CHAPTER 5

Sampling Strategies, Scaling, and Statistics

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5.1 Introduction

Many experiments aim to understand how some aspect of the aerial and soil environment of a plant influences its growth. Considerable biomass is often allocated to the root system, and it is the roots that absorb most nutrients and water

A.L. Smit et al. (Eds.), Root Methods

(Russell 1977). The property of the root system which is most appro-priate to measure depends on the objective of the experiment. Root length should be measured to calculate the inflow rate of water and nutrients (see Chaps. 6, 13, 14). Root dry mass indicates the carbon allocation to the root system. Branching patterns and the number and lengths of each class of root, together with the distribution of root diameters gives a more complete picture of root architecture, but requires a large investment of labour. Detailed information on root architecture may be of interest in constructing mathematical models of root growth (see Chap. 4), in comparing the structure and function of root systems of different species or genotypes (e.g. Fitter and Stickland 1991), and in evaluating root responses to environmental conditions (e.g. Robinson 1994).

It is not easy to design an appropriate scheme to adequately sample the root system, because of the complex and variably branched structure of roots (see Fig. 5.1), the variability of root distribution in space and, most importantly, the opaque growing environment, which means the experimenter works continually "in the dark". Root researchers face the peculiar problem of only being able to see or sample a small fraction of the root system at any time, using sampling procedures that are often destructive (e.g. see Chaps. 6 and 7). The design of sampling schemes for roots that are adequate and appropriate for different situations has received little study. This is despite the large effort that has been directed, and sometimes misdirected, towards sampling roots in the field. Many studies have considered different techniques for measuring roots, but the problem of where to sample, and how big a sample to collect, is rarely considered (van Noordwijk et al. 1985).

The "problem" of large variation between replicate samples of roots is widely known. This has often been seen as a nuisance, making it necessary to sample many times to obtain an accurate estimate of the mean. However, it is becoming recognized that the variation itself is worthy of study. This is also being seen in the soil sciences, where interest is increasing in the study of soil heterogeneity, and the resources that the root system is trying to exploit (e.g.

Fig. 5.1. Root systems vary widely in architecture and in spatial distribution. Examples are shown of: a A 54-year-old Austrian pine (*Pinus nigra*) in sandy soil in the Netherlands – roots weighed a total of 30 kg and extended about 5 m from the base of the tree. b An excavated horizontal lateral root of a 3-year-old rubber tree (*Hevea brasiliensis*) in sandy soil in the Ivory Coast. c The thick vertical taproot and horizontal lateral roots near the base of the trunk of a 3-year-old rubber tree. In deeper horizons the lateral roots are finer and less horizontal. d An excavated nodal axis of maize (*Zea mays* L.) at silking, in a loamy clay soil at Colmar. e Maize grown for 690 Kd (above base temperature 6 °C; seven visible leaves present) in aeroponics. f and g Root systems of bean (*Phaseolus vulgaris*) and rye-grass plants grown in root boxes for 2 weeks under glasshouse conditions. Photos kindly supplied by Drs. F. Egas (a), Yannick Le Roux (b,c), S. Pellerin (d,e), and F. Meijboom (f,g)

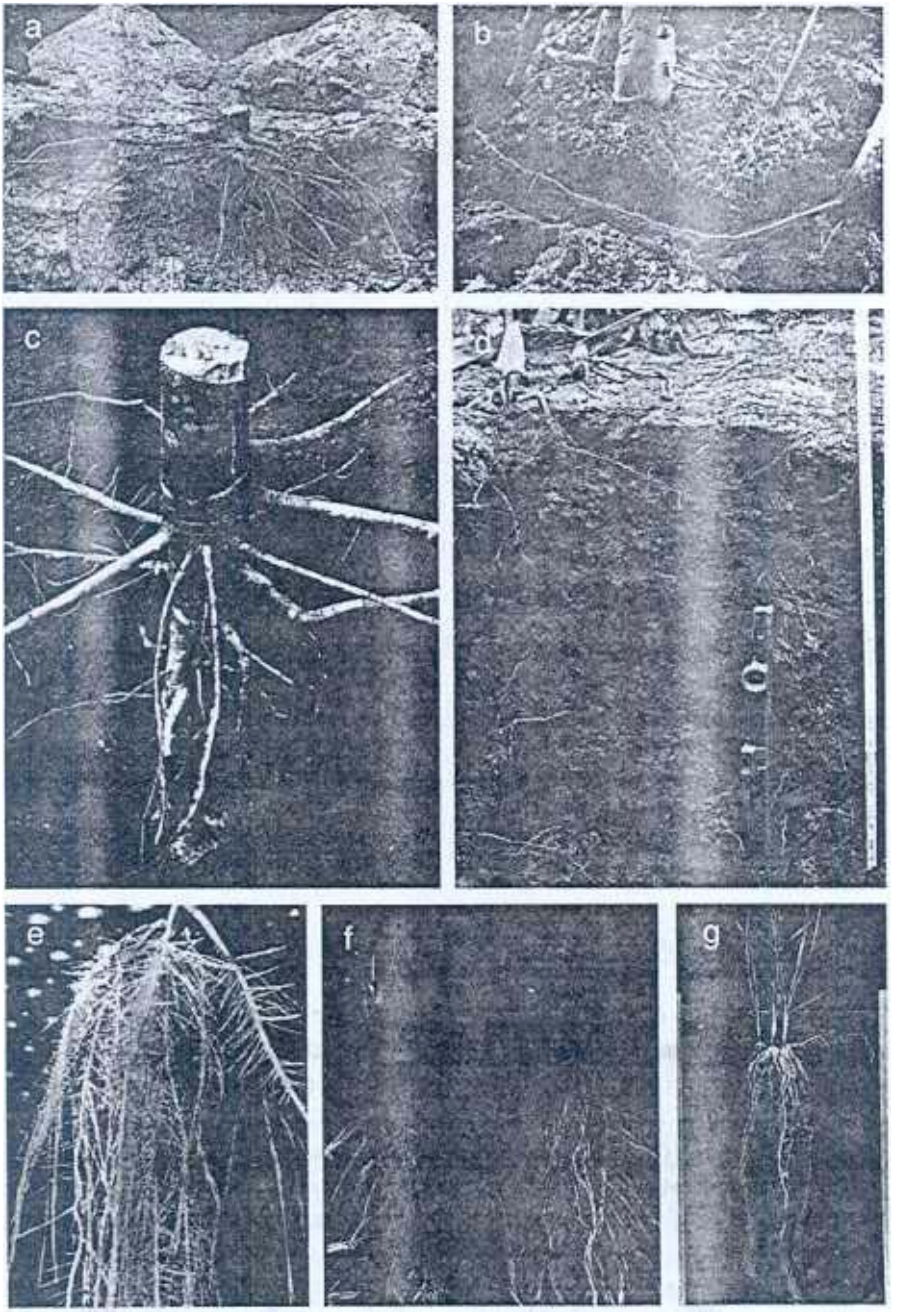

Fig. 5.1a–g

Jackson and Caldwell 1993a,b). A plant with a root system that explores an area of soil with a 50 cm radius may experience a variability in nutrient distribution equivalent to that across a 100 m^2 field plot (Jackson and Caldwell 1993b). A root system that is distributed non-uniformly can extract water and nutrients much faster from areas where the root length density is large. In contrast, resources may be largely inaccessible in regions of the soil that are rooted sparsely. The superposition of soil and root data in space promises to give new insights into how roots explore the soil environment.

The aim of this chapter is to guide the reader towards designing effective schemes for sampling roots in the field. Specific statistical analyses appropriate to particular methods are discussed in the appropriate technique chapter (e.g. cohort analysis in Chap. 9). We examine the spatial properties of root systems, and the problems associated with the techniques for measuring them. The coefficients of variation of root systems of different crops are detailed to help determine the likely number of samples required. The potential uses of root growth models in experimental design are described, together with how geostatistics can be used to study spatial variability.

5.2 Root System Structure and Spatial Distribution

In the following sections, we identify three main properties associated with the spatial distribution of root systems: (1) systematic trends in the root length density of root systems – i.e. large scale (several decimetres) gradual variations; (2) root clustering – variations at a small scale (several centimetres); and (3) root anisotropy – the non-uniform directional distribution of roots.

5.2.1 Systematic Trends

The form of a root system depends both on the plant genotype and on its interaction with the environment. Species may be grouped into classes that have broadly similar forms of root system. Monocotyledons generally form dense fibrous root systems. The seminal and nodal axes of cereals and other grasses produce several successive orders of fine lateral roots. The total root length of a single 16-week-old winter rye plant (*Secale cereale*) was measured to be more than 500 km long (Dittmer 1937). Dicotyledons form root systems often initially centred around a tap root from which many lateral roots emerge (e.g. sugarbeet, *Beta vulgaris*). Trees may also have a taproot system; a more surface "plateroot" system consisting of horizontal main root axes; or a "heartroot" system which consists of more evenly distributed main axes that split into smaller roots (Wilde 1958). Certain species of tree (e.g. *Populus tremuloides*) grow new shoots from

buds on roots in the surface layers of soil: Such groups of interconnected trees form very extensive root systems, exploring huge volumes of soil.

The depths and distributions of root systems vary enormously according to species, climatic zone, and soil type. In a comprehensive review of rooting depths around the globe, maximum rooting depths varied between <0.3 m in some tundra species, to 68 m for *Boscia albitrunca* in the Kalahari desert (Canadell et al. 1996). Maximum rooting depths averaged about 9 m in the desert, with only about half of that root biomass being in the top 0.3 m (Jackson et al. 1996). In contrast, tundra vegetation averaged 0.5 m maximum rooting depth, with 80–90% of root biomass being in the top 0.3 m. When plants are grouped across biomes by functional group, trees, shrubs and herbaceous plants had global average rooting depths of 7 m, 5.1 m, and 2.6 m respectively.

The length of root per unit ground surface area has been reviewed briefly by Newman (1969). Perennial grasses had the densest root systems, with 360–3400 cm/cm^2 of root in the top 10 cm layer of soil. This corresponds to 36 to 340 cm/cm^3 volume of soil. Cereals and non-*gramineae* herbs had smaller root lengths per unit area than grasses, but generally greater root lengths per unit area than woody shrubs and trees.

The spatial distribution of roots depends on the spacing and arrangement of individual plants in an ecosystem (van Noordwijk et al. 1985). In grassland, the plants are spaced closely in all directions, so that the root length density varies mainly in one dimension with depth. In cereal crops planted densely in rows, the pattern of root length density is essentially two-dimensional. As distance between plants increases, the pattern becomes three-dimensional. In agroforestry systems, trees, often arranged in rows, are interplanted with grass or an arable crop, leading to two interacting root system structures. Large variations in the height of the soil surface, as occurs across the ridges and furrows of the potato crop, create even more complex three-dimensional patterns (e.g. Parker et al. 1991). Natural ecosystems in which plants are randomly spaced or clumped on an undulating landscape may mean that the plants have to be treated as isolated individuals or small groups, without any of the symmetry afforded by agricultural practice.

Root systems originate from seeds, rhizomes or shoots that are near to the surface of the soil. The root system extends and branches gradually, extending away from this origin. This creates a pattern of root length density that is oriented centrifugally. Root length density decreases with increasing distance from the stem base, both in the horizontal and in the vertical directions (Gajri et al. 1994). Gradients in soil characteristics, such as soil-water content, soil strength, and temperature, interact with the development of the root system (e.g. Tardieu and Pellerin 1990). Such gradients often restrict the spread of the root system with depth but, in practice, it is very difficult to separate the effects of genotype and environment on root distribution.

The gradual decrease of root length density with depth is a feature that is general to most root systems. A negative exponential function has been used to model the relationship between root distribution (measured as length density or root mass) and depth for vegetable crops, cereals, and grasses (Gerwitz and Page 1974). The exponential model accounted for at least two thirds of the variation in 70 of 101 cases.

The variation in root length density across a crop has been measured by several authors (see Fig. 5.2; van Noordwijk et al. 1985; Gajri et al. 1994; Pellerin and Pagès 1996). The variation was greatest near the surface for crops grown in widely spaced rows. The variation was small in established forest stands (Persson 1978). The horizontal variation in root length density tends to decrease with increasing depth, because the "fan" shape of the root systems tends to smooth out fluctuations between neighbouring plants.

5.2.2 Clustering

Large variations in root length density occur across distances of several centimetres because of root clustering or clumping. Roots cluster in space because of their branched connections with daughter roots necessarily concentrated close to their mother root (Logsdon and Allmaras 1991). The constraint caused by the connection of roots, and the consequent aggregation of roots in space is exacerbated in root systems that have many short branches, with short link lengths between branches (e.g. Varney et al. 1991). A large percentage of the total root length is segmented into roots shorter than 10 cm, even in root systems that explore a total volume of several cubic metres (Lyr and Hoffman 1967). Such short roots are generally much thinner than the main axis, and can represent an economical way for the plant to increase the surface area of the root-soil interface, and perhaps exploit a nutrient source before its competitors.

Clustering of roots can also be induced by changes in the local environment of the root. Roots grow preferentially in cracks or biopores in compacted soil, such that the soil space is colonised irregularly (Wang et al. 1986; Tardieu 1988; Logsdon and Linden 1992). Patches of nutrients or residues from previous crops may also cause local increases in root length density, and this localised branching has become the subject of many studies (see review by Robinson 1994).

5.2.3 Anisotropy

Anisotropy is the non-uniform directional distribution of roots. It is the result of both morphogenetic and environmental factors, which interact during

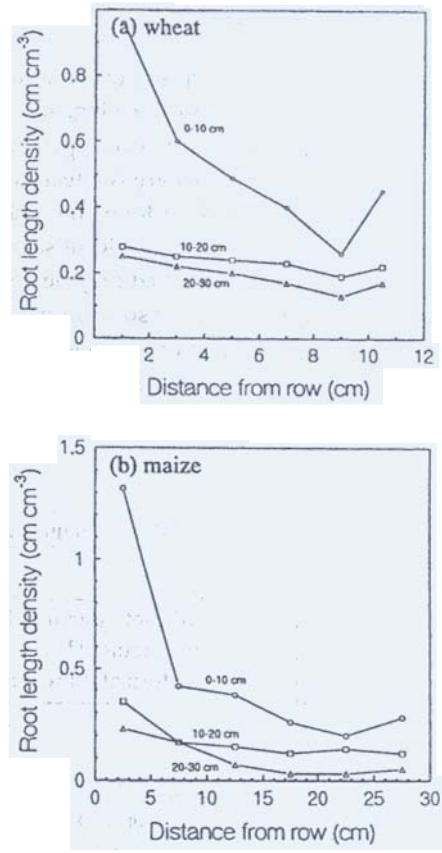

Fig. 5.2. Root length density at different distances from rows of crops of a wheat (row spacing 22 cm), and b maize (row spacing 60 cm). (Data from Gajri et al. 1994)

the development of the root system. Tropisms, and especially gravitropism, orient root growth in defined directions, according to the root type: the orientation is vertical for orthogravitropic roots, inclined at an angle for plagravitropic roots, and is horizontal for diagravitropic roots (Coutts 1989). The pattern of root branching also makes some root directions more likely. In some diarch surface roots of trees, for example, branching takes place only in a horizontal plane, resulting in a typically anisotropic pattern (Wilson 1964). Many soil properties (e.g. temperature, water content, strength) and morphological features, are structured in space, and contribute to root anisotropy (Logsdon and Linden 1992). Compact layers of soil, such as a tillage pan, can deflect roots horizontally for considerable distances, leading to anisotropy. This anisotropy is particularly marked when the main tap root of a plant encounters an impenetrable layer, as illustrated in Bennie (1996), for the tap root of cotton.

5.3 Choosing a Measurement Technique

The main steps in deciding on an appropriate sampling scheme are discussed in the following sections (see Fig. 5.3). Details of the various root system properties and measurement techniques are given in Table 5.1, with reference to appropriate chapters where the techniques are described.

It is first essential to focus on a clear research question, so that resources are used to maximum effect. Root sampling and measurement is always labour intensive, and the workload may be decreased by concentrating measurements on particular layers of the soil, or on particular samples. In the time that it takes to measure accurately the root length density in one sample containing 5 cm cm^{-3} of root, several samples containing 0.5 cm cm^{-3} may be measured. To determine, for example, the volume of soil from which a root system can extract water

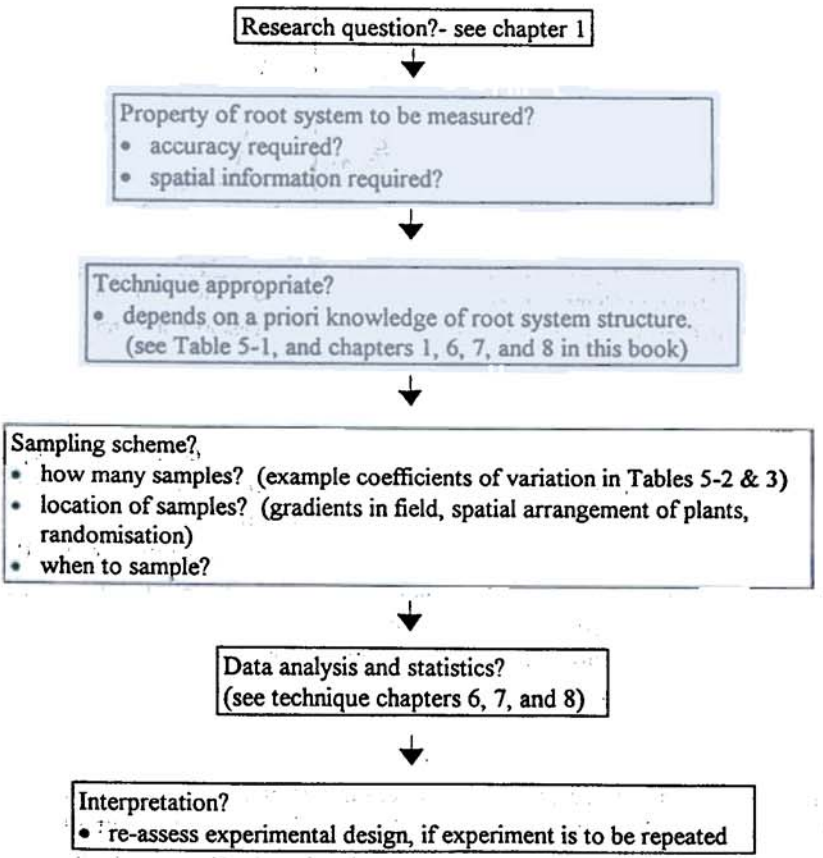

Fig. 5.3. Flow diagram of steps in deciding on experimental technique and sampling procedure

Table 5.1. Root system properties, measurement techniques, and their associated advantages and problems

Property	Technique and description	Advantages	Problems
Root length density in cores	Auger (Chap. 6). Soil cores sampled. Roots washed from cores in the lab	Most accurate method for root length density	Labour intensive. Many (e.g. 30%) roots lost during washing. Sample size determined by auger
Root end distribution in excavated planes	Trench wall and root mapping techniques (Chap. 7). Vertical or horizontal planes are excavated in the soil to expose root ends. Root end locations are plotted on transparent sheet, and number of ends per unit area calculated	Relatively fast. Good spatial resolution	Many fine roots go undetected. Problems converting measurements to root length density
Root end distribution in excavated cores	Core-break technique (Chap. 7). Root ends are counted on the face of a cut soil core	Relatively fast	Many fine roots go undetected. Problems converting measurements to the root length density. Sample size determined by auger
Root end or length distribution on cylindrical surface	Minirhizotron (Chap. 8): roots are observed with an endoscope around a transparent cylinder buried in the soil	Non-destructive, in situ. Can monitor temporal variation	Equipment expensive. Root grow preferentially along tube walls. Poor correlation with root length density

Table 5.1. (*cont.*)

Property	Technique and description	Advantages	Problems
Root diameter and branching patterns	Minirhizotron (above)	(as above)	(as above)
	Rhizotron (Chap. 8): Roots observed at a soil-glass interface	(as for minirhizotron, above)	Expensive facility. Roots growth preferentially along interface.
	Pinboard (Chap. 6): a board containing nails arranged in a regular grid is pushed into an excavated plane. The block of pinned soil is then then washed to expose the roots	Spatial distribution of roots in soil. Interactions between species	Roots disturbed and lost during washing
Whole root system architecture	Excavation and washing (Chap. 6) – with woody root systems, much of the soil may be washed or or blown from the root system to expose the main roots	Three-dimensional root distribution	Much fine root material lost. Roots moved during excavation
Root turnover	Rhizotron/minirhizotron (Chap. 9)	Non-destructive	Preferential root growth along interface. Equipment expensive. Difficult to determine live and dead roots

effectively, it may be more efficient to concentrate on measuring many samples that are rooted sparsely, than on measuring a few densely rooted samples accurately. Under some circumstances it may be appropriate to avoid measuring root growth and, instead, measure some function associated with roots: Gregory et al. (1978) monitored the advancing rooting front in wheat by measuring the movement of the drying front using a neutron probe.

Measurement of washed samples is very rapid using image analysis techniques (discussed in Chap. 10), but much time is required to spread root samples before analysis. Simple visual comparison of washed samples with standards of known length can provide faster but more approximate measurements of root length, with less preparation of samples than is required normally for image analysis (see Chap. 6 for visual comparison techniques).

It is impossible to set rigid rules for balancing all aspects of experimental design. The best approach depends crucially on the particular research question, the priorities and resources of the investigator, and a sound knowledge of the current literature.

5.4 Sampling Design

Two approaches based on different concepts are possible: firstly, we may excavate a given root system, individual root, or part of a root. We shall call this "architecture-guided" sampling. Secondly, we may determine the density distribution of the root system in some volume of soil. We shall consider these two approaches separately. In both cases, good prior knowledge is essential of the way that root systems are structured and distributed.

5.4.1 Architecture-Guided Sampling

It is possible to measure the length and branching density of individual roots, by gradually following and excavating them, starting from the base of the shoot (e.g. Tardieu and Pellerin 1990; Pagès and Pellerin 1994; Fig. 5.1b,d). This type of sampling requires an appreciation of root type, morphology, and behaviour (e.g. Waisel and Eshel 1991). Various criteria can be used to classify roots during this sampling procedure (e.g. Le Roux and Pagès 1994). Roots are often classified by some developmental criteria, such as the origin of the roots (seminal, nodal, branch), or branching order (primary axis; first second or third order lateral). Roots in some developmental categories continue to appear throughout the life of the plant. Age is an important additional criterion for classifying the sub-population, because it affects both root morphology and root physiology. For example, the proportion of primary lateral roots of maize that can be

classified as determinate or normal, depends on the age of the parent root to which they are attached (Varney and McCully 1991). This information can be was obtained by careful excavation and sampling of individual nodal axes, followed by histological examination.

Spatial and temporal variation in the soil is superimposed on the endogenous variability between roots. In the field it is often difficult to assess the variation in soil conditions that a root has experienced during its existence. By studying the spatial distribution of a root system, it should also be possible to identify regions of soil of particular importance. For example, regions of root proliferation, or places roots have failed to penetrate could be selected for further study.

For trees and other perennials root architecture can also be approached by a study of fractal branching patterns. The basic assumption is that secondary thickening of roots is based on the demands for transport and thus at any point in the branched system transport capacity is proportional to the amount of fine roots distal to it. The proximal root diameter of a tree root (at the stem base) may contain enough information to reconstruct the total branched structure – provided that a number of simplifying assumptions hold, and that a number of parameters (which probably depend on tree species, but can otherwise be treated as constants) are known. The basic assumption of a "pipe-stem" model is that cross-sectional area (or the sum of diameter squares) is conserved during a branching event. Van Noordwijk et al. (1994) and Spek and van Noordwijk (1994) relaxed this assumption by introducing a proportionality factor alpha, but assumed that alpha is independent of current root diameter. A second parameter, q, describes the share of the largest "daughter" root in the total sum of squares of "daughters". To test the assumptions, one has to trace individual roots and take measurements of diameters before and after branching points (Van Noordwijk and Purnomosidhi 1995), although this is obviously a labour intensive process. If regression of alpha and q against diameter does not reveal any dependency, fractal (self-repetitive) models can be used. To re-construct three-dimensional structures of root systems, additional information on link ("inter node") length and branching angles is needed. Spek (1997) has since developed a visualisation routine by using a model originally developed for complex molecules. The overall prospects for this approach are relatively "quick and not-too-dirty" statements about tree root distribution, which can, if necessary, be backed up by detailed destructive sampling, but which can at least be used to specify sampling schemes in situations where tree roots are important.

5.4.2 Measuring the Distribution of Root Length Density

The root length density can be measured by separating the roots from a soil sample of known volume. Another method of assessing density relies on count-

ing the number of intersections that roots make with a surface of known area in the soil. The properties measured, with a brief description of the techniques, are listed in Table 5.1, together with some advantages and problems associated with the techniques. Fuller information about each technique is given in the appropriate chapters of this book as indicated in Table 5.1.

None of the techniques are entirely satisfactory: the auger technique is the standard method for measuring root length density although, using this technique, up to a third of the fine root length may be lost during washing. The root-mapping technique is better for measuring the spatial distribution of individual roots. It is possible theoretically to calculate the root length density from the density of root intersections with a plane, although correlations obtained from field data suggest that a large proportion of the fine roots are often overlooked using the root-mapping techniques, leading to underestimates in the root length density – this is discussed in Bengough et al. (1992).

5.4.3 Sample Location

When designing any field experiment, it is important to assess the major sources of variation in data. Standard texts on experimental design discuss these sources of variation (e.g. Pearce 1983), which can be divided into patterned and non-patterned sources. Factors, such as slope, soil fertility, soil depth, shading, and variation in soil texture, are sources of patterned variability, and their relative importance will vary with weather conditions between seasons. Much more information is now becoming available on the spatial variation of yield in arable crops, due to the advent of precision agricultural equipment, which makes use of global satellite position references (Robert et al. 1996). The yield data from such systems gives an indication of plant performance, but there is no fixed relation between yield and root system development. Sources of non-patterned variability include errors such as loss of root material during washing and measurement.

When sampling real root systems, it is not possible to recover the whole root system of individual plants. Special consideration must be given to deciding which part of the root system should be sampled. Root systems of neighbouring plants are often intermingled, although this depends on the species concerned: Nelson and Allmaras (1969) found that maize roots intermingled with neighbouring soybean roots to a much greater extent than with neighbouring roots of the same species. It is possible to define a "unit soil area" for crop plants that are spaced regularly (Fig. 5.4, after van Noordwijk et al. 1985). The soil below each unit area is expected to contain a total root length equal to the mean root length per plant. Many of the roots within the unit area may belong to neighbouring plants but, similarly, an equal number of roots from the plant may have extended outside the area.

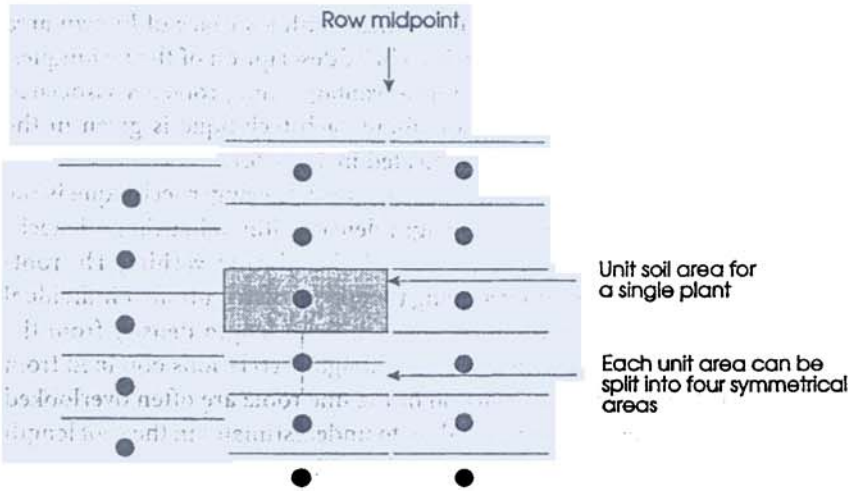

Fig. 5.4. Plan view showing unit area for single plants, represented by *black circles*, in a row crop. By symmetry, each unit area consists of four smaller representative areas. (After van Noordwijk et al. 1985)

The unit area can be divided into four equal parts which are equivalent, because of symmetry. The quarter area represents the fundamental unit in which the distribution of root length density should be studied. The most appropriate sampling schemes have been modelled for a range of crops (Fig. 5.5; after van Noordwijk et al. 1985). The assumptions of root distribution that underlie the choice of these sampling schemes are discussed in detail in van Noordwijk et al. (1985). Systematic trends in the root length density can bias the estimates of mean root length density, if the way that the quarter area is sampled over-represents either the dense or sparsely rooted areas (Fig. 5.5). Simply averaging the root dry weight in the row and the inter-row of cereals can overestimate the total root dry weight by as much as 30%.

5.4.4 How Big a Sample, and How Many Samples?

Clustering of roots, and the associated variability in root length density is an important characteristic of the root system (Tardieu 1988; Logsdon and Allmaras 1991). The size of the sample taken using an auger determines the minimum scale on which the variation of the root length density will be detected. Clustering increases the variance between samples and so, to achieve a given precision, more samples are required.

The average root length density measured for two populations can be compared using a T-test, provided that the root length density data are normally

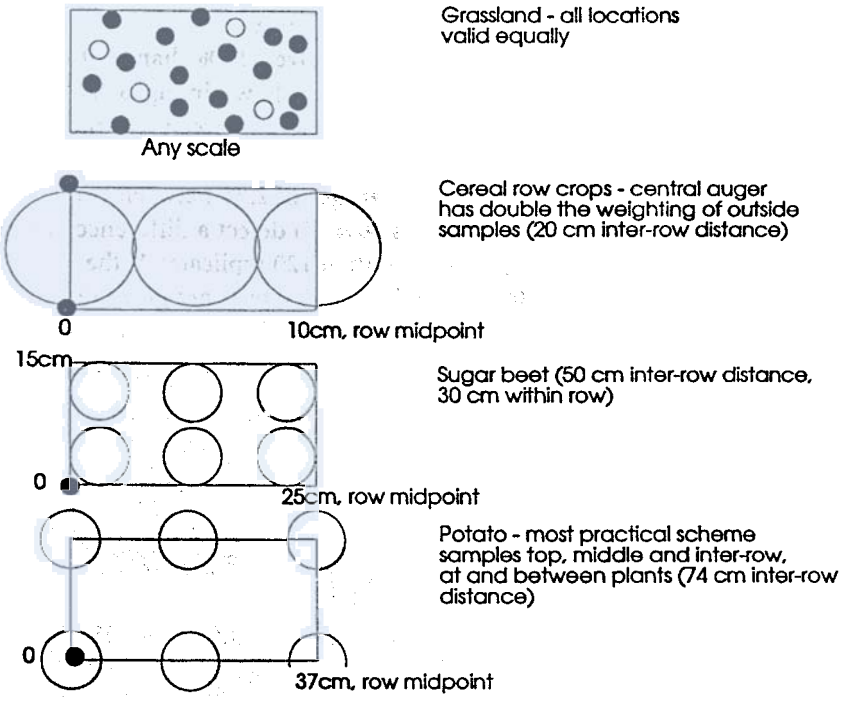

Fig. 5.5. Plan view of locations where samples should be taken in grassland and in crops of cereals, sugar beet and potato. *Black circles* represent the plants, *open circles* represent sampling locations using a cylindrical auger. (After van Noordwijk et al. 1985)

distributed, and that the variance for the two data sets is similar (see Box 5.1). The number of replicates required to give a good chance of finding a significant difference between treatments is discussed in Box 5.1 for a T-test. Approaches to estimating sample number for more complex experimental designs, for example involving blocking, are discussed in Mace (1964, especially Chap. 3) and Cochrane and Cox (1957, especially Chap. 2).

If the assumptions associated with parametric statistics are invalid (e.g. the quantity is not normally distributed), it may be possible to transform the data so that the transformed values can be tested. Failing this, or for rank or root count data, it is necessary to use non-parametric statistical tests, such as the Chi squared test. These tests can be relatively simple to perform, although they use less information than the parametric tests, and so are weaker.

In planning an experiment it is necessary to have some prior estimate of the degree of variation that is expected. In Tables 5.2 and 5.3 the coefficients of variation are listed for measurements made using the auger technique from crops grown under a variety of conditions. In the crops studied the coefficient

BOX 5.1. Number of Samples Required for T-test

The number of replicates that is required to give a 50% chance of detecting the difference between two means is shown in Fig. 5.6. More replicates are required to detect small differences between treatments, or for populations with a large coefficient of variation. For example, at least 25 replicates are needed to detect a difference of 22% between two means, if the coefficient of variation is 40%. To detect a difference of just 10% between two means requires more than 120 replicates, if the coefficient of variation is 40%. More details of T-tests are given in standard statistical texts (e.g. Sanders 1990)

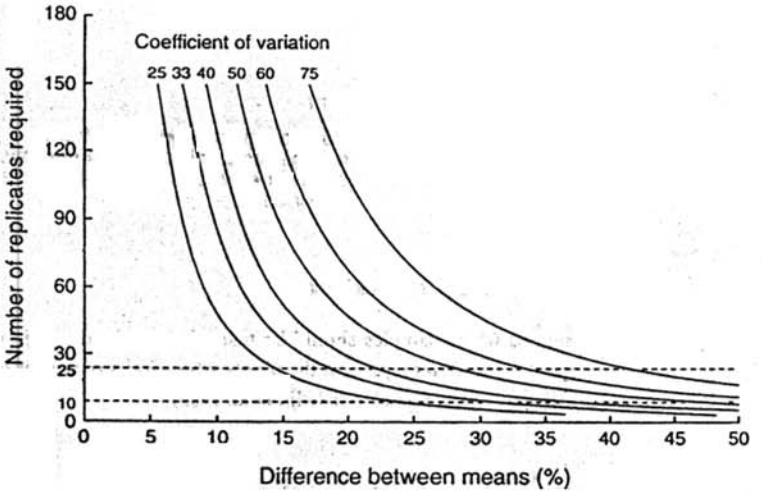

Fig. 5.6. Plot of the number of replicates required to give a 50% chance of distinguishing between two means in a two-sided t-test at 95% significance level. (After van Noordwijk et al. 1985)

of variation increased with depth of sampling (Tables 5.2 and 5.3). The reason for this may be partly associated with the sparse rooting of these deeper layers, with relatively few main axes present, surrounded by associated clusters of lateral branch roots (Grabarnik et al. 1998). Another contributing factor is changes in the soil structure with depth – in compacted subsoils, roots may be confined to continuous cracks and biopores in the soil to a much greater extent (e.g. Ehlers et al. 1983).

The coefficients of variation for root dry weight in auger samples from grassland are typically between 30 and 50% (Table 5.3). There is considerable variation between the coefficient of variation of root dry weights measured in

Table 5.2. **a** Coefficients of variation for root length density from Kucke et al. (1995). Samples were taken from fields of sugar beet, wheat and rye, using an auger (6.5 cm diameter by 15 cm long) from soils of different texture. **b** Coefficients of variation for the root length density of maize. Samples taken using an auger (5.1 cm diameter by 15 cm deep)

a

Depth (cm)	Coefficients of variation (%)		
	Sand[a]	Loam[a]	Clay[b]
0–15	12	27	
15–30	28	8	54
30–45	76	12	57
45–60	129	60	
60–75	74	71	69
75–90	113	35	60

[a] Sugar beet followed by winter wheat.
[b] Sugar beet followed by rye.

b

Depth (cm)	Coefficients of variation (%)	
	45 Days after emergence	59 Days after emergence
15	42	30
30	42	22
45	33	49
60	71	61
75	54	45
90	69	53

different studies – they average 38% for studies j to l in Table 5.3, but 59% for m1 and n1. Data from samples taken in the row should not be pooled with data from samples taken between the rows, as the root samples represent distinct populations. This is shown by the increased variance when the data is pooled (see columns headed m3 and n3 in Table 5.3). The root weight is large in the surface layer of soil immediately around the stem base. The ratio of the mean root dry weight in the row to that between rows is generally bigger than for the ratio of root length densities. This is because the root mass per unit length is normally greatest in the topsoil at the base of the stem. The coefficient of variation for root length density of cereals is often between 30 and 70% (Tables 5.2a,b), depending on the particular study.

Table 5.3. Coefficients of variation of root dry weight in auger samples of grassland and cereals.[a] (After van Noordwijk et al. 1985)

Depth (cm)	Grassland									Cereals								
	Ref	a	b	c	d	e	f	g	h	i	j	k	l	m1	m2	m3	n1	n2	n3
	Samples	100	100	20	20	20	20	20	50	50	25	25	20	4	4	8	47	32	103
	Diameter	7	4	7	7	7	7	7	4	4	7	7	7	7	7	7	105	36	70
0–5				34	30	51	45	41	29	43	30	43		41	89	100	36	31	33
5–10		33	38	34	36	33	48		29	37				83	56	73	42	44	43
10–20				30	45	44	30	55	36	50	34	27	47	40	32	37			63
20–30				36	41	40	38	75	43	56	31	28			64	65			59
30–40				35	41	55	38	53	35	38	36	37	43	45		69			46
40–50		35	41	52	31	48	47	59	46	31	35	29				78			45
50–60				44	44	54	54	76	51	53	39	35	50	54		46			62
60–70								100	56	39	56	47				48			106
70–80								85	75	46	53	53				43			125
80–90								76			61	63				72			
90–100								76			44	50				54			

[a] The key to column headings is as follows: a, b homogeneous grassland (1949); c, d young grassland (at Gilze, 1966); e, f same fields as c and d, 4 years later (June 1970); g established grassland (1976); h, i established grassland: root dry weight and root counts (estimates from Schuurman and Knot 1957); j, k oats: root dry weight and root counts (estimates); l winter wheat on cracking clay soil (Biddinghuizen, May and June 1977); m, n spring wheat on clay loam and sandy loam, respectively (Ulrum 1957); m1, n1 samples in row; m2, n2 samples between rows; m3, n3 equal number of row and between row samples combined.

5.5 New Developments

5.5.1 Using Root Growth Models to Improve Sampling Strategies

Schemes for sampling root systems must be designed carefully to obtain good estimates of the total dry weight or root length density in a layer of soil below a crop. Schemes used traditionally for cereal crops, for example, can result in a bias of up to 30% in the total root dry weight (van Noordwijk et al. 1985). Experiments that compare different sampling schemes are labour intensive and subject to experimental errors that are difficult to quantify and are associated with the excavation, washing and measurement of roots. An alternative approach, which is complementary to experimental comparisons, is to use a model of root distribution based on existing knowledge. Examples are becoming more common in the literature of using models to evaluate the sampling procedure for root systems, and this is a promising area in experimental design.

The types of model used range from simple exponential functions describing root distribution (van Noordwijk et al. 1985) to simulations of biomass fluctuation with time (Singh et al. 1984), and sophisticated simulations of root architecture in three-dimensional space (Nielsen et al. 1997; Pagès and Bengough 1997; Grabarnik et al. 1998). The basic approach using these models is the same, although the sophistication and applications of the models may vary: the model is used to generate a theoretical root distribution in space or time. This root distribution is then sampled or measured in a particular way, to simulate some sampling procedure. The simulation can be repeated many times very rapidly, as it is performed numerically on a computer. The sensitivity of sampling schemes to changes in the root distribution can be quantified by systematically varying model-input parameters.

The effect of sample timing and frequency on estimates of root biomass production was investigated using a model of root biomass for prairie grasses (Singh et al. 1984). The model was used to simulate variations in biomass with time, and the effects of sample timing, variability, and replication were studied. The estimate of biomass production was compared using two methods of calculation: firstly, from the difference between maximum and minimum biomass values and, secondly, from the summation of increments in biomass across the season. Estimates of biomass production were between 1.8 and 7 times greater than the actual production, suggesting that existing sampling schemes were inadequate. The use of a model allowed the effects of sample timing and replication to be investigated in more detail than is practical in most field experiments, although the details of the study were controversial (Lauenroth et al. 1986; Vogt et al. 1986).

Relations between root length density and root intersections with planes have been investigated with models that simulate root architecture in three-dimensions (Bengough et al. 1992). The use of minirhizotron tubes to measure rooting depth in a maize crop has been simulated using a three-dimensional model of root architecture of a small plot of 51 maize plants (Pagès and Bengough 1997). It was shown that the maximum rooting depth measured using minirhizotrons was very variable and could strongly underestimate the true rooting depth. Underestimation was a particular problem if the tube radius was smaller than 3 cm, and when the tube was close to the vertical. In this simulation study, however, the interactions between the growing root and the tube were not modelled. Some aspects of root growth and orientation alongside the minirhizotron tube may be modified by the presence of the tube itself, but there was insufficient detailed information on root-tube interaction to enable a realistic model of the interaction to be developed.

The fractal dimensions of two-dimensional projections of root systems have been compared with the three-dimensional fractal dimension of simulated bean root architecture (Nielsen et al. 1997). The model showed that the three-dimensional fractal dimension differed from that in two-dimensions, suggesting that the washing and flattening of the root system is not an acceptable way of measuring the fractal dimension of root systems in situ. The use of root intersection data with horizontal and vertical planes was found to give accurate estimates of the fractal dimension in three-dimensions, suggesting that this is a much better way of characterising root distribution in soil.

5.5.2 Applications of Geostatistics to Study Spatial Variability

Geostatistics is a relatively new technique that can be used to study the spatial variation of roots in a particular depth layer across a field site. It can also be used to study spatial variation in root systems with depth, although any systematic trends in vertical root distribution must first be subtracted. A major disadvantage of geostatistical techniques is that large numbers of samples (typically >100, but sometimes 300 or more; Jackson and Caldwell 1993b) are required, each at a known location, and at a range of separations.

The traditional statistical tests used in biology assume that the data in the populations being tested have the same distribution, and that each datum is independent of all other data. Roots form interconnected branched structures that may be correlated spatially and temporally: the presence of a root in a particular volume is often more likely if a root is present in a neighbouring volume, or was present when the volume was sampled previously. The scale and timing at which the sampling is performed determine whether such spatial and temporal correlations are present.

Geostatistics is a branch of applied statistics that can be used to detect, model and estimate spatial patterns. It was developed originally for mining and geology, but has been used more recently in the plant and soil sciences (e.g. Castrignano and Lopez 1988; Castrignano et al. 1994; Bourgault et al. 1997). In this chapter we give a very brief introduction to some geostatistical techniques. Concise introductory works that use examples from soil science and agronomy are those by Vieira et al. (1983), Burgess and Webster (1980) and Webster (1985) while Rossie et al. (1992) is a comprehensive review of geostatistical tools for ecology. Only a few studies have applied geostatistical techniques to root systems (Aiken 1992; Jackson and Caldwell 1993a,b). The techniques of variography and kriging are potentially very relevant: Variography models spatial dependence of variables, whilst kriging interpolates between the measured locations.

5.5.2.1 *Semivariograms*

Geostatistical techniques quantify spatial correlations and can be used to detect clustering of roots. Semivariance is a measure of the variability between pairs of observations and, in semivariograms, is plotted against the separation between those observations (Box 5.2).

Semivariograms have been used to study the spatial distribution of soil properties and roots (Jackson and Caldwell 1993a,b). A positive increase in semivariance with distance (Fig. 5.8) indicates clustering. The separation distance at which semivariance approaches a constant value is called the range, and is the length of spatial correlation, corresponding roughly to the dimension of clustering. Roots of an individual plant may experience very different conditions due to the heterogeneity of soil conditions. The distribution of nutrients (nitrate, ammonium, phosphate and potassium), has been studied on a scale of 0.1–10 m around perennial *Artemisia* and *Pseudoroegneria* plants (Jackson and Caldwell 1993b): nitrate and ammonium contents varied by between two and three orders of magnitude within the 120 m^2 plot, with phosphate and potassium showing smaller variation. Clustering of these nutrients occurred on a scale of <0.5 m (corresponding to the range), with no further increase in variance at scales greater than 1 m.

The semivariance at a separation equal to the range is known as the sill, and is an estimate of the population variance for observations separated by distances greater than the range. The semivariance extrapolated to zero separation is called the nugget, and represents all unaccounted for spatial variability at distances smaller than the smallest sampling distance.

The nugget includes both variation due to measurement errors and spatial variation that occurs over distances shorter than the sample spacing. The

BOX 5.2. Semivariance and Semivariograms

A semivariogram is constructed by plotting the semivariance as a function of the distance separating pairs of observations (Fig. 5.7). Semivariance is defined as

$$\gamma(h) = \frac{1}{2N(h)} \sum_{i=1}^{N(h)} [R(i) - R(i+h)]^2, \quad (5.1)$$

where $\gamma(h)$ is the semivariance at separation distance (h), $N(h)$ is the number of paired observations separated by distance h, $R(i)$ is some root variable (e.g. root number, length density or dry weight) at location i, and $R(i + h)$ is the same variable separated from i by a distance h (Vieira et al. 1983)

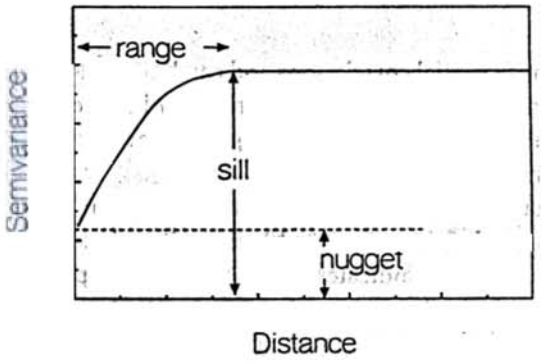

Fig. 5.7. Semivariogram showing idealised behaviour

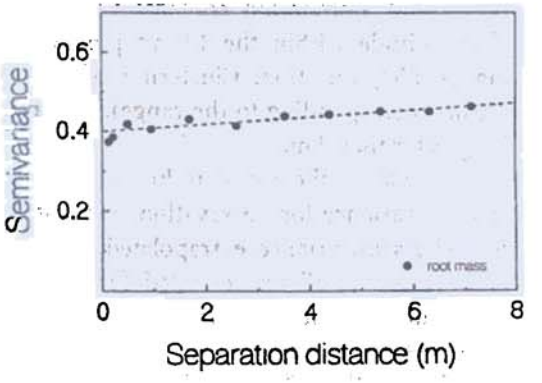

Fig. 5.8. Semivariogram from root mass data measured by Jackson and Caldwell (1993a)

difference between the semivariance at the sill and that at the nugget represents the variance that can be modelled as a spatial dependence, based on the available sampling grid. A small nugget/sill variance ratio indicates spatial correlation.

When values of semivariance are being compiled into a semivariogram, it is assumed that there are no systematic trends in the quantity measured (Aiken et al. 1991). If such trends exist, functions characterising these trends must first be subtracted from the data. For example, vertical trends must be removed before generating a variogram of root intersection density with minirhizotrons or washed root length density with depth. Semivariograms constructed from data containing trends show increasing semivariance with increasing distance between observations.

5.5.2.2 Kriging and Cokriging

Kriging is an interpolation procedure that uses the semivariogram to estimate values at locations where measurements were not made. The values estimated $[Z^*(x_o)]$ using kriging are linear functions of the known values $[Z(x_i)]$ measured at N other locations:

$$Z^*(x_o) = \sum_{i-1}^{N} [\lambda_i Z(x_i)], \tag{5.2}$$

where the weight factors (λ_i) are chosen such that the mean value of the difference $[Z^*(x_o) - Z(x_o)]$ is zero, and the variance of the difference is minimised. The semivariogram determines the values of λ_i and the relative weight of observations decreases with distance from the interpolation points.

Cokriging can be used where two or more variables are correlated in space. Measurements of one variable may be used to produce predictions of how a second variable is distributed. For example, very hard regions of the soil may be rooted sparsely, and so the root length density would be correlated negatively with penetration resistance. Measurement of one variable may be used to produce predictions of how a second variable is distributed.

5.5.2.3 Conditional Simulations

Kriging is a procedure for estimating optimal values at intermediate points on a map. However, no interpolation procedure can produce information that has not been surveyed. The real spatial variation in the quantity studied includes an extra component of variation that is not accounted for in the kriged values.

Conditional simulations show realistic images of the variability of the quantity studied. By running many of these simulations, the variance and mean values can be mapped and this map can provide a reliable basis for calculating root biomass, deciding on root sampling strategies, or for simulating water or nutrient uptake. Further details of the theory of conditional simulation are given in Matheron (1973).

5.6 Conclusions

Root systems are complex branched structures that vary in space and time. The structure of the root system must be appreciated before it can be studied in the field. It is important to consider the aims of any experiment carefully when choosing an appropriate measurement technique from the many available, as root sampling is very labour intensive. Auger sampling gives the most reliable estimate of root length density, but losses of about 30% can occur during washing procedures (see Chap. 6). Abias of about 30% for cereals can result in estimates of root length density, per plant or per layer of soil, made using traditional sampling schemes. The scientific basis of where to sample roots is still understood relatively poorly, and there is a need for new experimental and theoretical studies of root distribution to address this problem. Modelling is starting to become a useful aid in investigating the consequences of different temporal and spatial sampling schemes. Geostatistics is also providing new ways of studying spatial heterogeneity in roots, although large numbers of samples (e.g. 100 plus) are often required to provide sufficient data for the analyses.

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