from the soil in which they grew. Some of these methods are included in previous reviews (Kolesnikov 1971; Böhm 1979). The methods are either disruptive or totally destructive to the root system being studied and to the immediate environment (Taylor et al. 1991).

The spatial variability of root systems requires that a large number of replicates be sampled to obtain reasonably accurate estimates of root parameters. Intensive, destructive sampling disrupts the remaining roots, which may in turn affect results in the future. Consequently, due to the large variability of results, it is not possible, in practice, to estimate root turnover from frequent sampling schemes (Van Noordwijk 1993). Nevertheless, soil cores from auger sampling or pinboards often yield the best quantitative information on root system biomass and root length per volume of soil (Caldwell and Virginia 1991). These methods are therefore the basis for calibrating other techniques, including those for estimating root turnover.

Monolith samples can be taken using a pinboard, which enables the complete root system to be observed after washing the soil away. In contrast, augered samples are taken at discrete locations in relation to the horizontal and vertical distance in the soil profile from the plant (stratified sampling). Auger sampling provides a soil-root sample of a limited volume from the root zone.

The ingrowth core technique (Steen 1984; Fabião et al. 1985) is based on mesh bags filled with root-free soil, placed at different depths and removed at prescribed intervals. Differences between soil conditions inside the bags and in the surrounding undisturbed soil, such as soil strength and mineral nutrition status, can change the pattern and quantity of roots. However this method is particularly suitable for recording root response to localised fertiliser application or other heterogeneity in the soil (Cuevas and Medina 1988; Hairiah et al. 1991).

The destructive sampling methods described in this chapter are the basis for quantifying root length, diameter, surface area, volume, biomass, disease, mycorrhizal association and ion content. Depending on the goal of the further analysis, however, one may need correction factors to account for the changes in root properties during sampling, handling and storage.

Table 6.1 indicates the information that can be obtained from different methods of root measurement. Auger sampling, ingrowth cores and pinboard methods are described in this chapter. Core break and root mapping methods are described in Chapter 7. Minirhizotron and related methods are described in Chapter 8.

6.2 Methods of Root Sampling

6.2.1 Auger Sampling

The best sampling technique for obtaining volumetric soil-samples is the auger method. With this method soil samples are taken from the field using hand-operated or mechanical samplers and washed to separate roots from soil.

Table 6.1. Aim of different methods for root measurement[a]

Parameter	Auger sampling	Ingrowth cores	Pinboard	Core break method	Root mapping	Minirhizotron
Root length $cm\,cm^{-3}$	+++	+++	++(+)	+	+	+
Root weight $g\,cm^{-3}$	+++	+++	++(+)	–	–	–
Distribution pattern	+	–	+	–	++	–
Root growth and decay	–	++	–	–	–	+++
Root diameter	++(+)	++(+)	++(+)	–	–	+
Branching pattern	–	+	+	–	–	+
Time consuming d (8 hours)	7–20	5–15	15–30	2	4	4–10
Number of samples	120	60	2 Boards $0.5\,m^2$	120 = 12 Boreholes	6 Maps $0.5\,m^2$	1 Minirhiz./year[b]

[a] Symbols are as follows: – no information on parameter, + qualitative interpretation may be drawn, ++ semi-quantitative interpretation through a ranking, +++ fully quantitative information obtained.

[b] length of minirhizotron is 1–2.1 m.

6.2.1.1 Hand Sampling

The simplest method for taking soil samples is to use a hand-driven corer. One of the most commonly used corers is that described by Schuurman and Goedewaagen (1971; Fig. 6.1). It comprises a cylindrical tube 15 cm long with an inside diameter of 7 cm. A T-handle at the top of the auger shaft facilitates rotation of the auger to aid penetration into and removal from the soil. Many authors have modified this simple hand auger, mainly by varying the tube diameter, or by using mass impact to drive the auger into the soil.

Albrecht (1951) and Albrecht et al. (1953) describe an auger consisting of two halves held together by a metal ring. These halves can be separated to allow for recovery of intact soil cores. A new manual coring system has been proposed by Prior and Rogers (1992, 1994) which includes a manual driver of adjustable weight, a manual core extractor and steel core tubes with clear plastic liners which encase the soil core for retrieval and transport.

Various materials have been used for the augers but the most frequently utilised are metal (stainless steel) or Plexiglas, bevelled at one end to minimise soil disruption during sampling.

6.2.1.2 Mechanised Techniques

When core samples must be recovered from depth or from difficult soils, mechanised equipment is used. One of the simplest ways is to use an auger driven into the soil by a hand-held motorised drop-hammer and removed by a puller or a screw-jack (Böhm 1979). Soil core samplers can also be mounted on tractors, typically on the drawbar, which has hydraulic power for the movement (upward and downward). It is also possible to utilise special machines constructed for agricultural engineering purposes, such as for drainage studies and soil survey mapping. Hydraulic devices transported by large tractors have reduced the time and the labour required for taking soil cores but their use is limited to situations where damage of a large portion of the plot is not a problem.

Fig. 6.1. Design of a simple auger based on Schuurman and Goedewaagen (1971)

The method proposed by Baarstad et al. (1993), where a hydraulic probe is attached to a knuckle boom mounted on a truck, would reduce soil compaction, although its cost could be a problem.

6.2.1.3 Core Diameter

One of the most important factors in field sampling is the core diameter (see Böhm 1979). The core must be large enough to obtain a reasonable sample volume, yet small enough to enable the cores to be obtained by the sampling method to be used. With decreasing core diameter an increasing number of replicates must be taken to maintain sampling accuracy (see Chap. 5). Small diameter cores can be a particular problem where there are low rooting densities. Furthermore, Schuurmann and Goedewaagen (1971) reported average root length densities were lower using a 4 cm diameter core than with a 7 cm diameter core. The difference could not be accounted for by the actual soil volume sampled and was attributed to the increased frictional resistance between the soil core and the bore of the tube in the smaller diameter tube. They recommended a core diameter of 7 cm, and the most commonly used core diameters range from 5 to 8 cm (Van Noordwijk 1993).

In dry sandy soils, a smaller auger diameter may be required to avoid soil loss during core extraction. In very wet soils special augers may be needed which allow air entry into the mud (Schuurman and Goedewaagen 1971).

6.2.1.4 Sampling Strategy in the Field

Position of Sampling. Sample position depends on the purpose of the measurements. If the aim is to obtain an average value for the field, a form of stratified sampling may be desirable, as rooting density will vary with the spatial arrangement of plants. In grassland, or other systems where plants are not grown in rows, a completely randomised design may be followed, unless there is reason to expect specific spatial patterns in the field (e.g. due to distance to the nearest drain, or irrigation water patterns), when such factors must be considered in the sampling design. For row crops, sampling must be stratified within and between rows. Crozier and King (1993) suggest sampling in a transect perpendicular to the row, within the rooting zone, while Van Noordwijk et al. (1985) propose special sampling schemes, depending on row spacing. In more complex plant arrangements, for example in agroforestry systems, additional levels of stratification are required and in the case of very complex systems or in natural vegetation, samples are no longer stratified but rather collected along a larger number of randomly assigned transects. Within each stratum, core position must be assigned at random. With any stratified sam-

pling scheme care must be taken when estimating a field average value from the data. Serious biases may be introduced if the average value for all samples is assumed to be the average for the field.

For treatment or variety comparisons, one sampling position per treatment may be sufficient, though this would need to be replicated throughout the experiment. For more detailed process or model calibration studies it may be necessary to quantify the total root expansion or the root depth or the soil layers in which the root length density is above a certain threshold (Passioura 1980).

Depth of Sampling. Ideally the core should be driven to the limit of rooting depth but the deepest layers are difficult to reach, and within those horizons the variability is often high. The maximum sampling depth can be estimated in some cases by extrapolating from the root length densities in the upper soil using a negative exponential equation (Van Noordwijk 1993). In any case, all soils must be sampled to a minimum depth of 30 cm or the bottom of the plough layer because most of the roots will probably be concentrated in this layer.

If clearly differentiated soil horizons are present in the sampled soil, the first approximation is to separate the different soil layers and then to subdivide the cores in standard length intervals. The most widely used core length is 10 cm. However, in grasslands and no till systems, it may be worth separating the 0–10 cm layer into 0–5 and 5–10 cm. Shorter core lengths will increase sample variability and it is difficult to cut portions of the core shorter than the diameter of the core itself.

Number of Replications. Particular attention must be given to the number of replicates (see Chap. 5). It is clear that by increasing the number of replicates the variability of data decreases, but the standard error of 25 samples is only five times smaller than that of a single sample (van Noordwijk 1993).

The number of replications adopted by different authors varies from three to ten per experimental unit, similarly to what is done in aboveground measurements. Van Noordwijk et al. (1985) estimated a coefficient of variation in grassland root weight for individual auger samples of 385 cm^3 (10 cm height and 7 cm diameter) of at least 40%. Values up to 100% are, however, not uncommon. The number of soil cores required for each plot can be determined at the beginning of the sampling program by extracting the roots from a number of soil cores coming from the same plot (Vogt and Persson 1991). In this way it is possible to have an idea of the variability of the samples and optimise the sampling process.

In order to reduce the number of samples to be processed, Schroth and Kolbe (1994) propose a method consisting of the combination and homogeni-

sation of several cores from a plot, with consequent subsampling for root extraction. This method, similar to the composite sampling schemes usually followed for soil chemical analyses, allows an increase in the number of soil samples and/or a reduction of processing time. However, the method introduces a new source of error dependent on the homogeneity of the total sample before subsampling and it is not appropriate when the root turnover is studied.

6.2.1.5 Special Techniques for Tree Roots

Sampling tree roots requires special techniques because of the large range of root diameters within a tree root system and the large volume of soil occupied by the roots. Knowledge of the species' root system structure is necessary if it is desired to sample a particular portion of the root system (see Chap. 1). Veller (1971) proposed a sampling scheme consisting of concentric circles, centred on the stem of the tree, the diameter of each circle being chosen according to the nature and age of the tree under study.

Generally, for taking soil cores in tree root systems it is necessary to utilise mechanised sampling techniques because of the hardness of the large roots.

For some purposes it is relevant to investigate the finer parts of the woody roots (e.g. spatial distribution, biomass turnover). Methods defined by Kalela (1950) or Nielsen (1990, 1994) are suitable. The "Nielsen sieve" is 45 × 45 cm with 25-cm-high walls, and ribs, 0.5 cm thick, placed in two perpendicular layers, defining holes of 3 × 3 cm squares. Depending on the soil and the hypothesis to be tested, the soil material is extracted separately from a number of soil horizons to the sieve. Stones and soil material are sieved in the field, and the collected root material is washed, sorted, dried and weighed in the laboratory. Roots are sorted into biomass and necromass, the living roots being sorted to the following diameter classes (root material below 0.1 cm diameter is removed after drying): fine roots (<0.2 cm), thin roots I (0.2–0.5 cm), thin roots II (0.5–1 cm), thin roots III (1–2 cm) and coarse roots (2–5 cm). Coarse roots can not be adequately sampled with any auger sampling scheme and one may have to resort to methods predicting ratios of roots in different diameter classes based on fractal branching analysis (Spek and van Noordwijk 1994; Van Noordwijk et al. 1994; Van Noordwijk and Purnomosidhi 1995).

6.2.1.6 Drawbacks

Auger sampling requires a large number of samples since the volume of auger samples is small compared with the total soil volume. In very small plots problems may arise if coring damages a relatively large portion of the plots.

Sampling depth using hand-operated apparatus is seldom greater than 100 cm. On stony soils or soils with large amounts of woody roots, inserting the auger may be difficult. Also, on dry clay soils the penetration resistance presents a problem. Sometimes it is helpful to rewet the surface soil before coring, if this does not interfere with the purposes of sampling.

The use of heavy mechanised equipment for core sampling may be a risk since it can compact the soil cores, shortening the core itself. This must be taken into account when cutting the core and interpreting the results.

The total time needed for a single core depends on soil conditions and depth of sampling, but generally the time needed for taking samples in the field is much less than the subsequent processing in the laboratory.

6.2.2 Ingrowth Cores

6.2.2.1 Concept

The basic concept of the root ingrowth core technique is to present root-free soil to the growing root system for a fixed length of time, so that root growth during that period can be quantified (Steen 1984, 1991; Hansson et al. 1991). The method reduces uncertainty about the time interval during which the roots in a particular soil core developed, but may introduce biases, as it is not possible to create root-free soil which is identical to the undisturbed soil conditions. It is therefore best to use the method mainly as a *relative* indication of the main periods of root growth, by comparing mesh bags placed at various times or left in the soil for various time intervals.

Practical instructions for using ingrowth cores are given in Box 6.1.

6.2.2.2 Drawbacks

The main difficulty in interpreting data from soil ingrowth cores arises from the unknown degree of disturbance upon introducing the mesh bags. Some of the roots growing into the core may be branch roots of roots severed by the coring, and this may cause an overestimate of the root growth which would otherwise have occurred in this volume of soil. More importantly, however, the process of sieving and re-packing the soil modifies soil conditions. The alteration of sandy soil properties is small but on other soil types, the disturbance to the aggregate structure accelerates mineralization of soil organic matter and affects soil aeration. When results are compared for mesh bags placed at different time periods, one has to be cautious of the fact that the degree of disturbance may vary with soil moisture content, even if exactly the same

BOX 6.1. Procedure for Installing the Mesh Bags for the Root Ingrowth Cores Technique

The procedure is summarised in Fig. 6.2. Remove long cores of soil and sieve the soil to remove any roots. Then, place a tubular nylon or polypropylene mesh bag (0.5–0.7 cm mesh) in the hole, loosely attached to a plastic pipe which is slightly smaller in diameter than the soil core. Fill the hole with the sieved soil via this tube (using a funnel), re-packing the soil to the original bulk density. The filling procedure is critical and results depend on soil texture, structure and moisture content. The best results are obtained by measuring the amount of soil to be filled per 1 cm layer (or less) and pressing the soil layer by layer with a pestle which fits into the tube. After pressing a layer, scrape the surface to create roughness which allows good contact with the next layer of soil.

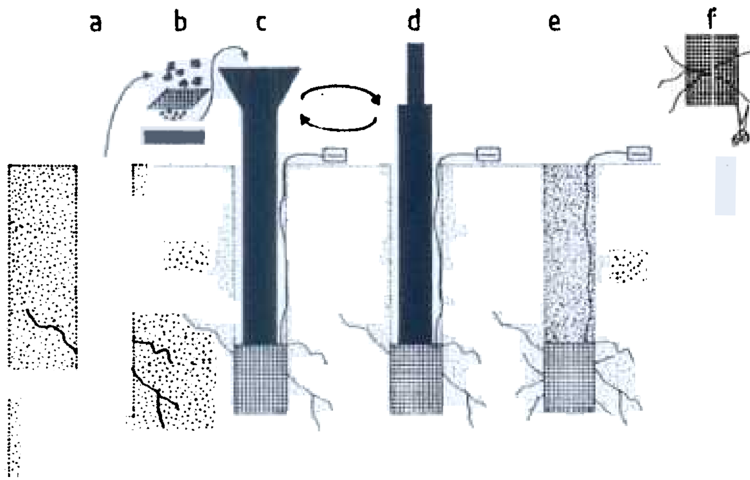

Fig. 6.2. Procedure for installing the mesh bags for the root ingrowth cores technique

It is convenient to attach a rope to the bottom of the mesh bag which can be used to pull out the mesh bag at harvest time. The other end of the rope is labelled and remains on the soil surface.

When the bags are recovered, it is often necessary to cut woody roots entering the bag with a knife (or auger of diameter slightly larger than the one originally used). Non-woody roots can be trimmed after mesh bag recovery. Wash the mesh bag contents as per a normal auger sample.

procedure is used for placing the mesh bags. If large variations in soil moisture contents are to be expected, it may be better to compare different intervals for root ingrowth into bags placed at the same time, rather than constant time intervals for bags placed at different times.

6.2.2.3 Results and Perspectives

With sufficient precautions, the method produces a reasonable estimate of the *relative* seasonal pattern of fine root growth. The results obtained from this method can usefully complement data from destructive sampling, which should be conducted at least once during the period of study. It may be worth collecting the roots sieved from the soil before placing it back into the mesh bag to correlate new root growth and previous root mass in the core, but sieving individual soil cores in the field is time consuming and often soil obtained from the same soil layer elsewhere in the experimental plot is used. Using the same, homogenised soil for all replicate ingrowth cores may reduce the variability of the results, but it depends on the purpose of the measurement whether or not this is desirable.

An alternative use of the ingrowth core technique is to compare different soil types, soil with different organic or inorganic amendments and/or packed to different bulk densities. For these applications one accepts that the soil inside the ingrowth core will be different from the surrounding bulk soil. Results may indicate the "local response" of root development to soil heterogeneity. Hairiah et al. (1991) used this method to investigate whether shallow root development of *Mucuna pruriens* on an acid soil was due to an inherent characteristic of the subsoil, or to the position of the subsoil. In other situations addition of specific soil organisms (symbionts, rhizosphere organisms, and rhizovores) may be compared. In this way "split-root" experiments can be conducted under field conditions with the advantage of keeping other conditions equal and focusing on local effects on root development. Most previous split-root studies have been restricted to pot experiments (e.g. De Jager 1982; Hairiah et al. 1993). The ingrowth core technique may be especially relevant for trees and other perennials where other experimental techniques are difficult to implement.

If dead roots are included in the ingrowth core, root decomposition can be studied under field conditions, but results may depend on whether the root material was naturally senesced or killed for the purposes of measurement. Root growth and senescence may be measured during root ingrowth studies when alive and dead roots are easily distinguished and a series of measurement are made over time.

6.2.3 Pinboard Method

6.2.3.1 Concept

Monolith samples can be obtained with pinboards ("fakir beds"), where the pins hold the root system in an approximately correct position during washing. The equipment needed to take a pinboard sample is shown in Box 6.2 and Fig. 6.3.

BOX 6.2. Pinboard Construction and Other Requirements

A pinboard ('fakir bed') can be made by inserting U-shaped stainless steel pins through plywood, to form a regular grid of steel pins (Fig. 6.3; Schuurman and Goedewaagen 1971; Böhm 1979). These pins can be made from bicycle/motorbike spokes bent into a U-shape (*A*) with a 5-cm-base and upright length equal to the sum of the required length plus the thickness of the board (a convenient total upright length is 12 cm). Before inserting the pinboard in to the soil, slide a plastic coarse-mesh screen over the pins. Other requirements (Fig. 6.3) are trowels (*B*), metal blades to smooth the profile wall (*D*), a rubber hammer, a car jack (*C*) adjusted to support the board, metal string or a steel cable with a diameter of 2 mm (e.g. motorcycle brake cable) with two handles (*E*) for cutting the soil.

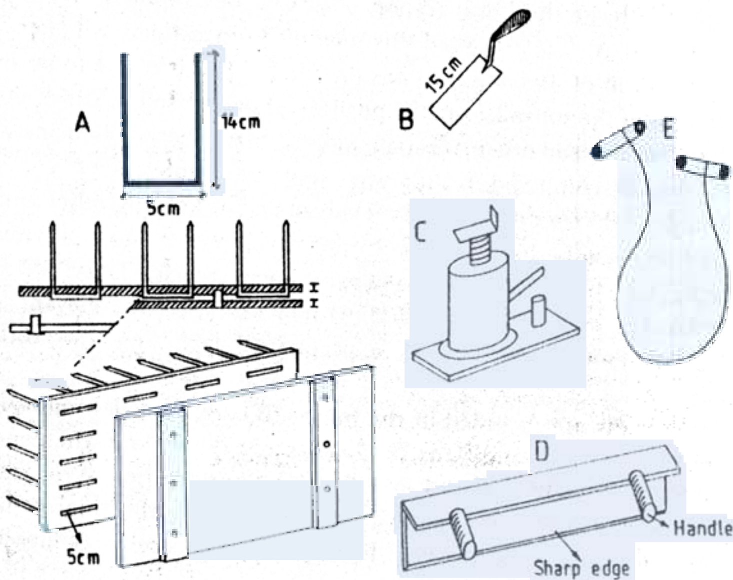

Fig. 6.3. Construction of a pinboard and some of the other equipment needed for taking samples. *A* pins, *B* trowel, *C* car jack, *D* blade, *E* string

The method has recently been improved by putting a coarse mesh screen (e.g. with openings of 0.5 cm) on the pins before the board is pushed into the soil, and gradually lifting this screen while washing away the soil. This screen prevents the movement of roots by "surface runoff" during the washing process.

Pinboard samples can be taken of individual plants, of row crops (usually perpendicular to the crop row), or of grassland vegetation. The size of the pinboard is determined by the vegetation rooting habit and practical considerations (samples of 100 × 60 × 10 cm of soil weigh about 100 kg).

6.2.3.2 *Procedure*

Practical guidelines for taking a sample are detailed in Box 6.3 and Fig. 6.4.

A set of pictures showing different steps of the pinboard method for sampling crop root systems is presented in Fig. 6.5.

An alternative sampling device for monolith samples, which avoids the need for a soil pit, is described by Floris and Van Noordwijk (1984). A metal

BOX 6.3. Procedure for Taking Pinboard Samples

Select and mark an appropriate sample location either at random or according to specified criteria (e.g. restricting samples to plants of a certain size).

Dig a soil pit just outside this sample location; the size of the soil pit depends on the dimensions of the pinboard. For a 60-cm-wide pinboard, a pit 100 cm wide allows for sufficient manoeuvrability to set and recover the pinboard. When plants are grown in rows, the pit is normally dug perpendicular to the row.

Smooth off the profile wall at about 5 cm from the plant (for 12 cm pins) so that the plant is in the middle of the pins. Harvest the aboveground parts of the plant and describe the soil profile.

Place the pinboard vertically with the pins against the profile face, and adjust so that the top row of pins is at ground level, and then push the pinboard into the soil by hammering it in. Alternatively, a jack or similar hydraulic device can be used to push it in smoothly.

Support the pinboard with a car jack (Fig. 6.4) and remove about 15 cm (a few centimeters beyond the tips of the pins) of soil underneath the pinboard with a knife. Cut away soil on both sides of the board, also a few centimeters further than the tips of the pins and put the steel cable in place (Fig. 6.3). Tie the plastic mesh screen around the sample to prevent soil loss during the next step. In a sawing movement, cut the last surface connecting the monolith to the surrounding soil mass, leaving the pinboard supported by the jack. Normally one person

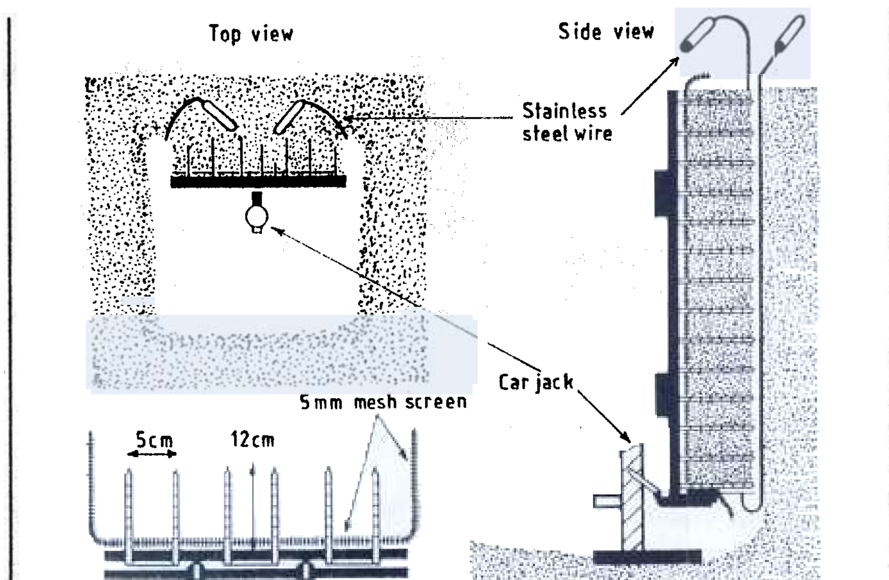

Fig. 6.4. Procedure for taking pinboard samples: after pushing the pinboard into a smoothed profile wall, the board is supported on a car jack; soil below and on both sides of the board is cut away by sawing movements with a stainless steel wire

stands in the soil pit to control the process while two people pull the cable. When loose, pull the board backwards and carefully lift it from the pit. Cut away the edge of the soil monolith to the level of the tips of pins.

Label the sample and cover it with a plastic bag for transport to the laboratory.

box is driven into the soil by a hydraulic cylinder and supported by a strong metal frame, anchored into the soil. When the desired depth is reached, the bottom is cut by pulling a cable (as in the pinboard sample) and a pair of claws is inserted into the soil by tightening screws on top of the box. A small metal tube provides air and thus prevents suction on the soil while the box is pulled to the soil surface. In constructing the box a delicate balance must be maintained between keeping the walls thin and smooth enough to allow easy insertion into the soil, and providing enough grip on the soil that the sample will not be lost from the box while it is pulled up. In practice, the equipment described by Floris and Van Noordwijk (1984) is no more time efficient than the pinboard method, but it involves less destruction of the surrounding soil (except for the soil anchors) and may thus be desirable in long-term experi-

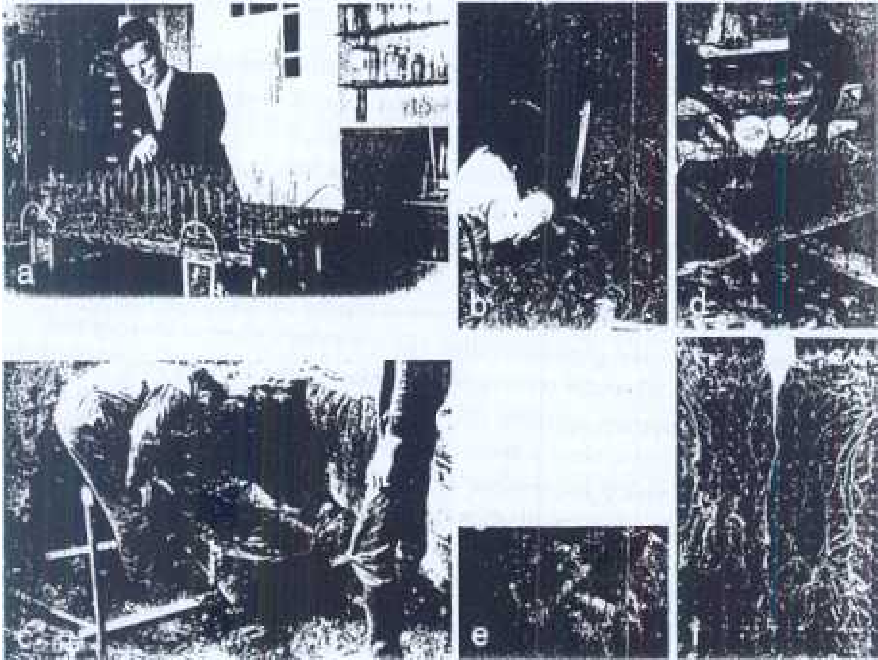

Fig. 6.5. Pinboard method for sampling crop root systems. a M.A.J. Goedewaagen (author of a classical description of root research methods) in the 1930s with a washed out sugarbeet (*Beta vulgaris*) root system. b Pinboard just pushed into a soil profile. c Cutting away redundant soil after the monolith has been cut free from the profile wall. d Washing a pinboard sample directly in the field (a small stream in the forest). e Washing a pinboard sample in the laboratory with a rotating sprinkler. f Sugarbeet root system washed free. (Photographs a and f courtesy of archive AB-DLO, Haren, the Netherlands, b–e Meine van Noordwijk)

ments with a restricted plot size. If a similar box is mounted on a tractor's hydraulic power system, the weight of the tractor is generally sufficient to push the box into the soil. This reduces the destruction of the experimental field to that caused by a tractor.

6.2.3.3 Drawbacks

Taking the pinboard samples requires some skill and takes more labour than sampling with augers. The major drawbacks for many situations are probably the destruction caused by the soil pit and the limits to sample size by the weight of the soil. Compared with auger samples, larger losses of fine roots during the washing process can occur, especially for roots entering the sample volume from the outside, instead of from the plant in the centre of the board.

6.2.3.4 Results and Perspectives

Overall, the pinboard method provides useful information per unit effort. The distinction between live and dead roots is easier with pinboard sampling than with methods where the root system is not sampled in its entirety. In comparison with other sampling methods, based on blocks of soil or auger samples, pinboard samples contain more coherent parts of a root system and can thus be washed on a coarse mesh screen rather than on a fine mesh sieve. This facilitates the washing process and leads to root samples without much organic debris. The main gain in time is during the sample cleaning stage. Apart from being faster, the pinboards also allow a more coherent view of the branching pattern and allow the tracing of individual roots to their origin. For an analysis of intercropping systems or in situations with a prominence of "weed" roots, this can be a clear advantage. The washed system can be photographed to obtain a visual impression or can be conserved as such. If combined with a resin cast of a small slice of the soil profile (Schuurman and Goedewaagen 1971) a visual link can be made between soil conditions and root response. Examples of root systems washed free on pinboards are presented in Fig. 6.6.

To save time when quantifying roots after washing, a visual estimate of the amount of roots per block of soil ($5 \times 5 \times 12\,cm^3$) can be made, with a representative selection of samples recovered to calibrate the visual ranking. Results then depend on the consistency of the person doing the visual assesment and

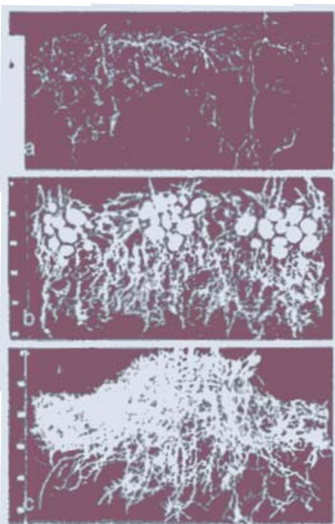

Fig. 6.6. Examples of root systems washed free on pinboards. **a** *Calopogonium mucunoides*, a legume cover crop on acid soils in Nigeria. **b** Potato (*Solanum tuberosum*) in a pinboard sample taken along the ridge. **c** *Helianthus tuberosus* in a pinboard sample perpendicular to the ridge. (Photographs courtesy of Meine van Noordwijk and archive AB-DLO, Haren, the Netherlands)

on the characteristics of the roots. Where large differences in root diameter occur it is more difficult to obtain accurate estimates. If root data are collected for a well-specified purpose, the analysis may concentrate on root densities in a certain range, and a visual estimation procedure can prove helpful. For example, models of nitrate uptake suggest that root length densities above 0.5 cm cm^{-3} are redundant for uptake of available N from a soil layer. If nitrate uptake is the main interest, analysis of the pinboard samples can focus on the layers or zones around and below this critical point. Generally, cleaning and measuring the upper soil layers with large amounts of roots takes a large share of the total time, and for certain questions it may be enough to rank root densities as few, intermediate or many.

6.3 Procedures for Root Washing

Once sampling in the field is finished the roots must be separated from the soil. This is done in different ways but usually soil is washed away from the root samples. Ideally, the best approach is to wash roots from the samples immediately upon arrival from the field but core samples may be stored in sealed polyethylene bags under refrigeration, or frozen until processing.

Roots may be separated from soil by dry-sieving but this method is only appropriate for very sandy soils, and its use is limited to tree root studies, where roots with a diameter greater than 2 mm are to be investigated (Böhm 1979).

6.3.1 Hand Washing

Soil Cores. Roots must be washed gently to minimise loss or damage. The water flow of the sprinkler must be very low because the roots are easily damaged by high water flows. Large roots may be removed by hand.

If roots and soil have not separated with sprinkling, it may be necessary to soak and gently agitate the sample. The soil particles should then settle while the roots and other organic matter float to the top and can be poured into another bucket or sieved for further cleaning. The procedure must be repeated as many times as necessary until a clean root sample is obtained. The sieve size used is critical to minimising root loss during washing (see Sect. 6.3.4.1), though the sieve size used will depend on the type of root material and the objective of the research. If there is a lot of organic matter, a coarse sieve (e.g. 0.2 cm mesh) can be used to split the sample into a first fraction, with roots

and large organic particles, and a second fraction, with fine short roots and debris.

When the main purpose of the research is chemical analysis of roots, it is important to reduce the washing time and a coarse sieve only can be used to save time.

Washing Pinboard Samples. Washing the soil from pinboard monoliths requires a different procedure than that described for auger or ingrowth samples.

Washing pinboard samples is facilitated by soaking overnight in water, deep freezing (for clay soils), soaking in oxalic acid (for soils with free calcium carbonate) or soaking in hexametaphosphate, preferably under vacuum. Whatever the pretreatment used, gentle washing must follow; a rotating or oscillating sprinkler is ideal, but pinboard samples can also be washed under more primitive conditions with a hose attached to pressurised water or a locally constructed "water tower" or by pouring small buckets of water over the sample. The pinboard is put at a slight angle and washing starts at the lower part. During the procedure some caution is need to avoid the formation of sharp ridges in the profile, which can cause the breaking of roots. Even when the roots do not break, there is a change in their positions, moving downwards which affects root distribution. To avoid this, the coarse mesh screen is lifted, and supported with wooden strips when the soil layer becomes very thin. The roots then become fixed on the mesh screen and are not pushed downwards by the water. Root losses from a pinboard can be collected by placing a large sieve under the waste-pipe. This indicates how carefully washing is being conducted and how well the root system is secured. Some losses are acceptable if the main purpose is the rooting pattern, but for quantitative studies care must be taken to minimise root displacement.

During the washing process large-sized organic debris can be carefully removed from the board using forceps. Soil layers or other feautures which become visible during the washing may be marked with string using the pins as points of reference.

After washing away the soil, the root system is lifted by the mesh screen, photographed (on a black cloth as background) and/or cut according to soil layers, depth zones and/or distance to the plant to obtain root biomass and/or root length. Total plant root biomass can be estimated by integrating root weight densities measured per zone and depth over the relevant soil volume.

6.3.2 Automatic Washing

Various root wash devices have been developed (Böhm 1979). The first to be commercially available (the Gillison root washer), was a hydropneumatic elutriation system (Fig. 6.7) developed by Smucker et al. (1982).

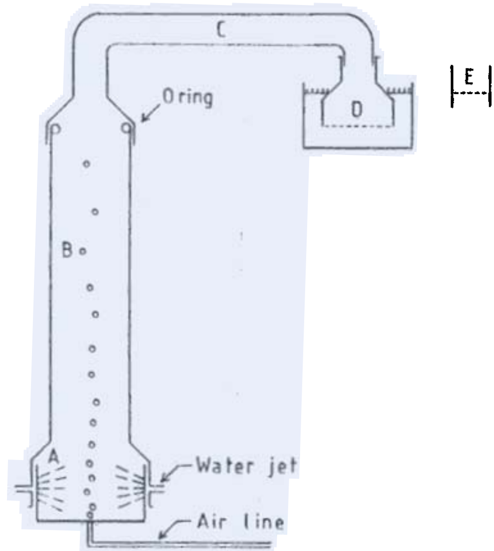

Fig. 6.7. Schematic representation of Smucker's system. *A* High kinetic-energy washing chamber, *B* elutriation chamber, *C* transfer tube, *D* low kinetic-energy primary sieve, *E* secondary sieve

This system consists of a high-kinetic energy water vortex, generated in a chamber in which roots and organic plant residues are separated from soil. Floating roots and associated materials pass onto low-kinetic energy separation screens (multiple screens may be stacked) submerged in water. The mechanical separation of roots is achieved by a closed system using water pressure and compressed air to isolate and deposit roots on a submerged sieve (Byrd et al. 1976; Smucker et al. 1982; Mackie-Dawson and Atkinson 1991).

According to Smucker et al. (1982) and Smucker (1992) this method retains almost all roots >0.05 mm diameter, with attached root hairs and nodules (if present), as well as some fruiting bodies of fungal pathogens. Nevertheless, roots can sometimes remain somewhere in the system and a periodic check of washing water and residues should be made to see if fine roots are being lost. Such losses should be kept less than 5% of root weight (Van Noordwijk 1993).

More recently another device, based on a design from the CSIRO Cunningham Laboratory, in Australia, has become commercially available (Delta-T root washer). The method proposed by Pallant et al. (1993) is also suitable for collecting very fine roots, with a minimum diameter of 0.025 mm, and is an inexpensive method.

The main advantage of automatic washing is the standardisation of the process, even when different operators do this work. Labour time is also saved by this washing system, but after elutriation roots must still be sorted by hand, and this may take several hours (Van Noordwijk 1993). Another advantage is that automatic washing can be used in research with radioisotopes, since the system is totally closed.

6.3.3 Chemical Dispersing Agents

For soils with a high clay content, chemical dispersing agents can be used to help disperse the soil particles and facilitate root washing. A list of some of these chemicals is presented in Table 6.2. Some form of sodium metaphosphate is the most commonly used chemical. Weak acids can be used on soils containing calcium carbonates as binding agents.

Chemical dispersing agents can damage the root system, creating problems for further observations. For example, Van Noordwijk (1993) mentions that soaking the samples in a 5% sodium hexametaphosphate solution speeds up the process of washing roots from clay soil, but the roots become discoloured, particularly in soil with high organic matter content, making the subsequent identification of live roots more difficult. So, the decision of when to use these chemicals must depend on the objectives of a given study.

6.3.4 Errors

The errors that occur during the washing and cleaning of samples are loss of fine roots, loss of dry weight; changes in nutrients content of the roots; incomplete separation of root, soil and debris; poor distinction between live and dead roots; and personal screening differences. Precautions to solve the problems can lead to conflicting situations. To quantify errors, samples can be split or a few samples analysed as a control, checking the losses.

6.3.4.1 Loss of Fine Roots

It is almost inevitable that not all the roots in the soil-root sample are recovered during the washing and cleaning stages. Most of the losses during this stage

Table 6.2. Listing of some dispersing chemicals used for root studies by different authors

Chemicals	Reference	Concentration
$Na_4P_2O_7$	Schuurman and Goedewaagen (1971)	0.27%
Na_3PO_4	Cassel et al. (1995)	0.2 M
$(NaPO_3)_6$	Van Noordwijk (1993)	5%
NaCl	Tardieu and Manichon (1986)	Sat. solution
HCl	Böhm (1979)	3–5%
$(COOH)_2$	Heringa et al. (1980)	10 g/l
CH_3COOH	Mitchell et al. (1993)	0.25–2.5 M
$NaHCO_3 + CH_3COOH$	Pallant et al. (1993)	Not given
Detergents	Kücke et al. (1995)	Not given

are fine roots, which can comprise a major loss in terms of root length but are not so critical in terms of root weight.

The mesh size of screens used to collect roots plays an important role in determining the amount and size of the recovered roots. Amato and Pardo (1994) compared 2 and 0.2 mm mesh sieves (4 and 0.04 mm^2), and found that recovered root length for wheat and field beans tended to be an order of magnitude higher when using the smaller mesh sieve. Roots collected on the 2 mm sieve represented on average 55% of the weight but only 10% of the total length collected using a 0.2 mm sieve. With a 1 mm sieve the recovered root weight was 75%, but the recovered root length was still only 34% of the 0.2 mm sieve. Caldwell and Fernandez (1975) recovered more than twice as much root biomass using a mesh of 0.03 mm^2 instead of 0.2 mm^2. The use of coarse screens is therefore more acceptable for root weight than for root length determinations and reduces the time needed for cleaning the samples from organic debris.

6.3.4.2 Loss of Dry Weight

Loss of root dry weight during washing and storage of root samples has been observed for several crops grown in nutrient solution (Van Noordwijk and Floris 1979; Floris and De Jager 1981; Grzebisz et al. 1989). Losses were typically 20–40% of the original root dry weight. Some combinations of washing and storage treatments resulted in up to 50% loss of the original root dry weight. Five to 10% losses in dry weight was reported over the 24 h immediately after harvest (stored at 20 °C in a moist environment), prior to any washing or longer term storage treatments being imposed. This initial loss was attributed to root respiration (Floris and De Jager 1981). Using sodium pyrophosphate as a dispersing agent tended to increase losses at the washing stage by 10–15% compared with washing without a dispersing agent.

Grzebisz et al. (1989) found that, for most of their washing and storage treatments, loss of cell wall material was limited. They therefore concluded that most of the dry matter loss comprised cell contents, not root tissues such as the epidermis or cortex. This agrees with the lack of change in root diameter during washing and storage reported by Floris and De Jager (1981).

It has been suggested that a correction factor in the range 1.4 to 2.0 could be used to account for dry matter losses of 30–50% due to handling of root samples (Grzebisz et al. 1989). As the authors point out, these correction values were obtained from roots grown in nutrient solution, which may result in a different root structure and chemical composition from field-grown roots. However, Grzebisz et al. (1989) suggest relative root dry weight losses from field-grown crops could be even higher, particularly when grown in conditions of limited water or nutrient availability.

Variation in dry weight losses can be reduced by treating all samples uniformly. When working with a large number of samples it is recommend that the samples be frozen after washing. This treatment gives high dry weight losses but extra handling of the roots after thawing does not involve new dry weight losses.

6.3.4.3 Change in Chemical Composition

There are conflicting reports concerning the importance of nutrient loss from roots during washing. Böhm (1979) cites work showing nutrient loss during washing of agricultural root systems was not significant. In contrast, G. Brouwer (unpubl. data) has observed that the nutrient content (N, P, Ca, K), as a percentage of dry matter remaining, can change considerably in a short time. For calcium, manganese and iron contents, Evdokimova and Grishina (1968) report a decrease of 10–15% in samples that were washed for 2 h compared with samples which were washed quickly. Grzebisz et al. (1989), comparing different washing and storage treatments, found that N losses were smaller than dry matter losses. Consequently, the root N content (as a percentage of dry matter remaining) in some cases increased by up to 10% of the original value. This can lead to overestimates of N content.

Böhm (1979) suggested that nutrient loss during root washing depends mainly on how old the roots are. Brown, decaying roots lose more nutrients than white, young roots.

Lignin content typically increases as a percentage of dry matter during decomposition of organic residues (Parr and Papendick 1978; Jawson and Elliot 1986).

When the root biomass is used for chemical analyses, quick washing on a coarse sieve, with some control samples is preferred. Samples should not be frozen before the effect of freezing on the analytical results is known. Nutrient contents are generally only reliable if sample handling is completed within 1 day (Van Noordwijk 1993).

6.3.4.4 Presence of Debris and Soil Particles

The amount of debris and soil particles present in washed roots is variable, depending on the conditions under which roots are grown. In the last stage of cleaning the distinction between small roots and debris can be difficult and for this reason it is better to try to standardise the cleaning procedure as much as possible.

When washing by floatation (as opposed to sprinkling) it is possible to separate the majority of debris from roots by repeating the decanting process.

Debris which are lighter than living roots float more readily than roots and can be picked up. Some care during this process is needed because some fine roots can come together with the debris.

The type of soil can modify the amount of roots separated from the soil itself. In general, the coarser the soil the easier is the recovery of roots. Small particles can adhere more closely to the roots, especially when the roots have an abundance of root hairs. This can break the root hairs during washing.

The adherence of fine soil particles to the fine root fraction in particular, despite careful washing, can be a problem and results in an overestimate of root dry weight. Böhm (1979) reported that soil particles could account for up to 50% of the sample weight. This error can be avoided, or at least checked, by determining the weight of the ash-free organic root matter (see Sect. 6.5).

In organic soils, where the microbial load is usually high, the roots present in the soil cores can be degraded rapidly.

Separation of soil particles and organic debris from roots still remains a big problem which has led some investigators to concentrate on developing techniques to determine accurately the total length of roots in samples without removing the debris. Dowdy et al. (1995) developed a technique for partitioning live root images from debris using imaging techniques, based on the assumption that the length : width ratio for root segments differs greatly from that of soil and organic debris.

6.3.4.5 Presence of Dead Roots

It can be difficult to distinguish between dead and live roots. Probably the best method is to check root elasticity during the washing procedure. Annual roots, which break when stretched a little, are dead and those with some elasticity are alive. With the auger method it is useful to have a fallow treatment to test if roots are still present from the previous crop, especially if sampling early in the growth of the current crop.

Many staining techniques are available (see Chap. 10), not only for increasing the discrimination between live and dead roots but also for increasing the contrast for automatic measurement of roots. The most appropriate technique for any particular experiment should be determined by testing a few samples taken before the main sampling date.

6.3.4.6 Operator Differences

Differences between operators in sample handling, mainly during the stages of decantation and cleaning up the root samples, can cause errors. The resolution of the human eye, time pressure, operator fatigue, different criteria for dis-

tinction between dead and live roots, the decision to stop cleaning, etc. are reasons for these errors.

It is preferable if the same person does the work, since this gives more comparable data. When several people are involved in the same work care should be taken to avoid bias in the measurement results. It is advisable to record the operator for each sample, to allow calculation of a correction if necessary.

6.3.5 Time Investment

The time invested in cleaning roots, often recognised as a problem in root systems studies, is highly variable. It depends on: the procedure used; the crop itself; the stage of development of plants; and on the content of organic debris and other materials in the sample. Whereas for the upper layers of a grass stand it can take 1 day to clean up one or two samples, in the same period of time it is possible to wash and clean 40 samples of leek (G. Brouwer, unpubl. data).

Böhm et al. (1977) found that 9.5 person h were required to collect, wash out and measure soybean roots from twelve 15 cm increments of a 180 cm core from a silt loam soil.

T.C. Kaspar (pers. comm.) and Kaspar et al. (1995), working with core samples 10 cm in diameter and 15 cm long, for maize roots grown in a silty clay loam soil, has estimated that it required 0.16 person h/sample to take a core with a hydraulic sampler and an eight person crew, 0.30 person h/sample to wash out the soil using a hydropneumatic elutriator based on Smucker's design and a four person crew, and 0.30 person h/sample to remove organic debris.

6.4 Methods of Storage

Ideally, root measurements should be taken immediately after sampling. However, the logistics of processing the large number of samples that are required from an experiment mean that it is often necessary to store roots before measurement, sometimes for long periods.

6.4.1 Storage Before Washing

As a rule, samples should be washed immediately after being taken from the field and then stored in a suitable manner. This minimises the period for root respiration and microbial degradation of the roots after sampling. If it is not possible to wash samples immediately, they can either be cold-stored or dried.

Cold-storage should only be considered for temporary storage (<2 to 3 days) because the treatment does not stop root decay but only slows it down. Drying at 60–75 °C (Schuurman and Goedewaagen 1971) offers a longer-term storage option. An alternative long-term storage option is to freeze the samples (e.g. Kücke et al. 1995), but the sheer bulk of material that would usually need to be stored often precludes this in practice. Whichever option is used, because of their bulk it will probably take at least several hours before the samples attain the desired temperature, during which time some root degradation will occur.

6.4.2 Storage After Washing

Cold Storage. Washed fresh roots can be stored for up to 48 h in a refrigerator (Gregory et al. 1992). During storage, the roots should be kept moist to avoid desiccation. This can be achieved either by wrapping samples in a damp paper towel (Gregory et al. 1992), or by storing the samples in suspension with water. If the storage period is longer than 2 days, a chemical preservative should be added to the suspension (Table 6.3).

Freezing. Freezing at −20 °C (Schuurman and Goedewaagen 1971) provides a long-term storage option. Samples can be stored in small plastic bags or boxes, with a little water. The addition of water helps to avoid root damage during freezing.

Drying. Drying the root samples is another long-term storage option. Samples should be dried at 60–75 °C to avoid the pulverisation that can occur when roots are dried at 100 °C (Schuurman and Goedewaagen 1971).

Table 6.3. Listing of some chemicals used for root storage by different authors

Chemicals	Reference	Concentration
Formalin and thymol	Schuurman and Goedewagen (1971)	3–5% and 3%
Ethanol, formalin, acetic acid and water (FAA)	Tennant (1976)	50%; 6.5%; and 2.5%
Ethanol	Böhm (1979)	15–20%
Methanol	Henry and Deacon (1981)	70%
Formalin	Tanaka et al. (1993)	10%
Thymol	Marcum et al. (1995)	3%
Isopropanol	Kücke et al. (1995)	15%

Chemicals. A range of chemical agents have been reported in the literature as satisfactory for long-term root storage (Table 6.3). Formalin and alcohol are the most commonly used preserving agents. A 5% formalin solution or a 15–20% alcohol solution can preserve roots satisfactorily for several months at 10 °C (Böhm 1979). The alcohol concentration required is proportional to the storage temperature. The higher the storage temperature, the greater the alcohol concentration required. Combinations of low temperature with chemical preserving agents seem to prove most satisfactory.

6.4.3 Effect of Storage Methods on Root Properties

Storage methods can affect the condition of the roots. Ideally, a storage method should be chosen that does not influence the root property that is to be measured. All samples must be stored in the same manner.

For chemical analyses, roots need to be dried as quickly as possible before storage. When this is impossible, it is recommended that a zero control be taken to quantify the changes prior to drying. Samples must not be stored in a freezer because soluble nutrients can be lost from the roots when subsequently thawing the sample for analysis. Nutrient content, as a percentage of dry matter, begins to change after a few days in cold storage at 4 °C.

For physical analyses, a wide range of storage methods is acceptable, though the best method is to store the fresh samples in a freezer. Alternatively, samples can be dried at 60–75 °C (Schuurman and Goedewaagen 1971) and then stored. On re-wetting, the roots more or less completely recover their original root length and diameter. Any effect of drying and re-wetting on the physical parameters should be similar for all the samples. Williams and Baker (1957) report that storing roots in formalin solution had no significant effect on herbaceous root dry weight.

6.5 Root Quantification

A number of parameters may be estimated from roots obtained with the methods described in this chapter, the most frequent being root weight, length, diameter, surface area and branching pattern. The parameters measured depend on the purpose of the experiment and how the researcher intends to use the root data. For example, when studying the absorptive capacity of the root system, root length and surface area are important parameters. Net ion influx into roots is influenced by root diameter. In other studies, it might be pertinent to know the proportion of below-ground dry matter relative to the above-ground dry matter of the plant, and hence the weight of the root samples would

be recorded. Whatever parameter is measured, it is usually related to the volume of the soil sample, which can be the volume of the entire monolith obtained with the pinboard, or a smaller volume, such as that obtained with auger sampling.

Root Weight

Root weight is estimated either from fresh roots or, more frequently, from oven-dried roots. Fresh weight is often used in plant pathology studies, for example investigating nematodes and fungi in roots (Böhm 1979). For determination of root fresh weight, it is recommended that a standardised procedure be applied to remove all the water adhering to the roots, before weighing. This can be achieved by submitting the roots to low-speed centrifugation, typically for 30 s (van Noordwijk and Floris 1979; Grzebisz et al. 1989).

Root dry-matter is a useful measure of plant investment in the root system and also of the contribution of roots to the soil humus, particularly when related to shoot biomass. However, it is not valid to assume that root weight is correlated with root activity (Böhm 1979). Root length, particularly of fine roots, is generally considered a better indication of root activity.

Root dry weights are generally recorded for root samples which have been oven-dried at 65–75 °C until there is no further change in weight (typically 24 h). Adherence of fine soil particles to the roots is sometimes a problem. In these situations the weight of the ash-free organic root matter is determined. The procedure involves placing the weighed oven-dry roots in a muffle furnace at 650 °C for 5 h to burn off the organic matter. The weight of the residue is then recorded. The difference between root dry weight and the weight of ash residues represents the ash-free organic dry weight.

Root Length

Various techniques, both manual and automated, are available for determining root length. This section considers primarily manual methods. Automated methods, in particular the use of image analysis software, are considered separately in Chapter 10.

6.5.2.1 *Direct Measurement*

Direct measurements of root length can only practically be made on large diameter roots, e.g. tree roots. Direct measurements of herbaceous plant root lengths

are rarely made, and then only primary roots (e.g. Sivakumar and Salaam 1994). It is simply not feasible to undertake the direct measurement of the total root length (including fine roots) of a plant, or even of a root sample.

6.5.2.2 *Line Intersect Method*

The root length of a sample can be estimated using intercept counting techniques (Newman 1966; Tennant 1975). Newman's technique is based on the relationship between root length and the number of intercepts between roots, spread over a surface of defined area, and randomly arranged lines of known length in that area. Marsh (1971) simplified Newman's method, and Tennant (1975) tested and popularised this modified method.

The modified line intersect method has become the standard manual technique for estimation of root length (See Box 6.4). The line intersect method gives an estimation of the root length of the sample, and is not a direct measurement. Sources of error arise from the random arrangement of the roots on the grid, root visibility, the definition of an intersection, and operator bias and fatigue. With care, it is possible for a single individual to obtain coefficients of

BOX 6.4. The Modified Newman Line-intersect Method

Spread the roots, with the minimum overlap possible, over a regular grid of indeterminate dimensions, but of known regular grid units (e.g. 1 cm grid squares). Where necessary, tease the root material apart or cut it into smaller pieces, to avoid overlap. Placing the samples on the grid in a shallow layer of water in a dish (or on a glass plate) aids separation of the roots.

Count the total number of intersections between the roots and the horizontal (H) and vertical (V) grid lines. Assign a count of 1 to a root crossing a line, a root ending touching a line and a curved root portion touching a line. Curved root portions which lie on or along a grid line are assigned a count of 2 (Tennant 1975).

The relationship between the root length and the number of root intersections with the grid lines is given by:

$$\text{root length (cm)} = \pi/4 \times \text{no. of intersects} \times \text{grid unit (cm)}$$

To improve the contrast of roots it can be helpful to stain them. Alternatively, place a coloured background under the roots. To optimise working efficiency at an acceptable random error level, choose the grid size to obtain about 400 intercepts (200 H + 200 V) per sample (Van Noordwijk 1993).

variation of the root length estimate of 5% or less (Tennant 1975). However, this is the minimum likely error. Coefficients of variation for the line intersect method are typically 10 to 15% (Böhm 1979; Bland and Mesarch 1990; Farrell et al. 1993). Bland and Mesarch (1990) reported a bias in root length estimates by observers, with some observers yielding consistently low or high estimates relative to the mean.

Steps can be taken to minimise the error associated with the technique. These include: training new observers and comparing their results against experienced observers; ensuring adequate lighting; using a magnifying glass to aid root visibility; improving the colour contrast between the roots and the background; and ensuring the observers have regular rest breaks to avoid fatigue.

The line intersect technique can be mechanised, using photoelectric counters such as those of Rowse and Phillips (1974), Richards et al. (1979) and Wilhelm et al. (1982). The automation eliminates operator bias from the estimation and should therefore reduce error. However, root samples do need to be cleaned more carefully since it is difficult for automated systems to distinguish between roots and debris. Details of automated methods for root analysis are given in Chapter 10.

6.5.2.3 Visual Estimation Method

The visual estimation of the root length is a rapid low-cost method for estimating root length. At its simplest the sample root length can be described by a subjective rating where, for example, 1 indicates low root length and 9 indicates high root length. This method has been used to screen for cucumber root length as part of a cultivar evaluation programme (Walters and Wehner 1994). Visual estimation may also be used to obtain a quantitative root length estimate of samples recovered using the pinboard method or from auger samples (Naab 1994; Gaze 1996). This essentially involves visual comparison of root samples of unknown length with standard root samples of known length, enabling a reasonable estimate of the unknown root length to be made (see Box 6.5).

BOX 6.5. Visual Estimation of Root Length

After washing, store the roots in a chemical preserving solution (Table 6.2) until required. Comparison of samples for root length estimation is easier when the roots are suspended in solution.

Select a sub-set of the washed samples which covers the range of root lengths in all the samples, based on a visual subjective rating. Determine the root length of the samples in this sub-set as accurately as possible using the line intersect method (see Box 6.4). These samples comprise the standard samples of known root length.

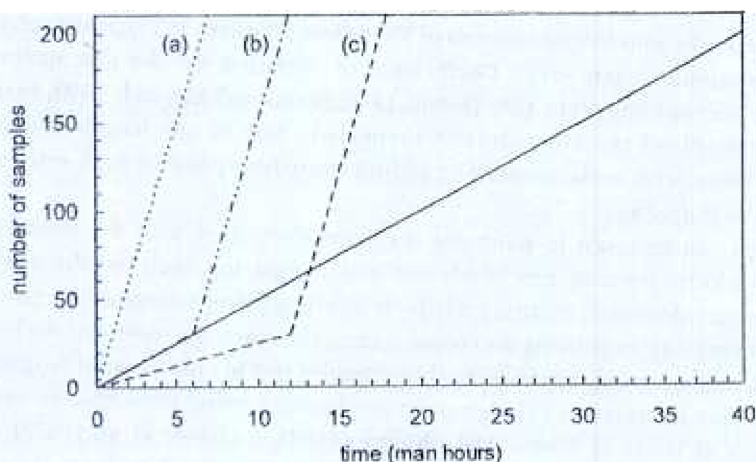

Fig. 6.8. Time required to determine root length using the line intersect method (*solid line*) and visual estimation (*broken lines*). The comparisons assume that 5 samples per h can be counted using the line intersect method, and that 30 samples per h can be estimated using the visual estimation method. For the visual estimation method: line *a* assumes standard samples are already available; line *b* assumes 30 standard samples are first counted once using the line intersect method; line *c* assumes that 30 standard samples are counted twice before visual estimation of the remaining samples

Estimate the root length of each of the remaining samples by comparing them against the standard samples. If necessary, estimate the root length by interpolation between the two standard samples which appear most similar to the root length of the sample being estimated. If the roots are stored correctly to prevent degradation, it is possible to use the same standard samples from one sampling date to the next. Standards should be for the same species (and possibly cultivar), have roots of similar condition and size, and should be stored in an identical solution volume and sample container to those samples against which they will be compared.

The major advantage of the visual estimation of root length is the time saved when compared with the line intersect method. Gaze (1996) reported that a single person could score 30–40 samples per h, compared with counting about five samples per h using the line intersect method. Figure 6.8 illustrates the major time-saving that can be achieved using the visual estimation method.

There is inevitably some loss in the accuracy of the estimation of root length when using a visual estimation method as opposed to the line intersect method. However, other measurements of root length, such as the line intersect method and image analysis techniques, are themselves in fact only root length estimates. For visually estimated root lengths of millet [*Pennisetum glaucum* (L.) R.Br.], the coefficient of variation was reported as just over twice that of the line intersect method (Gaze 1996). No systematic bias was found between observers using the visual estimation method. There can, however, be a tendency to underestimate root length using the visual method at high root lengths (Walters and Wehner 1994; Gaze 1996). This upper limit is probably a function of root length density in the sample solution volume, rather than absolute root length.

6.5.3 Root Diameter and Surface Area

Root diameter can be measured on samples spread out on a grid, using a binocular microscope with an ocular micrometer. According to Van Noordwijk (1993) at least 20 readings per sample are required.

Total root surface area has been estimated from other root parameters, like root length and diameter, by solution adsorption measurements, or by using photoelectric devices (Böhm 1979).

Recently, the availability of image analysing computers has made the determination of root length, diameter or surface area possible with more accuracy and with less time investment (see Chap. 10).

6.5.4 Presentation of Root Data

Root length and root weight are often related to soil volume and are referred to as root length density – L_v ($cm_{root}\,cm^{-3}_{soil}$) or root weight density ($g_{root}\,cm^{-3}_{soil}$). These are particularly useful for describing the spatial distribution of the root system, and are also useful data for soil-vegetation-atmosphere transfer models.

Root length and weight can also be related to soil surface area – L_a ($cm_{root}\,cm^{-2}_{soil}$) and ($g_{root}\,cm^{-2}_{soil}$). This facilitates comparison of root data with measurements of above ground plant production, which are typically made per unit soil surface area (e.g. yield, leaf area index).

The specific root length ($cm_{root}\,g^{-1}_{root\ dry\ weight}$) is used to give an indication of root thickness.

Comprehensive summaries of published rooting data have been made by Van Noordwijk and Brouwer (1991), Canadell et al. (1996) and Jackson et al. (1996).

6.6 Conclusions and Perspectives

Rapid developments in computing hardware and software hold much promise for assisting in the measurement of root length, root diameter and root branching patterns and architecture. However, to use these systems, the researcher must already have taken the samples from the field and washed the soil away from the roots. For the foreseeable future, this aspect of root sampling is likely to remain relatively time consuming and tedious when compared with taking measurements of the aerial parts of plants. Nevertheless, quantifying the location, amount and role of plant roots is essential to understanding plant growth on an individual plant basis, as well as understanding the competition and complementarity between plants and between flora and fauna in the wider ecosystem. The methods described in this chapter (together with those in Chapter 7) are likely to remain the basis of quantitative root research for many years to come.

References

Albrecht D (1951) Verbesserung der Spatendiagnose. Dtsch Landwirtsch 2: 41–43

Albrecht D, Fritzsche KH, Winkler S (1953) Weitere Entwicklung des Strukturbohrers. Dtsch Landwirtsch 4: 206–208

Amato M, Pardo A (1994) Root length and biomass losses during sample preparation with different screen mesh sizes. Plant Soil 161: 299–303

Baarstad LL, Rickman RW, Wilkins D, Morita S (1993) A hydraulic soil sampler providing minimum field plot disruption. Agron J 85: 178–181

Bland WL, Mesarch MA (1990) Counting error in the line-intercept method of measuring root length. Plant Soil 125: 155–157

Böhm W (1979) Methods of studying root systems. Ecological Studies: Analysis and Synthesis, vol 33. Springer, Berlin Heidelberg New York

Böhm W, Maduakor H, Taylor HM (1977) Comparison of five methods for characterizing soybean rooting density and development. Agron J 69: 415–419

Byrd DW Jr, Barker KR, Ferris H, Nusbaum CJ, Griffin WE, Small RH, Stone CA (1976) Two semi-automatic elutriators for extracting nematodes and certain fungi from soil. J Nematol 8: 206–212

Caldwell MM, Fernandez OA (1975) Dynamics of Great Basin shrub root systems. In: Hadley NF (eds) Environmental physiology of desert organisms. Dowden, Hutchinson and Ross, Stroudsburg, Pennsylvania, pp 38–51

Caldwell MM, Virginia RA (1991) Root systems. In: Pearcy RW, Ehleringer J, Mooney HA, Rundel RW (eds) Plant physiological ecology: field methods and instrumentation. Chapman and Hall, London, pp 367–398

Canadell J, Jackson RB, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) Maximum rooting depth of vegetation types at the global scale. Oecologia 108: 583–595

Cassel DK, Raczkowski CW, Denton HP (1995) Tillage effects on corn production and soil physical conditions. Soil Sci Soc Am J 59: 1436–1443

Crozier CR, King LD (1993) Corn root dry matter and nitrogen distribution as determined by sampling multiple soil cores around individual plants. Commun Soil Sci Plant Anal 24 (11&12): 1127–1138

Cuevas E, Medina E (1988) Nutrient dynamics within Amazonian forests: Part II. Fine root growth, nutrient availability and litter decomposition. Oecologia 76: 222–235

De Jager A (1982) Effects of localized supply of H_2PO_4, NO_3, SO_4, Ca and K on the production and distribution of dry matter in young maize plants. Neth J Agric Sci 30: 193–203

Dowdy RH, Smucker AJM, Dolan MS, Ferguson JC (1995) Root length measurements of segments washed from soil cores without removal of extraneous debris. Proc 14th Long Ashton Int Symp: Plant roots – from cell to systems. Sept 1995. Long Ashton, Bristol, England

Evdokimova TI, Grishina LA (1968) Productivity of root systems of herbaceous vegetation on flood plain meadows and methods for its study. In: Methods of productivity studies in root systems and rhizosphere organisms. Int Symp USSR 1968. USSR Academy of Sciences, Nauka, Leningrad, pp 24–27

Fabião A, Persson HA, Steen E (1985) Growth dynamics of superficial roots in Portuguese plantations of *Eucalyptus globulus* Labill. studied with a mesh bag technique. Plant Soil 83: 233–242

Farrell RE, Walley FL, Lukey AP, Germida JJ (1993) Manual and digital line-intercept methods of measuring root length: a comparison. Agron J 85: 1233–1237

Floris J, De Jager A (1981) Een schatting van het verlies aan drogestof en van de verandering in diameter van wortels van engels raaigras (*Lolium perenne*) dor bemonsteren, bewaren en spoelen. Inst Bodemvruchtbaarheid Haren-Gr Rapp: 1–81

Floris J, Van Noordwijk M (1984) Improved methods for the extraction of soil samples for root research. Plant Soil 77: 369–372

Gaze SR (1996) Water balance of farmer-managed millet and fallow-savannah on sandy soils in south west Niger. PhD Thesis, Department of Soil Science, University of Reading, Reading

Gregory PJ, Tennant D, Belford RK (1992) Root and shoot growth, and water and light use efficiency of barley and wheat crops grown on a shallow duplex soil in a Mediterranean-type environment. Aust J Agric Res 43: 555–573

Grzebisz W, Floris J, Van Noordwijk M (1989) Loss of dry matter and cell contents from fibrous roots of sugar beet due to sampling, storage and washing. Plant Soil 113: 53–57

Hairiah K, Van Noordwijk M, Setijono S (1991) Tolerance to acid soil conditions of the velvet beans *Mucuna pruriens* var. *utilis* and *M. deeringiana*. I. Root development. Plant Soil 134: 95–105

Hairiah K, Van Noordwijk M, Stulen I, Meijboom FW, Kuiper PJC (1993) P nutrition effects on aluminium avoidance of *Mucuna pruriens* var. *utilis*. Environ Exp Bot 33: 75–83

Hansson A, Andrén O, Steen E (1991) Root production of four arable crops in Sweden and its effect on abundance of soil organisms. In: Atkinson D (ed) Plant root growth: an ecological perspective. Blackwell, Oxford, pp 247–266

Henry CM, Deacon JW (1981) Natural (non pathogenic) death of the cortex of wheat and barley seminal roots, as evidenced by nuclear staining with acridine orange. Plant Soil 60: 255–274

Heringa JW, Groenwold J, Schoonderbeek D (1980) An improved method for the isolation and the quantitative measurement of the crop roots. Neth J Agric Sci 28: 127–134

Jackson RB, Canadell J, Ehleringer JR, Mooney HA, Sala OE, Schulze ED (1996) A global analysis of root distributions for terrestrial biomes. Oecologia 108: 389–411

Jawson MD, Elliot LE (1986) Carbon and nitrogen transformations during wheat straw and root decomposition. Soil Biology and Biochemistry, 18 (1): 15–22

Kalela EK (1950) On the horizontal roots in Pine and Spruce stands. Acta For Fenn 57 (2): 62–68

Kaspar TC, Logsdon SD, Prieksat MA (1995) Traffic pattern and tillage system effects on corn root and shoot growth. Agron J 87: 1046–1051

Kolesnikov VA (1971) The root system of fruit plants. Mir Publishers, Moscow

Kücke M, Schmid H, Spiess A (1995) A comparison of four methods for measuring roots of field crops in three contrasting soils. Plant Soil 172: 63–71

Mackie-Dawson LA, Atkinson D (1991) Methodology for the study of roots in field experiments and the interpretation of results. In: Atkinson D (ed) Plant root growth, an ecological perspective. Blackwell, London, 25–47

Marcum KB, Engelke MC, Morton SJ (1995) Rooting characteristics of buffalograsses grown in flexible plastic tubes. HortScience 30 (7): 1390–1392

Marsh B.a'B (1971) Measurement of length in random arrangements of lines. J Appl Ecol 8: 265–267

Mitchell AR, Shouse PJ, Rechel EA (1993) Using acetic acid to wash roots from the soil. Soil Sci Plant Anal 24: 15–16

Naab JB (1994) Interaction of canopy and root system in water use of potato. PhD Thesis, University of Reading, Reading

Newman EI (1966) A method for estimating the total length of root in a sample. J Appl Ecol 3: 139–145

Nielsen CCN (1990) Methodische und ökologische Untersuchungen zur Sturmfestigkeit der Fichte. In: Einflüsse von Pflanzenabstand und Stammzahlhaltung auf Wurzelform, Wurzelbiomasse, Verankerung sowie auf die Biomassenverteilung im Hinblick auf die Sturmfestigkeit der Fichte, Schriften aus der Forstl Fak Uni Göttingen ud Niedersächs Forst Versuchsanst. JD Sauerländers, Frankfurt

Nielsen CCN (1994) Aspects of sustainability by afforestation of agricultural set-aside areas: Development of roots and root/shoot-ratios (AIR3-ct93-1269), 1st Progress Report, Royal Vet Agric University, Kirkegaardsvej 3A, DK-2970 Hoersholm

Pallant E, Holmgren RA, Schuler GE, Mccracken KL, Drbal B (1993) Using a fine root extraction device to quantify small diameter corn roots (≤0.025 mm) in field soils. Plant Soil 153: 273–279

Parr JF, Papendick JR (1978) Factors affecting the decomposition of crop residues by mircooorganisms. In: Iscwald WR (ed) Crop residue management systems. American Society of Aoronomy, Madison, pp 101–129

Passioura JB (1980) The transport of water from soil to shoot in wheat seedlings. J Exp Bot 31: 335–345

Prior SA, Rogers HH (1992) Portable soil coring system that minimizes plot disturbance. Agron J 84: 1073–1077

Prior SA, Rogers HH (1994) A manual soil coring system for soil-root studies. Commun Soil Sci Plant Anal 25: 517–522

Richards D, Goubran FH, Garwoli WN, Daly MW (1979) A machine for determining root length. Plant Soil 32: 69–76

Rowse HR, Phillips DA (1974) An instrument for estimating the total length of root in a sample. J Appl Ecol 11: 309–314

Schroth G, Kolbe D (1994) A method of processing soil core samples for root studies by subsampling. Biol Fertil Soils 18: 60–62

Schuurman JJ, Goedewaagen MAJ (1971) Methods for the examination of root systems and roots, 2nd edn. Pudoc, Wageningen

Sivakumar MVK, Salaam SA (1994) A wet excavation method for root shoot studies of pearl-millet on the sandy soils of the Sahel. Exp Agric 30: 329–336

Smucker AJM (1992) Contemporary analytical methods for quantifying plant root dynamics. In: Hübl E, Lichtenegger E, Persson H, Sobotik M (eds) ISSR Symp. Root ecology and its practical application. Klagenfurt, Austria, pp 721–726

Smucker AJM, McBurney SL, Srivastava AK (1982) Quantitative separation of roots from compacted soil profiles by the hydropneumatic elutriation system. Agron J 74: 500–503

Spek LY, Van Noordwijk M (1994) Proximal root diameters as predictors of total root system size for fractal branching models. II. Numerical model. Plant Soil 164: 119–128

Steen E (1984) Variation of root growth in a grass ley studied with a mesh bag technique. Swed J Agric Res 14: 93–97

Steen E (1991) Usefulness of the mesh bag method in quantitative root studies. In: Atkinson D (ed) Plant root growth: an ecological perspective. Blackwell, Oxford, pp 75–86

Tanaka S, Yamauchi A, Kono Y (1993) Cultivar difference in response of root system to nitrogen application in rice plant. Jpn J Crop Sci 62: 447–455

Tardieu F, Manichon H (1986) Caractérisation en tant que capteur d'eau de l'énracinement du maïs en parcelles cultivées. I. Discussion des critères d'étude. Agronomie 6: 345–354

Taylor HM, Upchurch DR, Brown JM, Rogers HH (1991) Some methods of root investigations. In: Mc Michael BL, Persson H (eds) Plant roots and their environment. Elsevier, London, pp 553–562

Tennant D (1975) A test of a modified line intersect method of estimating root length. J Ecol 63: 995–1001

Tennant D (1976) Root growth of wheat. I. Early patterns of multiplication and extension of wheat roots including effects of levels of nitrogen, phosphorus and potassium. Aust J Agric Res 27: 183–196

Van Noordwijk M (1993) Roots: length, biomass, production and mortality. Methods for root research. In: Anderson JM, Ingram JSI (eds) Tropical soil biology and fertility, a Handbook of Methods. CAB International, Wallingford, pp 132–144

Van Noordwijk M, Brouwer G (1991) Quantitative root length data in agriculture. In: McMichael BL, Persson H (eds) Plant roots and their environment. Proc ISRR-Symp, Aug 21–26, 1988, Uppsala, Sweden. Elsevier, Amsterdam, pp 515–525

Van Noordwijk M, Floris J (1979) Loss of dry weight during washing and storage of root samples. Plant Soil 53: 239–243

Van Noordwijk M, Purnomosidhi P (1995) Root architecture in relation to tree-soil-crop interactions and shoot pruning in agroforestry. Agrofor Syst 30: 161–173

Van Noordwijk M, Floris J, De Jager A (1985) Sampling schemes for estimating root density in cropped fields. Neth J Agric Sci 33: 241–262

Van Noordwijk M, Spek LY, De Willigen P (1994) Proximal root diameters as predictors of total root system size for fractal branching models. I. Theory. Plant Soil 164: 107–118

Veller F (1971) A method for studying the distribution of absorbing roots of fruit trees. Exp Agric 7: 351–361

Vogt KA, Persson H (1991) Measuring growth and development of roots. In: Lassoie JP, Hinckley (eds) Techniques and approaches in forest tree ecophysiology. CRC Press, Boca Raton, pp 477–501

Walters SA, Wehner TC (1994) Evaluation of the US cucumber germplasm collection for root size using a subjective rating technique. Euphytica 79: 39–43

Wilhelm WW, Norman JM, Newell RL (1982) Semiautomated x-y-plotter-based method of measuring root length. Agron J 74: 149–152

Williams TE, Baker HK (1957) Studies of root development of herbage plants. I. Techniques of herbage root investigations. J Br Grassl Soc 12: 49–55