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THE ESTIMATION OF ROOT LENGTH IN SAMPLES AND SUB-SAMPLES

COMPARISON OF A VISUAL AND AN AUTOMATIC METHOD

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KEY WORDS

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SUMMARY

Methods for estimating the root length in a sample using the line intersect principle were compared. One method involved visual techniques and used simple equipment. Another method introduced a new machine designed to estimate root length automatically. Either method had a high degree of accuracy, comparable with or better than other reported methods. Furthermore, the methods were tested over a wide range of root lengths up to 50 m. Even larger samples could be estimated using a reliable sub-sampling technique. The development of the root machine enabled the estimation of root length to become a simple laboratory routine.

INTRODUCTION

Newman[2] suggested the use of a line intersect principle for estimating the total length of root in a sample. He derived the formula.

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

such that the total length (R) of a sample of roots when spread over a flat surface of area A can be estimated by counting the number of intersections (N) between the roots and a superimposed set of randomly placed lines of total length H.

Various modifications of Newman's method have been reported[1,3,5,6]. However calibration of the various methods have often relied on a minimum number of pre-measured root samples[1,2,3,5]. Instead cotton thread, which is easily measured, has been used as a more substantial test over the range of interest[3,5]. Tennant[6] however did test his method using a substantial number of root samples over the range 0–10 m.

Most methods are only concerned with the measurement of whole root samples of 15 m or less. Bigger samples have been sub-sampled on a weight basis[1,2] even though it is acknowledged that this may result in major errors[2].

The present paper describes a modified method of root length determination based on Equation (1). Furthermore, following the development of a machine to determine root length, reported in an earlier paper[4] we are able to make a comparison between the visual and the automatic method. Both methods are calibrated in detail up to 50 m of roots.

A simple method of sub-sampling roots from samples as big as 200 m is also described. The proposed method of sub-sampling does not rely on weight determinations.

MATERIALS AND METHODS

Root material

The root system of young peach (*Prunus persica* L. Batsch) seedlings was used for all measurements. Plants were maintained in aerated nutrient solution without any solid medium. Framework roots greater than 2 mm in diameter were not included in the length determinations. The diameter distribution of the remaining roots was not quantitatively determined, however all root samples contained a typical range of root diameters (0.1 to 2.0 mm). Root samples contained all types of root including suberised, unsuberised, secondary thickened and fine fleshy laterals as long as they complied with the diameter limit. For all calibration purposes roots were directly measured on glass overlying centimetre graph paper. Rather than cutting roots into short straight segments and removing laterals flush with the axis to help in direct measurement, roots were left as complete as possible. After direct measurement roots were cut indiscriminately into small pieces. For both the grid and the machine calibration ten 5 m samples were measured and during calibration samples were combined consecutively up to 50 m samples for calibration. After a number of initial tests sufficient 50 m samples had accumulated to be used in a similar way to give 50, 100, 150 and 200 m samples for testing the sub-sampling method.

Grid method

A galvanized iron tray (300 × 400 × 45 mm) with 5 mm thick poly-vinyl foam on the bottom was connected through a hole in the bottom to a suction pump. For each root sample a blotting paper dish was made and inserted in the tray. Roots were suspended under water in the tray and, after they had been teased apart and dispersed uniformly, the water was withdrawn leaving the roots on the moist blotting paper. The blotting paper, with roots, was removed from the tray and laid flat on a sheet of glass illuminated from beneath. Another sheet of glass (300 × 400 mm) etched with a 1 cm grid was placed over the root sample. With a hand tally counter all intersections of roots and grid lines were counted in first the vertical and then the horizontal axis, averaged and used in the formula.

$$R = \frac{\pi N}{2} \qquad (2)$$

to determine root length directly in centimetres. Equation (2) is a special case of Equation (1) where $A/H = 1$.

For each sample the roots were retrieved and redistributed on another blotting paper tray three times.

The time taken to arrange the roots in the tray and then to scan in both the vertical and the horizontal axes was recorded.

Sub-sampling method

Often in plant growth experiments, even with seedlings, root samples longer than 50 m are encountered. Even though the grid method was calibrated successfully up to 50 m the calibration became difficult and tedious above this range. With this in mind, a sub-sampling technique was developed and tested on a further 50 m sample and then on subsequent 50 m increments up to 200 m.

With the results of the initial grid calibration as a guide it was decided that a 10 per cent sub-sample for each of the total lengths considered would be adequate.

To obtain sub-samples, the total root sample was cut into small pieces and spread out uniformly under water on a 300 × 400 × 45 mm blotting paper tray in the same way as already described. After the water was withdrawn, the blotting paper with roots was removed and located on a glass sheet illuminated from beneath and marked with 50 equal rectangles of 40 × 60 mm. The outline of the rectangles and a numeral within each was clearly visible through the moist blotting paper. A series of five numbers 1 and 50 was selected at random and the roots within rectangles having those numbers were removed. A sharp scalpel or razor blade was used to cut roots flush with the edge of the marked area. The bulked roots from the five rectangles represent the 10 per cent sub-sample. The sub-sample was then redistributed on another blotting paper tray, covered with a 1 cm grid and intersections counted as described for the grid method.

For each of the total samples considered ten 10 per cent sub-samples were taken to compare the variability.

Root length machine

A description of the root length machine and an explanation of how it scans the sample has already been described in a previous paper[4]. To prepare root samples for measurement by the machine the root pieces were suspended in 400 ml of water containing a few drops of wetting agent in a glass-bottom tray (375 × 375 × 10 mm) already located on the machine. The roots having first been teased apart and spread-out uniformly remained submerged in the water throughout the measurement. At the end of the run the total root length (in metres) is displayed numerically by light emitting diodes. For the purpose of calibration each root sample was retrieved and redistributed three times.

RESULTS AND DISCUSSION

The calibration curves for both the modified grid method and the root machine are shown in Fig. 1. Values for the actual length were taken as those measured directly on graph paper. Each point on the graph represents the average of three readings on the same, but rearranged sample. The graph shows that the root machine calibration curve was linear over the entire range whereas the grid calibration was curvilinear. Each line had a very high correlation coefficient

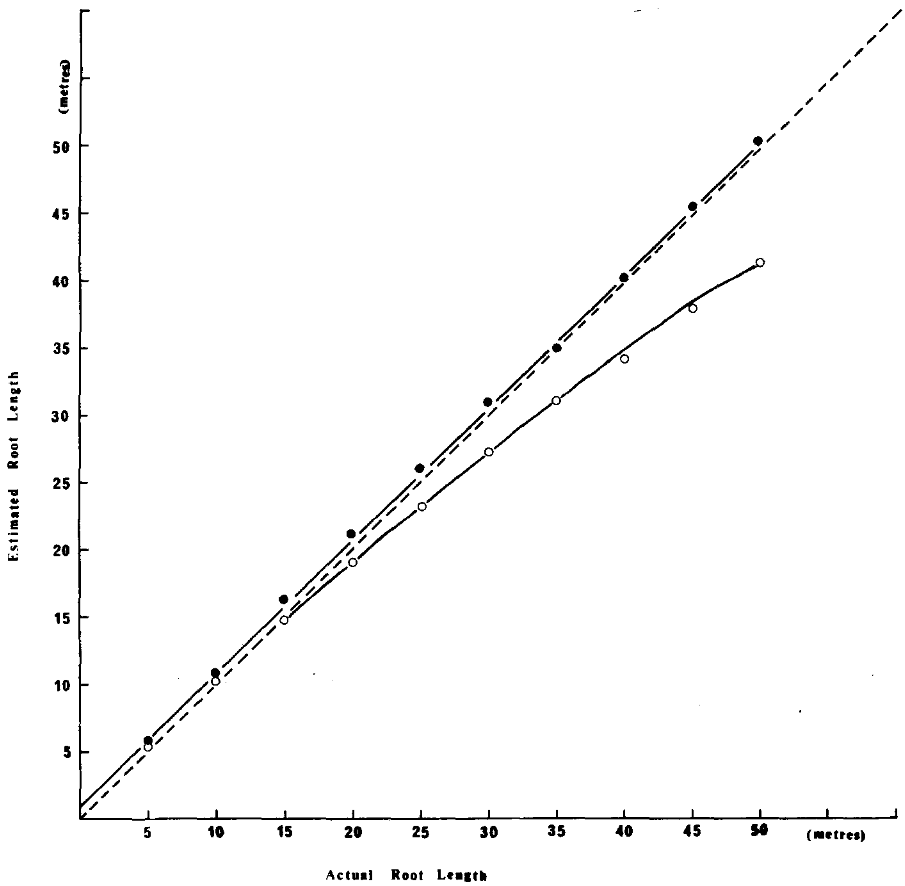

Fig. 1. The relationship between actual (A) and estimated (E) root length for each method.
• root length machine, $A = 1.014E - 0.978$, $r = 0.9996$;
o grid method, $A = 0.706 + 0.848E + 0.008E^2$, $r = 0.9977$.
Dotted line is the theoretical (1 : 1) relationship.

indicating a close relationship between the direct measurement and the estimates. Over the range of root length tested, the machine overestimated while the grid underestimated. This was probably due, in part, to the small particles of organic matter, such as bark, which readily sloughed off the sample. In an automatic method all contaminants are counted along with the roots whereas in the grid method they are easily distinguished and avoided. Fig. 2 shows the percentage error of each technique over the range of lengths considered. Taking Fig. 1 and Fig. 2 together it appeared that the machine gave the best estimate (± 5

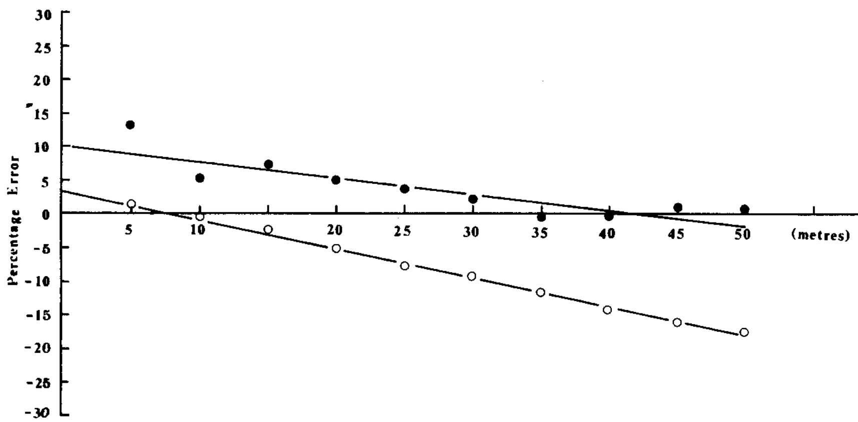

Fig. 2. The relationship between the percentage error of each technique and the actual root length. • root length machine; o grid method.

per cent of the actual length) for the longer samples (20–50 m) and the grid method the best estimate for shorter samples (5–20 m). The linear fall off in this percentage error in both cases was probably due to the increasing incidence of overlapping as the sample length increased. The fall off was faster in the visual method suggesting that operator fatigue and loss of resolution was involved. There was a rapid increase in time to finish counting a sample as the length

Table 1. Standard deviations, coefficients of variation and time taken for the grid method and the root machine with increasing root length. For each root length there were three estimates

Actual root length (m)	Grid method			Root machine		
	SD	CV	T	SD	CV	T
5	0.04	0.08	5	0.06	1.02	3*
10	0.06	0.64	9.5	0.15	1.45	
15	0.17	1.15	13	0.10	0.62	
20	0.24	1.28	17	0.15	0.73	
25	0.12	0.51	21	0.40	1.54	
30	0.16	0.57	25.5	0.51	1.67	
35	0.12	0.39	29	0.21	0.60	
40	0.30	0.87	31	0.29	0.72	
45	0.21	0.56	34	0.58	1.27	
50	0.65	1.58	37	0.56	1.11	

SD = standard deviation (m).
CV = coefficient of variation (%).
T = time taken (minutes).
* time taken for root machine was 3 minutes for all samples.

increased (Table 1). Beginning with cut root pieces the time to prepare and arrange a sample in either method was always less than 3 minutes.

Table 1 also shows standard deviations and coefficients of variation (standard deviation/mean × 100) of the length estimates. Both methods had very low coefficients indicating a high level of precision. A 5 per cent coefficient of variation has been considered acceptable[6]. The coefficient of variation for both the grid method and the machine were lower than those reported by Newman[2], Reicosky *et al.*[3] and Rowse and Phillips[5] but comparable with those of Tennant[6]. In both methods the standard deviation tended to increase with increasing root length. This probably reflects the tendency for roots to clump together as the sample size increase. In neither method does there appear to be a trend in the coefficients of variation.

Results for the sub-sampling method are shown in Table 2. In this case each measurement is the mean of ten equal sub-samples each representing 10 per cent of the total sample. It can be seen from Fig. 2 that the sub-samples (5, 10, 15, 20 m respectively) were of an ideal length for determination by the grid method. It is recommended that for the best estimate sub-samples should not be longer than 20 m. The sub-sampling itself was very successful in that the mean estimated length was always within 6 per cent of the actual length of the total sample. Taking the 50 m sample it can be seen that the estimate was closer to the actual length when using sub-samples (3.0 per cent underestimate) than when using the whole sample (17.5 per cent underestimate). However, the standard deviation and coefficient of variation was considerably higher when using a sub-sample. For each length the standard deviation was similar but the coefficient of variation fell in magnitude as the sample size increased. For a sample size of 135 m Newman[2] reported a coefficient of variation of 16 when the sample was estimated from five weighed sub-samples. For a similar size sample using the present sub-sampling technique the coefficient of variation was reduced to about 4. The

Table 2. Comparison of actual root length with the root length estimated from a 10 per cent sub-sample obtained by the suggested sub-sampling technique. Figures in brackets are estimates from 10 per cent sub-samples taken on a fresh weight basis. For each root length there were ten estimates measured by the grid method

Actual root length (m)	Estimated root length (m)	Standard deviation (m)	Coefficient of variation (%)
50	48.5 (61.0)	3.7 (25.0)	7.69 (40.9)
100	98.7	5.2	5.27
150	144.4	5.7	3.94
200	188.7	5.2	2.73

results of sub-sampling 50 m of roots on a fresh weight basis (figures in brackets, Table 2) confirm that this method of sub-sampling is subject to large errors and inaccuracy.

From our experience in handling roots, and in the absence of an automatic method, it is considered essential to sub-sample roots when samples are larger than 50 m. The only way of knowing how large a sample is before measurement comes from experience and crude fresh weight : length relationships. If a standard procedure of washing and of selecting roots of acceptable diameter is followed the relationship between fresh weight and length is at least sufficient to know whether to sub-sample or not.

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