RELATIONS BETWEEN THE DIMENSIONS OF THE BARLEY ROOT SYSTEM: EFFECTS OF MUTILATING THE ROOT AXES*

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Abstract

A previous study indicated that intact cereal root systems develop in such a manner that relations between the total number, length, surface area, and volume of their members remain roughly constant during the vegetative growth period. To investigate this phenomenon further, barley plants were grown in water culture and were given one or other of the following treatments: root systems left intact, or root systems mutilated by removing the tip of each axis once it was 10–20 cm long. As expected, the intact and mutilated root systems developed markedly different patterns of branching. Surprisingly, however, the relations between root dimensions were almost identical in the two groups of plants. It is concluded that the extension and branching of cereal root systems are coordinated to an extent hitherto unappreciated. The implications of this discovery for the study of root systems are discussed.

I. INTRODUCTION

The total number, length, surface area, and volume of the members of cereal root systems increase roughly in proportion during a substantial part of the vegetative growth period despite the great increase in branching complexity which occurs during normal development. This property of root systems was first identified in barley grown in water culture (Hackett 1969) but was manifest earlier in wheat grown in the field (Table III of Weaver, Kramer, and Reed 1924) and in barley grown in perlite (Fig. 1 of May, Chapman, and Aspinall 1965). It is of interest (1) because it constitutes another example of coordination in the development of plant structures, in this case a remarkable balancing of rates of root initiation, branching, and extension; and (2) because it suggests that one root dimension might be inferred from another, which, since root dimensions differ greatly in the ease with which they can be measured, could be of practical convenience.

To help assess the significance of this phenomenon for the study of root growth and performance, it was important to know whether the coordination could be maintained under adverse conditions. Moderate degrees of nutrient deficiency had already been found to have relatively small influence (Hackett 1969), so the effects of a more drastic treatment were examined, namely removal of the tip of each root axis once it was 10–20 cm long. Such treatment is known to alter root morphology dramatically (e.g. Weaver and Bruner 1927; Wilson and Horsley 1970), though no detailed quantitative studies of the long-term effects have been carried out.

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II. Methods and Materials

(a) *Terminology*

With the exception of a new term (average cross-sectional area), the morphological terms applied to the root systems described below are used in the sense defined earlier (Hackett 1968, 1969; Hackett and Bartlett 1971). The new term will be defined in context.

(b) *Plant Culture*

Seed of *Hordeum vulgare* L. cv. Maris Badger was soaked in water for 2 hr and then left in the dark on moistened filter paper. Selected seeds were transplanted next day on to stainless steel wire mesh supported over jars of nutrient solution (36 jars, four plants per jar). After a further 2 days in the dark, two plants per jar were eliminated and the remaining plants grown on under low light intensity. Two days later, the jars were transferred to the glasshouse (summer conditions—minimum temperature 15°C).

Eight days from germination, one plant per jar was harvested, leaving one plant per jar for the experiment proper. Immediately following this harvest, half of the remaining plants were removed one at a time, their roots spread out in a tray of nutrient solution, approximately 3 mm cut from the tip of each seminal axis, and the plants immediately returned to their jars. The remaining plants were then handled identically except that the tips were left intact. Harvests of six intact and six mutilated plants were taken subsequently at 13, 16, and 23 days from germination, and on the first two of these occasions the tips of nodal root axes more than 10 cm long on mutilated plants were also removed.

The volume of nutrient solution provided per plant was 160 ml for 1–3 days, 320 ml for 3–7 days, 640 ml for 7–14 days, and 2200 ml for 14–23 days. The concentration (m-equiv/l) of the major nutrients was Ca^{2+} 1·7, K^{+} 1·5, Mg^{2+} 0·75, $H_2PO_4^{-}$ 0·25, NO_3^{-} 2·0, and SO_4^{2-} 1·7; minor nutrients were given at concentrations used previously (Hackett 1968). The solutions were replaced each week. Aeration was provided throughout growth, and pH was maintained between 5·0 and 6·7.

(c) *Plant Measurement*

Upon harvesting, three of the replicate root systems were dried and weighed, together with all the shoot systems. The weights were required to check on the growth of the plants and will not be given here. The remaining root systems were preserved in a formalin–acetic acid–ethanol mixture (Johansen 1940). The number and length of the root members were determined from photographs, and the diameter of the root members was measured directly under the microscope. Since the degree of lateral development at comparable points along the root axes depended on whether the roots had been mutilated, all records were related to 2·5-cm intervals of axis length, measured from the base.

Determinations of number and length being relatively simple to make, all laterals on all roots were included in these measurements. It was impossible, however, to determine diameters comprehensively, so records were made of (1) the diameter of the axis at the centre of each interval; (2) the diameter of the primary lateral originating nearest the centre of each interval, measuring the lateral midway along its length; and (3) the diameters of the secondary and tertiary laterals originating nearest the mid-point of the measured primary lateral, also measuring midway along their length. The determinations were made by spreading the root out in water in a shallow dish with the root axis parallel to a line scored at 2·5-cm intervals. Successive intervals of axis length were then brought under the objective by gently sliding the dish across the microscope platform.

The determinations of number, length, and diameter obtained in this fashion were accumulated and related to one another to give a variety of information. This included estimates of surface area and volume, calculated on the assumption that the root members were smooth cylinders.

Relations between the dimensions were examined by the approach followed previously (Hackett 1969). The number and length of the members were compared by calculating their average length l (total length/total number). Length and surface area were compared by calculating the average diameter of the members d (total surface area/$\pi \times$ total length), and length and volume by calculating the average cross-sectional area of the members a (total volume/total length). Hackett (1968, 1969) used d to relate length and volume as well as length and surface area, but this

practice is not now recommended since there is no unique relation between the length, surface area, and volume of a family of cylinders whose members differ in diameter. The structure of the root systems described by Hackett (1968, 1969) was such that the conclusions reached in those papers remain sound but the reader should calculate a from the tables therein if an accurate expression of the relation between length and volume is required.

Working in 2·5-cm intervals of axis length proved satisfactory for most purposes, but when the change in length of the primary laterals along the root axis (called the root profile) came to be summarized, the data proved insufficient. The technique developed for describing root profiles (Bartlett and Hackett 1968) is based on polynomial regression analysis, and there were often too few intervals of axis length for acceptable curves to be fitted. The prints of the roots were therefore re-worked, with the length of every third lateral being measured as had been done when the method was first extensively applied (Hackett and Bartlett 1971). The same deficiency was found when data for the diameter of the primary laterals were submitted to regression analysis, but in this case no further measurements could be obtained, so only gross effects on lateral diameter are reported. These difficulties in no way affect the validity of the other determinations reported in this paper.

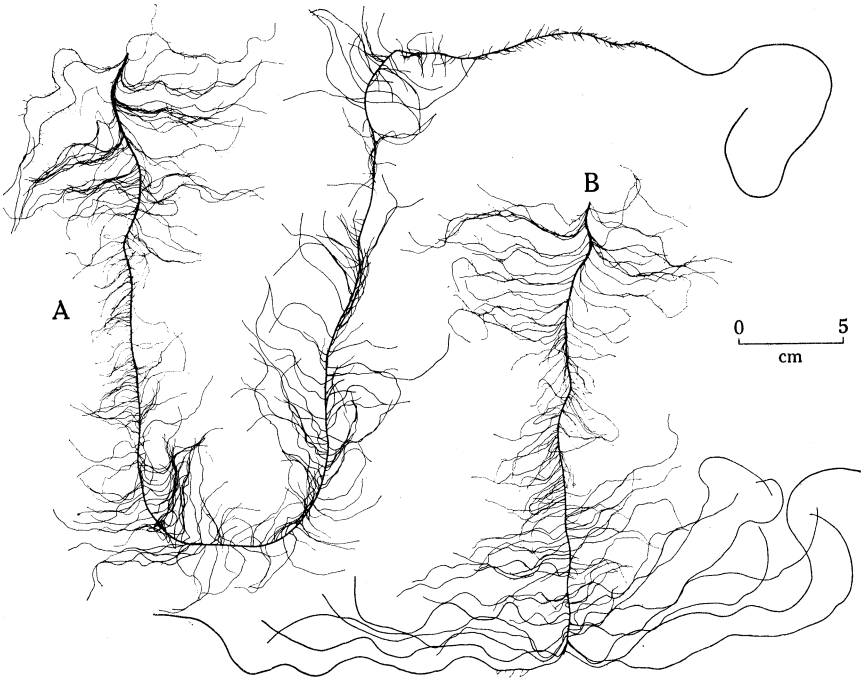

Fig. 1.—Twenty-three-day-old roots of barley (*Hordeum vulgare*) after fixing in formalin–acetic acid–alcohol and staining with azo black. Root A was intact until harvesting; root B had the tip of its axis removed at 8 days. The basal end of each root is nearest its label. *Note:* Entire roots have been photographed for this figure but when roots of this complexity are to be photographed for measurement, every second and third primary lateral is removed to permit accurate interpretation of the print.

III. Results

Effects of mutilating the roots were seen only in the root systems themselves. There were no significant effects on leaf development, tillering, or shoot dry weight.

The most striking responses to mutilation are illustrated in Figure 1. The intact roots developed a normal profile, whereas the mutilated roots produced

abnormally long primary laterals from near the distal end of the axis. These laterals were much thicker than those produced at comparable positions on the intact plants (i.e. at a similar distance from the base of the axis). Since the effects were similar in the seminal and nodal root systems, attention is mainly confined hereafter to the seminal roots.

The root profiles determined for the harvests at 8, 13, and 23 days are shown in Figure 2. Variation between replicate plants in their root profiles at 16 days was such that no representative mean profile could be obtained.

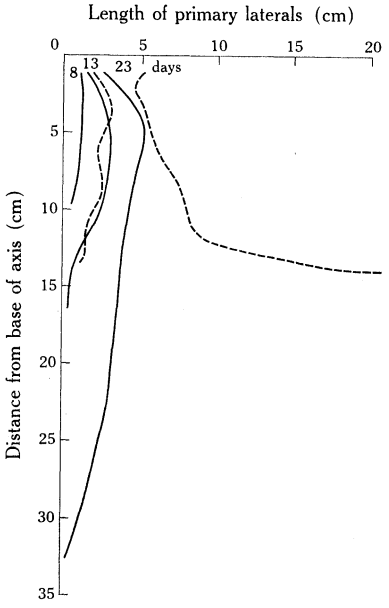

Fig. 2.—Effect on the length of the primary laterals of seminal roots of barley of removing the tips of the root axes at 8 days from germination. Curves are not shown for the harvest at 16 days because of high variation between the replicates. —— Intact roots. – – – Mutilated roots.

Figure 2 shows that at 23 days the primary laterals originating close to the distal end of the mutilated axes were about five times longer than comparable laterals. Progressing basally along the mutilated axes, the lengths of primary laterals became of similar length to those on the intact axes. None of the differences were apparent at 13 days because the most distal laterals on the mutilated axes had only recently emerged, but by 16 days these laterals were significantly longer than those on the intact axes (approximately 4·5 *v.* 2·5 cm—values taken from the primary data).

Considering the 13-, 16-, and 23-day harvests together, the mean diameter of the primary laterals formed near the distal end of the mutilated axes was 0·28 mm; at comparable points on the intact axes, the mean was 0·20 mm. The region in which such unusually thick laterals were formed on the mutilated axes extended at least 2 cm from the distal end of the axis.

As well as giving rise to abnormally long and thick primary laterals, the most distal portion of the mutilated axes produced more primary laterals per centimetre than were formed on the intact axes. Counts of primary laterals emerged were made in successive 1-cm zones from the distal end of the mutilated axes and at comparable points on intact axes. Considering again the last three harvests together, the value for

the intact axes was 3·1 laterals per centimetre; progressing basally, values for successive zones of the mutilated axes were 6·5, 4·8, 4·1, 3·6, and 3·2.

The foregoing results establish that the morphology of the intact and mutilated root systems contrasted greatly by 23 days from germination. Relations between the root dimensions will now be examined.

Table 1 shows the number of primary, secondary, and tertiary laterals emerged per seminal root at all four harvests (a root is defined as an axis with its accompanying laterals). The greatest difference between the intact and mutilated roots was seen at 23 days, by which time there were nearly three times more primary laterals on the intact roots ($P < 0{\cdot}001$). This difference was more than compensated for by an increase

TABLE 1

EFFECT OF MUTILATING THE ROOT AXES OF BARLEY ON THE NUMBER OF MEMBERS (PER ROOT) IN THE SEMINAL ROOT SYSTEM

Days from Germination	Condition of Axes	No. of Members per Root				Total No. of Members
		Axes	Primary Laterals	Secondary Laterals	Tertiary Laterals	
8	Intact	1	25	0	0	26
13	Intact	1	53	9	0	63
	Mutilated	1	50	8	0	59
16	Intact	1	62	87	0	150
	Mutilated	1	53	66	0	120
23	Intact	1	121	405	16	543
	Mutilated	1	47	626	23	697
Coefficient of variation*		—	16	30	60	17

* The values represent the mean of the coefficients calculated individually for each harvest.

in the number of secondary laterals on the mutilated roots ($P < 0{\cdot}05$). The additional secondary laterals were formed largely on the primary laterals arising near the distal end of the mutilated axes, a region where secondary laterals on the intact roots were still relatively few (180 secondary laterals per root in the distal 7·5 cm of the mutilated axes versus 60 in the comparable region of the intact axes). The density of secondary lateral branching (i.e. number per centimetre of branched primary lateral) was similar in both treatments.

The lengths of the seminal axes and laterals are shown in Table 2 together with the average length (l) of the members of the seminal and the total root system. The total length of primary lateral per root was similar in both groups of plants, indicating that the greater length of the laterals arising near the distal end of the mutilated axes compensated for the cessation of primary lateral production by these axes after 13 days. Secondary laterals, which were 50% more numerous in the mutilated plants, were 85% greater in total length. Despite these modifications in growth, l was almost identical in both groups of plants at 13 and 23 days. Temporarily, around 16 days, l was greater in the mutilated than the intact root systems ($P < 0{\cdot}01$).

At this time the primary laterals formed near the distal end of the mutilated axes had become longer than their counterparts on intact axes (see an earlier paragraph)

TABLE 2

EFFECT OF MUTILATING THE ROOT AXES OF BARLEY ON THE TOTAL LENGTH AND AVERAGE LENGTH OF THE SEMINAL ROOT MEMBERS AND THE AVERAGE LENGTH OF ALL ROOT MEMBERS

Days from Germination	Condition of Axes	Length (mm/root)				Total Length (mm)	Av. Length (mm)	
		Axes	Primary Laterals	Secondary Laterals	Tertiary Laterals		Seminal Root Members	All Root Members
8	Intact	150	170	0	0	320	12·3	12·3
13	Intact	220	890	10	0	1120	17·8	18·0
	Mutilated	140	920	10	0	1070	18·1	18·6
16	Intact	300	1810	320	0	2430	16·2	16·4
	Mutilated	180	2180	170	0	2530	21·1	20·9
23	Intact	380	3390	2530	30	6330	11·7	13·4
	Mutilated	150	3310	4740	40	8240	11·8	13·2
Coefficient of variation		19	15	42	n.d.	11	11	12

but had not begun the branching which, by 23 days, compensated for this more vigorous growth (no secondary laterals were recorded at 16 days in the distal 7·5 cm of the mutilated axes and negligibly few on the intact axes).

TABLE 3

EFFECT OF MUTILATING THE ROOT AXES OF BARLEY ON THE AVERAGE DIAMETER AND AVERAGE CROSS-SECTIONAL AREA OF THE SEMINAL ROOT MEMBERS AND ALL MEMBERS OF THE ROOT SYSTEM

Days from Germination	Condition of Axes	Average Diameter (mm)		Average Cross-sectional Area (mm^2)	
		Seminal Root Members	All Root Members	Seminal Root Members	All Root Members
8	Intact	0·29	0·29	0·303	0·303
13	Intact	0·24	0·26	0·054	0·062
	Mutilated	0·25	0·26	0·051	0·061
16	Intact	0·22	0·24	0·045	0·053
	Mutilated	0·23	0·24	0·047	0·053
23	Intact	0·18	0·22	0·030	0·054
	Mutilated	0·19	0·22	0·031	0·052
Coefficient of variation		6	4	12	10

The average diameter (d) and the average cross-sectional area (a) of the members of the seminal and total root system are shown in Table 3. As was found previously (Hackett 1969), d declined with time in the seminal root system, but at each harvest

the values for the intact and mutilated roots were almost identical despite the contrasts in form which have been described. Calculated over all root members, d declined less with time, and having regard to the range of diameters observed at 23 days (*c.* 0·10–1·10 mm), the effect was small. A very similar pattern of results was observed with respect to a.

IV. Discussion

The chief finding from the experiment was unequivocal and unexpected. Mutilation of the root systems, whilst highly successful in altering the pattern of branching, had almost no effect on relations between the root dimensions. Taken together with the earlier finding (Hackett 1969) that relations between cereal root dimensions remain roughly constant with time, the results imply that the extension and branching of root systems is a highly coordinated process.

The discovery of this phenomenon may have some valuable consequences. The phenomenon is marked enough to invite enquiry into its causes, and through this, some new understanding of root development may emerge. One line of enquiry being followed is to ask what latitude a root has in its pattern of growth if it is to accommodate an exponentially increasing supply of assimilate and yet maintain constant relations between its dimensions. An attempt is being made to answer this question by manipulating models of root growth based on data from the present experiment.

In a more practical context, knowledge that such homeostatic processes operate during root growth may diminish the conceptual difficulties faced by experimenters contemplating structural analysis of cereal root systems. The high degree of apparent randomness in the growth and branching of cereal root systems discourages their quantitative study, but if it is confirmed that development follows a theme, albeit one that can be expressed in a variety of forms, a framework for the analysis of root measurements will be available, so making the labour involved less forbidding.

A third consequence may be a slight alleviation of the problems of root measurement. It is common in studies of root growth and performance to take interest in more than one root dimension, and if within any one experiment the relationships between the root dimensions remain roughly constant, it must be possible to infer one dimension from another. Root dimensions differ very greatly in the ease with which they can currently be measured, so some advantage might be taken of the phenomenon in question if limited accuracy is acceptable and suitable precautions are observed. It should be pointed out, however, that l, d, and a may differ substantially between experiments (cf. data in this paper with that of Weaver, Kramer, and Reed 1924; May, Chapman, and Aspinall 1965; Hackett 1969), so their value should be determined afresh for each experiment.

The reasons for the much greater variation in l, d, and a between than within experiments will not become clear until more investigations are made. Fine control of the shoot and root environment is certainly not required for the phenomenon to manifest itself, since all examples so far come from glasshouse or field experiments in which major environmental fluctuations undoubtedly occurred. Gross discontinuities within the root medium, such as fertilizer placements or layers of com-

pacted soil, may upset the relations between root dimensions, but the outcome of the present experiment suggests that it would be wiser to prove this than to assume it.

In short, the phenomenon brings to light a number of intriguing possibilities for the study of root systems. Currently these seem relevant more to enquiries into growth than performance, because uptake from soil depends on many root characteristics unrelated to overall root dimensions. However, the degree of exploration of the soil medium enters into considerations of performance (Barley 1970), and in this respect too the phenomenon may be of interest.

V. Acknowledgments

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