QUANTITATIVE STUDIES OF ROOT DEVELOPMENT

II.* GROWTH IN THE EARLY STAGES OF DEVELOPMENT

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Summary

The growth of the root systems of barley seedlings was measured at daily intervals for the first 2 weeks after germination. The lengths and numbers of primary and higher-order branches were recorded and relative rates of extension and multiplication together with mean extension rates were derived from the data.

An earlier suggestion that root growth rates are high early in plant development but then fall to a lower level was supported by the data which also demonstrated changes in growth rate associated with the initiation of secondary and tertiary roots. A high nutrient concentration reduced root growth both by a transient effect on germination rate and a continuing reduction in mean extension rate. Nutrient effects on branch root production were traceable to these effects on elongation of the parent root.

I. INTRODUCTION

In the previous paper in this series (May, Chapman, and Aspinall 1965) it was demonstrated that the average relative rates of both extension and multiplication of roots of plants growing in a tenfold range of nutrient concentration were the same and, moreover, essentially constant over a period of 6 weeks beginning 2 weeks after seedling emergence. There were, however, substantial differences in both numbers and lengths of roots from different nutrient concentration at the time of the first sampling. Furthermore, extrapolation of the root growth curves, both of extension and of multiplication, back towards seedling emergence indicated that the relative rates of root development must have been considerably greater in the first 2 weeks of growth than in the period under observation. These data suggested the importance of this early period of development in establishing the pattern of root growth.

This early period of seedling root growth has been directly explored in the present investigation. The changing rate of root growth in this period and the origin of the effects of nutrient concentration have both been examined.

II. EXPERIMENTAL METHODS

In general, the techniques used in the work reported in this paper were identical with those previously described (May, Chapman, and Aspinall 1965). Barley plants (cv. Piroline) were grown singly in perlite-filled cylinders (5·5 cm diameter by

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15·2 cm high). The plants were watered with nutrient solution* of two concentrations (10 and 100%) but, in view of the smaller volume of perlite available for root exploration in this experiment, the amount of solution supplied (300 ml) was less than in the previous experiments. This volume was applied in amounts of 160 ml on the day of planting and 20 ml on each day of the succeeding week. During the final 11 days, the solution was recycled through the rooting medium daily when losses due to evaporation and transpiration were replaced with distilled water.

The plants were grown in a controlled environment at 20±1°C with a 12-hr photoperiod of fluorescent light (intensity 2000 f.c. or 6·67 g-cal/sq. cm/hr). Two plants from each treatment were sampled daily for the first 14 days after planting and further harvests were taken on days 17 and 19. The roots were washed from the perlite and floated on water in a petri-dish over a millimetre grid. The lengths and numbers of all primary, secondary, tertiary, and quarternary roots were recorded. The relative extension rate, relative multiplication rate, and mean extension rates were derived from the data according to the methods previously described (May, Chapman, and Aspinall 1965).

III. Results

(*a*) *Total Root Length*

The total length of the root system, considered on a logarithmic basis in Figure 1, increased rapidly during the first 3 days from planting but then entered a phase of substantially constant relative growth for the duration of the experiment. That is, the relative extension rates initially were very high but decreased to lower effectively constant rates within 3 days. Each of the successive orders of root branches (primary, secondary, tertiary) showed the same pattern. The initiation of secondary branches and their early high relative rate of growth were reflected by slight inflections in the growth curves of the total root system—most noticeable in the diagram showing relative extension rates—but the effect was not large because of the small size of the secondary root system compared with the total root system at this time. This general pattern of root growth was evident at both nutrient concentrations.

A tenfold increase in the nutrient level resulted in shorter root lengths, both of the whole system and of the several components. This difference in length of the primary roots was evident as early as 3 days after planting and in the secondary and tertiary roots at the time of first measurement. Statistical analysis of the root length data revealed no significant interaction between the effects of time and of nutrients, suggesting that the nutrient effect on root length arose very early in plant development and was perpetuated by the normal growth pattern of the root system. This is supported by the absence of any statistically significant effect of nutrient level on the relative extension rates. The large initial differences in root length in the secondary and higher-order branches suggest that the nutrient level affected either the time at which branches were first initiated or the number of branches growing

* Nutrients supplied in 100% solution (mg/plant): $NaNO_3$ 304, KNO_3 264, $Ca(NO_3)_2.4H_2O$ 854, $NaH_2PO_4.2H_2O$ 528, $MgSO_4.7H_2O$ 308, Fe-EDTA 3·4, H_3BO_3 2·4, $MnSO_4$ 1·2, $ZnSO_4.7H_2O$ 0·2, $CuSO_4.5H_2O$ 0·06, MoO_3 0·08.

simultaneously. The first suggestion is directly supported in the case of the quaternary roots where this class of roots were present at least 1 day earlier on low nutrient plants. With secondary and tertiary roots the difference, if any, must have been less than 24 hr.

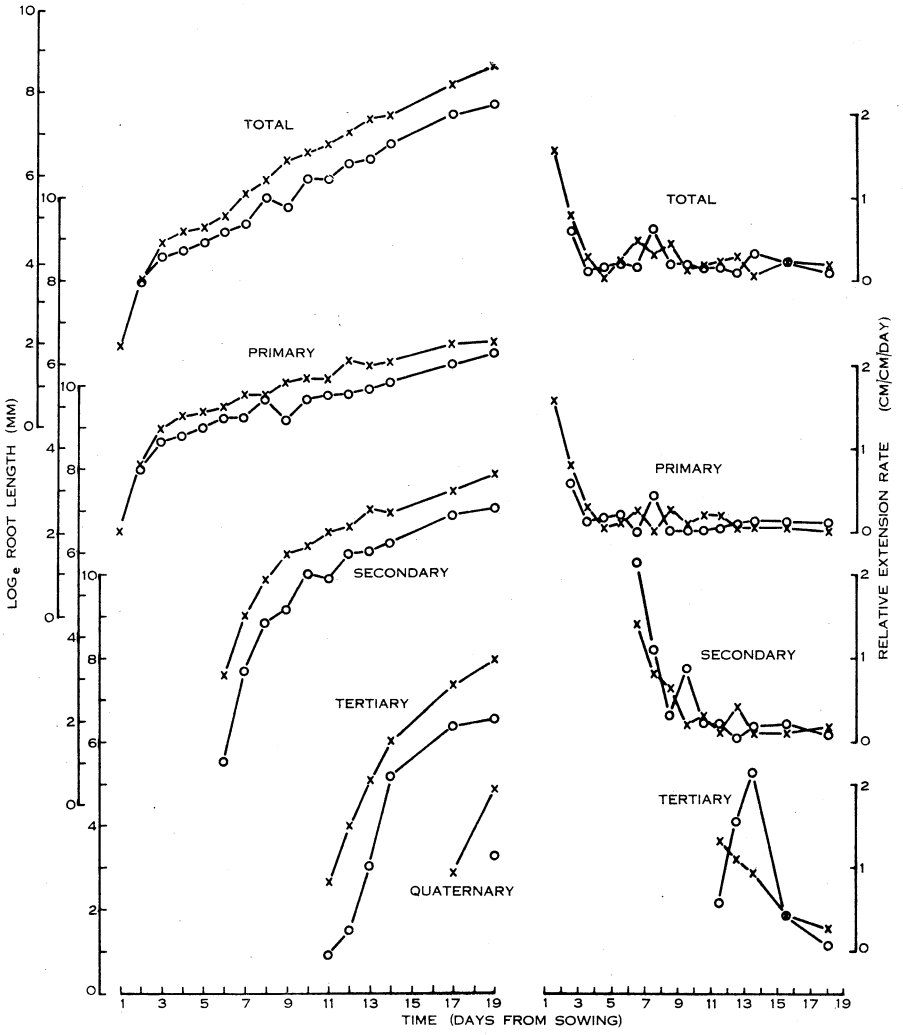

Fig. 1.—Logarithms of the lengths of the root system and its various components and the relative extension rates during the first 19 days of growth. × 10% nutrient concentration. ○ 100% concentration.

(*b*) *Total Root Numbers*

The production of new roots (Fig. 2) was more cyclic in nature than was the elongation of the root system; pronounced inflections in the curve correspond to the initiation of secondary and tertiary roots, these being reflected in peaks in the relative

multiplication rate of the total root system. Primary roots were relatively few throughout the period (13–14 present after 19 days), accounting for the large inflection in the curve for total root number at the time of initiation of secondary roots. Six primary roots were present after 3 days growth and no more were formed until 9 days after germination. These initial six roots presumably represented the seminal

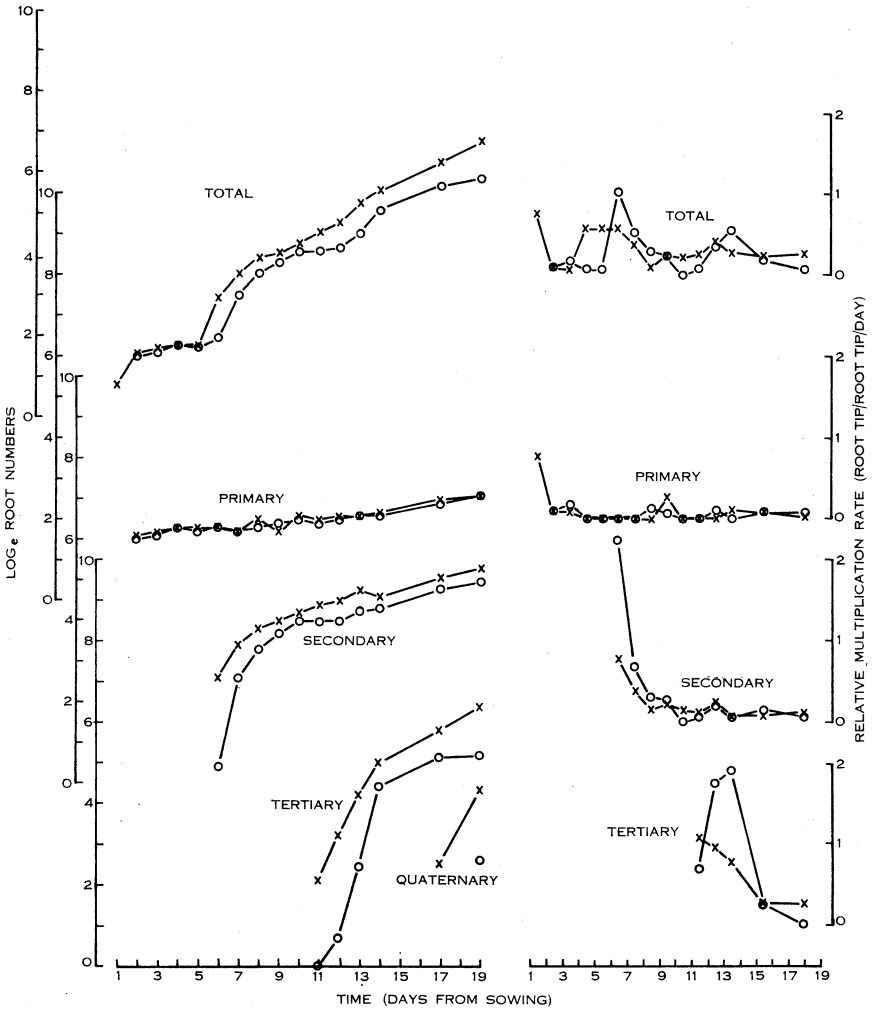

Fig. 2.—Logarithms of the numbers of roots and the relative multiplication rates during the first 19 days of growth. × 10% nutrient concentration. ○ 100% concentration.

root system although no distinctions were made between the seminal and adventitious roots during sampling. In contrast to the slow increase in primary root numbers, both the secondary and tertiary branches demonstrated a rapid initial increase in relative rate of root production followed by a constant relative rate of increase.

These patterns in root initiation were unaffected by nutrient supply but, parallel with the effects on the total length of the roots, there was a greater number of roots at the lower than at the higher nutrient concentration. This difference was entirely due to the greater number of secondary and higher-order branches at the lower nutrient level. Again there was no interaction between nutrition and time supporting the suggestion that nutrient level influenced the time of initiation of the first branch, or the number of branches formed initially.

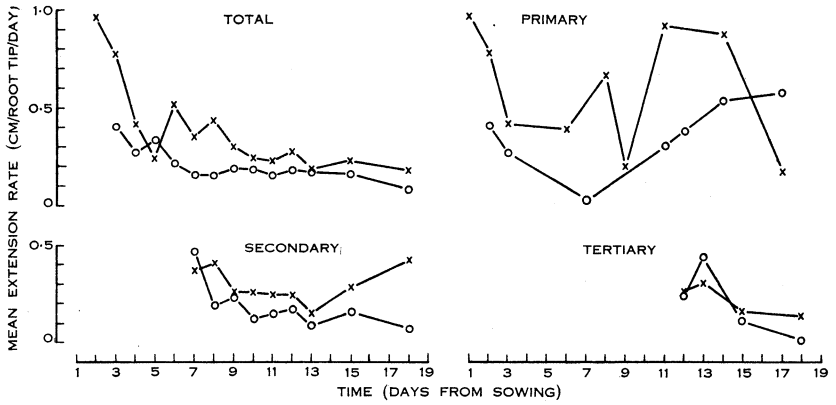

Fig. 3.—Mean extension rates of the root system and its components. × 10% nutrient concentration. ○ 100% concentration.

(c) *Elongation of Roots*

It has been previously established that a useful measure of root elongation, independent of the effects of branching, is provided by the mean extension rate, i.e. the average rate of elongation per root-tip.* For the whole root system (Fig. 3), the mean extension rate declined with time, particularly over the first 4 days of growth. Values for the individual components of the root system were much more variable, however (due principally to sampling variation), and no time trend in the data can be discerned with certainty. The time trend in the data for the total root system was probably a result of the relatively high mean extension rate of the primary root system.

The major interest in these data, however, is that an increase in nutrient concentration depressed the mean rate of root elongation. This effect was statistically significant with the whole root ($P > 0{\cdot}01$) and primary root ($P > 0{\cdot}05$) data but not with the branch root systems. As the relative extension rate was comparatively

* Where L is the total length of the root system, n the number of root tips, and l ($=L/n$) the mean root length, then the relative rate of extension of the whole system ($1/L.\mathrm{d}L/\mathrm{d}t$) may be considered as the product of the mean absolute rate of extension of each root ($1/n.\mathrm{d}L/\mathrm{d}t$) and the reciprocal of the mean root length (May, Chapman, and Aspinall 1965). Also, since $L = nl$, $1/L.\mathrm{d}L/\mathrm{d}t = 1/n.\mathrm{d}n/\mathrm{d}t + 1/l.\mathrm{d}l/\mathrm{d}t$, where $1/l.\mathrm{d}l/\mathrm{d}t$ is the mean relative rate of extension of each root.

unaffected by the nutrient concentration, and the mean extension rate is a function of the relative extension rate and the mean root length, it was to be expected that mean root length would be affected by nutrient concentration. The mean lengths of the primary and secondary roots (Fig. 4) showed a significant effect of nutrient concentration; this difference increasing with time with the primary roots.

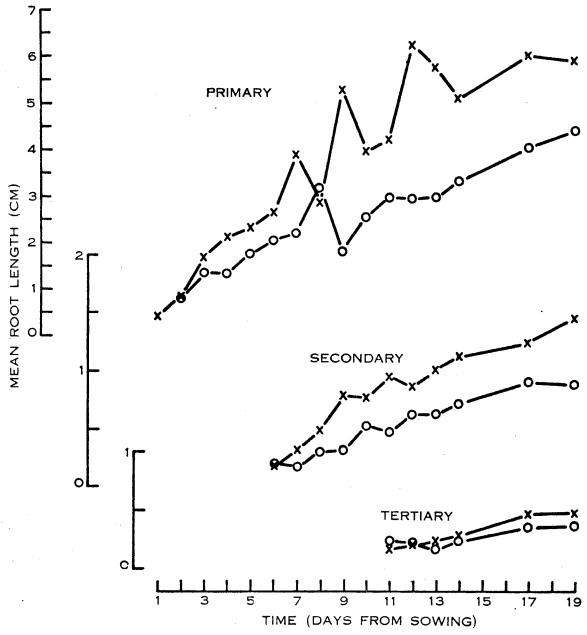

Fig. 4.—Mean root lengths of the primary, secondary, and tertiary root systems. × 10% nutrient concentration. ○ 100% concentration.

The mean root lengths calculated for each root component is of necessity the average of a large number of roots of widely differing lengths. A nutrient effect on mean length might be due to all of these roots being slightly longer or to a few of the roots being considerably longer. To test these alternatives the distributions of root lengths in the primary, secondary, and tertiary classes at the final harvest were examined (Fig. 5). There were insufficient primary roots to obtain a reasonable distribution, but the indication was that the greater mean length of the primary roots growing in the 10% nutrient concentration was due to the presence of a few long roots. However, the difference in mean length of the secondary roots was mainly due to the greater abundance of longer roots. In the 10% nutrient concentration, 32% of the roots were longer than 2 cm compared with 11% of that length in the higher nutrient concentration. The distribution of tertiary root lengths was similarly affected by nutrient supply.

Evidence presented in the previous paper suggested that new roots are not initiated between pre-existing branches, so it would appear likely that the branches along a primary or secondary root from the tip to its point of attachment represent

a series of roots of increasing age. As one effect of low nutrient concentration appears to be the promotion of the production of a few long roots, it might be supposed that the branches mainly affected would be the older ones, i.e. those nearest the point of attachment of the main root. This in fact was not so; the distribution of branch length along individual primary roots was examined with plants from the final harvest and, although there was great variation, the longest branches tended to occur near the centre of the primary root (Fig. 6). This distribution did not appear to be influenced by nutrient concentration.

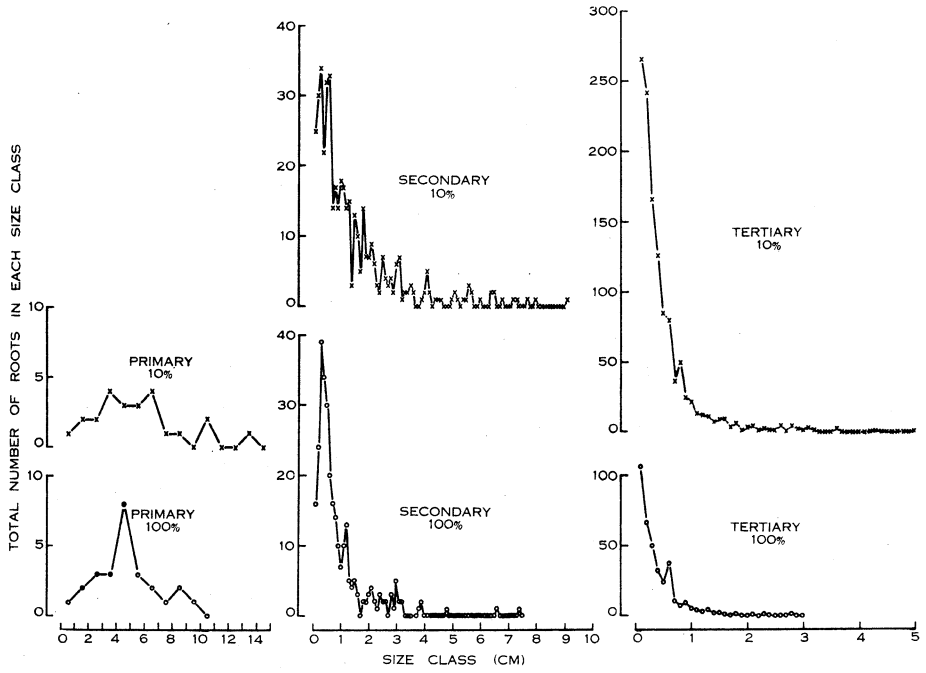

Fig. 5.—Distribution of roots among different length classes at the final harvest.

(*d*) *Branching of Roots*

The total root numbers data indicated an effect of nutrient concentration initiated early in the development of each branch system (Fig. 2). Three situations could account for this response. The parent root may have elongated more rapidly and so provided more branch sites per unit time at the low nutrient concentration. This we have seen was likely (Fig. 3). Secondly, the parent root may have commenced to initiate branches at a shorter length, and finally, the distance between branches may have been less at the lower nutrient concentration. These last two possibilities were explored by evaluating the relationship between number of branches and length of parent roots (Fig. 7). Linear relationships between these two parameters were found for both secondary and tertiary branches indicating that the spacing between branches was substantially constant with length of the parent roots. It is possible to estimate the length of the parent roots at the time of initiation of the

first branch by extrapolating the lines back to the point of zero branches. Nutrient concentration did not significantly affect this distance in either secondary or tertiary roots and indeed, with the tertiary roots the value was higher for roots grown at the low concentration of nutrients. The slopes of the lines are estimates of the reciprocal of the distance between branches, and again no statistically significant effect of nutrient concentration can be shown. It would appear, therefore, that the major influence of nutrient concentration on branching resides in the effect on elongation of the parent root.

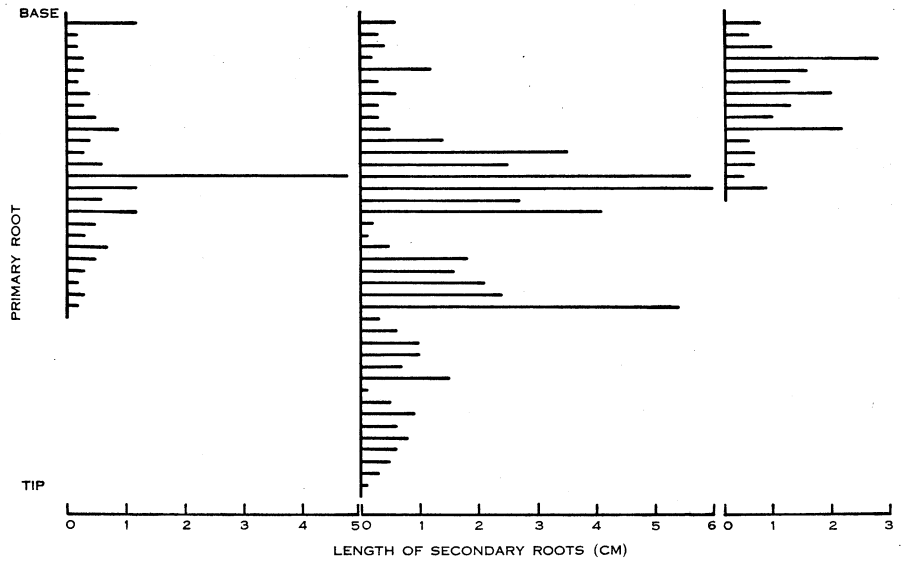

Fig. 6.—Lengths of successive secondary branches along three representative primary roots at the final harvest.

IV. Discussion

(*a*) *Pattern of Root Growth*

In the previous paper, root growth rates (RER, RMR, and MER) were found to be essentially constant over the period from 2 to 5 weeks after germination. The position of the growth curves, however, suggested that these rates were substantially higher during the earlier phase of growth. This has now been confirmed; indeed, it has emerged that the growth rates are very high at first but decrease during the first 4 days of growth, and are thereafter effectively constant with time although demonstrating some cyclic changes associated with the initiation of the various orders of branches.

The time-dependent fall in the mean extension rate of the total root system may result from two features, i.e. the depletion of endosperm reserves and a fall in the proportion of primary root tips to total number of root tips after the initiation of branching. The latter factor is important because primary roots have the highest mean extension rate. The difference in mean extension rate between orders of root tips (Fig. 3) may have been due to intermeristem competition for carbohydrate and

other substances, the primary root tips possibly being more effective competitors than the branch roots. The constant rates of extension following the first few days of growth were coincident with large increases in the numbers of presumed competing root tips, however, and does not lend support to a concept of substrate-limited extension growth. It must be emphasized that the calculated mean extension rates are derived from a large and changing population of root branches. Nothing is known

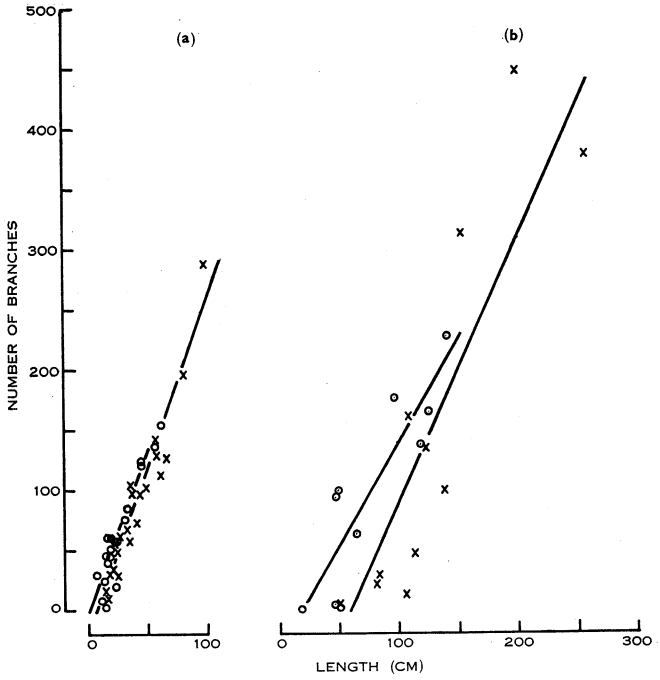

Fig. 7.—Relationships between (*a*) number of secondary branches and length of primary roots and (*b*) number of tertiary branches and length of secondary roots. × 10% nutrient concentration. ○ 100% concentration.

of the extension patterns of individual roots, these being obscured by the necessity of averaging extension rates of branches of different ages; indeed, the patterns of branch lengths along a random selection of primary roots (Fig. 6) indicate that the growth of these root branches is not likely to be describable by any simple model. The average extension rates presented in this paper greatly simplify what is undoubtedly a complex situation.

The initiation of lateral branches, although not necessarily their subsequent growth, appeared to be subject to a degree of internal control in that the distance between branches was relatively unaffected by either nutrient concentration or time. The principal factor governing the rate of production of new branch roots was the rate of growth of the parent root. The control of branch initiation in isolated roots of pea has been investigated by Pecket (1957) and Torrey (1956, 1959). Both found an inhibitory influence of the root tip and Torrey (1956) suggested that this, together with factors translocated from the cotyledons, accounted for the characteristic

acropetal production of branch initials. In addition, Pecket suggested that there was a promotive effect of the older parts of the root. In intact plants of *Acer saccharinum*, however, Richardson (1958) found evidence that the initiation, but not the elongation, of roots was dependent upon the presence of the shoot apex. There is ample data, therefore, to support the thesis that root initiation is subject to internal control by other plant organs.

TABLE 1

GERMINATION AND ROOT GROWTH OF BARLEY GERMINATED IN CONTACT WITH VARIOUS NUTRIENT SOLUTIONS

Germination Medium	Percentage Germination			Total Root Length per Germinating Seed (cm)			Mean Root Number per Germinating Seed (> 1 mm)		
	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr
Distilled water	90	100	98	0·23	4·00	20·50	1·0	4·9	6·5
10% solution	70	100	98	0·13	5·15	10·38		5·4	6·2
20% solution	34	86	96	>0·10	1·49	3·81		3·3	5·2
100% solution	0	54	66	>0·10	0·16	0·15			1·0

(*b*) *Nutrient Solution Concentration*

The trends in the data for root length and number suggest that a major portion of the effect of concentration of the nutrient solution occurred during the first 3 days of growth and were then perpetuated by the normal semi-exponential growth of the root system. This indicates an influence on germination, and the possibility was

TABLE 2

GERMINATION AND ROOT GROWTH OF BARLEY GERMINATED IN WATER FOR 24 HR AND THEN TRANSFERRED TO VARIOUS NUTRIENT SOLUTIONS

Germination Medium	Percentage Germination		Total Root Length per Germinating Seed (cm)		Mean Root Number per Germinating Seed (> 1 mm)	
	48 hr	72 hr	48 hr	72 hr	48 hr	72 hr
Distilled water	100	100	6·14	18·02	5·3	6·3
10% solution	100	100	6·49	13·85	5·4	6·2
50% solution	98	98	2·66	4·64	4·0	5·4
100% solution	96	98	0·97	1·09	2·0	2·6

explored by germinating barley (cv. Piroline) in the dark at 20°C with either 10, 50, or 100% nutrient solutions, or water. The data (Table 1) indicate a profound effect of the two highest nutrient concentrations on germination and early root growth; germination was considerably delayed and root growth, particularly in length, was severely restricted on the seedlings which did germinate. Even in the lowest nutrient concentration root elongation was less than in distilled water.

This severe effect of nutrients on germination could conceivably be restricted to the early stages of water imbibition and embryo activation. This possibility was evaluated by imbibing grains for 24 hr in distilled water before transferring them to the various nutrient solutions. Germination was virtually complete in all treatments (Table 2) but subsequent root growth was affected by the nutrient solutions. Once more both root elongation and root initiation was considerably retarded by the highest concentration of nutrients and elongation was reduced by the lowest concentration (in comparison with grains grown in water throughout). This suggests that the nutrient ions do not necessarily act solely during the early stages of germination. An osmotic effect of the ions restricting water uptake is unlikely to be of major significance as the osmotic pressure of the concentrated nutrient solution was only 2·1 atm and Gingrich and Russell (1957) reported only a slight reduction in maize radicle elongation in mannitol at this osmotic pressure. The inhibition is more likely to be due to effects of specific ions in the nutrient solution; for example, Collis-George and Sands (1962) found considerably different levels of germination inhibition with different solutes in solutions of the same osmotic pressure.

Many of the subsequent effects of nutrient concentration on root growth, including effects on branching, can be traced to this initial delay in development. Other effects appear to be more persistent, however, and an effect on mean extension rate throughout the course of the present experiment has been noted. Due to the close correlation between the various aspects of the growth of the root system as a whole, several other effects apparently stem from this difference. In particular, the rate of branching appears to depend upon the rate of elongation of the parent root. The difference in mean extension rates of plants in different nutrient concentrations also may be confined only to the relatively early stages of plant growth because there was no evidence of its persistence after 2 weeks of growth (May, Chapman, and Aspinall 1965).

V. Acknowledgment

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