THE EFFECTS OF DEFOLIATION AND SHADING ON ROOT GROWTH OF *LOLIUM PERENNE* L.

A thesis submitted for the degree
of Doctor of Philosophy to
Massey University

P. S. Evans

1971

ABSTRACT

The effects of defoliation, shading and dark on the growth and morphology of roots of Lolium perenne L. plants have been examined using glass fronted containers and a technique developed for measuring root lengths and numbers of apices.

A single defoliation caused a rapid drop in root elongation followed by a more gradual recovery with the most severe defoliation treatment having the greatest effect. Repeated defoliation caused a prolonged depression of root elongation but some recovery occurred. The most severe treatment resulted in considerable root death. With shading, root elongation fell over the first 8 - 10 days and then recovered to near the control level. Both defoliation and shading caused an increase in the length per unit weight of the root systems. Root elongation of plants placed in the dark fell rapidly to near zero, the effect being comparable with that of defoliating plants to 2.5 cm or less. Defoliation of plants placed in the dark caused a more rapid fall in elongation.

Supplying glucose or sucrose to the roots of plants defoliated to the extent that root elongation would otherwise have ceased maintained elongation at up to two-thirds of the level of undefoliated plants. Sucrose was marginally more effective than glucose with little difference between concentrations 1-6%. Benzyladenine and indole-acetic-acid marginally increased elongation in the presence of sucrose. Dark-treated plants responded in a similar manner to defoliated plants to sucrose. The level of soluble carbohydrates in the roots of plants defoliated or placed in the dark was seen to be low after root elongation ceased and recovered as root elongation recovered. However the level at the time most roots ceased elongating was higher than in other experiments where root elongation was near optimum. That under these conditions the addition of sucrose or glucose maintains elongation at up to two-thirds of the control level suggests that translocation of soluble carbohydrates to the root apex may be the limiting factor. This possibility is supported by the difference in levels of soluble carbohydrates in various parts of the plant following defoliation at two different times of the day. The tech-

nique used to measure soluble carbohydrates was not sensitive enough to permit analysis of the root tips and thus check the hypothesis. Apart from the requirement for soluble carbohydrates and the apparent associated translocation factor there is evidently some other factor (s) limiting root elongation of defoliated and dark-treated plants. The nature of this factor was not determined.

ACKNOWLEDGEMENTS

The guidance of Professor R. G. Thomas and Dr. E. D. Penny, Botany Department is gratefully acknowledged. Appreciation is expressed to Mr. V. J. Thomas, Applied Mathematics Division, D.S.I.R., for helpful discussion and to Mr. P. Dilks and Mr. M. H. Olsen for technical assistance. The facilities for this study were provided by Grasslands Division, D.S.I.R.

TABLE OF CONTENTS

APPENDIX PAGE

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

The study of the root systems of pasture plants has lagged behind the studies of the shoots and leaves because of the difficulties involved. However, because of the increases in productivity and utilisation of pastures at present occurring in New Zealand as a result of improved management techniques and economic pressures, close attention must be paid to all factors likely to limit economic pasture production.

Besides the general functions of nutrient uptake, water uptake and anchorage, roots of pasture plants warrant study for their roles as sinks for photosynthate, modifiers of soil structure for future crop rotations, and for their possible food storage capacity. There are also in New Zealand at present three particular problems where a knowledge of root growth and function would be of considerable help. On dairy farms, particularly where stocking rates are high, pasture pulling occurs at certain times of the year. The animals when grazing remove the whole plant, the roots breaking off just below the soil surface. A likely explanation lies in the seasonal production and death of roots. Grass grub (the larva of a scarab beetle, Costelytra zealandica (White)) causes loss of pasture production, and in extreme cases pasture death, by eating the roots of the plants. An accurate assessment of the way in which the root systems of the various species are affected would assist in the study of this pest. Phosphate fertiliser is an important requirement for high pasture production, the quantity applied being determined by the requirements of the legume component of the sward which is a poor competitor for phosphate compared with grasses. Part of the explanation of the differences in competitive ability may be differences in root morphology and distribution.

A major difference between pastures and most other kinds of vegetation is the imposition of severe and regular defoliation on the former, either by grazing animals or by machines in hay and silage making. The management of this defoliation pattern is a prime factor determining pasture production. Closely related to the defoliation pattern is mutual shading of the plants, shading being greatest where long intervals occur between defoliations.

Because of their importance in pasture production, the effect of defoliation and shading on root growth and morphology has been chosen as the topic of this thesis.

CHAPTER 2

MATERIALS AND METHODS

2.1 INTRODUCTION

The difficulty of examining roots under field conditions is that even supposing they can be satisfactorily separated from the soil, there is no method except perhaps for the tetrazolium test (Goedewaagen, 1954; Jacques and Sohwass, 1956) of distinguishing between living and dead roots (Troughton, 1957; Garwood, 1967). At any time therefore the weight of roots in a given volume of soil represents the balance between root growth and decay. The presence of dead roots can be avoided by using young, vigorously growing plants (Troughton, 1956). With young plants, then, the proportion of photosynthate that is being used in root growth (commonly considered in the form of the balance between the shoot and the root i.e. the shoot/root ratio) can be measured.

The distribution of root weight according to the depth below the soil surface and lateral distance from the base of the shoot has been measured by a number of authors (see Troughton, 1957; 1961). This provides some indication of the potential exploitation of the soil by the root system. However the worth of such measurement is doubtful when the root system is confined within a container which places a restriction on root growth.

Root length is a worthwhile parameter to measure since it gives a better picture than root weight of the potential exploitation of the soil by the root system. This point is discussed by Wiersum (1961). For example, other things being equal a plant with a greater length of roots would be expected to have a greater capacity to utilise the minerals and water in a soil volume than a plant with a lesser length of roots. It is conceivable that two such plants could have the same root weight (i.e. one having thinner roots than the other) and hence measurement of root length would help explain what might otherwise appear to be a major anomaly. Pavlychenko (1937; 1942) measured root length directly. This work emphasies just how extensive the root systems of even comparatively small plants are and how much they may be affected by environmental factors. The possible importance of measuring root length as well as dry weight is also raised by Krassovsky

(1926) and Williams (1962). Williams compared the capacity of nodal and seminal roots to support young timothy plants when growing in nutrient solution. He observed on analysing the plants for mineral nutrients that the seminal roots were several times more active on a dry weight basis. Krassovsky obtained similar results for water in several cereals. The greater activity of the seminal roots can be explained by the observations of Weaver (1926), Weaver and Zink (1945), Brouwer and Locher (1965) and Brouwer (1966) that the seminal roots are thinner than the nodal roots and would therefore have a greater surface area per unit weight.

May (1960), considered that measuring the number of root apices would be worthwhile as far as activity is concerned because the region immediately behind the root apex is most active in uptake (Wiebe and Kramer, 1954; Kramer, 1956; Brouwer, 1965; Russell and Sanderson, 1967). Therefore changes in the number of root apices might be expected to indicate changes in the potential activity of the root system.

In excised root culture, elongation of individual roots has been routinely used as a measure of response to applied chemicals. Baldovinos (1953) measured elongation of bean radicles by marking with indian ink and measuring distance from the ink mark to the position of the root tip after different periods of time. Crider (1955), Asher and Ozanne (1966) and Head (1966) have achieved the same results by observing roots growing against glass and measuring elongation against reference points on the glass. This work demonstrates the sensitivity of root elongation to changes in the environment.

2.2 CHOICE OF EXPERIMENTAL MATERIAL

Monocotyledons, especially perennial grasses, have an advantage over most dicotyledons as experimental material for root growth studies in that they produce from the base of the shoot a succession of adventitious roots (nodal roots) of fairly uniform size and growth rate under glasshouse conditions. This is in contrast to the tap root and laterals of varying sizes and growth rates which develop in typical dicotyledons.

Lolium perenne L. was chosen because it is the most important pasture grass in New Zealand and its morphology and physiology are well documented. As with other perennial grasses however this species

does have a small seed and thin roots compared with cereals.

Being an outbreeding species L. perenne shows considerable genetic variability which is reflected in the high coefficients of variation in the single plant experiments (20-30%). Genetic variability could have been avoided by using clonal material but this would have introduced the problem of root senescence and death during experiments which is unlikely to occur using young plants grown from seed. Results from experiments in which clonal material is used are also open to the criticism that the response of the clone may not be typical of the response of the species to the experimental treatments. To decrease variability, seed of air dry weight 1.7-1.9 mg (modal 20% of sample) was used. The effect of seed weight on plant size is described in Appendix 6. Further selection for uniformity of plant size was made at the 20-30 tiller stage except where experimental treatments commenced at an earlier stage (Appendices 3,5, 6 and 7).

2.3 PARAMETERS STUDIED AND METHODS USED

Root dry weight, length, number of apices, and elongation rate have all been measured in some experiments in this thesis. Greatest emphasis has been placed on elongation rate since this parameter is the most sensitive to environmental changes and it can be measured non-destructively.

The direct measurement of root length is time consuming and difficult in a species with fine roots such as L. perenne. Therefore a modification of the sampling technique published by Newman (1966a) has been used and in conjunction with this a technique has been developed for measuring numbers of root apices. The procedures are detailed in Appendix 1.

The weight of root systems has been measured after washing and drying overnight at 100°C.

Root elongation has been measured on plants growing in glass fronted containers (Appendix 2).

Other plants were grown in 40 cm x 10 cm unglazed pipes of the

type used in field drains, coated outside with aluminized bituminous paint to reflect heat and reduce moisture loss. These were used in preference to ordinary plant pots because their greater depth reduced the mat of roots which commonly forms at the base of a pot.

Sand was used as a root medium because of the ease with which it could be washed from the roots at harvest. Nutrient solution of the following composition (mg/l) was used: $Ca(NO_3)_2.4H_2O$,590; KNO_3,253; $MgSO_4.7H_2O$,246; KH_2PO_4,68.0; Fe EDTA,41.7; KCl,3.15; H_3BO_3,1.43; $MnCl_2.4H_2O$,0.9; $ZnSO_4.7H_2O$,0.11; $CuSO_4.5H_2O$,0.04; $Na_2MoO_4.2H_2O$,0.01.
Each day an amount in excess of that required to restore the sand to field capacity was applied. A test of the suitability of the concentration of this nutrient solution for growing L. perenne is presented in Appendix 3.

Seed was germinated at 20°C in petri dishes and planted into sand in the unglazed pipe or else into 10 cm plastic pots for subsequent transplanting into the glass fronted containers. Experiments were performed in a glasshouse the temperature of which was controlled within the range 10-21°C but which occasionally exceeded this range or in a growth room at 22 $\pm$ 1°C. The significance of temperature fluctuations on root elongation are indicated in Appendix 4.

The most suitable plant size for the various experiments was determined on the basis of two-weekly harvests of plants grown from seed (Appendix 5) and from trial plantings in glass fronted containers used in many of the experiments. (Appendix 7). In the glass fronted containers, three plants each of 20-30 tillers provided the optimum number of roots against the glass. In later experiments where the experimental period was less than a week, four plants were used.

Elongation was measured on a random selection of primary nodal roots (see Appendix 8) the apices of which were more than 5 cm below the base of the shoot and 10 cm above the bottom of the container.

CHAPTER 3

EFFECTS OF DEFOLIATION AND SHADING ON ROOT GROWTH

3.1 INTRODUCTION

The relationship between root growth and shoot growth may be regarded as primarily competitive, the root being limited by the supply of carbohydrates and other growth materials from the shoot and the shoot being limited by water and minerals from the root (Loomis, 1953; Humphries, 1958; Troughton, 1960). This relationship may of course be complicated by the action of endogenous growth substances. Obviously too, when the input of a limiting factor is decreased, besides the shift in relative production of shoot and root, there will be an overall decrease in the total growth of the plant. Brouwer (1963) points out that in the field the effects of the roots terminates as soon as a closed cover is formed and photosynthesis becomes the limiting factor. Therefore root factors are more important during early development or where defoliation has occurred.

Effect of defoliation on root growth

The mass of data much of which is summarised by Weinmann (1948) and Troughton (1957; 1962), indicates that generally in pasture plants grazing and cutting reduce rooting depth and both root and shoot production, the reduction in the root being the greater. The effects of successive defoliations are cumulative and reduced frequency balances increased severity. Cessation or severe retardation of root elongation has been reported in a number of grasses (Parker and Sampson, 1931; Robertson, 1933; Baker, 1957; Beard and Daniels, 1965; Davidson and Milthorpe, 1966) and death of some roots has also been recorded (Robertson, 1933; Peralta, 1935; Weaver and Zink, 1946) and by Butler et.al. (1959) in three pasture legumes. Davidson and Milthorpe noted also an associated drop in respiration and ^{32}P uptake. Beard and Daniels found that the effect on root elongation was much less severe than on weight increase in the root mass. Reduced root growth following defoliation has also been recorded in apple and plum trees (Head, 1967) and in Acer saccharinum (Richardson, 1953b). New roots were noted to be thinner than those of undefoliated plants (Parker and Sampson, 1930; Robertson, 1933; Biswell and Weaver, 1933; Peralta, 1935; Beard and Daniels, 1965).

Differences between species in response to defoliation have been recorded. These are considered to be related to plant habit, the amount of shoot remaining for supplying stored food or for photosynthesis being the important factor (Harrison and Hodgson, 1939; Lowvorn, 1945; Julander, 1945; Weinmann, 1948). Nitrogen fertilizer enhances the inhibiting effect of defoliation by reason of the reduction of carbohydrate reserves in the formation of extra protein (Harrison, 1934; Johnson and Dexter, 1939; Haynes, 1943).

Consistent with the idea that the relationship between the shoot and the root is constant for a given set of environmental conditions (Troughton, 1960; Brouwer, 1963), Ennik (1966) showed that in L.perenne and Trifolium repens L. defoliated plants recovered to a constant shoot/root ratio. This is also implicit in the results of Mitchell (1954) where the differences in rates of growth of shoot and root decreased as plants of Lolium species recovered from defoliation.

Effect of Light Intensity

Consistent with the concept of a balance between shoot and root is the increase in shoot/root ratio on reducing light intensity. The results of a number of experiments and observations on pasture plants are summarized by Troughton (1957). Similar results have been recorded more recently by Troughton (1960), Lebedev (1963), Evans et al (1964), Asher and Ozanne (1966). Kozlowski (1949) recorded an increase in the shoot/root ratio in Quercus and Pinus as a result of shading. Kramer and Decker(1944) concluded that loblolly pine seedlings failed to establish in shade because they did not produce enough carbohydrates to grow sufficient roots to absorb enough water during drought. Asher and Ozanne (loc.cit) found that shade reduced the rate of seedling root growth in all pasture species they examined, the degree of reduction being related to the degree of shading. Richardson (1953a) found in short term experiments that Acer saccarinum root growth exhibited a rapid drop on reducing light intensity from 5000 to 200 lux and a rise on returning to 5000 lux. In a later paper (Richardson, 1953b) the initial drop in elongation on reducing the light intensity was followed by a partial recovery. Wassink and Richardson (1951) found differences between species in response to reduced light intensity.

They associated these differences with differences in natural habitat, the species which grew naturally in shade being less affected by reduced light intensity.

Butler et al (1959) found that reducing the light intensity by 75% resulted in the death of some roots in three pasture legumes. In contrast to the effect of defoliation in these three species there was little subsequent new root growth.

Soper (1957) examined in several pasture species the effect of reduced light intensity accompanied by supra-optimal temperature. Roots of plants grown under the adverse conditions were thin and wavy while those in high light at a lower temperature were thick, straight, glossy and white. The change in existing roots was rapid. The thin roots had a lesser number of smaller cells contributing to the reduced diameter. She considered that under low light and high temperature the cortex was short lived. The change was considered to be due to a decrease in carbohydrate supply.

3.2 EFFECT OF A SINGLE DEFOLIATION

Methods

The experiment consisted of six replicates of four treatments. In one treatment group the plants were defoliated to 2.5 cm above the base of the shoot, in the second group to 5.0 cm above the base and in the third group to 7.5 cm above the base. The fourth group was left undefoliated as a control.

200 seeds were germinated in petri dishes and 140 were planted 4 per pot into 12 cm plastic pots of sand when the coleoptiles were approximately 1 cm long. The early and late germinating seeds were discarded. When the plants had reached the 20-30 tiller stage, all were sorted according to size and those from the middle of the range planted three per container into 24 glass fronted containers. The containers were arranged in a row along the front of the glasshouse bench all facing south. Six days after transplanting the positions of 20 root apices per container were marked on the glass with a fibre tip pen and the elongation for the following 24 hours recorded. The marks on the glass were then removed with a cloth moistened with acetone and the procedure repeated. Plants were then defoliated and root

elongation on all containers measured each day until harvest. The treatments were harvested separately when the elongation rate had recovered to the control level, the controls being harvested at the same time as the last defoliated group. At harvest the plants were washed free of sand and the shoots and roots weighed separately after drying at 100°C overnight. The shoot/root ratios were calculated.

Results

The mean daily elongation rates are presented in Fig. 1. The results are presented as percentages of the growth of the controls for the particular day because the growth of all plants fluctuated from day to day owing to fluctuations in environmental conditions, particularly temperature (see Appendix 4). In all defoliation treatments elongation dropped sharply on day 1 after defoliation with lesser reductions on days 2 and 3. The reduction in elongation was greatest in the 2.5 cm defoliation treatment and least in the 7.5 cm treatment. Subsequent to day 3 following defoliation all plants recovered steadily to slightly above the controls, the least severe defoliation treatment exhibiting the most rapid recovery. There were significant differences at the 5% level between all treatments except 7.5 cm defoliation and control from days 8 to 11. No standard errors could be presented in the figure because of the transformation of the data.

In the 2.5 cm treatment most of the roots ceased elongating altogether on day 3 but a few continued to grow at rates up to 30% of the control mean. In a preliminary experiment it was noted that as a result of defoliation the apices of some roots which ceased to elongate turned brown and showed no further activity. These roots were presumed to have died. In the main experiment a record was kept over the first 8 days of the state of individual roots. In the 2.5 cm treatment, two root apices turned brown and were presumed dead. The figures for these two roots were excluded from the results.

The shoot weights, root weights and shoot/root ratios of the plants at harvest are presented in Table I. The 4 groups did not differ significantly in shoot/root ratio although varying amounts of shoot had been removed. The three defoliation treatments did not

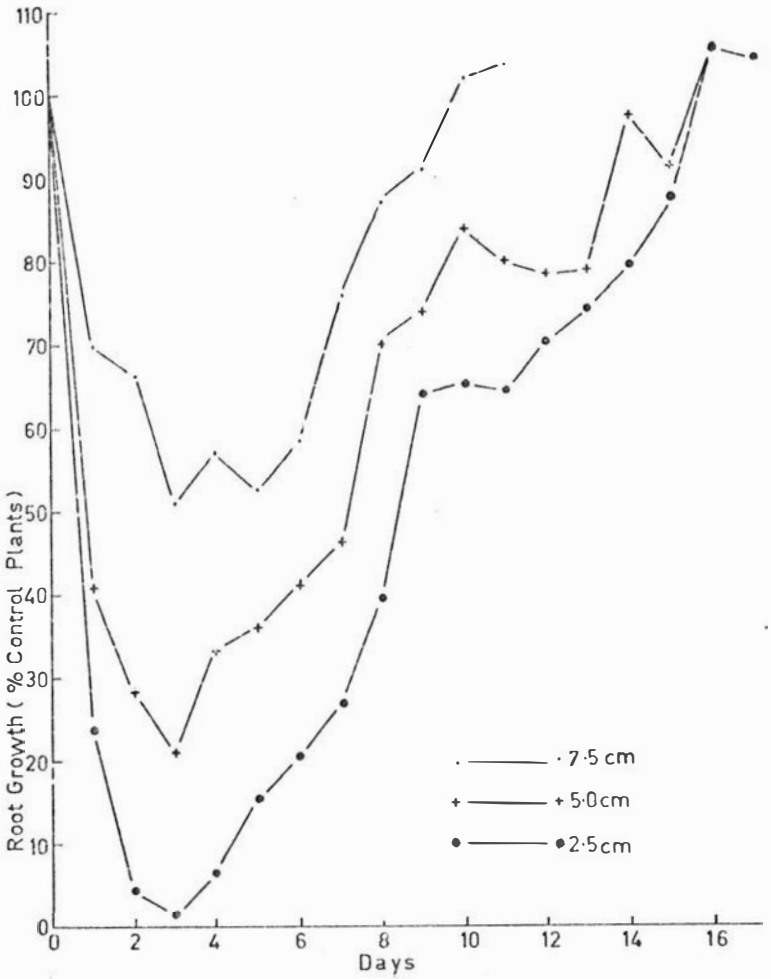


Fig. 1 - Effect of a single defoliation to three different levels on root elongation. Results expressed as percentages of the elongation of the controls.

Table 1 - Effect of a single defoliation on plants harvested when root growth had recovered to control plant level

Treatment	Shoot weight (g)	Root weight (g)	Shoot/root ratio	Days to harvest
2.5 cm	5.78	2.29	2.52	17
5.0 cm	5.56	2.31	2.43	16
7.5 cm	6.02	2.48	2.44	11
Control	14.71	5.91	2.56	17
S.E.±	0.30	0.27	0.088	-
D.05	0.64	0.59	0.19	-

differ significantly from one another in root or shoot weights however all were less than half the values for the controls. Note that the 7.5 cm treatment was harvested several days earlier than the 2.5 cm and 5.0 cm treatments.

3.3 EFFECT OF REPEATED DEFOLIATION

Experiment 1 - Plants grown in glass fronted containers.

Methods

The experimental layout and procedure were the same as for the investigation of the effect of a single defoliation except that the plants were defoliated every second day instead of once only. The experiment was terminated 14 days after the initial defoliation and 10 nodal roots were removed from each container. These roots were washed and a 5 cm length was taken from immediately behind the apex of each root. These pieces were dried overnight at 100°C and weighed. The length per unit weight of nodal root was calculated for each container.

Results

The results are presented in Fig. 2. As in the single defoliation experiment a record was kept of the growth of individual roots and the figures for any which died were excluded from the results. Elongation fell steeply on the first day after defoliation and reached a minimum on day 3. Thereafter there was an indication of recovery with time in all three defoliation treatments. In the 2.5 cm treatment an average of 60% (40-87%) of the roots died and in the 5.0 cm treatment 45% (25-68%) died. No deaths occurred in the other two treatments. Depression of root elongation was most severe in the 2.5 cm defoliation treatment and least in the 7.5 cm treatment. Differences between treatments were significant at the 5% level or almost so on all days except on days 9-11 between 7.5 cm defoliation and control, on days 13 and 14 between 5.0 cm and 7.5 cm defoliations and on day 8 between 2.5 cm and 5.0 cm. defoliations.

The length per unit weight of nodal root for each of the treatments is presented in Table 2. The values for the 2.5 cm. and 5.0 cm treatments are significantly greater than that of the controls.

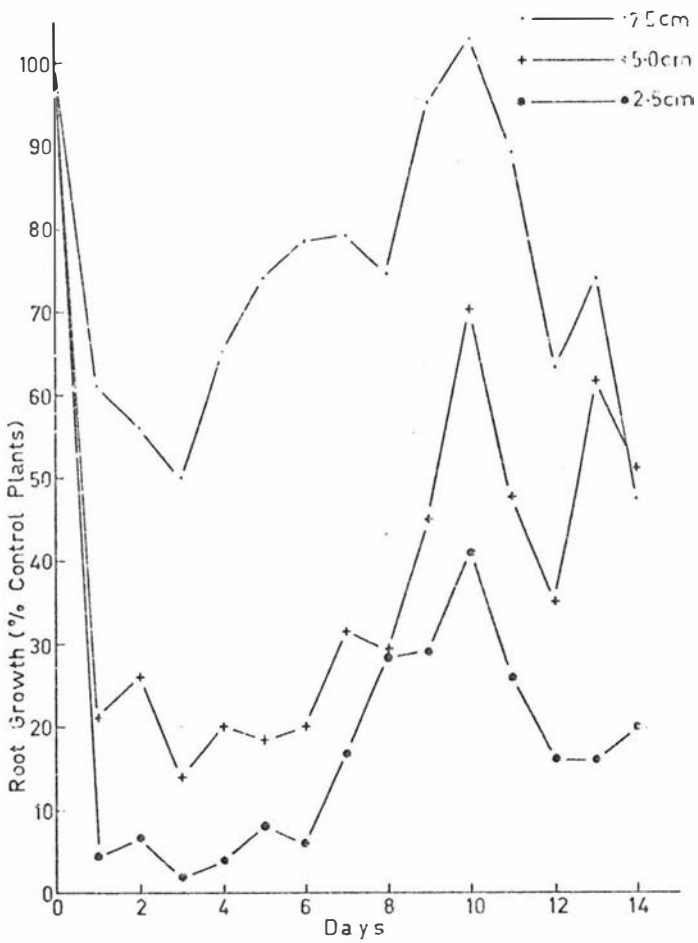


Fig. 2 - Effect of defoliation to three different levels every second day on root elongation. Results expressed as percentages of the elongation of the controls.

Table 2 - Effect of repeated defoliation on root morphology

Treatment	Experiment 2 Plants in pipes			Experiment 1 Plants in containers
	All roots below 7.5 cm		Primary nodal root. Length per unit weight (cm/mg)	Primary nodal root length per unit weight (cm/mg)
	Length per unit weight (cm/mg)	No. of apices per unit length (cm)		
2.5 cm	49.5	1.13	6.20	10.92
5.0 cm	43.9	1.15	4.87	7.58
7.5 cm	47.5	1.18	3.59	4.55
Control	38.9	1.08	2.44	3.12
S.E. ±	2.62	0.07	0.30	1.03
D.05	5.37	0.14	0.62	2.19

Experiment 2 - Plants grown in clay pipes.

Methods

The measurement of possible changes in root morphology caused by defoliation was not considered possible in the container-grown plants because the amount of new root growth in the defoliated plants would have been small compared with the amount present at the start of treatment and it was doubtful if this new root could be distinguished from the old. Therefore to assess the effect of repeated defoliation on root morphology 40 plants at the 20-30 tiller stage with the roots and shoots trimmed off to 7.5 cm from the base were planted singly into clay pipes. The experiment consisted of 10 replicates. One plant at random in each replicate had the shoot defoliated at 2.5 cm, 5.0 cm or 7.5 cm twice a week. The interval between successive defoliations was greater than in the previous experiment in order to ensure that sufficient new root grew within a reasonable time for sampling. The fourth group was allowed to grow on as a control. Several extra plants were treated in the same way and defoliated to 2.5 cm. One of these plants was harvested each week to ascertain the amount of new root below 7.5 cm from the base of the shoot. After 4 weeks by which time the amount of new root below 7.5 cm from the base of the shoot was considered sufficient for sampling, all plants from the experiment proper were harvested. Root length and apex numbers were estimated as detailed in Appendix 1. The dry weight of ten 5 cm lengths of primary nodal root from each plant was also measured in order to compare this experiment with the one in the glass fronted containers.

Results

The results are presented in Table 2 together with the length per unit weight of primary nodal root from Experiment 1. The length per unit dry weight of all root below 7.5 cm was greater in two of the three defoliation treatments than in the controls and was also greater in the 2.5 cm treatment than in the 5.0 cm treatment. The differences between these two treatments may not be important taking into account the figure for the 7.5 cm treatment. The number of apices per unit length of root in the defoliated treatments was not significantly different from the controls. In both experiments the length per unit weight of primary nodal root was increased by increasing severity of defoliation. The effect was greater in the case of the

experiment in the glass fronted containers where the plants were defoliated more frequently.

3.4 EFFECT OF SHADING

Experiment 1 - Plants grown in glass fronted containers.

Methods

The effect of shading was examined using screens consisting of one or more layers of two different grades of white paper giving light transmissions of 60, 40, and 20%. These screens came down 15 cm below the bases of the plants on all sides and were vented at the corners to allow air circulation without allowing a significant amount of light to penetrate. Twenty four containers were planted and set out as described for the defoliation experiments and the shade screens placed over the appropriate containers. The experiment consisted of 6 replicates, one treatment being an unshaded control. Root elongation was recorded each day, the experiment being terminated when the trends in elongation had become evident. The dry weights of ten 5cm lengths of primary nodal root harvested from each container at the conclusion were recorded and the length per unit weight calculated.

Results

The results are presented in Fig. 3. In all three shading treatments root elongation fell steadily, reaching a minimum 8 - 10 days after shading commenced. Elongation increased from day 10 to day 14 and then in the 20% and 60% treatments more slowly till the termination of the experiment. There was no trend in the 40% treatment from day 14 onwards. The 20% light treatment produced the greatest reduction in elongation. There was no consistent difference between the 40% and 60% treatments until after day 15. No root death occurred and a few roots only in the 20% treatment stopped elongating and then not for more than two days. The differences between the 20% treatment and the control on days 2 to 18 and between the 40% and 60% treatments and the control on days 7 to 13 were significant at the 5% level.

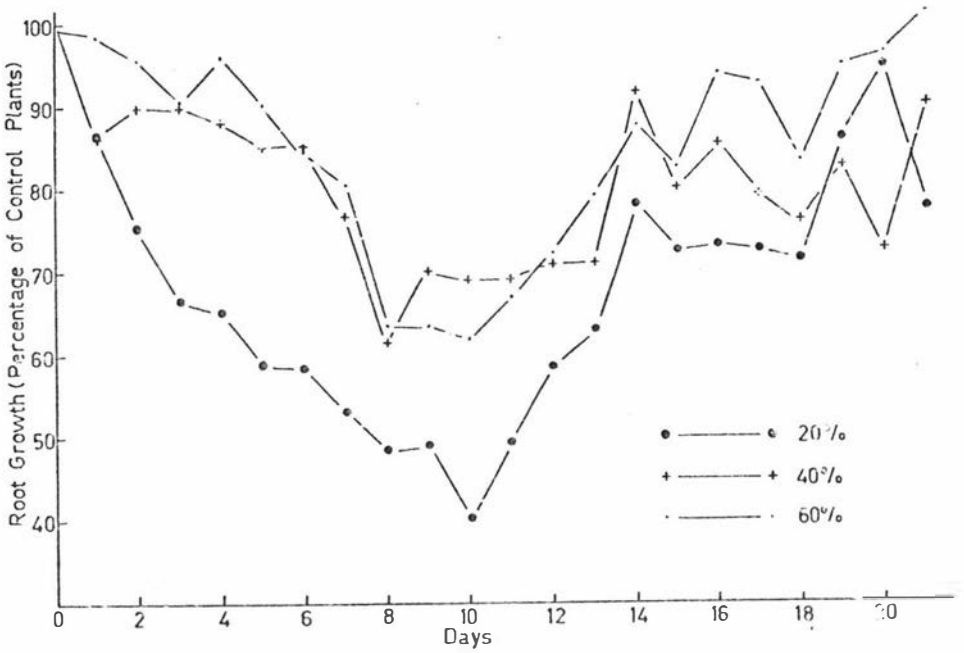


Fig. 3 - Effect of three levels of shading (% of full light in glasshouse) on root elongation. Results expressed as percentages of the elongation of the control.

Experiment 2 - Plants grown in clay pipes

Methods

This experiment consisted of ten replicates using the same shade screens. As in the defoliation experiment in the clay pipes, the shoots and roots were trimmed to 7.5 cm from the base of the shoot at transplanting. Several extra plants treated in the same manner were subjected to the heaviest shading treatment and one harvested each week to determine the growth of new root. All plants from the experiment proper were harvested together after 4 weeks. Root length per unit weight and apices per unit length were determined on the new growth below 7.5 cm from the base of the shoot. The weight of ten 5 cm lengths of primary nodal root per pipe were determined for comparison with the experiment in glass fronted containers.

Results

The results are presented in Table 3 together with the length per unit weight of primary nodal root from experiment 1. Length per unit weight of all root below 7.5 cm from the base of the shoot and number of apices per unit length were significantly greater in the 20% light treatment than in the controls. In experiment 2 the length of primary nodal root per unit weight was significantly greater in all shade treatments than in the controls and was significantly greater in the 20% treatment than in the 40% and 60% treatments. In experiment 1 the 20% and 40% treatments were significantly different from the 60% treatment and the controls.

3.5 DISCUSSION

The most widely reported effects of defoliation and shading are a reduction in total root weight and rooting depth. Root weights were reduced in the present study as a result of single defoliation (Table 1) but no information was obtained on the second point as in all treatments root growth occurred down to the bottom of the containers.

In Appendix 2 elongation rate of roots was shown to increase with increasing root length. In the defoliation and shading experiments the longest roots were seen to be the least affected

Table 3 - Effect of shading on root morphology

Treatment	Experiment 2 Plants in pipes			Experiment 1 Plants in containers
	All roots below 7.5 cm		Primary nodal root. Length per unit weight (cm/mg)	Primary nodal root length per unit weight (cm/mg)
	Length per unit weight (cm/mg)	No. of apices per unit length (cm)		
60% light	39.1	1.38	3.49	3.29
40% light	42.5	1.38	3.63	3.79
20% light	45.6	1.57	4.60	4.56
Control	40.7	1.37	2.49	3.20
S.E. ±	1.93	0.069	0.21	0.19
D.05	3.95	0.14	0.43	0.41

by the treatments. These observations support the contention of Jacques (1937) that new roots arise from the bases of young tillers. The shortest roots in general being the youngest would be associated with young tillers which were not well established and therefore lacking in food reserves. These roots would have a poorer food supply which would be more sensitive to any factor affecting photosynthesis.

Troughton (1960) and Brouwer (1963) considered that under any set of environmental conditions there was a balance between root and shoot. Brouwer (loc.cit.) and Ennik (1966) also believed that in the case of defoliation there is a return to an optimum shoot/root ratio. Ennik (loc.cit.) with perennial ryegrass and white clover found no increase in root weight following defoliation until the shoot/root ratio recovered to the level prior to defoliation. In the present experiment plants were harvested when the elongation rate of roots had recovered to the control level. At this time the shoot/root ratios were not significantly different from the controls. The significance of root weight in such a situation is uncertain. In the heaviest defoliation treatment at least, some root death occurred and in other treatments as well there may have been depletion of reserve substances. On the other hand there was new growth at least of the primary nodal roots. In contrast to the findings of Ennik (loc.cit.), in the present experiments the root weights of the 7.5 cm plants were significantly greater than those of plants harvested at the start of the experiment (1.80 ± 0.14 g).

In the plants repeatedly defoliated to 2.5 cm the reduction in root diameter was similar to that illustrated by Soper (1957) as a result of shading and high temperature. In other cases there was an increase in length per unit weight compared with the control although the decrease in diameter was not obvious. The figures given for length per unit weight of all root below 7.5 cm (Table 2) may mean that the whole root system was affected or may be a reflection of the change in the primary nodal roots only. The primary nodal roots comprise a large proportion of the total weight but only a small proportion of total length. Such a decrease in thickness or increase in length per unit dry weight is consistent

with the idea that the treatments applied would reduce the carbohydrate supply. Soper (loc.cit.) found that the decrease in diameter was due to decreases in both cell size and cell number.

Root death as observed in the case of repeated defoliation is most important in a pasture grass from the angle of photosynthate wasted in a situation where only that harvested from the shoot is important. Where the distribution of living root is not altered the death of some roots would not necessarily be detrimental to future growth of the plant since this would mean that the shoot needed to supply photosynthate to a lesser amount of root tissue. In the case of defoliation the size of the shoot would be reduced so the need for water and mineral nutrients would decrease. A decrease in rooting depth would be much more serious as this would reduce the volume of soil the plant could utilize. Oswalt, Bertrand, and Teel (1959) found that the defoliation of cocksfoot and bromus plants caused the cessation of uptake of ^{32}P from 6 in. and 10 in. below ground level. They considered that this was due to root death and that uptake only recommenced when new nodal roots grew down to the fertilizer level - a time interval of 19 and 30 days respectively. Davidson and Milthorpe (1966) with young cocksfoot plants found uptake of ^{32}P much reduced at about the time root growth ceased and uptake had not recovered at all three days after growth recommenced. No root death was reported in this study.

With repeated defoliation to 2.5 cm there was initially death of many roots present at the start of the experiment. There was no apparent replacement of dead roots so the number visible against the glass remained at a low level to the end of the experiment. There was however a suggestion of a recovery of root elongation with time. In plants defoliated to 2.5 cm all lamina was removed but new leaves were produced which had their laminae horizontal below the defoliation level. This would mean that the effective photosynthetic area of the plant would increase with time even under repeated defoliation. Jacques (1937) reported a reduced number of roots per tiller with defoliation. If this were the case in the present experiment an increase in mean growth rate could be expected on the grounds that each root was receiving photosynthate from an

increasing number of tillers. Although there was no such marked drop in the shaded plants, the same explanation might apply. The peak on day 10 in Fig 2 is probably due to the occurrence of three successive sunny days in a period of cloudy weather. This would have the double effect of raising photosynthesis in the defoliated plants and because of high transpiration, causing a temporary water deficit in the control plants.

Maximum depression of root growth due to defoliation occurred on the third day with most of the effect occurring on the first day. This corresponds well with the results of Davidson and Milthorpe (1966) working with oocksfoot. With shading however there was a steady decrease in root growth to the 8th or 10th day. In both situations severity of treatment had only a small effect on the pattern of root growth inhibition. Both treatments would decrease the synthesis of carbohydrates. The more rapid drop in the defoliated plants indicates that the leaf may be an important storage organ or else it synthesises some other substances required for root growth. A third possibility is that reduced transpiration suction in defoliated plants causes a physiological response in the roots.

The effect of defoliation and shading on elongation of primary nodal roots may be different to the effect on higher order roots which constitute the bulk of root length . It was observed however that where a primary root apex was removed the laterals increased in thickness and rate of elongation. This suggests that the primary apex is dominant in utilizing the carbohydrate supply from the shoot so if the primary apex is deprived the laterals are likely to be also so that a treatment such as defoliation or shading would be expected to affect laterals in the same manner as it would the primary apex.

The rapidity with which plants such as L. perenne recover from defoliation is extremely important in pasture production. From this angle the immediate response to defoliation in terms of root death and inhibition of function may be less serious than the long term effects. A single severe defoliation at a critical period of the year for example may reduce rooting depth sufficiently to affect production over a considerable period especially during summer when there is a moisture deficit in the upper layers of the soil.

CHAPTER 4

MECHANISM OF DEFOLIATION AND SHADING EFFECTS - LITERATURE REVIEW

4.1 INTRODUCTION

It is clear that root growth is dependent on photosynthesis in the shoot in the long term and that any factor which causes a reduction in photosynthesis must sooner or later have an effect on root growth but there is some doubt as to the short term mechanism whereby root growth is affected. The two most important points are the extent to which storage products substitute for photosynthesis and the possibility that either some other substances such as plant growth regulators may in some circumstances limit growth when photosynthate is adequate or in the presence of sub-optimal photosynthate limit growth more than it would be limited by sub-optimal photosynthate alone.

As with other aspects of root development, the greater part of the investigation of the importance of photosynthesis on root growth has been carried out on agricultural plants.

4.2 CARBOHYDRATE LEVELS IN ROOTS

Sullivan and Sprague (1943) found that in Lolium perenne a decrease in water soluble carbohydrates in the roots occurred following partial defoliation. If the plants remained in the light the water soluble carbohydrates increased again but if the plants were placed in the dark, the reserves continued to decline. They detected no change in the levels of cellulose, hydrolysable pentosans, or lignin, indicating that these substances did not act as reserves. Decreases in the soluble carbohydrates in roots following defoliation have also been reported by Weinmann (1948), Sprague and Sullivan (1950), Sullivan and Sprague (1953), and Alberda (1957, 1960).

The observed fall in carbohydrate levels in the roots following defoliation led to the assumption that these reserves were trans-

located to the shoot where they were used in regrowth (Weinmann, 1948). The use of root reserves in shoot growth in grasses is relevant to the theme of this thesis because if used in this way they are not available for root growth. However May and Davidson (1958) suggested that much of the observed decrease in root reserves could be accounted for by root respiration. Marshall and Sagar (1965) then showed by the use of $^{14}CO_2$ that carbohydrate which had been in the roots for a few hours was not available for regrowth of the shoot. Davidson and Milthorpe (1966) found that with young cocksfoot plants the reduction in soluble carbohydrates in the roots in the first two days after defoliation was insufficient to account for all respiration and new growth of the roots. There must therefore have been appreciable movement of reserves from the shoot and / or the utilization of other substances as an energy source. The increase in nitrate in the plants during this time (Sullivan and Sprague, 1943; Alberda, 1960) may indicate that protein was utilized as an energy source (Steward et al., 1958).

It appears that in some species not all the soluble carbohydrates in the roots are utilized before growth ceases. Davidson and Milthorpe (1966) found that in cocksfoot the level had fallen from 1.7% to 1.0% by the time root growth ceased.

Shoot carbohydrate reserves are also implicated in root growth. Davidson and Milthorpe (1965) reported that when shoot carbohydrate reserves were high, root growth was not as severely restricted by defoliation as when they were low. Brouwer (1966) also found this to be the case in Zea mays. Asher and Ozanne (1966) reached a similar conclusion with subterranean clover after observing the depressive effect of removing one cotyledon. Reserves in the shoot may not be as readily available for root growth as for shoot growth however as in Chapter 3 growth of most roots ceased in the most severely defoliated plants whereas leaf growth appeared to be continuous. Mitchell (1954) in explaining the effect of light intensity on the distribution of growth between root and shoot suggested that when a substance necessary for growth is in short supply the greater proportion of it will be used by the meristems nearest to the source of supply. In grasses the meristematic zones in the leaves are at the base and in the

vegetative state the shoot meristems are at the base of the shoot whereas many of the root apices are at a distance of 10 cm or more from the base of the shoot. Material stored in the shoot would therefore be closer to the shoot and leaf meristems. Sagar and Marshall (1967) found that in Lolium multiflorum there was no translocation to the roots for several days subsequent to the first 24 hours following defoliation.

Wassink and Richardson (1951) found that in Quercus borealis the initial drop in root growth on reducing the light intensity at the shoot was followed by a partial recovery which could only be explained by the plant adjusting to the utilization of stored carbohydrate. Richardson (1953b) showed this storage to be in the roots themselves as changes in shoot temperature which would be expected to affect translocation of any material stored in the shoot did not affect root growth. Jones (1944) also showed that root growth in Acer pseudoplatanus continued after leaf fall and so the energy must have been obtained from storage products. The rapid response of root growth of several species to factors affecting photosynthesis however emphasises the importance of current photosynthate at least in some species (Wassink and Richardson, 1951; Davidson and Milthorpe, 1966; Eliasson, 1968). Plaut and Reinhold (1969) showed that regardless of previous treatment, ^{14}C was translocated more readily when the plants were in light and an atmosphere containing CO_2. They suggested that the explanation was the ATP loading of the phloem.

4.3 IMPORTANCE OF SUBSTANCES OTHER THAN CARBOHYDRATES

The reports of reduced carbohydrate levels following defoliation or shading suggest that lack of a sufficient energy source is the most usual factor limiting root growth under these circumstances. However in some instances experimental evidence implicates other substances.

Richardson (1953b) having shown that the reserves utilized for root growth when the light level was reduced were in the roots, demonstrated by ringing the stem to prevent upward translocation that transfer of reserves from the root to the shoot of defoliated

plants was not the cause of reduced root growth. He considered that because the temperature of the shoot had no effect the factor was hormonal. This same author (Richardson, 1958) substantiated this postulate by demonstrating that defoliation inhibition of root elongation was similar in plants grown at 250 lux to that of plants grown at 4000 lux where produotion of photosynthate was presumably much greater. This is in contrast to the work of Brouwer (1966) who showed that in Zea mays plants with a high carbohydrate reserve took longer to show inhibition of root growth following defoliation than those whose carbohydrate status had been reduced by shading. Contrary also to his later work, Richardson (1953a) demonstrated the apparent importance of photosynthesis in Acer saccharinum by surrounding the shoot in an atmosphere devoid of CO_2. The results were much the same as those obtained when the light intensity was reduced.

Richardson (1953b) found that in Acer saccharinum root growth of defoliated plants recommenced when new leaves developed. This stimulus could also be supplied by non-dormant buds. Richardson (1958) found that roots of plants growing in humus did not react as markedly to defoliation due supposedly to the humus supplying some growth stimulus normally produced by the leaves. He speculated that the stimulus might be from vitamins of the B group since several workers had demonstrated the presence of these in the soil and they had been demonstrated as a requisite for growth of excised roots of a number of species.

4.4 EXCISED ROOT STUDIES

As can be seen from the previous sections, the information available is meagre and fragmentary. In the findings discussed, the only part of the plant removed has been the leaves or leaves plus some shoot tissue. The shoot apices and most of the shoot tissue have remained. The physiology and chemical requirements of root growth have been extensively studied in excised root culture. The difficulties that have been encountered in attempts to grow excised roots and the varying nutrient requirements of the comparatively small number of species which have been successfully cultured emphasise the complexity of the whole subject of the extent

to which the roots depend on the shoot for the various classes of organic compounds necessary for metabolism and growth (Butcher and Street, 1964; Torrey, 1965; Ferguson, 1967; Street, 1969). In particular there has been diffioulty in growing monocotyledon roots. Ferguson (loc.cit.) demonstrated that even within a single species there may be differences in the chemical requirements for growth. There does not appear to be any record of the successful culture of roots of Lolium.

In wheat the early work suggested that low intensity light was necessary for satisfactory growth (Street et al.1961). Later, autoclaved tryptophane was shown to substitute for this requirement and that auxin was the substance involved (Sutton et.al.,1961; Carter and Street, 1963). Butcher and Street (1964) suggested that in the entire plant, the roots depend on the photochemical synthesis of auxin in the leaves for their supply. However it must be emphasised that in many species successfully cultured, external auxin is not required.

The complexities of the auxin requirements of roots are discussed by Street (1969). He obtained from root extracts several substances which showed activity in auxin bioassays. Some of these substances were indoles and some were not.

The situation regarding gibberellins and cytokinins is even less well understood. The most striking effects of gibberellins are on shoot growth (Morgan, 1968) but responses to smaller concentrations have been recorded in roots (Manos, 1961). The effect of cytokinins on root growth has also been reported (Srivastava, 1967). There is evidence that gibberellins (Holm and Kay,1969) and cytokinins (Letham, 1967; Sitton, Itai and Kende, 1967; Mullins, 1967) are produced in the root system.

CHAPTER 5

RESPONSE TO SUGAR IN DEFOLIATED PLANTS

5.1 INTRODUCTION

It was not considered possible to grow plants of the size used in previous experiments in sterile oulture in order to test responses to energy sources so any such experiments attempted had to be as brief as possible to avoid a massive build up of micro-organisms as would be inevitable under such circumstances. In preliminary trials (see Appendix 9) no such build up was observed until the fifth day from the start of the sugar treatment so a three day experimental period was adopted as standard. The short experimental time meant that only root elongation could be measured. No attempt was made to prevent infection by microorganisms of the sand used which would have had a relatively low concentration, being from a recent river deposit. The decline in elongation over the three days which occurred in some experiments where sugar was applied to the roots may have been caused by microorganisms. On the other hand, such a decline is seen to be typical of plants which have been defoliated or shaded (see Chapter 3). In the single experiment where the response to sugar of undefoliated plants was examined (fig. 6) the sugar treatments did not differ significantly from the controls on day 2 or day 3.

5.2 EFFECT OF GLUCOSE

According to Butcher and Street (1964) glucose as an energy source for the growth of monocotyledon roots is equal or superior to sucrose, which is the acceptable source for dicotyledons. Ferguson (1967) reported that the Hilgendorf oultivar of wheat would only utilize glucose as an energy source although sucrose was released into the medium. Because of the above information, the first experiments were performed using glucose.

Experiment 1 - Application to the Root Apices

Confining of the glucose to the vicinity of the root apices

whose elongation was to be measured was attempted in order to reduce the build up of microorganisms. Although there was an apparent response in root elongation in defoliated plants, the high variation between individual roots, the time required, and the difficulty experienced in applying the glucose to the root apices resulted in this technique being abandoned. An account of this experiment appears in Appendix 10.

Experiment 2 - Application to the Whole Root Mass

Glucose at the appropriate concentration was added to the inorganic nutrient solution being used. The experiment consisted of six replicates. Plants were established in glass fronted containers as described in Chaper 2. Three glucose concentrations widely mentioned in excised root studies (0.5, 1.0, and 2.0 per cent w/v) were used. The appropriate quantity of glucose was dissolved in the inorganic nutrient solution immediately before application to the plants. Fresh solutions were made up each day. On the first morning the plants were defoliated to 2.5 cm at 8 am and 1 litre of the appropriate solution added to each container. This was sufficient to flush all previous solution from the container. On the second and third mornings 500ml of solution was applied to each container. Root elongation was measured as previously described. In a fourth group of containers, the plants were defoliated to the same level but received inorganic nutrient solution only. The results are presented in Fig. 4. In all three glucose treatments the elongation on each of the three days was significantly greater than that of the controls ($p < .05$).

The relatively small differences between the three glucose levels indicates that concentrations greater than 2% would not have given a significantly greater elongation. An undefoliated control was not included in this experiment because glasshouse space available at the time was only sufficient for 24 containers. Therefore the extent to which glucose supplied to the roots was replacing the photosynthetic function of the leaf laminae and sheaths could not be accurately assessed. However the mean elongation for all containers for the 24 hours immediately prior to defoliation was 6.1 cm and to the extent that temperature determines

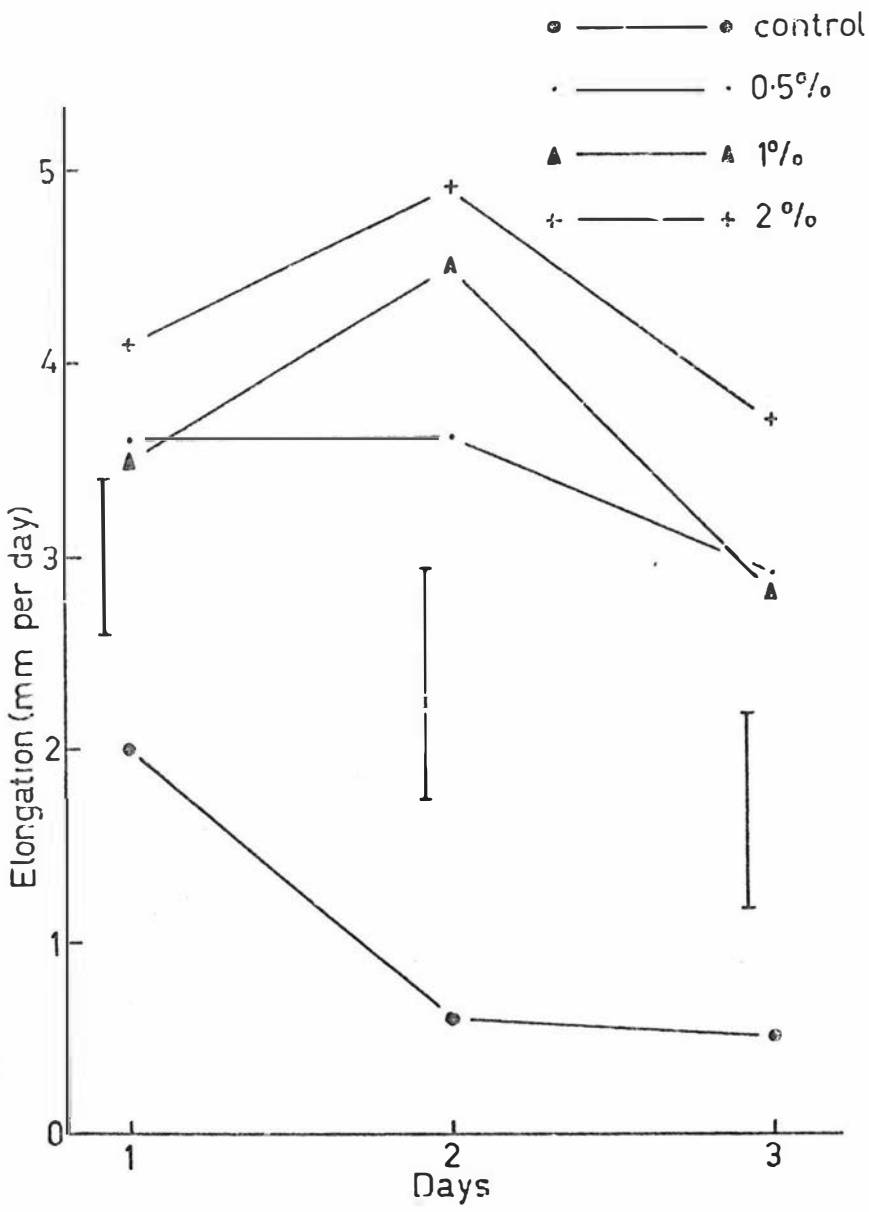


Fig. 4 - Response of root elongation of plants defoliated to 2.5 cm to three different glucose concentrations. Vertical lines indicate twice the standard error.

elongation (see Appendix 4) the figure would have been less than this as the temperature fell by 3°C over the four days. The elongation rates for the glucose treatments were therefore approximately two-thirds of the probable rate for undefoliated plants.

5.3 COMPARISON OF GLUCOSE AND SUCROSE AS ENERGY SOURCES

Although glucose was thought to be the preferred source of energy in monocotyledons, because of the variation between the relatively few species reported on in the literature and the complete lack of relevant information on the genus Lolium, glucose and sucrose were compared. The experimental layout and procedure were the same as in the second glucose experiment. The concentration tested was 2%.

The results are presented in Fig. 5a. Sucrose gave a greater mean elongation than glucose. The difference was significant at the five percent level for the first day and for the three days combined. Because the results appeared contrary to the reports in the literature, the experiment was repeated. The results of this second experiment are presented in Fig. 5b. On the second and third days the percentage difference between the sucrose and glucose treatments was greater than in the first experiment. However neither for the individual days, nor for the three days combined, were the differences significant at the five per cent level.

Clearly the elongation of roots was lower in the second experiment than in the first. This is unlikely to have been the result of environmental factors as the elongation of undefoliated plants not supplied with sucrose, included in the experiments, (results not presented in the figures) was 10 mm and 12 mm respectively for the first and second experiments. In the glucose concentrations experiment (section 5.2 Experiment 2) the actual elongation of the 2% treatment was intermediate between those of the two glucose -sucrose experiments but since the probable level of undefoliated plants was much lower than in these latter experiments (6 mm compared with 10 mm and 12 mm) the comparative response to glucose appears to be much greater. The differences between

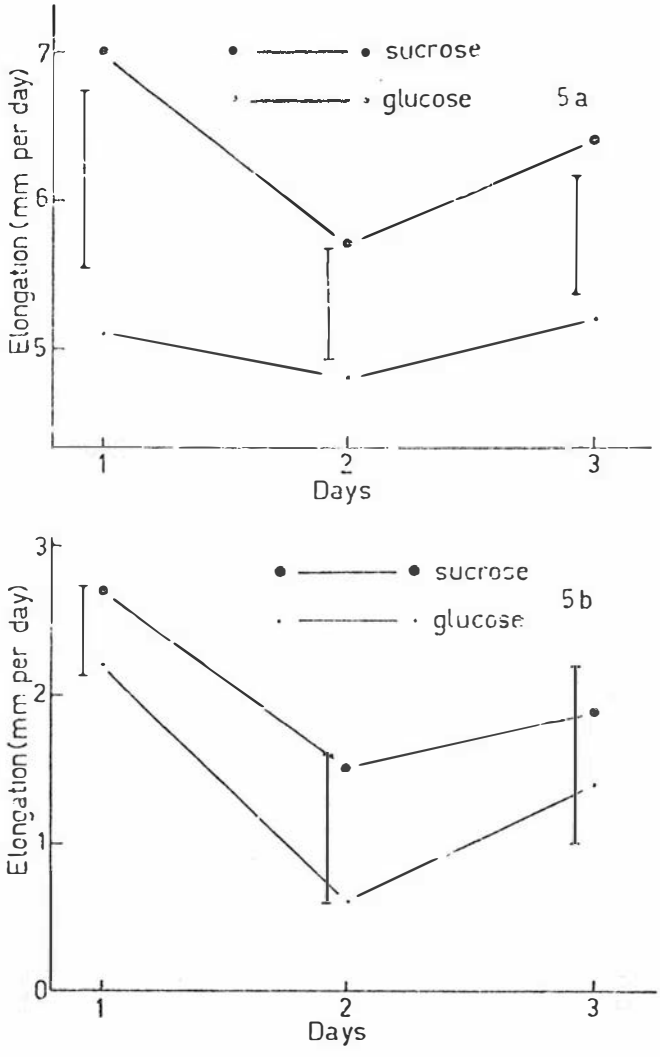


Fig. 5 - **Comparison of the effects of 2% sucrose and glucose on root elongation of plants defoliated to 2.5 cm. Vertical lines indicate twice the standard error.**

undefoliated plants can be explained by differences in growing conditions during the experiments. The varying response to 2% glucose could also have been caused by differences in growing conditions. The effect of various concentrations of glucose was examined in June and the glucose-sucrose comparisons were made in August and October. Plants grown in the glasshouse during the winter months were noticeably more prostrate and shorter in the leaf sheath than those grown in the spring and summer so that defoliation to 2.5 cm above the base would leave much more leaf blade tissue on winter-grown plants. Therefore the differences between experiments could have been caused by differences in the contribution of photosynthesis or growth substances from the shoot to root elongation. There may also have been differences in the levels of reserves in the plants.

5.4 OPTIMUM SUCROSE LEVEL

In the preceding experiments in which a sugar was supplied in the nutrient solution, it was observed that the roots which had grown between the expanded aluminium sheet and the glass for some distance appeared to respond less to sugar application than those which had more recently emerged from the sand through the aluminium sheet. As previously mentioned, the aluminium was coated with bituminous paint which is hydrophobic and may have acted as a barrier to water movement. In addition, the normal diurnal temperature fluctuations resulted in moisture from within the sand in the container condensing against the glass. These two factors in combination are likely to have resulted in a lower concentration of sugar in contact with the roots growing against the glass than that applied to the sand in the container. Therefore with the exception of two experiments in the following chapter, all experiments from this point onwards where sugar was applied were conducted in containers without the expanded aluminium sheet. To compensate for the greater tendency of the roots to grow back into the sand, the angle of the glass was increased from 12 degrees to 20 degrees by placing a block of wood under the back edge of each container base. Placing of the root masses of three plants directly against the glass gave an undesirably high concentration of roots making measurement of elongation of individual roots difficult.

This problem was overcome by transplanting the entire contents of a four inch plastic pot, used to raise the plants, into the container. This did not have any detectable effect on variation between containers in root elongation.

Experiment 1 - Comparison of Two and Four Per Cent Sucrose

The aim of this experiment was to determine if increasing the concentration to above 2% would increase elongation and if there was a response in undefoliated plants comparable with that in plants defoliated to 2.5 cm. The experimental procedure was the same as outlined for the previous sugar experiments with the exception of the changed planting procedure outlined above. There were six replicates. Because of the increased glasshouse space available at the time, five treatments were possible. The treatments were:

defoliated to 2.5 cm. 2% sucrose applied
defoliated to 2.5 cm. 4% sucrose applied
undefoliated. 2% sucrose applied
undefoliated. 4% sucrose applied
undefoliated. Inorganic nutrients only applied

The results are presented in Fig.6. All five treatments were analysed together; however lines representing the separate standard errors for the undefoliated and defoliated treatments are included in the figure to facilitate comparison of sucrose levels. The only significant difference between sucrose levels was in the undefoliated plants on day 1, the 2% treatment being higher than the 4% treatment and control treatment ($p < .05$). On all three days the undefoliated treatments had a significantly higher elongation than the defoliated treatments ($p < .01$). In the defoliated plants, elongation increased from day 1 to day 2 whereas in the undefoliated plants the level decreased or did not change.

In the undefoliated plants, sucrose initially increased elongation but subsequently decreased it by comparison with the controls so that elongation over the three days was the same in all three treatments. A possible explanation is that some other factor became limiting over the three day period, the supply of this factor being

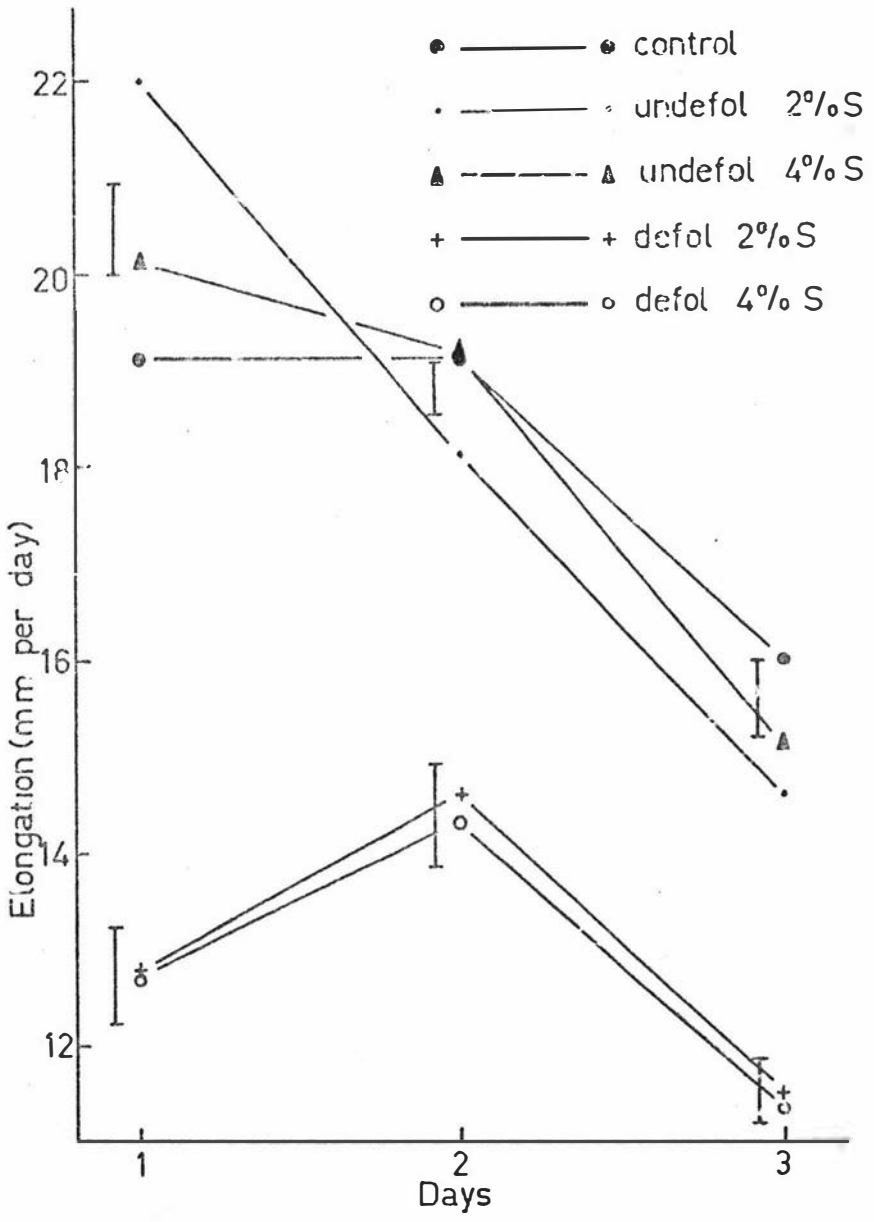


Fig. 6 - Comparison of the effects of 2% and 4% sucrose on root elongation in undefoliated plants and in plants defoliated to 2.5 cm. The control plants were undefoliated. Vertical lines indicate twice the standard error.

depleted by the additional growth on the first day in the sucrose treatments. A significant osmotic effect on growth appears unlikely since at both sucrose levels the elongation was initially higher than the controls. The increase in elongation in defoliated plants from day 1 to day 2 is in contrast to the decrease in the undefoliated sucrose treatments. No explanation of this difference is offered. The drop from day 2 to day 3 in the sucrose treatments is of the same order as that in the control treatment so is assumed to be due to environmental conditions. No comparable rise from day 1 to day 2 occurred in the two previous experiments where sucrose was supplied (Figs. 5a & 5b), however, these experiments were conducted during winter and early spring when temperatures and light levels would have been lower than in December when the present experiment was conducted. These differences are reflected in the different elongation rates of the three experiments. As in the previous experiments, sugar applied to the roots was not a complete substitute for the presence of the leaves in promoting root elongation. Although there was no evidence of an osmotic effect on the root elongation of undefoliated plants, in the glucose concentration experiment and in subsequent experiments in which one treatment consisted of defoliated plants which were not supplied with sugar a phenomenon occurred which may have indicated an osmotic effect. Where sugar was absent, drops of water formed overnight on the cut ends of the leaves but not where sugar was applied. In view of this observation and the certainty of a build up of microorganisms around the roots, the lowest possible sugar concentration consistent with a reasonable level of root growth should be used.

Experiment 2 - Comparison of Four Sucrose Concentrations

This experiment was conducted to determine the lowest sucrose concentration which would give optimum or near optimum elongation in roots of plants defoliated to 2.5 cm. The concentrations tested were 1%, 2%, 4%, and 6%. The experimental procedure was the same as in the previous experiments.

The results are presented in Fig. 7. The 1% treatment gave a significantly greater elongation than the 6% treatment on day 1

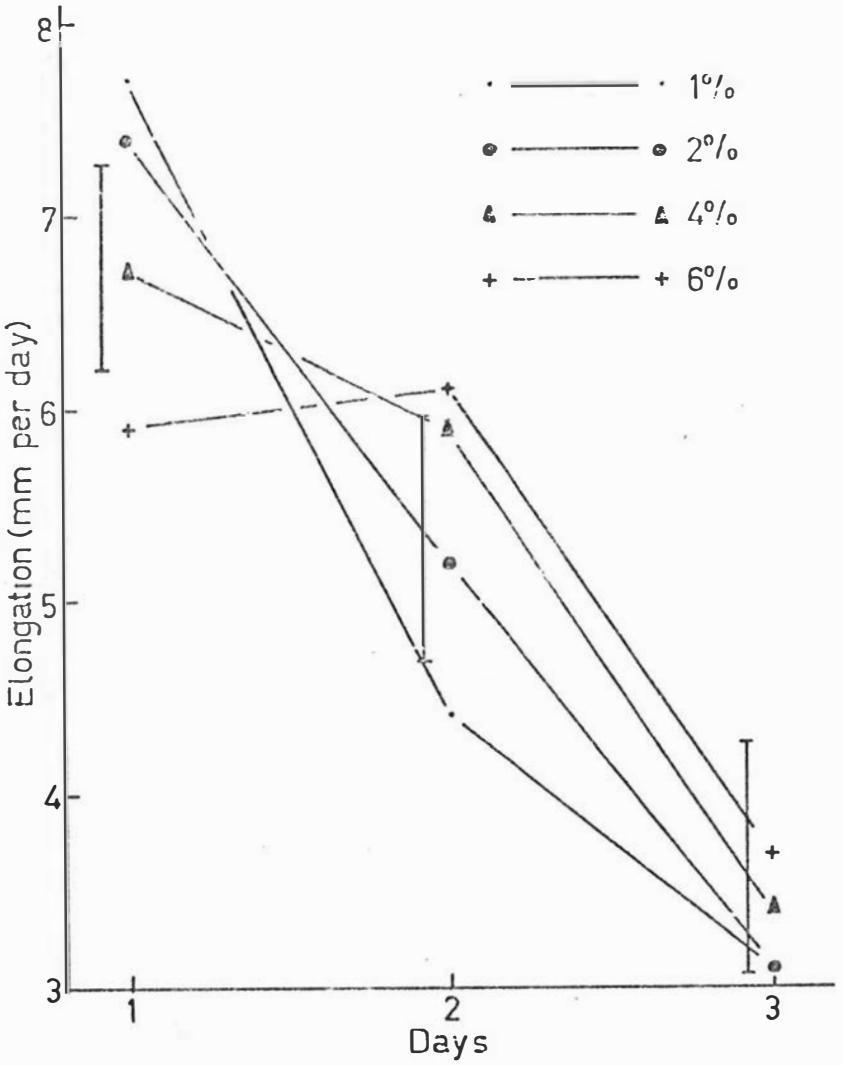


Fig. 7 - Comparison of four sucrose concentrations on root elongation of plants defoliated to 2.5 cm. Vertical lines indicate twice the standard error.

with the 2% and 4% treatments giving intermediate values. However, over the three days, the elongation in all four treatments was the same.

The elongation of the plants used on the day prior to defoliation was 19 mm which was similar to that of undefoliated plants in the previous experiment, however the elongation of the defoliated plants was much lower. As suggested in the previous section, differences in defoliation and growing conditions are probably responsible.

The higher elongation of the 1% level on the first day and the subsequent drop relative to the other treatments is similar to the situation in undefoliated plants in the first experiment. However in the first experiment no such phenomenon occurred in the defoliated plants. It is therefore regarded as being of doubtful significance.

The possibility of an osmotic effect as already discussed with respect to the first experiment was not recognised at the time this second experiment was conducted so the following experiments were conducted using 2% sucrose rather than 1% or less as may have been advisable on the basis of this observation. The concentration was not subsequently lowered as this would have confounded comparisons between experiments.

5.5 RESPONSE TO SUCROSE AT SEVERAL DEFOLIATION LEVELS

In Chapter 3 it was shown that elongation was depressed to the greatest extent by the most severe defoliation treatment. In the previous section of this chapter it was shown that in the case of plants defoliated to 2.5 cm, up to approximately 70% of the control plant root elongation could be maintained by applying sugar to the roots. That there was no response in undefoliated plants indicates that in that case the energy supply was not the limiting factor in root growth. That the elongation level in the control plants could not be achieved in the 2.5 cm defoliated plants supplied with sugar indicated that some other factor(s) may be supplied by the shoot. In order to obtain further information the effects of sucrose on plants defoliated to different levels was examined.

Experiment 1 - Defoliation to 7.5 cm or Complete Removal of Shoot

The greatest defoliation height known from previous experiments to depress root elongation was 7.5 cm. The most severe defoliation possible is complete removal of the shoot. The effect of 2% sucrose on root elongation of plants defoliated to these two levels was examined. The experimental procedure was as previously described. In the complete removal treatments, the roots were cut immediately below the junction with the shoot. Care was taken to ensure that the roots were not disturbed more than was absolutely necessary.

The results are presented in Fig. 8. As in Fig. 6 although all treatments were analysed together, separate standard error lines are shown in the figure. The complete removal of the shoot resulted in complete cessation of root elongation on the third day in the absence of sucrose whereas some elongation occurred on all three days where sucrose was supplied. The difference was significant on all three days ($p < .01$). In the plants defoliated to 7.5 cm, there was no response to sucrose application, both groups of plants having a lower elongation than the undefoliated ones on all three days ($p < .01$).

The elongation of the plants in which the shoot had been completely removed was comparable with that of plants in previous experiments which were defoliated to 2.5 cm. The response to sucrose in this instance was greater than the response to glucose in Fig. 4. The level of elongation in the 7.5 cm treatments was higher relative to the undefoliated plants than in the defoliation experiments in Chapter 3.

Defoliation to 7.5 cm resulted in the removal of approximately 60% of the leaf blade tissue (eye estimate) hence photosynthesis would have been considerably reduced. That the addition of sucrose produced no response indicates more clearly than previous experiments that some other factor may be required and that sucrose alone can only produce approximately 70% of the maximum growth in defoliated plants. The possibility of an osmotic effect as mentioned in the previous section cannot be excluded at this stage however. The suction pressure of the plants may have been reduced sufficiently through the vessels in the vascular bundles being severed on defoliation

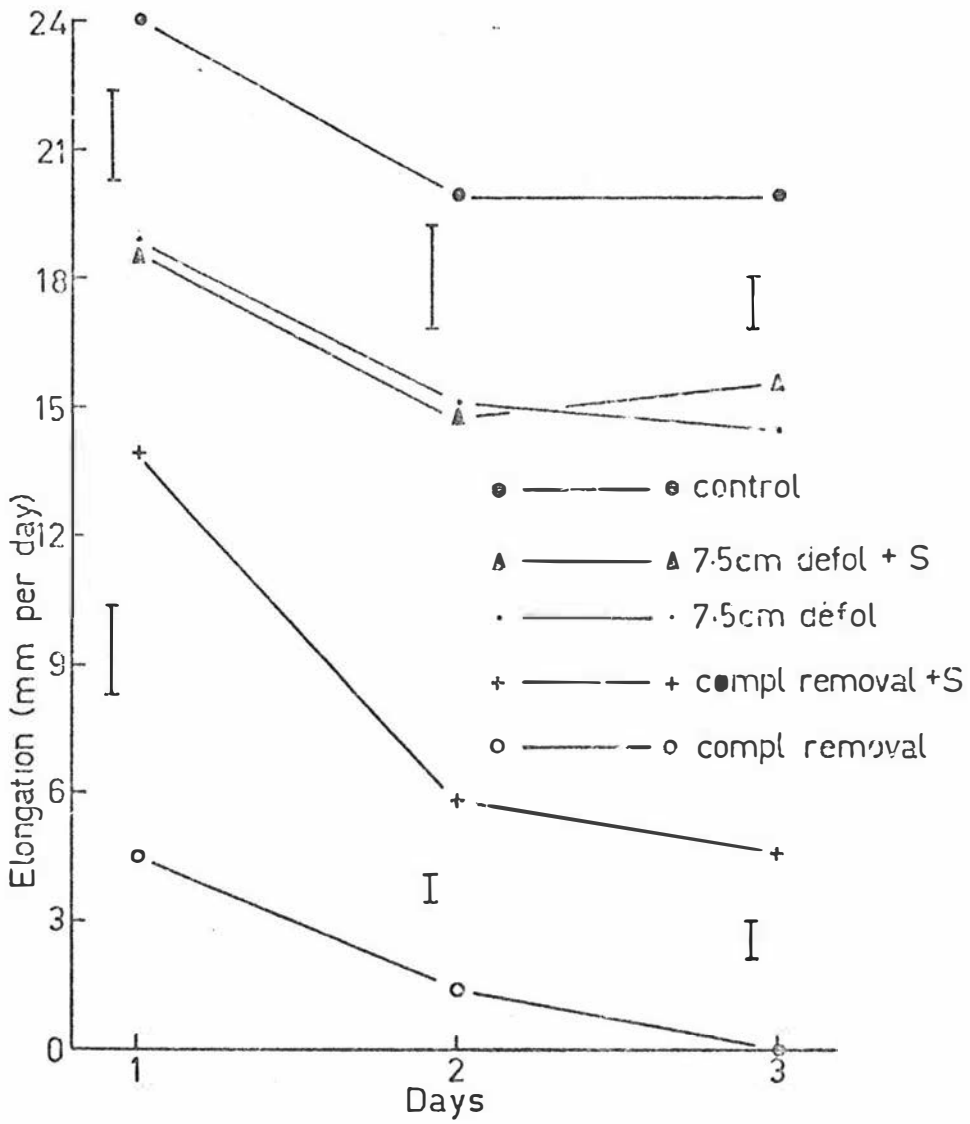


Fig. 8 - **Effect of 2% sucrose on root elongation of plants defoliated to 7.5 cm or with the shoot completely removed. The control plants were undefoliated. Vertical lines indicate twice the standard error.**

to inhibit the uptake of water from the root medium where the osmotic pressure had been raised by the addition of sucrose. This may have cancelled out any potential increase in elongation due to an increased energy supply.

Experiment 2 - Comparison of Defoliation to 7.5 cm and 15 cm

In order to test the possibility of a suction pressure effect, the response to sucrose of plants defoliated to 7.5 cm was compared with the response in plants defoliated to 15 cm above the base which in addition had the apical 2 cm removed from all leaves shorter than 15 cm. The veins in L. perenne leaves run parallel to each other along the entire length of the blade except in the last 1 - 2 cm where they join one another or cease as the blade narrows to the apex. Removing the apical 2 cm or more should have cut the vessels in all veins in all leaves and thus should have produced a similar effect on the suction pressure of the leaves to defoliating to 7.5 cm. In the 15 cm treatment approximately 20% (eye estimate) of the leaf blade tissue was removed compared with approximately 60% in the 7.5 cm defoliation. The direct effect on photosynthesis should therefore have been much less.

The results are presented in Fig. 9. In the 15 cm defoliation treatments, sucrose gave a significantly higher elongation on days 2 and 3 ($p < .01$) and did not differ from the undefoliated control. At the 7.5 cm defoliation level also, the sucrose treatment had a significantly greater elongation on days 2 and 3 ($p < .01$ and $p < .05$ respectively).

Clearly the results of the 7.5 cm defoliation treatments are different from those of the previous experiment. In the present experiment there was also a sucrose effect at the 15 cm defoliation level. That this was so suggests that the photosynthetic effect is the major one. There does not appear to be an osmotic effect since elongation was increased at both defoliation levels to a similar amount by the addition of sucrose. The growth of undefoliated plants was higher in the first experiment due to higher temperatures and light levels. Under these circumstances transpiration would have been higher so it is possible to have had an osmotic effect there but not in this second experiment.

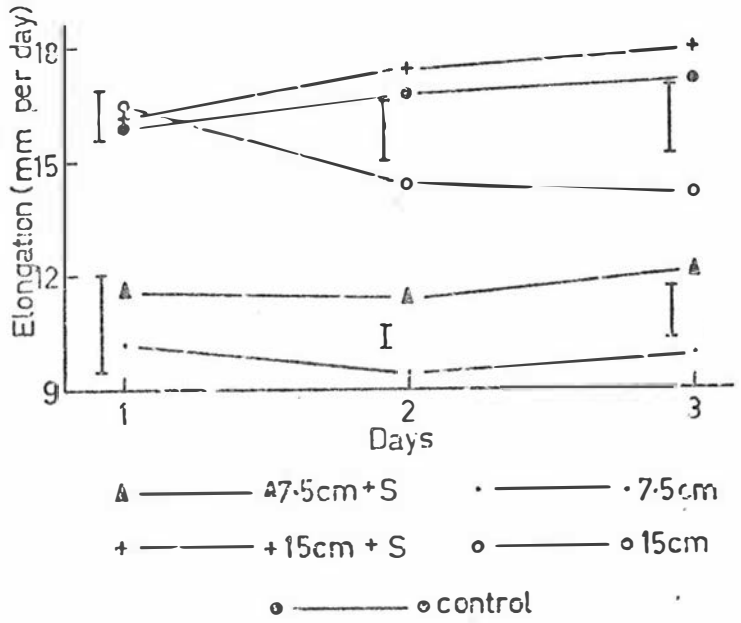


Fig. 9 - Effect of 2% sucrose on root elongation of plants defoliated to two different levels. The control plants were undefoliated. Vertical lines indicate twice the standard error.

Experiment 3 - Defoliation to Four Different Levels

In this final experiment, the effect of sucrose on plants defoliated to 5 cm, 2.5 cm, 0.6 cm and in which the shoot was completely removed was examined. The aim was to test the possibility that particular portions of the shoot might have specific roles regarding root growth. Defoliation to 0.6 cm would leave only the stem and a large part of the meristems at the bases of the leaves while at the 2.5 cm level all of the leaf meristems and most of the leaf sheaths would be retained. In the 5 cm defoliation treatment all of the leaf sheaths and part of the leaf blades would remain. The techniques and procedure were the same as described earlier.

The results are presented in Fig. 10. There were no significant differences between the complete removal and 0.6 cm treatments which were both lower than the 2.5 cm treatment, the trend reaching significance level only on day 3 ($p < .05$). Also the 5 cm treatment showed a significant increase over the three day experimental period and the higher elongation compared with the 2.5 cm treatment reached significance level on day 3 ($p < .01$).

The significant increase over the three days in the 5 cm treatment has no counterpart in other experiments where there was frequently a decline. This increase is therefore attributed to environmental conditions. The difference between defoliation treatments together with the results of the two previous experiments suggests that the effect of the shoot on root elongation is proportional to the amount of shoot tissue retained as in the case where no sugar was added (Chapter 3). The presence of the shoot apices and part of the leaf meristems does not appear to have had an effect as over the three days the plants whose shoots were completed removed tended to have the greater elongation. Again, photosynthesis as such cannot be the major factor or the plants of the 5 cm treatment would have shown a much greater elongation relative to the 2.5 cm treatment since there is little photosynthetic tissue in the leaf sheaths. It appears that some additional substance (s) necessary for root growth supplied by the shoot is synthesises and/or stored in the leaf sheaths and blades. There is no evidence that it is a

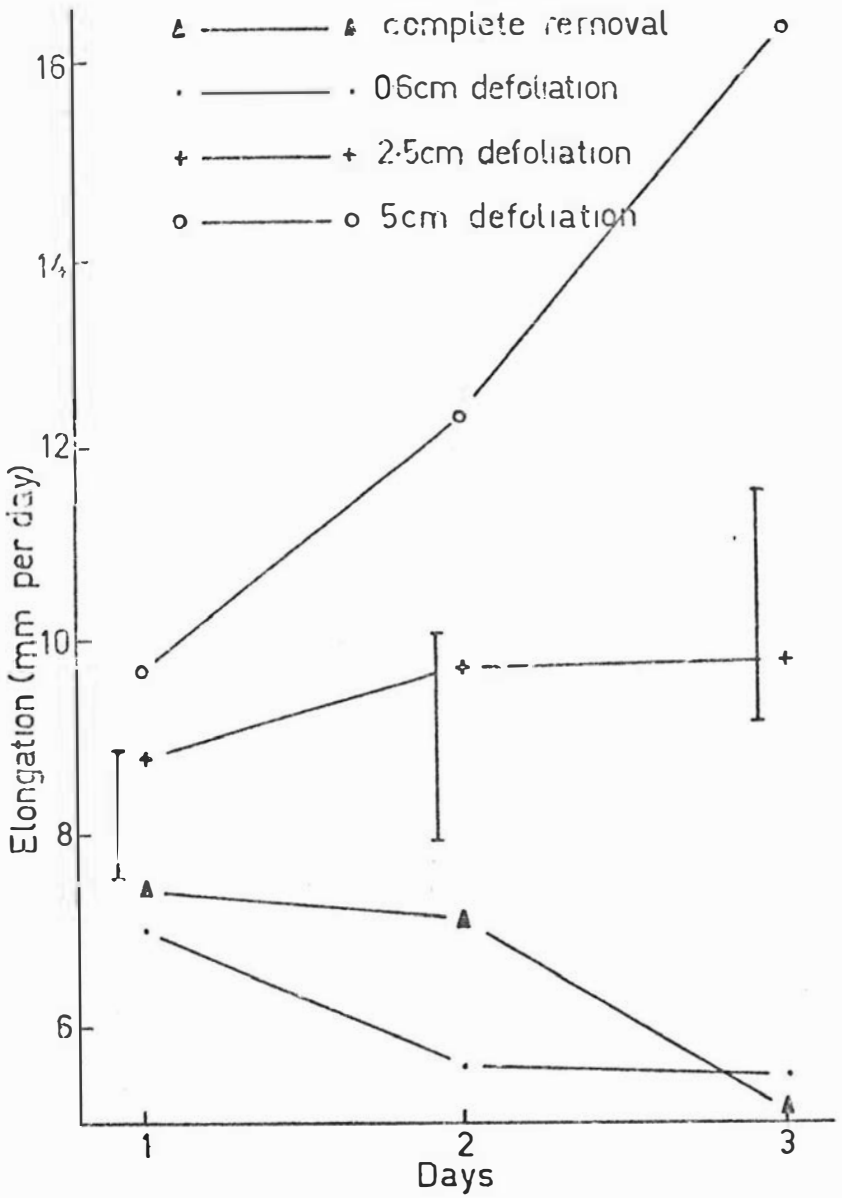


Fig. 10 – **Effect of four defoliation treatments on root elongation of plants supplied with 2% sucrose. Vertical lines indicate twice the standard error.**

substance produced in the meristematic tissue.

5.6 DISCUSSION

The difference in the apparent facility with which glucose and sucrose can be used as energy sources by L. perenne roots is of minor importance to the investigation of the mechanism of root growth inhibition by defoliation. The important feature is that applied sugar, presumably acting as an energy source, can maintain elongation at a high proportion of the rate in undefoliated plants under a defoliation regime where growth would otherwise cease. The apparent superiority of sucrose as an energy source in a monocotyledon appears contrary to the literature on excised roots. This is not necessarily significant however as the use of sucrose may depend on some other substance a proportion of which at least is supplied by the shoot.

Variation between experiments in root elongation and response to applied treatments caused by differing environmental conditions is a major drawback when conducting a series of experiments since it complicates comparisons between experiments. Unfortunately, controlled climate facilities were not available to the author.

CHAPTER 6

EFFECT OF GROWTH SUBSTANCES

6.1 PRELIMINARY EXPERIMENT

This was designed to test some likely combinations of growth substances in association with 2% sucrose. It was assumed that the energy source was the prime requirement of the roots of defoliated plants and that growth substances would be most likely to exhibit an effect in conjunction with an energy source. From the literature it appeared that of the three major classes of growth substances, auxin was the one most likely to be involved (Butcher and Street, 1964), as gibberellic acid has been widely recorded as inhibiting root growth (Brian, 1959) and gibberellins and cytokinins appear to be synthesised in the roots (Letham, 1967; Mullins, 1967; Sitton, Itai, and Kende, 1967; Carr and Reid,1969; Holm and Kay, 1969). Accordingly, the effects of various concentrations of indole acetic acid (IAA) were measured on seminal root growth of L. perenne seedlings. This test is detailed in Appendix 11.

The concentration tested on defoliated plants was 10^{-4}ppm, this being lower than the lowest concentration which did not inhibit seminal root elongation. Gibberellic acid (GA) was tested at 10^{-5}M (3.3 ppm) this being the highest concentration which did not inhibit root growth in Pisum sativum (Manos, 1961). Benzyladenine (BA) was used at a concentration of 0.20 mg/l (2×10^{-1}ppm) which was the concentration found by Skoog and Miller (1957) to promote root growth in tobacco callus tissue. The organic constituents of White's medium (White, 1943) were also included in some treatments. These ingredients were glycine, 3.0 mg/l; thiamine, 0.1 mg/l; pyridoxine, 0.1 mg/l; and nicotinic acid, 0.5 mg/l. Since an initial search of the literature had failed to reveal any instances where gibberellic acid enhanced root growth and L. perenne seedlings had failed to show a positive response to any of the indole acetic acid concentrations tested, the preliminary experiment was primarily a test of the effects of benzyladenine. The experimental layout and procedure were

the same as detailed previously. This experiment was performed immediately after the glucose-sucrose comparisons (Section 5.3) using containers with the expanded aluminium against the glass. The growth substances were dissolved in sufficient water to give working solutions of the required concentrations when added to the inorganic nutrient solution at the rate of 10ml/1. Where necessary, additional water was added to the inorganic nutrient solution so that all treatments received solution of the same concentration. The treatments were as follows:

Control
BA only
BA, IAA and White's nutrients
BA, IAA, GA, and White's nutrients
IAA, GA, and White's nutrients

The results are presented in Fig. 11. Benzyladenine alone gave an increase in root elongation on days 2 and 3 ($p < .01$). There was also a trend for the treatment which had all factors except gibberellic acid to have a greater elongation on days 2 and 3, however this difference failed to reach significance level.

The elongation of the roots of the plants in this experiment during the two days prior to defoliation and treatment was approximately 14 mm per day. The elongation of all plants during the experimental period was lower relative to the probable level of undefoliated plants than in most of the experiments previously described. This must be kept in mind when considering the apparent response to benzyladenine. That the response increased with time indicates that the supply of cytokinin in the plants was not the limiting factor initially. Apparently the effect of benzyladenine was partly inhibited by indole acetic acid and White's nutrients and completely inhibited by these together with gibberellic acid.

6.2 EFFECT OF BENZYLADENINE WITHOUT SUCROSE

Having indicated that the roots of defoliated plants responded to, 0.2 ppm benzyladenine in the presence of sucrose in the nutrient solution, this same concentration was tested in the absence of sucrose.

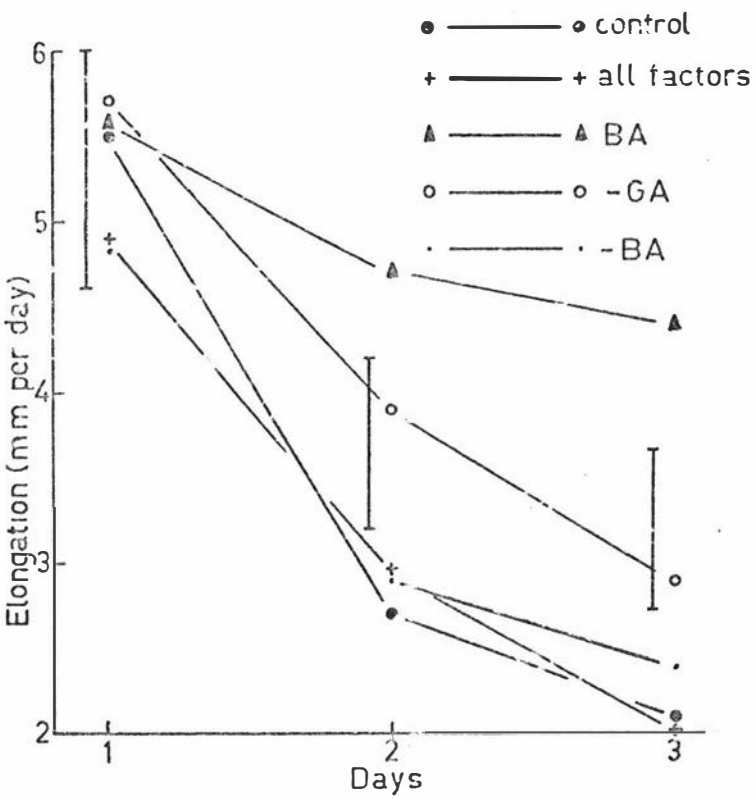


Fig. 11 - Effect of benzyladenine alone, or in combination with other growth substances on root elongation of plants defoliated to 2.5 cm and supplied with 2% sucrose.

Control = sucrose only;

All factors = benzyladenine, gibberellic acid, indole acetic acid and White's nutrients;

BA = benzyladenine;

-GA = all substances except gibberellic acid;

-BA = all substances except benzyladenine.

Vertical lines indicate twice the standard error.

The results of this experiment are presented in Fig. 12.

On no day did the benzyladenine-treated plants differ from the controls which were defoliated to the same level. From this experiment it was concluded that benzyladenine acts only in the presence of an energy source. This is consistent with the literature on cytokinins which are shown to act primarily through promoting cell division which is an energy requiring process (Letham, 1967).

6.3 OPTIMUM BENZYLADENINE CONCENTRATION

At this point in the series of experiments, the change was made to growing plants in containers without the expanded aluminium sheets. Several experiments were conducted using the modified technique. Two per cent sucrose was applied in all experiments.

Experiment 1. 1 ppm, 10^{-1} ppm, 10^{-2} ppm, and 10^{-3} ppm.

This group of concentrations spans that used in the preliminary experiment. The experimental procedure and layout were the same as those detailed previously. The results are presented in Appendix 12 Table 1.

At the highest rates there was a depression of root elongation on days 2 and 3 ($p < .05$). At the two lowest rates there was an increase on day 3 ($p < .05$).

Experiment 2. Repeat of Experiment 1.

Although it was suspected at this stage that the presence of the expanded aluminium sheets in the earlier experiments may have reduced the concentration of the substances under study in contact with the roots whose elongation was being measured and that the difference in results between experiment 1 and the preliminary experiment may have been caused by this factor, the experiment was repeated. The results of this repeat experiment are presented in Appendix 12 Table 2.

Only the highest concentration resulted in inhibition on days 2 and 3 ($p < .05$). There was no increase in elongation at the lower

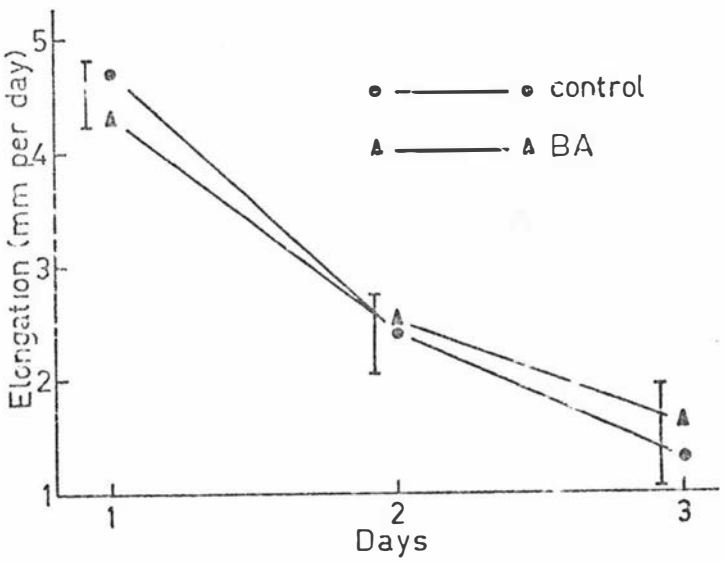


Fig. 12 - Effect of benzyladenine in the absence of sucrose on root elongation of plants defoliated to 2.5 cm.
Control = without benzyladenine.
BA = benzyladenine supplied.
Vertical lines indicate twice the standard error.

concentrations on day 3.

Experiment 3. Several Concentrations without Expanded Aluminium Sheets.

Elongation levels were approximately the same in Experiments 1 and 2 as in the preliminary experiment so it appears probable that the difference in results was due to the effect of the expanded aluminium sheets. To determine if the presence of the aluminium sheets had an effect, three concentrations spanning that used in the preliminary experiment were used. These were: 2ppm, $2x10^{-1}$ppm, and $2x10^{-2}$ppm. As well as a 'sucrose only' treatment there was an undefoliated control with which the growth of the defoliated plants could be compared.

The results are presented in Appendix 12 Table 3. All three benzyladenine levels tended to increase elongation compared with the 'sucrose only' treatment on days 2 and 3, with the $2x10^{-2}$ppm treatment giving the greatest response ($p < .05$ on day 2). The results of this experiment therefore confirm that of the preliminary experiment that root elongation of defoliated plants is enhanced by benzyladenine in the presence of sucrose. Also the effect of the expanded aluminium is confirmed.

Experiment 4. Concentrations 1ppm, 10^{-2}ppm, 10^{-4}ppm, and 10^{-6}ppm.

In this experiment and in experiment 5 no aluminium sheets were used. The aim of these two experiments was to determine the optimum concentration in the presence of 2% sucrose. The experimental layout and procedure were the same as described previously.

The results of experiment 4 are presented in Appendix 12 Table 4. As in experiments 1 and 2, 1 ppm depressed elongation ($p < .05$ on day 1); however the two lowest concentrations increased it ($p < .05$ for 10^{-6}ppm on day 3). 10^{-6}ppm appeared to be the most suitable concentration of those tested.

Experiment 5. Concentrations 10^{-2}ppm, 10^{-4}ppm, 10^{-6}ppm and 10^{-8}ppm.

The results of this experiment are presented in Fig. 13. As in experiment 4 the trend was for the 10^{-6}ppm treatment to give a

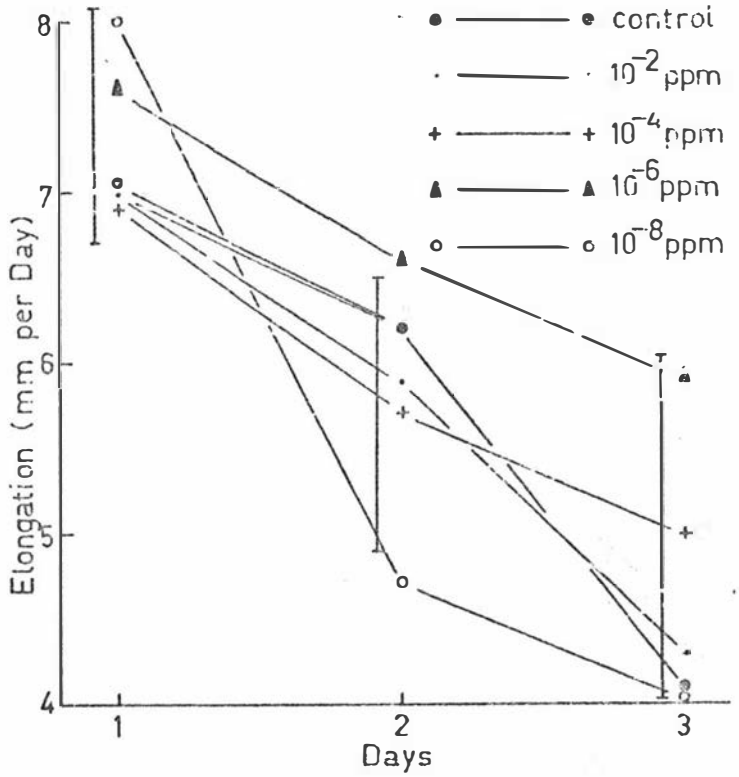


Fig. 13 - Effect of various concentrations of benzyladenine in the presence of 2% sucrose on root elongation of plants defoliated to 2.5 cm. Vertical lines indicate twice the standard error.

higher elongation than the control with a slight indication of a response from the 10^{-4}ppm concentration. The 10^{-8}ppm concentration while giving a higher level on day 1 fell to below the control level on day 2. In this instance the differences were not significant.

From these last two experiments it would appear that 10^{-6}ppm was the optimum concentration of benzyladenine for the promotion of elongation in the presence of 2% sucrose. However the increase attributable to benzyladenine was small compared to the depression in all treatments relative to the elongation of the plants on the two days prior to defoliation in these experiments (11 mm and 15 mm respectively). Hence it appears that cytokinin is of minor importance in limiting the elongation of roots of defoliated plants.

6.4 EFFECT OF GIBBERELLIC ACID

Although gibberellic acid has been recorded as inhibiting root growth (Brian, 1959) and in the preliminary experiment its presence appeared to partly counter the promotive effect of benzyladenine the possibility of promotion by low concentrations was investigated.

Experiment 1. 10 ppm, 10^{-1}ppm, 10^{-3}ppm and 10^{-5}ppm.

The highest concentration used was within the range reported by Brian (1959) to enhance shoot growth. The other three concentrations were lower than that used in the preliminary experiment.

The results are presented in Appendix 13 Table 1. The three highest concentrations tended to depress root elongation although none of the differences were significant. At 10ppm and 10^{-1}ppm shoot growth appeared to be enhanced although no measurements were made.

Experiment 2. 10^{-3}ppm, 10^{-5}ppm, 10^{-7}ppm, and 10^{-9}ppm.

In this experiment the range of concentrations was extended downwards. The results are presented in Fig. 14. The three highest concentrations tended to increase elongation on days 2 and 3. However, the results were not significant. Also the lowest concentration depressed elongation on day 1 ($p < .05$).

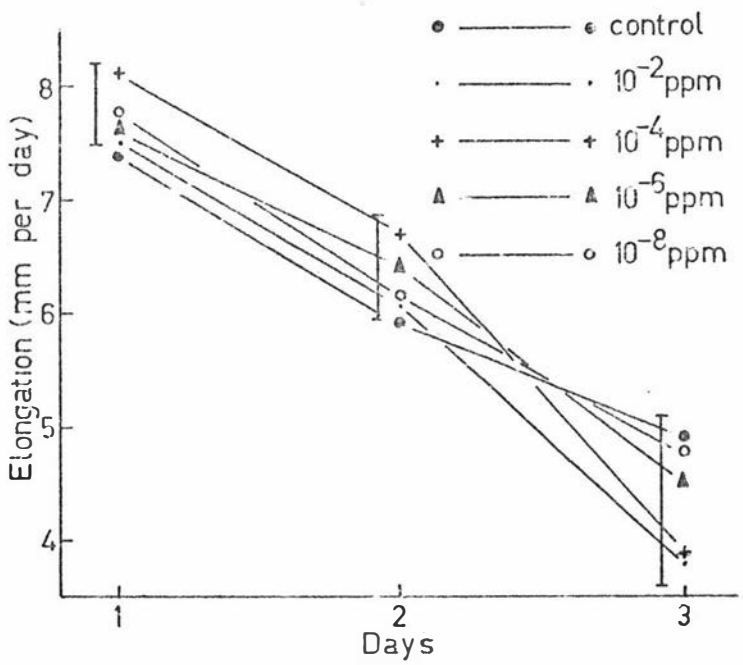


Fig. ~~14~~ 15 - Effect of several concentrations of ~~gibberellic~~ indole-acetic-acid in the presence of 2% Sucrose on root elongation of plants defoliated to 2.5 cm. Vertical lines indicate twice the standard error.

The possibility that gibberellic acid at low concentrations may enhance root elongation in defoliated plants appears doubtful on the basis of these two experiments. The differences between the two experiments in response to the 10^{-3}ppm and 10^{-5}ppm concentrations if it is real can be explained by differences in the environmental conditions. In particular, these resulted in a difference of 4 mm per day in average root elongation in plants in the two experiments on the two days prior to defoliation (14 mm and 18 mm per day respectively in experiments 1 and 2). That the significant depression of elongation on day 1 by 10^{-9}ppm is a real effect is highly unlikely in view of the trend towards enhancement by higher concentrations in the same experiment. This also increases the doubt as to the possibility of enhancement by the higher concentrations.

6.5 EFFECT OF INDOLE ACETIC ACID

It was established previously (Appendix 11) that indole acetic acid at greater than 10^{-2}ppm inhibited root elongation in intact L. perenne seedlings. In this experiment the range of concentrations used covered the range seen to have had no effect on the seedlings. The concentrations were 10^{-2}ppm, 10^{-4}ppm, 10^{-6}ppm, and 10^{-8}ppm. The standard experimental procedure was used.

The results are presented in Fig. 15. IAA at 10^{-4}ppm tended to increase elongation over the first two days but depressed elongation on day 3. On no day was the difference significant.

This initial boost to elongation followed by a depression is similar to the pattern in two of the sucrose experiments in Chapter 5. The explanation advanced there was that the initial increased elongation caused a more rapid depletion of some other factor which then limited growth. The pattern is different from that of the benzyladenine and gibberellic acid responses. With these substances the elongation increased relative to the control over the three day period of the experiment indicating that the supply diminished over the three days in the control plants.

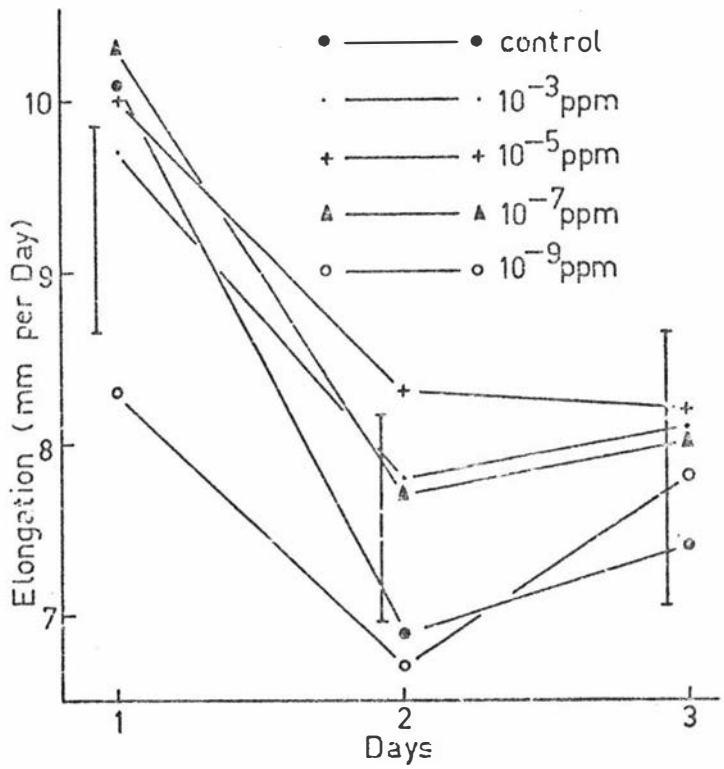


Fig. ~~15~~ 14 - Effect of several concentrations of ~~indole-acetic-acid~~ gibberellic acid in the presence of 2% sucrose on root elongation of plants defoliated to 2.5 cm. Vertical lines indicate twice the standard error.

6.6 COMBINATIONS OF BENZYLADENINE AND OTHER SUBSTANCES

To finish the series of experiments on growth substances, the apparent optimum concentration of benzyladenine was tested with the apparent optimum concentrations of gibberellic acid and indole acetic acid, and with White's nutrients. The concentrations were benzyladenine, 10^{-6}ppm; indole acetic acid 10^{-4}ppm and gibberellic acid 10^{-5}ppm. The White's nutrients were used at the concentrations already detailed in section 6.1. The treatments were:

BA only
BA + IAA
BA + GA
BA + White's nutrients
BA + IAA + White's nutrients

The experimental layout and procedure were the same as in the previous experiments. No aluminium sheets were used.

The results are presented in Fig.16. IAA + White's nutrients appeared to lift elongation on the second day compared with benzyladenine alone. On the first day White's nutrients reduced elongation. Neither result was significant.

6.7 DISCUSSION

Of the growth substances tested only benzyladenine produced a clear response. This indicates that cytokinin or cytokinin precursor from the shoot may be necessary for root elongation even though cytokinins have been recorded as emanating from roots. However since there was no response when no sucrose was applied it may merely be acting in the utilization of the applied sucrose.

As discussed by Street (1969) the effect of benzyladenine appears to be antagonized by indole acetic acid and gibberellic acid in the preliminary experiment. This is not evident in the final experiment where there were no clear trends.

As in the previous chapter, there appears to be large differences

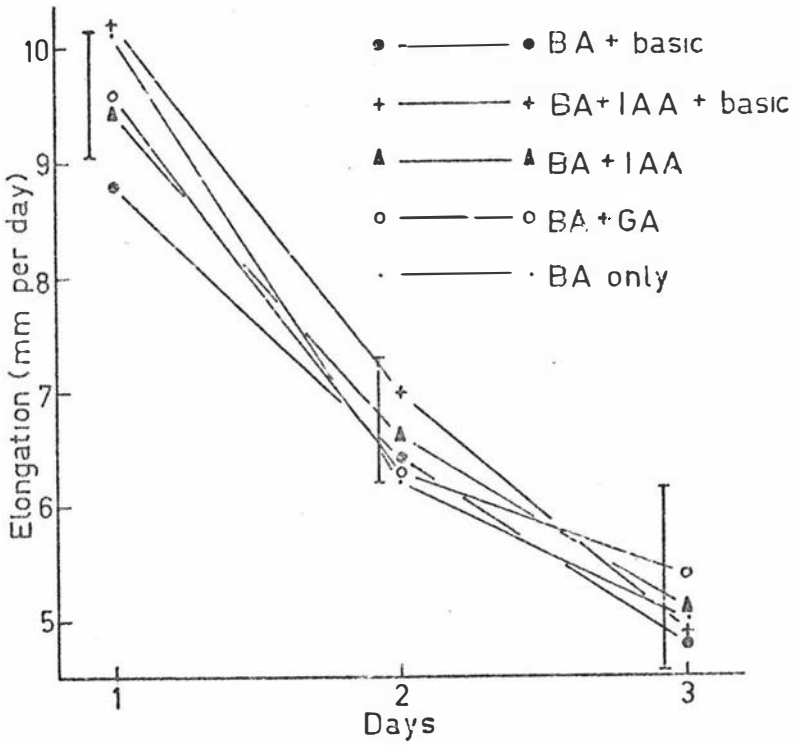


Fig. 16 - Effect of optimum concentration of benzyladenine in combination with basic White's nutrients and optimum concentrations of indole-acetic-acid and gibberellic acid on root elongation of plants defoliated to 2.5 cm.

BA = benzyladenine;

IAA = indole-acetic-acid;

GA = gibberellic acid;

basic = basic White's nutrients.

Vertical lines indicate twice standard error.

between experiments which can be attributed to environmental factors. The presence or absence of the aluminium sheets has added to the differences in this instance.

Of the four organic constituents of White's nutrient, glycine is a nitrogen source and the other three are B vitamins. Because of the complexity of the requirements of roots for these substances (Butcher and Street, 1964) no further investigation of their effects was carried out other than their inclusion with benzyladenine in one treatment in the final experiment. Such an investigation was deemed to be outside the bounds of this study.

Only one cytokinin, one auxin, and one gibberellic acid preparation have been tested in the presence of one sucrose concentration at one defoliation level. This only constitutes a superficial investigation of the importance of growth substances and no firm conclusions can be drawn. In view of the variations between experiments which are attributed to differing environmental conditions it is doubtful if this line of investigation is worth pursuing further in the absence of controlled climate facilities.

CHAPTER 7

GROWTH IN THE DARK

7.1 INTRODUCTION

A comparison of Figs. 2 and 3 indicates that the response of plants to heavy shading was not as great as the response to defoliation. Furthermore the maximum response was not achieved for several days. Since it was considered inadvisable to continue sugar feeding experiments for more than three days because of the likely build-up of microorganisms, shading experiments investigating the effects of sugar and growth substances were not considered worth while. Instead, total exclusion of light, which was believed likely to produce a larger and more rapid response, was investigated.

All experiments in this section were carried out in a room with a temperature of 22 $\pm$ 1°C. A light bank of six 5ft Philips type TLF80w/55 fluorescent tubes provided a light level of 1200 fc at plant height. A 12 hour photoperiod was used throughout. Dark treatment plants were placed under a frame covered with several layers of black cloth.

In experiments in this chapter which included a light treatment a randomized block design as used elsewhere in this study was not possible. The analysis of the results has been altered accordingly.

7.2 GROWTH AT CONSTANT TEMPERATURE

If photosynthate translocated direct to the root apices is more important than stored material for root growth, diurnal fluctuations are likely to occur in roots grown at a constant temperature. To test this possibility, containers were set up under the light bank for a seven day pre-experimental period and then root elongation was measured every 4 hours for 48 hours. In order to increase the accuracy of measurement, the mark on the glass over the root apex was made approximately 1 mm behind the

apex and the distance from the edge of this mark measured using a 16x stereomicroscope with a gratioule in one eyepiece. From these readings the average elongation for each 4 hour period was calculated. Because of the time taken to make the measurements, 4 was the maximum number of containers it was deemed practical to measure. The individual root was taken as the experimental unit. Twenty roots were marked in each container and the results from any which could not be observed for the full 48 hours ignored. The experiment was repeated using a second group of plants.

The results are presented in Fig. 17. In the first experiment root elongation fell throughout the 48 hours, most of the decrease ocurring during the dark periods. In the second experiment root elongation fluctuated with no clear trend evident.

The first experiment was performed in December when light levels, and hence presumably the quantity of photosynthetic products, when the plants were growing in the glasshouse prior to transfer to the growth room would have been higher than in the second experiment in April. Possibly in the first experiment seven days pre-treatment at the higher temperature and lower light level was not sufficient and during the 48 hour experimental period the supply of some substance stored during growth in the glasshouse prior to transferring to the growth room was depleted. None of the fluctuations in the second experiment was significant. They are believed to have been the result of difficulties experienced in focusing the reference mark on the glass and the root tip at the same time together with the gratioule. From the second experiment it appears that there is no regular diurnal fluctuation in elongation when the roots are growing at a steady daily rate. To avoid the possibility of a decline in root elongation such as occurred in the first experiment affecting results, the pre-treatment period was increased to 10 days and experiments designed as far as possible so as to avoid any drop during the dark period.

7.3 EFFECT OF DARK AND RETURN TO LIGHT

Having ascertained that at a steady daily elongation rate there was no consistent difference in growth between light and

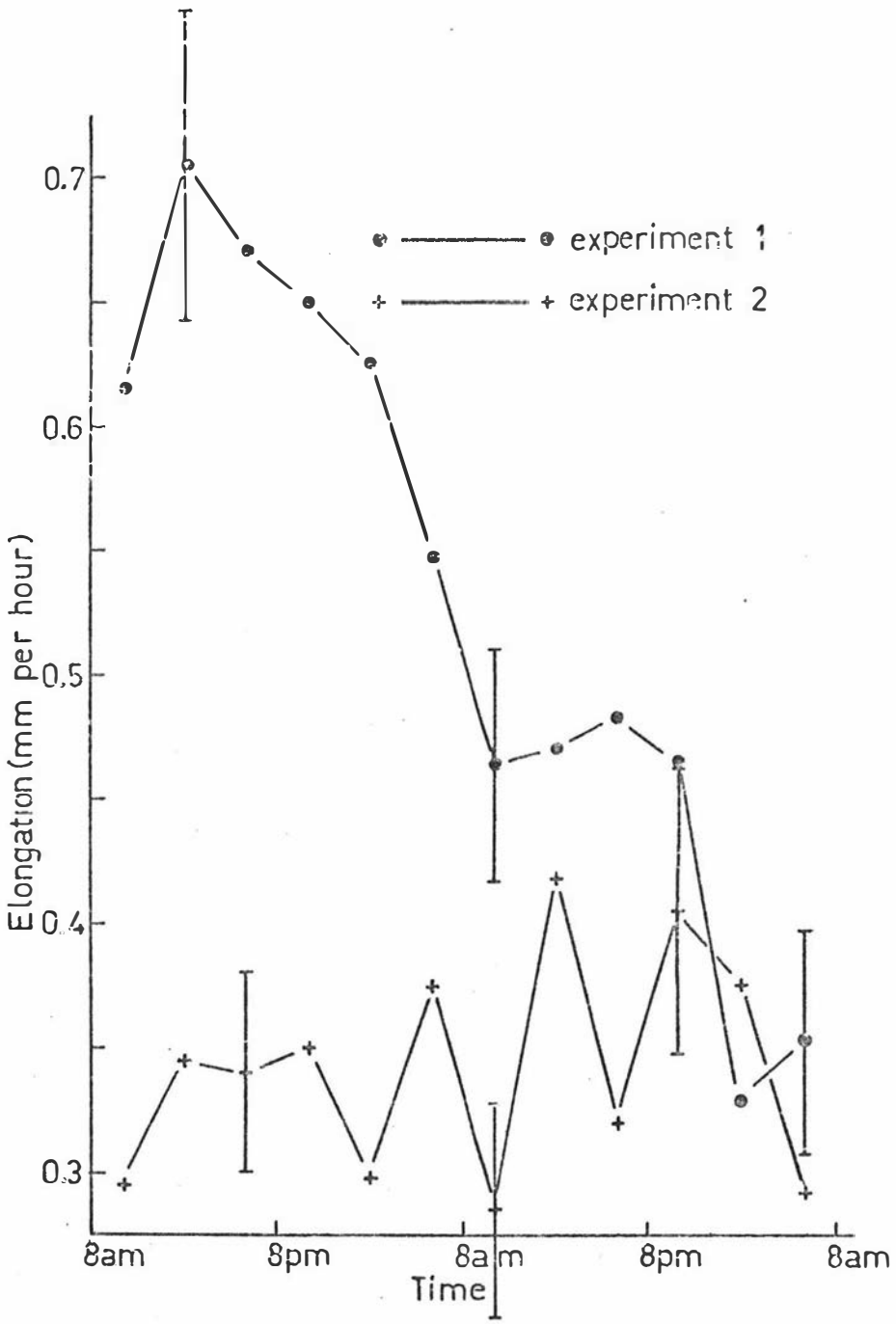


Fig. 17 - Four hourly root elongation of plants in growth room at constant temperature and 12 hour photoperiod of 1200 f.c. Vertical lines indicate twice the standard error.

dark periods at a constant temperature, the effect of total darkness was tested.

Experiment 1. Three Days Dark.

Six containers were used. They were placed in the growth room for 10 days in a 12 hour photoperiod and the lights were then switched off. Root elongation was measured by the standard method for each 24 hours commencing at 8 am (start of the light period). After three days of dark the lights were switched on again and elongation measured for a further five days.

The results are presented in Fig. 18. Growth during the first day of darkness fell to one-eighth of the level of the previous day and on the second day fell almost to zero. After the lights were switched on again, growth rose after two days to a steady level slightly below the level prior to switching off the lights.

Experiment 2. One Day Dark.

A second experiment was performed during which elongation was measured every 12 hours during the depression and recovery period. In this instance the lights were switched on again after 24 hours by which time elongation had dropped to a very low level.

The results of this experiment are shown together with those of the first experiment in Fig. 18. Recovery was slow during the first 12 hours (light period) but more rapid during the following dark period. Subsequently, elongation rose steadily to the level of the 12 hours prior to the imposition of the dark treatment.

The initial recovery was more rapid in experiment 2. This may be related to the shorter period of dark treatment. Recovery was slower in the following 48 hours however.

These experiments show that although in the previous experiment elongation did not vary from light to dark, continued dark caused a rapid drop in root elongation, most of this effect occurring in the first 24 hours. That initial recovery was faster in experiment 2 in which the period of dark was shorter (one day

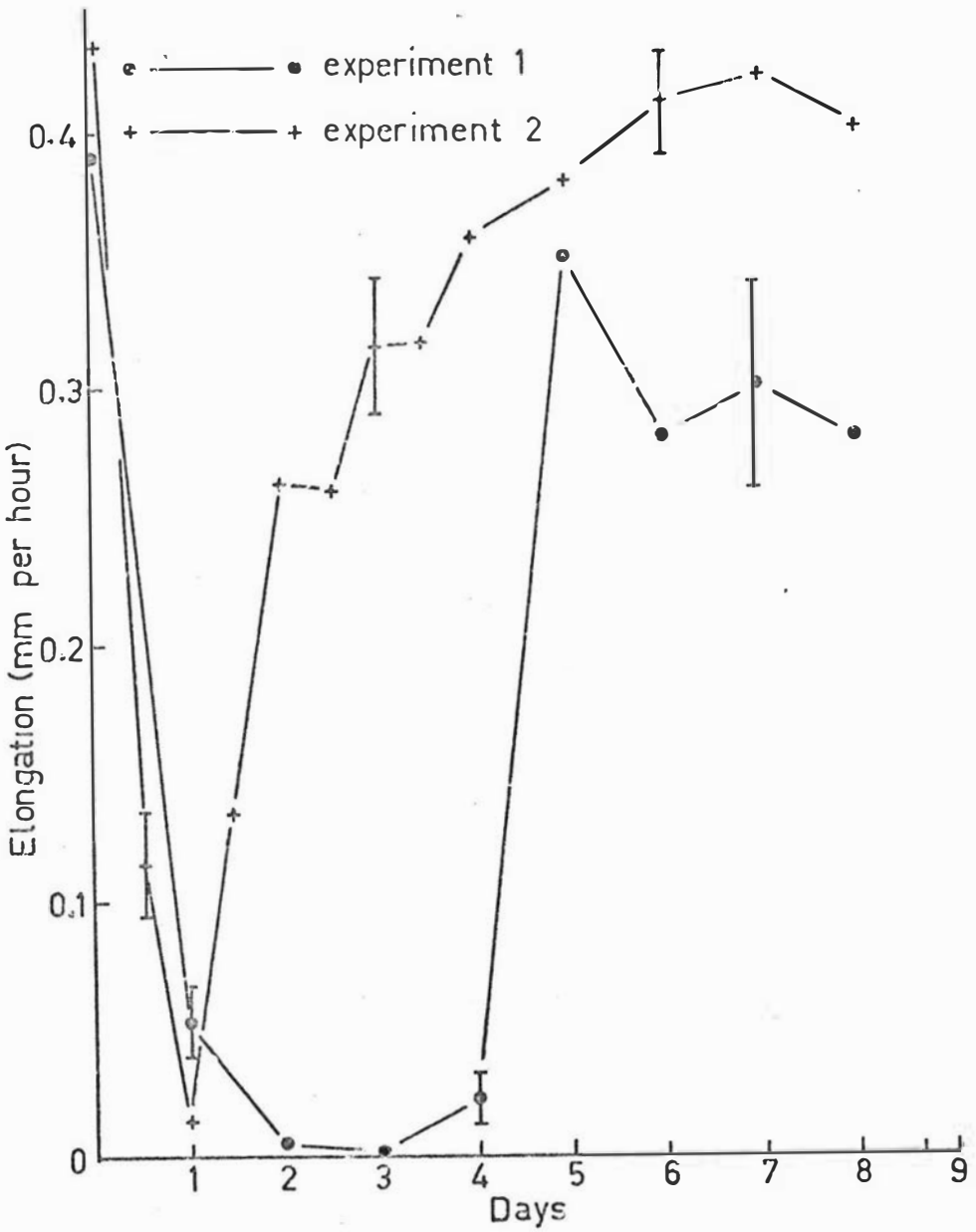


Fig. 18 - **Response of root elongation to placing plants in dark and returning to 12 hour photoperiod at constant temperature. Vertical lines indicate twice the standard error.**

compared with three days) indicates that a substance used in respiration as well as growth may have been involved, i.e. the supply was depleted beyond the level at which root elongation ceased.

7.4 TIME COURSE OF ROOT GROWTH DEPRESSION

The aim of this experiment was to examine the rapidity with which root elongation fell following defoliation and placing in the dark. The measurement of root elongation using a stereo-microscope as in the four-hour experiments (Fig. 17) was rejected because of the difficulty and time involved. Instead measurements were made by the usual method but to the nearest 0.25 mm. The three treatments each of which consisted of six containers were:

Undefoliated in dark

Defoliated in dark

Defoliated in light

It was not possible to place more than 12 containers under the light bank so no pre-treatment period in the growth room was possible. Considering the speed with which elongation fell in the previous two experiments, this lack of pre-treatment which would have served primarily to deplete reserves, is not likely to have markedly affected the results. The probable effect would have been to increase the total amount of growth following treatment. The experiment was conducted in June. Plants were shifted into the growth room at 5 pm (dusk) and root elongation measured from then to 5 am, every two hours thereafter until elongation ceased, and for the following 12 hours. Two groups of plants were defoliated to 2.5 cm at 5 am. One of these groups and the undefoliated plants were placed under the light-proof covers. The second defoliated group was placed under the lights which were set to operate from 5 am to 5 pm.

The results are presented in Fig. 19. In the undefoliated plants there was a slight rise in elongation rate recorded at 9 am but then a drop to zero in the two hour period ending at 7 pm. Over the following 12 hours there was a small amount of growth suggesting that in the dark some periodicity of growth occurred. The increase from the 7 am reading to the 9 am reading

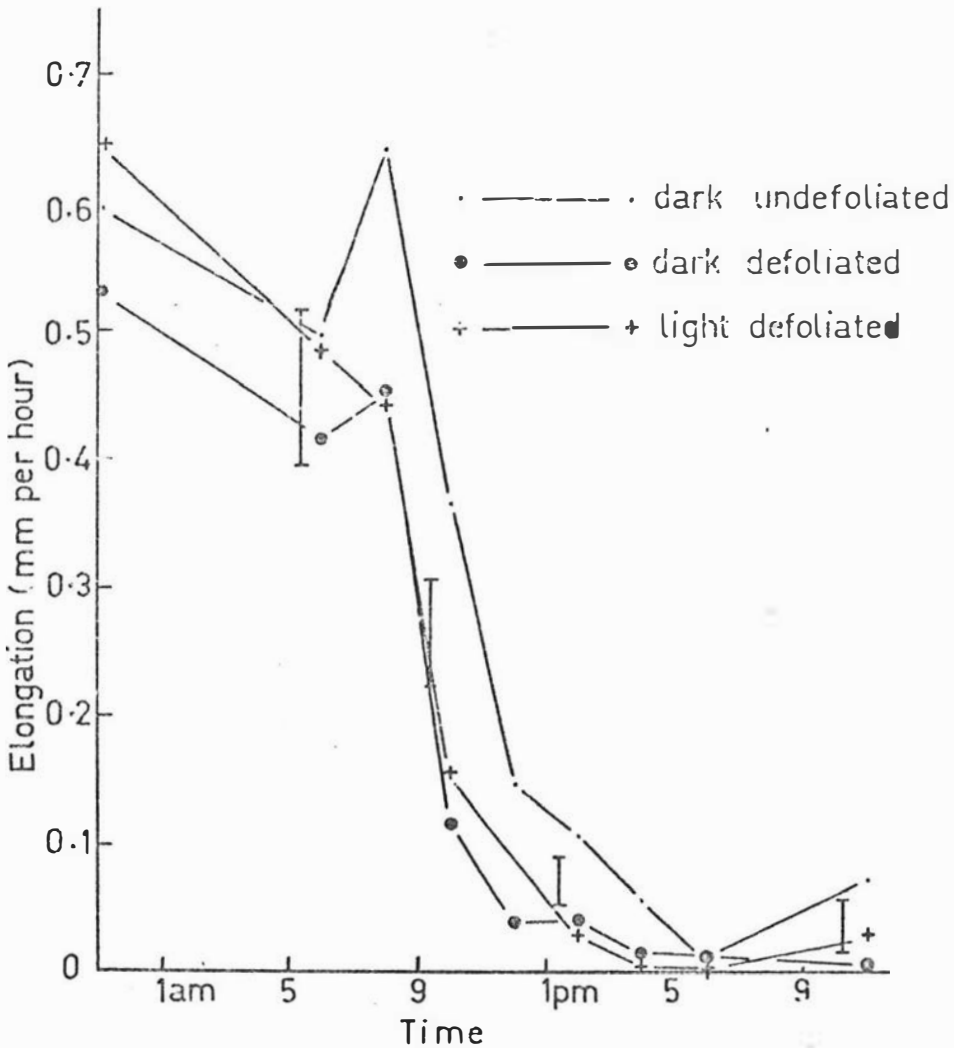


Fig. 19 - **Fall in root elongation of defoliated and undefoliated plants placed in dark and of defoliated plants in a 12 hour photoperiod. Vertical lines indicate twice the standard error.**

is similar to the fluctuations in experiment 2, Fig. 17. As in that experiment, this difference did not reach significance level. There was no difference between the two defoliation treatments. In both, growth fell from the initial rate to the 9 am reading. Elongation in both treatments was less than in the undefoliated plants from the 9 am reading to the 5 pm reading. Growth after 7 pm was also less than in the undefoliated plants.

The results of this experiment indicate that elongation falls rapidly after approximately 16 hours of dark and that defoliation accelerates this decline but not the time at which the onset occurs. Light did not appear to have any effect on elongation in defoliated plants.

The rapid drop in elongation after a period of 16 hours in the dark at a constant temperature suggests that some substance(s) necessary for elongation, produced in the presence of light, is depleted to a critical level. That defoliation produced an additional effect suggests that at least part of the supply of this substance is stored in the shoot. It seems reasonable on the assumption that the factor concerned is produced in the shoot in the presence of light, that light would have little effect in the first 12 hours after defoliation because little shoot tissue would be present. When root growth was being recorded on the plants under the light-proof covers, the shoots of the plants would have received some illumination. This was kept to a minimum by using a portable lamp directed downwards on to the glass face of the containers and was less than 1 fc (the lowest level which could be recorded on the meter used).

7.5 EFFECT OF SUCROSE IN THE DARK

Having established that dark causes virtually complete cessation of root elongation, comparable to the effects of defoliation to 2.5 cm or less, the response of dark-treated plants to the addition of 2% sucrose was investigated. The experiment consisted of six replicates. The plants were moved from the glasshouse to the growth room at 6 pm (dusk). One group was placed under the light bank and received 12 hours light per day. The other two

groups were placed under the light-proof covers. One group of plants kept in the dark was supplied with 2% sucrose in the nutrient solution.

The results are presented in Fig. 20. On day 1 the dark-plus-sucrose plants had a mean elongation only slightly lower than the plants under the light bank. However, elongation on days 2 and 3 fell steeply while in plants under the light bank, the fall was not as great. The dark-minus-sucrose plants exhibited a sharp reduction in elongation on day 1 comparable with that exhibited by the plants in Fig. 18. Elongation then fell to zero on day 3.

The response to sucrose in this experiment decreased with time. This again suggests that sucrose is not the only factor involved. That elongation is only slightly less in the dark-plus-sucrose treatment than in the light treatment on day 1 indicates that the roots are taking up sucrose in adequate quantities from the nutrient solution. However it is possible that a build-up of sucrose to above the optimum level may have occurred over the three days. The fall in elongation in the light treatment plants from day 1 to day 2 indicates that a pre-treatment period in the growth room would have been desirable. This was not possible for the reason previously stated.

7.6 EFFECT OF DEFOLIATION ON DARK-PLUS-SUCROSE-TREATED PLANTS

In Fig. 19 defoliation was shown to decrease the subsequent elongation of the roots of plants grown at constant temperature in the dark. It was postulated that part of the supply of the factor(s) necessary for root elongation was stored in the shoot. The results presented in Fig. 20 indicate that besides an energy source, some other factor is required. Further information on the source and nature of this substance was sought by examining the effect of defoliation to 2.5 cm and completely removing the shoot on root elongation in the dark. As previously, the experiment consisted of six replicates. Plants were transferred from the glasshouse to the growth room at 6 pm and elongation measured from 8 am the following morning. Other details of the experimental procedure are as previously described.

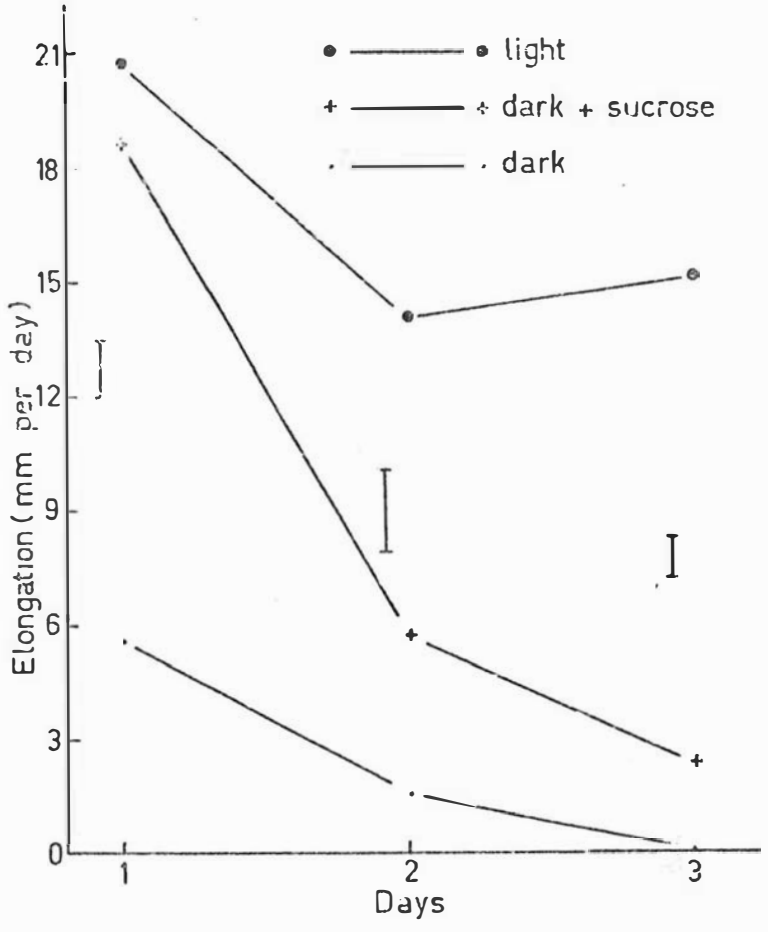


Fig. 20 - **Effect of 2% sucrose on root elongation of plants placed in the dark. Vertical lines indicate twice the standard error.**

The results are presented in Fig. 21. Plants defoliated to 2.5 cm had a significantly lower elongation rate on all three days than the undefoliated plants. Where the whole shoot was removed elongation was the same as in the undefoliated plants on day 1 and was thus significantly greater ($p < .01$) than the 2.5 cm defoliation treatment as it was also on day 2. In all three treatments elongation fell over the three days.

The results of this experiment indicate that the factor(s) required by the root and contributed by the shoot, other than an energy source, is stored mainly in the leaves. No stem tissue and little or no meristematic tissue from the leaves would have been removed by defoliating to 2.5 cm yet this tissue apparently had a negative effect on root elongation. There are two possible explanations for this. The first is that the shoot tissue remaining produced some substance which inhibited root elongation and the second is that part of the sucrose absorbed by the roots was translocated to the shoot possibly together with substances stored or synthesised in the roots. The results of this experiment are contrary to those where the same defoliation treatments were applied to plants growing in the glasshouse in full light (Fig.10). In that case complete removal of the shoot considerably depressed root elongation by comparison with defoliation to 2.5 cm. This suggests that the postulated substance was synthesised or stored to a significant extent in the tissue remaining after defoliation to 2.5 cm.

7.7 EFFECT OF LOW INTENSITY LIGHT

Although 2% sucrose promoted elongation in dark-treated plants this elongation was below the level of plants grown in the light. This situation was similar to that in defoliated plants. No major response was obtained to the application of representative growth substances in the case of defoliation. Non-photosynthetic responses to low light levels, similar to responses to growth regulators, have been reported by Street (1953), Hillman (1957), Powel and Griffith (1960), Burstrom (1961) and Lockhart (1961). Accordingly the effect of low intensity light was investigated in the growth room.

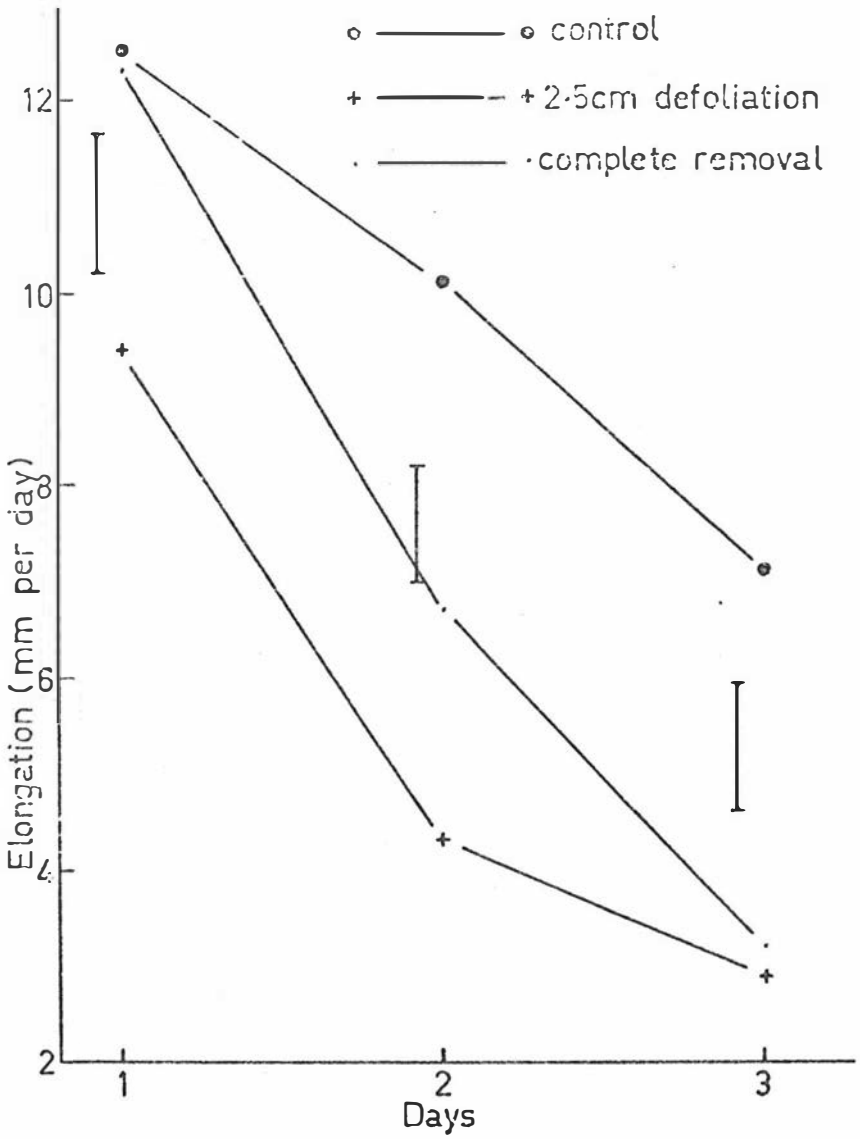


Fig. 21 - **Effect of defoliation to 2.5 cm and of complete removal of the shoot on root elongation of plants placed in the dark and supplied 2% sucrose. Vertical lines indicate twice the standard error.**

Experiment 1. Comparison of 150 fc and Dark

The light bank in the growth room was raised as far as possible above the bench and all tubes but one disconnected. As a result, a light intensity of 150 fc at plant height was obtained. This level is higher than those used by the authors mentioned above so should be capable of producing any low-level responses observed by them. The elongation at this light level with a 12 hour photoperiod was compared with elongation in the dark. Both groups of plants received 2% sucrose. There were six containers per treatment. Plants were given ten days pre-treatment under the full light bank (1200 fc).

The results are presented in Fig. 22. The low intensity light produced a greater root elongation, the difference being significant on days 2 and 3 ($p < 0.01$).

Experiment 2. Comparison of 1200 fc, 150 fc and Dark

Having demonstrated that the low light produced a significant increase in elongation over dark in the presence of 2% sucrose, the effects of low light, full light and dark were compared. The light bank was lowered to its former position and the tubes reconnected. A single flourescent tube of the same make and type was set up above another bench in the growth room so as to give a light intensity of 150 fc at plant height. No pre-treatment period in the growth room was possible. Two per cent sucrose was supplied to all treatments.

The results are presented in Fig. 23. It appears that 150 fc of light for 12 hours per day although increasing elongation compared with the dark treatment was still not sufficient to maintain the elongation level obtained at higher light levels. The increase in elongation in 150 fc compared with the dark treatment was uniform over the two experiments although the actual levels were higher and decreased by 4 mm over the three days in experiment 2.

Experiment 3. Comparison of 150 fc and dark in the absence of Sucrose

The photosynthetic effect of 150 fc light level was examined

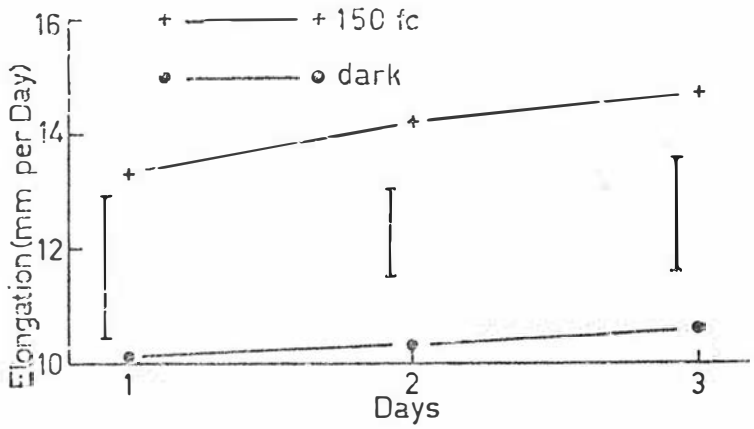


Fig. 22 - Response of root elongation of plants supplied 2% sucrose to low intensity light for 12 hours per day. Vertical lines indicate twice the standard error.

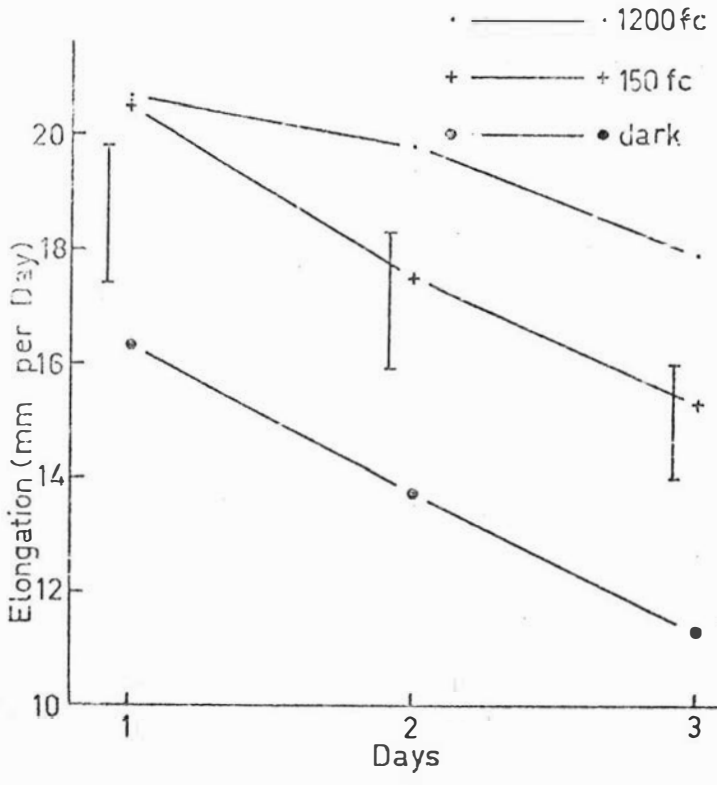


Fig. 23 - **Comparison of high light, low light and dark on root elongation of plants supplied 2% sucrose. Vertical lines indicate twice the standard error.**

next by comparing the root elongation of plants grown in the dark and at 150 fc in the absence of sucrose. In order to compare this experiment with the previous one, plants were not given a pre-treatment period in the growth room. The plants were placed in the growth room at 5 pm and the elongation measured from then to 5 am, every two hours to 5 pm and for the three following 12-hour periods.

The results are presented in Fig. 24. The elongation of the dark-treated plants fell steadily to near zero at 5 pm on the first day of treatment. The rate in plants growing at 150 fc light fell during the first day at a lesser rate and then remained steady for the final 36 hours. This final rate was approximately 4 mm per day which is the same as the difference between dark and 150 fc treatments in Figs. 22 and 23 in which sucrose was added.

7.8 DISCUSSION

The rapid drop in elongation in plants retained in the dark is similar to that in plants defoliated to 2.5 cm or less and is in contrast to the steady drop over several days in shaded plants. This indicates that the mechanism may be different in the two cases. Reduced root growth under low light is the logical means whereby the shoot/root ratio is adapted to the changed growing conditions since the alternative, increased shoot growth, is unlikely under these conditions and is probably brought about by a steady decline in carbohydrates and perhaps other substances in the plants. The rapid drop on placing in the dark appears more likely to be due to the action of some specific factor than to the depletion of carbohydrates.

The reduction in root elongation to an apparently steady, low level in plants growing under 150 fc light without sucrose is interesting. The most obvious assumption is that the plants synthesised the critical factor whatever its nature at a reduced level. It appears that even at this low level, the elongation was maintained at the apparently steady level during the dark period. That there occurred 4 mm per day elongation attributable to 150 fc light both in the presence and absence of sucrose suggest that

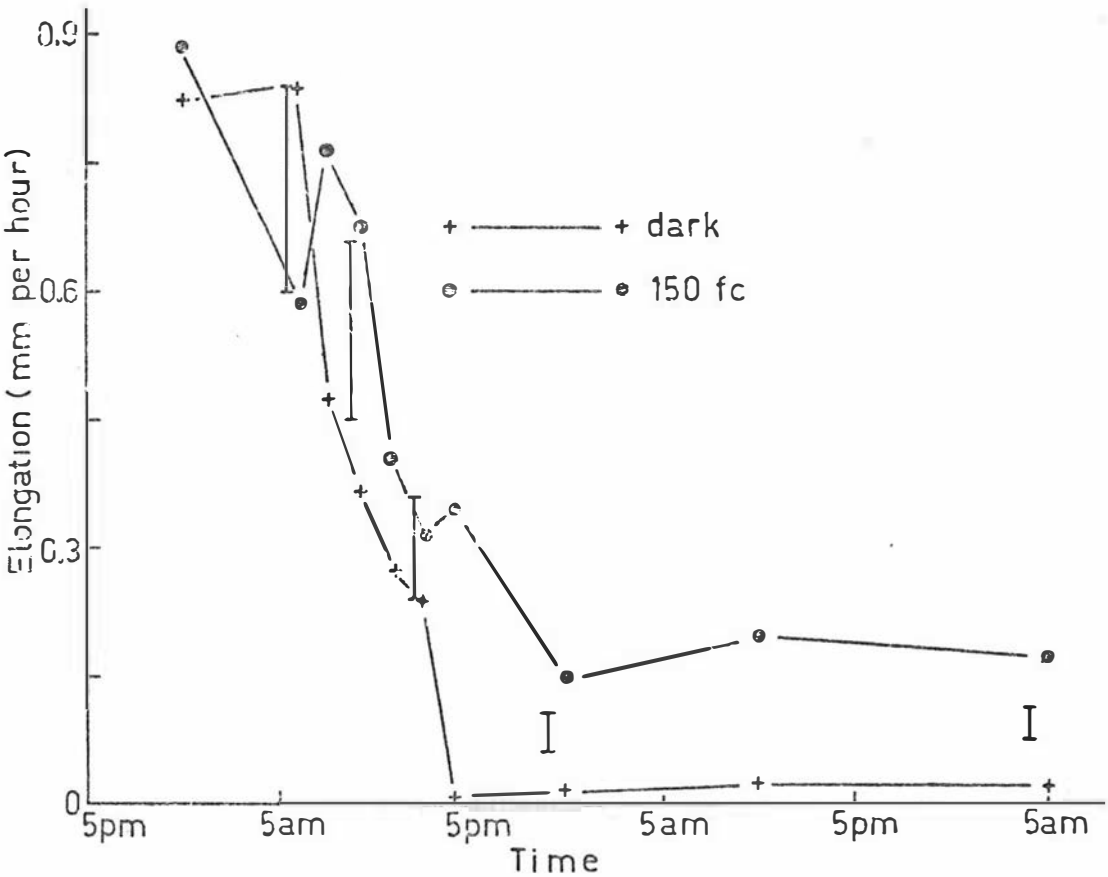


Fig. 24 - **Fall in root elongation of plants ~~supplied 2% sucrose~~ placed in the dark or in 150 fc light for 12 hours per day. Vertical lines indicate twice the standard error.**

the factor involved is not an energy source although this light level would provide an appreciable proportion of the maintenance requirements of the plants, this species having a compensation point of approximately 300 fc (D. Wilson, pers.comm.). That defoliation caused a more rapid drop in elongation than merely placing in the dark indicates that the postulated factor is stored in the shoot.

In the heaviest shade treatment described in chapter 3 the light level was approximately 1500 fc at mid day in full sunlight so the average level would have been rather less than that under the light bank in the growth room. In contrast to shade treatments in chapter 3 root elongation under the light bank was steady for several days at a higher level than occurred in most experiments in the glasshouse where the mean temperature would on all occassions have been lower. However after a few days the root elongation was noted to fall.

CHAPTER 8

SOLUBLE CARBOHYDRATE LEVELS

8.1 INTRODUCTION

In chapters 5 and 7 it was demonstrated that depression of root elongation following defoliation or placing in the dark could be partly countered by supplying 2% sucrose in the nutrient solution. This is consistent with reports in the literature that the levels of soluble carbohydrates in the roots fall following defoliation and shading. Measurements of soluble carbohydrates reported in this chapter have been made to determine (1) the extent to which the levels fall following defoliation and placing in the dark (2) the rate of recovery (3) whether there is a critical level for root elongation and (4) the mobility of carbohydrates within the plant.

8.2 EFFECT OF A SINGLE DEFOLIATION

Experiment 1. Soluble Carbohydrate Levels Following a Single Defoliation.

Reduction in soluble carbohydrate levels in the roots of defoliated plants has been reported by a number of authors and recovery following defoliation was associated with increasing carbohydrate levels by Sullivan and Sprague (1943). Accordingly the following experiment was set up to measure the relationship between root elongation rate and soluble carbohydrate levels. The experiment consisted of six replicates and four treatments. In three treatments, plants were defoliated to 2.5 cm. Root elongation was measured each day on all containers. After two days from the commencement of treatment, by which time the daily elongation had dropped below 1 mm in the defoliated plants, one of the defoliation treatments was harvested. The second and third defoliation treatments were harvested when elongation had recovered to approximately one-third and two-thirds respectively of the level of the undefoliated treatment which was harvested at the same time as the third defoliated group. At harvest, the roots of all plants were washed free of sand and freeze-dried. They were then ground in a Cassella mill to pass through a 1 mm screen. Total soluble carbo-

hydrates were estimated on boiling water extracts. The procedure is described in Appendix 14.

The results are presented in Table 4. After the two days the soluble carbohydrates had dropped to a low level. The level increased again as root elongation recovered.

It should be realized that elongation rate and soluble carbohydrate level cannot be related for a particular point in time as elongation measurements were for the 24 hours prior to the time when the plants were harvested for soluble carbohydrate determination. However since recovery occurred over a number of days (12 days to final harvest. See also Fig. 1) the discrepancy is not great.

The levels of soluble carbohydrates are low in all treatments compared with the results of some other workers. Sullivan and Sprague (1943) for example recorded that following intensive defoliation, levels fell from 5.0% to 3.5%. Weinmann (1943) recorded levels of 3 to 5% depending on frequency of defoliation. The low carbohydrate levels in the present experiment are probably due to the high nitrogen status of the plants (Nowakowski, 1969).

Sullivan and Sprague (loc.cit.) reported that partial defoliation of L. perenne resulted in a rapid decrease in water soluble carbohydrates for 11 days. The level remained constant for another 10 days and then increased. Sprague and Sullivan (1950) obtained similar results with Dactylis glomerata. Presumably in the present experiment environmental conditions were conducive to a more rapid recovery.

Experiment 2. Soluble Carbohydrate Level when Root Elongation Ceased.

The root elongation figures presented in this chapter and elsewhere in this thesis are the means of up to 20 roots per container. In containers where plants were defoliated to 2.5 cm or less, most of the roots ceased elongating after the first day but a few continued to elongate at a low rate. The carbohydrate level in Table 4 column 2 represents the situation in the roots some time

Table 4 - Level of soluble carbohydrates and elongation of roots of plants harvested at different times after defoliation

	Control	Defoliated		
Days to harvest	12	2	5	12
Elongation for 24 hours prior to harvest (% of control)	100	0	35	65
Soluble carbohydrates (% freeze dried wt.)	0.91 ±0.11	0.24 ±0.03	0.45 ±0.05	0.79 ±0.15

after most root elongation had ceased and because of respiration losses was probably a figure much lower than that at the time elongation ceased in the majority of roots. The fall in root elongation occurred mainly in the first 12 hours (see Fig. 19). Most of the roots measured in that instance had ceased growth completely within that time. Use was made of this information to measure soluble carbohydrate levels near the time when elongation ceased in most roots.

The plants in six containers in the glasshouse were defoliated to 2.5 cm at 8 am. The positions of 20 root apices per container were then marked and these apices were inspected every two hours for elongation using a 16x steromicroscope and re-marked if still growing. When the growth of most roots had ceased (4 pm) the plants were harvested and the soluble carbohydrate levels determined as described for the previous experiment.

The mean level for the six containers was 2.0 $\pm$ 0.20%

i.e. there was a higher percentage of soluble carbohydrate present at about the time most roots ceased elongating than in all treatments in the previous experiment (Table 4). This suggests that although changes in soluble carbohydrates may accompany reduction and cessation of root elongation induced by defoliation, these changes are not necessarily the determinant. That under such conditions, in which soluble carbohydrates would not be expected to be limiting growth, the application of sucrose increased elongation (Chapter 5) suggests that the defoliation may cause some kind of translocation blockage and hence restrict the soluble carbohydrate level at the root apex.

Experiment 3. Test of Sucrose Absorption from the Nutrient Solution.

The amount of sucrose absorbed by the roots when applied in the nutrient solution may not have been sufficient to raise the concentration within the roots to the level required for maximum growth. This was tested in the following experiment. Plants were grown in glass fronted containers in the glasshouse. One treatment consisted of plants defoliated to 2.5 cm and supplied

with 2% sucrose and the second of undefoliated plants supplied with inorganic nutrient solution only. Root elongation was measured on all containers. After three days the roots were harvested, washed clean of sand, rinsed and freeze dried. They were later analysed for soluble carbohydrate.

To ensure that the results of the comparison had not been falsified by sucrose adsorbed on the root surface the test detailed in Appendix 15 was carried out.

The results of the analyses together with the mean elongation rates of the two groups of plants are presented in Table 5. The soluble carbohydrate level was higher in the defoliated plants supplied with sucrose than in the undefoliated plants yet the root elongation was significantly lower. This shows that sucrose entry into the roots as a whole was not limiting.

8.3 EFFECT OF DARK

Having demonstrated that defoliation resulted in a large reduction in soluble carbohydrate levels in the roots and that these levels increased again as root elongation recovered, the effect of dark on the soluble carbohydrate level of the roots was investigated.

Experiment 1. Soluble Carbohydrate Levels following Placing in the Dark.

The experiment consisted of six replicates and four treatments. All plants were placed in the growth room at 5 pm (sunset). One group (controls) was placed under the light bank in a 12 hour photoperiod and the remainder under the light-proof covers. Root elongation was measured on all containers. After two days by which time elongation had ceased in most roots in the plants held in the dark, the plants under the light bank were transferred to the glasshouse and two of the groups held in the dark were placed under the light bank. The fourth group was harvested. After one day under the light bank, the second group of plants was harvested. The control plants were then returned to the growth room and placed under the light bank with the remaining treatment group. The final two groups

Table 5 - Effect of defoliation to 2.5 cm plus 2% sucrose on elongation and level of soluble carbohydrates of roots

Treatment	Elongation (mm/day)			Soluble carbohydrates (% freeze dried wt.)
	Day 1	Day 2	Day 3	
Control	19.9	15.5	15.0	1.48
Defoliation plus sucrose	18.6	4.8	4.9	2.48
S.E. ±	0.96	0.66	0.98	0.10
D.05	3.00	2.05	3.07	0.32

of plants were harvested two days later by which time the root elongation in the dark-treated plants had recovered to approximately two-thirds of the control plant level. This transfer of the control plants back to the glasshouse for one day was necessary because of the limited number of containers which could be placed under the light bank. As a result of the variability in soluble carbohydrate levels between replicates of the same treatment in the two defoliation experiments, it was not considered practical to use four replicates in this experiment and thus avoid the necessity of shifting the control plants. Elongation during the pre-treatment period of plants used for the experiment presented in Fig. 18 was steady so the assumption was made in the present situation that growth of the control plants during the first two days of the experiment could be used as a standard against which to judge the recovery of elongation in the treatment groups on the first day under the light bank. At harvest, the roots of all plants were washed free of sand and freeze-dried. They were then analysed for soluble carbohydrates.

The results are presented in Table 6. The soluble carbohydrates fell to a low level after elongation had ceased. After only one day under the light bank, by which time elongation had recovered to approximately 15% of the estimated control level, the soluble carbohydrates had risen to the control level.

These results are in contrast to those of the comparable defoliation experiment. It must be kept in mind that the elongation rates shown are the totals for 24 hour periods. From Fig. 18 it can be seen that the recovery in elongation is rapid after placing the plants under the light bank. This would mean that the rate at the time the second group of plants was harvested would be much higher than the mean for the 24 hours. Even taking this into account, the recovery of soluble carbohydrates in dark-treated plants is more rapid relative to recovery in elongation than for comparable plants defoliated to 2.5 cm. Note that the control treatment levels were lower in the defoliation experiment.

Table 6 - Level of soluble carbohydrates and elongation of roots of plants harvested at different times after placing in dark and returning to light.

	Control	Dark Treatment		
Days to harvest	5	2	3	5
% of control elongation for 24 hrs. prior to harvest	100	0	15	71
Soluble Carbohydrates (% of freeze dried wt.)	1.63 ±0.09	0.30 ±0.02	1.85 ±0.05	1.62 ± 0.11

Experiment 2. Soluble Carbohydrate Level when Elongation Ceased.

The level at the time that elongation in most roots ceased was also examined as in the case of defoliation. The procedure was the same as in that experiment. Plants were placed in the growth room at dusk (5 pm) and were harvested at 6 pm the following day. The soluble carbohydrate level was 2.27 $\pm$ 0.29% which is marginally higher than the level in the control treatment in experiment 1 where root elongation for the 24 hours prior to harvest was 9.9 mm.

The results of these two experiments confirm those of the defoliation experiments of the previous section. These are (1) that soluble carbohydrate levels appear to be adequate at the time when most root elongation ceases and (2) that recovery of root elongation is paralleled by a recovery of soluble carbohydrate levels. The presence of the whole shoot provides for a more rapid recovery in the root system. This can be attributed to the greater storage and photosynthetic capacity of the intact shoots.

Experiment 3. Test of Sucrose Absorption from the Nutrient Solution.

Again as in the case of defoliation, an experiment was conducted to check that the soluble carbohydrate level in the roots supplied with sucrose was not likely to be the limiting factor in elongation in the dark. Twelve containers were used. Six were placed under the light bank and the others under the light-proof covers. The plants in the dark were supplied with 2% sucrose. Root elongation was measured on all containers for three days and the roots were then harvested and analysed for soluble carbohydrates.

The results are presented in Table 7. The soluble carbohydrate level in the dark-plus-sucrose treatment was significantly higher than in the control treatment yet the root elongation was significantly lower. The results are similar to those in the comparable defoliation treatment and show that as in that case, the soluble carbohydrate level in the roots is not likely to be the limiting factor in elongation.

Table 7 - Effect of dark plus two per cent sucrose on elongation and level of soluble carbohydrates of roots.

	Elongation (mm per day)			Soluble carbohydrates (% freeze dried wt.)
	Day 1	Day 2	Day 3	
Control	21.4	23.5	19.0	0.88
Dark plus sucrose	10.3	11.9	11.1	2.20
S.E. ±	1.26	0.71	0.85	0.13
D.05	3.92	2.22	2.26	0.42

8.4 SOLUBLE CARBOHYDRATE MOVEMENT FOLLOWING DEFOLIATION

In one dark experiment (Fig.21) translocation of sucrose supplied to the root up into the shoot was indicated since greater elongation occurred in plants with the shoot completely removed than in plants defoliated to 2.5 cm. There is no indication of this occurrence in an earlier experiment carried out in the glasshouse (Fig.10). To obtain an understanding of movement of carbohydrates within the plant after defoliation, the following experiment was conducted.

The experiment consisted of six replicates and six treatments. It was carried out in the growth room to eliminate any possible differences due to changes in temperature over the experimental period. Plants were moved into the growth room at 5 pm. The six treatments were:

1. Harvested at 5 pm.
2. Defoliated to 2.5 cm at 5 pm. Root elongation measured 5 pm to 7 am and then harvested.
3. Shoot completely removed at 5 pm. Root elongation measured 5 pm to 7 am and then harvested.
4. Undefoliated, harvested at 5 am.
5. Defoliated to 2.5 cm at 5 am. Root elongation measured 5 am to 7 pm and then harvested.
6. Shoot completely removed at 5 am. Root elongation measured 5 am to 7 pm and then harvested.

At harvest the plants were removed from the containers, washed clean of sand, separated into shoot and root where necessary, and freeze-dried. The dried material was ground to pass through a 1 mm screen and analysed for soluble carbohydrates. Note that the plants of treatments2, 3, 5, and 6 were harvested 14 hours after defoliation because it was not possible to record root elongation on treatments 2 and 3, harvest treatments 2, 3 and 4, and defoliate and mark the root apex positions on treatments 5 and 6 all at the same time. The work was therefore divided between 5 am and 7 am. In order that treatments could be compared, treatments 5 and 6 were harvested a similar period of time after defoliation to treatments 2 and 3. Also because of the shortage of time root elongation was not measured in treatments 1 and 4.

The results are shown in Table 8. In plants defoliated to 2.5 cm (treatments 2 and 5) root elongation in the following 14 hours was significantly greater than where the shoot was completely removed (treatments 3 and 6). Elongation was greater in the plants defoliated to 2.5 cm at 5 pm than at 5 am but there was no difference where the shoot was completely removed. The levels of soluble carbohydrates in the roots were higher in treatments 2 and 5 than in treatments 3 and 6 respectively. Although the level was higher in treatment 2 than in treatment 5 there was no difference in the level in the stubble. This is in contrast to the situation in the whole shoot in plants harvested earlier (treatments 1 and 4). In all cases the level in the shoots was much higher than that in the roots.

Although there was less elongation in the roots of plants where the shoot was completely removed than where a 2.5 cm stubble remained, the soluble carbohydrate level was reduced slightly. This indicates that in this case as shown by Sagar and Marshall (1967) movement occurred from the shoot to the root during the first 24 hours after defoliation. Certainly there is no indication that carbohydrate was translocated from the root to the shoot. The level in the roots of undefoliated plants harvested at 5 am (treatment 4) was also much higher than that of either group of defoliated plants harvested at 7 am as would have been the elongation, judging from previous experiments. That the level in the stubble did not fall between harvests suggests that the material here consists of relatively long term storage compared with the material in the leaves as demonstrated by the marked drop in the whole shoot (leaves plus stubble) between 5 pm and 5 am. The overall pattern appears to be that carbohydrates synthesised during the day are translocated down into the stubble and roots during the night so that the level in the leaves is depleted. The movement into the root system in intact plants may be greater during the day than in the dark as evidenced by the lower level in the roots of intact plants harvested at 5 am. However this interpretation is open to doubt as the plants were at a higher temperature in the growth room so respiration losses would have been greater. Defoliation reduced the translocation to the roots as the level at 7 am in plants defoliated to 2.5 cm was only

Table 8 - Effect of defoliation to two different levels at the start or finish of the dark period on subsequent root elongation and level of soluble carbohydrates. (% freeze dried weight).

Treatment	1	2	3	4	5	6
Soluble carbohydrates in roots	5.60 ± 0.35	2.88 ±0.32	2.58 ±0.18	4.28 ±0.43	2.02 ±0.11	1.70 ±0.16
Soluble carbohydrates in shoots	13.53 ± 1.13	9.74 ±1.66		7.61 ±0.81	9.90 ±0.47	
Elongation (mm) in the 14 hrs. prior to harvest		5.5 ±0.53	2.8 ± 0.24		4.2 ±0.14	2.8 ±0.15

Treatments

1. Undefoliated, harvested at 5 p.m.
2. Defoliated to 2.5 cm at 5 pm, root elongation measured 5 pm to 7 am and then harvested.
3. Shoot completely removed at 5pm, root elongation measured 5 pm to 7 am and then harvested.
4. Undefoliated, harvested at 5 am.
5. Defoliated to 2.5 cm at 5 am, root elongation measured 5 am to 7 pm and then harvested.
6. Shoot completely removed at 5 am, root elongation measured 5 am to 7 pm and then harvested.

two-thirds of the level in the roots of undefoliated plants harvested two hours later. It is unlikely that this great a difference would have occurred in the intervening two hours considering that the drop in the previous 12 hours was less than the difference between defoliated and undefoliated plants. These levels were all well below the level in the stubble of plants defoliated to 2.5 cm. This indicates that the soluble carbohydrates in the stubble are less mobile than the recently synthesised material in the leaves.

8.5 DISCUSSION

It has been shown that following defoliation or placing in the dark root elongation ceases when the soluble carbohydrate level is above the lowest level seen to support root elongation in untreated plants. That under these circumstances there is a response to applied sucrose indicates that there may be a shortage of soluble carbohydrates at the root apex. This fits in with the picture obtained of movement in the whole plant where there was seen to be a difference between the level in the shoot and root. The investigation of the soluble carbohydrate level in the root apices was considered but was decided against. The cells reach their mature length approximately 4 mm from the apex (Appendix 8 Fig. 1.). With approximately 100 root apices per container suitable for harvesting and with a dry weight of primary nodal root of 0.35 mg / cm the amount of tissue available for analysis would have been 14 mg per container. The analytical technique used required a 0.5 g sample.

CHAPTER 9

CHANGES IN THE ROOT APEX

9.1 INTRODUCTION

In Appendix 8 the cell length pattern in the cortex at the root apex was examined in roots of different elongation rates. It can be seen in Appendix 8 Fig 1 that the pattern is typically sigmoid and that in the roots with the greatest daily elongation the cells elongated at a greater distance from the root cap and achieved a greater final cell length than cells of roots with a low daily elongation. In this chapter the effects of defoliation and dark on this pattern have been investigated.

9.2 EFFECT OF DEFOLIATION ON THE ROOT APEX

In the preceding chapters it has been shown that defoliation to 2.5 cm above the base of the shoot or less resulted in the complete cessation of elongation in most primary nodal roots. In this section the cell pattern after root elongation ceased and during recovery has been examined.

Experiment 1. Cell Length Pattern when Elongation Ceased and During Recovery.

Ten containers were planted and allowed to establish. The positions of all primary nodal root apices were marked on the glass. Twenty four hours later, the apices of two roots having a high elongation rate and two having a low rate were harvested from each container by removing the glass. These root apices, whose individual elongation rates were recorded, were fixed and preserved in formalin-aceto-alcohol (see Appendix 8). The plants in the containers were then defoliated to 2.5 cm above the base. The positions of the remaining apices were marked 24 hours after defoliation. Forty eight hours later, the roots were examined and the marks removed from any roots which had not ceased to elongate. In each container, the apices of two roots which had ceased to elongate were removed and preserved. On the following days, the remaining marked roots were observed and the amount of new growth measured. From each container, two roots with a regrowth of 2-3 mm and two each with a regrowth of 5-6 mm

and 9-10 mm were removed and preserved. One root apex from each pair was dehydrated, embedded and sectioned as detailed in Appendix 8. The second was retained in case of loss or damage to the first during processing. The sections were stained with safranin and fast green (Johansen, 1940) rather than with azo black as in the former case in order that cell divisions could also be observed.

The results are shown in Fig. 25. The curves were fitted to the data by eye. As in Appendix 8 roots with a high elongation rate differed in cell length pattern from those with a low elongation rate. The pattern in the roots that had ceased elongating was identical with that of the roots with a low elongation rate harvested prior to defoliation although the mean elongation of these roots prior to defoliation would have been intermediate between those of the two pre-defoliation groups. The roots at the three stages of recovery following defoliation-induced cessation of elongation showed increased cell length near the root cap compared with the two pre-defoliation groups. In the 2-3 mm and 5-6 mm groups there was a striking depression of cell length beyond 1 mm from the root cap. This depression appears to start at a cell length of approximately 50 μ. In the roots in which growth had ceased this corresponds to a point about 1700 μ from the root cap.

The similarity between the cell patterns in the roots which had ceased elongating and those harvested prior to defoliation indicates that all stages of cell growth in the root apex are affected to a similar extent by defoliation. This suggests that the limiting factor is one which affects a wide range of processes in a similar way. That cell length was 'frozen' when elongation ceased also indicates that the factor concerned must move rapidly within the root apex relative to the rate at which it is used. If this were not so then the cells nearest the root cap would cease elongating before those nearer the base of the root. Such a situation would show as an increase in cell length near the root cap compared with roots that are growing normally. This situation would appear to occur on recovery following defoliation. This could be explained on the assumption that the factor is translocated down from the shoot and that initially the supply is inadequate. Under these circumstances, the cells nearest the source of supply would use most of the supply

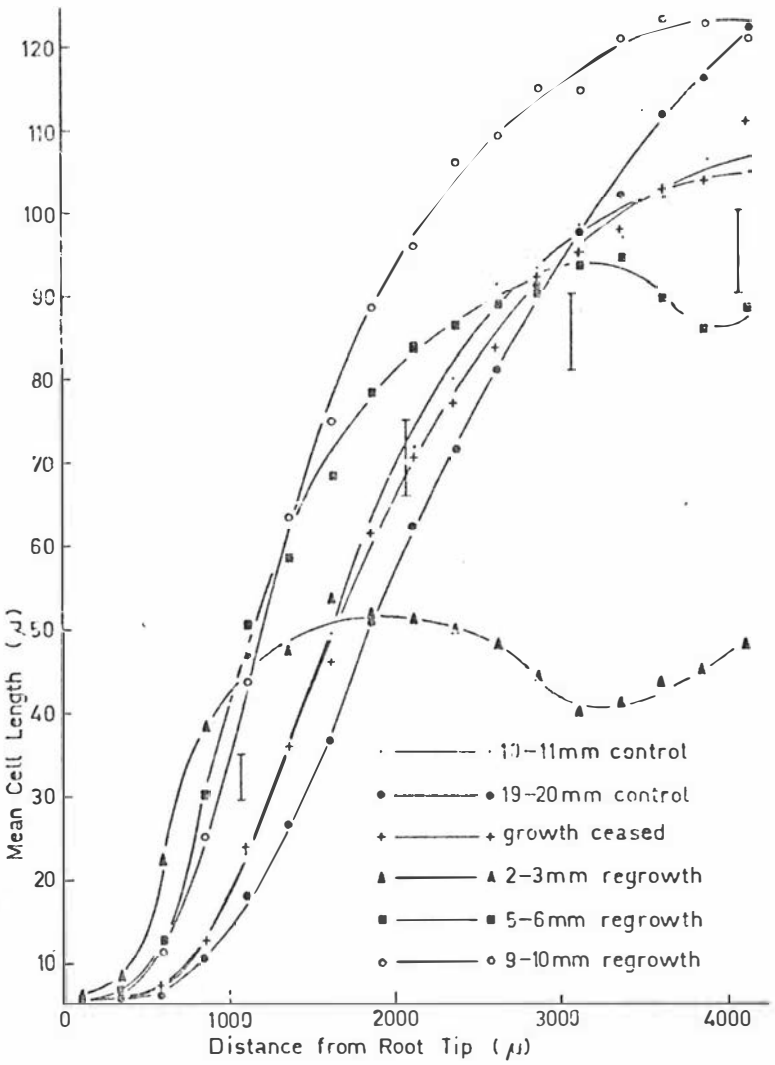


Fig. 25 – **Cell length pattern in the cortex of roots growing at a normal rate and after elongation had been affected by defoliating the plants to 2.5 cm. Vertical lines indicate twice the standard error.**

and little would reach the cells nearer the root cap. This situation arises because the phloem, in which translocation occurs, does not differentiate until approximately the position where the cells attain their final length. Beyond this point, movement must be by diffusion from cell to cell, there being few intercellular spaces through which movement could occur.

Experiment 2. Nature of the Cell Length Depression.

The depression in cell length when elongation recommenced following defoliation may be an effect of some change during the time when no growth occurred or may be part of the pattern of the resumption of growth. In an attempt to elucidate this point, plants in a further five containers were defoliated to 5 cm above the base. From previous experiments this level of defoliation was known to result in cessation of elongation in some roots but not others within a plant. The position of all primary nodal root apices were marked 24 hours after defoliation. Those roots which ceased to elongate were distinguished from those in which elongation was continuous. After a further five days by which time all roots had recommenced growth and most had elongated by 10 mm or more, the length of root which had grown since 24 hours after defoliation was measured for three roots which had ceased elongating and three in which elongation was continuous in each container. From Appendix 8 Fig. 1 and text Fig. 25 it can be seen that cell length reaches a maximum approximately 4 mm from the apex. Therefore the cells which showed a decrease in length following defoliation would at the time root elongation ceased be less than 4 mm from the apex. The glass was then removed and the required roots excised 4 mm behind the position of the apex on the day the positions were marked (24 hours after defoliation). For two of each group of three roots excised, the basal 5 mm was embedded and sectioned and the cortical cell lengths measured. The third root of each group was retained against the possibility of loss or damage to one of the other two during processing.

In all roots of both classes (growth ceased and growth continuous) there was a zone of reduced mature cell length. The position of this zone varied from root to root as did the minimum cell length. The minimum length was 20.1 $\pm$ 6.9 μ for the roots in which elongation ceased and 42.6 $\pm$ 6.5 μ for roots in which elongation was

continuous. Fig. 26 shows the cell length patterns of typical roots. The position of the root apex with respect to the data is indicated in the figure.

The variation in the position of minimum cell length relative to the point at which the root was excised is probably due to errors in marking the position of the root apex and in measuring the subsequent growth. Because of the apparent variation in the position of minimum cell length it was not possible to determine precisely at what stage in development cells were affected. The mean was approximately 3000 μ from the reference point for roots which had ceased growth and slightly more than this for roots in which growth was continuous. This would place these cells approximately 1000 μ from the apex when elongation ceased. In each group of plants, cell length throughout the 5 mm length examined was below the mature length of the roots of plants which had not been defoliated (Fig.25 and Appendix 8 Fig. 1). This difference may however have been caused by differences in environment.

The permanent depression in cell length in cells which would have been elongating when the plant was defoliated points to the occurrence of some irreversible reaction to the cessation of elongation which is presumed to have been brought about by the depletion of some factor. That this depression of cell length occurs to a lesser extent in roots whose elongation was reduced but not stopped indicates a differential effect on various stages of growth or else the presence of more than one factor.

That the cell length pattern in the apices of roots that had ceased to elongate was similar to that of normal elongating roots indicates that the mechanism must be a fundamental one. That no cells in the dividing zone were observed in a state of cell division in contrast to the frequent cell divisions in the elongating roots suggests that there was some differential effect according to the stage in the cell division cycle. The greater cell length close to the root apex in roots which had resumed growth compared with those which had ceased (Fig. 25) signifies some change from the pre-defoliation condition.

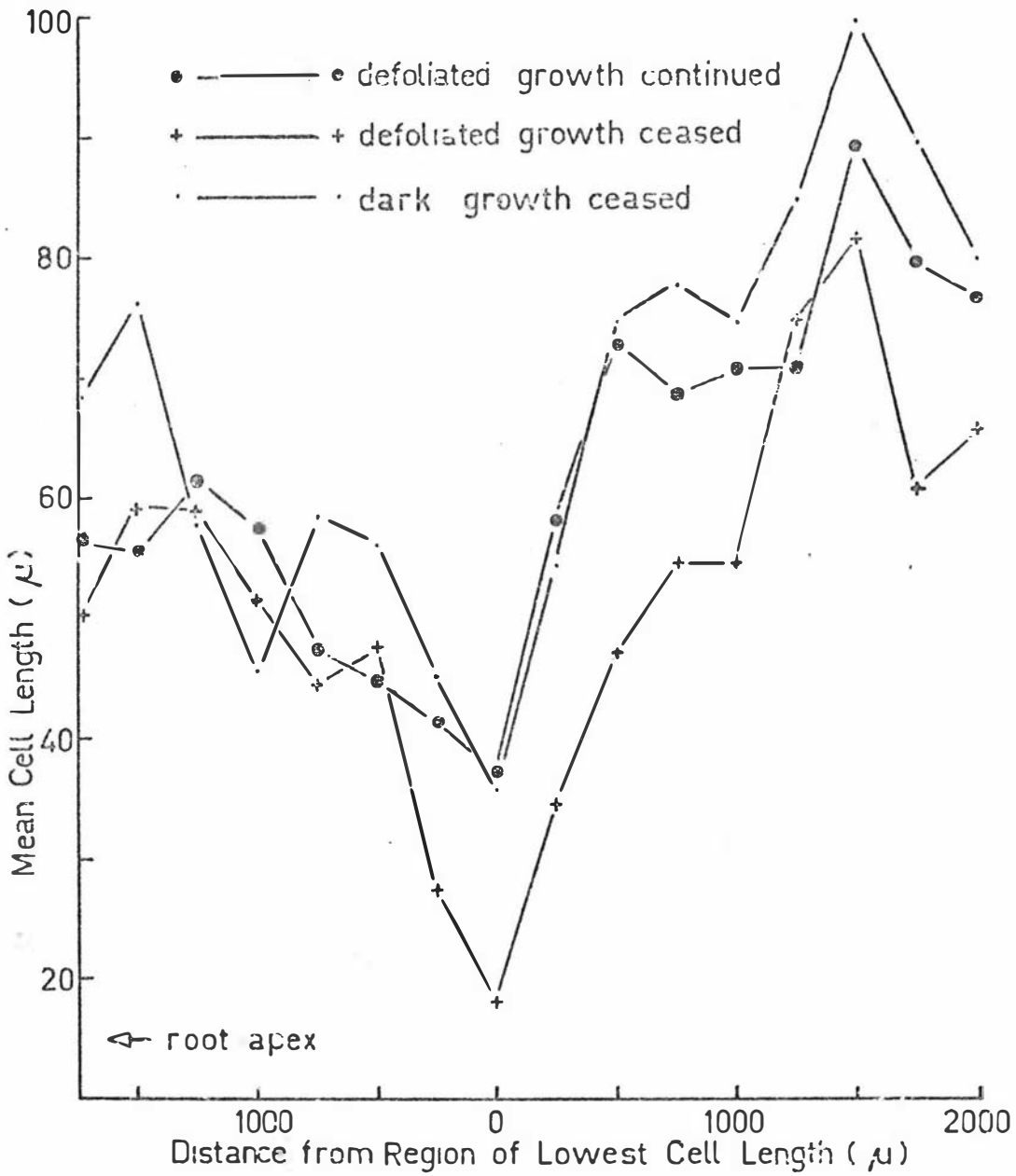


Fig. 26 – **Typical mature cortical cell length patterns in the region of roots affected by defoliation and dark.**

The sharp depression noted in most roots with a minimum cell length of 13 μ in two roots which ceased elongating may be a separate feature from the generally depressed cell length. Cell length does not increase with distance from the minimum in both directions but rather rises steeply within 1000 μ to a steady but still depressed level on the side nearest the apex. The final length of only 13 μ in two roots indicates that in these roots the cells affected would have been 1000 μ or less from the root apex. This corresponds well with the estimate from the position of maximum depression relative to the position at which the roots were excised. The most reasonable explanation would appear to be that the cells affected were all at about the same stage of development but that the severity of the inhibition differed from one root to another. This postulated difference in severity is consistent with the observation in earlier experiments that some roots of defoliated plants took longer than others to recover from defoliation induced stoppage and that some continued to elongate.

9.3 CELL LENGTH DEPRESSION IN ROOTS OF DARK-TREATED PLANTS

Roots of plants kept in the dark until elongation ceased were also examined for permanent depression of cell length. The procedure was the same as in the case of defoliation. This experiment was carried out in the growth room.

A pattern of cell length depression similar to that in defoliated plant roots occurred with a minimum cell length of 44.0 ± 4.7 μ. A typical result is presented in Fig. 26 with those of the two roots from defoliated plants.

That the cell length pattern in roots that had ceased elongating in the dark was similar to that in roots that continued to elongate following defoliation rather than the pattern in roots that had ceased elongating points to the mechanism responsible differing from that which causes the stoppage of root elongation.

9.4 DISCUSSION

The factor postulated as being responsible for the depression of cell length may have been one of the complex discussed by Brown

(1959, 1963). From observations on enzyme activity aooompanying cell expansion and differentiation, Brown advanced the hypothesis that during these phases of growth an increasing proportion of the cellular protein is enzymatio protein and that a succession of protein states are sequentially established. Suoh a succession implies a succession of RNA complexes (Street, 1966). Such RNA changes were found in Pisum (Heyes, 1960, 1963). Street suggests that on this assumption, interference with RNA synthesis could either prevent expansion and bring about differentiation or prolong expansion by blocking transformation to the state which forecloses expansion and initiates differentiation. Yeoman (1962) in Pisum and Zea increased elongation by treating with a low concentration of ribonuclease. Woodstook and Brown (1963) achieved a similar result with 2-thiouraoil in Vicia and Zea.

Burstrom's studies of excised roots (Burstrom, 1954) led him to advance the hypothesis that cell expansion takes place in two phases, one promoted by auxin and the other inhibited by it. An auxin imbalance in either of these two phases might be expected to have a widespread effect on cell length such as the depression over several thousand /u in the mature root. More recently Morre and Eisinger (1969) and Ray and Abdul-Baki (1969) have shown the relatinnship between auxin, oell wall synthesis and oell elongation.

Woodstock and Skoog (1962) suggested that in Zea the amount of RNA formed in the oell while in the meristematic region (region of cell division) determines the amount of elongation and hence the final size.

Several authors (Jaoobs, 1947; Denne, 1960; Evans, 1965; Bucknall and Sutcliffe, 1965) have demonstrated that in an organ with a meristem in which growth is polarised as is the case in roots, there are different patterns of cell division and elongation in different tissues. The results shown in Fig. 25 and Appendix 8 Fig. 1 are measurements for one particular tissue, the oortex, and the pattern would be different in other tissues. The oell division and elongation patterns in the various tissues are however closely related. In any such organ, the product of the longitudinal components of cell division and expansion must have the same value for all tissues. For example, if the final cell length in one tissue is only half that

in a second tissue then the number of cell divisions (i.e.the number of cells) in a longitudinal row must be double that of the second tissue. If this relationship did not hold then the tissues would be torn apart as described for stem development in some monocotyledons (Miltenyi, 1931; Golub and Wetmore, 1948; Kaufman, 1959; Evans, 1965). It was observed that the zone of short cells in the corter had an adjacent counterpart in other tissues of the roots. An additional factor supporting the contention that all tissues were affected in a similar manner was the collapse of all tissues in the region of cell elongation in roots whose elongation had ceased. This collapse occurred during fixation and could be due either to a reduction in osmotic pressure of the cell contents or to the reduced strength of the walls. The fixative used is a standard one for plant tissue so the phenomenon cannot be regarded as being caused by unusual conditions in the preparation of the roots for examination.

CHAPTER 10

DISCUSSION

Some time was spent at the start of this study on the experiments listed in the appendices referred to in the methods and materials chapter. This work which was a necessary preliminary to the main body of the thesis is also seen to be useful as the groundwork of a wider study of root growth of New Zealand pasture plants. In particular, the technique developed for measuring root length and number of apices is likely to be a useful tool for investigating the effects of insects such as grass grub which attack the roots, the drought resistance and nutrient requirements of the various species and competition between species. The usefulness of the glass fronted containers for examining the effects of defoliation have already been indicated in Chapter 3. The rapidity with which root elongation responds to treatments applied to the shoot of the plant points to the value of such containers in the testing of other applied treatments on root growth. In the agricultural situation the treatments which could be tested in this way, besides defoliation, are soil moisture, soil and air temperature, soil aeration and nutrient concentration. The demonstration of the effect of seed size on root length in young plants indicates that selection for large seed may be worth while as a means of improving seedling survival which is an important aspect of pasture establishment.

In Chapter 3 the effects of defoliation and shading on root elongation were examined. The results are broadly comparable with those reported by other authors. The level of defoliation at which root elongation ceases and root death occurs is above the minimum level to which sheep can graze so that under farm conditions, hard grazing could lead to root death. The effect of a reduction of root elongation and any possible related reduction in nutrient and water uptake on subsequent pasture growth is unknown but may be an important consideration in the recovery of pastures following defoliation. The effect of levels of shading comparable with those which may occur within pastures do not appear likely to cause cessation of root elongation or root death over short periods of time. There may

be such an effect if these treatments were maintained for longer than 3 weeks (the longest period studied).

Defoliation if it occurs repeatedly over a sufficient time must result in the depletion of the supply of all substances necessary for root elongation which are synthesised in the shoot apart from those synthesised specifically in the shoot apices which in the vegetative state would be below defoliation level. This situation approaches that of excised root culture in which nutritional requirements are seen to be complex and variable. The later chapters in this thesis have been concerned rather with investigating the deficiencies which cause root elongation to fall or cease during the first three days after defoliation. The effects of dark have been examined in the same way.

Importance of an energy source for continued root growth

It is clear here as shown by other authors that the level of soluble carbohydrates in the roots is depleted following defoliation or placing in the dark. The differences in the actual levels recorded have been attributed to differences in the growth habit of the species studied and in growing conditions. In particular it appears that soil nutrient levels have a major effect (Nowakowski, 1969). In addition, the authors have not all used the same analytical technique. The relationship between root growth and the level of soluble carbohydrates in the roots is not well understood however as only Davidson and Milthorpe (1966) have recorded both items in the same experiment. They found that the level fell from 1.7% to 1.0% at about the time root elongation ceased. It is unfortunate that the experimental technique used in the present study limited the duration of experiments as longer experiments may have produced evidence on the minimum levels at which elongation could take place. In the experiments conducted the lowest level recorded at which elongation was not believed to have been inhibited was 0.9% (Table 4). Of course not all of the soluble carbohydrate as analysed may constitute a reserve which can be mobilised for root growth.

From the response of root elongation of defoliated and dark-treated plants to applied sugar it is evident that lack of soluble

carbohydrate is the main cause of the reduction. This is supported by the demonstration that recovery of root elongation was slower following three days dark than following one day dark, indicating that the factor the depletion of which limits elongation is further depleted through respiration losses.

The demonstration that the level of soluble carbohydrates in the roots at the time most root growth ceased following defoliation or placing in the dark was higher than in other experiments where elongation was continuing at a high level points to some mechanism affecting the utilisation of carbohydrates within the roots. That under these circumstances root elongation responds to the application of both glucose and sucrose indicates that the form of the carbohydrate in the roots is unlikely to be the explanation. Rather it appears that the transport of soluble carbohydrates to the root apex is limiting. The possibility of this occurring is supported by the differences in levels between the shoot and the root (Table 8). There it can be seen that the levels in the shoot are higher even after a 12 hour dark period indicating that the plants control the distribution of these materials.

The analytical technique used for determining soluble carbohydrates was not sensitive enough to allow analysis of the root apices to determine if there was a depletion there relative to the rest of the root system as is indicated by the response to applied sugar. This response to applied sugar points to the presence of a translocation factor the nature of which has not been determined. Benzyladenine has been shown to influence the translocation of assimilates towards the site of application (Quinlan, & Weaver 1969) however in the present study, this substance only gave a response in the presence of applied sugar. Plaut and Reinhold (1969) suggested that light induced loading of the phloem occurs. This is considered unlikely to be the case in L. perenne since root elongation continued at a steady rate throughout the dark period.

This postulated translocation factor may be important in the survival of plants subject to grazing. Where grazing is severe a major proportion of the photosynthetic tissue of the plant may be removed. The root system must draw on storage products for respiration

energy until the photosynthetic tissue regrows and recommences export of photosynthate to the roots. Under these circumstances a mechanism aimed at reducing the depletion of reserves through the formation of new root tissue would aid in the survival of the plants by ensuring that sufficient reserves are maintained for respiration and the growth of new photosynthetic tissue.

Requirement for some factor other than an energy source

In no experiment was the application of sugar able to maintain the control level of root elongation in plants which had been defoliated or placed in the dark. In general two-thirds of the control elongation was achieved in plants in which elongation would otherwise have ceased. The question of sugar uptake from the root medium being adequate to achieve optimum elongation has been answered by demonstrating that the level of soluble carbohydrates in the roots of plants supplied with 2% sucrose was higher than in the control plants where elongation was at a higher level. As with soluble carbohydrates occurring naturally in the roots however, the distribution within the roots is uncertain. It seems likely however that uptake would be greater at and near the apex than elsewhere on the basis of findings regarding the uptake of water and inorganic nutrients. There was no evidence of an osmotic effect of 2% sucrose on elongation. However as already mentioned, sucrose did appear to have an effect on the exudation of water from the cut ends of leaves in the defoliation experiments.

The internal concentration of soluble carbohydrates in defoliated and dark-treated plants supplied with 2% sucrose was higher than in control plants so the cellular osmotic pressure was not likely to have been limiting elongation through reducing water uptake from the external solution. Even so, some interaction between osmotic pressure and auxin action on cell expansion of the type mentioned by Burstrom (1954) and Cleland (1959) may have occurred.

If some additional factor apart from carbohydrate and the associated translocation factor is required, this factor could be hormonal even though experiments with representatives of the three major classes of plant growth substances failed to produce a major

response. (Benzyladenine produced a response in the presence of applied sucrose but this was small by comparison with the depression which occurred in these particular experiments.) The scope of these experiments was limited and the requirements of roots for growth substances is seen to be complex.

The additional factor if one exists would appear to be produced generally in the shoot, not only in the shoot meristems, as is shown by the similar root elongation levels in plants defoliated to 0.6 cm and where the shoot was completely removed. It is synthesised in light but it appears doubtful if adequate quantities are produced in low light. In some experiments where sucrose was applied to defoliated or dark-treated plants, root elongation while lower than in the control plants on day 1 fell further on days 2 and 3 indicating further depletion. In other experiments, although the initial reduction in elongation compared with the untreated plants was similar, no further reduction occurred.

The permanent reduction in cell length of roots affected by defoliation or dark implies some specific response in cells at a certain stage of development and although this response may be activated by a change in soluble carbohydrate levels of the cells it is likely to be caused by some other factor which is operative only in cells at a particular stage of development. It is not possible to determine if this response occurs as the roots cease elongating or afterwards. If it occurs as elongation ceases it could indicate the nature of the additional factor postulated.

The direction of supply of substances reaching the root apex may have an effect on elongation. In a plant, the organic substances are translocated from the shoot to the root apex. In the experimental treatments, the materials under test were supplied to the root medium. Wilkins and Scott (1968), Kirk and Jacobs (1968), Hertel, Evans and Leopold (1969) showed that in a number of species auxin moved towards the root apex more readily than towards the base. Raggio and Raggio (1956) devised a technique for testing the effects of substances applied to the cut ends of excised roots as compared to including the substances in the medium surrounding the root. Torrey (1963) using this technique showed that auxin gave responses at much

higher concentrations in the medium applied to the base of the root than in the medium surrounding the root. There were also differences with sugar but elongation was not increased by application to the base of the root compared with the optimum concentration provided in the medium surrounding the root. In the present study an attempt was made to supply substances through the out leaves but no satisfactory means was established of sealing around the shoot bases and thus avoiding the loss of solution. Considering the length of root through which sugar could be absorbed and then translocated to the root apex (average of approximately 15 cm) the question of movement of sucrose and growth substances applied does not appear to be important since the material could be absorbed and translocated downwards to the root apex. The length of root used by Raggio and Raggio (loc.cit.) and by Torrey (loc.cit) was probably much shorter. This leads back to the possibility of a translocation factor as mentioned earlier.

APPENDIX 1

MEASUREMENT OF TOTAL ROOT LENGTH AND APEX NUMBERS

Lengths of root samples were measured by a modification of Newman's (1966a) technique based on Buffon's needle problem (Kendall and Moran, 1963).

If a needle of length L is placed at random on a plane on which are ruled parallel straight lines unit distance apart, the probability of the needle intersecting the lines is:-

$$\frac{2L}{\pi} \quad \text{for} \quad L < 1$$

For M throws of the needle or M needles each of length L the expected number of hits scored is:-

$$\frac{2LM}{\pi}$$

A root of length R although not normally straight, can be divided into M' pieces of length L', each short enough to be considered straight. The number of hits, N, in this case would be:-

$$\frac{2L'M'}{\pi} \quad \text{or} \quad \frac{2R}{\pi}$$

Therefore $R = \frac{\pi N}{2}$ is an estimate of the root length.

This is a special case of the formula $R = \frac{\pi NA}{2H}$ of Newman (loc.cit.) where A is the area over which the root mass is spread and H the total length of straight line used. Since the parallel lines are by definition unit distance apart and so divide the plane into strips of unit width, each unit area of each strip is associated with a unit length of line, i.e. $\frac{A}{H} = 1$.

The use of a series of parallel straight lines means that the area over which the sample is spread does not have to be defined thus simplifying the measuring and eliminating the possibility of error due to non-random arrangement of roots near the edge of the area mentioned by Newman (loc.cit).

The root sample to be measured was spread out in 5-10 ml of water on a 30cm x 40cm sheet of window glass so that there was little

or no chance of one root overlying another. Two sets of parallel straight lines 1 cm apart at right angles were scratched in black paint on a second 30cm x 40cm sheet of glass before the paint had completely hardened. The second sheet of glass was placed paint downwards on top of the root sample and the whole carefully turned over so that the clear glass lay uppermost and placed on four 9cm high wooden blocks over a sheet of white paper. When illuminated obliquely the roots showed up white against a black background over a series of fine white lines. With this technique even the finest grass roots showed up clearly although care had to be exercised to keep the sample and glass free of dust. The amount of water used to disperse the sample depended on the thickness of the thickest roots, the aim being to exclude air bubbles from the root sample without having water flowing out from between the sheets of glass and possibly carrying root pieces with it. With each experiment a few samples were counted on both sets of parallel straight lines to guard against error caused by the roots not being randomly orientated.

The use of the technique assumes that the roots lie in a plane parallel to the plane of the parallel straight lines and no account is taken of any component of root length perpendicular to this plane. Sandwiching the roots between two sheets of glass serves to flatten them but since in any one sample there was variation in root diameter there would be a component of root length perpendicular to the plane of the parallel lines, so the technique probably underestimates the actual length. L. perenne roots were too fine to measure accurately by the direct method as a check of the technique. However a check was made using two tree root samples (Table 1). In neither case did the mean estimated value differ significantly from the direct measurement.

Because measuring the whole root system of L. perenne plants would have taken too much time, three small samples were measured on each plant. It was noticed that the proportion of thick roots appeared to be much higher near the base of the shoot than elsewhere in the root mass and the proportion of fine roots appeared to be greatest at the periphery. Therefore, in order to obtain representative samples, the root mass was formed into a rope which was cut in

Appendix 1 Table 1 - Length (cm) of two tree root samples by direct measurement and by line intersection (means of 10 estimates)

	Line intersection	Direct Measurement
Sample 1	48.9 ± 0.94	50.1
Sample 2	62.1 ± 1.71	60.9

to approximately 5 mm pieces. The cut roots were dispersed in 200-800 ml of water, depending on the size of the root mass and samples of approximately 200cm total length removed for measurement. After several samples had been measured the size could be readily judged by eye. After measuring, samples were dried at 100°C overnight and weighed. To check that cutting the roots in to small pieces did not bias the results, three samples of L. perenne root were measured whole and again after cutting in to approximately 5mm lengths (Table 2). In no case did the difference between the means of 10 counts of the uncut sample and the mean of 10 counts when cut into 5mm lengths reach significance level.

The lateral roots were very fine (approximately 0.1mm diameter) and the apices could not be readily distinguished from out ends. The number of apices was therefore estimated by counting the number of branches in the sample spread out on the unpainted glass sheet before the painted glass was applied and the number of roots arising from the base of the plant. The relationship is as follows:- A single unbranched root has a single apex. If it produces one lateral (i.e. has one branch) it has two apices. If the lateral itself produces a lateral the whole root has two branches and three apices. If the whole root has B branches it has B + 1 apices. If there are R roots with a grand total of N branches the total number of apices is R + N. It is probable that in cutting the roots into small pieces some laterals would have been removed cleanly so that estimation of root apices by this method gave a low result but the error is considered to be small.

Appendix 1 Table 2 - Comparisons of lengths (cm) of three ryegrass root samples as whole root and cut into pieces (means of 10 estimates)

	Sample 1	Sample 2	Sample 3
Whole Root	110.1	90.9	100.9
Pieces	110.2	92.1	99.7
Av. S.E. ±	1.6	1.1	1.6
D.05	4.8	3.2	4.6

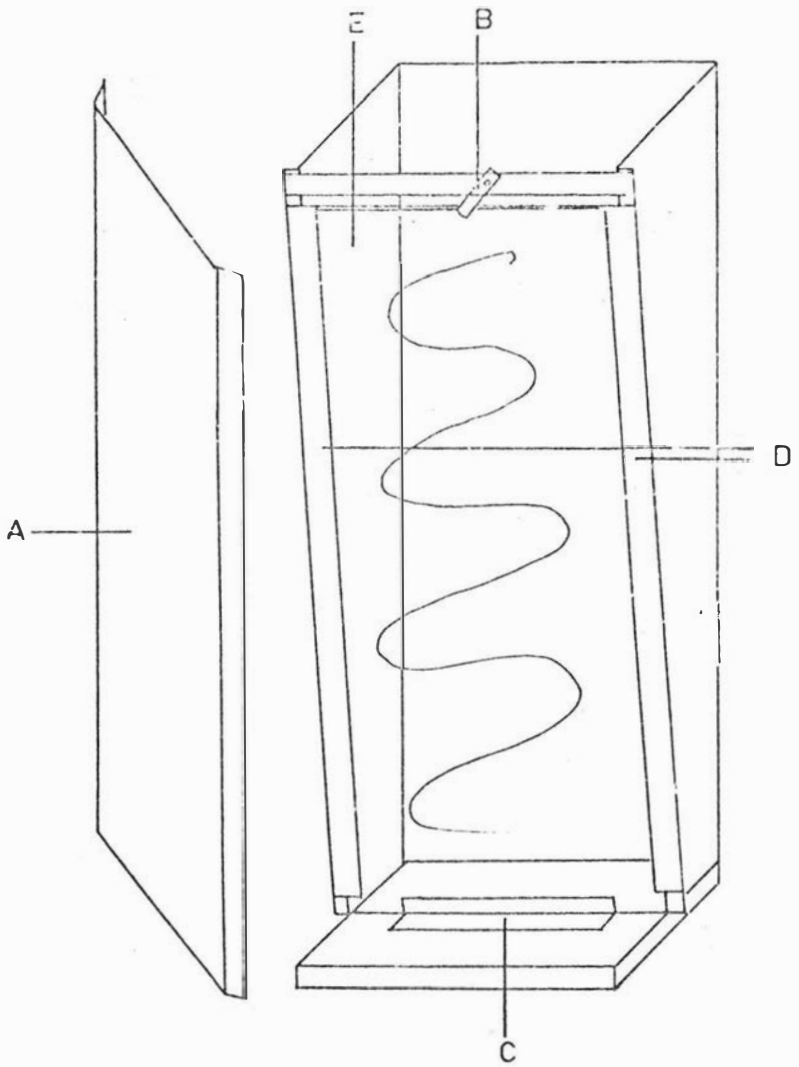
APPENDIX 2

GLASS FRONTED CONTAINERS

A number of authors have used containers with a glass side to observe root growth in a number of plants, (e.g. Rogers, 1939b; Crider, 1955; Salim et al, 1965; Butler et al, 1959; Lavin 1961; Beard and Daniels, 1965; Asher and Ozanne, 1966). The same principle has been used in the field by fitting glass panels into the side of a trench (Rogers, 1939a; Head, 1966; Garwood, 1967). Newman (1966b) used clear plastic tubing in the same manner. The technique enables individual roots to be examined without disturbance. Only a small proportion of the total root system can be observed and in order to increase this proportion some of the authors mentioned above had the glass face of the containers sloping in order to intercept some of the roots growing vertically downwards. Of the above authors only Lavin (loc.cit.) considered the possible effect of growth against the glass surface. He found the growth rate against the glass to be comparable with that in the field. Asher and Ozanne (loc.cit.) were careful to use a standard technique in filling their containers in order to obtain uniform conditions since root elongation is affected by the resistance of the root medium. Lavin (loc.cit.) also mentions this aspect briefly.

Several authors have also emphasised the necessity for excluding light from the glass face other than when observations or measurements are being made. Lengthy exposures to daylight severely checked root growth in apples although the short exposure necessary for recording root growth did not cause a significant check. (Rogers, 1939b). Goodwin and Avers (1956) found that in Phleum pratense roots would tolerate continuous, relatively high levels of illumination without exhibiting inhibitions or marked phototrophic responses.

The containers constructed (Fig. 1) were 15 cm wide, 15 cm deep and 40 cm high. They were made of galvanised iron coated inside with bituminous paint and outside with white paint to reflect heat. Filled with wet sand they weighed approximately 11 kg so could be conveniently moved about in the glasshouse by one person. Mobility was



Appendix 2 Fig. 1 - Glass fronted containers.

A, light proof cover;

B, clip for retaining cover;

C, bracket for retaining cover;

D, removable metal strips for holding glass in place;

E, glass.

considered important because of the possible position effects in the glasshouse. Subsequently, movement of the containers was found to be impractical as the sand tended to slump and the glass to crack. This could not be tolerated since the roots being measured were disturbed. Instead the containers were set up in blocks, each block consisting of one container for each treatment. Containers were then allocated to treatments at random and adjustments made within blocks, where necessary, to give uniform initial elongation rates for treatments. The glass side sloped at 12 degrees to the vertical. This angle was the greatest that could be achieved without affecting the strength and stability of the containers. Twelve degrees is less than the angle used by most of the authors quoted but was considered justified because of observations that the nodal roots of L. perenne grew at all angles from vertical to near horizontal so adequate numbers would grow against the glass. It was considered too that in spite of the finding of Lavin (1961) that growth at an angle to the vertical against the glass face did not affect root elongation, the angle should be the least that was consistent with satisfactory observation. The glass is held in place by metal clips which can be readily removed allowing direct access to the roots growing against the glass. In early tests with these containers it was found that the nodal roots of L. perenne were not strongly geotrophic and even with the glass at up to 30 degrees to the vertical would not grow against the glass for any distance so a piece of expanded aluminium sheet with holes 9mm x 3mm, coated with bituminous paint, was placed against the inner side of the glass providing vertical channels down which the roots could grow. Even with the expanded aluminium sheet in place the roots frequently grew back into the sand. This may be caused by nutational movements (Fisher, 1964; Head, 1965). When transplanting into the containers, a sheet of stiff paper was laid against the inside of the aluminium sheet and the roots of three plants were spread out against the paper. Damp sand was poured in and firmed by hand. The paper was then carefully removed leaving the roots of the plants spread out against the aluminium sheet. Any sand which lodged between the aluminium sheet and the glass was removed by tipping the container on its back, removing the glass, and brushing.

Daily elongation of primary nodal roots was measured by marking on the glass the position of the root tip with a fibre-tip pen and

subsequently measuring the distance between the mark and the new position of the root tip. In a preliminary experiment the roots in the top 5 cm were observed to grow more slowly and roots in the bottom 10 cm more rapidly than others against the glass so no roots within these regions were measured. Table 1 shows the relationship between mean elongation in 24 hours and the distance of the root tip from the surface of the sand. It can be seen that there is a steady increase in elongation rate with depth apart from the first 5 cm and bottom 10 cm, however it has not been considered practical to select within this region because of the relatively small number of nodal roots of a particular length against the glass at any time. Because of the way the containers were planted a majority of roots appeared against the upper half of the glass at first and grew downwards during the experiment. The increase in elongation with increasing root length would therefore tend to magnify the differences between experimental treatments since the treatment which most inhibited root elongation would result in a lower mean root length. However this effect is small compared with treatment differences in the various experiments.

No attempt was made to determine the possible effect of growth against the glass face but it was noted that roots growing against the face on no occasion appeared to reach the bottom of the container later than those growing through the sand and then out under the bottom of the glass and in most cases they reached there earlier. This is probably due to the lack of resistance usually afforded to the roots by the root medium. To this extent the roots being observed were growing in an artificial situation but since all the measurements of root elongation made were comparative this only affects the results in so far as they are related to conditions in the field.

The effect of indirect sunlight and the removal of the glass were examined (Table 2). The experiment consisted of six replicates. The control treatment containers had the light cover removed only for the time required to measure elongation and mark the new position of the root apices. The light treatment containers had the glass exposed to indirect sunlight all day. In the third group the glass was removed by tipping the containers on their backs and removing the retaining clips. The roots were left exposed for approximately half a

Appendix 2 Table 1 - Elongation of roots of different lengths.

Root Length (cm)	Number of roots measured	Elongation in 24 hrs. (mm)	S.E. ±
0 - 1	6	5.8	1.3
1 - 2	40	8.1	0.6
2 - 3	30	8.5	0.6
3 - 4	48	8.1	0.6
4 - 5	50	9.1	0.6
5 - 6	28	9.9	0.9
6 - 7	29	10.1	0.5
7 - 8	22	10.7	1.1
8 - 10	35	9.1	0.7
10 - 12	33	9.0	0.7
12 - 14	30	9.9	0.7
14 - 16	34	9.6	0.6
16 - 18	26	10.2	0.9
18 - 20	26	10.7	0.8
20 - 22	23	10.7	1.0
22 - 24	18	11.6	1.1
24 - 26	14	13.7	1.2
26 - 28	17	12.9	0.9
28 - 30	18	13.5	0.8
30 - 32	12	14.3	1.8
32 - 34	3	19.0	2.6

Appendix 2 Table 2 - Effect of exposure to indirect sunlight and removal of glass on root elongation (mm per day)

Date	Light	Removal of glass	Control	S.E. ±	D.05
3.10.67	15.4	15.4	16.9	0.43	1.28
4.10.67	15.3	15.8	16.2	0.49	1.46
5.10.67	13.3	14.8	15.2	0.68	2.00
6.10.67	12.8	14.3	15.8	0.61	1.81
29.5.68	15.8	16.7	15.6	0.35	1.10
30.5.68	13.6	13.7	13.4	0.59	1.86
31.5.68	12.9	14.7	13.3	0.68	2.14
5.6.68	9.7	11.0	11.3	0.51	1.61
6.6.68	8.9	10.5	9.6	0.27	0.85
7.6.68	11.3	10.9	11.3	0.45	1.42
11.6.68	8.8	9.2	8.4	0.58	1.83
12.6.68	9.1	9.8	9.3	0.83	2.62
13.6.68	8.6	9.2	8.7	0.89	2.80
14.6.68	8.0	7.8	7.7	0.88	2.77
18.6.68	11.6	10.5	9.8	1.28	4.04
19.6.68	10.3	9.1	8.9	0.93	2.93

minute, this being the time estimated to be required to remove several root apices for microscopic examination. The main effect being sought was the disturbance of the root system rather than the drying effect of exposure. On two out of sixteen days the exposure to light caused a significant reduction in elongation and on one day out of sixteen removal of the glass caused a significant reduction. It is not possible to decide if the brief exposure to light necessary in order to record and mark the containers had an effect but this effect can be considered small. Rogers (1939b) was able to measure the effect of a brief daily exposure to indirect sunlight by measuring other plants once per week. He found that this daily exposure caused a small reduction in growth compared with once per week exposure. Such an experiment was not possible with L. perenne as the roots seldom grew against the glass for more than 4-5 days and frequently for a much shorter time.

APPENDIX 3

EFFECT OF NUTRIENT CONCENTRATION

Although nutrient solutions of the type used in this study have been extensively used to grow a wide variety of plants, an investigation of the effects of nutrient concentration was undertaken in order to ensure that this was not going to be a limiting factor in plant growth and to determine if the amount used could be safely reduced by using water at the early stages of growth and for flushing the containers periodically.

Methods:

The experiment consisted of 10 replicates. The nutrient concentrations used were 0.05, 0.25, 1.0, and 4.0 times the standard solution. Seed was germinated in petri dishes and planted singly into sand in 10 cm x 36 cm unglazed pipes of the type used in field drains, painted outside with aluminised bituminous paint to reflect heat and reduce water loss. Pipes were spaced 10 cm apart. Plants were supplied each day with an excess of the appropriate nutrient solution and harvested 8 weeks after sowing. No mutual shading was considered to have occurred by this time. Plants were washed free of sand. Shoot and root dry weights and the number of nodal roots were measured on all plants. Root lengths and the number of branches were measured on three sub-samples per plant. From the primary data total root lengths, number of root apices, length per unit weight of root, root apices per unit weight and per unit length of root, and shoot/root ratios were calculated.

Results:

The results are presented in Table 1. There were no differences in apices per unit length of root between nutrient concentrations. The differences between the 0.05 and 0.25 levels were significant at the 5 per cent level in all other characters. In addition there were significant differences between the 0.25 and 1.0 levels and between the 1.0 and 4.0 levels in several characters.

Discussion:

As judged by dry matter production the standard nutrient

Appendix 3 Table 1 - Effect of nutrient concentration on shoot weight, shoot/root ratio and root details (means of 10 plants).

	Nutrient Concentration				S.E. ±	D.05
	0.05	0.25	1.0	4.0		
Shoot wt.(g)	0.44	2.41	3.54	2.43	0.23	0.68
Root wt.(g)	0.23	0.80	0.87	0.60	0.075	0.22
Shoot-Root Ratio	2.2	3.2	4.1	4.1	0.18	0.52
Root length (m)	160	360	360	200	46	134
No.of Apices	18,000	41,000	41,000	23,000	4,200	10,400
Length/ unit wt. (cm/mg)	70	46	40	33	3.0	8.7
Apices/ mg	79	51	46	38	4.2	12.1
Apices/ cm	1.14	1.12	1.16	1.16	0.041	0.12

solution was optimum for growth. At the lowest nutrient concentration, shoot weight was depressed more than root weight and hence shoot/root ratio was lowered but at the highest concentration, shoot and root weight were depressed to the same extent. The total root length and number of apices followed the same trend as root weight but the drop from 1.0 to 4.0 concentration was more marked. The length and number of apices per unit weight of root dropped with increasing nutrient concentration but the number of apices per unit length of root remained constant (i.e. the roots became thicker but the frequency of branching did not alter).

Various workers have reported increases in shoot/root ratio on increasing the nutrient supply to plants (see Weinmann, 1948; Troughton, 1957; 1962; Allsopp, 1965). Increased nutrient concentration usually produced thicker roots though there are exceptions, particularly in the case of single nutrients. May et al (1965) studied the components of root growth in young barley plants at three nutrient concentrations. They found that the lowest concentration gave the greatest length of roots. However the weight of roots was least at the lowest concentration. They considered that carbohydrate supply was not the cause of differences in total length of root since the weight of the first 10^3 cm of root in the highest nutrient concentration was almost twice that of the lowest concentration. May et al (1967), also working with barley, discounted carbohydrate as limiting extension growth pointing out that the primary root continues to elongate at a steady rate despite the development of competing secondary roots back nearer the source of supply. They do suggest however that the lower growth rate of the secondary roots may be a function of the competition of the primary root for carbohydrates. In plants grown in glass fronted containers the growth rate of the primary roots was observed to be higher than that of the laterals and growth on average increased with increasing length. Where a primary apex was removed, the laterals increased in diameter and rate of growth suggesting that carbohydrate may have been limiting either directly or indirectly.

May et al (1965) found that mean spacing between branches on the primary roots but not on the secondary roots was greater at low nutrient concentration. In the present study the primary and secondary roots were not analysed separately. From observation of the

root samples it would appear that a large proportion of the branches are on secondary roots so any differences between treatments in branching on the primary roots would be unlikely to markedly influence the overall figure.

The results of this experiment showed that plant growth and root morphology could be markedly altered by altering the nutrient concentration and that the safest procedure was to use standard nutrient solution throughout the growth of the plants.

APPENDIX 4

EFFECT OF TEMPERATURE

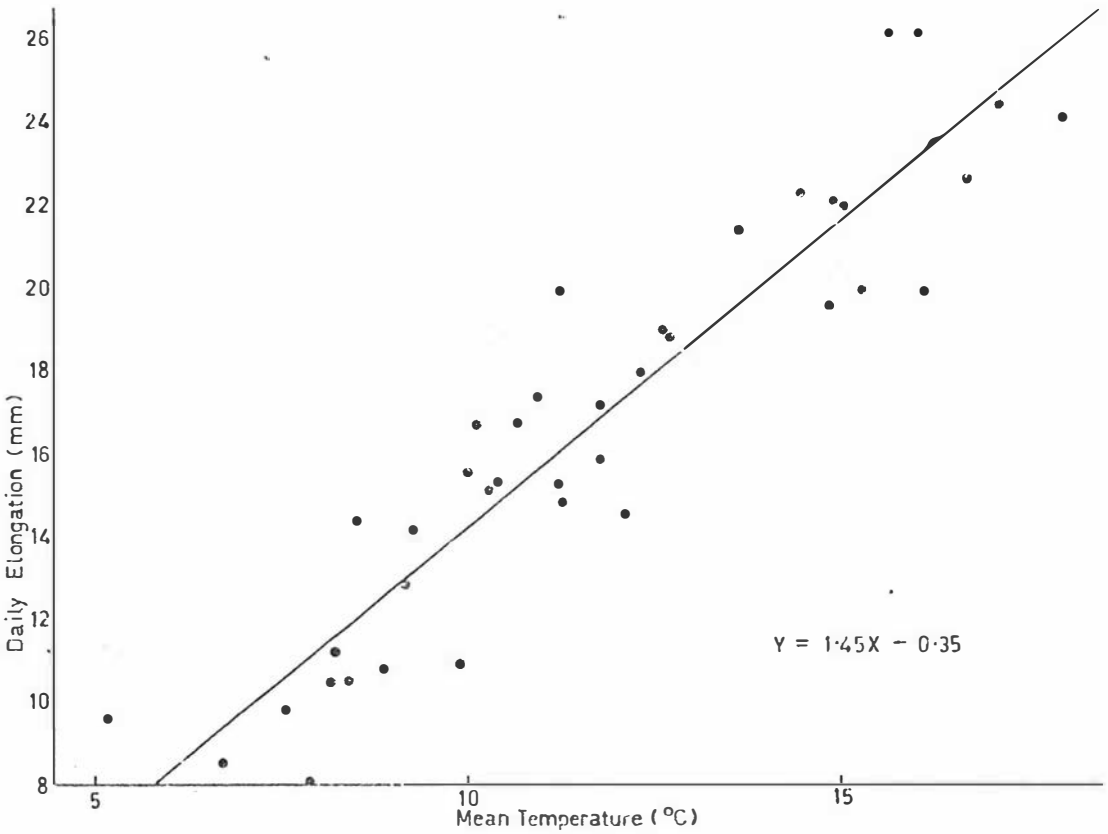
All experiments except those described in Chapter 7 were conducted in a glasshouse the temperature of which was controlled by a heater and exhaust fan set to operate at 10°C and 21°C respectively. However the control units were inadequate to keep the temperature within this range under all conditions so as the experiments were conducted throughout the year there was a general seasonal trend in temperature levels. The root elongation data presented (Fig. 1) are from control treatments of a series of experiments conducted between 4/3/69 and 15/10/69. Plants measured were all of approximately the same physiological age (40–60 tillers at the time the measurements were made). The temperature figures used were the means for the 24 hours up to 8 am on the day on which the elongation was measured (i.e. covering the same time period). They were obtained from a thermograph located on the bench next to the plants with the sensor shielded from direct sunlight. The straight line regression of temperature on elongation (i.e. of the form $y = a + bx$) was calculated from the formula

$$b = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

using mean values of x and y to obtain a. No more than 5 points were from a single experiment.

Discussion:

The data presented cover 8 months only, no figures for November - February being included. Experiments prior to March 1969 were conducted using aluminium stretch metal sheets against the inner face of the glass whereas from March 1969 onwards the stretch metal was absent so the roots being recorded were growing through sand rather than in an air space. Even though the effect of the aluminium sheets was considered to be small it was not deemed wise to include the results from any experiment where they were used. Lack of root growth figures for November - February is not considered serious for the comparison of



Appendix 4 Fig. 1 – Regression of temperature on root elongation for untreated plants over the period 4.3.69 – 15.10.69.

various experiments since the highest mean temperature recorded over this time was only two degrees above the highest figure included.

Over the range investigated root elongation shows a high positive correlation with temperature. This result is comparable with those of Rosenquist and Gates (1961) who obtained increases in elongation with increasing temperature up to at least 30°C in Agropyron inerme Scribn. and Smith, Festuca ovina L., Poa ampla Merr., and Dactylis glomerata L. In Agrostis palustris Huds. however, elongation was unaffected by temperature within the range 15 - 27°C (Beard and Daniels, 1965).

Temperature has also been shown to have an effect on the shoot/root ratio. Much of this work on grasses is reviewed by Troughton (1957) and Brouwer (1966). In general the shoot/root ratio increases with increasing temperature. The situation is complex however as shown by the conflicting results of Mitchell (1954) and Troughton (1961) in L. perenne.

Darrow (1939) and Stuckey (1942) working with temperate climate grasses noted that roots of plants growing at low temperatures were white and succulent while at higher temperatures they tended to be darker coloured and more finely branched. Stuckey related her findings to observations in the field, roots being coarse and white in winter and fine and brown in summer. Soper (1958) noted that in L. perenne a combination of low light and high temperature not only resulted in changes in root thickness and colour but that also under these conditions death and sloughing off of the outer tissues occurred. In the present series of experiments there were insufficient data to examine the effect of temperature on root appearance. The only feature observed was a noticeable difference between winter and summer in the rapidity with which browning of the root surface occurred.

APPENDIX 5

DEVELOPMENT OF YOUNG PLANTS

Methods:

Seed was germinated in petri dishes and planted out singly into clay pipes of sand (See Appendix 3). The experiment consisted of 10 replicates of 6 pipes which were spaced 10 cm apart within replicates so that no mutual shading was considered to have occurred by the end of the experiment and the replicates were 30 cm apart to facilitate harvesting. One plant from each block selected at random was harvested every 14 days from planting and washed free from sand. Shoot and root dry weights and the number of nodal roots were measured on all plants. Root lengths and numbers of branches were measured on three sub-samples per plant (See Appendix 1) except at the first harvest when because of the small size the whole root mass was measured. From the primary data total root lengths, number of root apices, length per unit weight of root, root apices per unit weight and per unit length of root, root length and number of apices per unit weight of shoot and shoot/root ratios were calculated. Standard errors were calculated for each harvest for shoot weight, root weight, root length and root apex numbers. For other characters the standard errors were obtained from analyses of variance of the six harvests.

Results:

In Table 1 are presented the shoot weights, root weights, root lengths and number of root apices for the six harvests. Note the length of root (1816m) at the final harvest within the 2.7l of sand in the pipe. At this stage a mat of roots was starting to form at the periphery. The ratio data are presented in Table 2. The shoot/root ratio showed an overall increase with time. The length per unit weight of root was high at first but then dropped with a recovery at 10 weeks. The number of root apices per unit weight of root showed a similar trend. Root length and number of apices per unit of shoot dry weight also shows this trend.

Discussion:

The high value for length per unit weight of root at 2 weeks represents mainly seminal root which has been described as finer than

Appendix 5 Table 1 - Shoot and root weights, root lengths and apex numbers of ryegrass plants harvested at two week intervals (means of 10 plants)

Harvest	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks	12 weeks
Shoot weight (g)	0.0046 ±0.0003	0.067 ± 0.003	0.854 ±0.109	3.83 ± 0.40	12.16 ± 0.51	21.66 ± 1.09
Root weight (g)	0.0015 ±0.0001	0.021 ± 0.001	0.253 ± 0.036	1.08 ± 0.10	3.13 ± 0.15	5.98 ± 0.48
Root length (cm)	56.6 ± 4.2	566 ± 23	7,590 ±1,200	32,430 ± 3,060	115,720 ± 10,340	181,570 ±15,070
Number of apices	70.3 ± 4.8	597 ±34	8,140 ±1,510	36,470 ± 3,750	163,020 ±14,880	262,780 ±21,080

Appendix 5 Table 2 - Root details and shoot/root ratios of ryegrass plants harvested at two week intervals (means of 10 plants)

Plant Age	Length per unit Root Dry Wt. (cm/mg)	Apices per mg Root Dry Wt.	Apices per cm Root Length	Length per unit Shoot Dry Wt. (cm/mg)	Apices per mg Shoot Dry Wt.	Shoot/ Root Ratio
2 weeks	37.8	47.2	1.26	12.3	15.3	3.1
4 weeks	27.8	27.7	1.01	8.5	9.0	3.2
6 weeks	30.3	32.2	1.06	8.9	9.5	3.6
8 weeks	30.3	33.7	1.12	8.5	9.5	3.5
10 weeks	36.8	52.0	1.43	9.5	13.4	3.9
12 weeks	31.2	45.3	1.48	8.4	12.1	3.8
S.E. ±	1.9	2.4	0.05	0.6	1.5	0.6
D.05	5.3	6.8	0.15	1.6	4.2	1.7

nodal roots (Weaver, 1926; Weaver and Zink, 1945; Brouwer and Locher, 1965; Brouwer, 1966). The marked drop at 4 weeks coincides with the appearance of the thick nodal roots which are at first unbranched. The values increase again as the first formed nodal roots start to produce laterals. The variation in apices per unit weight of root can be explained in the same way.

The problem of distinguishing between live and dead roots and the contribution that dead material makes to root weight measurements has been discussed by Jacques and Schwaas (1956), Troughton (1956) and Garwood (1967). Troughton emphasises the advantage in this respect of using young, actively growing plants. In the present experiment death and decay of root tissue on a large scale would have been manifest as a decrease in weight per unit length of root (i.e. an increase in length per unit weight). Discounting the 10 weeks sample, this does not appear to have happened or else has been balanced by growth of new nodal roots the main axis of which would have a much higher weight per unit length than the laterals which constitute the bulk of root length.

Observations made on plants grown in glass fronted containers showed that no root ceased to elongate for more than 4-5 days without the apex turning brown. In plants of the final harvest many of the roots were brown for part of their length but almost all the root apices were still white and therefore presumably still growing. It was not possible to determine just how much decay may have taken place in older roots but since in most cases the root apex was still alive the stele at least must still have been functional. Death may have occurred in the cortex and epidermis but there was no sloughing off as has been recorded in older roots of this species by Jacques (1956) and Soper (1957; 1958).

May (1960), Alcock (1964), May et al (1965) and Brouwer (1966) have pointed out the shortcomings of using root weight as an indicator of the functional size of the root system. For water and nutrients (Wiebe and Kramer, 1954; Kramer, 1956 ; Brouwer, 1965; Russell and Sanderson, 1967) the region immediately behind the apex is most active in uptake so an increase in the number of apices per unit weight of root might be expected to increase the efficiency of the root system. An

increase in length per unit weight could also be expected to increase efficiency by increasing the volume of the root medium in close proximity to the root surface. In the present experiment the shoot/root ratio increased with time, i.e. the weight of root supporting unit weight of shoot decreased. After the first sample however the root length and number of apices per unit weight of shoot showed a marginal increase. The two-weeks sample which was mainly seminal root may be a special case since much of the growth would have been made from seed reserves. Rapid proliferation of seminal roots through the surface soil which is most susceptible to drying out would be of importance to survival.

The changes in root morphology with plant age pose a problem when a treatment such as defoliation, which is likely to slow down development, is applied. Such a treatment would result in lower length per unit dry weight and apices per unit length in plants within the age range 4-10 weeks. This limitation had to be accepted however as any increase in plant age would have introduced the possibility of root death and the need to increase the size of the glass fronted containers above that which could be comfortably handled.

APPENDIX 6

EFFECT OF SEED WEIGHT ON PLANT SIZE

The effect of seed weight on plant size was studied in conjunction with the development of young plants. (Appendix 5). Included in each replicate of that experiment were two seedlings from seed of air dry weight 1.2 mg or less and two from seed of 2.4 mg or more. Each of the two seed weight groups represented approximately 20% of the original sample as did the seed used in that experiment. (1.7-1.9 mg). One plant each from high weight and low weight seed from each replicate was harvested at the first and fourth harvests (two and eight weeks from planting respectively). Measurements made on these plants were the same as for Appendix 5 and the results were compared with those of the plants from that experiment harvested at the same time, thus giving three seed weight groups.

Results:

The results are shown in Table 1. At both the two weeks and 8 weeks harvests the plants from low weight seed were significantly lower in root and shoot weights, root lengths and number of apices than plants from high weight seed. In some instances the differences between the high and low weight seed plants respectively and the medium weight seed plants were also significant. The low weight seed plants had a higher shoot/root ratio than the high weight seed plants at two weeks but not at eight weeks. The plants from high and low weight seed did not differ significantly at either harvest in root length per unit weight or number of apices per unit weight or per unit length but at 8 weeks plants from medium weight seed had a significantly lower length and number of apices per unit weight than plants from high or low weight seed.

Discussion:

In shoot weight, root weight, shoot/root ratio, root length and number of apices the difference between seed weight groups as a proportion of the mean value was lower at eight weeks than at two weeks indicating that plants from low weight seed had a higher rate of growth.

Appendix 6 Table 1 - Effect of seed weight on shoot weight, shoot/root ratio and root details of plants harvested at two weeks and eight weeks (means of 10 plants).

Seed weight	Shoot weight (g)	Root weight (g)	Shoot/ Root Ratio	Root length (m)	No.of Apices	Length per unit root wt. (cm/mg)	Apices per mg. root weight	Apices per cm root length
2 Weeks								
2.4mg	0.0056	0.0026	2.7	0.75	107	34	48	1.4
1.7-1.9mg	0.0046	0.0015	3.1	0.57	70	38	47	1.3
1.2mg	0.0025	0.0008	3.5	0.27	36	37	47	1.3
S.E. ±	0.00044	0.00012	0.24	0.049	8.0	2.2	3.5	0.069
D.05	0.0013	0.00037	0.72	0.15	24.0	6.5	10.0	0.2_1
8 Weeks								
2.4mg	4.9	1.4	3.6	480	57,000	35	42	1.2
1.7-1.9mg	3.8	1.1	3.5	320	36,000	30	34	1.1
1.2mg	3.3	0.9	3.8	320	38,000	38	45	1.2
S.E. ±	0.34	0.10	0.14	27	1,600	1.7	2.6	0.056
D.05	1.02	0.30	0.41	79	4,700	4.9	7.8	0.17

These results are consistent with those of Black (1957) and Harkess (1965). Harkess found that although dry matter yield differences ceased to be significant after 30 days, the difference in organ size persisted to the end of the experiment (8 weeks). Black found differences in plant size were still present after 6 months. The differences in shoot/root ratio at two weeks in the present experiment may be a result of luxury root production in the high weight seed plants due to the presence of large seed reserves.

The marked difference in plant size from different weight seed indicates that in this species selection for uniform seed weight may considerably reduce variability in experiments.

APPENDIX 7

TRIAL PLANTINGS OF GLASS FRONTED CONTAINERS

Five germinated seeds of L. perenne were planted in sand against the top edge of the glass in each of six glass fronted containers (Appendix 2) to determine the optimum amount of plant material in a container for measurement of root elongation. The most satisfactory amount was that which gave 30 - 40 primary nodal roots against the glass. This number was visible when there were 120 - 180 tillers. Where 30 - 40 primary nodal roots were visible at least 20 could be marked for elongation measurements. Some could not be marked because they were too close to the top, bottom or sides of the glass or to other roots. Growth in a few was also distorted. Where more roots were present it became difficult to distinguish between roots when measuring elongation. This estimate was used as the basis for all subsequent experiments.

It was found to be practical to transplant plants into the glass fronted containers thus reducing the time the containers were in use in any particular experiment and increasing the number of experiments that could be conducted. In practice, three plants per container were found to give a better distribution of roots against the glass than occurred with one plant. With more than three plants, transplanting became difficult and plant growth was affected in some instances. It was not possible to distinguish between the roots of individual plants in a container so the container was considered as the experimental unit.

In transplanting trials, 6 - 12 days, depending on temperature, elapsed before a satisfactory distribution of primary nodal roots against the glass occurred. Plants were therefore transplanted at the 20 - 30 tiller stage and the tiller number (120 - 180) found to correspond to the optimum number of primary nodal roots against the glass achieved at about the time the satisfactory distribution against the glass occurred.

APPENDIX 8

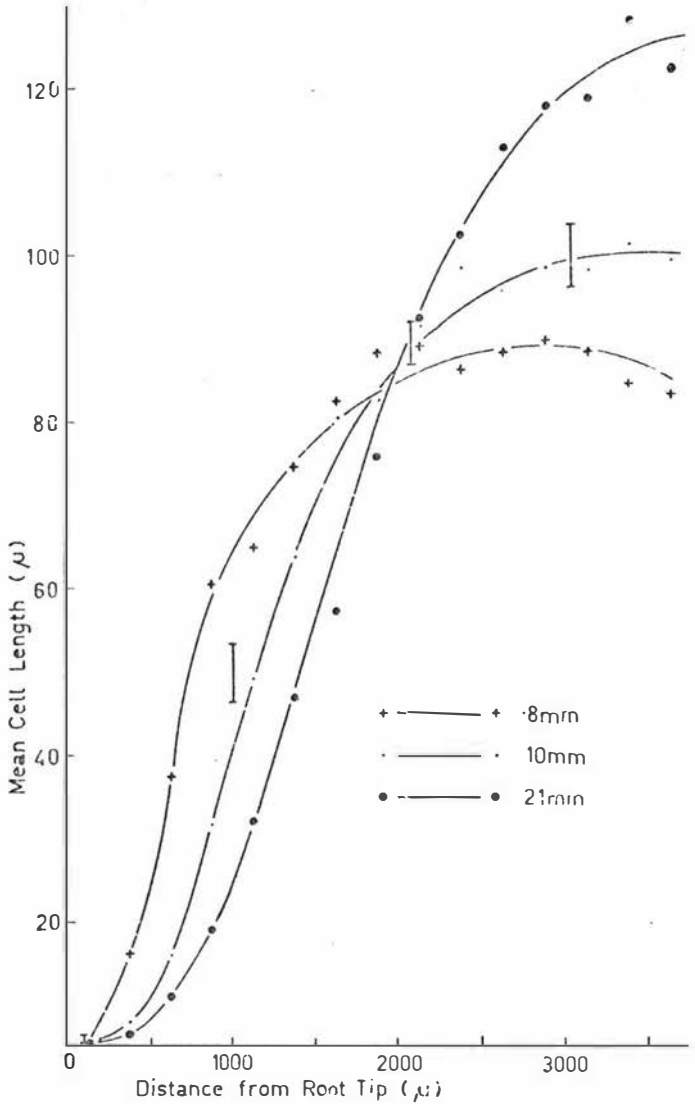
ROOT DIAMETER AND GROWTH RATES

The most obvious variation in elongation in L. perenne roots is between the primary nodal roots and the laterals. Where the primary nodal roots had a daily elongation of 6-30mm, that of the laterals was seldom more than 2-3mm and frequently less than 1mm. Within the primary nodal roots there was a considerable variation under any given set of environmental conditions. Because of their much higher elongation rates the primary nodal roots were selected for study and the basis of the variation was examined.

Fifteen plants selected for uniformity of size and each consisting of approximately 25 tillers were planted three each into five glass fronted containers. After seven days by which time approximately 40 primary roots were growing against the glass in each container the position of all primary nodal root apices visible was marked on the glass. Root elongation was measured for the subsequent 24 hours and the apices of three roots each of high elongation (approximately 20 mm) and of low elongation (approximately 10 mm) and also three thin primary nodal roots (elongation of approximately 8 mm) from each container were removed and fixed in formalin-aceto-alcohol. Two of each group of three apices were dehydrated and embedded by the method of Evans (1966), sectioned longitudinally at 10 μ and stained with azo black. The third apex from each group was preserved in ethyl alcohol in case of damage during processing to either of the others. Cell lengths were measured using a microprojector. Starting at the junction of the apex and the root cap, ten cells at random in the cortex were measured in each 250 μ segment to the position where the cells attained their mature size. The average number of new cells produced in 24 hours in each file within the cortex was calculated by dividing the daily elongation of the root by the mature cell length.

Results:

The cell length pattern at the apex is shown in Fig. 1. In the roots with a high elongation rate the cells elongated at a greater distance from the root cap than in the roots with a low elongation rate and attained a greater final length. In the roots with a low elong-



Appendix 8 Fig. 1 - Cell length pattern in cortex of roots of different elongation rates. Vertical lines indicate twice standard error.

ation rate cells elongated further from the root cap and reached a greater mature length than those from the thin roots. Mature cell lengths, daily elongation, number of new cells produced per day and root diameters are shown in Table 1. The high elongation rate roots had a higher rate of cell production as well as a greater final cell length than the low elongation rate roots. The thin roots had a lower final cell length than either of the groups of thick roots.

Discussion:

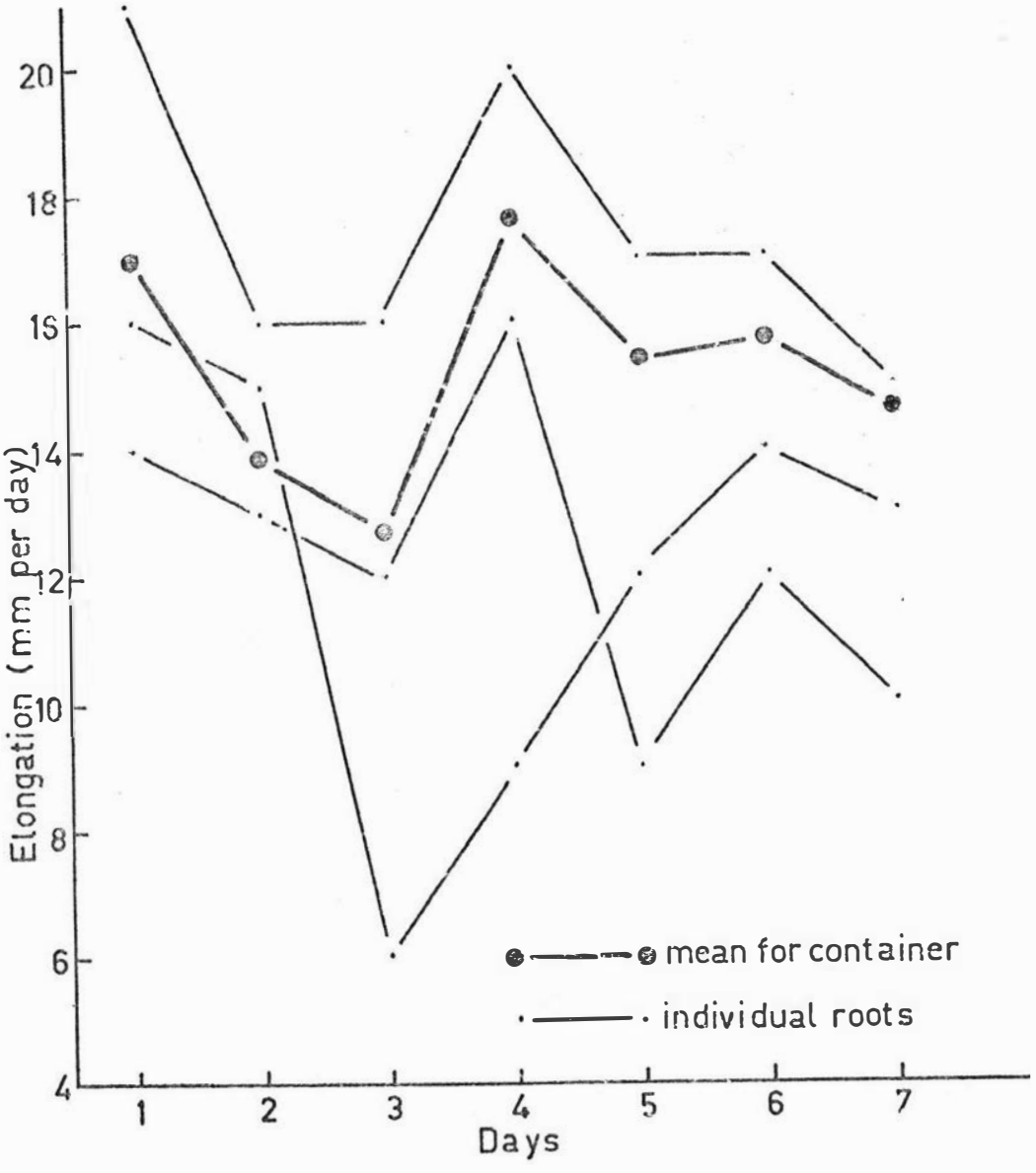
The size of the root is determined initially by the diameter of the primordium. Thus a secondary root is of lesser diameter than the primary root from which it arises and a tertiary root smaller than the secondary root from which it arises. Environmental conditions can however affect root diameter within this context as discussed elsewhere in this thesis. Also it was observed that where primary root apices were excised the laterals immediately behind the point of excision increased in diameter and in growth rate. This suggests that the determinant of root diameter and growth rate is food supply rather than the size of the primordium. As can be seen in Table 1 the thin roots had a lower elongation rate. They may have been roots which developed on weak tillers or else had restricted vascular connections with the shoot.

It was seldom possible to follow the elongation of individual nodal roots for sufficient time to determine if some were consistently slower growing than others or if the growth of all roots was periodic as might be expected as a result of fluctuating photosynthate supply. In Fig. 2. is shown the elongation of three nodal roots over a period of seven days together with the mean for the container. The data is from one of the controls in the single defoliation experiment (P 9) in which the elongation of individual roots was followed during the first eight days. One of the roots exhibits a marked drop on day 3 compared with the mean for the container. This may indicate that the elongation of individual roots is affected by the growth of the tillers to which they are attached.

Since there was a gradation in primary root thickness and since thin roots were not necessarily slow growing and vice versa a representative sample of roots was marked on all occasions for measurement of elongation.

Appendix 8 Table 1 - Elongation rate, mature cell length, rate of cell division and diameter of three classes of primary nodal roots.

	Class			S.E. ±	D.05
	Rapid	Slow	Thin		
Elongation in 24 hr. (mm)	21.2	10.0	8.6	0.82	2.30
Mature cell length (μ)	122.1	99.1	83.6	4.4	13.1
Cell Divisions per 24 hrs.	176.3	99.0	100.0	9.3	28.7
Root diameter (μ)	407	374	220	12.2	37.4



Appendix 8 Fig. 2 - An example of the relationship between elongation of individual roots and the mean rate for all roots in the container that were measured.

APPENDIX 9

THE EFFECT OF ONE PER CENT GLUCOSE ON SEEDLING ROOT GROWTH

This experiment was originally designed to determine if root growth of intact seedlings could be increased by applying sugar to the root medium.

Seed of air dry weight 1.7 - 1.9 mg was germinated in petri dishes and planted four per pot into 10 cm square plastic pots of sand. Plants were supplied with an excess of nutrient solution of the composition detailed in Chapter 1 each day. The experiment consisted of 20 replicates, the pot rather than the plant being the experimental unit. When the second leaf had started to appear in most plants, one per cent glucose was supplied in the nutrient solution to one pot at random in each replicate. After five days, these pots and one from each replicate which had received the inorganic nutrients only were harvested and the length of the longest root in each plant measured. This procedure was repeated three times on different groups of plants from the same planting at seven day intervals. The results for the four plants from each pot were averaged.

The results are presented in Table 1. At the first two harvests, root length was significantly lower ($p < 0.01$) in the plants which had been supplied with glucose. The sand in these pots had a visible fungal growth which made the washing of the sand from the roots of the plants difficult. This fungal growth had not been apparent when the plants were supplied with nutrient the previous day. This feature was constant through the four experimental periods. No such fungal growth occurred in the control pots.

From this experiment it appears that one per cent glucose may eventually inhibit the elongation of the seminal roots of L. perenne seedlings. The massive build up of microorganisms which occurred during the five-day periods when glucose was being supplied is presumed to be a direct result of the presence of the glucose. Whether the depression of root elongation was caused by this build up or by

Appendix 9 Table 1 - Effect of supplying 1% glucose solution for five days on the length (mm) of the longest root of L. perenne seedlings (means of 20 replicates of four seedlings)

	Root length (mm)			
	Harvest			
	1	2	3	4
Glucose	47.0	64.7	103.3	117.5
Control	56.5	82.8	103.9	125.8
S.E. ±	0.9	2.7	4.9	3.7
D.05	2.8	7.5	14.4	10.7

the glucose itself is not certain. From the results, it would appear that the effect is greatest in the youngest seedlings however with increasing seedling age, the five day period during which glucose was being supplied would be a decreasing proportion of the total growing period so the treatment effects of the third and fourth periods may have been masked by the high length of root present when treatment was applied.

APPENDIX 10

APPLICATION OF GLUCOSE TO INDIVIDUAL ROOTS

Glucose was dissolved in water and added to a solution of methyl cellulose (May & Baker) to give a 1% w/v glucose and 5% w/v methyl cellulose solution. This was coloured with a few crystals of methylene blue. A similar solution lacking glucose was prepared. Six containers were set up as described in Chapter 1 and a number written on the glass front beside the apices of selected primary nodal roots. Any roots which passed close to another root apex higher up the glass face were avoided. The containers were carefully tipped on their backs one at a time and the glass removed. Drops of the two solutions prepared were applied to alternate numbered root apices and the glass replaced. The positions of the root apices were marked on the glass and the plants defoliated to 2.5 cm. The methyl cellulose solution was sufficiently viscous to remain around the root apex between the aluminium sheet and the glass. The methylene blue permitted the applied solution to be readily distinguished from the drops of water which condensed against the inside of the glass. Root elongation was recorded for the following 24 hours.

The elongation of the glucose-treated roots was 3.86 $\pm$ 0.63 mm and for the controls, 3.04 $\pm$ 0.48 mm. The difference was not statistically significant.

The technique was unsatisfactory. In many instances, water condensing against the inside of the glass diluted the solution to the extent that it soaked into the sand. Any root growing at a rate of more than 2 - 3 mm per day quickly grew out of the methyl cellulose solution. Also, although care was taken to avoid roots which passed close to another root apex visible higher up on the glass face, the possibility that treating a root apex high in the container affected a root growing within the sand at that point, and hence not visible through the glass, but which grew against the glass lower down could not be excluded. The importance of this last factor could be reduced to an extent by applying one treatment only to a particular container

but there would still be the possibility that some roots were receiving the material being tested along a greater part of their length than others. In addition the technique involved disturbing the roots and although removing the glass was shown not to significantly affect root elongation (Appendix 2) it was considered an undesirable feature.

APPENDIX 11

EFFECT OF INDOLE ACETIC ACID ON SEMINAL ROOT GROWTH

Seed of air-dry weight 1.7 - 1.9 mg was germinated in petri dishes. When the main seminal roots were approximately 10 mm long the length of the longest root was measured and 20 seedlings transferred into each of seven petri dishes. To each petri dish was added a volume of a different ~~concentration~~ concentration of I.A.A. in water just sufficient to cover the seeds. The concentrations used were 20ppm, 1 ppm, 10^{-2}ppm, 10^{-4}ppm, 10^{-5}ppm, 10^{-6}ppm and 10^{-7}ppm. The petri dishes were placed in an incubator at a temperature of 15°C. Root length was measured three days later and the increase in root length calculated. The experiment was repeated.

The results of the two experiments are presented in Table 1. Individual seedlings were not identified so the figures presented are the differences between the means of root length at the start and finish of the treatment period, and no standard errors were calculated. There was little difference between concentrations below 10^{-2}ppm. Consequently 10^{-4}ppm was selected as being a concentration unlikely to depress elongation in defoliated plants.

Appendix 11 Table 1 – Response of seedling root growth to various concentrations of I.A.A. (Increase in length (mm) in three days).

Concentration	20 ppm	1 ppm	10^{-2} ppm	10^{-4} ppm	10^{-5} ppm	10^{-6} ppm	10^{-7} ppm
Experiment 1	0.6	17.0	32.8	35.2	36.3	36.3	34.0
Experiment 2	0.1	8.8	27.2	28.5	25.1	29.4	31.6

APPENDIX 12

EFFECT OF BENZYLADENINE ON ROOT GROWTH

Table 1 - Effect of various benzyladenine concentrations down to 10^{-3} ppm on root elongation (mm per day) of plants defoliated to 2.5 cm.
1st. Experiment.

	Day 1	Day 2	Day 3
Sucrose only	5.7	4.0	2.2
1 ppm	5.3	2.5	1.3
10^{-1} ppm	5.5	3.0	1.4
10^{-2} ppm	5.9	3.5	3.0
10^{-3} ppm	6.0	3.9	3.4
S.E. $\pm$	0.3	0.3	0.2
D.05	0.7	0.8	0.7

Appendix 12 Table 2 - Effect of various benzyladenine concentrations down to 10^{-3} ppm on root elongation (mm per day) of plants defoliated to 2.5 cm.

2nd Experiment.

	Day 1	Day 2	Day 3
Sucrose only	5.4	4.1	2.0
1 ppm	4.6	2.6	0.8
10^{-1} ppm	4.6	3.5	1.8
10^{-2} ppm	5.1	3.2	2.3
10^{-3} ppm	4.9	3.6	2.1
S.E. ±	0.3	0.3	0.3
D.05	1.0	1.0	0.9

Appendix 12 Table 3 - Effects of various benzyladenine concentrations down to 2 x 10^{-2} ppm on root elongation (mm per day). Experiment with expanded aluminium sheets in containers.

	Day 1	Day 2	Day 3
Sucrose only	4.3	2.8	1.0
2 ppm	4.6	3.4	1.2
2 x 10^{-1} ppm	4.3	3.2	1.2
2 x 10^{-2} ppm	4.4	4.4	1.9
Undefoliated	10.1	10.4	8.1
S.E. $\pm$	0.4	0.5	0.5
D.05	1.2	1.4	1.3

Appendix 12 Table 4 - Effect of various benzyladenine concentrations down to 10^{-6} ppm on root elongation (mm per day) of plants defoliated to 2.5 cm.

	Day 1	Day 2	Day 3
Sucrose only	6.1	5.5	4.0
1 ppm	5.2	3.2	2.5
10^{-2} ppm	6.0	5.8	4.7
10^{-4} ppm	5.8	5.1	4.9
10^{-6} ppm	6.5	6.1	5.6
S.E. ±	0.3	0.5	0.4
D.05	0.8	1.4	1.1

APPENDIX 13

EFFECT OF GIBBERELLIC ACID ON ROOT GROWTH

Table 1 - Effect of Gibberellic Acid concentrations down to 10^{-5} ppm on root elongation (mm per day) of plants defoliated to 2.5 cm.

	Day 1	Day 2	Day 3
Sucrose only	7.3	10.2	10.7
10 ppm	7.6	9.0	9.4
10^{-1} ppm	7.7	9.1	9.5
10^{-3} ppm	7.3	9.2	9.3
10^{-5} ppm	7.4	10.1	10.3
S.E. $\pm$	0.5	0.7	1.0
D.05	1.5	1.9	2.8

APPENDIX 14

SOLUBLE CARBOHYDRATE ANALYSIS

A 0.5 g ground, freeze-dried sample was washed into a 250 ml beaker with 70 ml of water. A drop of antifoaming agent (Dow-Corning) was added and the contents of the beaker boiled for 10 minutes and then filtered. The residue was washed with approximately 20 ml of water and the washings added to the filtrate which was then made up to 100 ml in a volumetric flask. After mixing, the filtrate was transferred to a conical flask which was sealed and stored at $4^{\circ}C$.

When a batch of samples had been prepared to this stage 5 ml from each sample was pipetted into a boiling tube with 1 ml of 2N H_2SO_4, covered to prevent splashing and heated for 15 minutes in a boiling water bath to hydrolyse the soluble carbohydrates to reducing sugars. The tubes were cooled in cold water and the contents transferred to a 10 ml volumetric flask with a drop of phenolphthalein, neutralised with 2N NaOH and made up to volume.

The solutions were analysed for reducing sugars by the method of Nelson (1944). Two ml of the hydrolysed sample was transferred to a graduated test tube. Standards containing 25, 50, 100, and 200 µg glucose were prepared by using the appropriate quantities of 100 µg / ml glucose solution and making up the volume to 2 ml with water where necessary. A blank containing 2 ml of water was also prepared. One ml of 25:1 copper reagent was added to each graduated tube. The tubes were then covered with foil and heated for 20 minutes in a boiling water bath. After cooling in cold water, 1 ml of arsenomolybdate was added to each tube which was then shaken and allowed to stand for five minutes before the contents were diluted to 20 ml.

Samples were analysed on a Unicam SP 600 spectrophotometer at a wavelength of 520 µ using the red photocell. A straight line was drawn through the plots of the readings of the glucose standards. Soluble carbohydrate levels were read from this graph and converted to percentages of the freeze-dried weight of the samples by multiplying by the reciprocal of the weight of the fraction analysed.

APPENDIX 15

TRIAL WASHING OF ROOTS

Four containers were used. Plants in two of these containers were defoliated to 2.5 cm and supplied with 2% sucrose in the nutrient solution. The other two which were not defoliated were supplied with the inorganic nutrient solution only. The experiment was conducted in the glasshouse.

After three days, the roots of the plants were washed clean of sand in cold water. A 5 g wet weight (0.3 g oven-dry weight) sample of root from each container was rinsed for five minutes in each of three 20 ml volumes of water and then for 30 minutes in each of three further 20 ml volumes. Surplus water was removed from the roots between rinses by pressing between layers of filter paper.

The soluble carbohydrate content of each rinse was determined by using the method outlined in Appendix 14.

The results are presented in Table 1. It is clear from these results that any sucrose present after the initial washing to remove sand was as strongly held as the soluble carbohydrate present in the roots of plants which had not been supplied sucrose.

On the basis of this test, a single five minute rinse following the initial washing was considered to be ample.

Appendix 15 Table 1 - Soluble carbohydrates extracted from 5 g fresh weight of roots by 3 successive 5 minute rinses followed by 3 successive 30 minute rinses (μg per sample expressed as reducing sugars).

	Rinse	Control	Sucrose
	1	82	82
	2	43	24
	3	40	24
	4	47	43
	5	31	34
	6	28	21

BIBLIOGRAPHY

ALBERDA, T. 1957: The effects of cutting, light intensity and night temperature on growth and soluble carbohydrate content of Lolium perenne L. Plant and Soil 8: 199-230.

_____ 1960: The effect of nitrate nutrition on carbohydrate content in Lolium perenne. Proc. 8th Int. Grassl. Congr.: 612-17.

ALCOCK, M.B. 1964: The physiological significance of defoliation on the subsequent regrowth of grass-clover mixtures and cereals. in "Grazing in Terrestrial and Marine Environments" edit. D. J. Crisp, Blackwells, London. pp 25-41.

ALLSOPP, A. 1965: The significance for development of water supply, osmotic relations and nutrition. in "Encyclopedia of Plant Physiology" XV / 1 edit. W. Ruhland, Springer-Verlag, Berlin. pp 504-52.

ASHER, C.J.; OZANNE, P.G. 1966: Root growth in seedlings of annual pasture species. Plant and Soil 24: 423-36.

BAKER, H.K. 1957: Studies on the root development of herbage plants, 2 . The effect of cutting on the root and stubble development, and herbage production of spaced perennial ryegrass plants. J. Brit. Grassl. Soc. 12: 116-26.

BALDOVINOS, G. de la P. 1953: Growth of the root tip. in "Growth and Differentiation" edit. W. E. Loomis, Iowa State Coll. Press. pp 27-54

BEARD, J.B.; DANIELS, W.H. 1965: Effect of temperature and cutting on the growth of creeping bent-grass (Agrostis palustris Huds.) roots. Agron. J. 249-50.

BISWELL, H.H.; WEAVER, J.E. 1933: Effect of frequent clipping on the development of roots and tops of grasses in prairie sod. Ecology 14: 368-90.

BLACK, J.N. 1957: Seed size as a factor in the growth of subterranean clover (Trifolium subterraneum L.) under spaced and sward conditions. Aust. J. Agric. Res. 8: 335-51.

BRIAN, P.W. 1959: Effects of gibberellins on plant growth and development. Biol. Rev. 34: 37-84.

BROUWER, R. 1963: Some physiological aspects of the influence of growth factors in the root medium on growth and dry matter production. Jaarb. Inst. Biol. schiek. Onderz. LandbGewass 1963: 11-30.

_____ 1965: Water movement across the root. Symp Soc. Exptl. Biol. 19: 131-49.

_____ 1966: Root growth of grasses and cereals. in "The Growth of Cereals and Grasses" edit. Milthorpe and Ivins. Proc. 12th U. of Nott. Easter Sch. Butterworths, London. pp 153-66.

_____; LOCHER, J.T. 1965: The significance of seminal roots in growth of maize. Jaarb. Inst. Biol. schiek. Onderz. LandbGewass. 1965: 21-28.

BROWN, R. 1959: The regulation of growth and differentiation in the root. Proc. 4th. Intern. Congr. Biochem.Vienna, 1958. 6: 77-94.

_____ 1963: Cellular differentiation in the root. Symp. Soc. Exptl. Biol. 17: 1-17.

BUCKNALL, R.A.; SUTCLIFFE, J.F. 1965: The nucleic acids and cellular differentiation in the root apex of Pisum sativum. II. The nucleic acids and cellular development patterns. J. Exptl. Bot. 16: 587-603.

BURSTROM, H. 1954: Studies on growth and metabolism of roots. XI. The influence of auxin and coumarin derivatives on the cell wall. Physiol. Plant. 7: 548-559.

_____ 1961: Physics of cell elongation. in "Encyclopedia of Plant Physiology" XIV. edit. W. Ruhland. Springer-Verlag, Berlin. pp 285-324.

BUTCHER, D.N.; STREET, H.E. 1964: Excised root culture. Bot. Rev. 30: 513-586.

BUTLER, G.W.; GREENWOOD, R.M.; SOPER, K. 1959: Effects of shading and defoliation on the turnover of root and nodule tissue of plants of Trifolium repens, Trifolium pratense, and Lotus uliginosus. N.Z. Jl. agric. Res. 2: 415-26.

CARR, D.J.; REID, D.M. 1969: The physiological significance of the synthesis of hormones in roots and of their export to the shoot system. in "Biochemistry and Physiology of Plant Growth Substances." edit. Wightman F. & Setterfield G. Runge Press, Ottawa. pp 1169-85.

CARTER, J.E.; STREET, H.E. 1963: Studies on the growth in oulture of excised wheat roots. IV. The activation of D,L-tryptophane by autoclaving. Physiol. Plant 16: 347-58.

CLELAND, R. 1959: Effect of osmotic concentration on auxin action and on irreversible and reversible expansion of the Avena coleoptile. Physiol. Plant 12: 809-25.

CRIDER, F.J. 1955: Root-growth stoppage resulting from defoliation of grass. U.S.D.A. Tech. Bull. 1102: 1-23.

DARROW, R.A. 1939: Effects of soil temperature, pH and nitrogen nutrition on the development of Poa pratensis. Bot. Gaz. 101: 109-27.

DAVIDSON, J.L.; MILTHORPE, F.L. 1965: Carbohydrate reserves in the regrowth of cocksfoot (Dactylis glomerata L.) J. Brit. Grassl. Soc. 20: 15-18.

_____ 1966: The effect of defoliation on the carbon balance in Dactylis glomerata. Ann. Bot. 30: 185-98.

DENNE, M.P. 1960: Leaf Development in Narcissus pseudonarcissus L. II. The comparative development of scale and foliage leaves. Ann. Bot. 24: 32-47.

ELIASSON, L. 1968: Dependence of root growth on photosynthesis in Populus tremula. Physiol. Plant. 21: 506-10.

ENNIK, G.C. 1966: Influence of clipping and soil fumigation on shoot and root production of perennial ryegrass and white clover. Jaarb. Inst. Biol. sohiek. Onderz. LandbGewass. 1966: 11-18.

EVANS, L.T.; WARDLAW, I.F.; WILLIAMS, C.N. 1964: Environmental control of growth. in "Grasses and Grasslands" edit. C. Barnard. MacMillan, London. pp 102-25.

EVANS, P.S. 1965: Intercalary growth in the aerial shoot of Eleocharis acuta R.Br.Prodr. 1. Structure of the growing zone. Ann. Bot. 29: 205-17.

_____ 1966: Preparation of specimens for microtomy using continuous dehydration. J. Roy.Microscp. Soc. 85: 375-8.

FERGUSON, J.D. 1967: The nutrition of excised wheat roots. Physiol. Plant. 20: 276-84.

FISHER, J.E. 1964: Evidence of circumnutational growth movements of rhizomes of Poa pratensis L. that aid in soil penetration. Can. J. Bot. 42: 293-9.

GARWOOD, E.A. 1967: Seasonal variation in appearance and growth of grass roots. J. Brit. Grassl. Soc. 22: 121-30.

GOEDEWAAGEN, M.A.J. 1954: Beoordeling van de vitaliteit van het wortelstelsel. Landbouwk. Tijdschr. Wageningen 66: 553-5. (Quoted by Troughton, 1957)

GOLUB, S.J.; WETMORE, R.H. 1948: Studies of development in the vegetative shoot of Equisetum arvense L. II. The mature shoot. Amer. J. Bot. 35: 767-81.

GOODWIN, R.H.; AVERS, C.J. 1956: Studies on roots. III. An analysis of root growth in Phleum pratense using photomicrographic records. Amer. J. Bot. 43: 479-87.

HARKESS, R.D. 1965: The effect of seed size on early growth of diploid and tetraploid Italian ryegrass. J. Brit. Grassl. Soc. 20: 190-93.

HARRISON, C.M. 1934: Responses of Kentucky blue-grass to variations in temperature, light, cutting and fertilizing. Pl. Physiol. 9: 83-106.

_____; HODGSON, C.W. 1939: Response of certain perennial grasses to cutting treatments. J. Amer. Soc. Agron. 31: 418-30.

HAYNES, J.L. 1943: Effects of pasture practices on root distribution. Ibid. 35: 10-18.

HEAD, G.C. 1965: Studies of diurnal changes in cherry root growth and nutational movements of apple root tips by time lapse cinematography. Ann. Bot. 29: 219-24.

_____ 1966: Estimating seasonal changes in the quantity of white unsuberized root on fruit trees. J. Hort. Sci. 41: 197-206.

_____ 1967: Effects of season changes in shoot growth on the amount of unsuberized root on apple and plum trees. Ibid. 42: 169-80.

HERTEL, R.; EVANS, M.L.; LEOPOLD, A.C.; SELL, H.M. 1969: The specificity of the auxin transport system. Planta 85: 238-49.

HEYES, J.K. 1960: Nucleic acid changes during cell expansion in the root. Proc.Roy.Soc. (London) Ser. B.152: 218-30.

_____ 1963: The role of nucleic acids in cell growth and differentiation. Symp. Soc. Exptl. Biol. 17: 40-57.

HILLMAN, W.S. 1957: Non-photosynthetic light requirement in Lemna minor and its partial satisfaction by kinetin. Science 126: 165-6.

HOLM, R.E.; KAY, J.L. 1969: Hormonal regulation of cell elongation in the hypoootyl of rootless soybean: An evaluation of the role of DNA synthesis. Plant Physiol. 44: 1295-1302.

HUMPHRIES, E.C. 1958: Effect of removal of part of the root system on the subsequent growth of the root and shoot. Ann. Bot. 22: 251-7.

JACOBS, W.P. 1947: The development of the gynophore of the peanut plant Arachis hypogaea L. 1. The distribution of mitoses, the region of greatest elongation, and the maintenance of vascular continuity in the intercalary meristem. Amer. J. Bot. 34: 361-70.

JACQUES, W.A. 1937: The effect of different rates of defoliation on the root development of certain grasses. N.Z. J. Sci. Tech. A19: 441-50.

_____ 1956: Root development in some common New Zealand pasture plants. IX. The root replacement pattern in perennial ryegrass (Lolium perenne). N.Z. J. Sci. Tech. A38: 160-5.

_____; SCHWASS, R.H. 1956: Root development in some common New Zealand pasture plants. VII. Seasonal root replacement in perennial ryegrass (Lolium perenne) Italian ryegrass (L. multiflorum) and tall fescue (Festuca arundinacea). N.Z. J. Sci. Tech. A37: 569-83.

JOHANSEN, D.A. 1940: "Plant Microtechnique" p. 80 McGraw-Hill , New York. 523pp.

JOHNSON, A.A.; DEXTER, S.T. 1939: The response of quack grass to variations in height of cutting and rates of application of nitrogen. J. Amer. Soc. Agron. 31: 67-76.

JONES, E.W. 1944: Biological flora of the British Isles. Acer L. J. Ecol. 32: 215-52.

JULANDER, O. 1945: Drought resistance in range and pasture grasses. Plant Physiol. 20: 573-99.

KAUFMAN, P.B. 1959: Development of the shoot of Oryza sativa L. II. Leaf histogenesis. Phytomorphology 9: 277-311.

KENDALL, M.G.; MORAN, P.A.P. 1963: "Geometrical Probability" Griffin & Co., London. p 70.

KIRK, S.C.; JACOBS, W.P. 1968: Polar movement of indole-3--acetic acid-^{14}C in roots of Lens and Phaseolus. Plant Physiol. 43: 675-82.

KOZLOWSKI, T.T. 1949: Light and water in relation to growth and competition of piedmont forest trees. Ecol. Monogr.19: 207-31.

KRAMER, P.J.; DECKER, J.P. 1944: Relation between light intensity and rate of photosynthesis of loblolly pine and certain hardwoods. Plant Physiol. 19: 350-8.

KRAMER, P.J. 1956: Roots as absorbing organs. in "Encyclopedia of Plant Physiology" III edit. W. Ruhland. Springer-Verlag, Berlin. pp 188-214.

KRASSOVSKY, I. 1926: Physiological activity of the seminal and nodal roots of crop plants. Soil Sci. 21: 307-23.

LAVIN, F. 1961: A glass-faced planter box for field observations on roots. Agron. J. 53: 265-8.

LEBEDEV, P.V. 1963: Effect of nitrogen and light intensity on correlative relationship during growth of roots and shoots of meadow grass. Soviet Pl. Physiol. 10: 294-99.

LETHAM, D.S. 1967: Chemistry and physiology of kinetin-like compounds. Ann. Rev. Pl. Physiol. 18: 349-64.

LOCKHART, J.A. 1961: The hormonal mechanism of growth inhibition by visible radiation. in "Plant Growth Regulation" 4th Intnatl. Conf. Iowa State University Press. pp 543-57.

LOOMIS, W.E. 1953: Growth and differentiation - and introduction and summary. in "Growth and Differentiation in Plants" edit. W. E. Loomis. Iowa State Coll. Press. pp 1-19.

LOVVORN, R.L. 1945: The effect of defoliation, soil fertility, temperature and length of day on the growth of some perennial grasses. J. Amer. Soc. Agron. 37: 570-82.

MANOS, G. 1961: The effects of growth substances on attached and detached root tips of Pisum sativum L. Physiol. Plant. 14: 697-711.

MARSHALL, C.; SAGAR, G.R. 1965: The influence of defoliation on the distribution of assimilates in Lolium multiflorum Lam. Ann. Bot. 29: 365-70.

MAY, L.H.; DAVIDSON, J.L. 1958: The role of carbohydrate reserves in regeneration of plants. I. Carbohydrate changes in subterranean clover following defoliation. Aust. J. agric. Res. 9: 767-777.

MAY, L.H. 1960: The utilization of carbohydrate reserves in pasture plants after defoliation. Herbage Abstr. 30: 239-45.

_____; CHAPMAN, F.H.; ASPINALL, D. 1965: Quantitative studies of root development. 1. The influence of nutrient concentration. Aust. J. Biol. Sci. 18: 25-35.

_____; RANDLES, F.H.; ASPINALL, D.; PALEG, L.G. 1967: Quantitative studies of root development. II. Growth in the early stages of development. Ibid. 20: 273-83.

MEYER, R.E.; GRINGRICH, J.R. 1964: Osmotic stress: Effects of its application to a portion of wheat root systems. Science 144: 1463-4.

MILTENYI, L. 1931: Histologisch-Entwetlungsgeschichtliche Untersuchungen an Getreidearten. Bot. Közl. 28: 1-51.

MITCHELL, K.J. 1954: Influence of light and temperature on growth of ryegrass (Lolium spp.) III. Pattern and rate of tissue formation. Physiol. Plant. 7: 51-65.

MORGAN, D.G. 1968: A quantitative study of the effects of gibberellic acid on the growth of Festuca arundinaceae. Aust. J. Agric. Res. 19: 221-5.

MORRÉ, D.J.; EISINGER, W.R. 1969: Cell wall extensibility: Its control by auxin and relationship to cell elongation. in "Biochemistry and Physiology of Plant Growth Substances" edit. Wightman F. & Setterfield G. Runge Press, Ottawa. pp 625-45.

MULLINS, M.G. 1967: Morphogenetic effects of roots and of some synthetic cytokinins in Vitis vinifera L. J. Expl. Bot. 18: 206-14.

NELSON, N. 1944: A photometric adaptation of the Sonogyi method for the determination of glucose. J. Biol. Chem. 153: 375-80.

NEWMAN, E.I. 1966a: A method of estimating the total length of root in a sample. J. App. Ecol. 3: 139-45.

_____ 1966b: Relationship between root growth of flax (Linum usitatissimum) and soil water potential. New Phytol.65: 273-83.

NOWAKOWASKI, T.Z. 1969: Effects of nitrogen and potassium fertilisers on contents of carbohydrates and free amino acids in Italian ryegrass. I. Effects on growth and soluble-carbohydrate contents of leaves, stubble and roots. J. Sci. Fd. Agric. 20: 666-70.

OSWALT, D.L.; BERTRAND, A.R.; TEEL, M.R. 1959: Influence of nitrogen fertilization and clipping on grass roots. Soil Sci. Soc. Proc. 23: 228-30.

PARKER, K.W.; SAMPSON, A.W. 1930: Influence of leafage removal on the anatomical structure of roots of Stipa pulchra and Bromus hordeaceus. Plant Physiol. 5: 543-53.

_____. 1931: Growth and yield of certain gramineae as influenced by reduction of photosynthetic tissue. Hilgardia 5: 361-81.

PAVLYCHENKO, T.K. 1937: Quantitative study of the entire root systems of weeds and crop plants under field conditions. Ecology 14: 62-79.

_____ 1942: Root systems of certain forage crops in relation to management of agricultural soils. "Publ. 1088 Can. Nat. Res. Coun. " 46pp.

PERALTA, F. de 1935: Some principles of competition as illustrated by Sudan grass Holcus sorghum sudansis (Piper) Hitch. Ecol. Monogr. 5: 355-404.

PLAUT, Z.; REINHOLD, L. 1969: Concomitant photosynthesis implicated in the light effect on translocation in bean plants. Aust. J. Biol. Sci. 22: 1105-11.

POWELL, R.D.; GRIFFITH, M.M. 1960: Some anatomical effects of kinetin and red light on disks of bean leaves. Plant Physiol. 35: 273-5.

QUINLAN, J.D.; WEAVER, R.J. 1969: Influence of benzyladenine, leaf darkening, and ringing on movement of ^{14}C-labelled assimilates into expanded leaves of Vitis vinifera L. Plant Physiol. 44: 1247-52.

RAGGIO, M.; RAGGIO, N. 1956: A new method for the cultivation of isolated roots. Physiol. Plant. 9: 466-9.

RAY, P.M.; ABDUL-BAKI, A.A. 1969: Regulation of cell wall synthesis in response to auxin. in "Biochemistry and Physiology of Plant Growth Substances" edit. Wightman F. & Setterfield G. Runge Press, Ottawa. pp 647-58.

RICHARDSON, S.D. 1953a: Studies of root growth in Acer saccharinum L. I. The relationship between root growth and photosynthesis. Proc. Kon. Ned. Akad. v.Wetensch. Amsterdam C56: 185-93.

_____ 1953b: Studies of root growth in Acer saccharinum L. II. Factors affecting root growth when photosynthesis is curtailed. Ibid. C56: 346-53.

_____ 1958: The effect of IAA on root development of Acer saccharinum L. Physiol. Plant. 11. 698-709.

ROBERTSON, J.H. 1933: Effect of frequent clipping on the development of certain grass seedlings. Pl. Physiol. 8: 425-47.

ROGERS, W.S. 1939a: Root studies. VIII. Apple root growth in relation to rootstock, soil, seasonal and climatic factors. J. Pomol. 17: 99-130.

_____ 1939b: Root studies IX. The effect of light on growing apple roots: A trial with root observation boxes. Ibid. 17: 131-40.

ROSENQUIST, D.W.; GATES, D.H. 1961: Response of four grasses at different stages of growth to various temperature regimes. J. Ra. Mgmt. 14: 198-202.

RUSSELL, R.S.; SANDERSON, J. 1967: Nutrient uptake by different parts of the intact roots of plants. J. Exptl. Bot. 18: 491-508.

SAGAR, G.R.; MARSHALL, C. 1967: The grass plant as an integrated unit - some studies of assimilate distribution in Lolium multiflorum Lam. Proc. IX Int. Grassl. Congr. 493-7.

SALIM, M.H.; TODD, G.W.; SCHLEHUBER, A.M. 1965: Root development of wheat, oats, and barley under conditions of soil moisture stress. Agron. J. 57: 603-7.

SITTON, D.; ITAI, C.; KENDE, H. 1967: Decreased cytokinin production in the roots as a factor in shoot senescense. Planta 73: 296-300.

SKOOG, F.; MILLER, C.O. 1957: Chemical regulation of growth and organ formation in plant tissues cultured in vitro. Symp. Soc. Exptl. Biol. 11: 118-42.

SOPER, K. 1957: Effects of changes in light and temperature on roots of perennial ryegrass (Lolium perenne L.) N.Z. J. Sci. Tech. A38: 783-92.

SOPER, K. 1958: Effect of flowering on the root system and summer survival of ryegrass. N.Z. J. agric. Res. 1: 329-40.

SPRAGUE, V.G.; SULLIVAN, J.T. 1950: Reserve carbohydrates in orchard grass clipped periodically. Plant Physiol. 25: 92-102.

SRIVASTIVA, B.I.S. 1967: Cytokinins in plants. Int. Rev. Cytol. 22: 350-387.

STEWARD, F.C.; BIDWELL, R.G.S.; YEMM, E.W. 1958: Nitrogen metabolism, respiration and growth of cultured plant tissue. J. Exptl. Bot. 9: 11-51.

STREET, H.E. 1953: Factors controlling meristematic activity in excised roots. III. Light as a factor in the 'location effect' noted with roots of Lycopersicum esculentum Mill. Physiol. Plant. 6: 466-479.

_____ 1966: The physiology of root growth. Ann. Rev. Pl.Physiol. 17: 315-44.

_____ 1969: Factors influencing the initiation and activity of meristems in roots. in "Root Growth" edit. W. J. Whittington. Proc. 15th. U. of Nott. Easter Sch. Butterworths, London: 20-39.

_____; CARTER, J.E.; SCOTT, E.G.; SUTTON, D. 1961: Studies of the growth in culture of excised wheat roots. I. The growth effects of an acid-hydrolysed casein and of light. Physiol. Plant. 14: 621-31.

STUCKEY, I.H. 1942: Influence of soil temperature on the development of colonial bent grass. Plant Physiol. 17: 116-22.

SULLIVAN, J.T.; SPRAGUE, V.G. 1943: Composition of the roots and stubble of perennial ryegrassfollowing partial defoliation. Plant Physiol. 18: 656-70.

_____1953: Reserve carbohydrates in orchard grass out for hay. Ibid. 28: 304-13.

SUTTON, D.; SCOTT, E.G.; STREET, H.E. 1961: Studies on the growth in culture of excised wheat roots. II. The growth -promoting activity of amino acids. Physiol. Plant. 14: 712-24.

TORREY, J.G. 1963: Cellular patterns in developing roots. Symp. Soc. Exptl. Biol. 17: 285-314.

TORREY, J.G. 1965: Physiological bases of organization and development in the root. in "Encyclopedia of Plant Physiology" XV/1 edit. W. Ruhland. Springer-Verlag, Berlin. pp 1256-1327.

TROUGHTON, A. 1956: Studies on the growth of young grass plants with special reference to the relationship between the shoot and root systems. J. Brit. Grassl. Soc. 11: 56-65.

_____. 1957: "The Underground Organs of Herbage Grasses". Commonwealth Agricultural Bureaux, Farnham Royal. 163pp.

_____. 1960: Growth correlations between the roots and shoots of grass plants. Proc. 8th Int. Grassl. Congr: 280-3.

_____. 1961: Studies on the roots of leys and the organic matter and structure of the soil. Emp. J. exp. Agric. 29:" 165-74.

_____. 1962: "The Roots of Temperate Cereals (Wheat, Barley, Oats and Rye)." Commonwealth Agricultural Bureaux, Farnham Royal. 91pp.

WARDLAW, I.F. 1968: The control and pattern of movement of carbohydrates in plants. Bot. Rev. 34: 79-105.

WASSINK, E.C.; RICHARDSON, S.D. 1951: Observations on the connection between root growth and shoot illumination in first -year seedlings of Acer pseudoplatanus L. and Quercus borealis maxima (Marsh) Ashe. Proc. Kon. Ned. Akad. v.Wetensch. Amsterdam. C54: 503-10.

WEAVER, J.E. 1926: "Root Development of Field Crops." McGraw-Hill, New York. 291pp.

_____; ZINK, E. 1945: Extent and longevity of the seminal roots of certain grasses. Plant Physiol. 20: 359-79.

_____.1946: Length of life of roots of ten species of perennial range and pasture grasses. Ibid. 21: 201-17.

WEINMANN, H. 1943: Effects of defoliation intensity and fertilizer treatment on Transvaal highveld. Emp. J. exp. Agric. 11: 113-24.

_____ . 1948: Underground development and reserves of grasses. A review. J. Brit. Grassl. Soc. 3: 115-40.

WHITE, P.R. 1943: "A Handbook of Plant Tissue Culture". p 103. Jacques Catell Press, Lancaster. 277pp.

WIEBE, H.H.; KRAMER, P.J. 1954: Translocation of radioactive isotopes from various regions of roots of barley seedlings. Plant Physiol. 29: 342-8.

WIERSUM, L.K. 1961: Utilization of soil by the plant root system. Plant and Soil 15: 189-92.

WILKINS, M.B.; SCOTT, T.K. 1968: Auxin transport in roots. Nature 219: 1388-9.

WILLIAMS, R.D. 1962: On the physiological significance of seminal roots in perennial grasses. Ann. Bot. 26: 129-36.

WOODSTOCK, L.W.; BROWN, R. 1963: The effect of 2-thiouracil on the growth of cells in the root. Ann. Bot. 27: 403-14.

_____; SKOOG, F. 1962: Distribution of growth, nucleic acids, and nucleic-acid synthesis in seedling roots of Zea mays. Amer. J. Bot. 49: 623-33.

YEOMAN, M.M. 1962: The effects of ribonuclease on the growth of pea roots. J. Exptl. Bot. 13: 390-96.

ROOT GROWTH OF LOLIUM PERENNE L.

1. EFFECT OF PLANT AGE, SEED WEIGHT, AND NUTRIENT CONCENTRATION ON ROOT WEIGHT, LENGTH, AND NUMBER OF APICES

P. S. EVANS

Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

Reprinted from the NEW ZEALAND JOURNAL OF BOTANY, *Vol. 8, No. 3, September 1970.*

ROOT GROWTH OF LOLIUM PERENNE L.

1. EFFECT OF PLANT AGE, SEED WEIGHT, AND NUTRIENT CONCENTRATION ON ROOT WEIGHT, LENGTH, AND NUMBER OF APICES

P. S. EVANS

Grasslands Division, Department of Scientific and Industrial Research, Palmerston North

(*Received for publication 23 May 1969*)

SUMMARY

The effect of plant age, seed weight, and nutrient concentration on root weight, root length, and number of root apices has been examined in young plants of *Lolium perenne* L. cv. 'Grasslands Ruanui'.

The size of the root system increased with age. At 12 weeks the root system had an average of 263,000 apices, 1,816 m total length, and weighed 6 g. Length of root, number of apices per unit of root, and shoot dry weight were initially high but dropped sharply and subsequently rose again. The sharp drop coincided with the growth of the first nodal roots.

Differences in plant size caused by differences in seed weight, while still present at 8 weeks, were smaller than at 2 weeks, indicating that plants from low-weight seed had a higher growth rate. There were no differences in length or number of apices per unit weight of root or in apices per unit length of root. The high-weight seed plants had a lower shoot/root ratio at 2 weeks, but this difference had disappeared by 8 weeks.

Root and shoot weights were both highest at the normal nutrient concentration. At the high nutrient concentration both shoot and root weight were depressed to the same extent whereas at low concentrations the effect on shoot weight was more severe. In contrast to weight, root length and apex numbers were not depressed at 0·25 standard nutrient concentration. Length and number of apices per unit weight of root decreased with increasing nutrient concentration.

The technique used for estimating root length and apex numbers is described.

INTRODUCTION

The study of the root system of pasture plants has lagged behind studies of the shoot and leaves because of the difficulties involved. However, with the increasing productivity and utilisation of pastures, greater knowledge of the root system has become imperative. Besides the general functions of nutrient uptake, water uptake, and anchorage, roots of pasture plants warrant study for their roles as a sink for photosynthate and as modifiers of soil structure for future crop rotations, and

for their possible food storage capacity. Basic to an understanding of function is a knowledge of structure, development, and response to environmental factors.

Under field conditions, the problem of distinguishing between living and dead roots becomes important as does the separation of roots from soil for detailed examination. The work reported in this paper, therefore, has been carried out on young plants, in which little or no root death is likely to have occurred, that have been grown in sand culture to facilitate the separation of roots from the growth medium. Dry weight, the common measure of root size, though defining the amount of photosynthate utilised, does not necessarily indicate the functional size. Therefore, besides measurements of root length, root apex numbers have been counted since the apex and the region immediately behind it are generally considered more active in mineral and water uptake than older regions. *Lolium perenne* L. was chosen because of its agronomic importance and because its morphology, ecology, and physiology are better documented than those of most other pasture species.

EXPERIMENTAL

Experiments were carried out in a glasshouse, the temperature of which was partially controlled within the range 15–21°C in summer-autumn 1967. Seed of *Lolium perenne* L. cv. 'Grasslands Ruanui' was germinated in petri dishes and, when the coleoptiles were approximately 1 cm long, was planted out singly 1 cm deep into sand in 10×36 cm unglazed clay pipes (the type used in field drains) painted silver outside to reflect heat and reduce water loss. Nutrient solution of the following composition (mg/l) was used:

$Ca(NO_3)_2.4H_2O$, 590; KNO_3, 253; $MgSO_4.7H_2O$, 246; KH_2PO_4, 68·0; Fe EDTA, 41·7; KCl, 3·15; H_3BO_3, 1·43; $MnCl_2.4H_2O$, 0·9; $ZnSO_4.7H_2O$, 0·11; $CuSO_4.5H_2O$, 0·04; $Na_2MoO_4.2H_2O$, 0·01.

Each day an amount in excess of that required to restore the sand to field capacity was applied.

EFFECT OF PLANT AGE AND SEED WEIGHT

Sixty seedlings from seed of weight 1·7–1·9 mg (mean of sample) were planted in clay pipes arranged in 10 blocks. Also included at random in each block were 2 seedlings from seeds of weight 1·2 mg or less and 2 from seeds of 2·4 mg or more. (Each seed-weight group represented approximately 20% of the original sample.) The clay pipes were spaced 10 cm apart within blocks so that no mutual shading was considered to have occurred by the end of the experiment, and the blocks were 30 cm apart to facilitate harvesting. One plant from medium-weight seed selected at random from each block was harvested every 2 weeks and carefully washed free of sand. In addition, one plant each from high- and low-weight seed from each block was harvested at 2 weeks and 8 weeks from planting.

Shoot and root dry weights were measured on all plants. Root lengths and numbers of branches were measured on 3 sub-samples per plant except at 2 weeks when the whole root mass was measured. The procedure followed is detailed below. From the primary data, total root length, number of root apices, length per unit weight of root, root apices per unit weight and per unit length of root, root length and number of apices per unit weight of shoot, and shoot/root ratios were calculated. For the plants from medium-weight seed, standard errors were calculated for each group of plants for shoot weight, root weight, root length, and root apex numbers. For other characters the average standard errors were obtained from analyses of variance of all six harvests. Standard errors for seed-weight comparisons were obtained from analyses of variance of the three seed-weight classes.

EFFECT OF NUTRIENT CONCENTRATION

This experiment consisted of 10 replicates with the clay pipes 10 cm apart. Nutrient concentrations used were 0·05, 0·25, 1·0, and 4·0 times the standard solution. All plants were harvested together after 8 weeks. Measurements made were as for the first experiment.

ROOT SAMPLING PROCEDURE AND MEASUREMENT

Lengths of root samples were measured by a modification of Newman's (1966) technique based on Buffon's needle problem (Kendall and Moran, 1963).

If a needle of length L is placed at random on a plane on which are ruled parallel straight lines unit distance apart, the probability of the needle intersecting the lines is:—

$$\frac{2\ L}{\pi} \text{ for } L<1$$

For M throws of the needle or M needles each of length L the expected number of hits scored is:—

$$\frac{2\ L\ M}{\pi}$$

A root of length R, although not normally straight, can be divided into M′ pieces of length L′, each short enough to be considered straight. The number of hits, N, in this example would be:—

$$\frac{2\ L'\ M'}{\pi} \text{ or } \frac{2\ R}{\pi}$$

Therefore $R=\frac{\pi\ N}{2}$ is an estimate of the root length.

This is a special case of the formula $R=\frac{\pi\ N\ A}{2\ H}$ of Newman (*loc. cit.*) where A is the area over which the root mass is spread and H the total length of straight line used. Since the parallel lines are, by definition, unit distance apart and so divide the plane into strips of unit width,

each unit area of each strip is associated with a unit length of line, i.e.,

$$\frac{A}{H} = 1.$$

The use of parallel straight lines means that the area over which the sample is spread does not have to be defined thus simplifying the measuring and eliminating the possibility of error due to non-random arrangement of roots near the edge of the area mentioned by Newman (*loc. cit.*).

The root sample to be measured was spread out in 5–10 ml of water on a 30×40 cm sheet of window glass so that there was little or no chance of one root overlying another. Two sets of parallel lines 1 cm apart at right angles were scratched in black paint on a second 30×40 cm sheet of glass before the paint had completely hardened. The second sheet of glass was placed paint downwards on top of the root sample and the whole carefully turned over so that the clear glass lay uppermost, then placed on four 9 cm high wooden blocks over a sheet of white paper. When illuminated obliquely the roots showed up white against a black background over a series of fine white lines. With this technique even the finest grass roots showed up clearly, although care had to be taken to keep the sample and glass free of dust. The amount of water used to disperse the sample depended on the thickness of the thickest roots, the aim being to exclude air bubbles from the root sample without having water flowing out from between the sheets of glass and possibly carrying root pieces with it. With each experiment a few samples were counted on both sets of parallel lines to guard against error caused by the roots not being randomly orientated.

The use of the technique assumes that the roots lie in a plane parallel to the plane of the parallel straight lines and no account is taken of any component of root length perpendicular to this plane. Sandwiching the roots between two sheets of glass serves to flatten them, but since in any one sample there was variation in root diameter there would be a component of length perpendicular to the plane of the parallel lines, so the technique probably underestimates the actual length. *Lolium perenne* roots were too fine to measure accurately by the direct method as a check on the technique. However, a check was made using two tree-root samples (Table 1). In neither sample did the mean estimated value differ significantly from the direct measurement.

TABLE 1—Length (cm) of two tree-root samples as determined by direct measurement and by line intersection. (Means of 10 estimates.)

	Line intersection	Direct measurement
Sample 1	48·9±0·94	50·1
Sample 2	62·1±1·71	60·9

Because measuring the whole root system of *L. perenne* plants would have taken too much time, three small samples were measured on each plant. To obtain representative samples, the root mass was formed into a rope which was cut into approximately 5 mm pieces. The cut roots were dispersed in 200–800 ml of water, depending on the size of the root system, and samples of approximately 200 cm total length removed for measurement. After several samples had been measured the size could be readily judged by eye. After measuring, samples were dried at 100°c overnight and weighed. To check that cutting the roots into small pieces did not bias the results, three samples of *L. perenne* root were measured whole and again after being cut into approximately 5 mm lengths (Table 2). In no case did the difference between the mean of 10 counts of the uncut sample and the mean of 10 counts when cut into 5 mm lengths reach significance level.

TABLE 2—Lengths (cm) of three ryegrass root samples measured as whole root and when cut into pieces. (Means of 10 estimates.)

	Sample 1	Sample 2	Sample 3
Whole root	110·1	90·9	100·9
Pieces	110·2	92·1	99·7
Av. S.E.	1·6	1·1	1·6

The lateral roots were very fine (approximately 0·1 mm diameter) and the apices could not be readily distinguished from cut ends. The numbers of apices were therefore estimated by counting the number of branches in the samples used for root length determination and the number of roots arising from the base of the plant. The relationship is as follows: A single unbranched root has a single apex. If it produces one lateral (i.e. has one branch), it has two apices. If the lateral itself produces a lateral the whole root has two branches and three apices. If the whole root has B branches it has B + 1 apices. If there are R roots with a grand total of N branches, the total number of apices is R + N. It is probable that in cutting the roots into small pieces some laterals would have been removed cleanly so that estimation of root apices by this method gave a low result but the error is considered to be small.

RESULTS

DEVELOPMENT OF YOUNG PLANTS

Table 3 presents shoot weights, root weights, root lengths, and number of root apices for the six harvests. Note the considerable length of root

TABLE 3—Shoot and root weights, root lengths and numbers of root apices of ryegrass plants harvested at 2 week intervals. (Means of 10 plants.)

Harvest (weeks)	2	4	6	8	10	12
Shoot weight (g)	0·0046 ±0·0003	0·067 ±0·003	0·854 ±0·109	3·83 ±0·40	12·16 ±0·51	21·66 ±1·09
Root weight (g)	0·0015 ±0·0001	0·021 ±0·001	0·253 ±0·036	1·08 ±0·10	3·13 ±0·15	5·98 ±0·48
Root length (cm)	56·6 ±4·2	566 ±23	7,590 ±1,200	32,430 ±3,060	115,720 ±10,340	181,570 ±15,070
No. apices	70·3 ±4·8	597 ±34	8,140 ±1,510	36,470 ±3,750	163,020 ±14,880	262,780 ±21,080

(1,816 m) at the final harvest within the 2·7 litres of sand in the tile. At this stage a mat of roots was starting to form at the periphery. The ratio data are presented in Fig. 1. The shoot/root ratio showed an overall increase with time. The length per unit weight of root was high at first but then dropped with a recovery at 10 weeks. The number of root apices per unit weight of root showed a similar trend. Root length per unit of shoot dry weight, and number of apices per unit of shoot dry weight also showed this trend.

EFFECT OF SEED WEIGHT

Shoot and root weights, shoot/root ratios, root lengths, and number of apices are presented in Fig. 2. At both 2-weeks and 8-weeks harvests the plants from low-weight seeds were significantly lower in root and shoot weights, root lengths, and number of apices than the plants from high-weight seeds. In some instances the differences between the high- and low-weight seed plants respectively and the medium-weight seed plants were also significant. The low-weight seed plants had a higher shoot/root ratio than the high-weight seed plants at 2 weeks, but not at 8 weeks.

The plants from high- and low-weight seed did not differ significantly at either harvest in root length per unit weight or number of apices per unit weight, but at 2 weeks plants from high-weight seed had a significantly higher number of apices per unit root length (data not presented).

NUTRIENT CONCENTRATION

The results are presented in Fig. 3. There were no differences in apices per unit length of root between nutrient levels. The differences

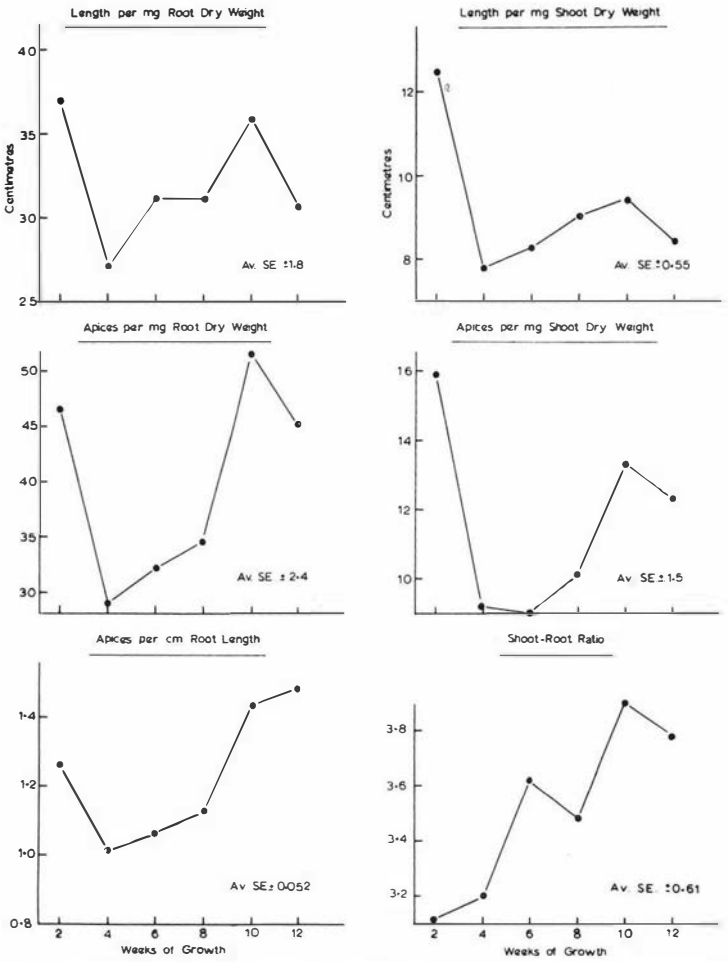


FIG. 1—Root details and shoot/root ratios of plants harvested at 2 week intervals.

between the 0·05 and 0·25 levels were significant at the 5% level in all other characters and between the 1·0 and 4·0 levels in all other characters except shoot/root ratio. In addition there was a significant difference in shoot weight and shoot/root ratio between the 0·25 and 1·0 levels.

DISCUSSION

DEVELOPMENT OF YOUNG PLANTS

The high value for length per unit weight of root at 2 weeks represents mainly seminal root which has been described as finer than the nodal

roots (Weaver, 1926; Weaver and Zink, 1945; Brouwer, 1966; Brouwer and Locher, 1965). The marked drop at 4 weeks coincides with the appearance of the thick nodal roots which are at first unbranched. The value increases again as the first formed nodal roots start to produce laterals. The variation in apices per unit weight of root can be explained in the same way.

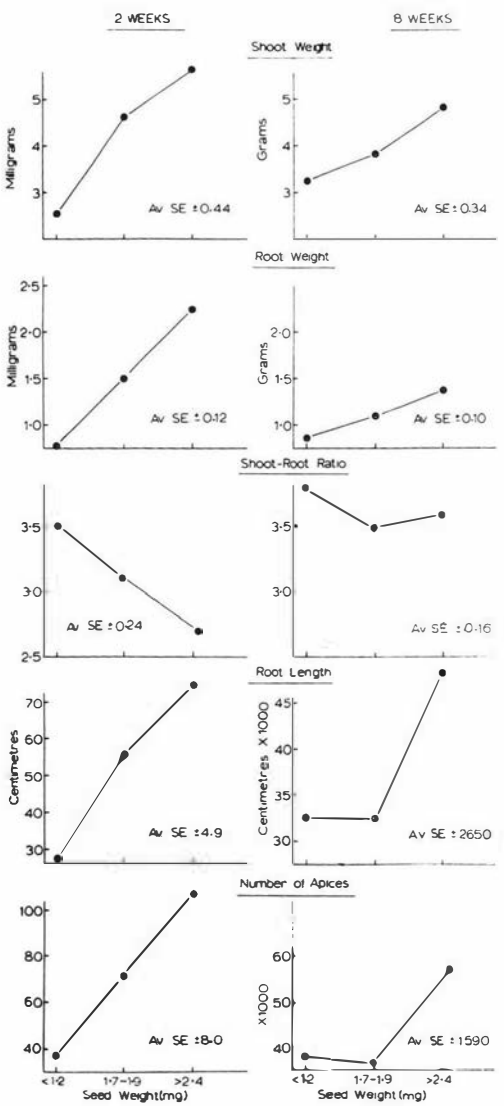


FIG. 2—Effect of seed weight on plants harvested after 2 and 8 weeks.

The problem of distinguishing between live and dead roots and the contribution that dead material may be making to root weight measurements has been discussed by Jacques and Schwass (1956), Troughton (1956), and Garwood (1967). Troughton emphasises the advantage in this respect of using young, actively growing plants. In the present

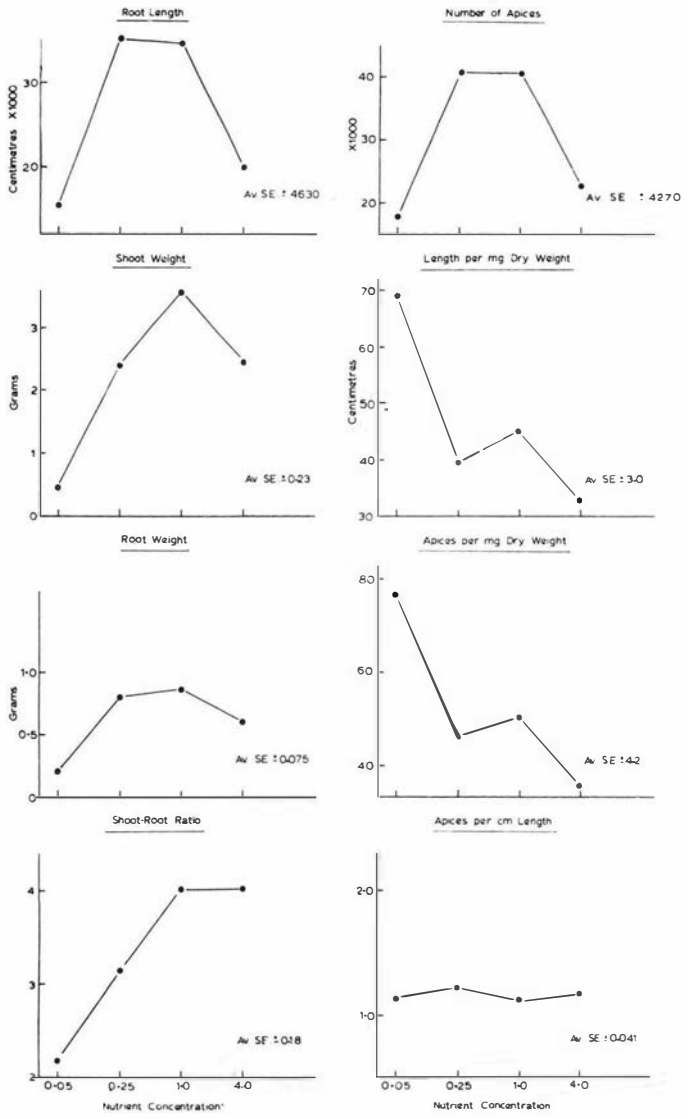


FIG. 3—Effect of nutrient concentration on root details and shoot/root ratios.

experiment death and decay of root tissue on a large scale would have been manifest in a decrease in weight per unit length of root. Discounting the 10-weeks sample this does not appear to have happened or else has been balanced by growth of new nodal roots, the main axis of which would have a much higher weight per unit length than the laterals which constitute the bulk of root length.

Observations made on plants grown in glass-sided containers showed that when root death occurred the apex turned brown. In plants of the final harvest many of the roots were brown for part of their length, but almost all of the root apices were still white and therefore presumably alive. It was not possible to determine just how much death and decay may have taken place in older roots, but if the root apices were still alive, the stele at least must have been functional. There was no sloughing off of the cortex and epidermis as has been recorded in older roots of this species by Jacques (1956) and Soper (1957, 1958).

May (1960), Alcock (1964), May *et al.* (1965), and Brouwer (1966) have pointed out the shortcoming of root weight as an indicator of the functional size of the root system. For water and nutrients (Wiebe and Kramer, 1954; Kramer, 1956; Brouwer, 1965; Russell and Sanderson, 1967) the region immediately behind the apex is most active in uptake so an increase in apices per unit weight of root might be expected to increase the efficiency of the root system. An increase in length per unit weight could also be expected to increase efficiency by increasing the volume of the root medium in close proximity to the root surface. In the present experiment shoot/root ratio increased with time, i.e. the weight of root supporting unit weight of shoot decreased. After the 2-weeks sample, however, the root length and number of apices per unit weight of shoot showed a marginal increase. The 2-weeks sample which was mainly seminal root may be a special case since much of the growth would have been made from seed reserves. Rapid root growth through the surface soil which is most susceptible to drying out would also be of importance to survival.

EFFECT OF SEED WEIGHT

In all characters the difference between seed-weight groups as a proportion of the mean value (Fig. 2) was lower at 8 weeks than at 2 weeks, indicating that the plants from low-weight seed had a higher rate of growth. These results are consistent with those of Black (1957) and Harkess (1965). Harkess found that, although dry matter yield differences ceased to be significant after 30 days, the difference in organ size persisted to the end of the experiment (8 weeks). Black found differences in plant size were still present after 6 months. The differences in shoot/root ratio at 2 weeks may be a result of an excess of root being produced in the high-weight seed plants due to the presence of seed reserves too large to be fully utilised by the small number of growing points in the shoot.

The marked differences in plant size from different weight seed indicated that in this species selecting for uniform seed weight may considerably reduce variability in single plant experiments.

NUTRIENT CONCENTRATION

Dry matter production indicated that the standard nutrient solution was optimum for growth. At the lowest nutrient concentration, shoot weight was depressed more than root weight and hence the shoot/root ratio was lowered, but at the highest concentration root and shoot weight were depressed to the same extent. The total root lengths and number of apices followed the same trend as root weight, but the difference between 1·0 and 4·0 concentrations was more marked. The length and number of apices per unit weight of root dropped with increasing nutrient concentration, but the number of apices per unit length of root remained constant (i.e. the roots became thicker but the frequency of branching did not alter).

Various workers have reported increases in shoot/root ratio on increasing the nutrient supply to plants (Weinmann, 1948; Troughton, 1957, 1962; Allsopp, 1965). Increased nutrient concentration usually produced thicker roots though there are exceptions particularly in the case of single nutrients. May *et al.* (1965) studied the components of root growth in young barley plants at three nutrient concentrations. They found that the lowest concentration gave the greatest length of roots. However, the weight of roots was least at the lowest concentration. They considered that carbohydrate supply was not the cause of differences in total length of root since the weight of the first 10^3 cm of roots in the highest nutrient concentration was almost twice that in the lowest concentration. May *et al.* (1967), also working with barley, discounted carbohydrate as limiting extension growth by pointing out that the primary root continues to elongate at a steady rate despite the development of competing secondary roots back nearer the source of supply. They do suggest, however, that growth of the secondary roots, which is considerably slower, may be due to the competition of the primary root apex. In plants grown in glass-sided containers the growth rate of the primary roots was observed to be higher than that of the laterals, and growth on average increased with increasing length to the bottom of the containers (40 cm). Where a primary root apex was removed, the laterals increased in diameter and rate of growth, indicating that carbohydrate had been limiting either directly or indirectly.

May *et al.* (1965) found that mean spacing between branches on the primary roots, but not on the secondary roots, was greater at low nutrient concentration. In the present study the primary and secondary roots were not analysed separately. From observation of the root samples it would appear that a large proportion of the branches are on secondary roots, so any difference between treatments in branching on the primary roots would be unlikely to markedly influence the overall figure.

ACKNOWLEDGMENTS

This paper is part of a study of root growth to be submitted for the degree of Doctor of Philosophy to Massey University. The guidance of Professor R. G. Thomas, Botany Department, is gratefully acknowledged. Appreciation is expressed to Mr P. Dilks for technical assistance and to Mr V. J. Thomas, Applied Mathematics Division, DSIR, for helpful discussion.

REFERENCES

ALCOCK, M. D. 1964: The physiological significance of defoliation on the subsequent regrowth of grass-clover mixtures and cereals. Pp. 25–41 *in* Crisp, D. J. (Ed.), "Grazing in Terrestrial and Marine Environment". Blackwells, London.

ALLSOPP, A. 1965: The significance for development of water supply, osmotic relations and nutrition. Pp. 504–52 *in* Ruhland, W. (Ed.), "Encyclopedia of Plant Physiology XV/1". Springer-Verlag, Berlin.

BLACK, J. N. 1957: Seed size as a factor in the growth of subterranean clover (*Trifolium subterraneum* L.) under spaced and sward conditions. *Aust. J. agric. Res. 8*: 335–51.

BROUWER, R. 1965: Water movement across the root. *Symp. Soc. exp. Biol. 19*: 131–49.

———— 1966: Root growth of grasses and cereals. Pp. 153–66 *in* Milthorpe & Ivins (Eds.), "The Growth of Cereals and Grasses". Proceedings 12th University of Nottingham Easter School. Butterworths, London.

BROUWER, R. and LOCHER, J. T. 1965: The significance of seminal roots in growth of maize. *Jaarb. Inst. Biol. schiek. Onderz. LandbGewass. 1965*: 21–8.

COOPER, J. P. 1948: Resistance to inanition in grass seedlings. *Nature, Lond. 161*: 894–5.

GARWOOD, E. A. 1967: Seasonal variation in appearance and growth of grass roots. *J. Br. Grassld Soc. 22*: 121–30.

HARKESS, R. D. 1965: The effect of seed size on early growth of diploid and tetraploid Italian ryegrass. *Ibid. 20*: 190–3.

JACQUES, W. A. 1956: Root development in some common New Zealand pasture plants. IX. The root replacement pattern in perennial ryegrass (*Lolium perenne*). *N.Z. Jl Sci. Technol. A38*: 160–5.

JACQUES, W. A. and SCHWASS, R. H. 1956: Root development in some common New Zealand pasture plants. VII. Seasonal root replacement in perennial ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*), and tall fescue (*Festuca arundinaceae*). *Ibid. A37*: 569–83.

KENDALL, M. G. and MORAN, P. A. P. 1963: "Geometrical Probability". Griffin & Co., London. p. 70.

KRAMER, P. J. 1956: Roots as absorbing organs. Pp. 188–214 *in* Ruhland, W. (Ed.), "Encyclopedia of Plant Physiology. III." Springer-Verlag, Berlin.

MAY, L. H. 1960: The utilization of carbohydrate reserves in pasture plants after defoliation. *Herb. Abstr. 30*: 239–45.

MAY, L. H., CHAPMAN, F. H., and ASPINALL, D. 1965: Quantitative studies of root development. I. The influence of nutrient concentration. *Aust. J. biol. Sci. 18*: 25–35.

MAY, L. H., RANDLES, F. H., ASPINALL, D., and PALEG, L. G. 1967: Quantitative studies of root development. II. Growth in the early stages of development. *Aust. J. biol. Sci. 20*: 273–83.

NEWMAN, E. I. 1966: A method of estimating the total length of root in a sample. *J. appl. Ecol. 3*: 139–45.

RUSSELL, R. S. and SANDERSON, J. 1967: Nutrient uptake by different parts of the intact roots of plants. *J. exp. Bot. 18*: 491–508.

SOPER, K. 1957: Effects of changes in light and temperature on roots of perennial ryegrass (*Lolium perenne* L.). *N.Z. Jl Sci. Technol. A38*: 783–92.

———— 1958: Effects of flowering on the root system and summer survival of ryegrass. *N.Z. Jl agric. Res. 1*: 329–40.

TROUGHTON, A. 1955: The application of the allometric formula to the study of the relationship between the roots and shoots of young grass plants. *Agric. Prog. 30*: 59–65.

———— 1956: Studies on the growth of young grass plants with special reference to the relationship between the shoot and root systems. *J. Br. Grassld Soc. 11*: 56–65.

———— 1957: "The Underground Organs of Herbage Grasses". Commonwealth Agricultural Bureaux, Farnham Royal, England. 163 pp.

———— 1962: "The Roots of Temperate Cereals (Wheat, Barley, Oats and Rye)". Commonwealth Agricultural Bureaux, Farnham Royal, England. 91 pp.

WEAVER, J. E. 1926: "Root Development in Field Crops". McGraw Hill, New York. 297 pp.

WEAVER, J. E. and ZINK, E. 1945: Extent and longevity of the seminal roots of certain grasses. *Pl. Physiol. 20*: 359–79.

WEINMANN, H. 1948: Underground development and reserves of grasses. A review. *J. Br. Grassld Soc. 3*: 115–40.

WIEBE, H. H. and KRAMER, P. J. 1954: Translocation of radioactive isotopes from various regions of roots of barley seedlings. *Pl. Physiol. 29*: 342–48.