QUANTITATIVE STUDIES OF ROOT DEVELOPMENT

I. THE INFLUENCE OF NUTRIENT CONCENTRATION

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Summary

The root growth of barley plants was examined at weekly intervals during the 5 weeks following emergence. The lengths of the separate components of the root system (primary, secondary, tertiary, etc.) were determined, as well as the number of branches and the distances apart of these branches.

The lowest nutrient concentration used produced the greatest length of roots, and growth analysis indicates that the mechanism producing this response operated at or shortly after germination. The mean extension rates of root tips in the separate components of the root system were different: the higher the order of the component, the smaller was its mean extension rate. Mean extension rates were not, however, influenced by nutrient concentration, at least between 2 and 5 weeks following emergence. The mean spacing between branches was influenced by nutrient concentration. Further, the mean spacing between branches on primary roots was different from that on secondary roots. The bearing of these results on the regulation of branching in roots is discussed.

I. INTRODUCTION

A major function of roots is the absorption of water and salts from the surrounding medium, and the surface area near root tips is commonly held to be the most active in these uptake processes, so that uptake is likely to be related to the number of root tips (or branches). Further, root tips are constantly changing their positions in relation to the surrounding medium, such that the rate of root elongation is also likely to be a factor controlling uptake processes. Hence, an analysis of root development in terms of number of root tips and rates of elongation of the roots might well be rewarding.

Roots have long been known to respond to changes in the external medium; for example, Weaver (1926) observed a stimulation of root growth and branching within certain bands of soil in a profile which he attributed to varying soil nutrient concentrations. Moreover, there is much evidence to suggest that root and shoot systems are closely interdependent, roots depending on the leaves for supply of photosynthate. Other workers, such as Went (1938) and Richardson (1957), claim that root elongation also depends upon hormonal factors originating in the shoot. It was decided, therefore, to establish in quantitative terms the pattern of root development in barley, and to determine if possible the extent to which various factors regulate root growth.

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II. Experimental Methods

Two sets of data were collected on the same barley variety (*Hordeum vulgare* L. cv. Piroline) as used by Aspinall (1961, 1963) to explore the influence of nutrient concentration on the branching of plant tops. Plants were grown in perlite-filled plastic cylinders (5·5 cm diameter by 37·5 cm high). In experiment 1, seeds were sown on April 5 and harvested weekly until May 17, and in experiment 2, sown on June 6 and harvested weekly until July 18. One seed was sown in each cylinder and the requisite number of plants was selected on the basis of uniformity of time to emergence of the shoot at the perlite surface. The plants were grown in a glasshouse, the temperature of which was partially controlled within the range 13–24°C.

The total amount of nutrients was supplied before emergence, and the standard solution, subsequently referred to as the 100% solution, contained (g/plant): $NaNO_3$, 0·455; $Ca(NO_3)_2.4H_2O$, 1·28; KNO_3, 0·395; $NaH_2PO_4.2H_2O$, 0·785; $MgSO_4.7H_2O$, 0·463; Fe–EDTA, 0·005; H_3BO_3, 0·0035; $MnSO_4$, 0·00175; $ZnSO_4.7H_2O$, 0·00025; $CuSO_4.5H_2O$, 0·0001; MoO_3, 0·000125; in 450 ml of distilled water. These amounts are less per plant than those used by Aspinall (1961, 1963) but the reduction was necessary to give the same concentration of nutrients in our smaller plastic cylinders as was present in the larger pots of the earlier work.

The volume of solution added exceeded the water-holding capacity of the perlite; the excess (1/9) drained into a lower container. This excess was recycled through the perlite daily when losses of water were replaced. Three nutrient treatments were employed: 100 (the standard solution), 50, and 10%. For each treatment there were five harvest occasions at approximately weekly intervals after germination, but because of the difficulties of measuring root systems only one plant was taken on each occasion. The perlite and roots were displaced from the cylinder and the perlite separated from the roots by careful washing in water. Individual primary roots and branches were then floated on water in a petri dish positioned above a black photographic plate bearing a white millimetre grid. The numbers and lengths, measured with the aid of a ×10 magnifying glass, of those primary (including both seminal and adventitious roots), secondary, and tertiary roots which were longer than 1 mm were recorded. The possibility that undeveloped initials were present but being overlooked was explored by the method recommended by Pecket (1957). Several thorough searches failed to reveal any undeveloped initials located between developing branches longer than 1 mm. However, in the region between the tip and the first branch there were many undeveloped initials. Therefore, many branch initials near the tip are excluded. Nevertheless, behind this zone all initials develop to or beyond 1 mm in length and have been catalogued. At the first three harvests the distance between each developing branch on each plant was also recorded, but the time involved was so great that thereafter this measurement had to be discontinued.

III. Experimental Results

Only results from the first experiment are presented in full; growth rates were lower in the second, when mean temperatures and light intensities were lower, but in other respects results were similar.

(*a*) *Primary Growth Data*

Throughout, both the total length of the root system (Fig. 1*A*) and the number of branches (Fig. 1*B*) were greatest in the 10% and least in the 100% solution. In contrast, the greatest weight and volume of roots were found at the 50% nutrient concentration (e.g. at the final harvest the dry weights were 0·42, 0·46, and 0·26 g in the 10, 50, and 100% concentrations respectively). It follows that the relationship between length and dry weight of roots was dependent upon the nutrient concentration surrounding the roots. Observations show that the dry weight (0·14 g) of the first 10^3 cm of root produced in the 100% concentration was twice that (0·07 g) of the first 10^3 cm in the 10%.

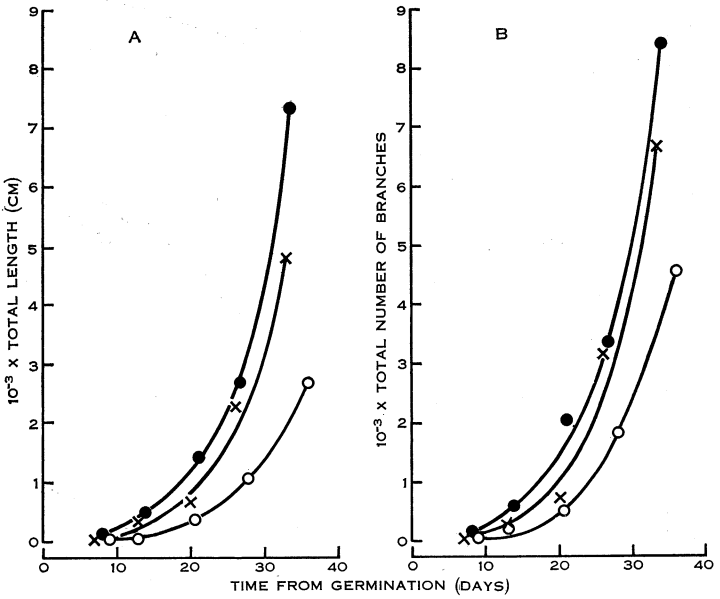

Fig. 1.—Relationships between total lengths (*A*) and total numbers of branches (*B*) and time for root systems growing in 10% (●), 50% (×), and 100% (○) standard nutrient concentration.

(*b*) *Relative Extension Rates and Relative Multiplication Rates*

The logarithms of the lengths (l) and of numbers (n) of the primary, secondary, and tertiary roots when plotted against time (Fig. 2) showed relationships which consisted of two phases. There was first an early phase of high relative rates of production and extension which decreased later to a phase of constant rates. Unfortunately, the second phase in the growth of the primary roots was already established by the time the observations commenced; the positions of the curves in relation to the origin, however, show that such a phase must have existed. The later stages of the first phase were evident in the development of the secondary and tertiary roots. Over most of the experimental period, however, the plants were in the second phase with the relative rates constant. There was some indication that the plants grown at the 50% nutrient concentration were later in attaining this constant phase than plants

in the other two concentrations. With omission of the data of the first harvest, analysis revealed no evidence of curvature of any of the lines; consequently, the average relative rates (Table 1) were calculated as the slopes of the straight lines of best fit for each attribute and treatment. The average relative rates of extension (R.E.R.) or of multiplication (R.M.R.) of roots of plants grown with the different

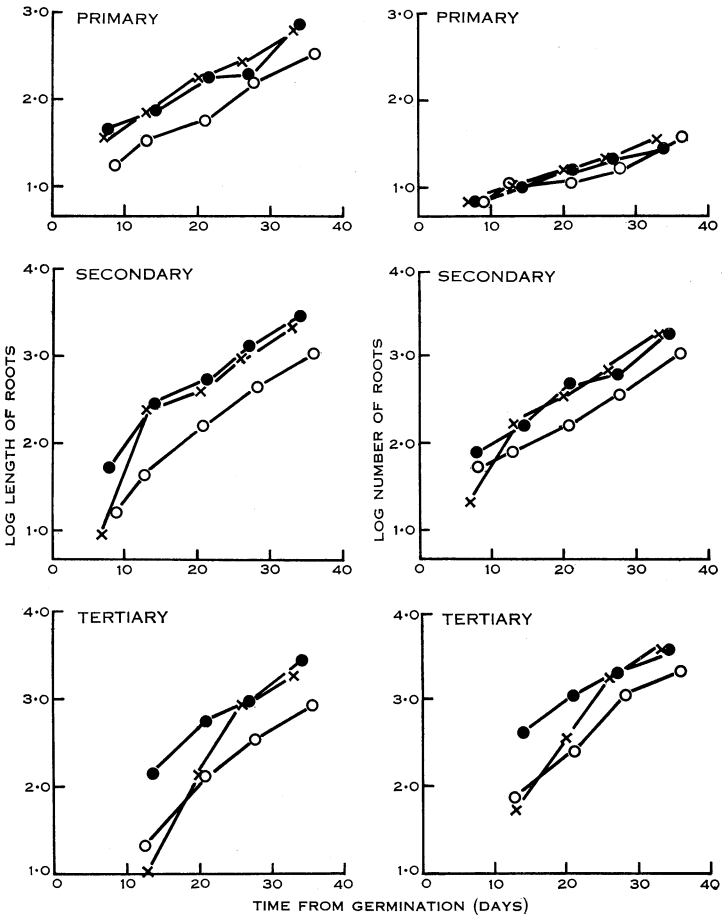

Fig. 2.—Relationships with time of logarithms of lengths and numbers of primary, secondary, and tertiary roots growing in 10% (●), 50% (×), and 100% (○) standard nutrient concentration.

nutrient concentrations were the same. That is, the differences in growth pattern of these roots were established during the early stages before observations commenced. There were similar numbers of primary roots in all treatments but these were significantly shorter in the full solution than in the lower concentrations; there were also fewer and a shorter length of secondary and tertiary roots in the high than in the lower concentrations (Table 1). With primary roots the relative rate of extension was about twice the relative rate of multiplication, but with secondary and tertiary roots these rates were of the same order.

TABLE 1

RELATIVE EXTENSION RATES, RELATIVE MULTIPLICATION RATES, AND LOGARITHMS OF LENGTHS AND NUMBERS OF ROOTS AT THE SECOND HARVEST (APPROX. 14 DAYS AFTER EMERGENCE)

Values are calculated from lines fitted to the data shown in Figure 2; in each case the first points on these lines have been omitted since roots were in the first growth phase at that time

Expt. No.	Root Component	Relative Extension Rate (cm/cm/day) at Nutrient Concentration:			Relative Multiplication Rate (No./No./day) at Nutrient Concentration:			$\log_e$ Total Length (mm) at Nutrient Concentration:			$\log_e$ Total Number of Roots at Second Harvest at Nutrient Concentration:		
		10%	50%	100%	10%	50%	100%	10%	50%	100%	10%	50%	100%
1	Primary	0·104 ±0·015	0·107 ±0·007	0·104 ±0·012	0·052 ±0·004	0·062 ±0·006	0·055 ±0·008	6·65	6·54	5·98	2·30	2·30	2·30
	Secondary	0·116 ±0·004	0·112 ±0·010	0·133 ±0·008	0·118 ±0·008	0·120 ±0·005	0·119 ±0·007	7·89	7·84	5·26	5·14	5·11	4·36
	Tertiary	0·122 ±0·001	0·196 ±0·059	0·159 ±0·018	0·098 ±0·002	0·176 ±0·028	0·148 ±0·012	7·36	4·65	4·86	6·05	3·93	4·32
2	Primary	0·047 ±0·007	0·071 ±0·016	0·070 ±0·010	0·031 ±0·016	0·041 ±0·006	0·032 ±0·005	6·65	6·37	6·30	2·08	2·20	2·08
	Secondary	0·095 ±0·008	0·100 ±0·015	0·110 ±0·013	0·074 ±0·009	0·085 ±0·005	0·090 ±0·014	7·28	6·78	6·28	4·84	4·74	4·47
	Tertiary	0·103 ±0·005	0·085 ±0·045	0·154 ±0·032	0·089 ±0·003	0·089 ±0·030	0·127 ±0·038	5·53	4·16	1·89	4·77	3·26	1·61

In contrast to the lack of effect of the concentration of the nutrient solution on the relative rates of extension and multiplication in this growth phase, the large differences in these variables between the two experiments (Table 1) suggests that other environmental factors may have a profound influence. As the two experiments were carried out at different times of the year, many factors, such as light intensity, photoperiod, and temperature, were changed and it is impossible to comment on the underlying cause of these differences in rates. The need for further investigation is, however, clearly indicated.

(c) *Mean Extension Rate and Mean Root Length*

During the second phase of growth, both the numbers (n) and total lengths (l) of roots in each category increased logarithmically with time. The mean rate of extension of each root $\left(\frac{1}{n} \cdot \frac{dl}{dt}\right)$ may therefore be regarded as the product of the mean root length and the relative extension rate:

$$\frac{1}{n} \cdot \frac{dl}{dt} \text{ (cm/root tip/day)} = \frac{l}{n} \text{ (cm/root tip)} \times \frac{1}{l} \cdot \frac{dl}{dt} \text{ (cm/cm total length/day).} \quad (1)$$

Thus, the relative extension rate is analysed in terms of the reciprocal of the mean root length and the mean rate of elongation of each root tip in a manner similar to the analysis of the relative growth rate in terms of the leaf area ratio and the net assimilation rate (Gregory 1926). The mean extension rate (M.E.R.) over a finite period of time, $t_2 - t_1$, is required, however, and, by analogy with the net assimilation rate, the following relationship provides the best measure:

$$\text{M.E.R.} = \frac{l_2 - l_1}{n_2 - n_1} \times \frac{\log_e n_2 - \log_e n_1}{t_2 - t_1}$$

$$= \frac{l_2 - l_1}{n_2 - n_1} \times \text{R.M.R.}, \quad (2)$$

where l_1, n_1 and l_2, n_2 are the length and number of roots at times t_1 and t_2 respectively. As demonstrated by Williams (1946), this relationship is only strictly valid where n and l are linearly related. This was so for the whole root systems and for the secondary and tertiary roots examined separately, but there was considerable departure from linearity in the primary root data. With all but the primary roots, therefore, the mean extension rate could be calculated from the relative multiplication rate, and fitted values of l and n taken from the regressions of $\log_e l$ and $\log_e n$ on time. For the primary roots, a close approximation to this mean rate could be made by calculating the mean extension rate over each sampling interval and obtaining a mean value over the whole period weighted for the time interval between each sampling. The shorter the time interval between samplings, the more accurate is the estimate of mean extension rate (Williams 1946); in the present case the intervals varied between 6 and 8 days, and the departure from linearity of the relationship between n and l was not great over such a time interval.

Primary roots had the highest mean extension rate (Table 2) and roots of successively higher order had successively lower rates. The rate for the total root system was determined primarily by secondary and tertiary roots, at least between the second and fifth weeks, because of their much larger numbers. Differences in rates in the three nutrient concentrations were not significant. The tendency for mean extension rates, especially of primary roots, to be lower in the second series compared with the first will have to be investigated further, as the indications are that light intensity, photoperiod, or temperature here exerted an appreciable effect.

The mean root length can be calculated from the data in Tables 1 and 2 (see equation (1) above), and only a few values need be cited to illustrate its magnitude. In series 1 at the 10% nutrient concentration, final harvest, the mean length of primary roots was 14·3 cm, of secondary roots 1·47 cm, and of tertiary roots 0·63 cm.

TABLE 2

MEAN EXTENSION RATES FOR THE WHOLE ROOT SYSTEM AND ITS SEVERAL COMPONENTS GROWING IN NUTRIENT AT THREE CONCENTRATIONS, FOR THE PERIOD 14–35 DAYS AFTER EMERGENCE

Expt. No.	Root Component	Mean Extension Rates (cm/root tip/day) at Nutrient Concentration:		
		10%	50%	100%
1	Whole	0·106	0·109	0·087
	Primary	1·448	1·155	0·770
	Secondary	0·170	0·143	0·133
	Tertiary	0·073	0·089	0·070
2	Whole	0·080	0·079	0·087
	Primary	0·547	0·703	0·532
	Secondary	0·131	0·101	0·090
	Tertiary	0·046	0·028	0·051

(*d*) *Spacing between Branches*

When the numbers of branches borne by individual root systems were plotted against their lengths (Fig. 3) and regression lines fitted to the data, there was found to be a significant tendency for curvature over the lower values, particularly in the secondary root data. This may have arisen from a varying number of roots with no branches, a varying distance from the last recognizable branch to the root tip, and a varying distance from the origin of the root to the first branch. For this reason lengths were corrected to exclude all but those parts of the root system *between* branches. The average spacings between branches on primary and secondary roots were then calculated for the first three harvests. This analysis (Table 3) established that there was a significant difference in average spacing between branches on primary roots in the 10% nutrient concentration on the one hand, and the 50 and 100% concentrations on the other. This difference was not evident on secondary roots. The overall mean spacing on primary roots (0·224 cm) was also significantly

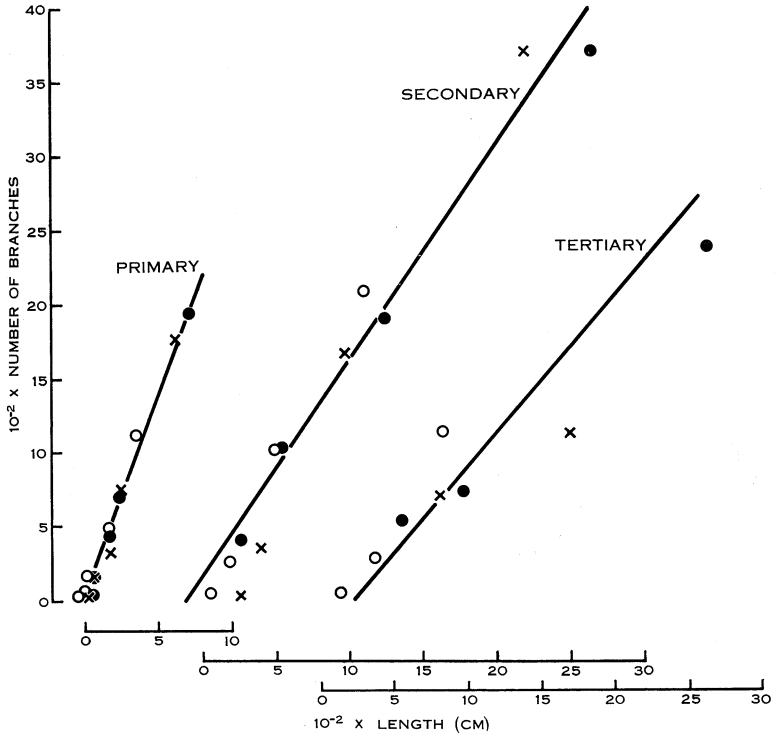

Fig. 3.—Relationships between: length of primary, secondary, and tertiary roots and number of branches on these roots. Three concentrations of nutrient solution were used: 10% (●), 50% (×), 100% (○). Measurements were made at approximately weekly intervals during the 35 days following shoot emergence for each nutrient condition.

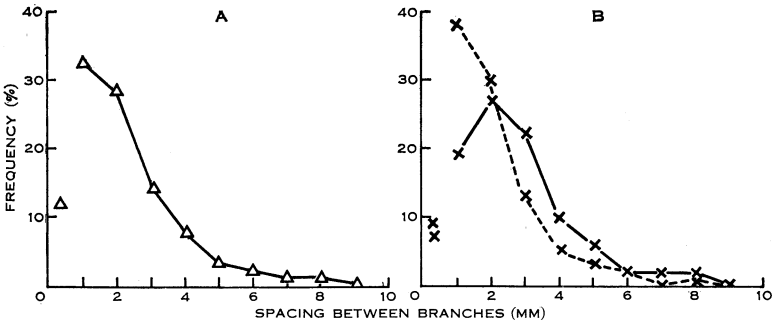

Fig. 4.—Frequency of occurrence of distances between branches when distances are grouped into 1-mm classes (e.g. 0·5–1·5, 1·5–2·5, 2·5–3·5 mm). The lowest class interval (0–0·5 mm) is only half the magnitude of the others, and hence not comparable. *A* presents all data accumulated from experiments 1 and 2. *B* presents data for primary roots (——) and secondary roots (- - - -) of a root system in 50% nutrient concentration at 21 days after shoot emergence.

greater than that on secondary roots (0·172 cm). When the distance between branches was measured directly, the frequencies, expressed as a percentage, with which selected class intervals occurred were as illustrated in Figure 4. Unfortunately the lowest class interval is only half the magnitude of the others and hence cannot be compared with them. The results of all data collected have been assembled in Figure 4*A*. In Figure 4*B* data from one treatment on one occasion have been presented. These confirm the result derived in Table 3 from other data, namely, that the mean spacing on secondary roots is lower than that on primary roots.

TABLE 3

MEAN DISTANCE (CM) BETWEEN BRANCHES BORNE ON EITHER PRIMARY OR SECONDARY ROOTS GROWING IN NUTRIENT AT THREE CONCENTRATIONS, AT THREE HARVEST OCCASIONS

Distances were measured at approx. 7, 14, and 21 days from shoot emergence

Expt. No.	Nutrient Concn. (%)	Primary Roots				Secondary Roots		
		Harvest 1	Harvest 2	Harvest 3	Mean ± S.E.	Harvest 2	Harvest 3	Mean ± S.E.
1	10	0·233	0·222	0·240	0·232±0·014	0·171	0·189	0·180±0·046
	50	0·167	0·140	0·229	0·162±0·014	0·337	0·244	0·290±0·046
	100	0·137	0·199	0·164	0·166±0·014	0·114	0·170	0·142±0·046
Mean		0·162	0·187	0·211	0·187±0·008	0·207	0·201	0·204±0·026
2	10	0·293	0·322	0·276	0·297±0·014	0·190	0·183	0·186±0·046
	50	0·208	0·265	0·261	0·245±0·014	0·100	0·193	0·147±0·046
	100	0·255	0·247	0·224	0·242±0·014	0·025	0·151	0·088±0·046
Mean		0·252	0·278	0·253	0·261±0·008	0·105	0·176	0·140±0·026
Overall mean					0·224±0·0057			0·172±0·0188

IV. DISCUSSION

A feature of these results was that the total length of the root system was greatest, at each time within the experimental period, in those plants grown in the 10% nutrient concentration. This concentration was suboptimal for growth of tops and also for growth of roots when this was assessed by either dry weight or volume measurement. Between 2 and 5 weeks there was no evidence for a consistent effect of nutrient concentration on either relative extension rate or relative multiplication rate, and it follows that, whatever the means whereby the 10% nutrient concentration induced the greatest length of roots, the mechanism operated only before 2 weeks from emergence. It is also clear that translocation of differing amounts of carbohydrate substrate from tops to roots cannot be the only mechanism for inducing the length differences, since the formation of the first 10^3 cm of root was accompanied, in the 100% nutrient concentration, by the entry of twice as much carbohydrate substrate into these roots as into roots growing in the 10% level.

The finding that the subsequent quantitative pattern of root growth was established during the first 2–3 weeks from planting is of considerable interest, and also indicates a system which can be readily analysed experimentally by exploring the responses to different environmental treatments. The mechanisms operating here are likely to be complex and related to the growth of the rest of the plant. These experiments do not indicate the length of time over which the constant relative rates of root production and extension operate; much published evidence would suggest that these are likely to decrease during the period of grain growth. Nevertheless, these data do suggest that the rate of root growth is under fairly rigid internal control, and perhaps large responses to variations in the environment occur only during the early stages of growth. This would be in keeping with the generally emerging concept that the responsiveness of plants to variations of the environment rapidly decreases with the age of the plant.

Attention is also drawn to the different mean extension rates for the several components of the root system. This observation is emphasized since, in the work relating to the uptake of water by roots, a single value is sometimes assigned to the rate of elongation of root tips. It is now evident that in barley this rate can vary within the whole root system by as much as 10-fold: from about 1·0 cm/day/tip in primary roots to about 0·1 cm/day/tip in tertiary roots. Further, there is no evidence for a change in the mean extension rate between 2 and 5 weeks after emergence. Brown (1959) has discussed data from isolated root cultures demonstrating a changing rate of cell division at the root tip which gives an extension rate that at first increases and then decreases with time. Whether this result differs because our estimates were made later in relation to the commencement of growth of the root tips, whether in this work with a population of root tips of different ages changes in extension rate with age balance each other, or whether root tips show different behaviour in this respect when intact and when isolated awaits more detailed examination.

The data suggested that there was a greater spacing between roots grown at the low than the higher nutrient concentrations. Other investigations have implicated mineral nutrients in root branching; for example, Wiersum (1958) claims that deficiencies of single nutrients, or the presence of single nutrients, can influence root-branching patterns. Clearly, there must be changes in the pericycle cell or cells at the site of root branch initiation, and hence external factors must influence these changes either directly or indirectly. Similarly, it may be supposed that internal factors such as the "shoot-apex factor" observed by Richardson (1957) or the "mature-root-tissue factor" of Pecket (1957) operate by initiating or modifying *in situ* changes. The data presented here allow the conclusions that mean spacing on primary roots is greater than that on secondary roots, and that on primary roots about five branch initials are formed per day whereas on secondary roots the number is one per day. Whether these differences arise from a varying distribution of internal regulatory factors or whether differences exist at the sites of initiation is uncertain, but clearly the factors concerned are internal. As other factors, both internal and external, are studied many may be found that influence branching, and already Torrey (1959) has listed many internal factors that can act in this way. The real challenge is, of course, to use this information to elucidate mechanisms operating in those cells

at the site of initiation of a branch root. The finding that at least two growth-regulating substances may be implicated (Goldacre 1959) emphasizes the undoubted complexity of the mechanism.

Procedures used in analysing primary data have been presented in some detail in the belief that they may be of use in other investigations. In its essentials these procedures involve separating the increase in size of the system (relative extension rate) into two components, mean root length and mean extension rate (an estimate of elongation of each root tip), and deriving an estimate of branching (mean spacing of branches). Determinations of these quantities in root systems of different varieties and genera, and in root systems growing in different environmental conditions, may well be rewarding.

V. Acknowledgments

Assistance by the Biometry Unit at the Waite Institute and especially by Mr. G. N. Wilkinson and Mrs. M. R. Atkinson is gratefully recorded.

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