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SOME EFFECTS OF RADIATION AND TEMPERATURE ON THE VEGETATIVE GROWTH OF LOLIUM PERENNE L.

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in the Faculty of Agricultural Science

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STATEMENT

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University and, to the best of my knowledge and belief, it contains no material previously published or written by another person, except when due reference is made in the text.

J. H. SILSBURY

SUMMARY

Growth, morphogenesis and the growth physiology of the vegetative grass plant are described with particular reference to the important pasture species, Lolium perenne. Aspects of leaf morphogenesis, leaf initiation, leaf appearance, the development of the leaf surface, tiller initiation and expansion and the role of growth analysis are reviewed.

Experiments are described in which the growth of individual leaves and tillers of Lolium perenne were determined by harvesting at two day intervals for 32 days plants grown in nutrient culture under the nine combinations of three levels of visible light energy, 31, 52, 161 cal. cm^{-2} day^{-1} and three temperature regimes, 10, 20 and 30°C produced by a single controlled environment cabinet.

It was shown that by using 'standard' seedlings, identical growth responses to a particular environment could be produced at different times.

Data were mostly examined by comparing the time trends of any attribute in each environment. Growth rate was influenced by the endosperm reserves until Day 4 so major comparisons were made after this time.

The distribution of total dry matter between root and shoot and between leaf lamina and 'stem' was little influenced by the level of radiation or temperature. Relative growth rate (R) of the whole plant fell with time in most environments, the rate of decline being greater the higher the value of R. R was maximal at 20°C and was positively related to radiation.

Tillering was maximal at 20°C, was depressed as temperature rose to 30°C and was minimal at 10°C. On a leaf appearance basis tillering was depressed by high temperature under low radiation only. The initial relative growth rates of tillers could not be shown to be influenced by the environment. High temperature delayed tiller appearance. The control of tiller expansion by the level of assimilate in the whole plant is discussed and it is proposed that tiller buds require a certain 'threshhold' of assimilate before expansion commences.

Individual leaf size was dependent on the level of leaf insertion. Leaf weight was maximal at 20°C at all levels of radiation and generally increased with radiation level. Leaf area was minimal at high radiation so that specific leaf area decreased as radiation increased. All leaves produced in a given environment displayed a common allometric relationship between their area and their weight according to the formular $L_1 = bW_1^k$. The exponent k was approximately equal to 0.78 in all environments; the constant b was characteristic of the environment and estimates specific leaf area. An hypothesis based on an optimal temperature of $20^{\circ}C$ for cell division and a direct morphogenetic effect of light intensity on cell size is proposed to explain the control of leaf size in ryegrass by radiation and temperature.

Rate of leaf appearance on any tiller was constant with time in each environment, was maximal at 20 and $30^{\circ}C$ and decreased slightly with decrease in radiation.

The relationship between total leaf lamina area and total plant dry weight was approximated by an allometric formula and the mean relative leaf area growth rate was linearly related to the mean relative growth rate of the whole plant. The ratio between these two quantities was close to unity under favourable conditions but increased with temperature and as radiation decreased.

It is concluded that the vegetative growth of *Lolium perenne* is dominated by leaf growth and that the plant can achieve the same relative growth rate in different ways in different environments.

Increase in leaf area is considered to be an integral part of increase in dry weight and there is no causal relationship between these two processes during the early stages of vegetative growth under non-competitive conditions.

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1.0.0 INTRODUCTION

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The rate and the pattern of growth of grasses, both as single plants and as swards, have long been known to vary between species and to respond to changes in climatic factors during the growing season and to vary with the particular species in question. A great deal of agronomic research is devoted to the study of the effects of climatic factors on plant growth so that agricultural productivity may be increased through a better understanding of the relationships between plants and their environments.

Two important factors of the environment are the quantity of radiation received by plants over a given period and the temperature at which growth occurs. In the field these two factors are usually highly correlated so that high radiation is associated with high temperature and *vice versa*. It is therefore difficult to measure the effects of one independently of the other under field conditions. With the advent of controlled environment facilities it is now possible to vary radiation and temperature completely independently and by this means to study how each factor separately influences the various aspects of plant growth.

Ability to vary independently environmental factors that are usually correlated is certainly a major advantage of controlled environments but these facilities also have their disadvantages and limitations (Evans 1963).

They are artificial environments and do not present to the plant conditions that are fully comparable to those in the field. Artificial light differs from the sun in its spectral composition and maximum intensity. Further it is difficult to reproduce in a controlled environment the infinite array of patterns of temperature change that operate in the field. Air movement, CO_2 gradients and humidity changes are also subject in the field to spatial and time variations which are difficult to measure accurately and almost impossible to reproduce artificially. Yet despite these limitations, controlled environments have proved a valuable tool in research into plant growth and undoubtedly their uses will continue to expand as technical improvements are made in the degree to which 'environment' can be defined, controlled and reproduced.

The study reported in this thesis is concerned with the effects of radiation and temperature on the growth of perennial ryegrass, Lolium perenne L. A controlled environment cabinet was used for this purpose.

Perennial ryegrass is widely used as a pasture grass throughout the temperate regions of the world. It is extremely polymorphic and is adapted to a wide range of conditions. In Australia it is mostly confined to the better watered agricultural regions of the south of the Continent. Recently, new genotypes have been introduced from the Mediterranean region, and these are considered to possess considerable potential for increasing the adaptive range of this species in Australia (Silsbury 1961).

In the course of a programme aimed at increasing our knowledge of the growth, physiology and ecology of perennial ryegrass (Silsbury 1964, 1965), it became apparent that although a considerable amount is known about the control of reproductive development of ryegrass (largely through the work of J. P. Cooper at Aberystwyth) there is still much to be learnt about the influence of climate factors on vegetative growth. Though a number of contributions to this field have been reported in the literature (Mitchell 1953 a, 1953 b, 1954; Alberda 1957; Patel and Cooper 1961; Soper and Mitchell 1956) they do not give a sufficiently detailed or comprehensive account of the growth of the whole plant under completely specified conditions.

Evans _et al_. (1964) suggested that 'a further understanding of the effects of environmental conditions on both (grass) swards and single plants will involve an increasing degree of resolution of these effects on the components of grass growth'. This statement is not to be construed as advocating an approach to dry matter yield in grass such as was attempted by Engledow and Wadham (1924) in cereals, an analysis of 'yield components'. Rather it suggests that the effects of environmental factors on the growth of the whole plant should be examined in relation to the rates of production of individual organs, their sizes and their relationships one with the other. The latter approach has been adopted in this study.

A descriptive account of the growth and morphogenesis of the vegetative grass plant is given in the following section (Literature Review, 2.0.0). Here it is shown that the segmented unit of structure of the vegetative shoot is the 'phytomer' which produces leaves at its apical meristem and an axillary (tiller) bud in each leaf axil. Plant growth was therefore analysed in terms of rates of production of these organs, and the changes in their dry weights at three different levels of radiation and at three temperatures.

The upper limit of radiation was set by the light source of the single controlled environment cabinet available for this work. This was about 3,400 lumens ft.$^{-2}$ intensity. By using a day length of 16 hours and knowing the proportion of the total radiation as visible light (400-700 mμ) it was found that an energy input of approximately 161 cal. cm.$^{-2}$ day^{-1} could be achieved. This amount is roughly the same as received at Adelaide during the months of September-October and represents radiation conditions during spring. The two lower levels (31 and 52 cal. cm.$^{-2}$ day^{-1})

were chosen after a consideration of the photosynthesis-light energy response curves given by Gaastra (1959) so that a reasonable range in dry matter could be expected as a consequence of the three levels of radiation. The medium level is a little lower than mid-winter radiation at Adelaide.

Temperatures of 10, 20 and 30°C were chosen partly for convenience and partly to represent winter, spring and summer conditions respectively.

2.0.0 LITERATURE REVIEW

2.1.0 GROWTH, MORPHOGENESIS AND THE GROWTH PHYSIOLOGY OF THE VEGETATIVE GRASS PLANT

2.1.1 Vegetative Growth in Grasses

The growth physiology and the morphology of grasses may be studied in relation to four growth stages defined by Friend (1966) as: (i) pre-emergence; (ii) post-emergence; (iii) vegetative; and (iv) reproductive. Growth and morphogenesis need to be examined on the basis of growth stage because different growth processes occur in each stage and the plant may react to environmental factors in different ways in each stage.

In the pre-emergence stage for example, growth (change in volume) is largely determined by the rate of water uptake by the seed and the rate of redistribution of the material of the endosperm into the developing root and shoot. Also, reproductive growth (change in dry matter) of many grasses is characterised not only by the elaboration of the floral parts but by the elongation of the internodes (Barnard 1964; Silsbury 1965). It is advantageous therefore for growth and morphogenesis to be studied according to the growth stage.

The experiments reported in this thesis are concerned with the effects of radiation and temperature on the vegetative growth of the perennial ryegrass shoot.

Friend (1966) suggested that the beginning of the vegetative stage is marked by 'the achievement of independence by the seedling (of the endosperm reserves) through photosynthesis'. This is a useful definition but is limited in value by the absence of any precise point at which independence begins. These studies are largely concerned with vegetative growth but the transition from post-emergence growth is also considered.

2.1.2 General Morphology of the Grass Plant

The characteristic structural features of the Gramineae have been summarised recently by Barnard (1964). This article also provides an excellent account of the anatomy of the grass plant. Descriptions of the general morphology of grasses may also be found in the now classical studies of Arber (1934); several pasture grasses have been described by Evans and Grover (1940) and Etter (1951). More recently Metcalfe (1960) has published a monograph on the anatomy of the Monocotyledons and Jewiss (1966) and Bunting and Drennan (1966) have reviewed the morphology of pasture grasses and cereals respectively.

Barnard (1964) lists the combination of features that characterise the grasses and briefly describes how the different growth forms of the temperate grasses mainly arise from the pattern of lateral shoot development and the characteristics of the stem internodes. Ryegrasses typically have a tufted habit, the initial seedling shoot normally branching into a large number of daughter shoots (tillers) which become increasingly crowded together as the plant grows. Since internode elongation does not usually occur in ryegrass until the reproductive stage, the shoot meristems are situated close to the ground at the base of the plant during vegetative growth.

Vegetative growth of the grass shoot is brought about by the initiation of lateral appendages (leaves and tillers) on the shoot apex and their subsequent expansion into new organs. The segmented unit of structure is the phytomer (see Evans and Grover 1940) which consists of an internode (compressed during vegetative growth) together with the leaf at its upper end and a bud at its lower. Roots may also be initiated at the lower end. New phytomers are produced only at the shoot apex, the developmental morphology of which has been examined by a number of workers, notably Sharman (1945, 1947), Evans and Grover (1940), Cooper (1951), Barnard (1964).

The vegetative apex is a minute, meristematic cylinder enclosed within the leaves that have developed from it. Leaves are initiated laterally on the apex in acropetal succession to give a distichous arrangement.

Thus the vegetative growth of the ryegrass shoot consists of the initiation of leaf and tiller primordia on the shoot apex and the subsequent expansion of these primordia into discreet, functional organs (leaves) on the one hand and new phytomers on the other. Aspects of leaf and tiller growth will be considered separately in the following sections.

2.1.3 General Problems Encountered in the Study of Leaf Growth in Grasses

It is useful in studying leaf growth to be able to recognise distinctive phases of growth (see 'Growth of Leaves', Ed. Milthorpe, Butterworths, 1956). The initiation of leaf primordia on the apical meristem and their subsequent expansion into mature organs are clearly two such phases. Sometimes a third phase, that of unfolding of the leaf from the terminal bud, is also recognised in Dicotyledonous species (Milthorpe 1959).

Friend *et al*. (1962) distinguished two stages in the growth of the wheat leaf, each affected differently by the environment. The first stage occurs whilst the developing leaf is enclosed within the leaf sheaths and whilst it is dependent on a supply of assimilates and other growth factors from more mature leaves. The second stage occurs when the lamina emerges from the protecting sheaths and commences photosynthesis.

The fact that on emergence, a leaf experiences a sudden change in its environment has been pointed out by Begg and Wright (1962) from their studies of leaf growth in *Phalaris arundinacea*. These authors suggest that this change may be responsible for the sudden cessation of the growth of cells influencing blade length and that differences in the red-far-red balance between the inside and the outside of the sheaths may be responsible.

Further support for the view that the growth pattern of the grass leaf inside the sheath is different from the pattern after emergence may be found in the changes in relative growth rate of the wheat leaf described by Williams (1960).

There thus seems to be reasonable justification on morphological and physiological grounds for recognising three stages in the growth of the grass leaf: (i) initiation; (ii) initiation to appearance; and (iii) post-appearance. However the structural features of the grass plant make a clear-cut distinction between pre-appearance and post-appearance growth extremely difficult.

The general morphogenesis of the grass leaf is considered below (2.1.4) but it is relevant to point out here that the lamina of a young, developing grass leaf does not emerge from the sheaths all at once - the leaf is gradually pushed out of the sheaths and unrolls to finally expose the whole of the lamina. The appearance of the leaf is then a continuous process occurring simultaneously with post-appearance growth. This contrasts with the situation in many Dicotyledonous species where an immature leaf may unfold from the bud relatively rapidly, and then expand to its maximum area.

It seems that pre- and post-appearance leaf expansion in the grasses may not easily be separated even though it is clear that: (i) a considerable degree of leaf development occurs between initiation and appearance; and (ii) leaf physiology may differ before and after appearance. However, the stage of appearance of the leaf tip is very useful as a measure of the time at which any particular leaf commences to contribute, but its continued expansion, to the photosynthetic surface of the whole plant as well as to its dry weight.

2.1.4 Leaf Morphogenesis in the Grasses

Foliar histogenesis in the grasses has been fairly extensively studied and the description given for wheat by Barnard (1955) may be taken as an example. Leaf primordia are formed entirely from the dermatogen and the hypodermis, the two outermost layers of the cells of the apex which together constitute the tunica. The first indication of leaf formation is the occurrence of periclinal divisions in the hypodermis; subsequent development is by further periclinal divisions of the original tunica cells. Leaf primordia first became visible as crescent-shaped protuberances which, growing laterally and vertically, form a hood enclosing the parent apex. Further meristematic activity by the young leaf appears to become progressively restricted to a zone of cells at the base so that the leaf tip is pushed upwards to emerge eventually from the top of the encircling leaf sheaths.

Initially the leaf lamina and leaf sheath are not distinguishable but, according to Jewiss (1966), 'a band of parenchymatous tissue eventually divides the inter-calary meristem into two: the upper part is responsible for the growth of the lamina and the lower for that of the sheath'. In the rye seedling De Ropp (1946) has shown that sheath growth is delayed until lamina growth has begun to slow down.

The leaf matures from the tip downward (Sharman 1942; De Ropp 1946; Soper and Mitchell 1956). Sharman considered that differentiation of the ligule marks the end of meristematic activity of the lamina. This has been supported by Begg and Wright (1962) who concluded that in _Phalaris arundinacea_ elongation of the emerged portion of the lamina had ceased by the time it had emerged from the encircling leaves. Aspinall and Paleg (1964) found no change in the lamina area of a barley leaf once the auricle and ligule were fully exposed.

A major contribution to an understanding of leaf growth in grasses was made by Williams (1960) who by serial construction and dry weight determinations was able to give a complete quantitative account of leaf growth in wheat from initiation to maturity under constant conditions. Williams showed that the early growth of each primordium is exponential but that there is considerable variation in the trend of relative growth rate before emergence. In general there was an increase in the relative growth rate to a maximum just prior to emergence with a final falling away asymptotically to zero after emergence. Recently Williams and Rijven (1965) have confirmed these results and have further analysed leaf growth in terms of cellular generation, cell growth and protein metabolism. These authors support the contention of Sunderland (1960) that leaf growth is much more complex than suggested by a simple phasic model based on cell number and cell size.

Humphries and Wheeler (1963) in their review stress the important contribution made to leaf growth by cell division. Analysis of leaf growth in terms of increase in cell size and increase in cell number requires sophisticated techniques so it is not surprising that relatively few such studies have been made.

Williams and Rijven (1965) estimated the numbers of cells in the fourth leaf of their wheat plants at different times and it appears from their data that cell number did not reach a maximum value until about day 25 when the leaf had also reached its maximum dry weight. This contrasts markedly with the situation shown for the first pair of leaves of Phaseolus by Dale (1964), where cell division had ceased by the time the area of the expanding leaf was only about 17% of the final value. Sunderland (1960) on the other hand estimated that in lupin and sunflower, cell division was the main determinant of leaf growth during the unfolding of the leaf. Dale's results may however not be typical of the leaf pattern in Phaseolus as he was dealing with the first pair of leaves only and at a time of considerable activity at the apex which could be associated with competition for available nutrients.

It thus seems that leaf growth is due both to increase in cell number and to increase in cell size and that a generalised distinction cannot be made between these two processes; leaf growth is a continuous process in which cells are formed and expanded possibly at different rates according to the growth stage of the leaf. The data given by Williams and Rijven (1965) do not suggest that the emergence of the wheat leaf is characterised by any discontinuity in the processes which they examined during its growth and development.

Blackman (1961) emphasised that leaves are extremely plastic organs responding readily to changes in the environment. Such effects, although most readily studied following leaf appearance, may be induced early in leaf history. Gregory (1956) stressed that environmental effects were most likely to predominate at an early stage and Matsushima and Tanaka (1963) consider that the most susceptible stage of leaf development occurs just after the sheath has been differentiated when the sheath and lamina are growing vigorously. Milthorpe (1956) considered that leaf size was determined very largely by occurrences in the primordial stage but later (Milthorpe and Newton 1963) this conclusion was modified and greater stress placed on processes occurring during leaf expansion.

2.1.5 Leaf Initiation

The time interval between the initiation of successive leaf primordia on the apex is termed the 'plastochron' (Askenasy 1880). Subsequent usage appears to have become ambiguous and Milthorpe (1956) has pleaded for a return to the original definition. Schwabe (1957) suggested the use of 'apparent plastochron' to denote leaf appearance. Bunting and Drennan (1965) introduced the term 'phyllochron' for this purpose. There seems to be little advantage in the use of new terminology when the effectively descriptive 'leaf appearance' is now well established in the literature.

Floral induction is, of course, the most dramatic response of the apical meristem to the environment and must be regarded as being of primary importance in controlling leaf initiation. Evidence in favour of a positive promotion of leaf initiation by floral induction has been presented by Langer and Bussell (1964) and by Schwabe (1963). The ultimate control of leaf initiation by the floral apex however is negative, since after the inception of double ridges, the apex is normally committed to reproductive development and further leaf initiation is prevented.

Other indirect controls of leaf initiation on established apices are provided by the phenomena of apical dominance and dormancy. The former is more appropriately discussed in relation to tillering; the latter, although considered to occur in some grasses (Laude, 1953; Silsbury 1961, 1964) does not seem to be of extensive occurrence. Vegis (1963) has reviewed the climatic control of dormancy and bud break in many species.

Confining attention then to the vegetative, non-dormant grass apex, there appear to be few data available on the effect of environmental conditions on the rate of leaf initiation. This conclusion, reached by Evans _et al_. (1964) in their review is supported by Humphries and Wheeler (1963), who in their more specific survey point out that most studies of the early ontogeny of leaves have been morphological (and histological) rather than quantitative. Clowes (1961) in his comprehensive monograph on apical meristems provides little comment on environmental effects on leaf initiation. Finally Cutter (1965) in her recent review states that 'with the exception of photoperiod these (environmental) factors have been relatively little studied in relation to the shoot apex itself'.

This situation may perhaps be attributed to the fact that shoot apices are small objects and require micro-techniques for chemical analysis as well as histological description. Biochemical studies such as those of Williams and Rijven (1965) as well as manipulative experiments such as those of Wardlaw (1956) have done much to elucidate the 'internal' physiology of the apex in relation to leaf initiation but there still appears to be a need for the effects of the whole plant environment to be more closely examined for effects on apex physiology. This need is emphasised by Cutter (1965) who points out that the growth of new primordia is controlled by the apex and existing primordia and is 'probably a complex combination of hormonal relationships and requirements for nutrients and other substances', and that it is mediated through the organisation and physiological activity of the apex as a whole and the distribution of growth within it.

In cereals, study of the environmental effects on leaf initiation is made difficult by the early onset of floral initiation. Thus Friend et al. (1962) found in wheat that the rate of leaf initiation could not be followed for longer than about ten days. Nevertheless it was found that the rate of primordium formation increased by more than 50% when temperature was increased from 15°C to 20°C and that the maximum rate was attained at 25°C. Positive responses to increased light intensity (200 to 1750 lumens $ft.^{-2}$) were also recorded although these were of a lower magnitude than those due to temperature. Aspinall and Paleg (1964) working with barley also obtained responses to increased light intensity in the rate of leaf initiation on the vegetative apex. Leaf initiation in the tomato has been found to increase with temperature (15°C to 25°C) and with light intensity (Hussey 1963).

2.1.6 Leaf Appearance

It is necessary to define 'leaf appearance'. In grasses it is convenient to take the appearance of the leaf tip above the encircling leaf sheaths or the appearance of the ligule rather than the attainment of maximum area as a suitable criterion. The former is adopted.

It is now well established that, under constant environmental conditions, the rate of leaf appearance on a single shoot of many plants is constant with respect to time. This has been shown for ryegrass - Cooper (1951), Mitchell (1953 a), Patel and Cooper (1961); barley -

Aspinall and Paleg (1964); wheat - Friend _et al_. (1962); cucumber - Milthorpe and Newton (1963).

The constancy of the rate of leaf appearance is not attributable to the plastochron since unexpanded primordia may accumulate on the apex in advance of the expanding leaves (Cooper 1951), i.e. the plastochron may be shorter than the leaf appearance interval. This implies that the rate of leaf appearance is independent of apex size which is known to increase with age (Abbe and Phinney 1951).

The fact that the leaves of many grasses appear on any one shoot in a linear temporal sequence under constant conditions is considered to be of importance in relation to the growth rate of single plants and of plant communities and will be discussed further when considering overall responses to environmental factors and to plant density.

Temperature and light intensity appear to be the two environmental factors exerting the most marked effect on rate of leaf appearance. Hussey (1963) examined growth and development of the tomato under controlled conditions and recorded increased rates of leaf production with increased light intensity and temperature. Responses to these factors by cucumbers were also demonstrated by Milthorpe (1959).

In wheat, Friend _et al_. (1962) found that an increase in light intensity from 200 to 2500 lumens ft.$^{-2}$ increased the rate of leaf appearance from approximately 1.1 to 1.7 leaves per week, a relatively small response for a ten-fold increase in light intensity. Perennial ryegrass, in the study reported by Mitchell (1953 a) responded to an increase in light intensity from 700 to 2000 lumens ft.$^{-2}$ and a temperature increase from 50°F to 65°F, the 15°F rise in temperature having a greater effect than the trebling in light intensity. This dominant effect of temperature confirms the earlier data for ryegrass given by Cooper (1951). The rate of leaf appearance in timothy is also known to respond to increased temperature (Langer 1954).

Variation in the level of nutrient supply according to Langer (1966) does not markedly influence the rate of leaf appearance. Cooper (1948, 1951) found that when tillering of ryegrass was prevented by low nitrogen status the rate of leaf appearance in the main stem remained unaltered. Ryle (1964) recorded only a small difference in the number of leaves on the main stems of a number of perennial pasture grasses grown at 150 ppm of nitrogen and 15 ppm. At low levels of nitrogen however, Gott (1961) found that the rate of leaf appearance of wheat was reduced.

Photoperiodic effects on leaf appearance are known to occur independently of their effects on floral initiation (Cooper 1951; Friend _et al_. 1962).

Seasonal trends in leaf production by Ipomea were examined in a comprehensive analysis by Ashby and Wangermann (1950). The only environmental factor to which these trends could be related was the length of day.

2.1.7 Leaf Size

The final size attained by each leaf is related to the level of insertion on the shoot. Ashby (1948) demonstrated a heteroblastic change in leaf size with the node of insertion in Ipomea and Borrill (1959) and Saha (1960) recorded an increase in the sizes of successive laminae along the main stem of several grasses. Changes in the character of leaves emerging from successively higher nodes on a shoot of winter wheat have been demonstrated by Maeda (1959).

Borrill (1961) related differences in lamina width in Lolium temulentum to differences in epidermal cell number; differences in lamina and sheath length were related to differences in epidermal cell length. These findings suggest that leaf size is related to cell number and cell size in the way indicated but caution must be adopted in extrapolating from the epidermal cells to those of the whole leaf. Increasing leaf size up a shoot is also likely to be a consequence of the increasing size of each leaf primordium associated with enlargement of the apical meristem with age (Abbe, Randolph and Einset 1941;

Ashby and Wangermann 1950; Yamazaki 1964 b).

A positive relation between the size of the leaf initial and final leaf size was shown by Abbe and Phinney (1951); the width of the initial itself was related to the circumference of the stem apex. Yamazaki (1963) concluded from a study on rice that the size and structure of the leaf were primarily controlled by the size of the shoot apex on which it was initiated - the length of the mature leaf corresponds to the height and the width to the basal diameter of the shoot apex.

Leaf shape and form as well as size are known to change with plant age and to be influenced by a number of environmental factors (Jones 1956; Schwabe 1963; Humphries and Wheeler 1963).

Mineral nutrition, especially the level of nitrogen, affects leaf size in many grasses (Ballard and Petrie 1936; Yamazaki 1964 a). Ryle (1964) found a relatively small promotive effect of nitrogen on leaf size in a number of perennial grasses but this could have been due to low radiation at the time the study was conducted.

It is well known that the size, shape and structure of the leaves of a large number of species may be markedly influenced by the level of radiation, responses to this factor often being described in terms of "sun" and "shade" leaves. This field is not reviewed extensively here; attention is confined to a few studies in which quantitative responses to radiation level have been recorded in terms of leaf area and leaf weight rather than leaf anatomy and leaf structure. Light intensity (and temperature) effects on the leaves of Monocotyledons appear to be less well documented than those of Dicotyledons.

A distinction must be made between the effects of radiation and temperature (or indeed any other factor) on leaf size _per se_ and the leaf area (weight) of the whole plant. Unfortunately these separate effects of environmental factors are not always clearly defined in experiments reported in the literature with the result that it is often impossible to determine whether the responses recorded are due to changes in one or more of leaf size, leaf production or the rate of expansion of each individual leaf.

Responses in leaf size to radiation level have often been determined by shading experiments in natural daylight but with the advent of controlled environment facilities it is now common for radiation and temperature to be varied independently of each other.

Njoku (1956) recorded small and non-significant increases in the mean areas of individual leaves of _Ipomea caerulea_ up to the seventh node on plants grown in 0.74 daylight compared with full daylight. Leaves at the eighth and ninth nodes were smaller in area at the lower than at the higher intensity. Reduction to light intensities of 0.56 daylight and lower, generally reduced the areas of all leaves at all nodes.

Small differences in the areas and in the weights of ryegrass leaves in response to differences in light and temperature have been reported by Mitchell (1954). The average of the areas of the sixth leaf on the main stem of Short Rotation and New Zealand perennial ryegrass increased slightly in area and leaf dry weight decreased (14.0 - 11.1 mgm.) when the light regime was decreased from 'full' daylight to 30% of 'full' daylight. In later experiments Mitchell (1956) recorded a general decline in the 'dry weight of leaf blade' of perennial ryegrass as the temperature increased from $55^{\circ}F$ to $95^{\circ}F$. These responses however were obtained from measurement of a single plant at the end of a growth period of 8 to 15 days and it is not clear whether the period of growth was similar at all temperatures for the one species.

Dale (1965) working with <u>Phaseolus</u> found that the first pair of leaves showed a marked optimum relation between area and radiation over the range 15 - 100 cal. $cm.^{-2}$ day^{-1}, but for the first three trifoliate leaves the relative depression at high levels of radiation was not as great as for the first pair. Newton (1963) also postulated an optimum relation between radiation and individual leaf area in the cucumber at a moderate level of plant nutrition.

Data of Friend *et al.* (1962) provide a comprehensive account of size responses by the wheat leaf to a wide range of light intensities and temperature levels (200–2500 ft.c. and 10–30°C). Taking the third leaf on the main stem as an example, leaf area was maximal at 20°C but leaf weight (at maximum area) was maximal at 15°C. Lamina thickness increased with increasing light intensity and lamina dimensions (length and breadth) were also influenced by light intensity and temperature. These responses were subsequently summarised by Friend (1966) and related to changes in the shape, size and number of the component cells. An increase in the light intensity in which the plant was grown resulted in a shorter, broader and thicker lamina; an increase in temperature over the range 10–30°C resulted in narrower and thinner leaves.

Changes in leaf size induced by changes in radiation and temperature are often recorded in terms of the leaf area to leaf weight ratio, the specific leaf area (S.L.A.). It is well known (Blackman 1956, 1961; Evans and Hughes 1961; Hughes and Evans 1962; Dale 1965; Friend *et al.* 1962) that S.L.A. is particularly sensitive to changes in radiation and also may be influenced by temperature. Generally S.L.A. increases as radiation decreases.

The clearest picture of this response is probably that provided for Salvinia natans by Blackman (1961). In experiments with this species the highest S.L.A. was obtained at the highest temperature (30°C) and the lowest light intensity (300 ft.c.); the lowest S.L.A. was obtained at the other extremes (20°C, 1800 ft.c.). In discussing this result Blackman drew attention to the plasticity of the response by the leaf to light intensity and temperature and inferred that it is the magnitude of the response to each factor that determines the resulting specific leaf area at any combination of light intensity and temperature.

In general it appears that the weights and the areas of individual leaves of different species display different patterns of response to radiation and temperature, each attribute following an optimum response curve with each factor. The positions of the optima vary between species and there is evidence of interaction between the effects of the two factors. A major problem remains however, in resolving how the different growth processes within the plant and within the leaf itself are separately influenced by the environmental factors to bring about variation in leaf size.

The level of radiation may influence leaf size in two ways: firstly through the level of assimilate available to the leaf primordium; and secondly through a direct morphogenetic effect of light on the leaf or on the whole plant.

A leaf, early in its development, is characterised by high rates of cell division with a consequent high demand for assimilate (Milthorpe 1959; Newton 1963) and any restriction in the supply of assimilate to the apex is likely to influence leaf size. Such a supply will undoubtedly be influenced by the overall level of radiation of the plant, by the competitive demands made by other plant parts and by the rate at which assimilate is transported to the apex. Thus any restrictions to leaf development imposed by assimilate supply to the apex are likely to follow a complex pattern depending on radiation, growth stage (plant size) and temperature. The extent to which this occurs will also depend on the relative importance of the primordial stage in determining final stage. In grasses it seems likely that the early stages are of considerable importance in view of the known relations between the dimensions of the apex and those of the mature leaf. Further, the young grass leaf is completely dependent on imported carbohydrate for the appreciable development that occurs prior to leaf appearance.

Direct morphogenetic effects of light on leaf size of wheat have been proposed by Friend _et al_. (1962) and Friend (1966) in terms of a light controlled hormonal system (possibly similar to the phytochrome reaction) and the possible destruction of gibberellins at high light intensities limiting cell expansion. The final size attained by a leaf could well be the resultant of both promotive and suppressive processes.

Gregory (1956) summarised what he considered to be the (internal) determinants of leaf size as: (i) the number of primordial cells; (ii) rates of cell division; (iii) duration of the phase of cell division; (iv) size of the mature cells. At this time Gregory apparently was not subscribing to what was then the more common view that leaf growth occurred in two distinct phases of cell division and cell enlargement. An analysis of variation in the size of the grass leaf in response to radiation and temperature in terms of the above determinants is not possible at present. In Dicotyledons, current opinion (Humphries and Wheeler 1963) is that cell number is of pre-eminent importance but the position in the Monocotyledons is by no means clear. Friend (1966) indicated that the greater length of wheat leaves grown under low light intensities was primarily related to increased cell division along the length of the lamina. Temperature effects were brought about primarily by increases in cell length, a result essentially in agreement with the conclusion of Davidson and Milthorpe (1965) that in <u>Dactylis</u> <u>glomerata</u> cell size increased with temperature.

2.1.8 Development of the Leaf Surface and Leaf Growth on the Whole Plant

The development of the leaf surface (leaf area) has received considerable attention from plant research workers in recent years, particularly in relation to crop productivity. Interest in this field has sprung largely from application of the techniques of growth analysis by D. J. Watson and his colleagues at Rothamsted and G. E. Blackman and co-workers at Oxford. These studies have been concerned more with the development of crop leaf area and community structure than with the physiology and morphology of leaf growth itself.

In his first study of the increase in leaf area and increase in the total leaf surface of the cucumber plant, Gregory (1921) remarked that 'no quantitative studies have yet appeared dealing with the increase in area of leaves from day to day'. Since that time the number of quantitative studies of leaf growth that have been made seem disproportionately small when, as stressed by Watson (1952, 1956), crop yield depends largely on the rate of development and maintenance of the leaf surface.

Leaves contribute to the photosynthetic surface of the plant by their size (area) and by their number. The rate of development of the first leaf on a seedling plant may be of considerable importance in relation to subsequent growth. Later on total leaf number is perhaps more relevant than leaf size.

The most comprehensive experiments conducted on the effects of environmental conditions on leaf growth are the studies on cucumbers initiated by Gregory (1921, 1928) and further developed by F. L. Milthorpe and his students at Nottingham.

Both the increase in area of individual leaves and the growth in area of the total leaf surface were examined by Gregory, largely in relation to temperature. There was no single curve that described the rate of expansion of the cotyledons with respect to time but the increase in area of single leaves was of S-form and could be represented by the formula for an autocatalytic reaction.

Gregory further showed that the relative growth rate of the total leaf area measured on a time scale as days from germination, conformed with the equation,

$$\frac{1}{A} \cdot \frac{dA}{dt} = \frac{r}{t^n}$$

where A is the leaf area, r a measure of the growth rate and t the time from germination. The value of the exponent 'n' was temperature dependent. In addition, temperature had a marked effect on the time of unfolding of the first leaf, due to a direct effect on the rate of metabolism and differentiation at the shoot apex. If expansion of the total leaf surface was considered as days from unfolding of the first leaf, a much more uniform series of curves for different temperatures was obtained. These were all closely approximated by a parabolic function, i.e. 'n' = 1 in the above equation.

Milthorpe (1956) in taking up Gregory's work again considered that the rate of expansion of the leaf surface could best be analysed in terms of two parameters: (i) time for a measurable area of leaf to appear; and (ii) the rate of growth of the leaf surface relative to that already present. In this paper considerable stress was laid on the importance of the primordial stage of leaf growth in influencing the final area. This conclusion was later modified (Milthorpe and Newton 1963) when evidence was presented which supported the finding of Sunderland (1960) that cell division is the dominant process contributing to leaf expansion after unfolding.

In a further examination of the effects of temperature on development of the leaf surface, Milthorpe (1959) was unable to confirm Gregory's earlier finding that the relative rate of expansion of the leaf surface was independent of temperature over the sub-optimal range. This difference was attributed to the relatively low light intensities employed by Gregory and the short term of the experiments. An optimal relation between relative leaf growth rate and temperature was proposed. Results however did support Gregory's conclusion that the relative growth rate of the leaf surface is largely independent of the net assimilation rate.

Effects of radiation (15-120 cal. cm.$^{-2}$ day^{-1} visible radiation) on the leaf growth of cucumbers were reported by Newton (1963). Here it was found that the total amount of radiation received was more important than light intensity or duration although photoperiod did have a significant effect on leaf expansion independently of the total amount of radiation. Photoperiod (or intensity) influenced the final area reached by the first three leaves. The maximum rate of expansion of a leaf at any one node was attained at an intermediate level of radiation although the rate of leaf production and appearance of unfolded leaves increased with radiation. The relative rate of expansion of the total leaf surface followed an optimum curve with radiation, reaching a maximum at about 80-90 cal. cm.$^{-2}$ day^{-1}.

The above effects of temperature and radiation on leaf production and leaf expansion and their resulting effects on plant growth were interpreted in terms of the demand for, and the supply of, mineral nutrients and assimilates to the leaves during their various phases of growth and in terms of the growth of the whole plant.

The potential rate of development was considered to be set by the rate of unfolding of leaves from the terminal bud (leaf appearance). Up to unfolding, leaves have a high demand for carbohydrate but a relatively low demand for nutrients which is not influenced by a wide range in potential supply. Limitation of carbohydrate is due to poorly developed vascular connections at this stage. During subsequent expansion carbohydrate requirement can soon be met by assimilation so the leaf does not require an 'external' supply. Mineral nutrient demand is high during expansion and when a large number of leaves is expanding the demand may exceed the supply leading to competition between leaves and a reduced leaf size. Evidence in favour of a high nutrient demand by an expanding leaf was not provided by these experiments but later Hopkinson (1964) showed that the importation of phosphorus into a leaf reached a peak before the maximum area was attained.

Milthorpe and Newton (1963) report an attempt to examine further the interaction between nutrient supply and amount of radiation in determining final leaf size. Nutrient supply was governed by the pot size; increasing differences (in favour of the larger pot) in the areas of successive leaves were recorded at the higher amount of radiation. It is difficult to see why this difference should occur so early but the results do provide support for the view that low nutrient level limits leaf development under high amounts of radiation. However the authors point out that results should be interpreted with caution since anomalous behaviour in other respects was noted.

Caution should also be adopted in applying the above interpretation to other species. It appears that during the early growth of the cucumber a large number of leaves is _produced_ at the apex (up to 20), but a relatively small proportion (about 25%) of these unfold and actually expand. A competitive situation such as described above could thus easily develop in the cucumber but this may not always be the case in other species. Further, it is difficult to reconcile the relatively large number of leaves produced with the concept of limitation of growth at the apex by carbohydrate supply unless it is postulated that the demand for assimilate for leaf production differs from that for actual unfolding. Differences between the rates of leaf production and unfolding (though each was constant with respect to time in any environment) were recorded (Milthorpe 1959) but the data did not permit an explanation of this difference to be attempted. It seems that this point would be well worth exploring.

Friend _et al_. (1962) in their study of leaf growth in wheat did not agree with the above hypothesis but regarded the morphogenetic effects of temperature, light intensity and photoperiod as being probably related to changes in the concentration of growth substances. However the experimental plants used by these authors were grown under a completely favourable nutrient regime and responses to nutrient levels were not examined.

2.1.9 Initiation and Growth of Tillers

Axillary tiller primordia are initiated at the shoot apex at almost the same time as are the leaf primordia that subtend them. Sharman (1942) regarded the axillary bud as the last stage of a phase of growth that starts with the initiation of a leaf primordium on one side of the apex and that passes down the internode and concludes with the formation of a bud on the other side of the stem. A bud is thus associated with the leaf above rather than with the leaf that subtends it.

Clowes (1961) has remarked that little has been published on the physiology of bud initiation. Certainly bud histogenesis and early morphogenesis appear to be poorly documented for the Gramineae as a whole in contrast to the relatively large number of studies that have been made of the leaf and of the floral primordia. A notable exception is the work of Sharman (1945).

It seems generally agreed that tillers arise on the apical meristem in acropetal succession in a similar manner to leaves except that they originate from the sub-hypodermal cells rather than from the cells of the tunica (Hamilton 1948; Sharman 1942, 1945; Barnard 1955). Buds appear to be initiated in the axils of almost all leaves but the proportion that develops further, depends on genotype, the environment and the nutritional status of the plant.

Tiller primordia differ from leaf primordia in being virtually a duplication of the original apical meristem and capable of producing new phytomers. Theoretically, therefore as Gregory (1956) has pointed out, tiller primordia are capable of unlimited growth whereas leaf primordia have a limited growth potential and life span. Tillers are then extremely important to the plant as a means of increasing the number of meristematic centres, i.e. of vegetative propagation. In many grass species daughter tillers may be readily separated from the parent stem and established as independent units.

Tillers, like leaves, arise within the protected environment of the leaf sheaths and the first organ that is produced on the tiller must eventually emerge outside. This is achieved (Arber 1934) by the tiller bud bursting through the enclosing sheath at its base (extravaginal tillering) or by an upward growth in the same way as a normal leaf (intra-vaginal tillering). The ryegrasses tiller intra-vaginally.

The first organ produced by a tiller does not differentiate into lamina and sheath and is therefore usually termed a prophyll (Etter 1951; Soper and Mitchell 1956). McCall (1934) examined this structure in wheat and concluded that the prophyll is completely analogous with the coleoptile. In ryegrass the prophyll is a short, translucent, leaf-like organ which does not emerge from the leaf sheaths; it grows initially at about the same rate as the first true leaf.

The morphology of the ryegrass seedling has been described by Soper and Mitchell (1956). Mitchell (1953 b) proposed a system of notation to identify the various organs of the plant as leaf growth and tillering proceed. This system identifies the primary tillers developed on the main stem by the subtending leaf - the tiller at the coleoptile node is termed the coleoptile tiller (C.); that in the axil of leaf one, tiller one (T_1) and so on. Similarly, the secondary tillers developed on the primaries are T_1CT (in the axil of the prophyll), T_1T_1 and so on. Tertiary tillers are denoted $T_1T_1T_1$, etc. This system seems more logical than that proposed for wheat by Friend (1965).

It should be pointed out that the tillering pattern displayed by any particular grass is influenced by the genotype and by the environment. For example, Langer (1956) has shown that in Phleum pratense tillers usually do not develop in the first and second leaf axils and up to five leaves may be produced on the main stem before the first tiller appears. Similarly, Mitchell (1953 b) noted that in ryegrass a tiller normally only appeared on any particular shoot when there was one fully expanded and one expanding leaf above it. The nodal position of the first tiller on seedlings of ryegrass and timothy has been shown by Patel and Cooper (1961) to be influenced by seasonal conditions and by genotype.

Since tillers are only produced in the leaf axils it is to be expected that the primary determinant of the rate of increase in tiller number on a seedling plant will be the rate of leaf appearance. Mitchell (1953 b) therefore proposed that 'leaf appearance age' should be used as a 'time' scale rather than chronological age when rates of tillering are to be examined. In critical studies this is an advantage, at least during early growth stages, but for general comparative purposes a normal time scale is probably adequate.

Tiller number per plant can increase very rapidly under suitable conditions. This was demonstrated by

Waldron (1921) working with Bromus inermis. More recently Friend (1965) has discussed a tillering model for 'Marquis' wheat based on a constant rate of leaf appearance and a constant rate of tiller appearance at a given leaf number interval. The numbers of tillers at different times then form a Fibonacci series so that tiller number can soon be expected to become exponential with respect to time. In the same paper Friend also presented data which show that tiller number increased more or less logarithmically for several weeks. An exponential increase in tiller number with time has also been shown to occur in ryegrass (Alberda 1957; Silsbury 1965; Cooper 1948; del Pozo 1963) and in barley grown under favourable conditions (Aspinall and Paleg 1964).

2.2.0 THE ROLE OF GROWTH ANALYSIS IN ASSESSING PLANT RESPONSE TO ENVIRONMENTAL FACTORS

2.2.1 Growth Analysis

The term 'growth analysis' has come to be used, mostly by British physiologists, to refer to certain growth functions relating plant dry weight, assimilatory surface and time.

An account of the early genesis of the concepts employed in growth analysis has been given by Blackman (1961).

In this review Blackman outlines the contributions made by the early workers in this field to the establishment from about 1920 to 1930 of the concepts of Relative Growth Rate (R), Net Assimilation Rate (E) and Leaf Area Ratio (L.A.R.). He then follows the subsequent history of the use of these functions, paying particular attention to that of the net assimilation rate, and describes more recent work at Oxford relating changes in R, E and L.A.R. of a number of species to both seasonal climatic changes and to artificial changes in radiation induced by shading. The use of growth analysis in assessing the whole plant's reactions to environmental factors is strongly advocated in this review.

Table 1 has been constructed to show how the three major functions employed in growth analysis are determined and the limitations associated with the application of any particular formula. The references considered to be the most relevant are also listed in the table. It remains to be recorded that Briggs _et al_. (1920-21) showed that the relative growth rate is the product of net assimilation rate and the leaf area ratio if L and W are increasing exponentially with respect to time,

$$\text{i.e.} \quad \frac{1}{L} \cdot \frac{dW}{dt} \cdot \frac{L}{W} = \frac{1}{W} \cdot \frac{dW}{dt} = R$$

The application of growth analysis techniques to agronomic and ecological problems of plant growth does not

TABLE 1.

SUMMARY OF FUNCTIONS USED IN GROWTH ANALYSIS

Symbols for Primary Data

W = Dry weight of whole plant or portion; Ws = Weight shoot; WR = Weight Root; WL = Weight Leaf;

L = Any leaf attribute used as a measure of the active growing material, i.e. LA = Leaf area; LW = Leaf weight; LN = Leaf total nitrogen.

t = Time.

All logarithms to base (e).

Derived Functions

Name	Symbol	Value at time t	Average over $t_2 - t_1$	Usual formula for derivation	Restrictions on formula	References
Relative Growth Rate Efficiency Index	R.	$\frac{1}{W} \cdot \frac{dW}{dt}$	$\frac{1}{t_2 - t_1} \int_{t_1}^{t_2} \frac{d \log W}{dt} \cdot dt$	$\frac{\log W_2 - \log W_1}{t_2 - t_1}$	Any form of curve	Blackman (1919)
Net Assimilation Rate Unit Leaf Rate	E.	$\frac{1}{L} \cdot \frac{dW}{dt}$	$\frac{1}{t_2 - t_1} \int_{t_1}^{t_2} \frac{1}{L} \frac{dW}{dt} \cdot dt$	(1) $\frac{W_2 - W_1}{L_2 - L_1} \cdot \frac{\log L_2 - \log L_1}{t_2 - t_1}$	$\frac{dW}{dL}$ constant for period of measurement	Gregory (1926) Williams (1946) Hughes and Evans (1962) Coombe (1960)
				(2) $\frac{W_2 - W_1}{t_2 - t_1} \cdot \frac{2}{A_2 + A_1}$	W increases as square of L	
Leaf Area Ratio	L.A.R.	$\frac{L}{W}$	$\frac{1}{2}\left(\frac{L_1}{W_1} + \frac{L_2}{W_2}\right)$	(1) $\frac{1}{2}\left(\frac{L_1}{W_1} + \frac{L_2}{W_2}\right)$	Methods 1 and 2 give almost same values	Blackman, Black and Kemp (1955)
				(2) $\frac{L_2 - L_1}{W_2 - W_1} \cdot \frac{\log W_2 - \log W_1}{\log L_1 - \log L_2}$		

appear to have been particularly wide, nor it may be argued, particularly fruitful. The former deficiency was commented on as early as 1938 by Heath and Gregory and the latter criticism has been challenged by Blackman (1961).

Watson (1952) in reviewing the use of growth analysis as applied to the study of field crops argued that 'the progress of dry matter accumulation and its end point yield at harvest can be completely described in terms of two attributes, E and leaf area'. He then showed that there were major limitations of the concept of E when applied to field crops and also that E displayed marked seasonal trends. The conclusion was reached that although E was shown to vary between species (in contrast to the earlier conclusion of Heath and Gregory, 1938) there seemed to be little opportunity for influencing dry matter yield through E and that much greater scope existed in this direction through varying leaf area.

The period since the publication of Watson's review has been characterised, quite logically, by the appearance of many papers devoted to the relation between leaf area and growth and relatively few to the relation between E and growth. The former studies have been concerned more with light interception, crop leaf area and community structure than with the development of leaf area by plants growing individually or in competition with each other. The plea by Watson at the end of his paper for more work on the physiology of leaf growth has not been entirely unfruitful but in the agronomic, as distinct from the physiological field, there is still a need for a closer examination of leaf production, leaf expansion and leaf senescence, particularly in the pasture grasses where the leaf is essentially the economic unit of dry matter production.

2.2.2 Relative Growth Rate

It seems that of the three major functions that constitute growth analysis, relative growth rate has been the most widely used. This is because the amount of dry matter produced after a given time interval can be analysed in terms of the initial amount present (the initial capital) and R the rate of relative increase (the rate of interest). Williams (1964) has advocated more extensive use of R because it records the multiplication of living substance.

Watson (1952) emphasised that all of the dry weight of a plant is not productive capital and it is probably a decline in the proportion of meristematic tissue to other tissue that best accounts for the general phenomenon of a decline with time in the relative growth rate of an entire isolated plant. This latter fact means of course, that care must be exercised in the use of R for comparative purposes, either between species or between environments. Also it is clear from the work of Williams (1960) that a decline of R with time cannot be held to apply too widely since in that paper it was shown that the relative growth rate of a single organ such as a leaf may show a complex pattern during development, rising, remaining constant or falling with time. In this case R is undoubtedly influenced by internal mechanisms and by developmental stage.

The general tendency for R to fall with time does not appear to have been intensively studied in plants. Thorne (1960) has shown that this occurs in a constant environment.

2.2.3 Net Assimilation Rate

This component of the relative growth rate has received considerable attention since it was first enunciated by Gregory (1917). Most of the limitations of the concept have been set out by Watson (1952).

(1) E is the resulting difference between photosynthesis per unit leaf area and the respiration of the whole plant similarly expressed. Changes induced by the environment, or those resulting from age effects, may influence these processes independently or in a complementary manner.

(2) No single, precise and easily determined measure of the essential 'growing material' has yet been suggested - leaf area, leaf dry weight, leaf protein nitrogen, cytoplasmic protein nitrogen all have their limitations. Watson urges the use of leaf area for purposes of uniformity. Chloroplasts or chlorophyll (see Brougham 1960) are attractive but Watson cogently points out that whilst photosynthesis occurs in chloroplasts, respiration occurs in all plant organs.

(3) E is underestimated when the root fraction is excluded.

(4) Photosynthesis occurs elsewhere in the plant apart from the leaves, in the ears of cereals and in the leaf sheaths (Thorne 1959).

(5) As plants become larger mutual shading of leaves occurs with the result that E has little meaning when applied to communities or even to large single plants.

Two major practical difficulties associated with the estimation of E are the accurate measurement of the leaf surface and the actual formula employed to calculate mean E. The former problem is no longer a major one as a suitable instrument is now available (Jenkins 1959) for the handling of large leaf samples.

The expression of E is more difficult. Williams

(1946) pointed out that the function,

$$\frac{1}{t_2 - t_1} \int_{t_2}^{t_1} \frac{1}{L} \cdot \frac{dW}{dt} \cdot dt$$

is not integrable unless the relation between L and W is known. If the relation is linear $\frac{dW}{dL}$ will be constant and the first equation for E given in Table 1 may be used. By using a method of graphical interpolation which was assumed to give the most accurate assessment of mean E, Williams was able to show that this equation gives a reliable estimate where the inter-harvest interval was less than four days.

It is probably not the absolute interval of time that is important but the relation between W and L during the period between harvests. Coombe (1960) quoting from the unpublished lectures of Briggs showed that if W increases as the square of L then the second equation given in Table 1 holds. Coombe further pointed out that the percentage difference between the values of Em (the mean of all the instantaneous values of E over a period) estimated by equations 1 and 2 is a function of the ratio L_2/L_1. If this ratio is small the difference between the two methods is negligible.

Hughes and Evans (1962) in further assessing the merits of the two equations suggest that the most appropriate formula can only be selected when detailed information on the relations between L, W and t is available. Even a general idea of these relations will assist in selecting between equations 1 and 2, or whether the mean between them is most appropriate.

A further practical problem is the level of accuracy that can be achieved in the measurement of the dry weight increments and mean leaf areas for the intervals between harvests. This may be reduced by the method employed by Goodall (1945).

2.2.4 Leaf Area Ratio

Leaf area ratio does not appear from the literature to have been intensively studied as a component of relative growth rate. It is difficult to see why this should be so when the foregoing discussion of E has made it clear that the relation between L and W is important in determining the best means of estimating E.

Further it seems likely that study of the extent to which the dry weight of a leaf is distributed spatially would prove extremely rewarding. Hughes (1959) introduced the term specific leaf area (S.L.A.) to denote leaf area/ leaf weight and Hughes and Evans (1962) considered that there were merits in regarding this as a separate component of L.A.R., i.e. L.A.R. = S.L.A. x leaf weight/plant weight.

2.2.5 Growth Analysis and Response to Environmental Factors

It was mentioned earlier that the techniques of growth analysis are considered by Blackman (1961) to be a valuable aid in the assessment of the whole plant's reactions to environmental factors.

An early approach, pioneered by Gregory (1926) and later adopted by Goodall (1945), Watson (1947), Blackman, Black and Kemp (1955) and Black (1955) was to relate variation in R, E and L.A.R. and other attributes to fluctuations in total radiation and temperature recorded over an interval by means of multiple regression analysis. This technique is open to the criticism that variation in radiation and temperature are positively associated so that it is difficult in nature to achieve independent variation in these factors over a suitable range. The results have therefore been of limited value in defining the precise effects of radiation and temperature on growth. Indeed the method can lead to erroneous conclusions. Black (1955), for example, concluded that the relative growth rate of _Trifolium subterraneum_ cv. _Bacchus Marsh_ is independent of temperature. Morley (1958) subsequently concluded from experiments conducted in controlled environments that in a number of cultures of this species R is significantly affected by temperature, although the effect of temperature was small in Bacchus Marsh. Morley further pointed out that Black's use of very small seedlings to reduce experimental error and minimise ontogenetic drifts with age may have in part been responsible for his (Black's) failure to obtain a temperature effect.

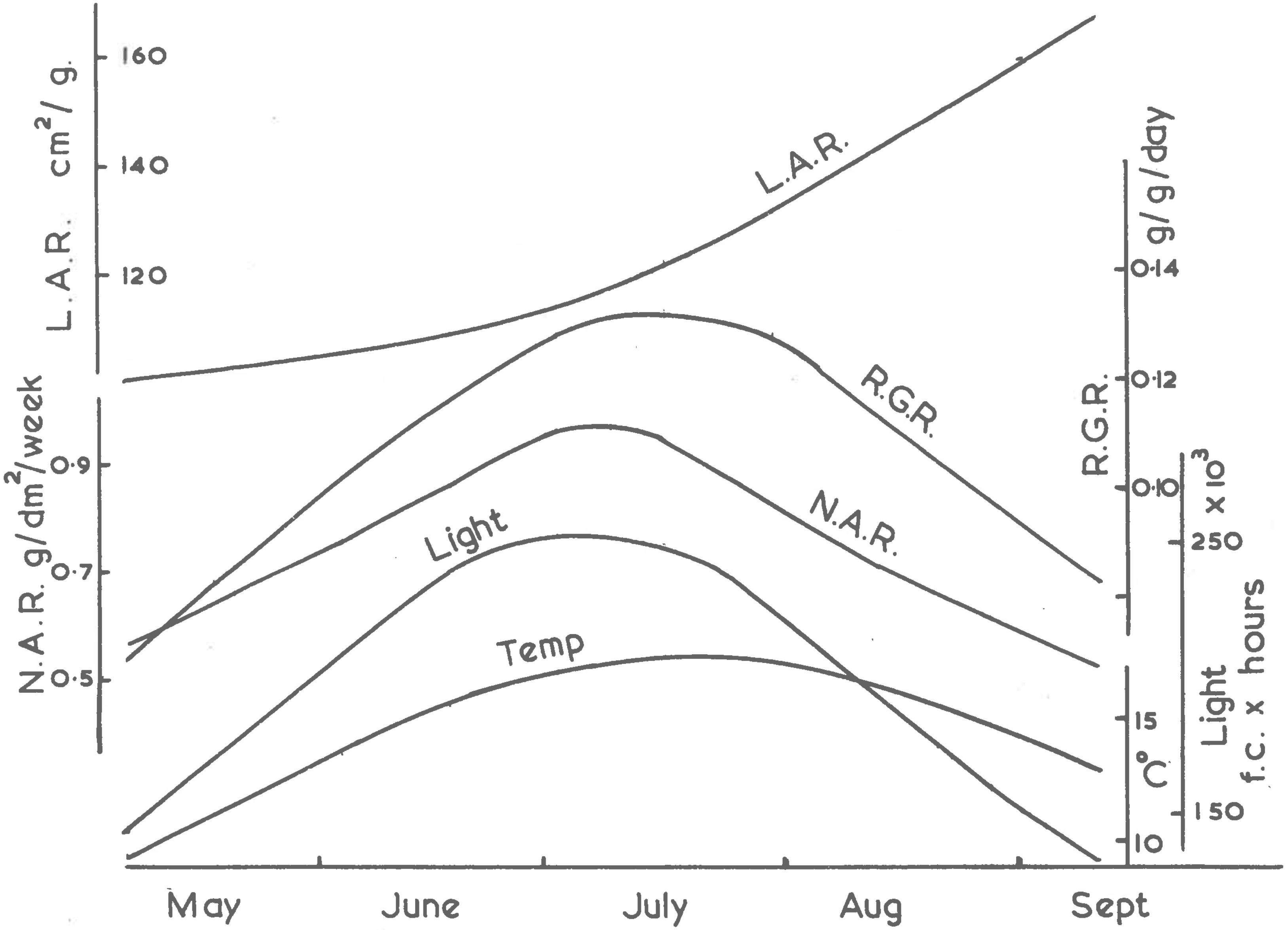
It seems then that multiple regression analysis, although useful for delineating trends in responses, is not a sufficiently accurate means of obtaining precise relations.

A superior approach for the analysis of radiation effects is to use artificial shading to vary the amount of light received by plants. This method has been used extensively at Oxford by Blackman and his colleagues, e.g. Blackman and Black (1959) to determine the response of a wide range of plant species to the light factor. A summary of the responses in R, E, and L.A.R. to seasonal changes is shown in Figure 1 which is taken from Blackman (1961). Evans and Hughes (1961) also used a similar technique to study <u>Impatiens parviflora</u>. Clearly this method permits response to radiation to be defined with much greater precision but it still does not completely preclude the possible occurrence of correlated temperature effects since reduction in light intensity may also reduce leaf temperature.

The most satisfactory method of resolving the independent effects of light energy and temperature on plant growth is by means of controlled environment facilities. The problems associated with the use of phytotrons and the dangers of extrapolation have been outlined in (1.0.0), but it is clear that this method is the best means of determining the response of the whole plant to environmental factors. Thorne (1960) has used controlled environments to determine age effects on net assimilation rate and, as reported by Blackman (1961), Achurch appears to have successfully employed the techniques to study plant growth under controlled conditions.

In this study controlled environment facilities are used to examine the effects of radiation and temperature on the pattern of vegetative growth of perennial ryegrass. Techniques of growth analysis are explored as an adjunct to this end.

Figure 1 Trends in relative growth rate (R), net assimilation rate (E), and leaf area ratio (L.A.R.) with seasonal changes in light and temperature for single plants of *Helianthus annus* at Oxford, U.K. (From Blackman 1961).


L.A.R. cm²/g.
160
140
120
N.A.R. g/dm²/week
0·9
0·7
0·5
L.A.R.
R.G.R.
N.A.R.
Light
Temp
g/g/day
0·14
0·12
0·10
R.G.R.
x 10³
250
150
Light f.c. x hours
15
°C
10
May
June
July
Aug
Sept

2.3.0 VEGETATIVE GROWTH OF THE GRASS PLANT IN RELATION TO LEAF AND TILLER DEVELOPMENT

It is necessary to pursue the subjects of the growth of individual leaves and tillers to their logical conclusion which is an examination of the manner in which these organs contribute to the increase in dry matter of the whole plant. The literature on this topic is not extensive and relevant papers will be referred to in Discussion.

It was stressed earlier that the vegetative growth of the grass plant consists essentially of the initiation of leaf and tiller primordia on apical meristems and the subsequent expansion of these primordia into new organs. The grass leaf is a determinate organ and as such is capable of limited growth: tillers on the other hand are indeterminate organs and have, theoretically, an unlimited growth potential through the continued production of new leaves. Tiller growth is then largely achieved, in the absence of internode elongation, by the production of leaves, and this may continue until floral induction puts an end to further leaf initiation. The dry matter produced by the shoot of a grass such as perennial ryegrass consequently may be regarded as arising from two sources: increase in tiller number and increase in tiller weight through leaf production. A major factor in the understanding of the vegetative growth of a grass plant is to evaluate the contributions to dry weight from each of these two sources.

Langer (1963) has drawn attention to the lack of critical studies of tillering in the field and was unable from his review to draw general conclusions as to the relative importance of tiller number and tiller weight as so called 'yield components' since both these 'components' are sensitive to a large number of environmental factors. Cooper (1948) demonstrated a positive relation between tiller number per plant and individual plant weight in ryegrass and Fejer (1958) showed that both tiller number and tiller weight relationships were useful for differentiating parents of this species. Lazenby and Rogers (1964) showed that during the vegetative stage the logarithm of green weight per unit area was linearly related to tiller number per unit area for four ryegrass cultivars grown at different densities in different years. The relations between tiller number and dry weight in ryegrass have been more precisely defined by Silsbury (1966). In this paper it is stressed that, although the relative growth rate (dry weight) is closely associated in the field with the relative rate of increase in tiller number, tillering is a dynamic process influenced by a large number of factors.

The review by Evans et al. (1964) draws attention to the inverse relation between environmental factors favouring leaf and stem growth and abundant tillering in many Festucoid grasses. The growth pattern of these grasses is discussed in terms of the degree of dependence or otherwise of the developing tiller on its parent tiller. Evidence in favour of such dependence is cited but it is also pointed out that a tiller is capable of acting as an independent unit once it has reached the stage of assimilate independence, although the whole plant may still function in an integrated manner with respect to nutrient and assimilate transfer between tillers. The question is then posed: 'If the growth pattern of these (Festucoid) grasses is determined by a hierarchy of sinks for translocated assimilates and nutrients, in what way are the priorities in this hierarchy determined?' The authors suggest that this is largely mediated through endogenous growth substance levels.

2.4.0 CONCLUSIONS

It is concluded from this review that although the growth and morphogenesis of the vegetative grass plant can be described in reasonable detail and although some of the effects of environmental factors on the growth pattern of plant parts can be defined, there is still a lack of basic knowledge of the way in which the successive organs contribute to increase in plant weight and how the responses of these organs to environmental factors are integrated in the phenomenon known as 'plant growth'. It is hoped that this thesis will contribute to this field.

3.0.0 EXPERIMENTAL PROCEDURES

3.1.0 INTRODUCTION

A single cabinet was available for the controlled environment experiments so these had to be conducted sequentially in time. This had the disadvantage of lengthening the total time of experimentation, but the advantage of reducing any 'between cabinet' error which could have arisen if experiments were run in parallel. It was however necessary to determine the within cabinet error, i.e. the ability of the single cabinet set for one environment to produce an identical plant response at different times. The data presented on this point below (3.6.0) show that excellent repeatability could be achieved with perennial ryegrass.

A major problem arising from the system of sequential experiments was the need to commence each experiment with an identical seedling population so that different growth time relations could subsequently be ascribed to the light and temperature regimes employed and not to initial differences in plant size. To this end a technique for producing a 'standard seedling' was developed.

3.2.0 PRODUCTION OF STANDARD SEEDLINGS

3.2.1 Germination

To obtain 'standard seedlings' it was considered necessary for germination to take place in a medium that:

(i) was physiologically inert;

(ii) was easily reproducible;

(iii) permitted good aeration of the seed;

(iv) provided even contact with the seed to facilitate water uptake;

(v) held water at zero or low potential;

(vi) allowed ready examination of root and shoot;

(vii) held seed in a uniform plane to permit easy measurement of the length of the seedling shoot;

(viii) allowed seedlings to be easily removed.

Sand, 'vermiculite' and 'perlite' were examined in relation to the above requirements and rejected in favour of a 0.5% (weight to volume) agar gel. Concentrations of agar above 0.5% (i.e. 1.0%, 2.5%, 5.0%) were found to retard germination rate or prevent germination completely. If a lower concentration was used, seed tended to sink into the gel and showed a slower germination rate, due presumably to reduced aeration. Seed germinated on 0.5% agar had a slightly better germination (total percentage and rate) than on moistened filter paper, provided the surface of the agar was kept moist for the first 24 hours. This was easily achieved by means of a hand atomiser.

Agar proved a most satisfactory germination medium giving reproducible results as shown by the data given in Table 2. Four lots of 200 seeds of New Zealand

TABLE 2

Mean Shoot Length and Percentage Germination of Four 200 Seed Samples of *L. perenne* Germinated for Six Days on 0.5% Agar at $20 \pm 1^{\circ}C$.

Lot Number	Mean Shoot Length	S.D.	% Germination
1	27.7	10.6	94
2	29.0	6.8	89
3	25.6	8.7	91
4	26.8	9.3	86

perennial ryegrass were germinated at different times for a period of six days on 0.5% agar at 20 ± 1°C. Shoot length is defined as the distance from the seed to the tip of the first leaf. The standard deviations of shoot length are not exceptionally large - coefficients of variation of 40% are not uncommon for single attributes of plants and individual seeds are known to vary in both their time and their rates of germination. Shoot length ranged from 0 to 60 mm. Construction of a frequency diagram with a class interval of 5 mm. for shoot length showed it to be normally distributed with a mean of approximately 28 mm. and a standard deviation of approximately 9.

The next problem that arose was to determine the relationship between seedling shoot length at planting and the dry weight of resultant plants.

3.2.2 Seedling Size and Subsequent Growth

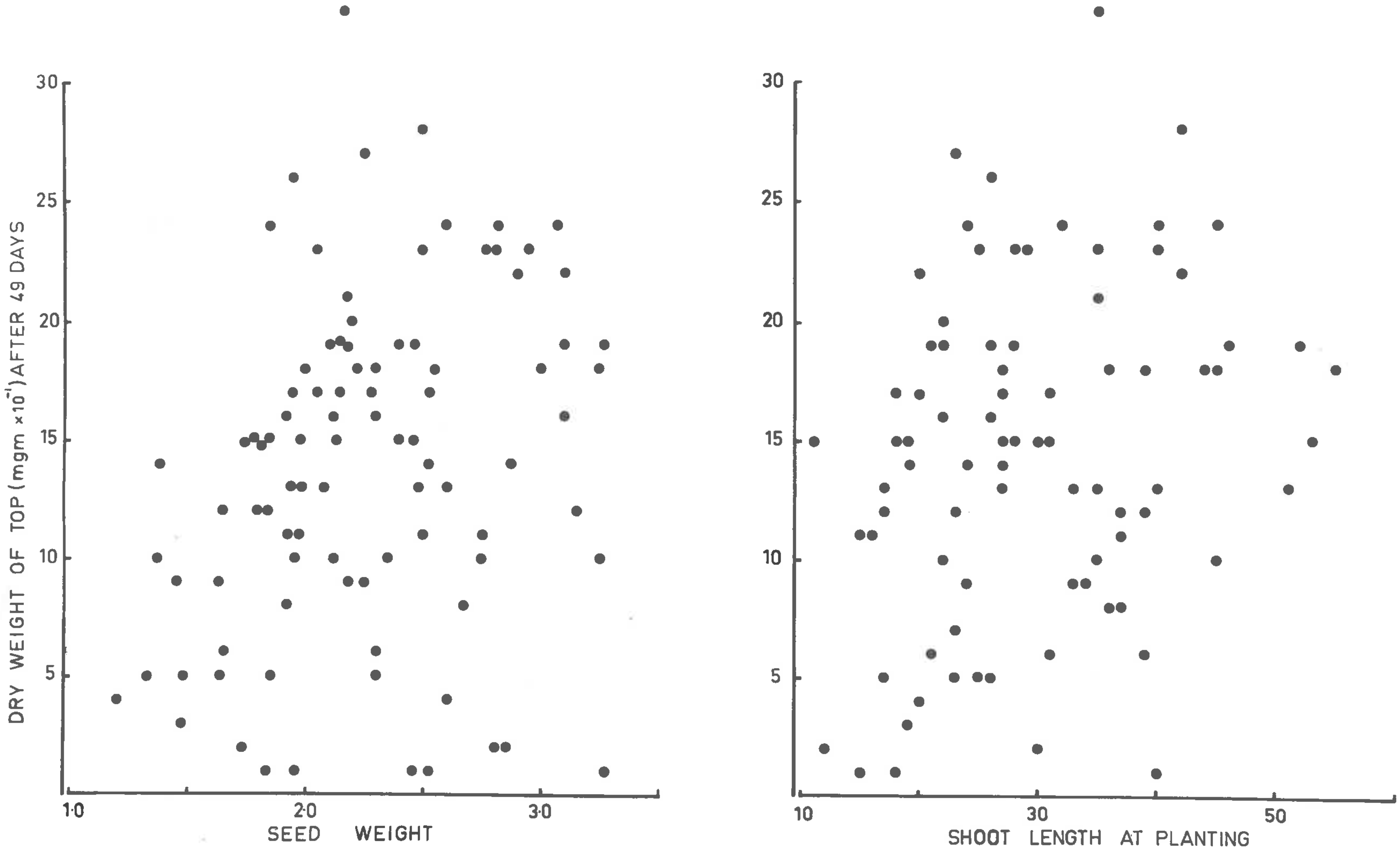
A number of tests were conducted to determine whether the length of the seedling shoot at planting (six days after commencement of germination on agar) influenced the dry weight of the plants at a later time. Approximately 300 seedlings derived from seed of known air-dry weight and varying in length from 10 to 60 mm. were grown individually in 'perlite' with a nutrient solution, or in potting compost for periods varying from 20 to 49 days. Plants were harvested separately, dried at 85°C, weighed and the dry weight then plotted against: (i) initial shoot length; and (ii) the weight of the original seed. Figure 2 shows typical results from one such trial. No relationship could be demonstrated and it was concluded that for the seed used, neither shoot length at planting nor seed weight provided an index of subsequent plant performance. This result is in sharp contrast to that reported recently by Thomas (1966) who concluded that plant weight was correlated with seed weight in perennial ryegrass.

Selection for shoot length was practiced nevertheless, firstly to reduce error at early harvests since it was noted that shoot length and shoot weight were positively related at planting; and secondly as a safe-guard against any major change in germination vigour of the seed during storage throughout the whole experimental period. The range of seedling size selected was 25-35 mm. (approximately 1 S.D. unit of the population). This range encompassed the mean (28 mm.) and included about 30% of the total seedling population.

3.2.3 Technique for Producing Standard Seedlings

Four opaque glass jars 14 x 12 x 15 cm. high were filled to within 9 cm. of the top with 0.5% agar which was then cooled to 20°C. 400 seeds of a single lot of New Zealand Mother Perennial

Figure 2 The relation between the dry weight of plant top (shoot weight) 49 days after planting and
(A) seed weight (mgm.); and
(B) shoot length (mm.) at planting.


DRY WEIGHT OF TOP (mgm ×10⁻¹) AFTER 49 DAYS
30
25
20
15
10
5
1·0
2·0
3·0
SEED WEIGHT
30
25
20
15
10
5
10
30
50
SHOOT LENGTH AT PLANTING

Ryegrass sieved to be retained by a sieve of 0.75 mm. diameter were spread as evenly as possible over the surface in each jar making sure that each seed rested fully on the agar surface. The jars, loosely covered with a flat lid were held in an incubator at 20 ± 1°C for 6 days. A small fluorescent lamp was held over the jar by the lid. This lamp gave an intensity of 137 lumen ft.$^{-2}$ at seed level for 16 hours each day. This ensured that the seedlings grew vertically and did not become etiolated. It had the added effect of raising the temperature inside the jars to 22.0 - 22.5°C. The surface of the agar and the seed was sprayed with a mist of water immediately after sowing and again 12 hours later.

After six days the agar block was slid out of the jar, seedlings exceeding 35 mm. in length picked off and those within the desired class height (25-35 mm.) carefully removed so that shoot, seed and root remained intact. 68 seedlings were removed from the seedling population in each jar; one of these was sown in each of 64 pots and 4 used immediately for an initial harvest (Day 0).

This technique proved quite satisfactory for producing 'standard seedlings' for eight of the nine major experiments of the whole investigation. Harvest 1 (Day 0) data for these nine experiments are shown in Table 3. GR34 was the last experiment conducted and it can be seen in

TABLE 3

Summary of Harvest 1 (Day 0) Data for all Controlled Environment Experiments

Experiment No.	Root	Shoot	Seed	Total Seedling
	(mgm. - means of 4 samples of 4 seedlings)			
GR 11	0.7	1.4	5.2	7.3
14	0.6	1.2	5.7	7.6
16	0.8	1.3	5.6	7.7
26	0.8	1.4	4.9	7.1
27	0.9	1.3	4.6	6.8
28	0.9	1.5	4.5	6.9
30	0.9	1.4	4.9	7.2
32	0.9	1.4	4.6	6.9
34	0.7	1.3	4.1	6.2
Mean	0.8	1.3	4.9	7.0
C.V.	0.21	0.09	0.14	0.11
L.S.D. 1%	0.32	0.24	1.30	1.50

the table that the mean seed weight at Day 0 of this experiment was significantly lower than it was in two of the earlier experiments. Root and shoot weights do not differ significantly between experiments but due to the lower seed weight in GR34 the total seedling weight is just significantly lower than it was in GR16. It is considered that as the root and shoot weights in GR34 were similar to those obtained in the other experiments all the subsequent growth data may validly be compared.

3.3.0 THE CONTROLLED ENVIRONMENT CABINET

3.3.1 General Specifications

Internal measurements of the cabinet are 120 x 120 x 180 cm. with a working area on a movable platform of approximately one square metre. The internal walls are covered with a reflecting plastic to maximise the area of uniform illumination.

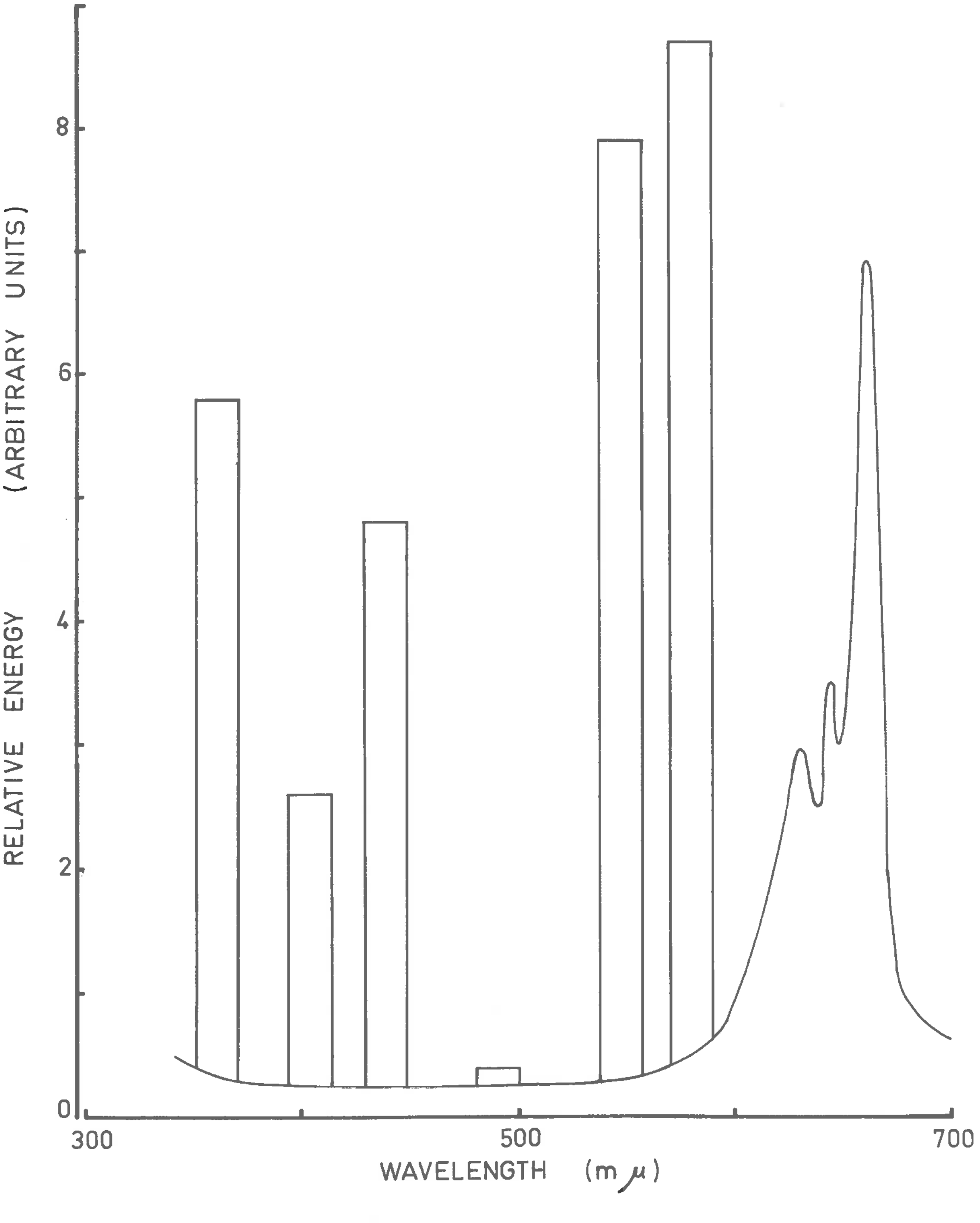
3.3.2 The Light Source

Light energy for plant growth is provided by a bank of 14 high pressure mercury vapour fluorescent lamps (MBF/U, 400 watt) separated from the rest of the cabinet by a sheet of rippled glass and ventilated by a forced draught to help dissipate sensible heat. The rippled glass was preferred to plain glass as it gave a more even (and probably a more diffuse) distribution of light at platform level. The high pressure mercury vapour lamps proved to be extremely efficient providing high intensity illumination (about 3000 lumens ft.$^{-2}$) for long periods without replacement.

According to van der Veen and Meijer (1959) the ultraviolet light emitted by the ordinary mercury lamp is absorbed by the glass of the bulb and is lost as heat. The high pressure lamps have an internal coating of fluorescent powder which absorbs this radiation and re-emits it as visible light, mostly in the red region of the spectrum. The emission spectrum of the MBF/U lamps is shown in Figure 3. There are four major emission bands within the range 400-700 mμ: 400, 440, 550 mμ with a background curve which reaches a maximum at about 660 mμ. There is also a band in the far-violet. There are thus three major wavelengths of visible light: (i) blue; (ii) green-yellow; (iii) red. The highest energy level is in the green-yellow.

Went (1961) suggested that green light at high intensities inhibits plant growth. Using fluorescent high-pressure mercury vapour lamps at two intensities, filters improved the 'growth' of peas and tomatoes at the high intensity (2000 lumens ft.$^{-2}$). Length was used as a measure of growth so it is not possible to determine whether dry weight

Figure 3 Emission spectrum of HPL (MBF/U) 400 watt high-pressure mercury vapour lamps.



RELATIVE ENERGY (ARBITRARY UNITS)
0
2
4
6
8
300
500
700
WAVELENGTH (mμ)

was affected as well. This claim does not appear to have been substantiated.

Gaastra (1959) determined the absorption by leaves of light (400-700 mμ) from different artificial sources. His figures for an average leaf (mean of bean, Swiss chard and tobacco) show that the number of Einsteins absorbed per incident erg and Einsteins absorbed sec.$^{-1}$ cm.$^{-2}$ per lumen ft.$^{-2}$ for high-pressure mercury vapour lamps (H.P.L. = MBF/U) are comparable to those for fluorescent tubes. Figure 10 of Gaastra's paper shows that photosynthesis in mercury light is up to 5% lower than in incandescent light but the mercury light referred to here is from HO-450 W lamps, not the HPL fluorescent type.

There thus appeared to be no reason for expecting growth under mercury lamps to be markedly different from that obtained under a fluorescent source. Initial tests revealed that ryegrass grew well in the cabinet.

3.3.3 Determination of the Level of Radiant Energy in the Cabinet

Routine determinations of light intensity in the cabinet were made with a 'Weston' barrier layer photocell fitted with a quartz filter (but not cosine corrected) and calibrated by the National Standards Laboratory, Sydney.

The spectral sensitivity of a selenium photocell such as the above is known (Gaastra 1959) to lie within the range of visible light (approximately 400-700 mμ) with a peak of sensitivity at about 560 mμ, the spectral region of highest relative energy output by the mercury vapour lamps and the least effective in photosynthesis. To obtain a more reliable measure of the radiation received by the plants, readings on the 'Weston' photocell were plotted against the output (measured on a 'Leeds and Northropp' milli-volt recorder) of a 'Kipp' thermopile. The thermopile and the photocell were placed side by side in the cabinet and variation in the light source achieved by successively turning off individual mercury vapour lamps or by raising or lowering the platform. The relation thus obtained between photometric units (lumens ft.$^{-2}$) and radiometric units (cal. cm.$^{-2}$ hr.$^{-1}$) was found to be essentially linear.

An estimate of the total radiation shorter than 700 mμ was obtained by placing a Chance-Pilkington HA1 filter (3 mm. thick) above the thermopile. According to the manufacturers this filter has an integrated transmission of visible light of 85% and a heat transmission of 9%. From these data and from the observations made, it was calculated that 52% of the total radiation occurred as 'visible light'. This 'visible light' was assumed from the known transmission characteristics of the filter to include wavelengths down to approximately 260 mμ. The first five lines of Table 4 could now be constructed for each of the

TABLE 4

Steps in the Determination of the Visible Radiation (400-700 mμ) at the Three Experimental Levels

			High	Medium	Low
1.	Weston photocell - uncorrected for source	lumens ft.$^{-2}$	3400	1200	680
2.	Weston photocell - corrected for C.I.E. Illuminant A	lumens ft.$^{-2}$	3000	1100	612
3.	Total radiation - from Kipp thermopile	cal.cm.$^{-2}$ hr.$^{-1}$	24.64	7.86	4.62
4.	Percentage of total radiation $<$ 700 mμ		52	52	52
5.	Radiation $<$ 700 mμ	(a) cal.cm.$^{-2}$ hr.$^{-1}$	12.60	4.09	2.40
		(b) ergs cm.$^{-2}$ sec.$^{-1}$ x 10^{-4}	14.6	4.7	2.8
6.	Radiation 400-700 mμ from Gaastra (1959), lumens ft.$^{-2}$ (Illuminant A) x 37.6	ergs cm.$^{-2}$ sec.$^{-1}$ x 10^{-4}	11.3	3.9	2.2
7.	Radiation 400-700 mμ calculated as 80% line 5	(a) ergs cm.$^{-2}$ sec.$^{-1}$ x 10^{-4}	11.7	3.8	2.2
		(b) cal.cm.$^{-2}$ hr.$^{-1}$	10.1	3.3	1.9
		(c) cal. cm.$^{-2}$ day^{-1}	161	52	31

three energy levels used in the experiments. Line (1) gives the actual reading of the Weston photocell and Line (2) this value corrected for C.I.E. Illuminant A, a tungsten filament lamp at a colour temperature of 2855°K. Line (3) gives the corresponding values for total radiation in cal. cm.$^{-2}$ hr.$^{-1}$ from the 'Kipp' thermopile. The percentage of the total radiation shorter than 700 mμ (Line 4) was not found to vary with different levels of radiation. Line (6) gives the radiation in ergs cm.$^{-2}$ sec.$^{-1}$ calculated from the factor of 37.6 given by Gaastra (1959) for converting photometric units into radiometric units for wavelengths between 400 and 700 mμ. It can be seen that lines (5 b) and (6) do not agree particularly well. It was considered that this descrepancy was due to the fact that a proportion of the radiation measured as $<$700 mμ was shorter than 400 mμ.

Estimates of the proportion of the radiation 400 mμ were obtained from two sources: (i) the emission spectrum of the lights; and (ii) by experimentally screening the thermopile with an ultra-violet photographic filter (Kodak Wratten Filter 1A) in addition to the HA1 filter. Both estimates gave a figure very near to 20% of the radiation less than 700 mμ as being below 400 mμ. The visible radiation (400-700 mμ) was therefore calculated as is shown in line (7). The figures shown in line (7 a) agree very well with the estimates from Gaastra's data, line (6).

For experiments at 'medium' and 'low' radiation the energy entering the cabinet was reduced by placing an appropriate sheet of woven plastic 'Sarlon' shade cloth, between the light bank and the glass. This method of reducing the light energy resulted in a more even light distribution than could be achieved by turning off a proportion of the lamps.

3.3.4 Temperature Control

Temperature control within the cabinet was achieved by a direct expansion refrigerator coil and a single bar heater resulting in a variation of ± 0.75°C with time at any one point, day or night. A similar variation was found between points in a horizontal plane at any one time with a wind velocity of 60 cm. $sec.^{-1}$. A marked temperature gradient occurred vertically so it was necessary to adjust temperature settings when platform height was altered since 'experimental' temperature was always taken as that of the ambient air at pot level during the light period.

Night temperatures were set 1°C lower than day so that the day-night changes would register on a thermograph. The only other record of the duration of the light period was the 'Venner' time switch controlling the day-night changeover. Both the thermograph and the clock of the time switch were used to check against short-term power failures or other breakdowns. Such failures were fortunately infrequent but when they exceeded four hours duration the experiment was repeated.

3.3.5 Air Movement

Air movement was maintained in an upward direction within the cabinet by ingress from one side and distribution across the lower part by a deflecting grille. Even air velocities in any one cross-sectional area are extremely desirable in a controlled environment cabinet but are difficult to achieve without carefully controlled entry via a suitable plenum.

Air velocity between the pots was measured with a vane anemometer or a hot-wire anemometer and in all experiments ranged from 30-70 cm. sec.$^{-1}$. Total plant dry weight per pot was found not to be correlated with wind velocity round the pot at 20°C or at 10°C so it was concluded that over this range, variation in wind velocity did not materially contribute to between-pot variation. At 30°C under high radiation (but not at medium and low) a positive correlation between dry weight and wind velocity was observed over the last four harvests. This effect is examined below (3.3.7).

3.3.6 Humidity

Humidification of the cabinet at a saturated water vapour pressure deficit of approximately 5.0 mm.

mercury was achieved in all except one experiment (see 3.3.7 below) by evaporation from a free water surface on the cabinet floor and a steam injection unit controlled by a 'Danfoss' humidistat, of which the sensing element was located above the inlet of the internal circulatory fan. The complete humidifying unit (similar to that used in the controlled environment cabinets of the C.S.I.R.O. Phytotron in Canberra) worked well giving, at each temperature, a range in relative humidity of about 6%. The major part of this variation was due to the dehumidifying action of the cooling coil.

3.3.7 Interaction Between the Effects of Humidity Level, Wind Velocity and Temperature on Plant Growth

The plant growth data given under (4.2.0) for 30°C high radiation were obtained at a saturated water vapour pressure deficit of 10.8 mm. mercury. As noted above (3.3.6) a positive correlation was found to occur between the dry weight of the plants in each of the four replicate pots and the wind velocity round each pot, at each of the last four harvests (Day 22-32) of experiment GR14. A similar phenomenon was not observed in any other experiment.

It was thought that this result could be explained in the following manner. At 30°C a high radiant energy level could raise leaf temperature to supra-optimal levels.

The cooling effect of transpiration would be favourably influenced by an increased rate of air movement.

To test this interpretation, copper-constantan thermocouples were attached to the under surfaces of a number of leaves to record their temperatures. The problems inherent in this technique are fully appreciated but it is considered that the technique employed resulted in a reasonable measure of leaf temperature.

It was found that leaf temperature followed that of the ambient air very closely (with a short time lag) and no effect of wind velocity of the range 20-100 cm. sec.$^{-1}$ was observed. The conclusion was therefore drawn that the favourable effects of increased wind velocity at 30°C were not due to the creation of a more favourable leaf temperature.

An alternative explanation for the wind velocity effect may be an increased CO_2 supply to the leaf surface with increased air movement but it is difficult to see why this should occur at 30°C but not at 20°C, especially as 30°C was supra-optimal for growth at all radiation levels.

The question also arises as to whether the pattern of growth and the growth rate at 30°C high radiation was unfavourably influenced by the low relative humidity at which this experiment was conducted. To examine this possibility a further experiment was conducted at 30°C, high radiation and a saturated water vapour pressure deficit of 5.0 mm. mercury. Figure 4 shows that the growth rate (GR38) under these conditions was indistinguishable from that of the earlier experiment (GR14) so it was considered that the GR14 data could legitimately be compared with those obtained from all other experiments.

3.4.0 VARIATION IN PLANT ATTRIBUTES

Before commencing any experimental programme it is useful to have an estimate of the variation likely to be encountered in the plant attributes to be measured. This was particularly necessary in the present study as a suitable compromise between the number of harvests and the degree of replication had to be reached due to the limited area available within the cabinet.

The most convenient pot size appeared to be one of 11 cm. diameter and it was considered that 4 plants could be grown satisfactorily in this for about 30 days under favourable conditions without inter-plant competition. Data on plant dry weight and the weights of various plant parts were obtained from preliminary experiments (20°C and high light and four replicates) conducted to determine a suitable plant culture technique. These data are shown in Table 5.

Coefficients of variation for total dry weight

TABLE 5

Coefficients of Variation for Total Plant Dry Weight and the Dry Weights of Selected Plant Parts Between Pots of Four Ryegrass Plants

Attribute	Days from Planting						
	6	9	12	15	18	21	24
Total Dry Weight	8.0	10.8	11.1	11.2	14.2	11.1	15.2
Leaf 3 on Main Stem			19.0	11.5	18.6	14.8	15.8
Leaf 4 on Main Stem				27.8	20.7	15.1	14.9
Tiller 1				8.3	8.7	19.7	7.2
Tiller 2					27.1	18.2	13.8
Tiller 3						29.9	19.5

ranged from 8 to 15% whilst those for single leaves on the main stem and individual tillers were higher at 8-30%. It appeared that errors were greatest when the plant parts were small.

The data for total plant dry weight indicated that, for any harvest of four replicates, differences of 13-24% between treatment means would be significant at the 1% level of probability. This was considered satisfactory but for plant parts a higher degree of replication would be desirable. Increased replication however could only be by reducing the number of harvests or increasing the harvest interval. Since a detailed record of plant growth was required it was decided that this could best be achieved by obtaining slightly less reliable data for each harvest at more frequent intervals.

3.5.0 PLANT CULTURE

Plants were grown in an artificial medium with a nutrient solution to eliminate variability due to soil. 'Perlite', an expanded silica, was selected since despite recent criticism (Wilson 1965) it has a low exchange capacity and a high water holding capacity (40% moisture content by volume at pF 2.4 and 18% at pF 4.2) (Morrison, McDonald and Sutton 1960). Further this material is relatively uniform, provides good aeration and is relatively easily separated from the root system. To facilitate drainage the fine fraction (< 0.028 in. diam.) was removed by dry sieving. A disadvantage of 'perlite' is that it is very light and does not provide a firm medium in which to establish small seedlings. However, if care is taken not to flood the pots when watering 'floating up' and consequent root disturbance can be avoided.

A comparison of the growth of seedlings in vermiculite, perlite and John Innes potting compost showed after seven weeks, the relative dry matter of tops to be:

John Innes = 100%
Perlite = 82%
Vermiculite = 33%

Perlite therefore appeared to be a satisfactory medium although not as good as potting compost.

The nutrient solution adopted was a modified Hoagland, almost identical with that used by Alberda (private communication) for the water culture of ryegrass at Wageningen. The solution was:

KNO_3	0.51 gm./litre
$Ca(NO_3)_2$	0.82 gm./litre
$MgSO_4.7H_2O$	0.49 gm./litre
KH_2PO_4	0.136 gm./litre

To each litre of the above was added 1 ml. of Fe-EDTA prepared as given by Jacobsen (1951) and 1 ml. of modified A-Z solution one litre of which contained:

$H_3\ BO_4$	2.86 gm./litre
$MnCl_2.H_2O$	1.81 gm./litre
$Zn\ SO_4.\ 7H_2O$	0.22 gm./litre
$Cn\ SO_4.\ 5H_2O$	0.08 gm./litre
$H_2\ MoO_4.\ H_2O$	0.09 gm./litre

Pots were flushed with nutrient solution each morning. During the first six days of each experiment flushing was repeated in the afternoon.

An experiment (experiment GR10) was conducted to compare solutions containing the main four salts in half ($\frac{N}{2}$) and double (2N) the above concentration (N) over a 32 day period with harvests every three days. Up to day 24 no significant differences between the effects of the three solutions on plant dry weight could be determined but after this time plants receiving $\frac{N}{2}$ grew less rapidly than those receiving N and 2N, which remained indistinguishable. The N concentration was therefore used for all experiments.

3.6.0 REPRODUCIBILITY OF PLANT RESPONSE IN ANY GIVEN ENVIRONMENT

The major experimental programme called for the establishment of 'standard seedlings' in different environments, with quantitative comparisons of growth responses over a period of time. Such comparisons are reliable only if it can be shown that a growth response is characteristic of the particular environment in which it is obtained, i.e. it is necessary to know whether the responses obtained at one environmental setting of the cabinet can be reproduced. Data are available which show that particular responses could be readily reproduced.

In Figure 4 the growth curve obtained at 20°C and 3400 lumens ft.$^{-2}$ (experiment GR11) is compared with that obtained previously in experiment GR10 at the same environmental setting using the data for the N nutrient concentration only (see 3.5.0). Also shown are the curves for two separate experiments at 10°C and 600 lumens ft.$^{-2}$ and two experiments at 30°C, 3400 lumens ft.$^{-2}$ with different levels of relative humidity. It is clear that in each case there is excellent agreement between the two growth curves obtained in like environments.

Table 6 shows data from four separate short-term experiments conducted at 20°C and 3200 lumens ft.$^{-2}$. Experiments were started four days apart, each from a separate population of 'standard seedlings'. Four harvests, to determine total plant dry weight were made, each four days apart. There were no significant differences between comparable harvests.

Results of a further experiment are shown in Table 7. Two experiments were conducted each lasting 19 days at 20°C and 2600 lumens ft.$^{-2}$ and starting a week apart. At Day 20 no significant difference between the

__Figure 4__ Growth curves obtained from six experiments under controlled conditions.

GR11	3400 lumens ft.$^{-2}$	20°C	5.1 mm.Hg,	S.W.V.P.D.*
GR10	3400 lumens ft.$^{-2}$	20°C	5.1 mm.Hg,	S.W.V.P.D.
GR14	3400 lumens ft.$^{-2}$	30°C	10.8 mm.Hg,	S.W.V.P.D.
GR38	3400 lumens ft.$^{-2}$	30°C	5.1 mm.Hg,	S.W.V.P.D.
GR32	600 lumens ft.$^{-2}$	10°C	5.0 mm.Hg,	S.W.V.P.D.
GR35	600 lumens ft.$^{-2}$	10°C	5.0 mm.Hg,	S.W.V.P.D.

* Saturated water vapour pressure deficit

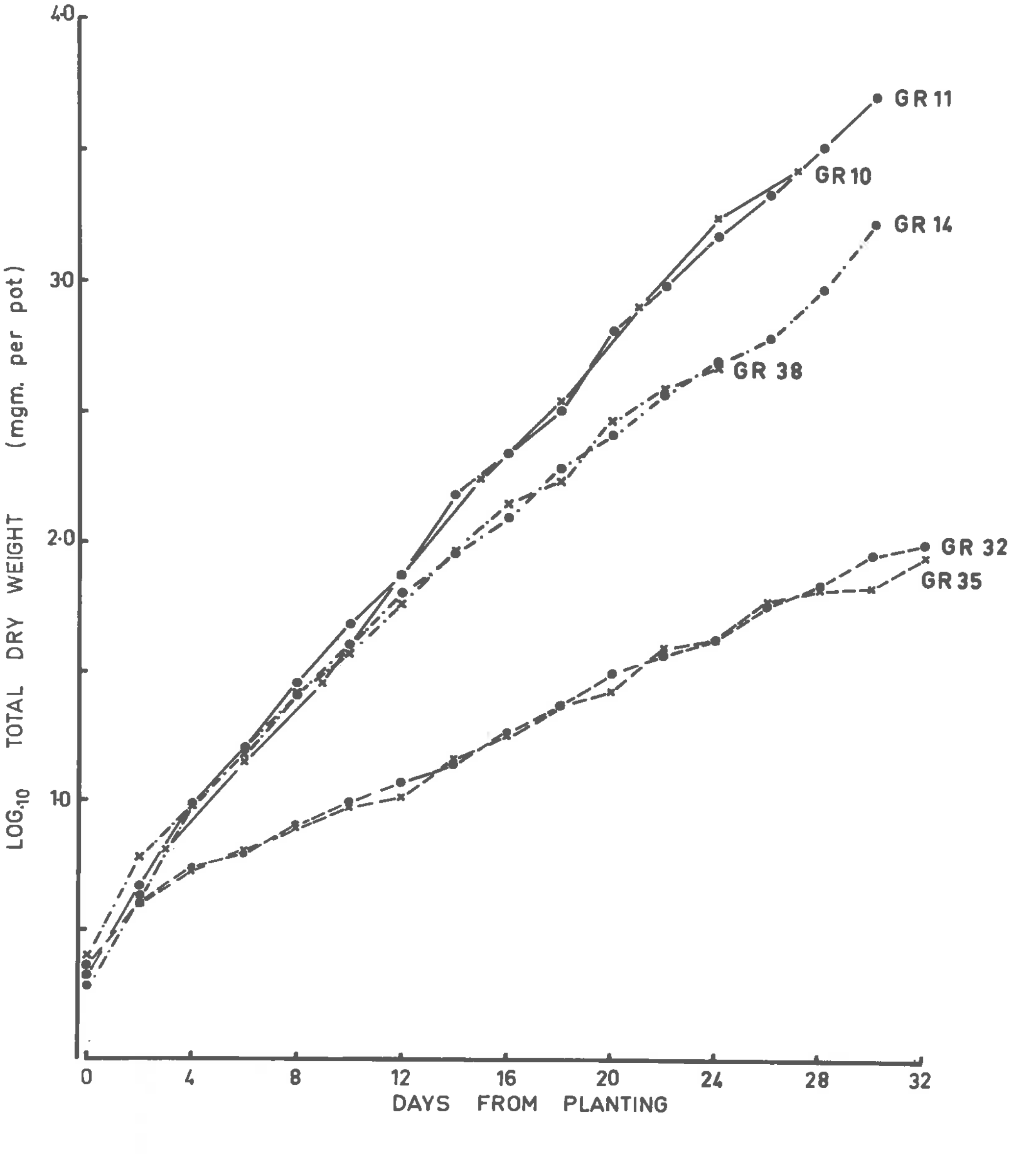

4·0
3·0
2·0
1·0
LOG10 TOTAL DRY WEIGHT (mgm. per pot)
0
4
8
12
16
20
24
28
32
DAYS FROM PLANTING
GR 11
GR 10
GR 14
GR 38
GR 32
GR 35

TABLE 6

Total Plant Weight Data for Four Growth Experiments (GR21-GR24) Started at 4-Day Intervals and Harvested Every 4 Days. 'Standard Seedlings' and Standard Culture Methods were Employed.

	Total Plant Dry Weight at			
	Day 1	Day 5	Day 9	Day 13
GR21	1.78	7.55	21.49	59.61
22	2.34	7.77	22.37	60.11
23	2.39	8.19	24.56	58.21
24	2.30	8.58	21.62	-*

* Not harvested

TABLE 7

Plant Components for two Growth Experiments Started one Week Apart and Lasting 19 days - 20°C, 2600 lumens ft.$^{-2}$.

	Sowing 1	Sowing 2
Tiller No.	10.4	10.7
No. of mature leaves	21.3	22.0
Root weight (mgm.)	15.3	15.8
Stem weight (mgm.)	91	95
Leaf weight (mgm.)	227	246
Total weight (mgm.)	471	498

tiller numbers, number of mature leaves, root, stem and leaf weights can be found.

It was concluded from the above data that, using standard seedlings it is possible to reproduce a given plant response with considerable precision. The similarity of the standard errors of the points on a growth curve obtained on separate occasions shows that the main source of variation was inherent in the plant material and that errors were not induced by unspecified changes in the environmental conditions. The different growth responses obtained in the different environments produced by a single cabinet may therefore be compared quite safely.

3.7.0 HARVESTING PROCEDURES AND DETERMINATION OF LEAF AREA

Harvests were made every two days during an experiment. Pots were removed from the cabinet at 0900 hours, one hour after the lights came on. Roots were washed free of perlite as rapidly as possible and the plants separated initially into roots and shoot. The shoot was subdivided into single tillers care being taken to ensure that each subtending leaf remained with its parent tiller. A tiller was separated from the parent when it had emerged above the subtending leaf sheath on any one of the 4 plants in each of the four pots. If no plant in any pot had a particular tiller emerged, then that tiller was regarded as absent at that harvest. When one plant in each pot had a particular tiller visible, it was recorded as present and the corresponding non-emerged tillers on the remaining plants were dissected out, dried and weighed.

This procedure was not adopted for the leaves due to the time required for dissection. A leaf was measured only after it had emerged from the enclosing sheaths of the preceding leaf and only the emerged lamina was recorded as 'leaf'. Entire leaves prior to appearance, the leaf sheaths and the stem apex, were classed as 'stem'.

Small containers were used to hold the plant material for drying in a forced draught oven at 90°C for 24 hours. Dried material was held in a desiccator before weighing on a torsion balance to 0.1 mgm.

The areas of fresh leaves were determined either by planimetering an outline on 'Ozalid' paper or from measurements of length x breadth. The latter method has been advocated by Lal and Subba Rao (1951). A wide range of leaf sizes were calibrated for LxB area against 'true' area as determined by planimetry. The appropriate conversion factor for LxB was then used to give the actual area.

3.8.0 EXPERIMENTAL DESIGN, DATA HANDLING AND DATA PROCESSING

The complete experimental programme involved the determination of the responses of seedlings of New Zealand Mother perennial ryegrass to nine combinations of radiation and temperature in a single controlled environment cabinet. The radiation levels were 161, 52 and 31 gm. cal. cm.$^{-2}$ day^{-1} and the temperatures, 10, 20 and 30°C. The radiation levels will subsequently be distinguished as high, medium and low.

'Standard seedlings' were established four per pot on Day 0 and harvests made every two days thereafter, i.e. Day 2, 4, 6, etc. for 32 days in all experiments except High 20°C and High 30°C when experiments ran until Day 30 only.

Sixty-four pots were sown on Day 0 and these were arranged 8 x 8 on the cabinet platform with two rows of 8 pots as a block, giving four-fold replication. Harvest numbers were allocated at random to the 16 pots of each of the four blocks. The blocks were located in the same positions in each experiment.

At each harvest the individual plants in each pot were separated into their component parts which were then dried and weighed as a unit and the weight of each part recorded as weight (mgm. per pot) of four plants. The question then arose whether to tabulate and process the data on this basis or per plant. The former course was adopted because it reduced calculation and facilitated checking against original records, even though it makes difficult comparison with data from other sources. It seemed likely however, that such comparisons would largely be in terms of rates and that in other respects the present data would be largely self-contained. No significant block effects could be demonstrated in any experiment (except for the last four harvests at High 30) the 'between pot' error being almost entirely due to variation between pots themselves rather than to their position. For this reason calculations were mostly based on the mean values.

Data were processed on a C.D.C. 3400 computer using in part 'Genstat', the Waite-Wellesbourne General Statistical Programme developed by Mr. J. A. Nelder of the Wellesbourne Vegetable Research Station and Mr. G. N. Wilkinson, Reader in Biometry at the Waite Agricultural Research Institute.

4.0.0 EXPERIMENTAL RESULTS

4.1.0 DISTRIBUTION OF TOTAL DRY MATTER BETWEEN MAJOR PLANT PARTS

It is commonly found that the distribution of dry matter between the root and the shoot system of a plant is influenced by the environment in which it is grown. To determine whether this occurred in ryegrass, the percentage of the total dry weight that occurred as root was calculated for each harvest of each experiment. These data recorded in Table 8, show quite clear trends.

Initially the mean root proportion over all environments was 0.33, but it steadily declined with time to a value of 0.26 at the final harvest. The rate of decline was most rapid over the first half of the experimental period, a trend common to nearly all environments.

The root percentage was not markedly influenced by the environment. A slight decline occurred with decrease in radiation; temperature also exerted an effect, 20° favouring shoot growth at the expense of root growth.

Within the shoot, effects of environment on the distribution of dry matter between leaf lamina and 'stem' (leaf sheath plus the unexpanded leaves surrounding the apex) can be detected. These effects are shown in Table 9 for the main stem only, since tillering was virtually precluded at both 10° and 30° under low radiation. Further the leaf

TABLE 8

Roots as a Percentage of Total Plant at Each Harvest

RADIATION:

Day	High			Medium			Low			Mean
	10	20	30°C	10	20	30°C	10	20	30°C	
4	36	34	37	35	33	27	33	29	29	33
6	35	36	40	23	28	32	35	28	33	32
8	34	35	41	33	30	32	32	24	32	33
10	31	37	42	30	27	34	30	25	31	32
12	33	34	40	29	30	32	30	25	31	32
14	30	31	39	30	31	32	29	25	33	31
16	30	31	39	27	28	33	30	26	28	30
18	31	30	35	30	28	32	27	25	28	30
20	33	27	36	31	25	33	27	23	29	29
22	31	28	33	33	27	31	28	25	28	29
24	30	27	36	29	26	30	27	22	27	28
26	29	27	32	28	24	30	28	24	26	27
28	31	25	31	31	24	28	26	24	24	27
30	31	34	30	30	24	28	28	25	28	27
32	31	-	-	28	24	26	28	23	26	26

Mean Values for all Harvests

Temperature	Radiation:			Mean
	High	Medium	Low	
10	32	30	29	30
20	31	27	25	28
30	36	31	29	32
Mean	33	29	28	

TABLE 9

Leaf Lamina as Percentage of Main Stem
(Mean percent by weight over all Harvests)

Temperature	Radiation: High	Radiation: Medium	Radiation: Low	Mean
10	71.2	66.9	68.3	68.8
20	75.5	72.5	74.1	74.0
30	72.7	75.5	77.3	75.1
Mean	73.1	71.6	73.2	

proportion did not vary appreciably between tillers in any one environment.

Radiation had no consistent effect on leaf proportion; the effect of temperature was more definite. There was, generally at each harvest, a lower leaf percentage at 10°C than at the two higher temperatures. Harvest data are not given but this effect was clearly discernible at nearly all harvests in each environment. Where comparisons could be made for other tillers the pattern was similar to that displayed by the main stem.

It is concluded that the range of environmental conditions to which ryegrass was subjected did not substantially alter the general morphology of the plant. The relative distribution of dry matter between root and shoot was influenced more by radiation than by temperature and that between leaf lamina and 'stem' was influenced more by temperature than by radiation.

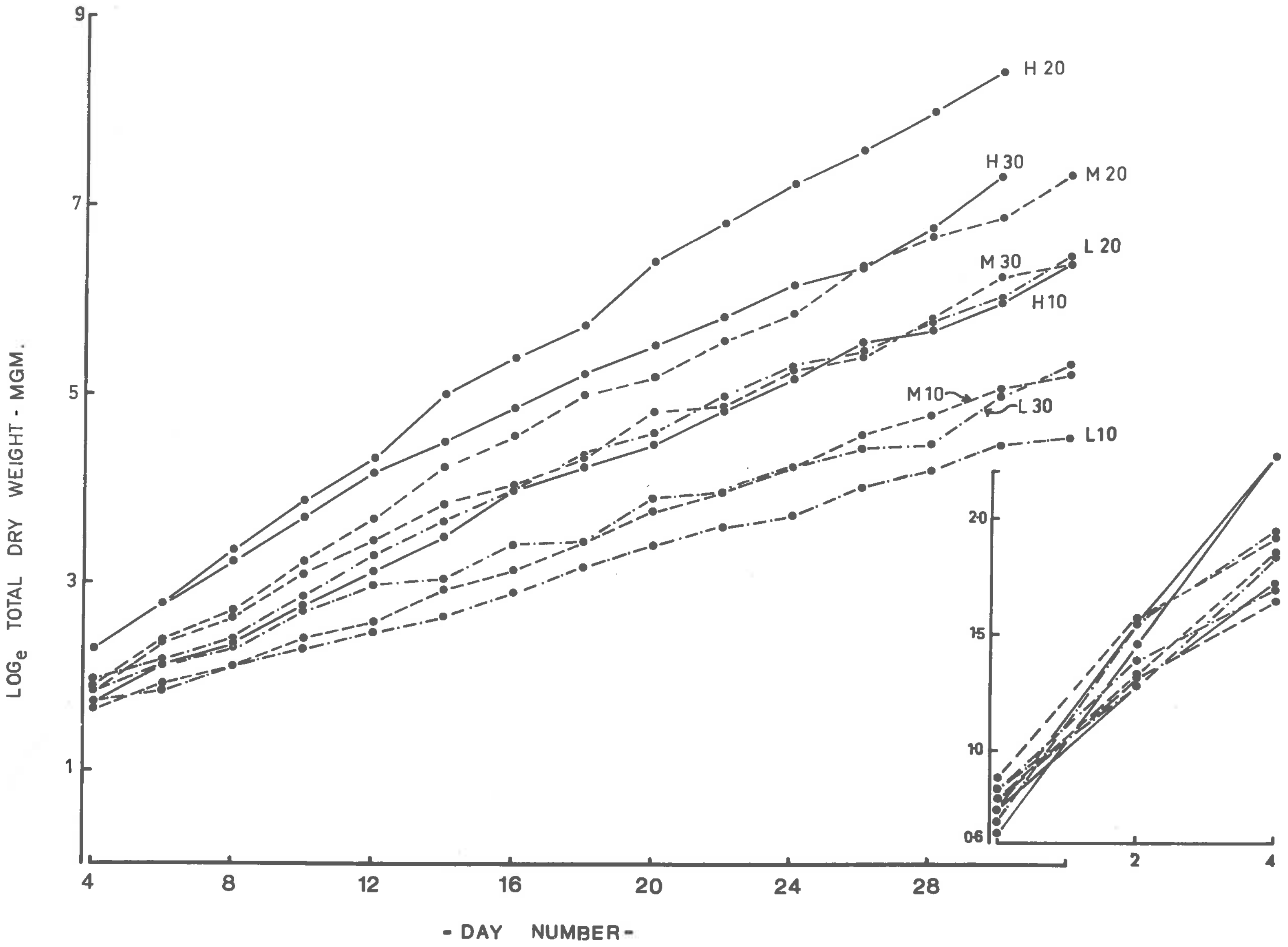
4.2.0 INCREASE IN TOTAL DRY MATTER AND WITH RESPECT TO TIME

4.2.1 Day 0 to Day 4

The accumulation of dry matter by the plants in each experiment with respect to time is summarised by Figure 5 in which the logarithm (base e) of the mean dry weight at each harvest is plotted against time.

<u>Figure 5</u> Growth curves ($\log_e$ total plant dry weight) obtained in each of nine environments.

High radiation:	H
Medium radiation:	M
Low radiation:	L
Temperatures:	10, 20, 30°C.



LOG$_e$ TOTAL DRY WEIGHT - MGM.
9
7
5
3
1
4
8
12
16
20
24
28
- DAY NUMBER -
H 20
H 30
M 20
L 20
M 30
H 10
M 10
L 30
L10
2·0
1·5
1·0
0·6
2
4

The resulting growth curves have, in general, two distinct phases. There is first a linear or curvilinear trend from Day 0 to Day 4 which is followed by a phase of different general slope from Day 4 until the end of the experiment. The major change in the slope of most of the curves at Day 4 appears to be due to exhaustion of the endosperm at about this time. A high initial relative growth rate is achieved by the seedling as a consequence of mobilization of the seed reserves and current photosynthesis; but when the endosperm is exhausted the plant depends entirely for assimilate on the latter source and a reduced relative growth rate ensues.

The dry weights of the seeds which were separated from the seedlings at each of the first four harvests were determined and are recorded in Table 10. A significant decline in the mean seed weight was found to occur between successive harvests. Between Day 4 and Day 6 however, this decline reached significance ($P = 0.01$) in only two of the nine individual experiments and it was small (0.34 mg.) in relation to the increase in total seedling dry weight (6.6 mg.) over this interval. Thus after Day 4, the growth of the seedling was negligibly affected by the amount of material translocated from the seed. It is therefore safe to assume that after Day 4 plant growth rate in all environments was determined by the prevailing conditions. Nevertheless, environment effects on plant

TABLE 10

Mean Seed Dry Weight at Day 0, 2, 4 and 6
(mg. per pot of 4 plants)

Radiation	Temperature	Day 0	Day 2	Day 4	Day 6
High	10	5.7	2.9	2.8	2.4
	20	5.2	3.4	2.6	2.2
	30	5.7	3.9	2.4	2.1
Medium	10	4.6	2.9	3.3	2.6
	20	4.9	3.4	2.5	2.2
	30	4.5	2.5	2.2	2.2
Low	10	4.1	3.4	2.5	2.6
	20	4.9	3.1	2.8	2.1
	30	4.0	2.9	2.3	2.0
Mean		4.8	3.2	2.6	2.3
Loss		-	1.6	0.6	0.3
Gain in Seedling Weight		-	1.5	3.1	6.6

growth during the first four days of each experiment cannot be disregarded; these are examined in Table 11.

Temperature appears to have a dominating influence on growth rate in this early period, the overall effect of radiation being small. Both temperature and radiation show an increased effect with harvest although the uniformity of all treatments at Day 0 undoubtedly contributes to this trend. The interaction between temperature and radiation is complex but it seems that at 10°C no response to radiation occurred while at 20 and 30°C, high radiation gave more dry weight than did medium or low radiation. This response was relatively greater at 20° than it was at 30°C.

It will be shown later (4.2.2) that after Day 4 the pattern of response to temperature and radiation differed from that during the intitial period and was not as complex. It is also apparent that in some cases, differences induced during this period persisted until the end of the period over which growth was examined. It is clear that the plants responded rapidly to the different environmental conditions imposed.

4.2.2 Growth after Day 4

The growth curves shown in Figure 5 were examined quantitatively by fitting quadratic equations of the form:

TABLE 11

Total Dry Weight of Seedlings (Excluding Seed) at Days 0, 2 and 4

(mg. per pot of 4 plants)

Radiation	Temperature	Day:		
		0	2	4
High	10	2.1	3.5	5.6
	20	2.1	4.7	9.7
	30	1.9	4.4	9.7
Medium	10	2.2	3.7	5.2
	20	2.2	3.7	6.4
	30	2.4	4.8	6.4
Low	10	2.3	4.1	5.4
	20	2.3	3.6	6.2
	30	2.1	4.8	6.7

Analysis of Variance of Logarithmically Transformed Data:

Source	d.f.	V.R.	
Total	26		
Temperature	2	19.78	***
Radiation	2	3.93	*
Harvest	2	823.34	***
Temp. x Rad.	4	5.39	***
Temp. x Harv.	4	10.61	***
Rad. x Harv.	4	11.24	***
T x R x H	8	2.32	*
Error	78		

$$\log_e w = a + b \quad \xi_1 (t) - c \quad \xi_2(t)^2$$ <u>Footnote</u>

to the harvest data to express total dry weight (w) as a function of time (t). The advantages of this procedure are: (i) the linear regression coefficient (b) is the average relative growth rate (R); and (ii) the coefficient (c) determines the rate of fall of R with time. The significance of the coefficient (c) can be determined by dividing by its standard error and looking up the resulting 't' value in standard tables. The equation derived for each experiment gave an excellent fit to the experimental data in each case. Table 12 shows the relevant coefficients and error terms.

Highly significant departure from linearity occurred at 20°C at all levels of radiation; there was a slight tendency for curvature at 30°C at high and medium radiation only. In the other experiments there was no significant decline in relative growth rate with time.

Expression of R on a daily basis is achieved by dividing the linear regression coefficient (the slope of the regression lines) by two since the harvest interval was always two days. In each case (see Table 12), the

---

<u>Footnote</u> $\xi_1 (t)$ and $\xi_2(t)$ are the orthogonol functions of time, i.e. the sum of products of their values is zero.

$$\xi_1 (t) = t - \bar{t}$$
$$\xi_2 (t) = t^2 - \bar{t}^2 - b_{21} t$$

where b_{21} is the linear regression coefficient in the regression of t^2 on t.

TABLE 12

Estimates of Average Relative Growth Rates (R) and Their Relation to Time from Quadratic Regressions of Log$_e$ Dry Weight on Time

Radiation	Temp.	Linear Coeff. (b)		R (1)	R (2)	Quad.Coeff. (c)		't'	d.f.	Significant level of 't'
High	10	0.3391	(0.0041)	0.169	0.170	-0.0029	(0.0010)	2.79	12	0.05
	20	0.4829	(0.0042)	0.241	0.240	-0.0068	(0.0011)	5.77	11	0.001
	30	0.3734	(0.0066)	0.187	0.185	-0.0057	(0.0018)	3.09	11	0.05
Medium	10	0.2669	(0.0031)	0.133	0.135	+0.0005	(0.0008)	0.61	12	n.s.
	20	0.3925	(0.0049)	0.196	0.195	-0.0062	(0.0012)	4.87	12	0.001
	30	0.3217	(0.0055)	0.161	0.160	-0.0041	(0.0014)	2.90	12	0.05
Low	10	0.2153	(0.0030)	0.108	0.110	+0.0001	(0.0078)	0.17	12	n.s.
	20	0.3354	(0.0043)	0.168	0.170	-0.0040	(0.0011)	3.64	12	0.01
	30	0.2367	(0.0066)	0.118	0.120	+0.0007	(0.0017)	0.41	12	n.s.

L.S.D. 1% 0.0265 for Linear Regression Coefficient

R (1) Linear Regression Coefficient (b)/2

R (2) Mean value from successive harvest intervals

S.E. given in parenthesis.

result R(1) agrees well (as indeed it should) with the estimate R(2) obtained by averaging the successive values for each harvest interval using the formula

$$R = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}$$

Comparison of relative growth rates may be made by considering the errors associated with each regression coefficient. Table 12 shows that these ranged from 0.0030 to 0.0066. For general comparisons it is simplest to take the highest error and determine the least significant difference between any two coefficients b_x and b_y as:

$$b_x - b_y = \text{'t'} \ (\text{on } n_1 + n_2 - 4 \text{ d.f.}) \times \quad 2(0.0066)^2$$

At $P = 0.01$ this is 0.0265.

The average values of R (gm. gm.^{-1} day^{-1}) obtained in each experiment are set out below to facilitate comparison. Values which do not differ significantly (at $P = 0.01$) have a common letter in parenthesis.

Radiation	Temperature 10	 20	 30°C
High	0.169 (C)	0.241 (A)	0.187 (B)
Medium	0.113 (D)	0.196 (B)	0.161 (C)
Low	0.108 (E)	0.168 (C)	0.118 (E)

The above results are presented graphically and will be considered further in (4.10.1). At this stage attention is drawn only to the fact that 20°C was clearly optimal for growth at all levels of radiation, and that at high and medium levels, 10°C was the least favourable. At each temperature each decrease in radiation caused a significant decline in the relative growth rate.

Re-examination of Figure 5 in relation to the above data shows that the final quantities of dry matter attained in the different environments were not directly related to the relative growth rate. This shows that initial weight (at Day 4) was also a determinant of the dry weight at the final harvest.

4.3.0 TILLER AND LEAF PRODUCTION

4.3.1 Growth at the Coleoptile Node

The system of nomenclature adopted to identify each individual tiller was that described in (2.1.9). The tiller developed at the coleoptile node was denoted Coleoptile Tiller (C).

A tiller was not produced at the coleoptile node on all plants even in the environment most favourable for tiller development (high radiation, 20°C). In addition it was noted that about 50% of the coleoptile tillers differed morphologically from all other tillers; they had small leaves, were of compact habit and often tillered rapidly themselves. Because of this irregular behaviour the growth pattern of the tillers arising from the coleoptile node was not followed in detail. Instead, all growth from this original source was bulked and weighed as 'coleoptile tiller'. This weight was <u>not</u> included in 'total dry weight' for the following reasons. Firstly, it would have been impracticable to measure the areas of all the minute leaves developed at this node and inclusion of the weight of 'coleoptile tiller' without an associated area would introduce errors into the area-weight relations. Secondly, weight of the 'coleoptile tiller' was extremely variable even though it contributed less than 2% to the weight of the whole plant. There thus appears to be sufficient ground for its exclusion.

4.3.2 General Characteristics of the Tillering Pattern

Since tillers were recorded only on a pot basis, the errors for tiller number per plant cannot strictly be expressed on a per plant basis and in the tables presented below the standard errors given are the 'between pot errors' and not the 'between plant errors' even though tiller number is expressed on the basis of the single plant.

This deficiency is perhaps not a major one as

cancelled

it was observed that the range in time for a particular tiller to appear on all the 16 plants at each harvest was rarely more than one harvest interval (two days). Further there was considerable regularity both within and between environments in the order in which primary tillers appeared on the main stem, secondary tillers appeared on the primary tillers and so on. There was thus a strong tendency toward a uniform sequence in tiller appearance independent of the environment. For example T_1 C nearly always appeared immediately after T_2, and T_1T_2 after T_4. This tendency weakened as the total tiller number increased but it was particularly strong in the early stages.

4.3.3 Effects of Radiation and Temperature on Tillering

Tiller number per plant at each harvest is shown in Table 13 for each of the nine environments. It is concluded from this table that at high and medium radiation the order of the tillering rates at each temperature was: 20 > 30 > 10°C and at low radiation, 20 > 10 and 30°C. At 20 and at 30°C tillering rates decreased with decreasing radiation but the reduction was only slight as radiation was reduced from medium to low. These effects are summarised in Table 14 which includes a column 'Total Tiller Number' at Day 30 with the associated between pot errors.

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TABLE 13

Tiller Number Per Plant, Excluding Coleoptile Tiller at each Harvest

Day	High Radiation			Medium Radiation			Low Radiation		
	10°	20°	30°	10°	20°	30°	10°	20°	30°
4	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
6	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
10	1.0	2.0	1.3	1.0	1.0	1.0	1.0	1.0	1.0
12	1.0	2.0	2.0	1.0	1.3	1.0	1.0	1.0	1.0
14	1.0	3.0	2.5	1.0	2.0	1.3	1.0	1.8	1.0
16	1.8	4.0	2.8	1.0	2.8	1.3	1.0	1.8	1.0
18	2.0	5.0	4.3	1.0	3.0	2.3	1.0	2.3	1.0
20	2.0	7.0	4.3	1.5	3.8	3.0	1.0	3.0	1.0
22	3.0	8.3	5.5	2.0	4.0	2.5	1.3	3.3	1.0
24	3.5	11.5	6.8	2.0	5.3	3.8	2.0	5.5	1.3
26	4.5	14.3	7.5	3.0	6.8	5.3	1.8	5.0	1.5
28	5.0	19.5	11.5	3.0	7.5	6.0	2.0	5.5	1.8
30	5.2	25.0	15.8	3.0	11.3	8.5	2.5	7.3	2.5
32	7.3	-	-	3.8	14.1	10.3	4.0	9.0	2.8

TABLE 14

Leaf Numbers and Tiller Numbers Per Plant at Day 30

Radiation	Temp.	Leaf No. on M.S.	Total Leaf No.	Tiller No. on M.S.	Total Tiller No.	Tillers per Leaf on M.S.	Tillers per Leaf on Whole Plant
High	10	5.0 (0.20)	11.3 (0.3)	3.3	5.2 (0.3)	0.65 (0.05)	0.46
	20	8.0 (0.06)	58.0 (1.0)	5.0	25.0 (0.3)	0.67 (0.01)	0.43
	30	7.5 (0.40)	39.7 (4.2)	4.5	15.8 (2.1)	0.64 (0.02)	0.40
Medium	10	4.0 (0.13)	7.0 (0.0)	2.0	3.0 (0.0)	0.55 (0.02)	0.43
	20	6.0 (0.10)	24.8 (1.0)	3.8	11.3 (0.7)	0.65 (0.05)	0.46
	30	6.8 (0.20)	21.0 (1.8)	4.5	8.5 (0.4)	0.68 (0.03)	0.40
Low	10	4.0 (0.15)	5.7 (0.5)	1.5	2.5 (0.3)	0.33 (0.07)	0.44
	20	5.8 (0.15)	16.0 (1.1)	3.8	7.3 (0.5)	0.69 (0.03)	0.46
	30	6.0 (0.14)	8.0 (0.7)	1.5	2.5 (0.5)	0.31 (0.04)	0.31

Standard Errors are given in parenthesis.

Environmental effects on the growth rates of single tillers are quantitatively examined in (4.9.0). For the present, Table 15 has been compiled to show that although particular tillers may have appeared in a given environment at a given time, their weights at that time were dependent on the level of radiation and temperature. This table which sets out the tiller weights at Day 30 also shows how the tillering pattern was influenced by the environment. The coleoptile tiller was the most 'labile' of the early formed tillers - a reduction in radiation from high to medium completely prevented its development. T_1 was the next affected as it did not appear at low 30, and was markedly reduced in weight compared with T_2 at Medium 30.

4.3.4 Leaf Production

Rates of leaf production are best examined only on the main stem as total leaf number will be a function of tiller number and it is only the main stem that developed sufficiently in all environments to permit a full range of comparisons to be made. Leaf numbers on the main stem of individual plants are shown in Table 16. The trend for increase in leaf number with time in each environment appears to be discontinuous due to the regularity of leaf appearance but it can be seen that the rate was approximately constant in time at each level of radiation and temperature. Final leaf numbers on the main stem are compared in Table 14.

TABLE 15

Weights (mg. per pot) of Coleoptile Tiller (C), Main Stem (M.S.) and Other Tillers at Day 30

	C	M.S.	T_1	T_2	T_3	T_4	T_5	T_1 C	T_1 T_1	T_1 T_2	T_1 T_3	T_2 C	T_2 T_1	T_2 T_2	T_3 C	T_3 T_1	T_4 C	T_1 C C	T_1 C T_1	T_1 C T_2	T_2 C C	T_2 C T_1	T_1 T_1 C	T_1 T_1 T_1	T_2 T_1 C	T_1 C C C
High Radiation																										
10°	26	160	73	41	10	-	-	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20°	145	670	491	443	344	178	38	302	252	129	26	223	138	29	96	32	11	115	62	5	43	11	61	11	13	10
30°	31	285	209	173	100	60	8	77	65	26	-	58	33	-	20	3	3	16	3	-	2	-	8	-	2	-
Medium Radiation																										
10°	-	88	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20°	-	293	164	142	71	7	-	61	30	-	-	30	3	-	-	-	-	4	-	-	-	-	-	-	-	-
30°	-	215	12	89	53	12	-	-	-	-	-	15	5	-	2	-	-	-	-	-	-	-	-	-	-	-
Low Radiation																										
10°	-	57	8	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20°	-	193	44	63	23	-	-	8	2	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30°	-	106	-	4	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

TABLE 16

Leaf Numbers on the Main Stem at Each Harvest

Leaf Number is Expressed on a Single Plant Basis

Day	High Radiation			Medium Radiation			Low Radiation		
	10	20	30°C	10	20	30°C	10	20	30°C
4	1.0	1.3	2.0	1.0	1.0	1.8	1.0	1.0	1.8
6	1.3	2.0	2.0	1.0	2.0	2.0	1.0	2.0	2.0
8	1.5	2.5	2.3	1.0	2.0	2.0	1.3	2.0	2.0
10	2.0	3.0	3.0	2.0	3.0	3.0	2.0	2.0	2.0
12	2.0	4.0	3.0	2.0	3.0	3.0	2.0	3.0	3.0
14	2.8	4.0	4.0	2.0	3.8	3.3	2.0	3.0	3.0
16	3.0	4.5	4.0	2.0	4.0	4.0	2.0	3.5	3.0
18	3.3	5.0	4.5	3.0	4.0	4.0	2.3	4.0	3.8
20	4.0	5.0	5.0	3.0	4.3	5.0	3.0	4.0	4.0
22	4.0	6.0	5.3	3.3	5.0	5.0	3.0	4.5	4.0
24	4.5	6.5	6.0	3.8	5.0	5.5	3.0	5.0	5.0
26	5.0	6.8	6.0	4.0	5.8	6.3	3.0	5.0	5.0
28	5.0	7.3	7.0	4.0	5.8	6.3	3.8	5.0	5.3
30	5.0	8.0	7.5	4.0	6.0	6.8	4.0	5.8	6.0
32	6.0	-	-	4.8	6.5	7.3	4.0	6.0	6.3

It can also be seen in this table that the total leaf number developed on the whole plant is a direct function of the tiller number and followed similar trends to tiller number in relation to the environmental factors.

Major differences in leaf number on the main stem occurred only between high radiation vs. low and medium; and between 10°C vs. 20 and 30°C.

The mean leaf appearance interval (days per leaf) was calculated using all data from all tillers, the time interval between successive leaves ($L_2 - L_1$ etc.) on any tiller being averaged. There was no evidence that this rate differed between tillers in the one environment. These data are summarised in Table 17. The interval decreased as the temperature was increased from 10°C to 20°C but was not affected by a further increase of 10°C. It is noteworthy that the optimal range extended over 20-30°C in contrast to the responses by other growth processes where 20°C was optimal.

The interval lengthened with each decrease in radiation level at each temperature. There is no indication of any interaction between the effects of radiation and temperature on the rate of leaf appearance.

TABLE 17

Leaf Appearance Interval (Days per Leaf) for all Leaves Produced on all Tillers

Radiation	Temperature	Days per Leaf	
High	10	6.2	(0.39)
	20	3.9	(0.13)
	30	3.9	(0.27)
Medium	10	7.3	(0.98)
	20	4.9	(0.39)
	30	4.3	(0.31)
Low	10	9.5	(0.50)
	20	5.3	(0.30)
	30	5.3	(0.42)

Radiation Means:		
	High	4.7
	Medium	5.5
	Low	6.7

Temperature Means:		
	10°C	7.7
	20°C	4.7
	30°C	4.5

4.4.0 INCREASE IN AREA OF THE TOTAL LEAF LAMINA SURFACE

4.4.1 Day 0 to Day 4

Examination of the growth of the leaf surface shows that the total lamina area raises problems similar to those for total dry weight. From Day 0 to Day 4 seedling growth was influenced both by translocation from the endosperm and by current photosynthesis.

The curves obtained from an initial plotting of $\log_e$ leaf area against time showed one phase for Day 0 to Day 4 and a phase of different slope after Day 4, as also occurred for $\log_e$ total dry weight. The differences in the slopes for these two phases were not as marked for area as for dry weight. The errors associated with the early area measurements are also quite large. It seems reasonable therefore to treat the leaf area data in a similar manner to the dry weight data and use the area at Day 4 as the base value - the initially measured leaf area for subsequent harvests.

The leaf areas at Day 4 show the effects of radiation and temperature on the rate of expansion of the leaf surface up to this time (Table 18). This area was almost entirely provided by leaf one, leaf two having appeared only at High 20. It should be noted that even leaf one was not fully expanded in all environments by Day 4.

TABLE 18

Leaf Area ($cm.^2$) at Day 4

Radiation	Temperature: 10	20	30°C
High	0.93	1.96	1.46
Medium	1.60	2.66	2.67
Low	1.75	2.89	3.05

Temperature x Radiation Interaction significant at $P = 0.01$

L.S.D. (0.01) Body of Table 0.38

Despite the short time interval of only four days from planting, leaf area was strongly influenced by the environment. A larger area developed at each temperature with each decrease in radiation level, differences between High and Medium being more marked than between Medium and Low.

Plants reacted differently to temperature at each level of radiation. At high radiation 20°C was optimal for leaf expansion but at low radiation the largest area developed at 30°C.

The above results show that the responses of leaf area to variation in radiation and temperature were rapid, substantial differences being recorded after four days.

The rate of expansion of the total leaf surface will depend on: (i) the rate at which leaves appear on the whole plant; (ii) the rate of expansion of individual leaves; (iii) the final area attained by each leaf. These components of the total leaf area growth rate will be discussed in more detail later but it is pointed out that between Day 0 and Day 4 only component (ii) was operative. It will be shown subsequently that the environment influenced all three components so that if the response of the whole plant is to be examined it should be done over a period when all components are contributing to the expansion of the total leaf surface. This situation is more nearly reached at Day 4 than at Day 0 which is a further reason for using Day 4 as the starting point for a consideration of the expansion of the leaf surface.

4.4.2 Increase in Area After Day 4

Quadratic regressions of the form

$$y = a + bt + ct^2$$

relating $\log_e$ area (y) to time (t) were examined and were found to give an excellent fit to the data as shown in Figure 6.

Inspection of Figure 6 shows that the degree of curvature in the relation between $\log_e$ area and time varied with the environment. This is examined quantitatively in Table 19 which also shows the coefficients of linear regression of $\log_e$ area on time, the average Relative Leaf Area Growth Rates (R_L) in each environment. These rates expressed as $\text{cm.}^2\ \text{cm.}^{-2}\ \text{day}^{-1}$ are shown below.

	Temperature:		
	10	20	30°C
High radiation	0.162	0.221	0.164
Medium radiation	0.115	0.175	0.138
Low radiation	0.099	0.146	0.091

It may at once be remarked how closely these figures parallel those for mean relative growth rate (R);

Figure 6 The relation between $\log_e$ leaf area and harvest number in each environment.

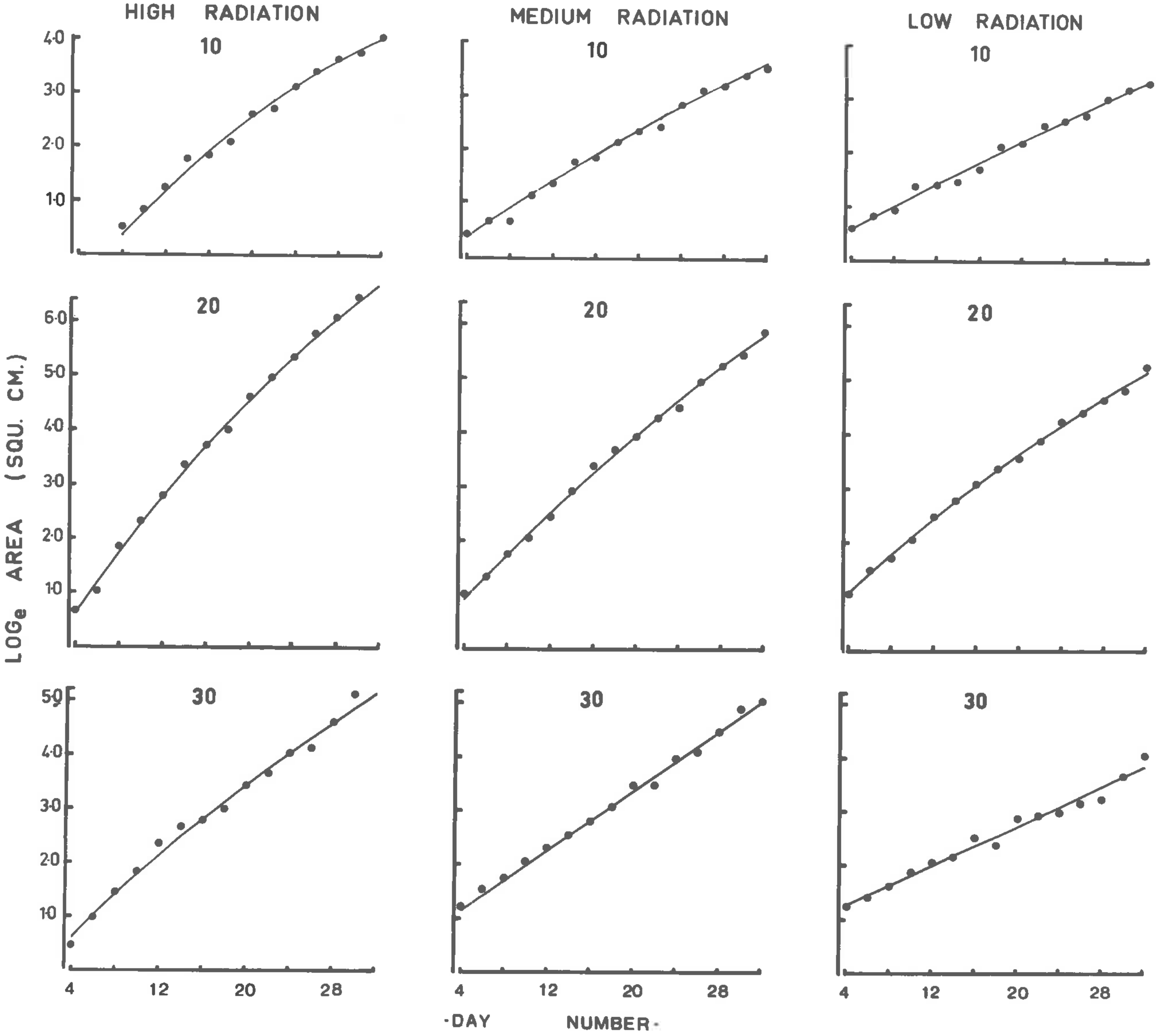

HIGH RADIATION
MEDIUM RADIATION
LOW RADIATION
10
20
30
4·0
3·0
2·0
1·0
6·0
5·0
4·0
3·0
2·0
1·0
5·0
4·0
3·0
2·0
1·0
LOGe AREA (SQU. CM.)
4
12
20
28
DAY NUMBER

TABLE 19

The Relative Rate of Increase of the Leaf Surface (R_L) and its Rate of Decline as Estimated from Quadratic Regressions Fitted to Log$_e$ Area on Time from Day 4 Onwards

Radiation	Temp.	Linear Coeff. (b)		R_L	Quad. Coeff. (c)		't' Quad. Coeff.	d.f.	Significance Level of 't'
High	10	0.3240	(0.0069)	0.162	-0.0110	(0.0018)	6.09	12	0.001
	20	0.4415	(0.0059)	0.221	-0.0096	(0.0016)	5.84	11	0.001
	30	0.3270	(0.0099)	0.164	-0.0050	(0.0027)	1.82	11	n.s.
Medium	10	0.2308	(0.0058)	0.115	-0.0027	(0.0015)	1.83	12	n.s.
	20	0.3491	(0.0047)	0.175	-0.0047	(0.0012)	3.89	12	0.001
	20	0.2751	(0.0054)	0.138	+0.0009	(0.0014)	0.63	12	n.s.
Low	10	0.1987	(0.0058)	0.099	-0.0005	(0.0015)	0.37	12	n.s.
	20	0.2925	(0.0037)	0.146	-0.0047	(0.0009)	4.95	12	0.001
	30	0.1827	(0.0079)	0.091	+0.0007	(0.0020)	0.35	12	n.s.

S.E. given in parenthesis.

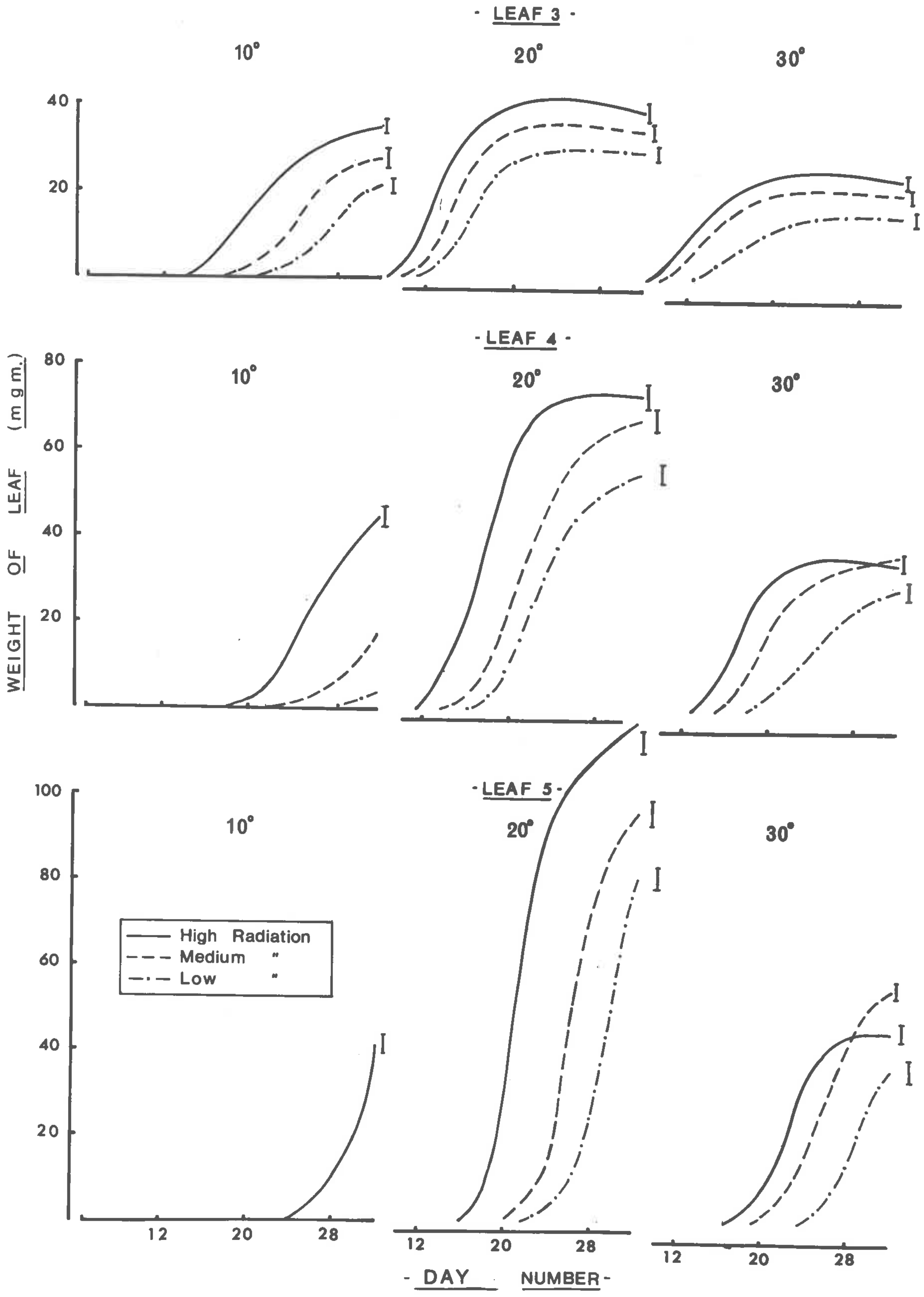
this point is amplified in Discussion. As with dry weight a significant decline with time from a constant relative growth rate occurred at 20°C at all levels of radiation. Expansion of the total lamina area was therefore exponential or slightly less than exponential under all conditions.

4.5.0 INCREASE IN WEIGHT OF THE SINGLE LEAF LAMINA

In each environment each leaf on the main stem or on any tiller reached, or approached, a greater weight than the leaf that preceded it. After each leaf appeared, its weight followed a more or less S-shaped curve with respect to time. The general form of these curves was similar but they varied in detail and in asymptotic value according to the environment. In some cases the phase of rapid growth tended to be linear with no apparent point of inflexion. Growth curves for leaves 3-5 on the main stem are shown in Figure 7 to illustrate the general pattern displayed by all leaves in all environments.

Comparisons of the effects of radiation and temperature on leaf growth are limited because only a small number of leaves reached full expansion in the least favourable environments. Tillering was also virtually prevented by these treatments so the effects of environments are confined to leaves 3, 4 and 5 on the main stem

Figure 7 Growth curves (mg. of leaf) for leaves 3, 4 and 5 on the main stem in each of nine environments.



- LEAF 3 -
10°
20°
30°
40
20
- LEAF 4 -
80
60
40
20
WEIGHT OF LEAF (m g m.)
- LEAF 5 -
100
80
60
40
20
High Radiation
Medium "
Low "
12
20
28
- DAY NUMBER -

(Figure 7). The three major features of these curves are:

(i) the time at which a particular leaf appears is strongly influenced by temperature and radiation, in keeping with results already given for environmental effects on the rate of leaf appearance. At each level of radiation a leaf first appeared at 20°C and either at the same time or slightly later at 30°C. Decreasing the temperature to 10°C markedly delayed leaf appearance. A decrease in the radiation level at any one temperature also resulted in a delay in appearance.

(ii) The ultimate weight attained by each leaf was also influenced by temperature and radiation. At each radiation level the greatest weight was always reached at 20°C. An increase to 30°C resulted in a lighter leaf, the difference increasing with the point of leaf insertion. To illustrate this point the following shows the maximum weights of main stem leaves 2, 3, 4 and 5 at 20 and 30°C (high radiation) and the ratios between them.

	Leaf Number:			
	2	3	4	5
Asymptotic Weight 20°	17.5	42.5	75.0	121.4
" " 30°	15.0	25.0	35.0	44.6
Ratio 20/30°	1.2	1.7	2.1	2.7

The way in which low temperature influenced ultimate leaf weight is not completely clear. For leaf 2 on the main stem (not shown in Figure 7) 10°C appeared optimal but for all other leaves 20°C was optimal. At 20°C the highest radiation always resulted in the heaviest leaf and low radiation the lightest but at 30°C the relative positions of final weights under high and medium radiation varied with the particular leaf.

(iii) The shape of the growth curve of a leaf after it appeared was largely determined by temperature, the curves at each radiation level being quite consistent at any one temperature. There is thus an indication in Figure 7 that the form of the growth curve of a leaf is unaltered by the level of radiation, this factor influencing only the time of leaf appearance and the upper asymptote.

The best means of approaching this question would be to regard the leaf growth curves as autocatalytic curves of the form

$$\log \frac{A}{x} = k(t_2 - t_1)$$

where A is the upper asymptote, x the extent to which this is reached, k a constant and t time. An analysis along these lines is particularly attractive but it has not been attempted for two reasons. Firstly, it must be remembered that although the data have been treated as though a single leaf was being examined, in actual fact the values obtained are the weights of a <u>population</u> of leaves (e.g. all third leaves on main stem) each member of which may be at a slightly different stage of development. Measurement of the behaviour of a population in time therefore may not truly reflect the behaviour of an individual leaf. The parameters of the above equation cannot therefore be estimated accurately. Secondly, in view of the relatively infrequent sampling in time, it is doubtful whether such parameters would yield much more information from the present data than can be deduced from Figure 7 or from the procedure given below.

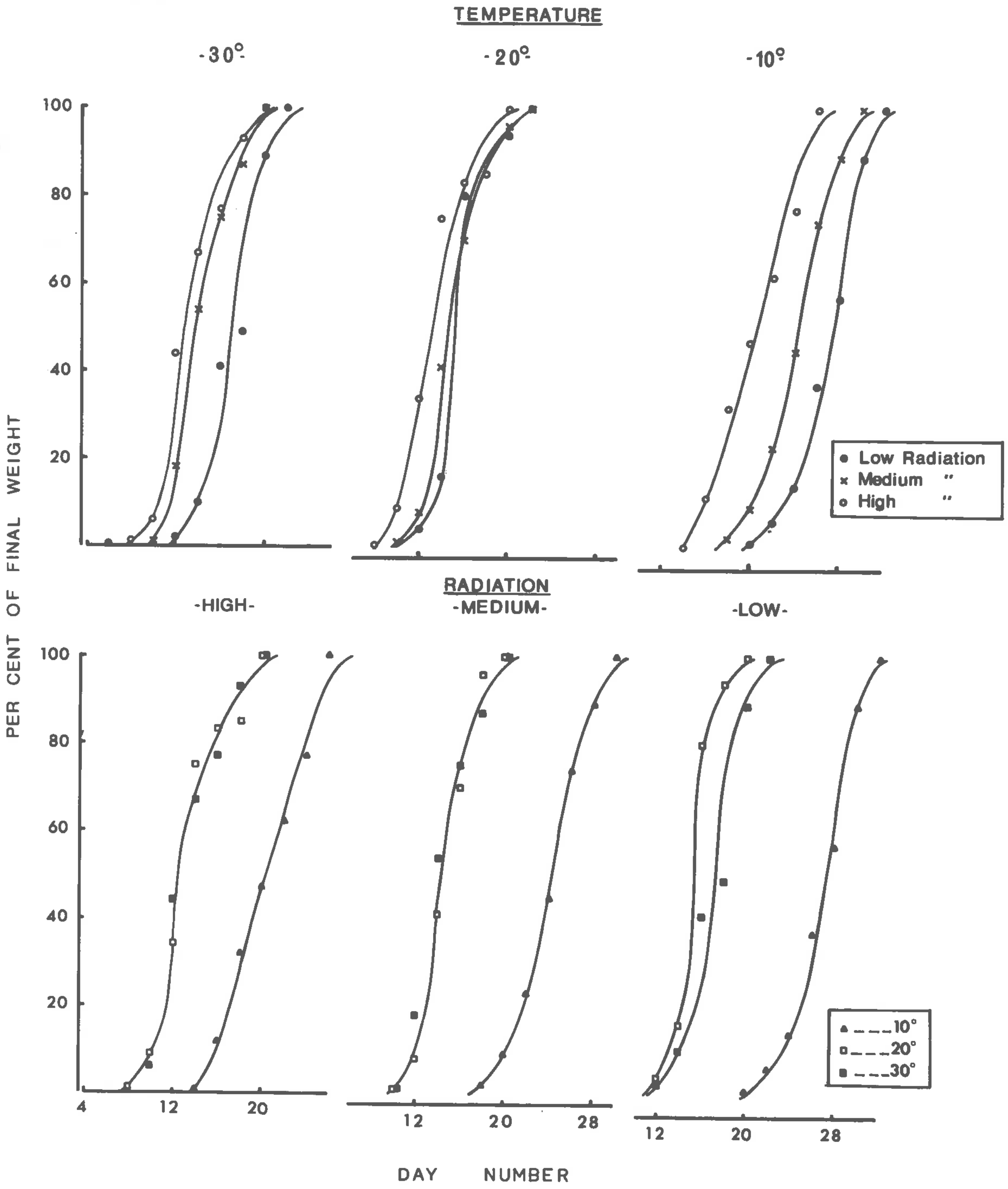
To facilitate comparison of the curves in Figure 7 it is necessary to eliminate variation in the ultimate weight attained and the time of leaf appearance. This is partly achieved by equating the maximum weight reached to 100 and expressing the weight at each harvest as a percentage of the maximum. Determination of the maximum proved a little difficult since leaves showed some variation in maximum weight at successive harvests after a maximum appeared to have been reached. To improve the accuracy of the estimate of this value, the means of the weights recorded after the apparent maximum was reached were calculated except where there was an obvious downward trend in which case the actual maximum weight was used. Weights at earlier harvests were then calculated as a percentage of the 'mean maximum' weight. Results of this procedure for leaf 3 and leaf 4 on the main stem are shown in Figure 8. The curves were drawn by eye and must be

Figure 8 Percentage of final leaf weight attained at each harvest for:

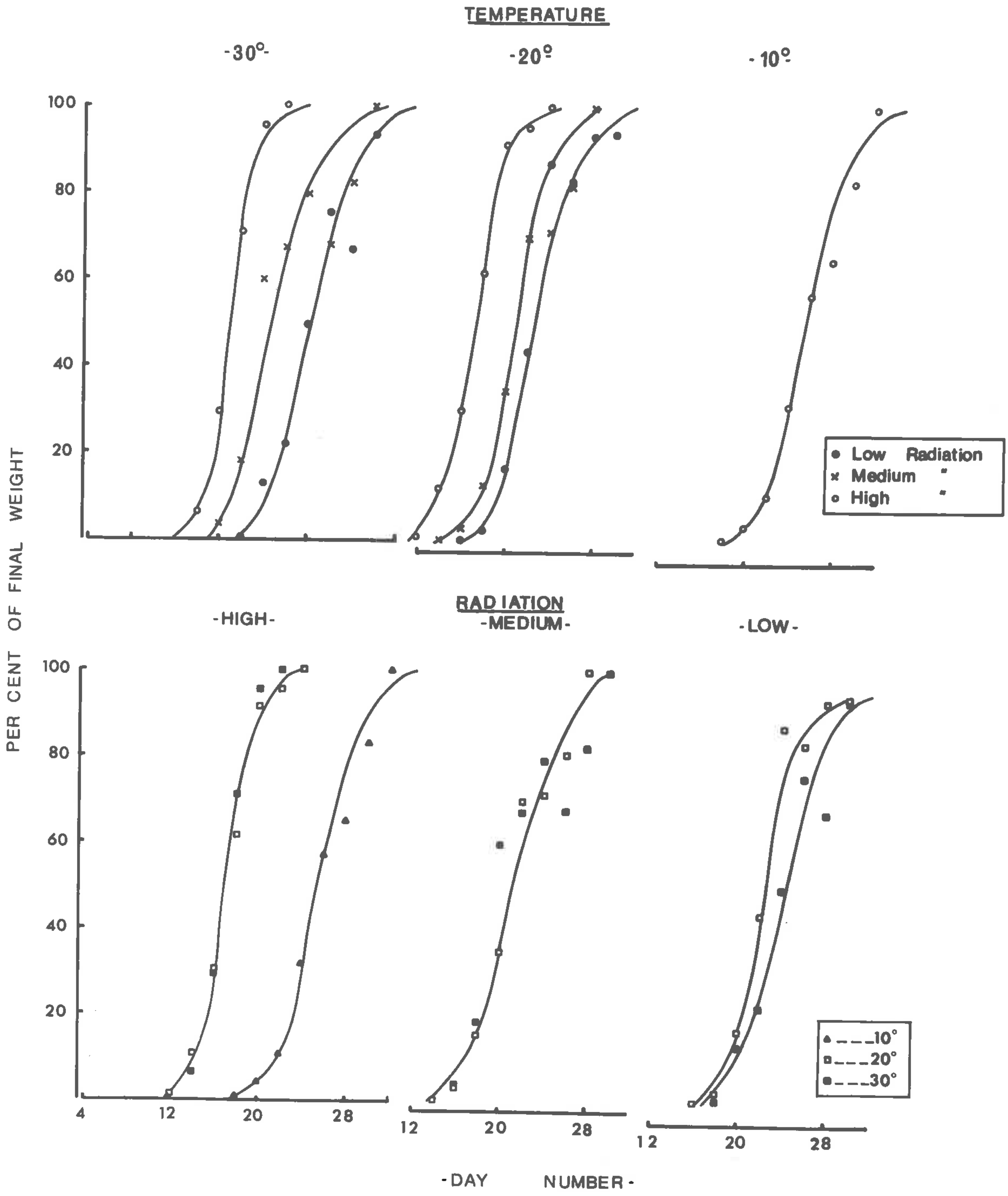
First Part: Leaf 3 on main stem;

Second Part: Leaf 4 on main stem.

LEAF 3


TEMPERATURE
-30°-
-20°-
-10°-
100
80
60
40
20
● Low Radiation
× Medium "
○ High "
PER CENT OF FINAL WEIGHT
RADIATION
-HIGH-
-MEDIUM-
-LOW-
▲ ---10°
□ ---20°
■ ---30°
4
12
20
28
DAY NUMBER

LEAF 4


TEMPERATURE
-30°-
-20°-
-10°-
100
80
60
40
20
● Low Radiation
× Medium "
○ High "
RADIATION
-HIGH-
-MEDIUM-
-LOW-
PER CENT OF FINAL WEIGHT
100
80
60
40
20
4
12
20
28
12
20
28
12
20
28
▲ ---10°
□ ---20°
■ ---30°
-DAY NUMBER-

regarded as an approximate representation of leaf growth under the different regimes.

The upper part of each figure shows the effects of radiation at each temperature; it appears that decreased radiation delayed leaf appearance and that the time taken for a leaf to reach maximum weight increased with decreasing radiation.

The lower part of each figure shows how leaf growth was influenced by temperature at each level of radiation. At high and medium radiation, 20 and 30°C are indistinguishable in their effects but at low radiation leaves took slightly longer to reach maximum weight at 30°C compared with 20°C. At 10°C leaf appearance was delayed appreciably so that leaf 4 fully expanded within 32 days under high radiation only.

A feature of the leaf growth curves as represented in Figure 8 is the apparent similarity of their shapes which suggests that leaves reached their maximum weights at similar rates under the wide range of conditions employed. This was assessed in the following way.

The mean maximum weights of all leaves (excluding leaf one) that reached a maximum weight by the time of the final harvest were determined and the time taken for 90% of this weight to be produced (5% to 95%) was calculated. The number of leaves available for examination depended on the environment and in some cases the number was very small. Nevertheless the results (Table 20) are informative.

Firstly in any environment the range in days for full (weight) expansion is small in relation to the range in the final weights attained - the time to reach maximum weight was relatively independent of that weight.

Secondly, between environments expansion to maximum weight was fastest at 20°C; at high radiation was slightly retarded by a temperature rise from 20°C to 30°C; and was also retarded by a decrease in radiation from High to Low. The effects of radiation and temperature on leaf expansion to maximum weight are in good agreement with those on rate of leaf appearance (Table 17).

4.6.0 INCREASE IN THE AREA OF THE SINGLE LEAF LAMINA

The pattern of increase in the area of single leaves after appearance is essentially the same as that of increase in weight - a series of S-shaped curves displaced along the time axis and reaching different asymptotic values. The effects of radiation and temperature on the area of single leaves were however different from their effects on leaf weight.

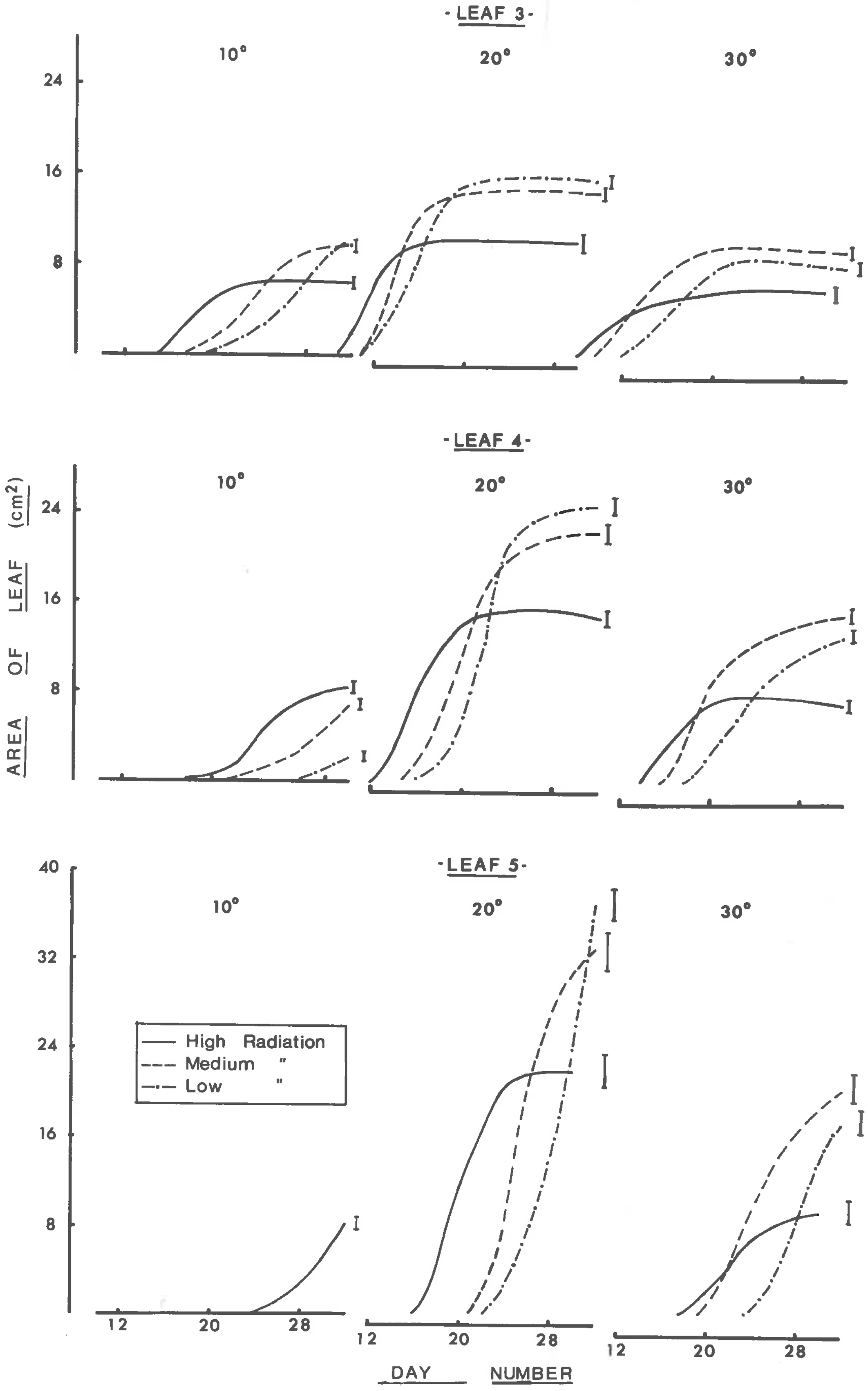
Figure 9 shows that at any radiation level the ultimate area is determined by the temperature. 20°C is optimal, a marked decrease occurring when the temperature

TABLE 20

Mean Number of Days After Leaf Appearance (taken as 5% of mean maximum weight) for a Single Leaf to Reach 95% of Mean Maximum Weight

Radiation	Temp.	No. Leaves	Days to Max. Weight		Range in Mean Max. Weight (mg.)
			Mean	Range	
High	10	6	11.8	11-15	21-44
	20	10	8.4	8-9	18-121
	30	9	9.7	8-12	13-55
Medium	10	2	12.0	11-13	6-26
	20	8	10.3	9-12	17-96
	30	7	11.0	9-13	8-54
Low	10	2	15.0	14-16	6-21
	20	5	10.0	9-11	12-80
	30	3	10.7	10-12	8-36

Figure 9 Increase in area of leaves 3, 4 and 5 on the main stem with time in each of nine environments.



-LEAF 3-
10°
20°
30°
24
16
8
-LEAF 4-
10°
20°
30°
AREA OF LEAF (cm²)
24
16
8
-LEAF 5-
10°
20°
30°
40
32
24
16
8
High Radiation
Medium "
Low "
12
20
28
12
20
28
12
20
28
DAY NUMBER

is raised to 30°C. The area attained at 10°C is probably intermediate between that reached at 20° and that reached at 30°C.

Radiation level had almost the reverse effect on leaf area to that which it had on leaf weight. At 10 and 20°C area increased with each decrease in radiation but at 30°C medium radiation appeared to be optimal with Low > High.

4.7.0 THE RELATION BETWEEN AREA AND WEIGHT OF SINGLE LEAF LAMINAE

In the two preceding parts, changes in the weight and changes in the area of single leaves were examined. These two quantities may now be considered in relation to each other. This can be done either by examining the ratio leaf area/leaf weight (specific leaf area) at each harvest or by examining leaf area as a function of leaf weight. It is considered necessary to adopt both procedures to explore the relation fully.

The specific leaf area (S.L.A.) of each leaf at each harvest is likely to show considerable variation since it is the ratio of two quantities each with an associated variance. The areas of very small leaves were difficult to determine accurately and leaf weight was determined only to an accuracy of 0.1 mg. Calculations of S.L.A. showed this quantity to be very variable especially when leaves were small, but nevertheless trends with time for individual leaves, differences between leaves in the one environment and effects of radiation and temperature are clearly discernible.

Specific leaf areas of all leaves on the main stem are set out in Table 21. It will be established later (Figure 10) that these data may be taken as representing the behaviour of leaves on other tillers. They are presented in full in the table as it is difficult to devise an alternative method of presentation that draws attention to all the following points.

(i) The S.L.A. of an individual leaf either decreases as the leaf expands until a nearly minimum value is reached, or it remains approximately constant with time.

(ii) The first three of four leaves on a tiller tend to reach successively lower final values of S.L.A. until an approximate common minimum is reached below which further decline with leaf insertion is small.

(iii) The environment, particularly the level of radiation, has a marked effect on S.L.A.

(iv) At each harvest the S.L.A. of the whole plant is not adequately described by taking the mean S.L.A.s of the constituent leaves which are at different stages of development.

TABLE 21

Specific Leaf Areas (cm.^{2} gm.$^{-1}$ x 10^{-1}) for Main Stem Leaves at Each Harvest

A. High Radiation

Day	10°C Leaf Number							20°C Leaf Number								30°C Leaf Number							
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
4	24							47								37							
6	29							44	32							40	41						
8	39							41	33							42	34	50					
10	32	30						42	41	59						42	33	35					
12	33	29						50	37	46	37					36	34	35					
14	28	42						45	35	35	34					36	35	33	31				
16	21	23	26					42	33	31	39					29	28	26	33				
18	20	20	27	40				41	33	29	28	51				32	21	20	20	30			
20	27	27	38	39				40	29	24	24	32				32	27	26	24	35			
22	30	20	28	23				-	29	25	22	23	34			31	22	22	22	24	50		
24	37	30	16	27	24			-	-	23	20	20	30			-	27	24	21	24	25		
26	22	19	20	25	25			-	-	26	21	19	23	48		-	27	22	16	19	21		
28	24	22	22	27	30			-	-	26	21	20	19	30	27	-	-	23	20	20	20	25	
30	28	18	18	21	26			-	-	26	20	18	13	23	31	-	-	-	18	19	18	21	30
32	-	21	18	18	20	41				x	x	x	x	x	x	x	x	x	x	x	x	x	x

TABLE 21 (continued)

B. Medium Radiation

	10°C							20°C							30°C							
	Leaf Number							Leaf Number							Leaf Number							
Day	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8
4	83							92							81							
6	66							69	74						80	133						
8	55							77	79						77	76						
10	49							60	56	60					71	61						
12	60	92						65	56	64					66	58	69					
14	66	70						60	51	59	85				60	55	48					
16	50	60						62	60	55	56				60	49	53	68				
18	53	53						57	49	47	61				59	53	51	62				
20	53	49	63					58	51	46	51				51	51	46	47				
22	38	45	45					54	51	40	45	63			67	44	41	41	57			
24	56	45	48	105				-	47	42	45	54			-	41	44	43	50	50		
26	55	44	32	55				-	53	41	37	40	52		-	40	47	44	49	55	97	
28	48	39	39	43				-	-	38	34	36	48		-	44	40	41	44	49	47	
30	40	33	35	49				-	-	42	36	37	41		-	53	45	41	37	42	66	
32	42	34	34	39	69			-	-	38	33	33	35	46	-	42	44	35	38	42	51	90

TABLE 21 (continued)

C. Low Radiation

Day	10°C							20°C							30°C							
	Leaf Number							Leaf Number							Leaf Number							
	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	8
4	80							90							92							
6	83							91							91	172						
8	68							84							92	110						
10	77							74	80						79	80						
12	65	148						72	73						84	79	68					
14	67	77						82	73	85					73	79	97					
16	75	52						78	71	74					62	70	80					
18	70	69						53	66	63	126				40	69	61	170				
20	56	55						60	74	56	69				42	69	64	75				
22	73	60	93					-	49	64	59				48	51	69	60				
24	70	58	68					-	69	56	51	130			-	46	57	53	53			
26	67	48	48					-	68	57	54	84			-	48	46	50	58			
28	69	55	56					-	58	54	49	59			-	-	55	53	59	180		
30	67	52	47	93				-	50	50	45	49	111		-	-	53	47	52	67		
32	67	52	45	73				-	-	47	45	46	59		-	-	42	46	48	52	43	

- Leaf area not measured due to leaf senescence.

* This harvest not made.

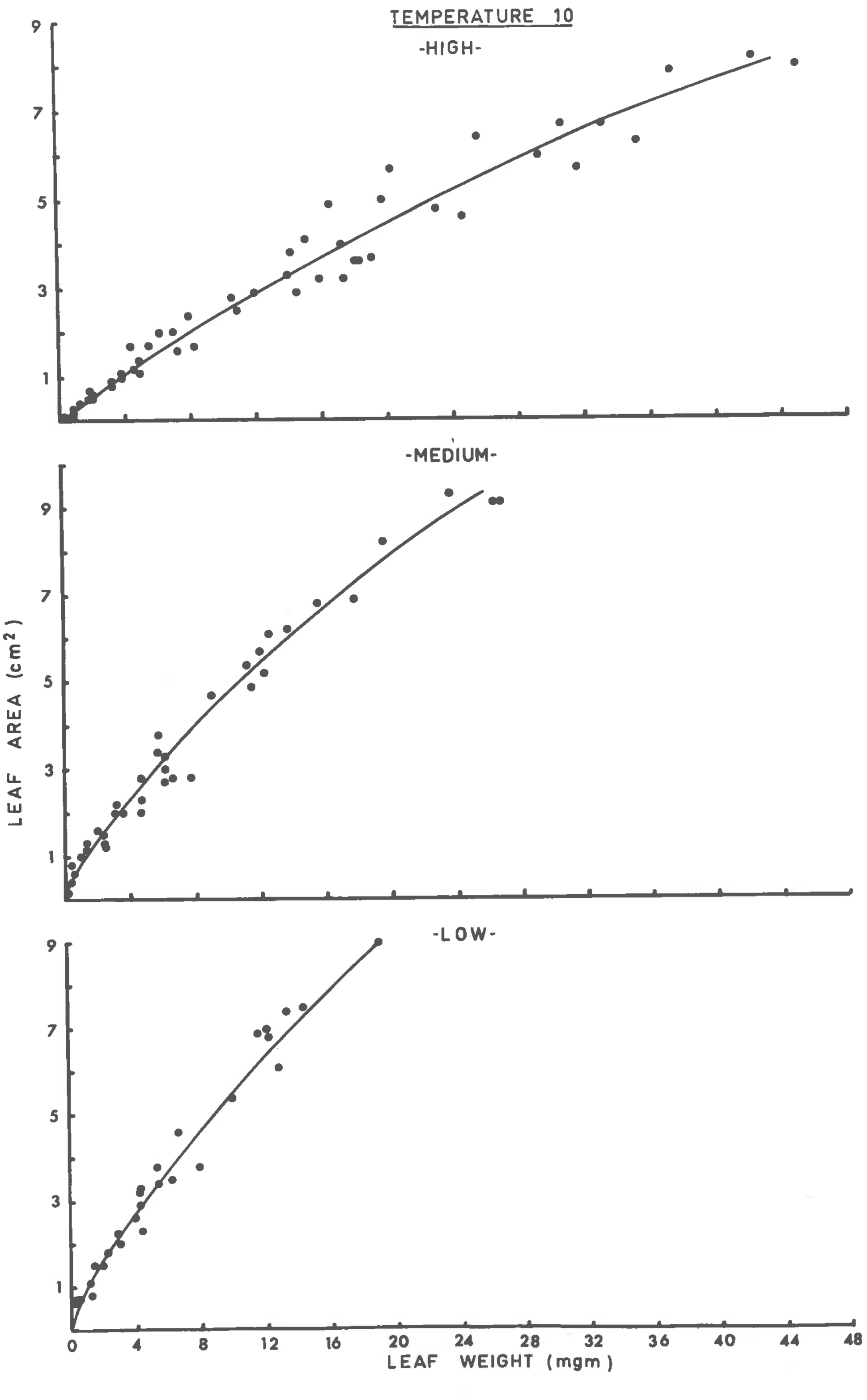
To examine these points further, it is useful to present the data in the alternative form by plotting leaf area against leaf weight (Figure 10). This method is much more comprehensive since it permits virtually all leaves to be included.

In drawing Figure 10 it was necessary to use a different scale for each temperature environment to achieve a comprehensible array of points. The fact that the values at different points of insertion on the one tiller or for leaves from different tillers lay on a common curve of area and weight is indicated on the figure.

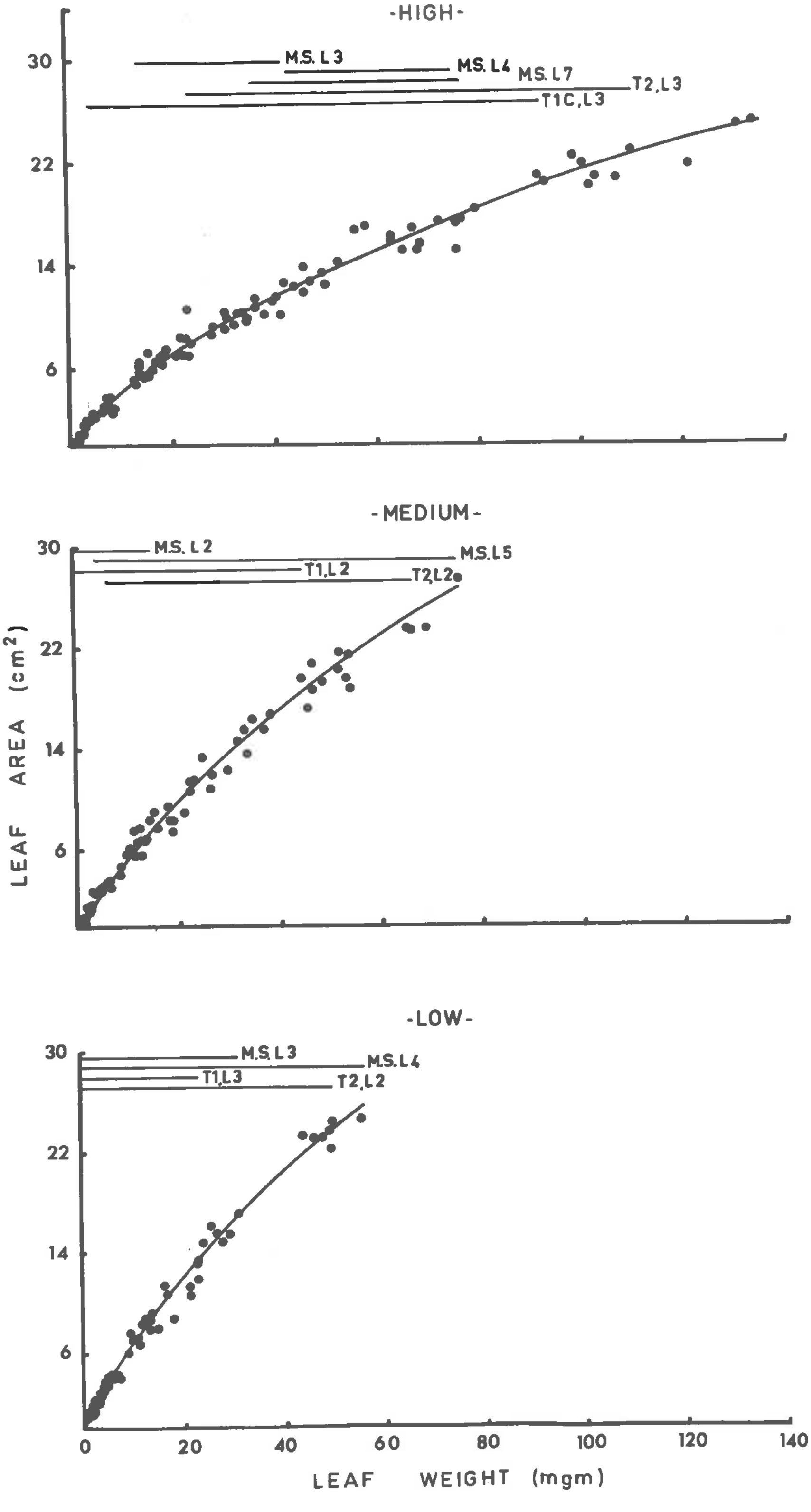
The conclusions to be drawn from Figure 10 are that in any one environment the area of a leaf is a function of its weight and that in that environment <u>all</u> leaves regardless of origin on the plant conform to a common relation between area and weight. Thus as each leaf expands the relation between its area and its weight is essentially the same and is independent of the final area (weight) attained. The change in S.L.A. shown by a large leaf as it increases in weight is the same as that shown by a small one, except that the small leaf ceases growth before its S.L.A. reaches the minimal value characteristic of the particular environment.

Care must be exercised in interpreting the above relation in detailed rather than in broad terms as the

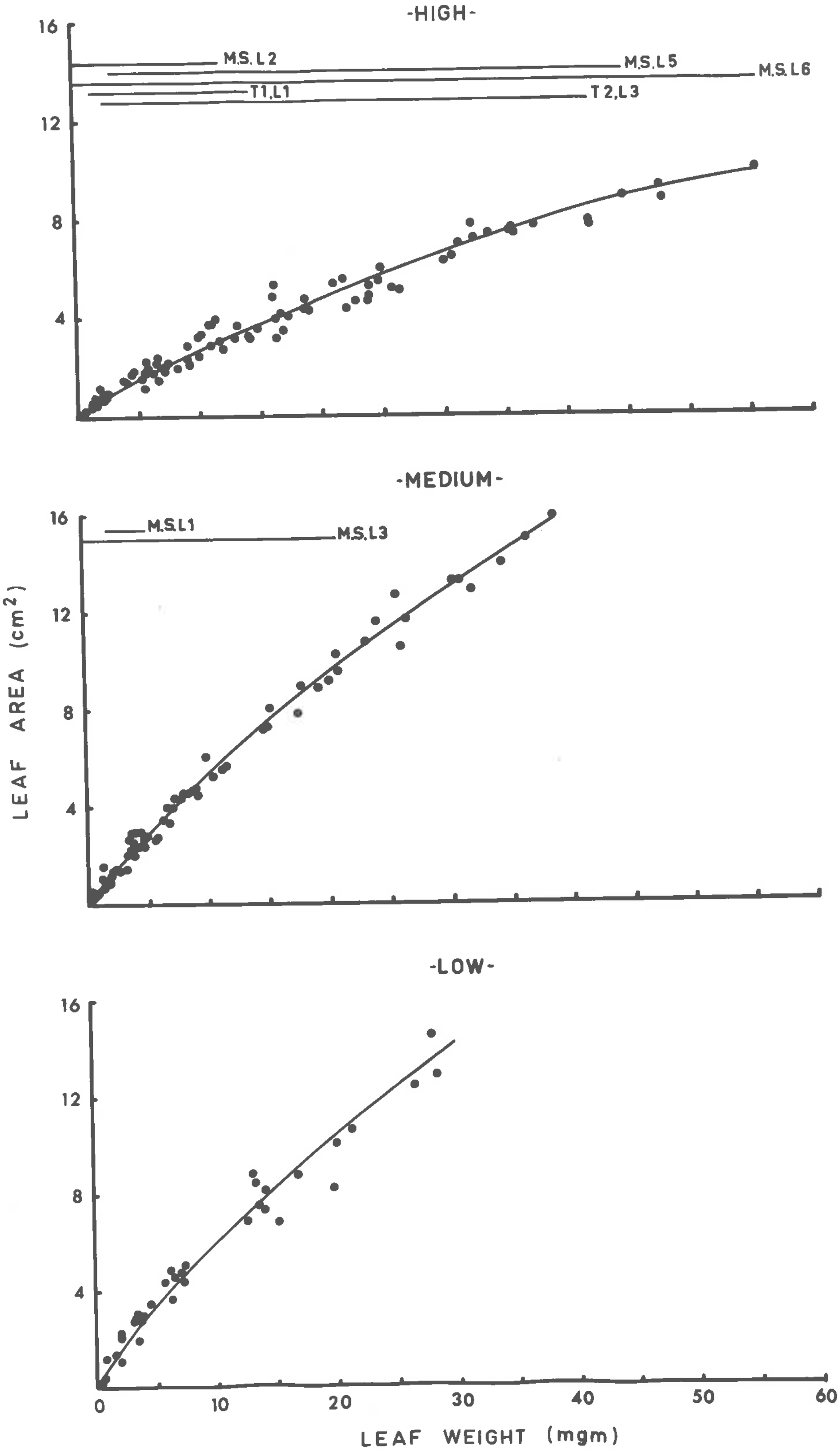
<u>Figure 10</u> The relation between individual leaf area and individual leaf weight for all leaves produced at each level of radiation and temperature. 'High', 'Medium' and 'Low' refer to radiation level.


TEMPERATURE 10
-HIGH-
-MEDIUM-
-LOW-
LEAF AREA (cm^2)
LEAF WEIGHT (mgm)
0
4
8
12
16
20
24
28
32
36
40
44
48
1
3
5
7
9

TEMPERATURE 20



TEMPERATURE 30



variation about a line drawn for any single leaf precludes a closer examination of the exact way that the curve for each leaf links up with that for the succeeding one. Initially the points in Figure 10 were plotted using a separate symbol for each leaf and the resulting array inspected for evidence of departure by any one leaf from the general curve. No evidence of this type of behaviour could be found and it therefore seems that all leaves conform to a generalised curve for any particular environment.

The leaf area and leaf weight data suggest that these attributes might be related allometrically. Log_e leaf area (L_1) was therefore plotted against Log_e (W_1) and a very good linear relation was found for each environment, i.e.

$$L_1 = bW_1^{\ k}$$

The values of the constant (b) and the exponent (k) were determined for each environment by fitting linear regressions of the form

$$\text{Log}_e\ L_1 = k \log W_1 + \log b$$

using as many pairs of measurements of L_1 ans W_1 as were available from each experiment. These data encompassed different leaves measured at different times during their development. In each case the variance ratio of the regression sum of squares was highly significant. Results are shown in Table 22.

The value of the exponent (k) varies from 0.71 to 0.83 and shows no consistent trend with change of temperature or radiation level: the mean value is 0.78. The constant (b) on the other hand increases as radiation decreases and at each radiation level is maximal at 20°C. Thus the most apparent effect of environment was to shift the intercept of the graph of $\log_e L_1$ on $\log_e W_1$ rather than alter its slope. In this, radiation had a much more pronounced effect than temperature.

A problem posed by the data given in Table 21 and the relations shown in Figure 10 is the best means of estimating an overall value for S.L.A. - the S.L.A. characteristic of a particular environment. Clearly it is unrealistic to use the mean of the specific leaf areas of individual leaves for this purpose since these varied with age and with the level of leaf insertion. Also the fact that the general relation between leaf area and leaf weight was curvilinear (Fig. 10) precludes the use of mean ratios at different leaf weights.

One approach to this problem is to consider only the mature leaves. If, in each environment, the means of the specific leaf areas of leaves at or near their mean maximum weights are calculated the figures recorded in

TABLE 22

Values of the Constant (b) and the Exponent (k) in the Equation $L_1 + bW_1^k$ Relating the Area (L_1) and the Weight (W_1) of Individual Leaves in Each Environment. The Mean Values for Specific Leaf Area (S.L.A.) of Mature Leaves are Also Shown.

Radiation	Temperature	b cm.^{2} mg.$^{-k}$	k	S.L.A. of Mature Leaves cm.^{2} mg.$^{-1}$
High	10	.37	.83	.21
	20	.80	.71	.22
	30	.52	.74	.21
Medium	10	.82	.76	.40
	20	.92	.78	.41
	30	.81	.81	.42
Low	10	.97	.77	.52
	20	1.01	.81	.55
	30	1.00	.76	.53

Table 22 for 'S.L.A. of Mature Leaves' are obtained. These figures show that the effect of temperature on S.L.A. was negligible but that of radiation was large, S.L.A. increasing as radiation decreased.

An alternative approach lies in the use of the constant (b) in the allometric relation between leaf area and leaf weight demonstrated above. The constant ($L_1 . W_1^{-0.78}$) is essentially the same expression as specific leaf area but is perhaps a more realistic measure of this attribute at all stages of leaf development.

The 'b' values are necessarily larger than the corresponding values for 'S.L.A. of Mature Leaves', show similar trends with radiation level but more apparent trends with temperature at high and medium radiation.

4.8.0 THE RELATION BETWEEN TOTAL LEAF AREA AND TOTAL PLANT DRY WEIGHT

The relation between total leaf area and total plant dry weight can be expected to be similar to that between individual leaf area and individual leaf weight since the leaf proportion on the whole plant was not markedly influenced by the environment.

Both total leaf area and total plant dry weight followed an exponential or slightly less than exponential rate of increase with respect to time in all experiments so the relation between these two quantities independently of time can be expected to be linear or curvilinear, depending on the coefficients of the terms in the equations relating each with time. When total leaf area was plotted against total plant dry weight, curves were obtained which were essentially similar to those for individual leaf area and individual leaf weight. $\log_e$ total leaf area was therefore plotted against $\log_e$ total dry weight and it was found that the resulting curves were consistently linear in each environment although there was more variation about the regression line the less favourable the environment, i.e. as the radiation level was reduced and as temperature departed from the optimum of 20°C. Linear regressions of $\log_e$ total area on $\log_e$ total weight were therefore calculated. The coefficients and constants are shown in Table 23.

Values of k generally decrease with decrease in radiation level and with rise in temperature but on the whole the variation between environments is not great. The constant 'b' increases as radiation decreases and is lower at 10°C than at 20 and 30°C.

4.9.0 THE GROWTH OF THE MAIN STEM AND OF SUBSIDIARY TILLERS

Growth curves of individual tillers were compared in as many of the nine environments as provided satisfactory data. The number of comparisons that could be made

TABLE 23

Linear Regression Equation Relating Log_e Total Leaf Area to Log_e Total Plant Weight

Equation: $L = bW^k$

Radiation	Temperature	k	S.E. of k	b
High	10	0.956	0.037	.148
	20	0.915	0.013	.272
	30	0.877	0.015	.236
Medium	10	0.865	0.022	.395
	20	0.889	0.009	.473
	30	0.853	0.017	.549
Low	10	0.922	0.026	.405
	20	0.873	0.007	.645
	30	0.774	0.017	.783

was limited firstly by the fact that the number of tillers produced dependent on the environment and secondly by the need for a minimum of four harvests to give a reasonable representation of the growth curve. Only the main stems could be compared in all environments and a limited number of other tillers in some of the environments. The particular tillers examined under particular conditions were as follows:-

		M.S.	T_1	T_2	T_1C	T_3	T_2C	T_1T_1
High Radiation	10°C	+	+	+	+	+		
	20	+	+	+	+	+	+	+
	30	+	+	+	+	+	+	+
Medium "	10	+	+	+				
	20	+	+	+	+	+	+	+
	30	+		+				
Low "	10	+	+					
	20	+	+	+				
	30	+						

The method of analysis was to calculate the linear and the quadratic regressions of $\log_e$ tiller weight (Day 4 onwards) on time for each replicate of each tiller and then conduct an analysis of variance on the linear and quadratic trends between environments. For the main stem it was possible to examine the 'radiation' and 'temperature' effects separately but for the subsidiary tillers the variables were undefined.

Table 24 shows the mean linear and quadratic coefficients for the main stem weights ($\log_e$) and the least significant differences between coefficients. Both radiation and temperature had highly significant effects on the linear and the quadratic coefficients. Since the main stem weights were measured from Day 4 onwards in all environments it is legitimate to compare the linear coefficients over the whole time range and to use this coefficient (divided by two) as the average relative growth rate of the main stem.

At 10°C and at 20°C the relative growth rate of the main stem was significantly reduced with each reduction in radiation level but at 30°C a reduction occurred only at low radiation. At each radiation level 20°C was clearly optimal for main stem growth and at the medium and low levels, 30°C   10°C. At high radiation, 10°C and 30°C did not differ. The interaction between radiation and temperature was significant at the 5% level of probability.

The values of the quadratic regression coefficients show that the relative growth rates of the main stem declined with time. The rate of decline decreased with decrease in radiation and was fastest at 20°C at each level of radiation. At Medium 10, Low 10 and Low 30

TABLE 24

Linear and Quadratic Trends in Log_e Dry Weight of the Main Stem from Day 4 Onwards

Linear Regression Coefficients

Temperature	Radiation: High	Medium	Low
10°C	0.290 (C)	0.243 (D)	0.212 (E)
20	0.355 (A)	0.312 (B)	0.288 (C)
30	0.284 (C)	0.275 (C)	0.236 (D)

L.S.D. (P = 0.01) 0.022

Quadratic Regression Coefficients

Temperature	High	Medium	Low
10	-0.997 (B)	-0.340 (C)	-0.362 (C)
20	-1.643 (A)	-1.437 (AB)	-1.113 (AB)
30	-1.491 (AB)	-0.949 (B)	-0.100 (C)

L.S.D. (P = 0.01) 0.559

Coefficients with the same letter in parenthesis do not differ significantly in each part of the table.

the quadratic coefficients were not significant so that in these environments the relative growth rates of the main stem were constant with time.

In general the rate of decline of relative growth rate increased with the average relative growth rate itself, i.e. the higher the relative growth rate the faster its decline with time. This is shown in Figure 11 in which the linear trend of the main stem (expressed as gm. $gm.^{-1}$ day^{-1}) is plotted against the corresponding quadratic coefficient.

Between environments the trends in relative growth rates of the main stem are related to those of the whole plant (Figure 12). This comparison is valid since both are the average relative growth rates over the same period of time. Figure 12 shows that at 10° and 20°C the mean R values for the main stem at different radiation levels follow a continuous curvilinear relation with the R values for the whole plant. A different curve of the same type occurs when the R values at 30°C are plotted. It is clear that where the relative growth rates are low, those of the main stem and the whole plant are similar but as conditions favour higher relative growth rates that of the main stem increases proportionately less than that of the whole plant.

Growth curves of T_1 and T_2 for seven of the nine environments are shown in Figure 13 and it can be seen

Figure 11 Quadratic coefficients of the equation

$$\log y = a + bt + ct^2$$

relating $\log_e$ weight of main stem (y) to time (t) plotted against the average relative growth rate of the main stem.

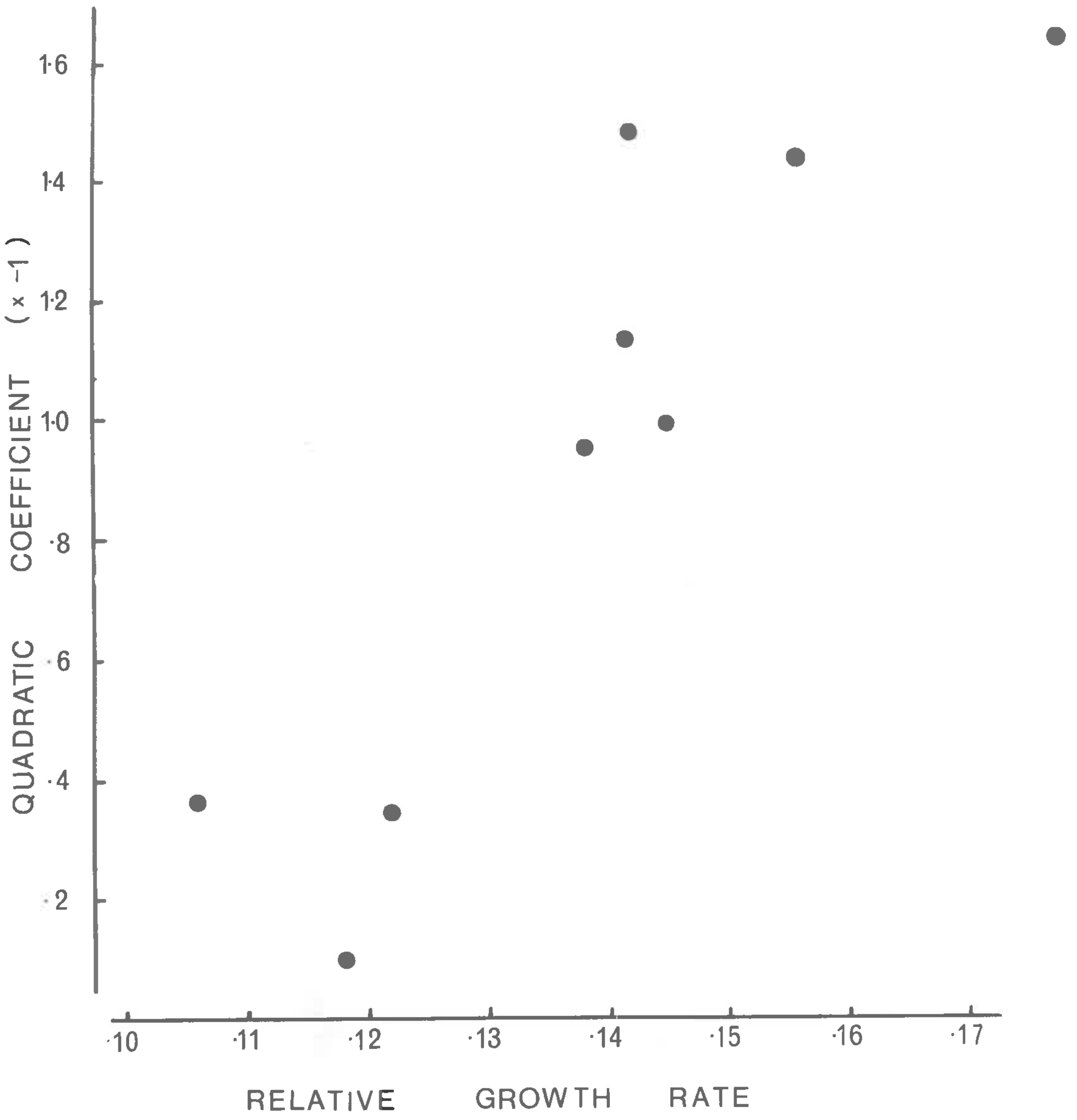
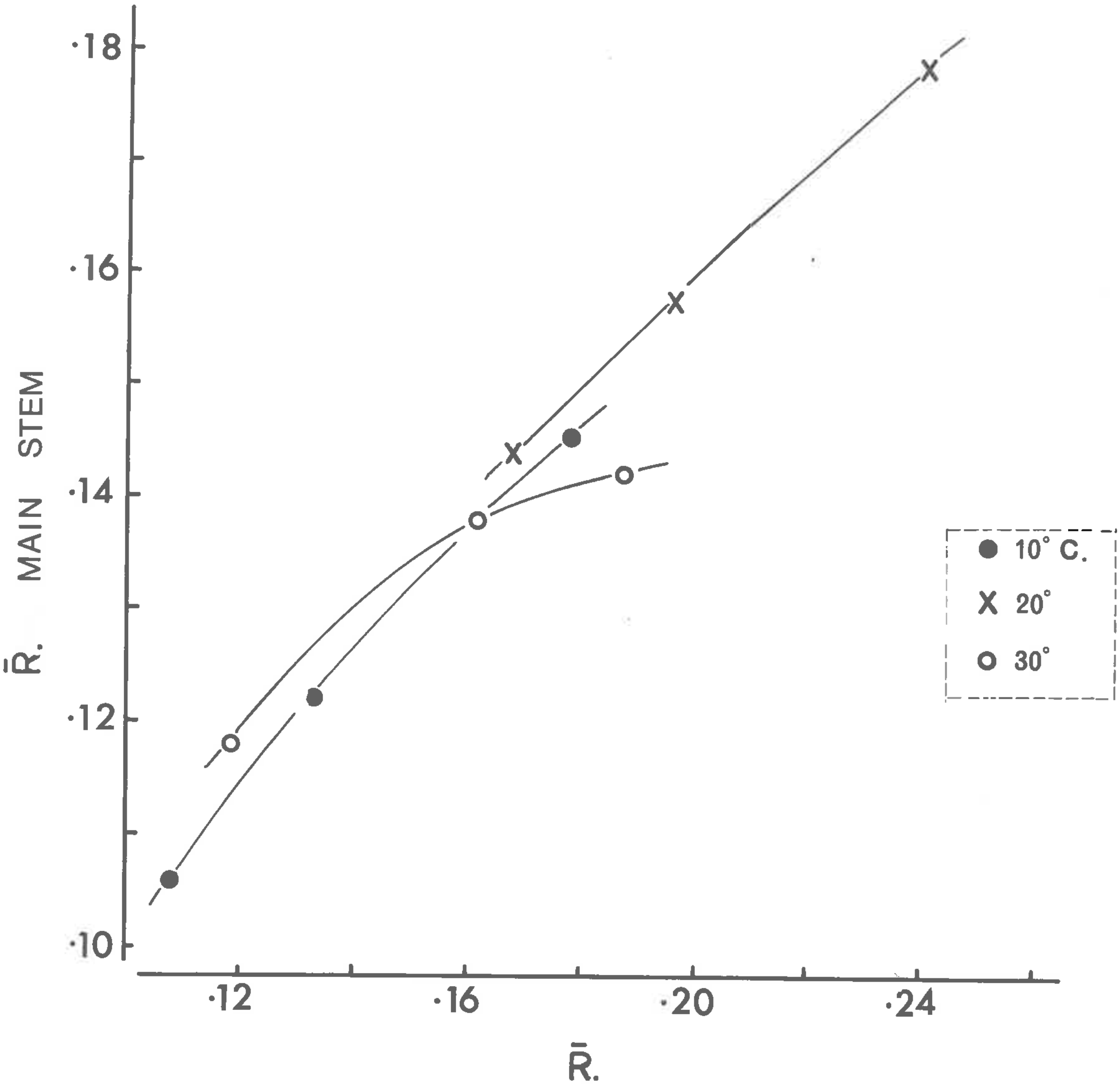
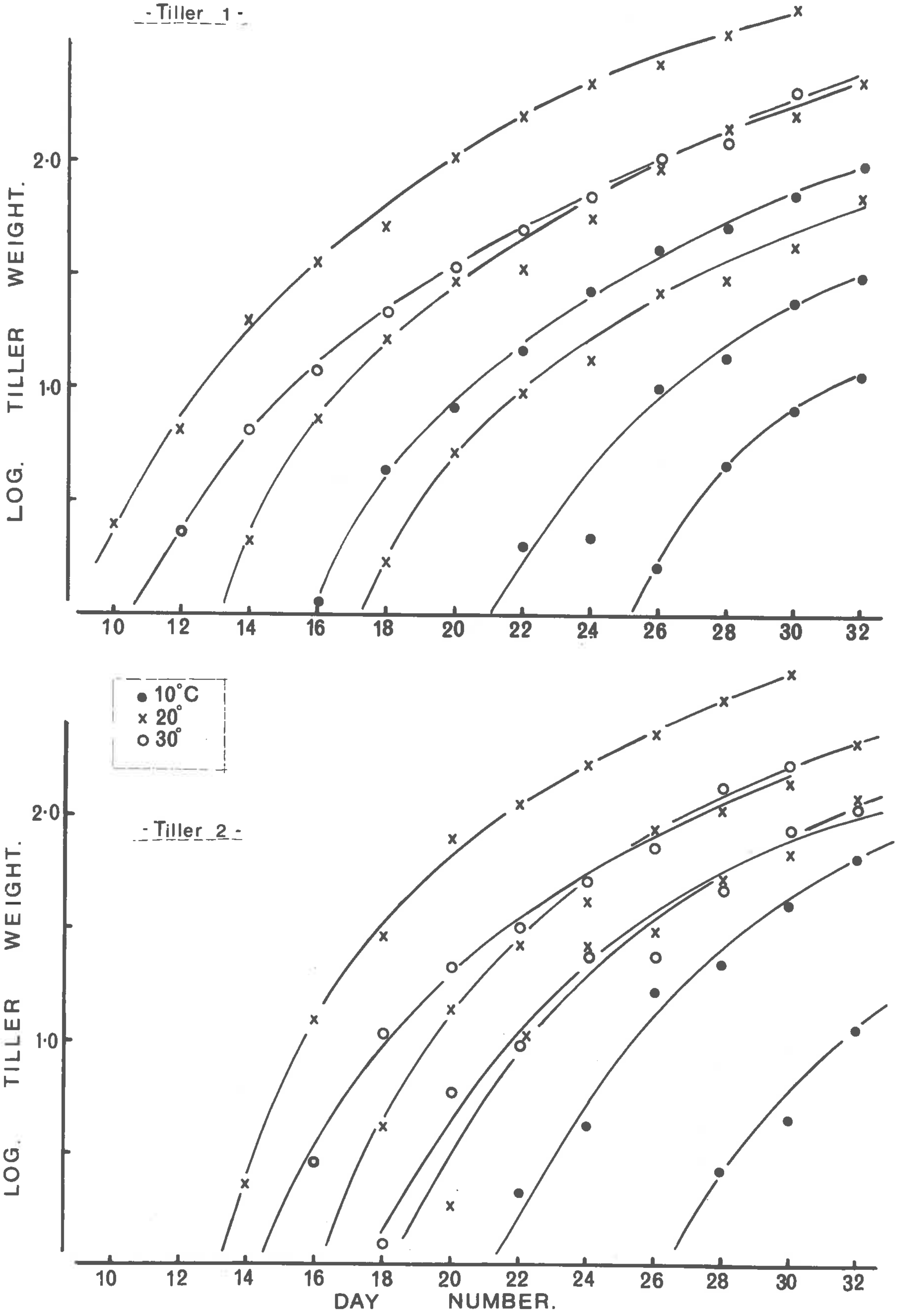

QUADRATIC COEFFICIENT (x -1)
1·6
1·4
1·2
1·0
·8
·6
·4
·2
·10
·11
·12
·13
·14
·15
·16
·17
RELATIVE GROWTH RATE

Figure 12 The relations between relative growth rates of the main stem and the relative growth rates of the whole plant at 10 and 20°C and at 30°C over all levels of radiation.



·18
·16
·14
·12
·10
R̄. MAIN STEM
·12
·16
·20
·24
R̄.
● 10° C.
X 20°
O 30°

<u>Figure 13</u> Growth curves for tiller one and tiller two in each of seven environments.


- Tiller 1 -
LOG. TILLER WEIGHT.
2·0
1·0
10
12
14
16
18
20
22
24
26
28
30
32
● 10°C
x 20°
o 30°
- Tiller 2 -
LOG. TILLER WEIGHT.
2·0
1·0
10
12
14
16
18
20
22
24
26
28
30
32
DAY NUMBER.

that the general shapes of the curves are similar. A major effect of environment appears to have been to displace the curves along the time axis; early in tiller development the curves appear to be similar in slope.

The effect of 'environment' on the shapes of the growth curves was determined by comparing the linear and quadratic trends in each 'environment' for $\log_e$ tiller dry weight on time by means of regression analyses. In these analyses 'environment' could not be specified as radiation or temperature. It was found that 'environment' significantly ($P<0.01$) affected the linear trends of $\log_e$ tiller weight but had no effect on the quadratic trends, indicating that the rates of decline of the relative growth rates of each tiller were consistent between environments. The significant difference between the coefficients of the linear trends both in T_1 and T_2 however is largely a consequence of differences in the extent of the curves in time between environments. It is clear from Figure 13 that the relative growth rates declined with time; the slope of the linear trend in each environment will therefore depend on the time range over which the regression is fitted. The more relevant parts of the growth curves of T_1 and T_2 are the initial slopes which represent the relative growth rates early in tiller development. An attempt was therefore made to compare the initial slopes of the curves.

The approach adopted was to determine the discriminant ($b^2 - 4ac$) of each of the quadratic equations calculated for each replicate of each tiller in each environment. If $y = \log_e W$ and t = time in

$$y = a + bt + ct^2$$

then at $\log_e W = 0$, i.e. tiller weight = 1 mg.

$$\frac{dy}{dx} = + \sqrt{b^2 - 4ac}$$

Means of the replicate values of the initial relative growth rates determined in the above manner are shown in Table 25. 'Environments' were significant at the 5% level of probability only in Tiller 2. No consistent effects of temperature or radiation are discernible but Tiller 2 appears to have a higher initial relative growth rate than Tiller 1. The conclusion is that effects of radiation and temperature on relative growth rate early in tiller history could not be demonstrated but it can be noted that these rates were exceedingly high at 40-80% per day.

Responses of T_1C, T_3, T_2C and T_1T_1 were also examined at certain temperatures at high and medium radiation only. Curves for these tillers are not presented. Linear and quadratic trends of $\log_e$ dry weight were determined as for T_1 and T_2. The number of harvests that contributed data to the curves for these

TABLE 25

Initial Relative Growth Rates of Tiller One and Tiller Two

		T_1	T_2
High Radiation	10°C	0.49	0.61
	20	0.60	0.73
	30	0.45	0.49
Medium Radiation	10	0.59	0.83
	20	0.47	0.67
	30	-	0.48
Low Radiation	10	0.54	-
	20	0.52	0.64
	30	-	-
L.S.D. (5%)		0.18	0.20

tillers was much smaller than in the case of T_1 and T_2 and it is considered that the linear regression coefficients were not influenced by small differences in harvest number. It was found that both the linear and the quadratic trends showed no significant effect of the environment. This result supports the earlier evidence from the behaviour of T_1 and T_2 that the relative growth rates of tillers early in their development are not influenced by radiation or temperature and that these environmental factors do not differentially effect the rate of decline of relative growth rate with time.

Inability to establish significant effects of radiation and temperature on the initial relative growth rates of tillers subsidiary to the main stem is surprising in view of the responses shown by the main stem and the whole plant to these factors. In view of the wide range in initial relative growth rates recorded in different environments (Table 25) this does not imply that such differences do not exist but rather points to the inadequacy of the present data. In this connection it may be noted that consistent trends of the dry weight of a tiller with time within each replicate were not always obtained, with the result that each separate regression coefficient had a relatively large associated variance.

Finally, it is useful to examine, as noted earlier, the effect of environment on the time of appearance of individual tillers. Although the shapes of the growth curves of tillers in each environment were similar they were characterised by displacement along the time axis in response to environmental change. T_2 may be taken as an example. Delay in the appearance of T_2 at High 30 compared with High 20 is partly due to a delay in the appearance of leaf two at this temperature but as shown in Table 26 the interval between the appearance of leaf two and that of T_2 increased as the radiation level decreased and as temperature departed from the optimum of 20°C.

4.10.0 GROWTH ANALYSIS

4.10.1 Relative Growth Rate (R)

It was shown earlier that the relative growth rate was either constant or declined with time in each environment. A comparison of the effects of the different environments on this attribute is best achieved by determining the mean value of R for each environment. This may be done either by averaging the several values of R obtained for each inter-harvest period or by using the coefficient of the linear regression relating $\log_e W$ to time. The estimate of R is the same in each case but it should be noted that the value is an average and does not fully describe plant behaviour throughout the entire growth period. It does however give a reasonable

TABLE 26

Effect of Environment on the Time of Appearance of Tiller One and Tiller Two

Radiation	Temp.	Estimated Day of Appearance of: T_1	T_2	Leaf 2	Interval, L_2 to T_2 (Days)
High	10	17	22	9	13
	20	10	14	4	10
	30	12	16	5	11
Medium	10	21	26	11	15
	20	14	17	6	11
	30	18	19	5	14
Low	10	24	32	11	21
	20	16	19	6	13
	30	-	27	5	22

description of the plant response since curvature in the relation $\log_e W$ to time was in no case excessive and the differences in R induced by the different environments were relatively large. There is then little danger that responses will be obscured by the use of the average value.

The average R values for each environment are set out in the growth analysis table, Table 27.

4.10.2 Net Assimilation Rate (E)

The problems of determining this attribute were outlined in (2.2.3). Values of E were calculated from the usual formula (the first in Table 1) between successive harvestsand these were averaged to give the mean value of E over the whole of the growth period in each environment. The successive values were found to show considerable variation since at each harvest the estimates of L and W had coefficients of variation of about 10%.

To improve the accuracy of the estimate of E new values for area and weight at each harvest were determined from the appropriate quadratic equations relating each attribute with time. These estimated values for area and weight were then used to calculate a new series of E values for each inter-harvest period which were then averaged to give the 'calculated E' shown in Table 27. The successive calculated E values showed trends with time,

TABLE 27

Estimates of Net Assimilation Rate (E), Leaf Area Ratio (L.A.R.) and Relative Growth Rate (R)

Radiation	Temp.	E* $mg. cm.^{-2} day^{-1}$ (1)	(2)	L.A.R. $cm.^{2} mg.^{-1}$	R** $gm. gm.^{-1} day^{-1}$ (1)	(2)	(3)
High	10	1.36	1.36	0.126	0.171	0.171	0.169
	20	1.35	1.41	0.173	0.233	0.244	0.241
	30	1.54	1.44	0.131	0.202	0.189	0.187
Medium	10	0.72	0.55	0.251	0.181	0.138	0.133
	20	0.73	0.70	0.283	0.207	0.198	0.196
	30	0.59	0.56	0.300	0.177	0.168	0.161
Low	10	0.33	0.34	0.319	0.105	0.108	0.108
	20	0.45	0.45	0.380	0.171	0.171	0.168
	30	0.38	0.34	0.367	0.139	0.125	0.118

* (1) from Experimental values
(2) from Calculated values

** (1) E(1) x L.A.R.
(2) E(2) x L.A.R.
(3) from Regression Coefficients (Table 12)

mostly this took the form of a steady increase but in the case of High 10, there was a fall followed by a rise.

Both the 'experimental' and the 'calculated' mean values of E are shown in Table 27. The latter can be seen to be within a few percent of the former except at Medium 10 and Low 30 where the calculated values are 24% and 12% lower respectively. It is likely that the means based on the experimental values are in error as the range of the successive values of E between harvests appears to be larger than normal in both instances. The calculated values are therefore used as the best measure of E for all experiments.

4.10.3 Leaf Area Ratio (L.A.R.)

The leaf area ratio ($\frac{L}{W}$) may be determined at each harvest by dividing leaf area by total plant dry weight. A constant value will be obtained only where L and W are linearly related over their entire range and the line relating them passes through the origin.

In each environment the ratio of L over W declines as time (or W) increased, as previously noted (4.8.0). The average value of L.A.R. was determined by using the successive values of $^L/W$ at each harvest. The 'experimental' data and those derived from the fitted regressions of L and W to time were used for this purpose;

the resultant mean values were almost identical in each case. The 'experimental' means are given in Table 27.

4.10.4 Environmental Effects on E, L.A.R. and R

Figure 14 shows the mean values of E, L.A.R. and R plotted against radiation and temperature.

Net Assimilation Rate. E increased almost linearly with radiation at all temperatures.

At high radiation, E was only slightly affected by temperature but at medium and low radiation E was 20-25% less at both 10 and 30°C than at 20°C.

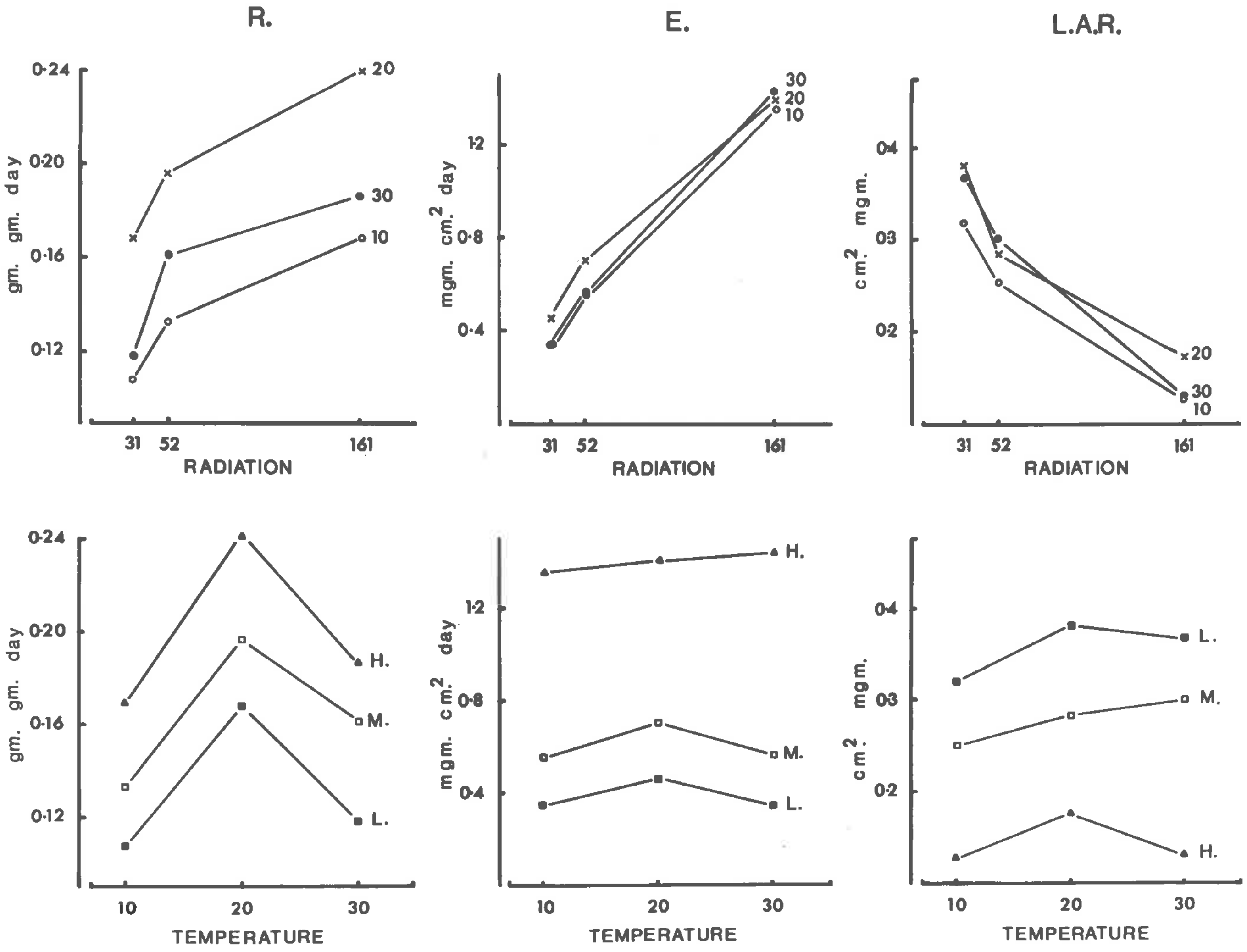
Leaf Area Ratio. L.A.R. decreased with increased radiation, the response probably being linear. Reduction of radiation by one third approximately doubled the ratio and a reduction to one fifth increased the ratio roughly two and one half times.

At high radiation L.A.R. was maximal at 20°C. At medium radiation the ratio increased with temperature rise from 10 to 30°C. At low radiation 20°C was optimal with only a slight decrease from this temperature to 30°C.

Relative Growth Rate. R increased with radiation at each temperature, the response being curvilinear.

At each level of radiation, R consistently increased with temperature rise from 10° to 20°C and fell again

<u>Figure 14</u> Average relative growth rate (R), net assimilation rate (E), leaf area ratio (L.A.R.) at each level of radiation and temperature.



R.

E.

L.A.R.

0·24
0·20
0·16
0·12
gm. gm. day
20
30
10
31
52
161
RADIATION
1·2
0·8
0·4
mgm. cm.2 day
30
20
10
31
52
161
RADIATION
0·4
0·3
0·2
cm.2 mgm.
20
30
10
31
52
161
RADIATION
0·24
0·20
0·16
0·12
gm. gm. day
H.
M.
L.
10
20
30
TEMPERATURE
1·2
0·8
0·4
mgm. cm.2 day
H.
M.
L.
10
20
30
TEMPERATURE
0·4
0·3
0·2
cm.2 mgm.
L.
M.
H.
10
20
30
TEMPERATURE

between 20° and 30°C. The parallelism in the response curves at each level of radiation is particularly striking. The difference in R between 10° and 30°C at each level of radiation was not great and depended on the radiation level.

Responses in R to radiation and temperature can be assessed in terms of the relative responses in E and L.A.R. to these factors.

Decrease in R with decreased radiation was due to a proportionately smaller decline in E than increase in L.A.R. At medium and low radiation E was decreased to about 43% and 27% of the high value respectively. L.A.R. on the other hand increased to about 190% and 250% respectively with a resultant decline in R to about 80% and 70% of the High value.

Responses of E and L.A.R. to temperature form a more complex pattern. The substantial increase in R with temperature increase from 10° to 20°C was due at each level of radiation to increase in both E and L.A.R. The decrease in R from 20° to 30°C was brought about in a different way at each radiation level: at high radiation there was a relatively greater decline in L.A.R. than increase in E; at medium radiation there was a relatively greater decline in E than increase in L.A.R.; at low radiation both E and L.A.R. declined.

4.10.5 Changes in E, L.A.R., R and S.L.A. with Time

In the preceding sections the above attributes were presented as average values over the 30 and 32 day experimental period. Since this period was (almost) constant between environments, the average figure gives an adequate description of the plant response to the environment. However, it is also necessary to examine how the different growth attributes changed with time and how they are inter-related in time. The extent of the changes in E, L.A.R. and R from Day 5 to Day 31 are shown in Table 28.

As noted previously E increased during nearly all experiments, the relative change from the beginning to the end being greater the lower the level of radiation. When the 'calculated' E values are plotted as a function of time the resulting curves have a positive general slope but are linear or slightly curvilinear, the particular shape probably depending on the fitted values of the curves relating leaf area and plant weight with time. The 'experimental' values for E are too variable to show other than an upward trend so it is not possible to determine the exact nature of the change of E with time.

L.A.R. declined with time in all experiments. When the successive 'calculated' values in each experiment are plotted against time the resulting curves, as for E, are linear or curvilinear but with negative slope. Less curvature is evident at low radiation than at Medium and

TABLE 28

Changes in E, L.A.R. and R from Day 5 to Day 31 in Each Experiment

Radiation	Temp.	E	L.A.R.	R
High	10	1.51 - 1.62	0.11 - 0.09	0.19 - 0.15 (.05)
	20	1.37 - 1.41	0.21 - 0.12	0.28 - 0.20 (.001)
	30	1.28 - 1.54	0.18 - 0.10	0.22 - 0.15 (.05)
Medium	10	0.45 - 0.76	0.29 - 0.17	0.13 - 0.13 (ns)
	20	0.59 - 0.72	0.39 - 0.21	0.24 - 0.15 (.001)
	30	0.42 - 0.58	0.47 - 0.24	0.19 - 0.13 (.05)
Low	10	0.32 - 0.39	0.35 - 0.28	0.11 - 0.11 (ns)
	20	0.40 - 0.50	0.50 - 0.27	0.19 - 0.14 (.001)
	30	0.21 - 0.49	0.52 - 0.24	0.11 - 0.12 (ns)

Figures in parenthesis show the significance level of the quadratic coefficient in the equations relating $\log_e$ dry weight to time and thus indicate the significance of the rate of fall of R with time.

High. 'Experimental' values show a generally linear downward trend.

Decline in L.A.R. with time is almost entirely attributable to decline in the S.L.A. This association was general but is shown for High 20 only in Table 29. In this Table S.L.A. is calculated at each harvest from total leaf area and total leaf weight. It can be shown further that variation in mean L.A.R. induced largely by radiation level is associated with corresponding changes in mean S.L.A. (Figure 15). At each temperature the proportionality between L.A.R. and S.L.A. is almost exact as radiation changes but at 10°C, L.A.R. was lower than it was at 20° and 30°C for the same S.L.A. This is undoubtedly due to the small reduction in percentage leaf that occurred at this temperature. Hughes and Evans (1962) working with Impatiens parviflora also concluded that differences in L.A.R. were due to differences in S.L.A.

Decline in L.A.R. with time is not attributable to increased root proportion as the general trend was for this to decline with time. In the particular case of High 20, no clear trend in the root proportion is evident.

Decline in R with time was analysed in Table 12 and for reference the extent of decline is indicated in Table 28. In general, decline in R is associated with a corresponding decline in L.A.R. as illustrated for

TABLE 29

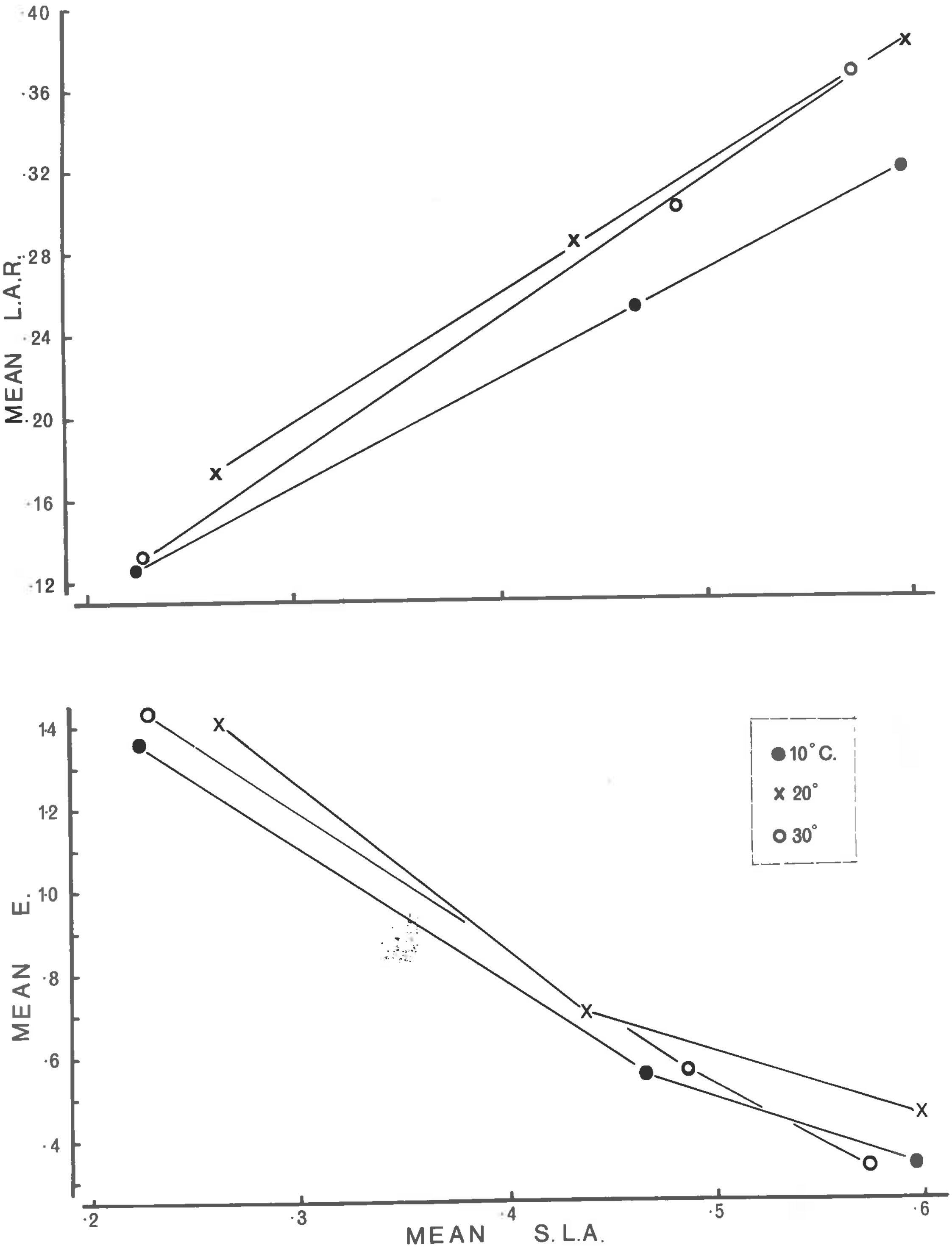
Relative Growth Rate (R), Leaf Area Ratio (L.A.R.) and S.L.A. Between Harvests, High Radiation 20°C

Day	R	L.A.R.	S.L.A.
5	0.29	0.21	476
7	0.28	0.21	406
9	0.27	0.20	471
11	0.27	0.20	457
13	0.26	0.19	418
15	0.25	0.19	360
17	0.24	0.18	335
19	0.24	0.17	323
21	0.23	0.17	280
23	0.22	0.16	263
25	0.22	0.15	256
27	0.21	0.14	244
29	0.20	0.13	229
31	0.20	0.12	216

Figure 15

Upper Part: Mean leaf area ratio plotted against mean specific leaf area at each temperature over all levels of radiation.

Lower Part: Mean net assimilation rate (E) plotted against mean S.L.A.



MEAN L.A.R.
·40
·36
·32
·28
·24
·20
·16
·12
MEAN E.
1·4
1·2
1·0
·8
·6
·4
● 10° C.
x 20°
o 30°
·2
·3
·4
·5
·6
MEAN S.L.A.

High 20 in Table 29. It appears that whether or not R falls during an experiment depends on the extent to which increase in E balances decrease in L.A.R. At high radiation (all temperatures) at 20°C (all radiation levels) and at Medium 30, relatively small increases in E were more than balanced by decrease in L.A.R. with a resultant fall in R with time. In the other environments changes in E and L.A.R. were compensatory so that R was constant with time.

Figure 15 also shows that mean E also declined with mean S.L.A.

5.0.0 DISCUSSION

The pattern of morphogenesis and the rate of dry matter production of perennial ryegrass were markedly influenced by radiation and temperature. It is difficult to devise a completely satisfactory basis for discussion of the data, but in relation to growth and morphogenesis it seems that a developmental approach is the most useful.

The objective of the study was to describe and to compare in different environments, the contributions made to the growth of the ryegrass plant by its separate vegetative organs. In this way it was hoped to reveal the manner in which two environmental components influence the pattern and the rate of vegetative growth of an important perennial pasture grass.

In the course of the experiments a considerable amount of data was obtained on the size attributes of individual leaves in different environments. It is considered that this data is most conveniently assessed separately from the objectives outlined above, and that its relevance to the growth functions used in 'classical' growth analysis should also be separately considered. Accordingly, the discussion will be conducted under three major headings:

(i) morphogenesis and dry matter production in relation to radiation and temperature;

(ii) relations between leaf area and leaf weight in different environments; and

(iii) increase in plant dry weight in relation to increase in leaf area.

5.1.0 MORPHOGENESIS AND DRY MATTER PRODUCTION IN RELATION TO RADIATION AND TEMPERATURE

It was stressed in the literature review that increase in the dry matter of the vegetative grass shoot is achieved in two ways - increase in tiller weight and increase in tiller number. Mitchell and Glenday (1958) suggest that in the grass sward the individual tiller is the fundamental unit of grass growth. There are also advantages in examining the growth of single tillers when considering the isolated plant, although the present data indicate that it may be profitable to regard the leaf as the more basic unit.

Before growth and morphogenesis of the shoot system is examined, it is pointed out that the lack of environmental effects on the partitioning of total dry matter between the root and shoot system evident from these experiments is somewhat unusual. It is commonly found that the shoot/root ratio is influenced by both light intensity and temperature (Mitchell 1954; Troughton 1960; Blackman 1961; Friend 1965).

del Pozo (1963) also found that the ratio, leaf lamina weight/total plant dry weight in ryegrass, was constant.

5.1.1 Aspects of the Growth of the Single Tiller

Increase in the dry weight of the ryegrass tiller is achieved by the initiation of leaf primordia on the apical meristem and the expansion of these primordia into mature leaves. Tiller growth rate will be influenced by the rate of leaf appearance, the final weight of each leaf and the rate at which that weight is attained. As the tiller ages its growth rate is also likely to be influenced by the extent to which the first formed leaves senesce after they have reached mature size.

Rate of Leaf Appearance. Evidence of a constant rate of leaf appearance on a single tiller in a constant environment is in general conformity with the published data.

The pattern of response of leaf appearance rate to temperature is in sharp contrast to that of the relative growth rates of both the main stem and the whole plant. A marked acceleration in leaf appearance brought about by a temperature rise from 10 to 20°C undoubtedly accounts for a large proportion of the response of the whole plant to temperature over this range. On the other hand decrease in the rate of plant dry weight increase between 20 and 30°C is not associated with decrease in the rate of leaf appearance, and it is clear that dry weight responses to this temperature change must be associated with temperature effects on other 'partial' processes.

At each temperature the response to radiation was relatively small and was negatively curvilinear in the same manner as indicated for the wheat leaf by Friend *et al.* (1962). Mitchell (1953 b) also found that the rate of leaf appearance in perennial ryegrass was increased more by a rise from 50° to 75°F than by a trebling of the light intensity. It can be noted that the rate of leaf appearance of 9.5 days per leaf at Low 10 is very close to the figure of 9.2 days per leaf obtained by Beevers and Cooper (1964) for Irish ryegrass grown at 12°C and 650 ft.c. light intensity.

Sato (1962) observed that in rice the mean leaf appearance interval of successive leaves on the main stem was a little longer than that of leaves produced on subsidiary tillers. Mitchell (1953 b) and Cooper and Edwards (1961) considered that such differences do not occur in ryegrass. No evidence to support Sato's view could be found in the present study and it therefore appears reasonable to express the leaf appearance interval as the time in days between the appearance of two successive leaves on any tiller.

The rates of initiation of leaf primordia were not recorded but it is reasonable to assume that the constancy of the rate of leaf appearance in any one environment was associated with constancy of the rate of initiation, and that both processes proceeded at an equal rate. It is possible that when primordia accumulate on the apex in advance of the expanding leaves (Cooper 1951), some other mechanism is involved in the control of leaf appearance. This situation did not seem to occur in these experiments as dissection of the main stem apices at the end of each experiment showed one, or rarely two, unexpanded primordia to be present.

Leaf primordia are initiated on the apex in a linear time sequence under a highly developed and integrated system of control to which growth substances, mineral nutrients, assimilates and indeed the whole apex itself appear to contribute. The physiology of the apex has been studied by many workers but at present there appears to be no satisfactory explanation as to why the initiation of leaf primordia should proceed at a constant rate. Clowes (1961) concluded that the positioning of leaves on the apex can often be explained in terms of space and shape and it could be that it is the spatial requirements for leaf inception that ensure a linear sequence with time. Yet it is clear that such a control is modified when the apex embarks on reproductive growth and an accelerated rate of primordium production. The role of the apex in tiller growth is discussed more fully in (5.1.2).

The rate of leaf initiation is believed to be of considerable importance in determining the growth rate of a single tiller. It also determines the potential tillering rate - the number of sites at which leaf growth may occur.

Leaf Weight. It has been contended in the literature (Newton 1963; Dale 1965) that plant dry weight attributes may follow a parabolic relation with increased radiation although, as pointed out by the latter author, there is no evidence that in either species high radiation levels were associated with a decrease in dry weight; a parabolic relation was assumed in the curve fitting. It is also known that net photosynthesis may asymptote to maximum values in response to radiation, the maximum depending on temperature, CO_2 supply, and the particular species (Gaastra 1959; Hesketh and Moss 1963).

There is evidence (Figure 7) that in ryegrass the dry weights of individual leaves follow an optimum curve with radiation at 30°C: at 10 and 20°C dry weight increased with each increase in radiation. Evidence to support this finding is found in the data presented by Blackman (1961) for Salvinia natans. In this species individual leaves decreased in weight with temperature increase above 20°C and showed a positive response to increased radiation. High light intensity appeared to be supra-optimal at 20°C - the lower rather than the higher temperature as in ryegrass.

Differences in the weights of ryegrass leaves obtained by Mitchell (1954) were discussed previously (2.1.7). It can be noted that the reduction in light intensity from 'full' daylight to 30% of 'full' daylight used by Mitchell in his experiments, was nearly the same percentage decrease in radiation as occurred from High to Low in the present study, yet the effect on leaf weight was smaller.

In general, responses in the weights of ryegrass leaves to radiation and temperature followed a similar pattern to that shown for the third leaf of the wheat plant by Friend _et al_. (1962), except that in wheat there appears to be no interaction between radiation and temperature.

The most marked feature of the effect of temperature on leaf weight in the present experiments is the substantial decrease in weight associated with a temperature rise from 20 to 30°C. This effect is clearly associated with a decreased growth rate at 30°C compared with 20°C.

An hypothesis concerning the control of leaf weight by radiation and temperature is presented in (5.1.2).

Growth Rates of Individual Leaves. It was suggested earlier (4.5.0) that the data on the increase in the weights of particular leaves with respect to time should not necessarily be taken as representing the behaviour of an individual leaf, as the data refer to a _population_ of leaves. Particular leaves on separate plants normally show variation in the time at which they appear and even if all leaves subsequently display identical growth curves to reach the same final weight, the curve of the population will depart from that of the individual leaf according to the range in time over which the leaves appear. Meaningful data on increase in leaf weight can therefore only be acquired when the members of the population exhibit a small range in time of appearance. This makes the construction of reliable growth curves for leaf weight extremely difficult.

In the present study weight attributes were determined after destructive harvests, and although the range in time for a particular leaf to appear on all plants was small it was considered sufficient to have influenced the form of the growth curve. It was largely for this reason that a more sophisticated approach to leaf expansion was not adopted and it should be noted that Figure 8 can be used only to give an approximate measure of the rates at which leaves reached their maximum weights.

Figure 8 shows that the time for full expansion was influenced more by temperature than by radiation. Support for this view is to be found in the data for wheat provided by Friend *et al*. (1962). Also Dale (1965) found that the time taken for full expansion of leaves of *Phaseolus* was essentially independent of the radiation level although the maximum rate was maintained for a longer period at high intensities.

In each environment the range in final leaf weight was appreciable, yet the range in time for full (weight) expansion was small. This suggests that in each environment the time taken for each leaf to reach its maximum weight was relatively constant and independent of leaf size. Thus the absolute growth rates of individual leaves are strongly biased for the ultimate size attained.

It is difficult to arrive at a satisfactory explanation of the above phenomenon. It is clear that the range in leaf weight in a given environment is associated with the level of leaf insertion, and it is therefore possible that the average relative growth rate (R) of each leaf and time (t) are constant in the equation

$$W_2 = W_1 e^{Rt}$$

W_2 (the final dry weight) being solely determined by W_1 (the weight of the primordium prior to expansion). Williams (1960) however has demonstrated a varying pattern of R during ontogeny of the wheat leaf and shown that maximal values of R vary from leaf to leaf. Average values of R for the whole course of leaf development may therefore have little meaning; but if maximal values during post-appearance growth could be determined they may be useful in elucidating the relative importance of the primordial stage in determining final leaf weight.

5.1.2 The Importance of the Apical Meristem in the Growth of the Single Tiller

The control of leaf size in ryegrass by radiation and temperature appears to be complex but as suggested earlier (2.1.7) is most likely to operate through growth relations at the apex and direct morphogenetic effects. An hypothesis to explain the response patterns observed is developed below. To help clarify the argument there is first given a summary of the effects of radiation and temperature on the various aspects of leaf production on a single tiller. Effects of radiation and temperature on leaf area are included in this summary even though these aspects of leaf growth have not been discussed up to this stage: it is convenient to consider weight and area attributes simultaneously. In the summary, a plus sign indicates the magnitude of a promotive effect; zero shows no effect; and minus signs a depressive effect.

Environmental Change	Leaf Appearance	Leaf Weight	Leaf Area
Temperature:			
From 10 to 20°C	+++	+	+
From 20 to 30°C	0	--	-
Radiation:			
From Low to Medium	+	++	-
From Medium to High	+	++	--

It is suggested that final leaf weight is primarily determined by the number of cells produced early in leaf ontogeny. Leaf area is also influenced by cell number but at any temperature is largely determined by cell size. The way in which the processes of cell division and cell enlargement are integrated in the ryegrass leaf is not known, nor yet the manner in which these processes are separately influenced by radiation and temperature. However, it is likely that early in leaf ontogeny,leaf growth is dominated by cell division and it is envisaged that this is especially so during pre-appearance growth.

Cell division in ryegrass may have an optimum temperature of about 20°C. This view is supported by the fact that relative growth rates were clearly optimal at this temperature. A temperature rise from 10 to 20°C has a strongly promotive effect on processes occurring at the apex and hastens the development of leaf primordia and the rate of increase in apex size. In line with the results of Hussey (1963) for the tomato, a higher temperature (in this case 30°C) does not further increase the rate of differentiation of leaf primordia and decreases the rate of increase in apex size. This effect, and the lower rates of cell division during subsequent development, result in a decreased final leaf weight at 30 compared with 20°C. The lack of a marked increase in leaf weight with the level of leaf insertion at 30°C favours the idea of a decreased rate of apex enlargement at this temperature. The promotive effects of increased radiation level on leaf appearance and leaf weight are due to an increased supply of assimilate for cell division.

After appearance, leaf area is a function of leaf weight, but the area over which any given amount of dry matter is distributed increases as the radiation decreases. It is suggested that cell size increases as the radiation decreases, the response representing a direct morphogenetic effect of this factor. Leaf area may also increase through cell size increasing with temperature. Davidson and Milthorpe (1965) showed that in cocksfoot, cell size in the leaf lamina increased linearly with temperature over the range 5-30°C, but this may not necessarily be the case in Lolium.

A 'heavy' leaf is produced where conditions favour cell division but limit cell expansion, i.e. under optimum temperature and high radiation. Decreased radiation at this temperature reduces cell division but increases substantially the size of the cells that are produced resulting in increased leaf area and an increased specific leaf area. A similar situation is envisaged at 10°C.

The above argument is tentatively proposed to explain present results. It is based on a number of assumptions, on limited experimental data and on evidence from other species, but it may serve as a model on which to base a more detailed analysis of the manner in which leaf growth in ryegrass is influenced by two important environmental factors.

The model does not envisage that competitive mechanisms for nutrients or for assimilate are operative at the apex. It is thus closer to the interpretation of the control of leaf size in wheat suggested by Friend _et al_. (1962) than to that suggested for cucumbers by Milthorpe (1959). However it does imply that processes at the apex are sensitive to the level of assimilate and admits the possibility of that level being influenced by demand in other plant parts. It is possible to explain a greater leaf weight at Medium 30 compared with High 30 as a response of the main stem apex to decreased demand for assimilate by developing tiller buds at the medium level of radiation.

The most important point with respect to temperature effects is that cellular processes resulting in primordium production may have a temperature response different from those resulting in increased apex size.

Substantiation of the above explanation will involve determination of cell number and cell size in the mature leaves of ryegrass plants grown at different levels of radiation and temperature. Also it will be useful to conduct transfer experiments between different regimes at different stages of leaf growth to determine the range of developmental stages over which the leaf remains responsive to the changes in the environment. It is unlikely that substantial differences in leaf size will be obtained by shading individual leaves. If hormonal mechanisms are involved in controlling leaf area it is probable that these will not be localised in a single leaf and that area response to radiation will be a 'whole plant' response. The extent to which individual leaf weight may be influenced by shading will depend on the overall radiation level and the extent to which each leaf is dependent on its own photosynthetic products for its own growth.

5.1.3 Increase in Tiller Weight

Increase in the weights of single tillers in different environments can be examined in three ways: (i) the average relative growth rates of the main stem; (ii) the initial relative growth rates of the subsidiary tillers; and (iii) the forms of the growth curves of tillers one and two.

Relative Growth Rates of the Main Stem. This responded to radiation and temperature in a similar way as did the relative growth rate of the whole plant except that response to radiation was more distinctly curvilinear.

Table 24 and Figure 14 show that at 20°C the relative growth rate of the main stem (R.M.S.) tended to reach a maximum value at high radiation and at 30°C a maximum was reached at medium radiation. There is thus clear evidence that R.M.S. is maximal at a much lower level of radiation than R.

Initial Relative Growth Rates of Subsidiary Tillers. It is difficult to accept the finding that the initial relative growth rates of these organs were unaffected by temperature; but it is conceivable that radiation level was of little importance at this stage. Early in its ontogeny it is likely that a developing tiller will draw on the parent tiller for its carbohydrate supply. R.D. Williams (1964) has shown that there is movement of labelled carbon from a mature leaf into tillers developing on the same tiller. It could be that very young tillers are adequately provided with assimilate from the 'pool' in the parent tiller over a wide range of radiation levels and that such tillers will therefore show little or no response to variation in radiation level. Two lines of evidence to support this view can be found.

Firstly, the main stem showed only a small response to radiation between Day 0 and Day 4 when it was growing with a 'pool' of assimilate available to it from the endosperm. Secondly, it is likely that the initial growth rate of a tiller will be determined by the rate of expansion of the first leaf and it has been shown that, although ultimate leaf size is influenced by radiation, the rate of attainment of this maximum is not markedly influenced by radiation. It seems then that responses in the initial relative growth rates of tillers to radiation are likely to be small.

Growth of Tillers One and Two. The effect of environment on the form of the growth curve of T_1 and T_2 is difficult to appraise accurately but it is clear that similarity between the curves shown in Figure 13 is more apparent than real. If the slopes of the tangents to the curves are estimated at a tiller dry weight of 10 mg., differences in the relative growth rates are apparent. Similarly, superimposition of the curves about a common origin reveals marked differences in curvature. This shows that, like the main stem, the relative growth rates of T_1 and T_2 were influenced by the environment. Failure to detect significant differences in the coefficients of the equations relating $\log_e$ tiller weight with time is attributed to the high variances associated with the fitting of separate regressions to the replicate data and the relatively low number of points available in some treatments.

A final point concerning the growth of single tillers is the marked decline in R with time. The exponential phase of growth lasts only for a short time after tiller appearance and plots of untransformed data for individual tillers show that there is a general tendency for growth absolute rates to become constant with time. This is illustrated for High 20 in Figure 16.

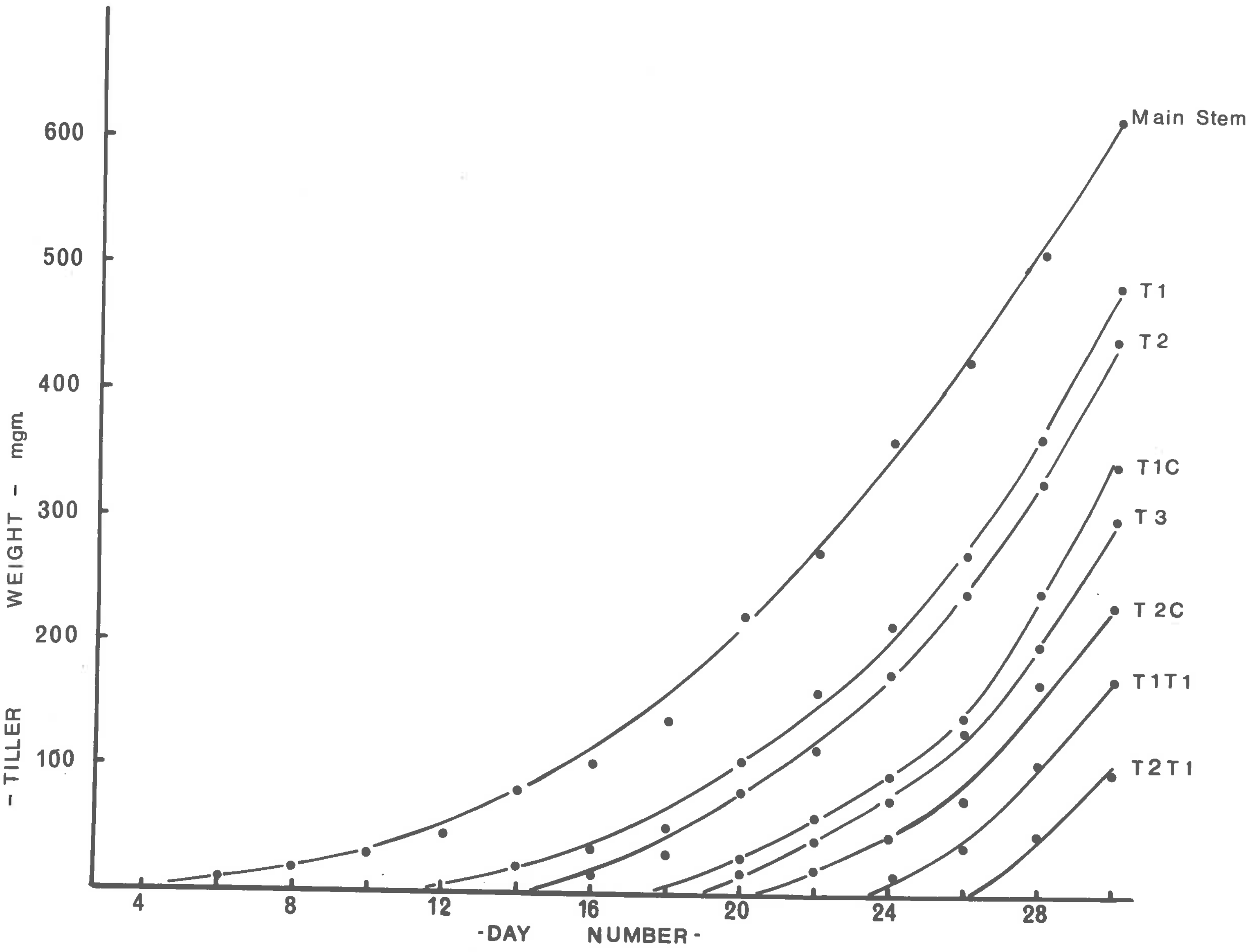
5.1.4 Increase in Tiller Number

Tillering in grasses may be examined directly as tiller number per plant or may be expressed in relation to leaf appearance age. The latter procedure separates the effects of environment on leaf appearance from those on tillering since the rate of leaf appearance imposes an upper limit to tillering. Both methods are adopted.

There is general evidence in the literature that high levels of radiation are associated with high tillering rates, and that tillering, particularly in Festucoid grasses, has a relatively low temperature optimum (Mitchell 1953 b, 1955, 1956; Alberda 1957; Peterson and Loomis 1949; Khalil 1956). The present results conform to this pattern but it is evident that on a leaf appearance basis, tillering was suppressed by high temperature only at low radiation.

In advocating the estimation of tillering in relation to leaf appearance age, Mitchell (1953 b, 1954) presented data on the effects of light and temperature on tiller number per plant at a common leaf appearance age for three ryegrass cultivars. The general conclusion was

<u>Figure 16</u> Increase in dry weight with time of all tillers produced at 20°C and high radiation.


600
500
400
300
200
100
- TILLER WEIGHT - mgm
4
8
12
16
20
24
28
-DAY NUMBER-
Main Stem
T 1
T 2
T1C
T 3
T 2C
T1T1
T2T1

drawn that a temperature rise decreased tillering. This conclusion does not appear to be completely valid as it is clear from Mitchell's data that the response to temperature depended on the level of radiation, in conformity with the present results.

The pattern of tillering induced by variation in radiation and temperature in ryegrass was consistent in these experiments, and it appears that tillering is reduced in a definite sequence up the main stem commencing with the coleoptile tiller, followed by tiller one and tiller two. This agrees with the pattern of inhibition of lateral bud development in ryegrass by low light intensity and high temperature shown by Mitchell (1953 b). Also Patel and Cooper (1961) have shown that the percentage of ryegrass plants which developed a coleoptile tiller varies seasonally with a peak frequency at the time of highest radiation.

A further aspect of tillering is the effect of environment on the time of appearance of a tiller after that of its subtending leaf. Friend (1965) showed that in wheat a general increase occurred in the leaf interval between tillering as light intensity increased and as temperature decreased between 30°C and 10°C. Also Mitchell (1953 b) found that conditions unfavourable to tillering caused a delay in the time of tiller appearance.

Present results (Table 26) agree with this conclusion. Environment had only a small effect on the potential for tiller development as shown by the near constancy of tiller number in relation to leaf number, but the time of realisation of that potential was affected by the environment, particularly by high temperature at low radiation.

Evans _et_ _al_. (1964) concluded that tiller initiation is not suppressed by unfavourable conditions, tiller buds being found in the axils of all leaves. Present experience confirms this conclusion and it therefore appears to be the time of commencement of tiller expansion that is influenced by environment.

It is contended in the literature that with adequate nutrition (as in the present experiment) tiller production during the early growth phase of a grass or cereal is limited by the supply of assimilates in response to the level of radiant energy although in barley, Aspinall (1963) and Aspinall and Paleg (1964), have expressed doubt that this is achieved by a direct auxin-mediated apical dominance system.

The current argument (Evans _et_ _al_. 1964) is that a system of priorities of 'sinks' for assimilate and nutrients exist but the way in which the priorities are determined is not known. Friend (1965) suggested that as the level of radiation declines there is less assimilate available for translocation to tillers and roots, and that this shortage of supply is accentuated as temperature increases above the optimum - in the present case from 20 to 30°C - as a consequence of an increased rate of primordium production.

This explanation is difficult to accept in the present study. Although primordium production was not examined, the rate of leaf appearance was not affected by a temperature increase from 20 to 30°C. Also leaf size was reduced at the higher temperature and the rate of growth of a single leaf was influenced only slightly over the same temperature range. Further, neither temperature nor radiation had substantial effects on the root proportion. A 'competitive' explanation is therefore unacceptable. An alternative may be found in the suggestion that a threshhold of assimilate may be needed to supply an initial stimulus to growth. It may be that with a higher respiration rate at the higher temperature this takes longer to achieve at 30°C than at 20°C.

5.1.5 Growth of the Whole Plant in Relation to Leaf and Tiller Production

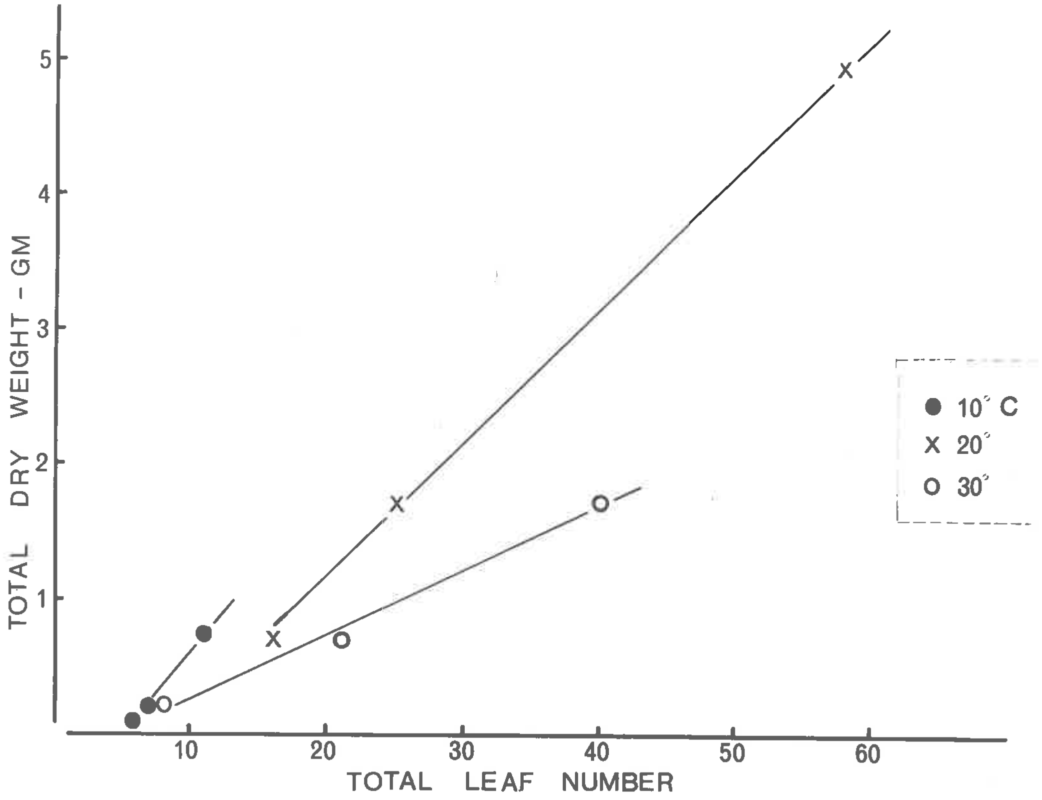
A major conclusion drawn from the data obtained in these experiments is that the vegetative growth of perennial ryegrass was dominated by leaf growth under the range of environmental conditions in which growth was examined. Environment influenced dry matter production largely through affecting the rate of leaf appearance and leaf size.

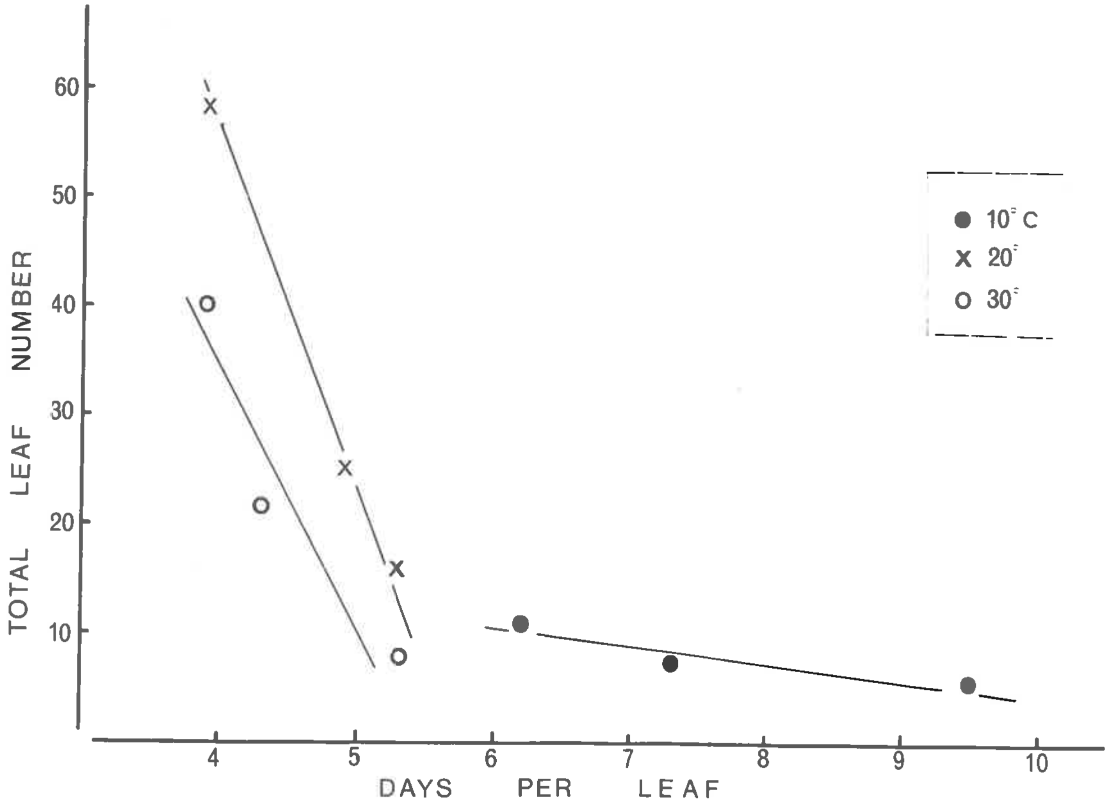
Figure 17 shows that, after growth for 30 days at each temperature, total plant weight was linearly related to leaf number as radiation increased, a different regression being evident at each temperature. The figure also shows that the average leaf weight increased as radiation increased since the total weight of the leaf laminae was a relatively constant proportion of the total plant dry weight over all environments. It may be noted that the regression lines in Figure 17 do not extrapolate to the origin since it is the responses to radiation after a given time that are plotted, not responses with time. Further, there is nothing intrinsic in the slope of each regression line as this will be determined by the point in time at which leaf number and plant weight are recorded. Average leaf weight increases with plant age; when data from an earlier harvest were plotted, the relation between leaf number and plant weight at each temperature was still linear and dependent on radiation but of decreased slope compared with those at 30 days.

The number of leaves present on the whole plant after a given time is determined by the rate of leaf appearance on each tiller and by the rate of tillering. Tillering, on a leaf appearance basis, was little affected

Figure 17 (Upper) Total plant dry weight at Day 30 plotted against total leaf number on the whole plant for each temperature at each level of radiation.

Figure 18 (Lower) Total leaf number on whole plant at Day 30 plotted against rate of leaf appearance for each temperature at each level of radiation.


TOTAL DRY WEIGHT - GM
1
2
3
4
5
10
20
30
40
50
60
TOTAL LEAF NUMBER
● 10° C
X 20°
O 30°


TOTAL LEAF NUMBER
10
20
30
40
50
60
4
5
6
7
8
9
10
DAYS PER LEAF
● 10° C
X 20°
O 30°

by environment except at high temperature and low radiation. Thus nearly all leaves had equal opportunity to produce a tiller, which itself then contributed to leaf number increase. Total leaf number is therefore directly related to the rate of leaf appearance at each temperature as shown in Figure 18. In this figure the discrepancy between leaf number at 20° and 30°C for the same rate of leaf appearance is undoubtedly due to the delay at the higher temperature in the time of tiller appearance after leaf appearance.

Increase in tiller weight and increase in tiller number can be seen to be aspects of the same phenomenon, increase in leaf number, in the first instance at a given site (the apical meristem) and in the second, at different sites. Both leaf appearance and tiller appearance are accelerated by increased radiation but are differently affected by temperature. Leaf appearance has a wide optimum temperature at all levels of radiation but tillering shows an increasing optimum at 20°C as radiation decreases. Both processes therefore appear to be sensitive to the level of the supply of assimilate which is governed by the radiation level, and probably the respiration rate in response to temperature. However tillering appears to be more sensitive than leaf appearance to a decrease in the overall supply of assimilate since it is the contribution to total leaf number from this source that is reduced most as temperature increases and radiation decreases.

Friend (1965) associated decreased tillering in wheat with a decreased supply of assimilate to the tiller buds and considered that this was due to the main axis exercising a priority in demand for assimilate. A similar situation could occur in ryegrass but as noted previously, a competitive explanation in terms of increased leaf appearance at higher temperatures does not seem to apply. A more acceptable explanation lies in a decrease in net photosynthesis at the higher temperature.

Increased dry matter production in response to radiation is achieved in ryegrass through increased growth rate of the single tiller and through increased tillering. It is clear that a single tiller becomes 'light saturated' at a given temperature before the whole plant. A radiation level greater than that required to meet the demand for assimilate of a single axis therefore promotes the growth of the whole plant by increasing the number of sites at which leaf growth can occur. The limited response to radiation shown by a single tiller is considered to be associated with the constancy of its rate of leaf appearance. The fact that leaves are initiated in a linear time sequence ensures that the amount of meristematic tissue expanding at any time is limited to that provided by each successive leaf primordium. Leaves are determinate organs, their size being determined by their level of insertion and by the environment. The rate at which they reach that size is relatively independent of the radiation level and their energy requirements for expansion are apparently met at moderate levels of radiation. This argument points to the importance of the apical meristem in controlling the rate of dry matter production.

It is also considered that a constant rate of leaf appearance on any shoot has a considerable bearing on the growth potential of a ryegrass community. As plant density increases, tiller number per plant decreases, and at very high densities, tillering on the main stem may be almost completely prevented (Donald 1957). Where this situation occurs, leaf production will be restricted to a constant number of apices per unit area of ground unless competitive effects reduce plant number with time. A limit to the rate of dry matter production will now be set by the rate of leaf production on the main stem, and as a consequence, total dry matter on the whole plant will increase linearly with time as the growth rate of each single tiller tends to become constant (see Figure 17). Evidence to support this view is provided by Alberda (1965) and Bean (1964) who found that the early vegetative growth of ryegrass and cocksfoot communities respectively, was essentially linear with time once complete light interception had occurred. Evidence that the shape of the growth curve is density dependent is also supported by unpublished evidence of the author; it was suggested by Donald (1956). This result may have important implications concerning the relationship between growth rate and leaf area index as proposed by Davidson and Donald (1958) and Black (1963).

5.2.0 RELATIONS BETWEEN LEAF AREA AND LEAF WEIGHT

The most marked feature of the data for individual leaf area is the increase in final leaf area with decrease in radiation. This kind of response may be regarded as typical of many plants and it has been shown in the literature review that leaf area often follows an optimum curve with radiation, the greatest areas being reached at moderate levels of radiation.

The present data indicate that the maximum area of a leaf was achieved at the lowest level of radiation employed. Friend (1966) found in wheat that the area of the second mature lamina was maximal at 20°C and (coincidentally) at a radiation level of 3.3 cal. $cm.^2$ $hr.^{-1}$. In ryegrass the maximal area appears to increasingly favour a still lower radiation level at successive points of leaf insertion.

Leaf area responses in ryegrass were also different from those observed in Salvinia (Blackman 1961) in that in this species, area per leaf increased with increasing light intensities, 300-1500 ft.c. There is a suggestion in this work however that intensities above 1200 ft.c. were supra-optimal for leaf area as well as for leaf weight.

It seems that the responses in leaf weight and leaf area obtained in the present experiments follow a unique pattern but are not at variance with the idea that these attributes may each follow an optimum curve with radiation and temperature. The range of environments was limited in the present case but it is likely that individual leaf area would decrease with radiation below about 31 cal. cm.$^{-2}$ day^{-1}.

Changes in the ratio of leaf area to leaf weight have been described and discussed by other workers, notably Evans and Hughes (1961) working with Impatiens parviflora. The present data are more extensive in that they show specific leaf area to be a function of leaf age as well as environment and the point of leaf insertion.

A decrease in S.L.A. during post-appearance leaf growth is evident in ryegrass and it seems that a common pattern of change occurred in all the leaves expanded in a particular environment. Decline in S.L.A. during leaf development, as pointed out by Evans and Hughes (loc. cit.), makes difficult the determination of an average value for this attribute for the whole plant at any one time. The value will depend on the proportion of expanding to mature leaves. This proportion will decline as the plant ages since only one leaf undergoes rapid expansion on any tiller at any time. Thus an average value of S.L.A. calculated from the total leaf area/leaf weight ratio must decline with age. Such a decline will be accentuated at first by the decrease in 'ultimate' S.L.A. of successive leaves but will continue to fall so long as the proportion of meristematic to mature tissue falls and as the mature leaves continue to increase in weight without an associated increase in area. Decline in S.L.A. with time will be directly associated with decline in leaf area ratio so long as the ratio between leaf weight and plant weight remains unaltered. In the present experiments L.A.R. fell with time in each environment (Table 28).

Expression of the effect of environment on S.L.A. appears to be best achieved by considering only the mature leaves or by using the constant 'b' in the allometric relation between leaf area and leaf weight. The latter method has much in its favour as it describes, for any environment, the relation between leaf area and leaf weight at all stages of leaf development. However it should be noted that the present data do not include measurements of mature leaves over a sufficiently long period to determine for how long a single leaf conforms to the proposed relation. It could be that the relation no longer holds as senescence is approached. In this connection it may be noted that Evans and Hughes (1961) state that 'even when the leaf has assumed its mature structure, a certain amount of long-term wall thickening still goes on bringing about a slow fall in the specific leaf area'. There is evidence of a decline in leaf weight in ryegrass after a maximum weight had been reached.

Allometric relations have been used (Huxley 1932) to relate two dimensional aspects of an organ during its growth; and to relate the growth of a part of an organism to that of the whole. Von Bertalanffy (1957) also used allometry to relate the metabolic rates of an organism to surface area and to body weight. The present usage relates the area of one surface of an organ to its dry weight and the range in values (0.71 - 0.83) of the exponent (k) suggests that the relation is essentially the same as that between the surface area of a regular body and its volume, i.e.

$$\text{Area}/\text{Volume}^{0.67} = \text{Constant}$$

if it can be shown that leaf volume is related to leaf dry weight.

The volume of a fully turgid leaf can be determined from its fresh weight if no inter-cellular air is present and if it is assumed that the density of the constituent cells is unity. Leaf fresh weight will, in turn, be directly related to leaf dry weight if the dry matter of the leaf is hydrated to a constant extent.

The likely proportion of air space in a grass leaf is difficult to estimate. Soper and Mitchell (1956) recorded that the proportion of inter-cellular space in the ryegrass leaf was 'considerable' and Sifton (1945) notes in his review that the proportion of air by volume in normal leaves ranged from 7.7% to 71.3% in a number of species. Sifton (loc. cit.) also showed that the proportion varies with the species, the degree of shading, the water supply and light intensity.

The relation between leaf dry weight and leaf fresh weight was examined in a separate experiment using plants grown in a single environment - namely 20°C, 3000 lumens ft.$^{-2}$ and 85% relative humidity. 24 leaves ranging in fresh weight from 4.3 mg. ('just appeared') to 260.4 mg. ('just mature' - ligule exposed) and 12 fully mature leaves ranging in size from 38.8 mg. to 175 mg., were excised from the plants and their fresh weights determined immediately. The leaves were re-weighed after drying.

The ratio of dry weight to fresh weight of all leaves ranged from 0.13 to 0.19 and there was no significant difference between the mean of the ratios of the mature leaves and the mean of the ratios of the expanding leaves. Also the ratio was unrelated to leaf dry weight in either class of leaf. It is concluded that under the conditions specified, leaf fresh weight was linearly related to leaf dry weight. A small amount of variation in the ratio may have been due to variation in leaf turgidity.

Three factors seem to be concerned in the relation between leaf volume and leaf dry weight: (i) the dry weight/fresh weight ratio; (ii) the percentage of the leaf volume occupied by air; and (iii) leaf turgidity. It is not possible to assess from the present data, the manner in which these factors determined the volume occupied by a given amount of dry matter both within and between environments. In one environment it has been shown that the dry/fresh weight ratio varies little during leaf development but it is possible that this ratio may be effected by radiation. Also the effects of shading on the air-space proportion cannot be ignored (Mitchell and Soper 1958).

Returning to leaf area and leaf weight it is concluded that a fundamental basis for the allometric relation between these two attributes cannot as yet be determined. The fact that the exponent (k) was close to 0.67 certainly suggests that an area volume relation is involved and the relatively small variation in this exponent with environment indicates that leaf shape was not markedly influenced by the environment.

5.3.0 INCREASE IN PLANT DRY WEIGHT IN RELATION TO INCREASE IN LEAF AREA

In 'classical' growth analysis, increase in plant weight per unit time is expressed in relation to plant dry weight (R); or in relation to leaf area (E), the difference between these two expressions depending on the relation between leaf area and plant weight. Essentially R and E estimate the same thing - increase in dry matter per unit of plant capital. A problem in the present study is to determine which of the two attributes, plant weight and leaf area, provides the best basis for characterising responses to environment.

The effects of radiation on the relative growth rate, net assimilation rate and leaf area ratio of ryegrass are consistent with the general pattern recorded by other workers (see Blackman 1961).

The existence of a marked positive relation between E and radiation is well established in the literature but the exact nature of this relation may be linear (Newton 1963) or logarithmic (Blackman and Wilson 1951; Dale 1965). In the present instance the relation appears to be curvilinear and it may be noted that a good linear fit to log radiation is achieved at 10 and 20°C but not at 30°C.

Evidence has been gained from the present study that response in E and L.A.R. to temperature is influenced by the level of radiation. Response in R to temperature is consistent at each level of radiation so that at all three levels of this factor, 20°C is clearly optimal. E on the other hand is optimal at 20°C at medium and low radiation, and L.A.R. is optimal at this temperature at low and high radiation. Thus decrease in R from 20 to 30°C is associated with responses in E and L.A.R. which are of similar or opposite sign to that of R, according to the radiation level. In general, the responses in E and L.A.R. to temperature were small.

It is considered that the less complex pattern displayed by R to environmental factors in the present study makes this function more suitable than E for assessing plant response to different environments. As pointed out by Williams (1964), R measures the rate of duplication of living matter and therefore provides information concerning fundamental growth processes. Also the known (see 2.2.3) limitations to the concept of net assimilation rate make this function less useful than R. This argument does not imply that increase in leaf area is an unimportant aspect of plant growth, but it is considered that increase in dry weight can be related to increase in leaf area by an alternative approach.

Whitehead and Myerscough (1962) suggested that the ultimate form of a plant depends on the kind and the extent of the relation between the mean relative growth rate ($\bar{R}$) and the mean relative growth rate of the leaf surface ($\bar{R}_L$). The ratio between these two rates was termed α (i.e. $\alpha = \bar{R}/\bar{R}_L$) and its value is the exponent of L in the allometric relation between plant weight and total leaf area represented by the equation

$$W = bL^{\alpha} + c$$

where b and c are constants. This equation is the generalised form of the relationship between W and L of which the linear and square assumptions (see Table 1) are special cases. The constant c was considered to be negligible, and of importance only during the early stages of germination. The equation therefore becomes identical with that proposed here, i.e. $L = bW^k$. Which of L and W is regarded as the independent variate is probably immaterial but it may be preferable to regard L as a function of W rather than *vice versa*, and α then becomes $\frac{1}{k}$.

The index α was calculated for each of the nine environments of the present experiments and the resulting values tabulated in Table 30 which also lists values for 'k'. In Figure 19, $\bar{R}_L$ is plotted against $\bar{R}$.

In general, α is closest to unity under high radiation and increases as radiation decreases and as temperature increased from 10 to 30°C. The reason for the relation between $\bar{R}_L$ and $\bar{R}$ at 10°C being different from that

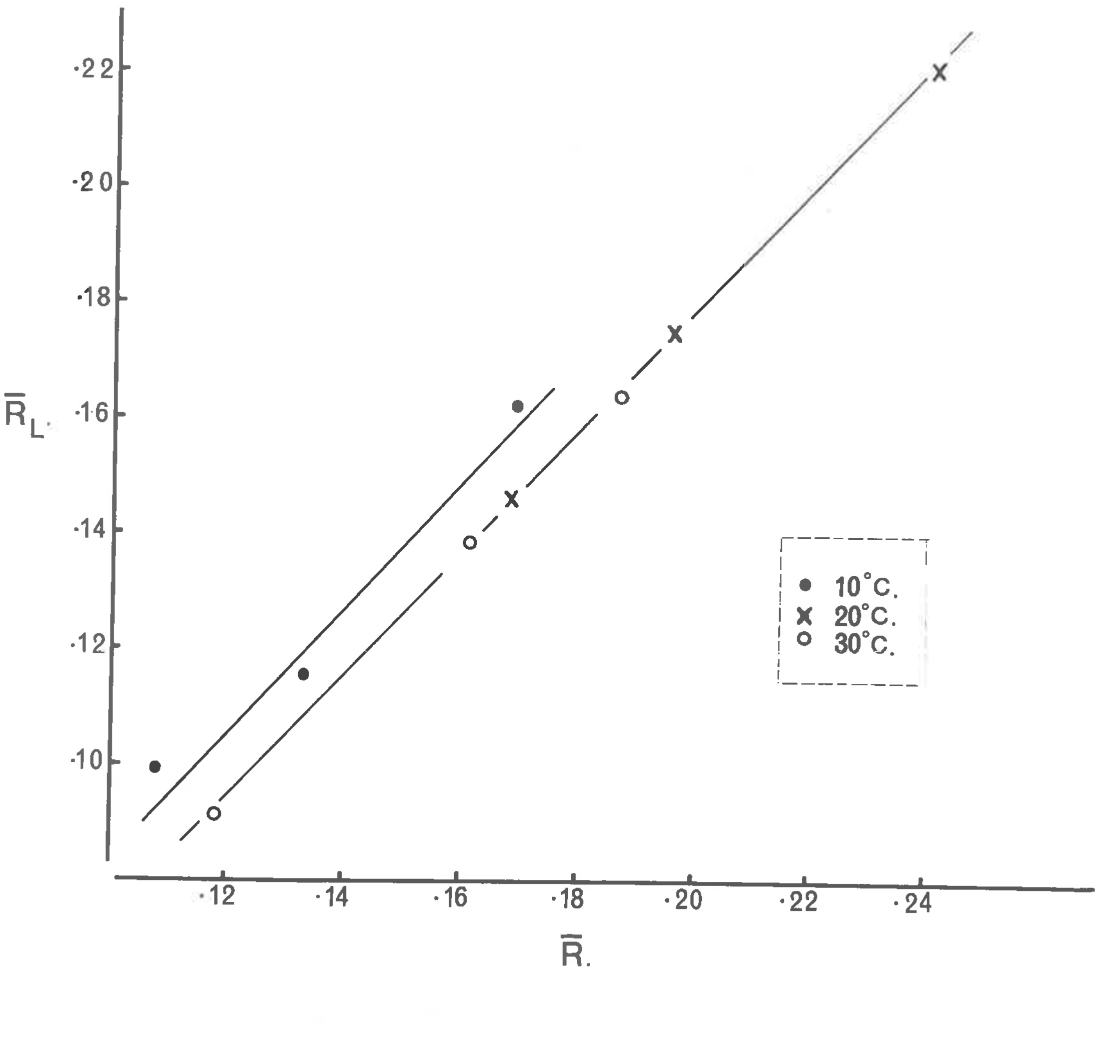
TABLE 30

The Ratio (α) Between Mean Relative Growth Rate ($\bar{R}$) and Mean Relative Leaf Area Growth Rate ($\bar{R}_L$) in Each Environment

Radiation	Temp.	α	k^*	$\frac{1}{k}$
High	10	1.04	0.96	1.05
	20	1.09	0.91	1.09
	30	1.14	0.88	1.14
Medium	10	1.16	0.86	1.16
	20	1.12	0.88	1.12
	30	1.17	0.85	1.17
Low	10	1.09	0.92	1.08
	20	1.15	0.87	1.15
	30	1.30	0.77	1.29

* k is the exponent in $L = bW^k$

<u>Figure 19</u> Mean relative leaf area growth rate ($\bar{R}_L$) plotted against mean relative growth rate ($\bar{R}$).


·22
·20
·18
·16
·14
·12
·10
$\overline{R}_L$.
·12
·14
·16
·18
·20
·22
·24
$\overline{R}$.
● 10°C.
x 20°C.
o 30°C.

at 20 and 30°C undoubtedly lies in the lower leaf lamina proportion at the lower temperature (Table 9).

The conclusion drawn from Figure 19 is that any response in the relative growth rate to radiation <u>or</u> temperature is associated with a parallel response in the relative rate of increase in leaf area provided that the environmental factor does not alter the proportion of leaf lamina to the rest of the plant. For a particular ratio, $\bar{R}$ increased proportionately less than $\bar{R}_L$ as conditions became more favourable for growth, and it appears that at very high levels of radiation (at 20°C) $\bar{R}_L$ would exceed $\bar{R}$. There is thus not only an intimate relation between leaf area and leaf weight within any given environment, but between environments the relative rates of increase in leaf area and in plant weight are also intimately associated.

It is suggested that the growth responses to different environments are best described by the relative growth rate, the relative leaf area growth rate and the specific leaf area. These functions provide information about the rate of duplication of living matter, the rate of increase in the photosynthetic surface and the area over which unit dry matter is distributed.

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