A METHOD OF ESTIMATING THE TOTAL LENGTH OF ROOT IN A SAMPLE

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Research in many fields of plant ecology could be helped by quicker and more satisfactory methods of determining the amount of root in a volume of soil. Most quantitative studies of roots have used weight as the means of assessing the amount of root; but it is generally accepted that the capacity to take up water and salts is usually more closely related to the surface area or total length of the root system than to its weight. Interest in root length has been stimulated by papers by Gardner (1960, 1964) and Cowan (1965) dealing with water uptake from soil, in which length of root per unit volume of soil is an important parameter. The main difficulties in determining root length arise from the great lengths which can occur in even small volumes of soil. Some root densities which have been reported for plants growing outdoors are: 364–3434 m of root per litre of soil under grass swards (data of Pavlychenko quoted by Troughton 1957), 66–548 m/l under Graminae (Dittmer 1938), up to 21 m/l under coffee trees (Nutman 1934), up to 18 m/l under sugar-cane (Evans 1938). Thus, even if it is practicable to use soil samples as small as 0·1 l., direct measurement of the roots may take a long time. Indirect methods have been used: for instance, measuring root diameters and then determining root volume (Evans 1938); or measuring the length of a small portion of the root sample, then weighing this portion and the remainder. However, these methods are often inaccurate because of variations in the ratios volume : length and weight : length.

This paper describes a new method of estimating the total length of root in a sample, the line intersection method. I believe that it will in many circumstances prove more satisfactory than any existing method.

DESCRIPTION OF TECHNIQUE

Fig. 1 shows a rectangular area within which some straight lines lie at random. If a root is laid within the area, we should expect that the longer the root the more intersections it will make, on average, with the straight lines. Thus the number of intersections can be used to estimate the length of the root. It is shown in the Appendix that whatever the shape of the root, an estimate of its length is given by:

$$R = \frac{\pi N A}{2H} \tag{1}$$

where R is the total length of root, N is the number of intersections between the root and the straight lines, A is the area of the rectangle, and H is the total length of the straight lines. In the technique described here the straight lines are provided by a hair-line in a microscope eye-piece.

I have found the following technique satisfactory. The apparatus used includes a shallow, transparent, flat-bottomed dish and a binocular dissecting microscope with a hair-line disk in one eye-piece. Through the base of the dish marks are visible whose

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functions are: (i) to provide areas of different sizes within which the roots can be arranged, and (ii) to mark positions for the hair-line. If the hair-lines are to be regularly spaced, a grid of squares performs both functions. If the hair-lines are to be randomly spaced one needs: (i) several rectangles of different sizes: (ii) one or more sets of random points within each rectangle, marked with different colours or symbols; and (iii) parallel guide lines to help in scanning the area. If the roots are light-coloured I have found it best to draw these marks on polythene sheeting, which is then attached to the base of the dish, and to stand the dish on a dark background.

The roots are separated from the soil by washing, and stored in a beaker of water. When the length is to be determined, the roots and water are poured into the dish. If the roots hold together as a single mass, a few scissor cuts are made through it, and the roots then float apart with some mechanical assistance. The roots are arranged to occupy the whole of an area marked out on the base of the dish. If the roots are springy they are

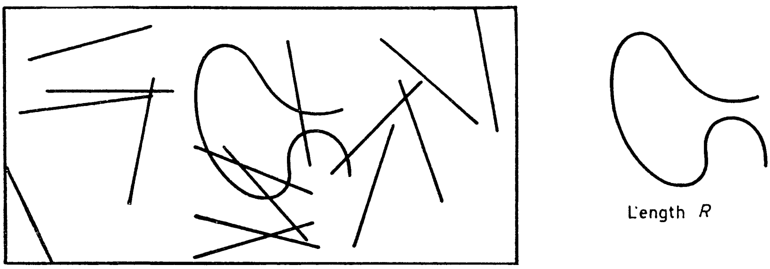

FIG. 1. Illustration of equation (1).

flattened by placing a sheet of Perspex on top of them. If all the roots are flexible they are not covered, but most of the water in the dish is removed by suction through a tube. Fields within the area occupied by the roots are then viewed through the microscope; the centre point of the hair-line is placed in turn over each random point or grid intersection. After each position has been reached, the microscope eye-piece containing the hair-line disk is rotated to give the hair-line a 'random' direction. Then a count is made of the number of intersections between the hair-line and the roots. An intersection is only counted if the hair-line crosses the centre line of the root. If the hair-line is randomly placed, sometimes part of it falls outside the area. When this happens, the proportion of the hair-line lying outside the area is estimated to the nearest tenth.

Examples of calculation

(i) *Microscope fields regularly spaced.* Roots arranged in area 10×20 cm; forty fields examined; apparent length of hair-line (= diameter of field) = 1·88 cm; 344 intersections. Using equation (1):

$$\text{Total length of root, } R = \frac{3{\cdot}14 \times 344 \times 10 \times 20}{2 \times 40 \times 1{\cdot}88} = 1436 \text{ cm.}$$

(ii) *Fields randomly placed; otherwise as above.* Total length of hair-line falling outside area = 19/10 hair-lines. Therefore effective total length of hair-lines = $(40 - 1{\cdot}9) \times 1{\cdot}88$ cm.

$$R = \frac{3{\cdot}14 \times 344 \times 10 \times 20}{2 \times 38{\cdot}1 \times 1{\cdot}88} = 1504 \text{ cm.}$$

PRACTICAL TESTS

The method was tested on six samples of root: two of bean (*Phaseolus vulgaris*), two of flax (*Linum usitatissimum*), one from a *Festuca elatior* sward, and one from an area of Duke Forest (North Carolina) where *Pinus taeda, Acer rubrum* and *Lonicera japonica* are the most abundant species. Five independent estimates of the length of each sample were obtained by the line intersection method, using regularly spaced microscope fields. For Bean A and Bean B five estimates were also obtained using random spacing. After each replicate estimation the roots were replaced in the beaker of water, then poured back into the dish for the next estimation. For random spacing a different set of random points was used for each replicate estimation. The three shorter samples (Flax A, Bean A and Forest) were also measured directly, by laying out each piece of root in a little water over graph paper. The three longer samples were not measured directly, but five sub-samples from each were measured. These sub-samples were then oven-dried and weighed, and so were the remaining roots. Each sub-sample thus provided an estimate of the total length of root, on the assumption that the ratio length : weight was the same in the sub-sample as in the whole.

Table 1. *Comparison of length estimates by line intersection method and direct measurement (lengths in metres with standard errors)*

Root sample	By line intersection: Random fields	By line intersection: Regular fields	By direct measurement
Flax A		3·76* ±0·07	3·43†
Bean A	7·57 ±0·18	8·05* ±0·18	7·31†
Forest		20·5 ±0·46	21·0†
Bean B	31·8 ±0·97	33·8 ±0·55	29·3 ±1·75‡
Flax B		36·5* ±0·55	41·1 ±0·98‡
Festuca		125·6 ±2·3	135·8 ±9·6‡

* Significantly different from result by direct measurement ($P = 0{\cdot}01-0{\cdot}05$).

† Whole root sample measured.

‡ Five sub-samples measured.

Table 1 compares the estimates of length obtained for each sample. The standard errors were calculated from the five replicate length estimates. Comparing first the results from the line intersection method using regularly spaced fields with the results from direct measurement, there is reasonable agreement in each case. However, for three of the samples there is a significant difference between the two estimates: for the two smallest samples (Flax A and Bean A) the line intersection method gave significantly higher values, whereas for Flax B it gave a significantly lower value. Considering the results from randomly spaced fields (Bean A and B), in neither case does the result differ significantly from the 'regular fields' result, but in both cases it agrees better with direct measurement than does the 'regular fields' result. This is particularly noteworthy for Bean A, where the 'regular fields' result differs significantly from that obtained by direct measurement, but the 'random fields' result does not. Strictly speaking, the equation $R = \pi NA/2H$ applies only when the hair-lines are randomly positioned. Initially I assumed that use of regular spacing would not lead to any appreciable error. The results in Table 1 throw doubt on the validity of this assumption, and suggest that, at any rate with small samples, using regularly spaced fields may sometimes lead to an over-estimate of the root length. This might be due to non-random arrangement of roots near the edge of the area.

One possible source of error in the line intersection method arises if some roots are obscured by others. This may have been the cause of the low estimate for Flax B, where the roots were fairly densely arranged. Provided the roots are well spread out, this error is probably small.

It is also possible for errors to occur in direct measurement. Many roots are not straight; it is not always practicable to hold each one absolutely straight for measurement, so subjective allowances have to be made for curved portions. Also, there may be many branch roots only a few millimetres long; if they are measured to the nearest millimetre, the error in measurement may be quite a large percentage of the total length. Both these errors can be greatly reduced by measuring an enlarged photograph or projection of the roots.

Table 2 shows, besides the coefficients of variation of the length estimates, the mean time taken to perform one of the five replicate estimations. This is made up of: (i) the time taken in arranging the roots in the dish, which is influenced by the amount of root, and (ii) the time taken in positioning the hair-lines and counting the intersections, which is influenced by the number of hair-lines used and also by the density of the roots. Eighty hair-lines were used for each replicate estimation. Using the same data, the coefficients of variation for forty hair-lines were estimated by considering each set of eighty as two independent sets of forty hair-lines. In regular spacing every alternate hair-line made up one set; in random spacing the forty hair-lines were selected randomly. The coefficients of variation for twenty hair-lines were obtained in a similar way. (For Flax A sixty-four, thirty-two and sixteen hair-lines were used.)

Table 2. *Coefficients of variation (%) and time taken (minutes, in parentheses) in line intersection and direct measurement methods*

	Line intersection Number of hair-lines*			Direct measurement
	80	40	20	
(a) Microscope fields regularly spaced				
Flax A	4·3 (24)	7·1 (14)	9·4 (9)	(67)†
Bean A	4·9 (24)	6·1 (14)	9·9 (9)	(77)†
Forest	5·0 (37)	5·9 (22)	10·5 (14)	(255)†
Bean B	3·6 (31)	4·7 (18)	8·8 (12)	13·4 (33)‡
Flax B	3·4 (39)	5·6 (23)	9·7 (15)	5·2 (37)‡
Festuca	4·0 (53)	7·7 (32)	11·8 (22)	15·9 (75)‡
(b) Microscope fields randomly spaced				
Bean A	5·4 (29)	6·8 (17)	11·3 (11)	(77)†
Bean B	6·8 (36)	7·6 (21)	11·0 (13)	13·4 (33)‡

* Hair-lines 1·88 cm long. For Flax A, 64, 32, 16 hair-lines.
† Whole sample measured.
‡ Five sub-samples measured.

The results indicate that the line intersection method is much quicker than direct measurement of the whole sample, even for quite small samples. Direct measurement of sub-samples plus weighing appears to be less precise (i.e. the coefficient of variation is larger) than the line intersection method, when about the same amount of time is spent on each. Alternatively, the line intersection method can achieve a given degree of precision in a shorter time. Comparing the results from randomly and regularly spaced fields, we see that for both samples the coefficient of variation was higher with random spacing; but it would be rash to generalize from two examples only. The time taken was a little longer with random fields: this was consistently true for every replicate. Nevertheless it is advisable to use random spacing, because use of regular spacing may lead to an error. Comparing the results for different samples using regular field spacing, it appears that the coefficient of variation is not influenced by the length of the root sample. The time taken, for a given number of hair-lines, does increase with sample length, and so the time required to achieve a particular degree of precision increases, though only slowly. For instance, to achieve the same coefficient of variation required rather more than twice as long for *Festuca* as for Flax A, although *Festuca* was more than thirty times as long.

Replicate estimates were made on each sample during these tests of the line intersection method, but in normal use of the method it would usually be necessary to make only one estimate of the length of each sample. Investigations in which an estimate of error is required would presumably include replicate root samples, and error from the line intersection counts would be incorporated with actual between-sample variation.

The line intersection method has several advantages besides those already mentioned. (i) Since the roots are examined through a microscope they can be clearly distinguished from non-root material, e.g. partly decayed remains of stems and leaves. (ii) The length estimate can, if desired, be confined to a certain type of root, e.g. unsuberized roots, or to the roots of one species where these are recognizable. (iii) Diameter measurements can easily be made while the roots are laid out in the dish, by mounting a micrometer disk in the eye-piece not containing the hair-line. If diameter measurements are made, the total surface area of the roots can be estimated.

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SUMMARY

The roots are laid out on a flat surface, and a count is made of the number of intersections between the roots and random straight lines. Then the total root length $= \pi NA/2H$, where N is the number of intersections, A the area within which the roots lie, and H the total length of the straight lines. Details are given of a technique in which a microscope hair-line provides the straight lines. In practical tests the method was compared with direct measurement, and with direct measurement of a sub-sample followed by weighing of the sub-sample and the remainder. The results from the different methods agreed well. The line intersection method was much quicker than direct measurement, and in a given time achieved higher precision than measurement of a sub-sample and weighing.

APPENDIX

Derivation of equation $R = \dfrac{\pi NA}{2H}$

A straight line PQ, of length ΔR, lies within a plane convex region of area A. PQ represents the central axis of a portion of root short enough to be considered straight. Another straight line, MN, of length h, lies within A. MN represents a hair-line. PQ and MN can intersect only if the mid-point, D, of PQ lies within $\frac{1}{2}\Delta R$ of MN, i.e. within a strip whose area is approximately $(\Delta R)h$ if $\Delta R/h$ is small (see Fig. 2). If MN is positioned at random relative to PQ, the probability that D lies within this strip is $(\Delta R)h/A$. Suppose

D does lie within $\frac{1}{2}\Delta R$ of MN, and that PQ makes an angle θ with MN. PQ and MN will intersect if the perpendicular distance of D from $MN \leqslant \frac{1}{2}\Delta R|\sin\theta|$. Therefore the chance, p, of an intersection is:

$$\frac{\frac{1}{2}\Delta R|\sin\theta|}{\frac{1}{2}\Delta R} = |\sin\theta|$$

If D can be anywhere in A

$$p = \frac{(\Delta R)h}{A}|\sin\theta|$$

Now suppose there are many hair-lines, lying randomly in position and direction within

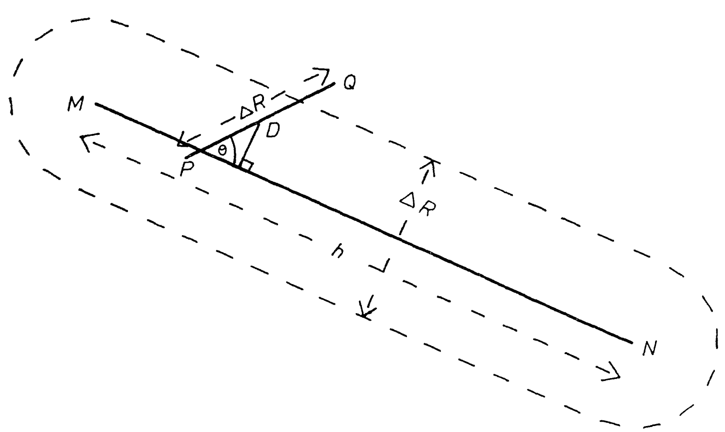

FIG. 2. Illustration of derivation of equation (1).

A, and their total length is H. Then the expected total number of intersections, N, between PQ and the hair-lines is

$$\frac{1}{2\pi}\int_0^{2\pi}\frac{(\Delta R)H}{A}|\sin\theta|d\theta$$

$$\therefore N = \frac{2(\Delta R)H}{\pi A}$$

If a root system of total length R and of any shape lies within A, it may be considered as made up of short straight sections each ΔR long. Therefore the expected number of intersections between the hair-lines and the centre line of the roots is given by:

$$N = \frac{2RH}{\pi A}$$

Therefore an estimate of the total length of root is given by:

$$R = \frac{\pi NA}{2H}$$

For further information see Kendall & Moran (1963, chapter 3).

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