ON THE GROWTH OF THE BARLEY APEX.

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by

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TABLE OF CONTENTS.

ABSTRACT.

An investigation was carried out on the growth and development of
the spring barley apex from shortly after germination to shortly before
heading. The influences of both daylength and increasing soil moisture
tension on the various measures, viz. (i) number of primordia (ii)
length of the apex, (iii) inflorescence, spikelet and early leaf
development were studied in controlled conditions of temperature and
humidity.

It was observed that there are two major changes in the pattern of
development of the apex viz. (i) the initiation of double ridges and
(ii) the initiation of internode elongation. The initiation of double
ridges was found to have occurred by the time the apex had reached
a specific length. Changes in the environment up to this time appeared
to have controlled the rate of increase in apex length in a quantitative
manner. The initiation of internode elongation was found to have
occurred at the first appearance of stamen initials on the most
advanced spikelet. The cessation of primordia formation was observed to
be correlated with the initiation of internode elongation. Both of
these changes were found to be initiated irrespective of the general
rate of growth of the plants in a particular environment, providing
that the necessary morphological conditions had been met. These observa-
tions have led to the conclusion that the apex has within its own
structure the necessary factors for initiating both double ridges
and internode elongation.

An examination of the cytological and hormonal changes surrounding
the initiation of internode elongation was carried out to obtain further
evidence on the mechanism of this event. It was found that cell

Abstract. (cont.).

division in the pith tissues of the internodes of the inflorescence
was initiated immediately following the appearance of stamen initials
and this phase of cell division had similar features to those which
had been described in some dicotyledonous rosette plants. These
observations were followed by the determinations of activities of
gibberellin-like substances in apices before, during and subsequent to
the initiation of internode elongation.

A new method of bioassay for gibberellins in plant material was
developed and was found to be considerably more sensitive than
previously described methods. This new technique enabled estimations
of gibberellin-like substances from small quantities of barley
inflorescences to be determined. It was found that a peak in the
specific activity of these substances occurred in the apex at the
initiation of internode elongation. This result is in agreement with
the previously known results from dicotyledonous rosette plants. But
it is still not known why a certain amount of floral development is
necessary in the spring barley (a monocotyledonous plant) before
internode elongation is initiated, in contrast with the dicotyledonous
plant, in which the initiation of internode elongation occurs at, or
prior to, flower initiation.

DECLARATION

The experiments reported in this thesis were carried out by myself under the supervision of Dr. L.H. May and Dr. L.G. Paleg except for one experiment described in the appendix which was carried out with Dr. L.G. Paleg.

Dated   Sixth Day August 1962

Signed

CHAPTER I   INTRODUCTION.

1.1.   Morphology and Histogenesis of Vegetative and Floral
Gramineous Shoot Apices.

1.1.1.   Developmental Morphology of the Shoot Apex.

The basic descriptions of the morphological changes of the
gramieous shoot apex during its growth are well documented, (Bonnett
1935, 1936, 1937, Evans and Grover, 1940, Sharman 1947 and Bremer-
Reinders 1958). It is generally agreed that the sequence of events
is as outlined below.

The vegetative stages are characterised by the lack of develop-
ment of axillary branch primordia on the apex. Typically, the early
vegetative stage has a short hemispherical apical dome with few
simple ridge primordia, while the late vegetative stage has an
elongating dome with more primordia.

The sequence of the developmental stages after floral initiation
is dependent on the genus under observation eg. lateral spikelet
initials in Hordeum spp. appear before glumes(Bonnett 1955), while
in Triticum spp. , Lolium spp. and Secale spp. there are no lateral
spikelets and therefore sterile glumes are the first new structures
to appear, (Bonnett 1936, Sharman 1945, Barnard 1955, Bremer-Reinders
1958). The lateral spikelet initials in barley appear simultaneously
and bear terminal sessile flowers. The glume, lemma, palea, stamen
and pistil initials arise in an acropetallous sequence, which is
similar to the sequence in Anthoxanthum odoratum (Sharman 1960), on
each of the three spikelet initials at each node of the spike. This
same sequence of appearance of floral parts is common to the other
above mentioned genera, except, however, the stamen and pistil
initials arise from a flower primordium which appears in the axil of

1.1.1.(cont).

a lemma. A third difference between barley on the one hand and
wheat and oats on the other is the absence of a terminal spikelet to
the inflorescence in barley and the presence of a spikelet terminating
the inflorescence in wheat and oats (Bonnett 1935, 1936, 1937). These
differences in the developmental morphology between barley and the
other genera commonly used for physiological studies tend to
support the present taxonomic idea of placing the genus Hordeum,
and allied genera in the subtribe Hordeinae and the genera Triticum,
Secale and Agropyron in the subtribe Titicinae (Bowden 1959). The
genus Lolium is placed in the tribe Festuceae as the orientation
of the flowers on the spikelets is quite different from the other
genera.

1.1.2. Histogenesis of the Vegetative and Floral Organs in the Gramineae.

The "tunica-corpus" theory of the zonation of the cellular layers
of the shoot apex (Clowes 1961 p 20), provides adequate concept for
the description of the initiation of leaf and branch primordia in
the gramineae (Barnard 1955, 1957, Sharman 1945). The various parts
of the gramineous flower can be classified into either those of
foliar origin or those of cauline origin on the basis of whether the
tunica alone or both tunica and corpus contribute to the floral part
(Barnard 1955, 1957). The glumes, lemmas and paleas are derived
from the tunica alone and hence are said to be of foliar origin as
the leaf is, in the vegetative apex, derived from the same cellular
layers. The spikelet, flower and stamen initials are derived from
both tunica and corpus and hence are said to be of cauline origin.

1.1.2. (cont.).

These structures are very similar to the tiller and main shoot meristems in their pattern of cellular zonation according to Sharman (1960 p. 197).

> "Once initiated, a stamen has the structure and general mode of growth of a typical grass bud primordium. It consists of a central core of cells, dividing in all phases, covered by a hypodermis and dermatogen."

The dermatogen and hypodermis are the two cellular layers comprising the tunica.

The ovary, surrounded by the carpels, is considered to terminate the flowering branch (Sharman 1960 a,b, Clifford 1961). The termination of fertile branches with anthers and ovaries is consistent with the "gonophyll" theory of the evolution of the angiosperm flower proposed by Melville (1960).

Hence in summary, the spike of a gramineous plant is a branched system, bearing fertile and infertile branches. In barley the main shoot axis does not differentiate into a terminal spikelet and the triad of primary branches are terminated by an ovary and bear fertile secondary branches (stamens). In contrast, in the wheat plant the main shoot axis, which differentiates into a terminal spikelet, and the primary branches are not fertile, ie. they are not terminated by an ovary (Barnard 1955). It is the secondary branches which are terminated by ovaries and these secondary branches bear fertile tertiary branches (stamens). A similar system occurs in rye, ryegrass and oats.

1.2.   Growth of the Shoot Apex.

1.2.1.   Correlation of Growth Parameters of the Shoot Apex.

The pattern of growth of the shoot apex in the gramineae has also been studied in relation to the influence of some environmental factor(s), eg. temperature, photoperiod and light quality. In particular the environmental control of the change from the vegetative to the reproductive state has been the main object of many workers. However, in studies by Cooper (1951, 1956), Gott et al. (1955), and Evans (1960 a,b) attempts have been made to correlate various parameters of apex growth with morphological development. Similar correlations can be derived from the data presented by Borthwick et al. (1948).

It has been shown by Evans (1960b) that there is a relationship between the development of the spikelets in Lolium temulentum L. and the apex length over a wide range of photoperiodic treatments. A similar correlation can be deduced from the data for Winter Barley reported by Borthwick et al. (1948) up to stage VI ("the individual structures of which the spikelet is composed could be recognised."). From stage VI onwards the relationship between stage of development and spike length is not linear, and the stem length also increases rapidly.

In vernalization studies on Triticum spp., the same apex length was observed by Cooper (1956) at the appearance of spikelet initials over a range of vernalization treatments. A similar result has been observed by Gott (1961) on a spring wheat grown either in short days or in continuous light. In photoperiodic studies on Lolium temulentum the apex length at the appearance of spikelet initials increases

1.2.1. (cont.).

with decreasing daylength (Evans 1960 b). If, however, this specie
is grown <u>under continuous light in a range of day and night temperatures</u>
the appearance of spikelet initials is observed at the same apex
length (Evans 1960 b). From the data of Borthwick et al. (1948) on
Wintex Barley a similar observation can be made, in that the same
apex length was observed at stage II ("slight swellings in the axils
of some leaf primordia indicated the earliest beginnings of the
structure that ultimately would form a group of three spikelet
primordia").

In the following:- (i) spring wheat grown in both short and
long days, (ii) wheat grown in continuous light after a range of
vernalization treatments, (iii) ryegrass grown in continuous light
over a range of temperatures, (iv) barley grown in a 11.5 hour
photoperiod and subjected to a range of night break treatments, the
observation of the same apex length at spikelet initiation suggests
a specific requirement for space on the apex for the initiation of
the reproductive state. Both Cooper and Evans have measured the apex
length from the base of the primordium immediately above the youngest
"cowl" leaf. But, as mentioned above, it is apparent from Evans
(1960 b), that the apex length at spikelet initiation increases as
the daylength decreases. However, it is also known that there is an
increasing number of leaf primordia in varying stages of development
up to the youngest cowl stage in decreasing daylengths and these
could be complicating the issue. In any event the relationship of
apex length and spikelet initiation needs further clarification.

It is apparent that log apex length increases smoothly (Not

1.2.1. (cont.).

necessarily linearly) with time, and it was shown for rye grass, by
Cooper(1951), that log apex length was related to the total number of
primordia during the period of primordia formation.   It appears from
the data presented by Cooper (1951, 1956) that primordia formation
ceases at the onset of internode elongation in the stem of Lolium
temulentum and Triticum vulgare, and this event occurs at a "late
spikelet bud stage" before the appearance of floret bud primordia.
The data of Gott et al. (1955) permits the conclusion that in Petkus
Rye a stage of spikelet development is associated with the cessation
of primordia formation over all environmental treatments employed.
This stage is between a "score" of 24 and 26 (Gregory and Purvis 1938),
which corresponds with the stage between the appearance of glume
initials and floret primordia. From recent studies on the influence
of Gibberellic Acid (GA_3) on unvernalized Petkus Winter Rye it is
apparent that the initiaiton of stem elongation occurs at the same
stage of spikelet development as the cessation of primordia formation
(Purvis 1960). A similar conclusion may be deduced from the data
of Borthwick et al. (1948) that the initiation of stem elongation in
Wintex Barley begins at stage VI of their developmental scale.

Hence it appears in the species cited in this section that the
onset of internode elongation is correlated with the cessation of
primordia formation. This change in the pattern of growth of the
shoot occurs at a definite stage of spikelet development. It is
perhaps more than coincidence that this stage of spikelet development
is the initiation of secondary branches on the inflorescence (section
1.1.2.).

1.2.1. (cont.).

In _Lolium temululentum_ the number of spikelets branches on the spike was constant over a wide range of photoperiodic treatments (Evans 1960 b). In contrast it has been shown for Petkus Rye that there are wide variations in the number of spikelets formed on the spike, eg. in the spring variety the number of spikelets increases with decreasing daylength (Gott et al. 1955). Since the number of spikelet branches formed is fixed by the cessation of primordia formation at a definite stage of spikelet development, then in Lolium sp.. the rates of primordia formation and spikelet development change in a parallel manner with changes in photoperiod while in Petkus Rye these two rates apparently change independently of each other.

1.2.2.   Influence of Daylength on Growth of Shoot Apices.

In the gramineae examples can be found of all types of photo-periodic responses, ie. both qualitative and quantitative short day or long day plants. However, in this work attention has been focused on those requiring, or promoted by, long days for flower initiation; ie. spring varieties or fully vernalized winter varieties. The results of Cooper (1951, 1956), Gott et al. (1955) and Evans (1960 a,b) show that the species studied either have a specific long day requirement for flower initiation (Evans 1960 a,b) or are promoted by long days. It is also apparent from Gott et al. (1955) that the rate of growth of the apex both in terms of length, number of primordia and rate of spikelet development , is increased in Petkus Winter Rye with increasing photoperiod. Similar results have been obtained for _Lolium temulentum_ (Cooper 1956, Evans 1960 b). Studies on spikelet

1.2.2. (cont.).

initiation and subsequent development in <u>Hordeum bulbosum</u> indicated

a strict requirement for long days for subsequent development of the

spike after flower initiation, (Koller and Highkin 1960). Similar

studies on <u>Poa bulbosa</u> (Younger 1960) indicated the necessity of

postulating a floret differentiating hormone whose production was

promoted by long days and temperatures above 20°C, as well as an inflo-

rescence initiating hormone. The inflorescence initiating hormone

was formed in both short and long days after a brief period of vernal-

ization.

Nightbreak experiments of Borthwick et al. (1948) Downs (1956)

indicated that small amounts of red light, given in the middle of a

12.5 hour dark period, were more effective in promoting flower

initiation and subsequent spikelet development in Wintex Barley than

no light. Also the effect of this red light could be reversed by a

small amount of far-red light immediately following the red light.

The results of similar experiments by Friend et al. (1959) using

either an incandescent source or a fluorescent source of nightbreak

light in the middle of a 16 hour dark period indicated that the

incandescent source was more effective in promoting flower initiation

and spikelet development in wheat and barley. Later experiments by

Friend et al. (1961) employing different ratios of red to far-red

energies during a nightbreak treatment showed that with increasing

amounts of far-red the rate of spikelet development and the rate of

stem elongation was promoted but there was no change in the time to

flower initiation for Marquis Wheat. Similar results have been

observed by Stroun (1958) for both wheat and barley on the influence

1.2.2. (cont.).

of light quality during the day on the rate of spikelet differentiation
while the earliness of the flower initiation was considered to be
related primarily to the daylength.

In experiments where a basic short day was lengthened by a
supplement of light from either an incandescent source or a fluorescent
source(Downs et al. 1958) it was shown that the incandescent source
was more effective for plants which require stem extension as an
integral part of the whole growth of the inflorescence (eg. many
species of the graminaceae). Later results (Downs et al. 1959)
indicated that a balance of red and far-red wave lengths is the most
effective in promoting the growth of the inflorescence of some
varieties of wheat and barley when compared with equal intensities
of illumination from either fluorescent or ruby red incandescent light
sources. In these latter two sources the mixture of red and far-red
wavelengths is shifted in favour of either the red or the far-red
respectively.

1.2.3.   Influence of Growth Regulators on Gramineous Shoot Apices.

Relatively little work has been done using growth regulators as
a means of inducing flower initiation in gramineous plants or stim-
ulating subsequent development. Alpha-naphthalene-acetic acid
applied to Petkus Rye and Wintex Barley appears to have no significant
influence on flower initiation and some promotion of post initiation
development of the spike, (Leopold and Thimann 1949, Hussey and
Gregory 1954).

In experiments involving the application of GA_3 to plants a

1.2.5. (cont.).

range of responses for a particular effect have been observed. GA_5
will (i) replace the cold and/or long day requirement for flowering
in some plants eg. Hyoscyamus niger, (ii) replace only the cold
requirement in Centaurium minus as long days are required for
continued stem elongation and flowering after the end of the GA_5
treatment, (iii) not replace the cold requirement in young
unvernalized Petkus Winter Rye or in young Minflor Winter Wheat
seedlings (Lang 1957, Carr and McComb 1957, Gott 1961). Recently it
has been shown that the failure of applied GA_5 to replace a specific
environmental requirement may be due to the gibberellin specificity
of the plant (Lockhart and Deal 1960, Brian et al. 1962, Michniewicz
and Lang 1962).

It has been shown by later workers (Caso et al. 1960, Purvis
1960) that the time of application was important for observing a
response to GA_5 on unvernalized Petkus Winter Rye. High concentrations
of applied GA_5 preceding floral induction stimulated development of
tiller buds in the apex (Caso et al. 1960), while the same concentations
applied after floral induction, promoted the development of the
spikelets in both short and long days. Applying GA_5 at the same
relative times to Hordeum spp (Koller et al. 1960) showed a promotion
of flowering following the early application and a promotion of post
initiation development of the spikelets following both times of
application. However continuous application of high concentrations
will interfere with the normal sequence of inflorescence development,
eg. male sterility, (Paleg and Aspinall 1958, James and Lund 1960).
Recent work by Aspinall (pte. comm) indicates that GA_5 applied to

1.2.3. (cont.).

young seedlings of Hordeum distichon L. cv. Prior increases the
rate of primordia formation, spikelet development and leaf elongation.

1.2.4. Effect of Drought on the Growth of the Shoot Apex.

"Detailed studies of the effect of drought on
flowering development in the cereals appears
to be lacking." (Gott 1961)

Most of the drought studies on cereals have been on the later
stages of development of the inflorescence, in which such parameters
as grain number per ear, pollen viability and stem elongation have
been studied (Skazkin and Zavadskaya 1957, Domanskii 1959, and Asana
1961).

It is known that tissues, which are immature, are capable of
tolerating higher intensities of dehydration than more mature tissues
which contain a large proportion of vacuolated cells (Milthorpe
1950, Amer and Williams 1958). It was considered by Gates (1955 a,b,
1957) that the effect of a drought on tomato plants was to interrupt
the translocation of materials from the stem to the younger leaves
at the shoot apex, while materials were still being translocated from
the older leaves to the stem. These observations are interpreted
by Gates as bringing about a "senescent" condition in the plant. On
watering up there was a return to a physiologically younger condition.
The immature tissues played a dominant role in the overall response
of the plants on exposure to extreme conditions.

The only published work bearing on the influence of soil water
shortage on flower initiation that came to notice was on apricots
Brown 1953). Results showed (i) a reduction in the rate of development

1.2.4. (cont.).

of the flowers already initiated, (ii) a delay in the initiation of
many flowers buds and (iii) a reduction in the total number of
flowers initiated. Hence it seemed desirable to determine the
influence of soil moisture tension on the rate of development of
the shoot apex in cereals

1.2.5.   Hypotheses on Flower Initiation in Long Day Cereals.

The original proposal of Gregory and Purvis (1937) and the
modification by Gott et al. (1955) is in essence the partitioning
of the product of vernalization (B), which is in high concentrations
in spring varieties, into either a leaf promoting substance (E) or
a spikelet initiating substance (C). The reaction of B to C is
reversible and its equilibrium is dependent on the combination of
(i) supply of B, (ii) the conversion of C into a further substance
(D), which is promoted by long days, and (iii) the night length. If
the nights are long, then the equilibrium is shifted towards C. A
nightbreak will shift the equilibrium towards B. If C is being
converted rapidly into D, as in continuous light, then the equilibrium
is shifted towards C. Both D, which is considered to control the
rate of development of the spikelets, and C are required to reach a
threshold concentration before their effect is observed.

These reactions were postulated to occur in the apex and as
indicated by Gott et al. (1955 p.123) the hypothesis would have to
be modified to include other substances, whose production is under
photoperiodic control, entering the system from outside the apex eg.
gibberellins (Brian 1959, 1961).

1.2.5. (cont.).

A simple hypothesis has been presented by Chaylakhyan (1959) for
the hormonal control of flowering in plants. It requires the combined
action of flower forming substances (anthesins) and stem forming
substances (gibberellins). In long day gramineous plants (spring
or summer varieties) the anthesins are present in all environmental
conditions, but long days are required for the formation of gibberellins.
However in both the above hypotheses no mention is made of the
possibility of inhibitors being present.

Evidence is put forward by Evans (1960 c) that the flower
initiation in Lolium temulentum is controlled by a balance of
transmissable promoting and inhibiting substances. When the plants
are grown in anaerobic conditions during a single long dark night,
spikelet initials are visible on the apex although an accelerated
rate of increase of apex length, characteristic of the promotive
stimulus, did not occur (Evans 1962).

Two possibilities are considered by Evans (1962 p. 289).

> "This difference could be due to incomplete
> suppression of the dark inhibitory process
> in anaerobic conditions, or it could be
> that long days, while not essential for
> inflorescence initiation in the absence of
> the inhibitory process, strongly accentuate
> the promotive process in the leaves of
> L. temulentum."

The latter of these two possibilities is favoured as there are
gramineous plants including wheat (Seidlova-Blumova 1961) and barley
which will form spikelet initials in an 8 hour photoperiod. The
development of these spikelets is very slow in short days and can
be accelerated by increasing the daylength. Hence, variations
among the annual long day gramineous plants in their response to

1.2.5. (cont.).

short days could be due to variations in their capability to synthesise an inhibitor. Also long days promote the synthesis of endogenous gibberellins (Brian 1959) which are considered to stimulate the growth of lateral meristems (Brian 1959, Caso et al. 1960). Gibberellins have been found in increased quantities during the early stages of floral initiation of several long day dicotyledenous plants following the exposure of these plants to long days (Harada and Nitsch 1959, Lang 1960, Reinhard and Lang 1961), however, the comparable experiments on monocotyledons have yet to be reported.

This thesis reports a study of the interrelationships of four paramenters of the growth and development, observable on shoot apices, of spring barley grown in different daylengths and in different soil moisture regimes. These parameters are:- (i) the rate of primordia formation (ii) the rate of growth of the early stages of leaf development (iii) the rate of spikelet development (iv) apex length, which in the floral state is the length of the inflorescence axis. The bearing of these results on our knowledge of factors regulating apex development in cereals is considered.

CHAPTER 2   MATERIALS AND METHODS.

2.1   Materials.

Two varieties of barley (Hordeum distichon L.), Prior "A" and C.I.5611 have been used for studies on the growth of the apex.   Prior is a relatively early flowering variety while C.I.5611 is late.   The Prior seed used in the experiments was from a single plant selection bulked and supplied by Dr. D. Aspinall of the Plant Physiology Department, Waite Agricultural Research Institute.   The original Prior seed and the C.I.5611 seed (1959 harvest) were supplied by Dr. K.W. Finlay of the Agronomy Department, Waite Agricultural Research Institute.   All the chemicals used in the experiments were of analytical grade and the Gibberellic Acid was supplied by Imperial Chemical Industries of Australia and New Zealand.

2.2.   Controlled Environment Cabinets.

2.2.1.   Cultural Practices.

All plants were grown in four inch polythene pots containing a loam-sand mixture.   Urrbrae Loam was mixed with Plympton Sand in the ratio of four parts of loam to one part of sand (v/v) and steam sterilised for fifteen minutes after complete penetration of the steam. After being air-dried the mixture was stored until required.   For each batch of one hundred pots 81.5 kg of air dry soil (80 kg dry wt.) were first mixed with a mineral nutient supplement and then moistened gradually up to about 14% soil moisture content with 11 litres of distilled water.   During moistening the soil was thoroughly mixed several times to prevent the formation of large, sticky, non porous lumps.   The final moistening was done after the soil had been added

2.2.1. (cont.).

to the pots and its moisture content determined.

The mineral nutrient supplement added to each batch of soil was as follows:

31.6 g	Potassium Nitrate
106.5 g	Calcium Nitrate (Hydrated)
36.4 g	Sodium Nitrate
55.0 g	Magnesium Sulphate (Hydrated)
63.0 g	Sodium diHydrogen Phosphate di-Hydrate
2.4 g	Ferric Citrate

In the water stress experiments the pots were civered with aluminium foil, which was pierced in two places, one hole for the plant and one smaller one for watering, to minimize evaporational loss from the soil. A half inch layer of sand was used in later experiments instead of the aluminium foil as larger numbers of plants were grown in each pot.

2.2.2.   Controlled Environment Cabinet.

The cabinet used has space for a hundred pots arranged in 10 rows on the floor. The light source, which is a bank of 54 80-watt "cool white" fluorescent tubes (Philips TLF 80/33), is separated from the cabinet by a sheet of plate glass to prevent the warm air from the tubes mixing with the air flow in the cabinet. The light intensity for all experiments was 2,500 foot candles in the centre of the floor and fell off to 2,000 foot candles in the corners. Temperature was regulated by a refrigerator air conditioning unit and maintained at $17^{\circ}C \pm 1.5^{\circ}C$ with a gradient of $1.5^{\circ}C$ between the air inlet and outlet.

2.2.2. (cont.).

Relative humidity was maintained at 85% ± 5% using an electric
frypan with an automatic water feed from a distilled water reservoir.
The same temperature was used in all experiments but humidity was
only controlled in the daylength- water stress series and in the
continuous light with and without incandescent supplement experiments.
In other experiments the relative humidity was approximately constant
at 40%.

2.2.3.   Daylength - Soil Moisture Stress Experiments.

In these experiments, the following experimental design was
employed. Every second day all the pots were re-randomised in three
replicates and the position of each replicate was changed on a cyclic
pattern. This procedure was adopted to reduce the variance for
positional effects arising from the known pattern of temperature and
light intensity gradients in the cabinets.

In each replicate the pots were allocated within a factorial
design among the two varieties, the three soil moisture stress
treatments, and five harvests. The soil moisture stress treatments
were applied as follows (i) no stress (W_0), (ii) an increasing soil
moisture stress up to 5 atmospheres (W_1) at which time harvest 2
was made and thenceforth no stress, (iii) an increasing soil moisture
stress up to 15 atmospheres at which time harvest 3 was made and
thenceforth no stress. Harvest 1 was made at an earlier date, and
harvests 4 and 5 were made at approximately equal intervals after
harvest 3.

Watering was usually done at the same time as the re-randomization
of the pots, but as the plantsgrew, daily watering had to be adopted.

2.2.3. (cont.).

Water was added to each pot to bring it up to a predetermined weight. From the fresh weight of the plants determined at each harvest a correction for the fresh weight of the plant was made.

2.2.4. Continuous Daylength Experiments With and Without an Incandescent Supplement.

The incandescent light supplement was added by replacing 4 pairs of fluorescent tubes with 4 150-watt incandescent strip lamps (B.G.E.). The light intensity at the top of the pots was 2,450 foot candles of fluorescent and 120 foot candles of incandescent light from measurement with a selenium photocell. The temperature and humidity were the same as in the water stress experiments. The light source in the experiment without the incandescent supplement was the same as that described for the daylength soil moisture stress experiments

The experimental design was 10 replicates of 10 pots, each pot containing two plants. The pots were re-randomised in each replicate and the replicates handled in a similar manner to the previous experiments. The pots were covered with aluminium foil pierced in two places for the plants and a smaller third hole for watering, which was carried out in the same manner as before.

2.2.5. Material for Gibberellin Analysis.

Two identical environment cabinets were used in this experiment, each containing one hundred pots. One of the cabinets was on an eight hour daylength cycle, the other operated on a continuous light basis. The temperature in both cabinets was maintained at 18°C $\pm$ 1.5°C, but no provision was made for humidity control. The light was from

2.2.5. (cont.).

the same type of fluorescent tubes as before and the light intensity
was 2,500 ft.candles at the top of the pots. The pots were sown
with 12 seeds per pot and thinned to 10 seedlings per pot. At the
first harvest, which was taken seven days from sowing, the pots were
thinned to 5 seedlings per pot. At subsequent harvests 3,1 and 1
plants were taken from each pot. The plants, from each daylength,
were harvested at the same stage of development, so the harvesting
schedule was arranged to meet this requirement. Since variable
numbers of plants would be in the pots during the experiment the
aluminium foil was replaced by a $\frac{1}{2}$" layer of sand as a barrier for
water loss.

2.5.   Dissection and Scoring Techniques.

2.5.1.   Daylength-Soil Moisture Stress Experiments.

The plants at each harvest were dissected by hand and the structures (leaf or spikelet) borne at each node were grouped into the various developmental classes, viz. expanded, expanding, differentiating leaves, simple ridges or spikelets. The stage of development of the inflorescence was assessed on the stage of floral development of the most advanced spikelet primordium. The vegetative stages of the apex were assessed on the shape of the apical dome.

The following scale was employed in describing the development of the apex.

Value	Stage	
1.	Early vegetative	(Apical dome short).
2.	Late vegetative	(Apical dome elongating).
3.	Double ridges	(Spikelet primordium visible)
4.	Triple mound	(Lateral spikelet primordia visible).
5.	Glume initials	
6.	Lemma initials	
7.	Stamen initials	
8.	Awn initials	
9.	Primordia further advanced than awn initials.	

The leaves were assessed as follows:- (i) Expanded leaves with the ligule exposed, (ii) expanding leaves were those greater than 0.65mm long and had not fully expanded, (iii) differentiating leaves were those less than 0.65mm long, but whose potential adaxial surface made an acute angle with the longitudinal axis of the shoot.

2.3.2. Continuous Daylength Experiment With and Without Incandescent Supplement.

In these and subsequent experiments (gibberellin assays) a fuller description of the developmental stage of each node was attempted. The scale of leaf development was as follows:-

Stage   I   was the same as the differentiating leaf described previously.

Stage   II   all expanding leaves which were not visible before dissection of the plant.

The scale of spikelet development was as follows:-

Value	Stage
1.	Simple ridge - undifferentiated and potentially capable of becoming floral or remaining vegetative.
2.	Double ridge
3.	Advanced double ridge (spikelet primordium swelling)
4.	Triple mound.
5.	Glume initials
6.	Lemma initials
7.	Stamen initials
8.	Awn initials
9.*	Anthers lobing (appearance of anther lobes).
10.*	Awns elongating.

* Stages used in material collected for gibberellin assay only. This last stage is a very subjective one in the absence of a quantitative measurement for discrimination.

2.3.3. Apex Length.

All apex lengths recorded are those measured from the top of the apex to the base of the oldest simple ridge primordium. The apex

2.5.3. (cont.).

length in the early vegetative stage is variable, because (i) the dome
is a considerable contributor to the length and its size is related
to the stage of plastochrone at the time of measurement, and (ii) the
oldest simple ridge primordium does not develop into a stage I leaf
at the same time as a new simple ridge primordium appears. The number
of simple ridges on such an apex varies between one and two in an
irregular manner.

2.5.4.   Developmental Phases.

The development of the apex was divided arbitrarily into three
phases, vis. vegetative, spikelet and elongating phases. The
vegetative phase is bounded by germination and the appearance of
double ridges. The spikelet phase starts at the appearance of
double ridges and ends with the appearance of stamen initials.
During these two phases there is active primordia formation and no
internode elongation. The elongating phase starts at stamen initials
and ends at the cessation of peduncle elongation. In this phase,
internode elongation is the characteristic feature.

2.4.   Sectioning Techniques.

The apices from the continuous daylength experiment without incandescent supplement were fixed in a formalin, acetic acid, ethanol and water mixture (5:5:45:45 v/v) for 24 hours and preserved in 70% ethanol. Dehydration was accomplished by using the graded series of tertiary butanol concentrations, (Johansen 1940 page 130), however the changes in butanol concentrations were made every 30 minutes for the solutions up to 100% tertiary butanol. The apices went through two changes at hourly intervals of 100% tertiary butanol, the second change contained 0.1% eosin.

The remainder of the procedure of infiltrating, embedding, sectioning and mounting was the same as prescribed by Johansen (1940 chapter 12); Paraffin wax (M.P.52°C) was used as the embedding medium.

Before the apices were dehydrated a short piece of nichrome wire was threaded through the stem to mark the plane of the spikelets. This section of the stem was pared away when the embedded material was ready for longitudinal sectioning. After sectioning at 10µ thickness on a Rotary Spencer Microtome, the ribbons were examined and only the twelve median sections were mounted, with Haupts adhesive to affix them to the glass slide. The most suitable stain for the purpose of staining cellulose cell walls and nuclei (including nuclei undergoing cell division) was found to be the iron mordanted Cellestine Blue stain described by Gray et al. (1956). The method of preparing the stain was adapted from the description given by Gray and Pickle (1956) for preparing the iron mordanted safranin and celestine blue stain, which may be used for staining skeletal elements in plant tissues. The affixed sections were handled by the same

2.4. (cont.).

procedures described by Johansen (1940 pp 151-154) for aqueous
stains. The sections were stained for 1-5 minutes and uniform
results were obtained, which gave clear sharp photgraphs.

2.5. Bioassay Technique.

2.5.0. Introduction.

A number of bioassay techniques are in use for the detection
of gibberellin-like substances in plant materials (West and Phinney
1960). Most of these techniques are based on the elongation of
some part of the test material induced by GA_3; stems in dwarf peas,
leaf sheaths in the various dwarf mutants of corn, excised portions
of the first leaf of the wheat or rice plant. The expansion of the
bean leaf disks has also been used by Wheeler (1958) and recently
Naylor and Simpson (1961) have published a technique based on the
germination stimulating property of GA_3 on after-ripened excised
embryos of _Avena fatua_ L.

The minimum amount of GA_3 required for a significant response
in most techniques is about 10^{-3} µg per replicate. Naylor and Simpson
(1961) claimed that in their technique 2.5×10^{-4} µg GA_3 per replicate
applied to 3 replicates, was sufficient for a significant response.
Thus activity of gibberellin-like substance equal to at least 10^{-3} µg
GA_3 is required from an extract of plant tissue.for a measurable
response.

The amounts of plant tissue readily obtainable from barley
shoot apices is 1-10mg (dry weight). The activity of gibberellins
reported in the literature is equal to a range of 10^{-3} to 5×10^{2} µg

2.5.0. (cont.).

GA_5/g fresh weight (Phinney and West 1960). Therefore the activities of gibberellin-like substances in barley shoot apices could be equal to a range of 5×10^{-6} to 1.5 µg GA_5 from a one mg sample of dried shoot apices.

A technique, which employed the discovery by Paleg (1960) that GA_5 applied to barley endosperm segments stimulates the enzymic breakdown of the stored starch into reducing sugars, appeared to be sufficiently sensitive to detect the expected small amounts of gibberellin-like substances. The preliminary experiments on the adaptation of the methods described by Paleg (1961) to a suitable bio-assay technique are reported in the appendix (subchapter 5.1). A significant response to applied GA_5 was found in this technique in 1.0 ml of a 5×10^{-5} microMolar (µM) solution at $\left(10^{-5} \text{µg } GA_5\right)$ per replicate for 5 replicates, ie. activity of gibberellin-like substances equal to 5×10^{-5} µg GA_5 in the extract.

2.5.1.   Materials and Methods of Bioassay Technique.

Hordeum distichon var Triple Awned Lemma (T.A.L.) (a huskless variety) from the 1960 harvest at the Winulta (S.A.) plant breeding site was used in all assays and was stored in the same manner as described by Paleg (1960). All solutions were made up in triple dis-tilled water. Samples of seed numbering two to three times the quantity required were taken and soaked for 24 hours in a freshly prepared 4% calcium hypochlorite solution at 24°C in a glass stoppered flask. No more than 150 seeds per 250 mls of solution were placed in a flask, otherwise precipitation of a white acid soluble substance took place. After soaking the seed was rinsed ten times with 100 mls of triple

2.5.1. (cont.)

distilled water each time and placed on several thicknesses of clean
white absorbent paper. Clean seeds, free of imperfections over the
distal 5 mm, were selected for the bioassay.

These prepared seeds, treated subsequently in groups of four,
were placed on clean mm squared graph paper and cut transversely,
with a clean razor blade, 5 mm from the distal end of the seeds. The
proximal ends, containing the embryos were then discarded. The
remaining fragments of each group were eighed and placed in a 2 x 1
inch plastic stoppered specimen tube, the particular tube being
selected at random.

The solution in the standard GA_3 tubes was as follows:-

(i) 0.5 ml of an aqueous GA$_3$ solution of one of the
following concentrations; $4 \times 10^{-5}\mu M$, $4 \times 10^{-4}\mu M$,
$4 \times 10^{-3}\mu M$, $4 \times 10^{-2}\mu M$ and $4 \times 10^{-1}\mu M$.

(ii)0.05 ml of an aqueous solution of streptomycin
sulphate containing 10mg/ml of streptomycin base.

(iii) 0.45 ml water to make a total volume of 1.0 ml.

The tubes containing an aqueous solution of the plant extract were
made up in a similar manner viz 0.7 ml of aqueous extract plus 0.05
ml of the streptomycin solution and 0.25 ml of water to make the
volume up to 1.0 ml. The endosperm fragments were arranged so that
the cut surface was not resting directly against a glass surface.

The tubes were then left for 48 hours at 30°C $\pm$ 1°C; zero time
being taken when half of the tubes were loaded. At the end of the
incubation 0.5 ml was taken from tubes containing less than $10^{-4}\mu$g

2.5.1. (cont.).

GA_5 and 0.2 ml from those containing 10^{-4} μg GA_5 or more. These samples were diluted to 10 mls and reducing sugars were assayed using the method described by Paleg (1960). As there was a large range of reducing sugar concentrations , ranging from that equal to 500μg to 18,000μg glucose per 1.0 ml original solution, the subsamples taken after filtration ranged from 0.5 ml to 0.1 ml and water was added to make them up to 1 ml before adding the alkaline copper reagent.

In fig. 2.5.1.1. the results for the standard GA_5 dosage-response curves are expressed as μg glucose equivalents / mg endosperm initial dry weight. The initial dry weight of the endosperm was obtained by taking two samples of four endosperm fragments after cutting and weighing, and drying for 72 hours in a forced draught oven at 80°C and re-weighing.

Each line in fig 2.5.1.1. was calculated from the results of an experiment in which one to three repeats of the same set of serial dilutions of a standard GA_5 solution (2 μM) were made and for each experiment a new standard solution was prepared. The analysis of variance (appendix table 5.5.1.1.) showed that there are significant differences between the mean responses and the slopes of the dosage-response curves. This result suggests that the activities of gibberellin-like materials should be estimated from the standard dosage-response obtained at the same time, and not from a mean curve derived from the results of a series of experiments. This suggestion was confirmed by experience.

2.5.2.   Extraction of Plant Material and the Application
of the Bioassay.

The following method of extraction was developed on inflorescences
obtained from plants which were grown in the glasshouse from March
25th 1961 and Harvested on May 14-16th 1961. The inflorescences
were dissected at the stage of awn elongation, and all leaves longer
than 0.65 mm were removed. The material was immediately quick frozen
on dry ice which was subsequently removed by evaporation at -25°C in
a deep freeze. The residue was transferred to clean brown paper and
placed in a dry ice box for storage until it was freeze dried. (In
later experiments (cf. 5.5.) section this step was changed and the material
quick frozen in a 25 ml glass stoppered flask surrounded by dry ice.)
After freeze drying all material was stored at -20°C.

The material was extracted, without grinding, with ethyl acetate
in a 10 ml glass stoppered tube (2.5 ml used each time, plant
material quantities 1 - 10 mg) for 2 hours at room temperature (not
above 25°C) and the ethyl acetate was decanted.   The residue was
re-extracted twice more for 3 hours and 17 hours respectively and the
extracts were combined together with the two 0.5 ml ethyl acetate
washings of the residue after the final extraction. The ethyl
acetate was evaporated to dryness under partial vacuum, at 30°C and
the residue was taken up in 2.5 ml of water overnight. A 0.25 ml
portion was serially diluted ten and one hundredfold and three 0.7
ml subsamples were taken from each dilution respectively. The
subsamples represented 28%, 2.8% and 0.28% of the original quantity
of extractable material for each dilution 1, 10 and 100 fold
respectively.

2.5.2. (cont.).

The slopes of the dosage-response lines for the plant extracts and the standard gibberellic acid concentrations were not significantly different at the 5% level. Therefore by entering the mean response for all dilutions of the plant extract at the appropriate points on the standard dosage-response line the mean activity of the gibberellin-like substances was ascertained. The standard error of activity was calculated from Bliss (1952 equation 52). The total activity of the sample was then a simple calculation.

The unit of activity is defined as the quantity of gibberellin-like substance having the same response in the bioassay as 1.0 $\mu\mu$g GA$_5$ and the unit of specific activity is defined as one unit of activity / μg dry weight of plant material.

The results of the extractions from the inflorescences are contained in Table 2.5.1.1. Since the standard dosage-response curve is linear when log response (fig 2.5.1.1.) is plotted against log mass GA$_5$ all the calculations of standard errors were performed using the log$_{10}$ transformation of the data. Hence log activity and log specific activity, which are tabulated (Table 2.5.1.1.), have the same standard error. Since partial purification of the material by obtaining the acidic ethyl acetate extract (cf Note 1. Table 2.5.1.1.) did not increase the activity of the extract, ie. there were no non-acidic interfering substances, the crude ethyl acetate extract was used in subsequent experiments.

Table 2.5.2.1.

Activity of Gibberellin - like Substances from a Glasshouse grown Sample of Inflorescences.

Subsample	Dry Weight mg	Extract	Standard Dosage-Response Line	Log. Total Extract Activity	Log. Specific Activity	Specific Activity
1	10	Crude	1	4.886 ± 0.206	0.886 ± 0.206	7.7
2	10	Acid Fraction	1	4.674 ± 0.199	0.674 ± 0.199	4.7
3	2	Crude	2	3.688 ± 0.284	0.387 ± 0.284	2.4
Mean					0.649 ± 0.233	4.46

Note 1. The acid fraction was obtained by extracting the crude ethyl acetate solution with a sodium hydroxide solution at pH 9.0 eight times, using 5 ml each time, and after bulking the aqueous layers and acidifying with 0.01 N hydrochloric acid, the aqueous solution was extracted with three 20 ml volumes of clean ethyl acetate.

2. The standard errors of estimation quoted in columns 5 and 6 were calculated from the formula developed by Bliss (1952). The standard error quoted for the mean is the pooled standard error of the individuals.

3. Subsamples 1 and 2 were assayed 12 - 19/12/61.
Subsample 3 was assayed 8 - 12/1/62.

Fig. 2.5.1.
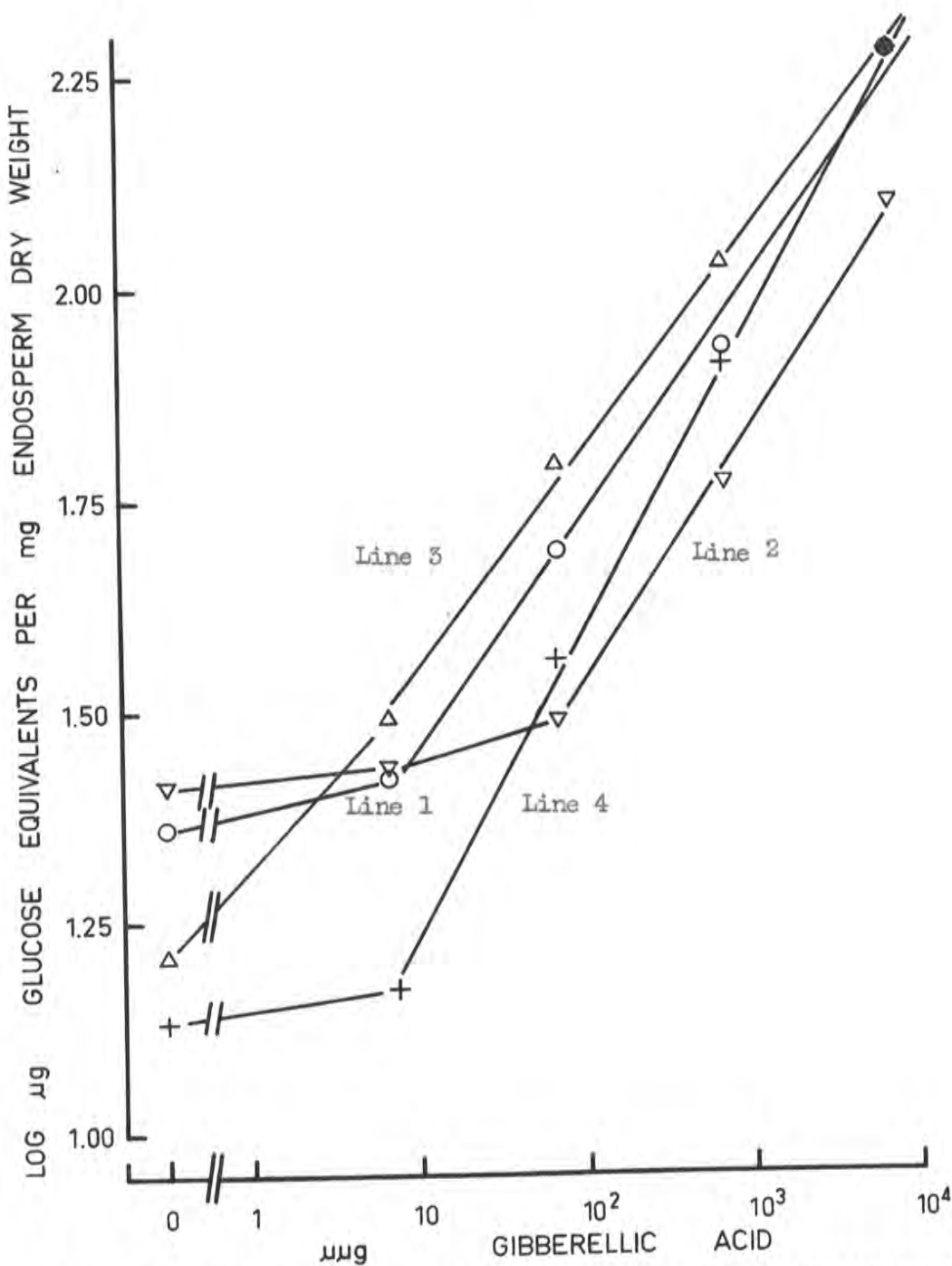
Dosage-response Curves for known Quantities of Gibberellic Acid.
(see appendix table 5.3.1.1. for Regression Lines.)

CHAPTER 3   RESULTS.

3.1.   Plant Growth

3.1.0.   Introduction.

A high light intensity (ca 2,500 ft. candles at the top of the pots) was used in all of the experiments conducted in the controlled environment cabinets. Thus there was a strong possibility that the difference between the growth rates of the spikes grown in each daylength may have arisen from the difference between the daily amounts of photosynthetic products. In other words the supply of carbohydrates in each daylength may have been limiting the growth of the spike. From the preliminary experiment conducted in the glass-house, in which a low level of incandescent supplement (0.5 - 5 ft. candles) to the natural daylight was applied, it was clear that the date of anthesis and the number of leaves on the main stem were linearly dependent upon the intensity of the supplemental lighting. These results suggest that some substance other than carbohydrates is accumulated at low light intensities and this is responsible for the earlier flowering of barley in long days. The following experimental results throw more light on the alternative suggestion.

3.1.1.   Results.

In Appendix table 5.2.1.1. the total dry weights of the plants, grown in the experiment described in sub chapter 3.2, are recorded. It can be seen that rates of gain in dry weight for the 8 hour daylength experiment were considerably less than those for the 16 and 24 hour daylength experiments. This prompted the plotting of the total plant dry weight against the number of hours of light received, (Fig. 3.1.1.). In wheat there is no gain in total plant

3.1.1. (cont.).

dry weight until after the sixth day (Williams R.F. 1960), and it was
observed that the barley endosperm had disappeared by the seventh
day when plants were being harvested in the continuous light
experiments (sub chapter 3.3.). Thus the number of hours of light
received was measured from the beginning of the seventh day from the
soaking of the seeds for germination. It appears (Fig. 3.1.1.)
that while the plants were in the vegetative and spikelet phases of
growth (cf. section 2.3.4), the dry weight is related to the number
of hours of light received and independent of the number of hours per
day. Subsequently, when the elongating phase of growth starts, the
rate of increase in dry weight differs between the various daylengths.
It is noted that the efficiency of the photosynthetic system of the
barley plant was unimpaired by the continuous high light intensity
while it was in the early phases of growth.

The total plant dry weight is the summation of the dry weights
of the individual parts; if the total is divided into the roots and
tops, the same relationship between the dry weight of the component
parts and the number of hours of light received is obtained, as the
ratio of tops to roots is relatively constant. In contrast, when
the growth of the spike, in terms of its length, is plotted against
the number of hours of light received, considerable differences are
observed (Fig. 3.1.2). All the points for C.I.5611 fall on the one
straight line; however, for Prior the rate of increase in length per
hour of light varies depending upon the stage of development and the
daylength.

Fig. 3.1.1.

Fig. 3.1.2.

NUMBER OF HOURS OF LIGHT
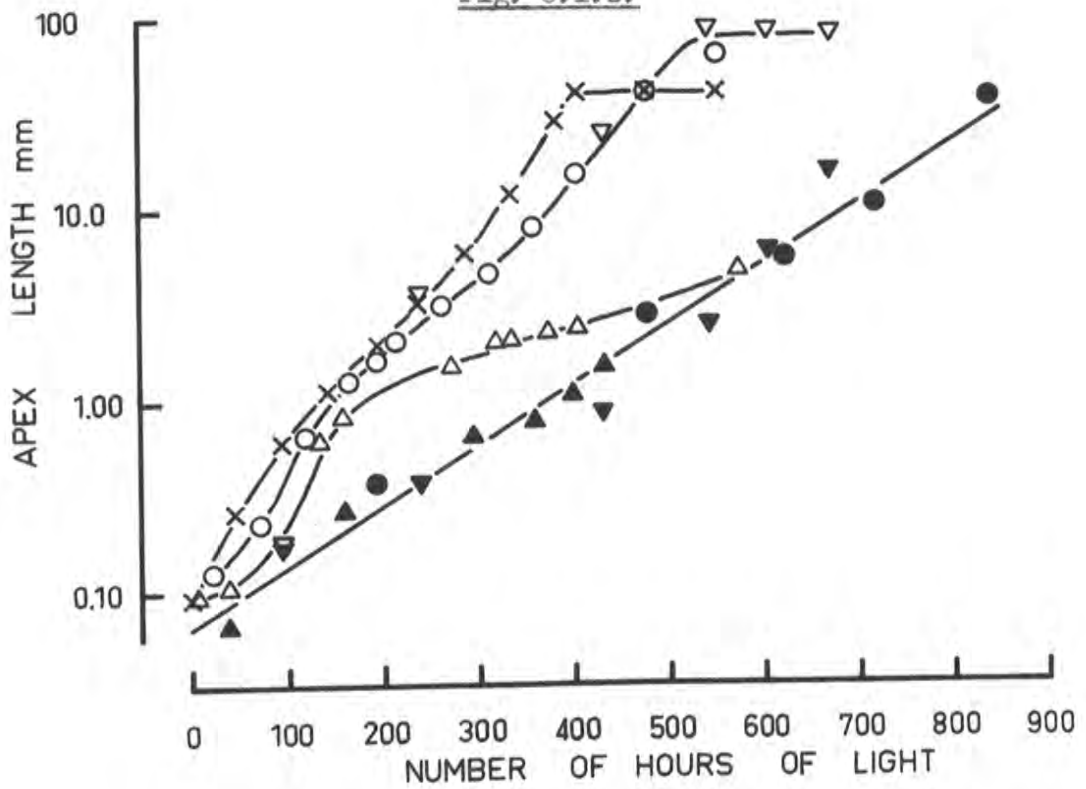
3.2. Daylength - Soil Moisture Stress Experiments.

3.2.0. Introduction.

The methods and experimental design for these experiments and the techniques for scoring the various measures of apical growth were described earlier (sub chapters 2.2 and 2.3). The times from germination to each harvest for each variety (Prior and C.I.5611) in each daylength are presented in table 3.2.0.1. below.

Table 3.2.0.1.

Time in days from germination to harvest.

Variety	Harvest Daylength	0	1	2	3	4	5
Both	24	-	14	26	32	36	41
Both	16	12	21	35	40	44	48
Prior	8	11	26	40	48	53	57
C.I.5611	8	11	26	45	51	56	60

The harvesting was determined by the soil-moisture tension in the treated pots, as Harvest 2 was made when the soil-moisture tension was in the pF range 3.3 to 3.6 and Harvest 3 was made in the pF range 4.0 to 4.3 (5.8 for C.I.5611 in the 8 hour daylength). The short soil moisture stress treatment (W_1) ended at Harvest 2 when water was added and the long soil moisture stress (W_2) at Harvest 3 while in the control (W_0) an adequate supply of soil moisture was maintained throughout the experiment. The mean soil moisture free energy (pF) is given for all combinations of variety, treatment and daylength at each harvest (excepting harvest 0, daylengths 8 and 16 hours) in table 3.2.0.2. below.

3.2.0. (cont.).

Table 3.2.0.2.

Soil Moisture Free Energy (pF).

Variety	Daylength	Harvest Treatment	1	2	3	4	5
Prior	24	W_0	2.2	2.3	2.5	2.6	2.7
		W_1	2.7	3.6	2.4	2.5	2.5
		W_2	2.7	3.6	4.2	2.3	2.4
	16	W_0	2.2	2.4	2.6	2.7	2.8
		W_1	2.9	3.6	2.1	2.2	2.4
		W_2	2.9	3.6	4.2	2.1	2.2
	8	W_0	2.2	2.3	2.5	2.5	2.6
		W_1	2.9	3.5	2.3	2.2	2.4
		W_2	2.9	3.6	4.3	2.2	2.2
C.I.5611	24	W_0	2.2	2.2	2.5	2.5	2.5
		W_1	2.7	3.3	2.4	2.5	2.5
		W_2	2.7	3.4	4.0	2.2	2.4
	16	W_0	2.1	2.4	2.6	2.7	2.7
		W_1	2.9	3.5	2.1	2.2	2.2
		W_2	2.9	3.5	4.2	2.2	2.3
	8	W_0	2.2	2.3	2.4	2.5	2.7
		W_1	2.8	3.3	2.1	2.2	2.3
		W_2	2.8	3.4	3.8	2.0	2.1

The data taken at each harvest were:-

1. Total Primordia Number

2. Number of leaves at each stage of development.

3. Number of Floral Primordia

4. Stage of Development of the most advanced Floral
Primordium.

5. Apex Length

6. Plant Top Fresh and Dry Weight.

7. Plant Root Fresh and Dry Weight.

The total primordia number is recorded as the total number of
lateral appendages on the main shoot axis. The fresh and dry

3.2.0. (cont.).

weights were obtained by usual techniques. The roots were washed as free as possible of adhering soil particles, surface dried with absorbent tissue paper and weighed. Both tops and roots were dried for 72 hours in a forced draught oven at 80°C and reweighed for dry weight determinations.

The results are presented as the response of the following processes to both daylength and soil moisture stress (i) rate of primordia formation, (ii) rate of increase in apex length (iii) rate of inflorescence development and (iv) the rate of appearance of differentiating and expanding leaves.

3.2.1.   Results.

3.2.1.1.   Rate of Primordia Formation.

In figs. 3.2.1., 3.2.2, and 3.2.5 the total numbers of primordia on the main shoots are plotted against time. The harvest programme, which was regulated by the soil moisture levels, was inadequate to present a useful picture of the unexpectedly rapid rates of primordia formation in the Prior plants grown in a 16 hour daylength or a 24 hour daylength and likewise for C.I.5611 in a 24 hour daylength (figs. 3.2.1. and 3.2.2.). The curves here between harvests 1 and 2 (harvests 2 and 3 for the treated C.I.5611 plants in fig. 3.2.1) are deduced from other results contained in the following sub sections. However, the following points are clear, (i) there is a pronounced increase in the rates of primordia formation with increasing daylength and (ii) the reduction in the rate of primordia formation due to the increasing soil moisture tension becomes significantly apparent by the time the pF has

Fig. 3.2.1.

24 HOUR DAYLENGTH
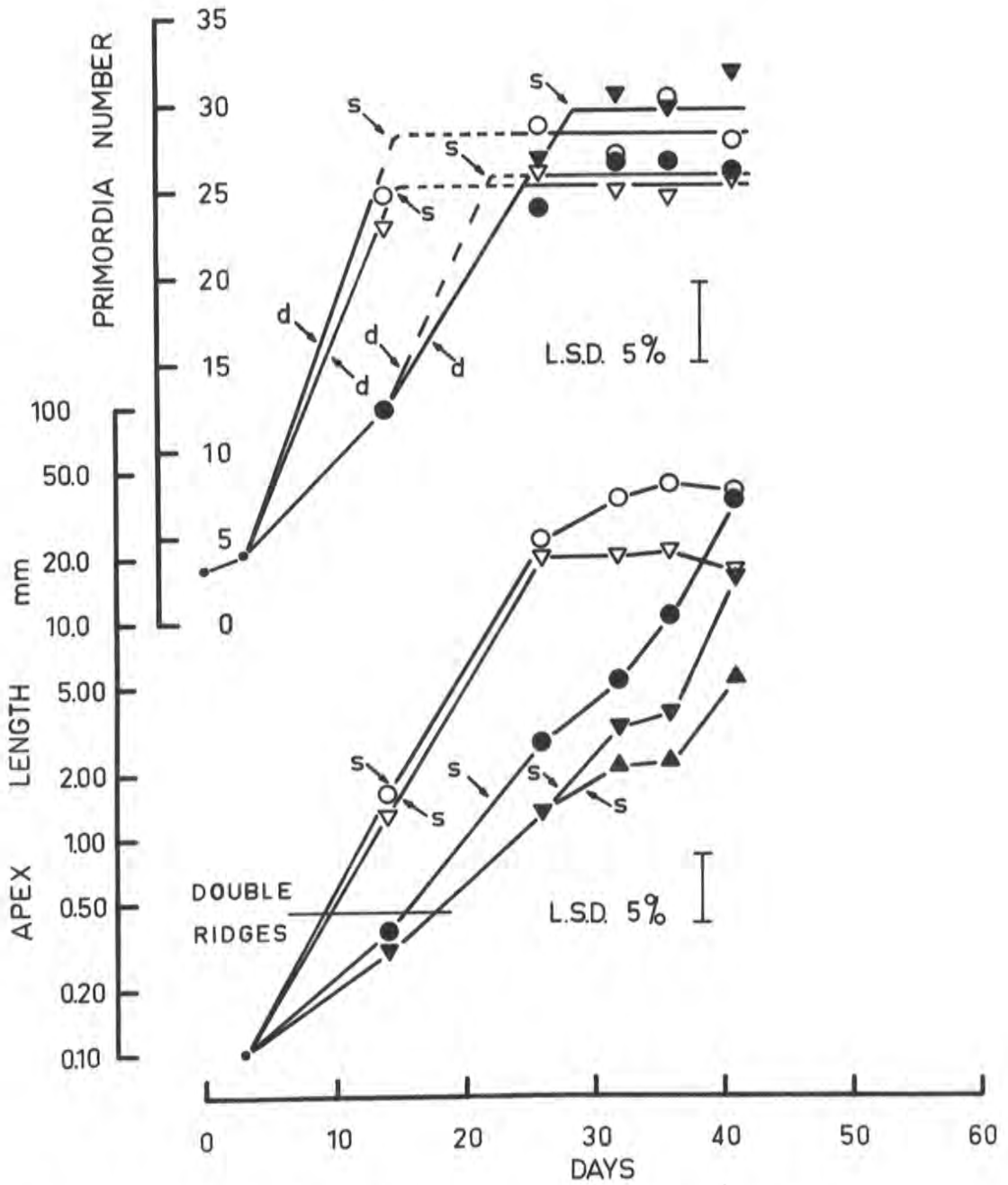
Relationship of Primordia Number and Apex Length with Time.

Prior   Open Symbols.   C.I.5611   Closed Symbols.
Treatment W₀ Circles,   W₁ Inverted Triangles,   W₂ Triangles.

Fig. 3.2.2.

16 HOUR DAYLENGTH

Relationship of Primordia Number and Apex Length with Time.

Prior   Open Symbols.   C.I.5611   Closed Symbols.
Treatment W₀ Circles,   W₁ Inverted Triangles,   W₂ Triangles.

8 HOUR DAYLENGTH

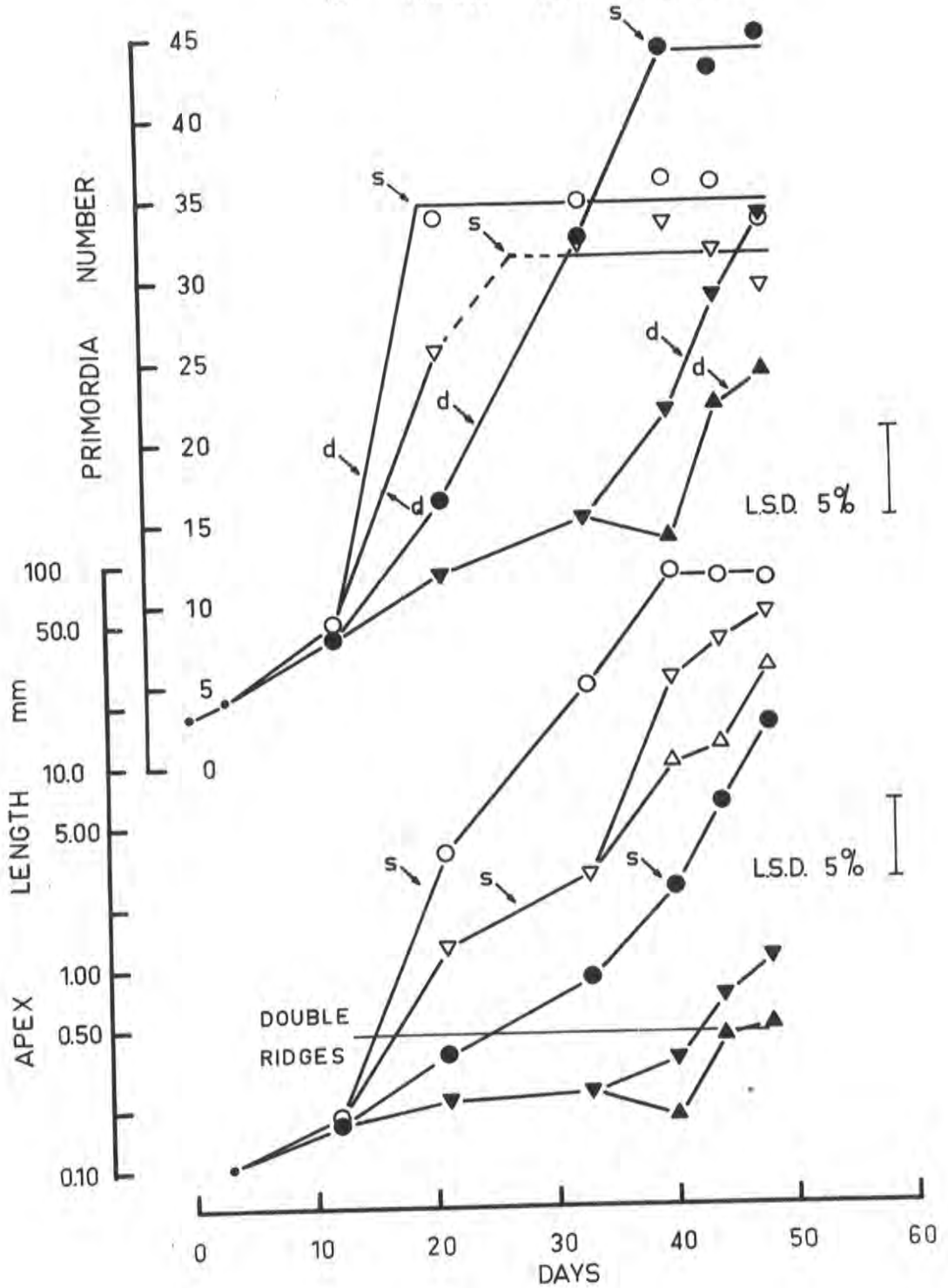Relationship of Primordia Number and Apex Length with Time.

Prior <u>Open Symbols.</u>   C.I.5611 <u>Closed Symbols.</u>
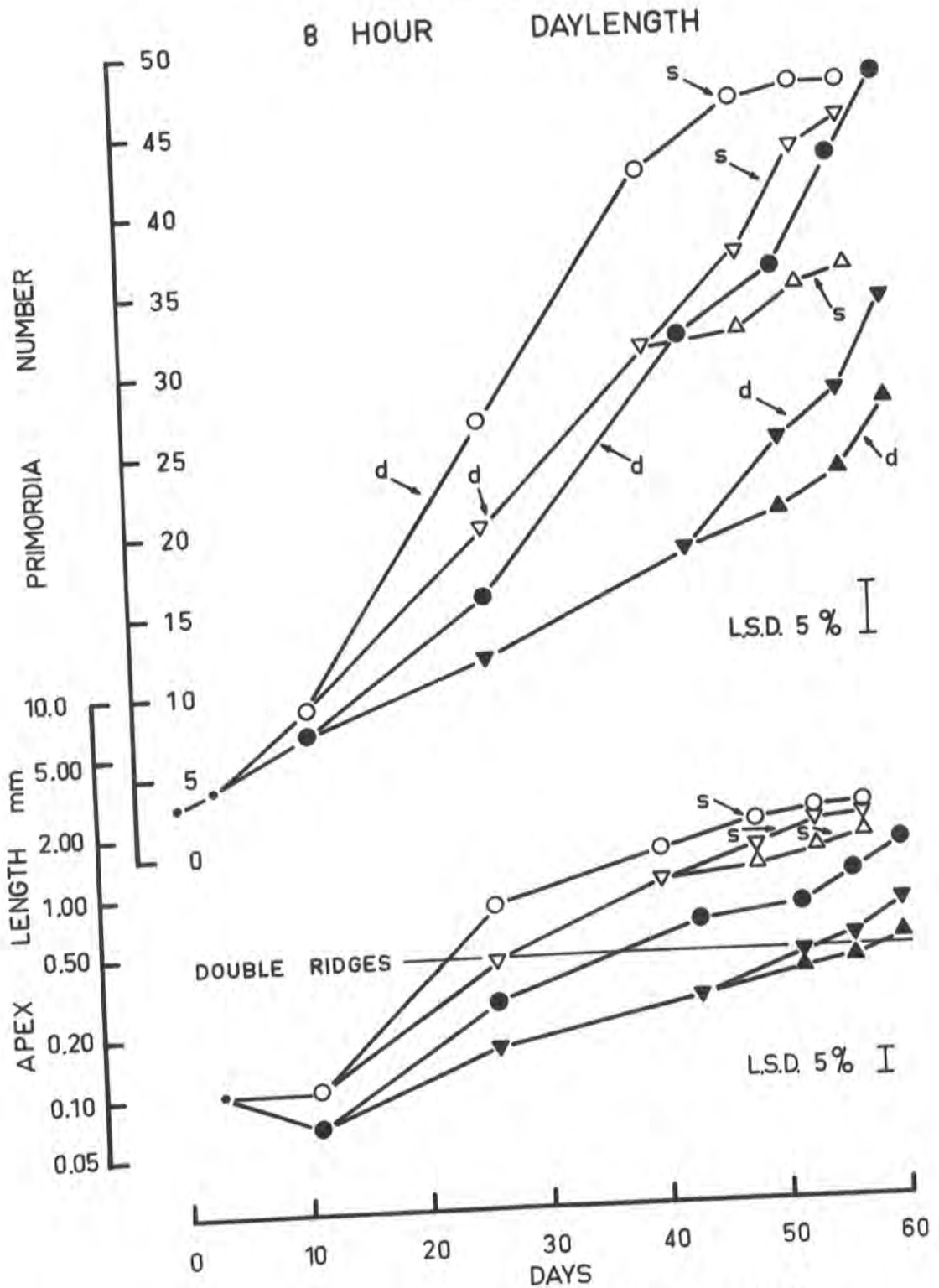Treatment W_0 <u>Circles,</u> W_1 <u>Inverted Triangles,</u> W_2 <u>Triangles.</u>

3.2.1.1. (cont.).

reached 3.0. (Harvest 1 in figs. 3.2.1, 3.2.2, 3.2.3).

In the following table (3.2.1.1.) the mean final primordia

numbers are presented for those treatments in which they could be

obtained.

Table 3.2.1.1.

Mean Final Primordia Number.
(Sample numbers in brackets)

Variety	Daylength	24	16	8
	Treatment			
Prior	W_0	28.42 (12)	34.67 (15)	47.0 (9)
	W_1 & W_2	25.29 (24)	30.20 (24)	-
C.I.5611	W_0	25.83 (12)	43.78 (9)	-
	W_1	29.92 (12)	-	-
	W_2	29.50 (12)	-	-

Prior   24 hour daylength   LSD $(P = 0.001)$ = 1.88
Prior   16 hour daylength   LSD $(P = 0.001)$ = 2.79
C.I.5611   24 hour daylength   LSD $(P = 0.05)$ = 3.71

In Prior the two treatments (W_1 and W_2) have been pooled as

primordia formation had ceased by harvest 2 up to which time these

two treatments were identical. The increased number of primordia

formed in the treated C.I.5611 plants grown in a 24 hour daylength

(Table 5.2.1.1.) appears to have arisen from the combination of a

relatively slight reduction in the rate of primordia formation

compared with a relatively greater increase in the time of

primordia formation for the treated plants compared with the controls

(Fig. 3.2.1.).

3.2.1.2.   Apex Length.

In figs 3.2.1, 5.2.2 and 3.2.3 the apex lengths are plotted

3.2.1.2. (cont.).

against time. Most of the apices of the Prior plants in all soil moisture regimes grown in a 24 hour daylength subsequent to harvest 2 ceased to elongate, turned yellow and died. The cause of the death of these apices is unknown and the only suggestion of a causal agent is that a temporary breakdown in the humidifier unit occurred during day 25 for 10 hours, giving rise to a sudden temporary atmospheric drought. Further experiments (subchapter 3.3) indicated that it was not an effect of daylength. It is apparent from these figures that the effects of the environment on the rate of increase in apex length are similar to those observed for primordia formation (preceding subsection). This suggested a relationship between apex length and the number of primordia on the apex (total primordia number less the total leaf number). In fig. 3.2.4 the mean apex length for all apices with the same number of primordia on the apex is plotted against the number of primordia on the apex for only those apices in the vegetative and spikelet developmental phases. It was found that the apices of both varieties from all soil moisture regimes grown in either 16 hour or 24 hour daylengths had the same relation- ship of apex length to the number of primordia on the apex, and hence the data from these apices could be pooled. A similar finding was found for all the 8 hour daylength apices. The regression lines are tabulated in the appendix (table 5.3.2.1.) and the difference in slopes is significant (P is less than 0.001). The slope represents the interprimordial distance and it appears that this paramenter is controlled solely by daylength.

Fig. 3.2.4.
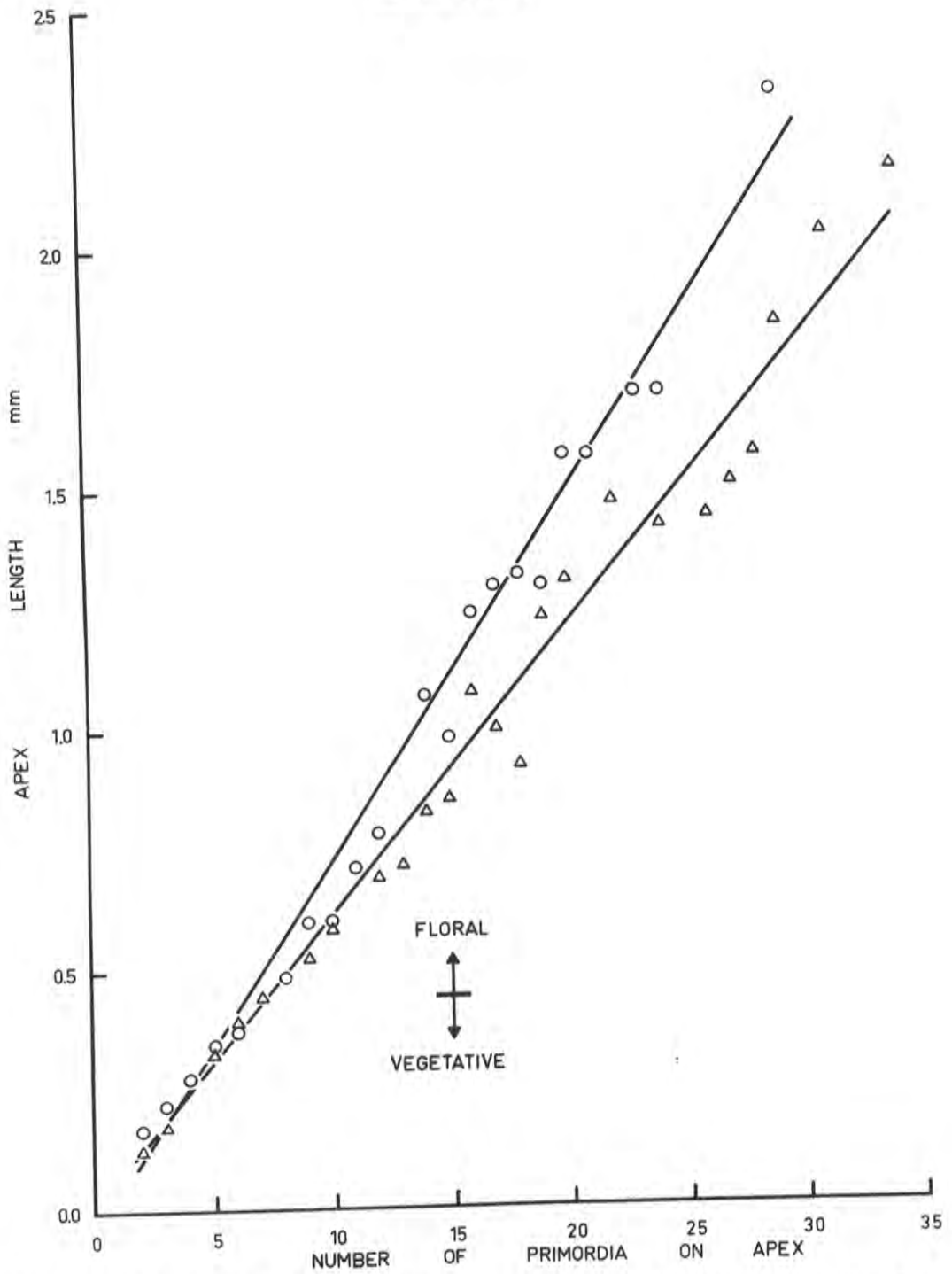
Relationship between Apex Length and Number of Primordia on the Apex.

24 and 16 Hour Daylengths   Circles.   8 Hour Daylength   Triangles.

3.2.1.3.   Inflorescence Development.

The stage of inflorescence development is given in table 3.2.1.2 for all treatments at all harvests. Again the environment has a similar effect on the rate of inflorescence development as that noted for the increase in apex length and the rate of primordia formation. The inflorescence development scale is described in subchapter 2.3, and the important morphological marker points in this scale are the stage of double ridges which has a value of 3 and the stage of stamen initials which has a value of 7.

Double ridges were observed on apices whose lengths were greater than 0.45 mm irrespective of (i) the daylength and (ii) the soil moisture regime. The appearance of stamen initials is correlated with the initiation of internode elongation and the cessation of primordia formation and again this correlation is independent of the environmental history of the plants. It was observed for the Prior plants in treatment W_2 grown in an 8 hour daylength that primordia formation had almost ceased during the harvest interval $2 - 3$ (fig 3.2.3), but that this change was reversible, for on supplying water at harvest 3 the rate of primordia formation was increased and continued to harvest 5. In this treatment insufficient harvests were taken after the appearance of stamen initials to determine whether primordia formation had ceased, but it was observed that the inter-primordial distance of these plants at harvest 5 was significantly greater than those at the preceding harvests, and hence it may be concluded that primordia formation had ceased by this time. A consequence of these apparently invariable correlations is that times of the appearance of double ridges and stamen initials as indicated

3.2.1.3(cont.).

Table 3.2.1.2.

Inflorescence Development.
(Means from samples of 3)

A   24 Hour Daylength.

Variety	Treatment	1	2	3	4	5
Prior	W_0	6.0	>8	>8	>8	>8
	W_1	6.0	>8	>8	>8	>8
	W_2	5.7	>8	>8	>8	>8
C.I.5611	W_0	2.0	7.7	8.3	8.7	>8
	W_1	2.3	4.7	7.7	8.0	>8
	W_2	1.7	6.0	6.7	7.3	8.3

B   16 Hour Daylength.

Variety	Treatment	0	1	2	3	4	5
Prior	W_0	1.0	8.0	>8	>8	>8	>8
	W_1		5.7	7.3	>8	>8	>8
	W_2		5.3	8.0	>8	>8	>8
C.I.5611	W_0	1.0	1.7	5.3	7.0	7.7	8.7
	W_1		1.3	1.3	2.3	3.7	4.3
	W_2		1.3	1.3	1.0	2.3	2.7

C   8 Hour Daylength.

Variety	Treatment	0	1	2	3	4	5
Prior	W_0	1.0	3.0	5.3	7.7	8.0	8.0
	W_1		2.7	5.0	6.0	7.0	8.0
	W_2		3.0	3.0	4.0	6.0	7.0
C.I.5611	W_0	1.0	2.0	3.0	3.0	3.3	4.0
	W_1		1.3	2.0	2.7	3.0	3.0
	W_2		1.7	2.0	2.0	2.0	3.0

5.2.1.3. (cont.).

on figs. 3.2.1., 3.2.2 and 3.2.3, can be calculated. The description

of the method of calculation is given in the appendix sub chapter 5.4.

3.2.1.4.   Leaf Development.

The total numbers of leaves at all stages of development are

given in table 3.2.1.3 and the number of expanding and expanded

leaves in table 3.2.1.4. From these tables the rates of appearance

of differentiating leaves and expanding leaves respectively can be

estimated.

When comparing the data in the control plants for each daylength

in both of these tables for harvest 0 in the 8 and 16 hour daylengths

and harvest 1 in the 24 hour daylength it appears that the rates of

appearance of both the differentiating and expanding leaves are

higher in the 24 hour daylength than in the other daylengths where

the rates are comparable. (The age of the plants at the particular

harvests were 11, 12 and 14 days respectively, table 3.2.0.1). Thus

the data for leaf development in the 16 hour daylength are closer to

the 8 hour daylength data than to the 24 hour daylength data, a

fact which is in contrast to the data in the 16 hour daylength

experiment for (i) rate of primordia formation, (ii) rate of increase

in apex length and (iii) rate of inflorescence development. On

the other hand the effect of soil moisture tension has been to

reduce the rates of appearance of both differentiating leaves and

expanding leaves in those treatments where these processes have

continued in all soil moisture regimes up to harvest 2 (particularly

in C.I.5611). The lowered value for the treated plants is significant

at a soil moisture pF of 3.3 - 3.6.

Table 3.2.1.3.

Total Leaves

Daylength	Variety	Harvest Treatment	0	1	2	3	4	5	LSD (P = 0.05)	Mean Leaf Number	LSD (P = 0.05)
24	Prior	W₀		7.3	7.0	7.0	7.0	7.3	7.13 (15) 0.41	7.13 (15) 6.93 (15)	0.18
		W₁		7.0	7.0	7.0	7.0	6.7			
		W₂		7.0	7.0	7.0	7.0	7.0		7.0	
	C.I.5611	W₀		7.7	8.7	9.7	9.0	10.0	1.23	9.33 (12)	0.62
		W₁		7.7	9.7	9.7	10.3	8.7		9.58 (12)	
		W₂		7.7	9.7	9.0	9.0	9.3		9.25 (12)	
16	Prior	W₀	6.7	7.0	7.3	7.3	7.3	7.7	1.19	7.33 (15)	0.53
		W₁		8.7	9.3	7.7	8.0	7.7		8.27 (15)	
		W₂		8.3	8.3	8.0	8.0	8.0		8.13 (15)	
	C.I.5611	W₀	6.0	11.0	18.3	15.0	14.0	13.0	1.86	14.00 (9)	
		W₁		9.0	11.0	15.3	16.0	16.7			
		W₂		8.3	11.7	11.3	15.3	15.7			
8	Prior	W₀	7.0	13.3	15.7	15.0	14.7	15.3	0.88	15.17 (12)	0.44
		W₁		12.0	15.0	14.7	14.3	15.0		14.75 (12)	
		W₂		13.0	15.0	14.7	14.7	14.3		14.67 (12)	
	C.I.5611	W₀	6.3	11.3	20.0	21.3	21.7	21.7	1.58	21.56 (9)	
		W₁		8.0	14.0	18.3	19.3	20.3			
		W₂		10.0	14.0	15.3	17.3	19.3			

(Values in the body of the table are the means of samples of three)

Table 3.2.1.4.

Expanding and Expanded Leaves

Daylength	Variety	Harvest / Treatment	0	1	2	3	4	5	LSD (P = 0.05)
24	Prior	W_0		6.7	7.0	7.0	7.0	7.3	0.44
		W_1		6.0	7.0	7.0	7.0	6.7	
		W_2		6.0	7.0	7.0	7.0	7.0	
	C.I.5611	W_0		5.0	8.7	9.7	9.0	10.0	1.06
		W_1		5.0	6.7	9.3	10.0	8.7	
		W_2		5.0	7.0	9.0	8.7	9.3	
16	Prior	W_0	4.0	7.0	7.3	7.3	7.3	7.7	1.11
		W_1		6.7	8.7	7.7	8.0	7.7	
		W_2		6.7	8.3	8.0	8.0	8.0	
	C.I.5611	W_0	4.0	6.3	11.3	14.0	13.7	13.0	1.22
		W_1		5.0	6.7	9.3	11.3	12.0	
		W_2		5.3	6.7	7.0	9.3	10.3	
8	Prior	W_0	4.0	8.7	12.0	14.0	13.7	15.0	0.72
		W_1		7.3	10.7	11.7	13.3	14.0	
		W_2		8.0	10.3	11.3	12.0	13.3	
	C.I.5611	W_0	3.7	6.7	11.7	13.3	15.3	16.0	1.06
		W_1		4.7	8.7	10.7	11.7	12.7	
		W_2		6.0	8.7	9.0	10.7	12.0	

(Values in the body of the table are means of samples of three)

3.2.1.4. (cont.).

In the treatments where mean final leaf numbers were obtained in all soil moisture regimes, the effect of the soil moisture deficiency has not been consistent, (Table 3.2.1.5 for Prior in all daylengths and C.I.5611 in the 24 hour daylength). There are no signifi cant differences in the 24 hour daylength, since the final leaf number was, most probably, established before the increasing soil moisture tension affected the rates of appearance of the differentiating leaves. In the 16 hour daylength experiment there is a significant increase in the final leaf number for the treated plants (treatments W_1 and W_2) in Prior compared with that of the controls and in the 8 hour daylength experiment a decrease, which borders on the 5% level of significance is observed. Observations in other experiments, not recorded here, have shown a similar decrease for soil moisture stressed plants grown in short days.

3.3. Continuous Light Experiments.

3.3.0. Introduction.

In previous experiments the same pattern of development was observed in both long and short days. The prime effect of increasing the daylength was to increase the rates of the component processes to such a degree that the harvesting programme did not permit accurate estimations of these rates. Thus the following experiments were devised to determine more accurately in long day conditions:-
(i) the size of the spike at floral initiation, (ii) the stage of development at the cessation of primordia formation, (iii) the effect of altering the spectral balance of red/far red wavelengths on the rates of primordia formation and spikelet development, and (iv) the rates of development of the individual primordia in relation to their position on the spike.

3.3.1. Methods.

Two hundred germinated seeds were sown into one hundred 4 inch plastic pots and the pots were arranged into replicates of ten pots each, randomised and rotated as in the previous experiments. Harvests were made from 9 pots consecutively in each replicate, a single plant from each pot being taken. When the ninth harvest was complete the other plant in each pot was taken, again consecutively, from pot 9 to pot 1. Both plants from the tenth pot were included in the eighteenth harvest also, as a check on the influence of competition for nutrients between the two plants in each pot on their development. No differences between one plant per pot and two plants per pot at the final harvest were observed in the first experiment and so in the

3.5.1. (cont.).

second experiment twenty single plant harvests were made instead of eighteen.

In the first experiment continuous high intensity fluorescent lighting at 2,500 ft. candles was obtained from a bank of thirty TLF 80W/35 Philips fluorescent tubes, and in the second experiment, in which incandescent lighting was added to the fluorescent, twenty-two TLF 80W/35 Philips tubes were used, interspersed with four 150 watt incandescent strip lamps. The total light intensity in the se second experiment was 2,570 ft. candles of which 120 came from the incandescent lights.

Each plant was scored for:-

(i) Total number of primordia. This number was subdivided
   into leaves and number of primordia on the spike.
(ii) Apex Length.
(iii) Stage of Development of each primordium.
(iv) Stage of development of each leaf.

3.5.2.   Results.

3.5.2.1.   Primordia Formation.

The rate of primordia formation was slow for the first six to seven days after which there was a very marked increase in the rate from 0.5 to approximately 3.5 primordia per day (fig. 3.5.1). In the fluorescent plus incandescent lighting this change occurred between the sixth and the seventh days, whilst in the fluorescent lighting the change occurred after the seventh day. The cessation of primordia formation was extremely sudden both light regimes and it occurred $1\frac{1}{2}$ days earlier in fluorescent plus incandescent lighting

Fig. 3.3.1.
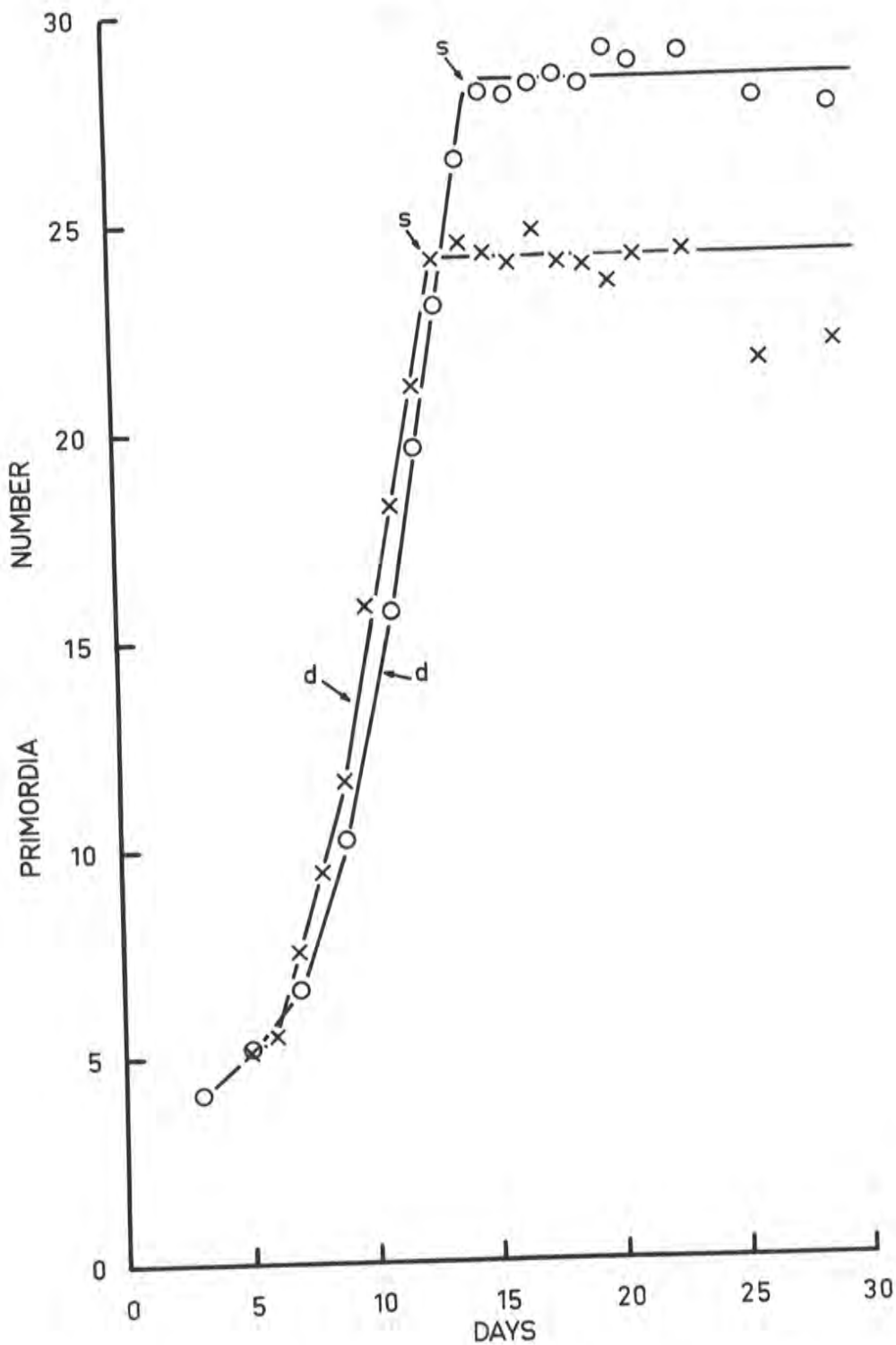
Primordia - Time Curves for Continuous Fluorescent Light (O)
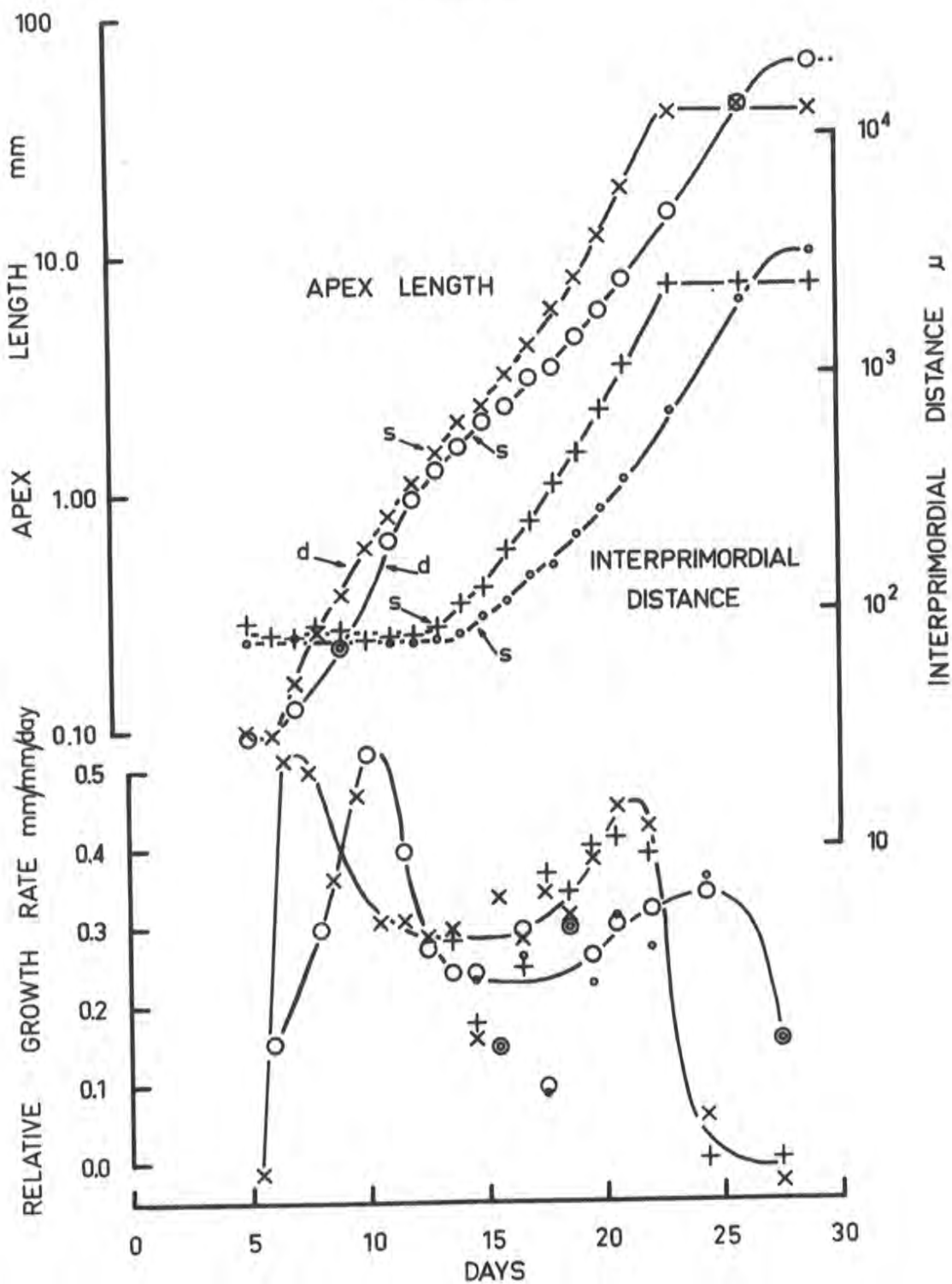and Continuous Fluorescent + Incandescent Light (X).

3.3.2.1. (cont.).

than in fluorescent alone.

3.3.2.2.   Apex Length.

The increase in apex length with time is shown in Fig. 3.3.2. The best description of the curves shown is that they are the summation of two overlapping sigmoid curves, as the relative growth rate curve for the apex length shows two peaks. Statistical analysis of the data (appendix table 5.3.3.2) indicated a highly significant scatter of means about a straight line, although the regression coefficient was itself highly significant (appendix table 5.3.3.1.). It is possible, therefore, if only a few samples are taken during the growth of the apex, to obtain a straight line relationship between the length of the apex and time. A double sigmoid curve is compatible with the hypothesis of two successive phases of growth in the development of the rachis. Two such phases of growth exist (Fig. 3.3.2). In the first the number of primordia in the apex is closely correlated with the length of the apex; that is, the increase in apex length is soley due to the net addition of primordia to the spike from the apical meristem (appendix table 5.3.3.3 cf. fig.3.2.4 and appendix table 5.3.2.1). In the second/increase in the apex length is due solely to the increase in length of the internodes, as the relative growth rate of the internode length is very closely correlated with the increase in apex length (Fig. 3.3.2). The change-over from the first to the second phase of growth is marked by the appearance of stamen initials, with which is correlated the cessation of primordia formation and the initiation of stem internode elongation.

Apex Length and Interprimordial Distance - Time Curves and
Corresponding Relative Growth Rate Curves for Continuous
Fluorescent Light (O) or (o) and Continuous Fluorescent +
Incandescent Light (X) or (+).

3.3.2.3.   Spikelet and Inflorescence Development.

In these experiments all the spikelets on the apex were scored for their stage of development, the values assigned to each stage were those tabulated in section 2.3.2. This scoring system differs from the inflorescence development scale used in the previous experiments(sub chapter 3.2), between double ridges and stamen initials, in that an extra stage, advanced double ridges, is inserted between double ridges and triple mounds (appearance of lateral spikelet primordia).

The rate of spikelet development from double ridges to stamen initials as stages/day for the individual spikelets is shown in fig. 3.3.4. There is a significant increase in the rate for plants grown in fluorescent plus incandescent light compared with those grown in fluorescent alone. It should also be noted that the rate of development of spikelets becomes progressively slower towards both the apical and basal ends of the inflorescence, the collar spikelet being the slowest.

The mean spikelet score for selected spikelets and the inflorescence status for a series of harvests is presented in table 3.3.2.1. It is to be observed that the spikelet at node 8 remains as a single ridge until day 13 in the continuous light experiment by which time the flag leaf (appendage at node 7) had reached stage II (fig. 3.3.5) in its development. In contrast in the incandescent supplement experiment spikelet development takes place before the leaf at node 7 reaches stage II. It is also to be noted that in both experiments the considerable number of days during which primordium 8

5.3.2.(cont.).

Table 5.3.2.1.

Progress of Individual Spikelets and the Inflorescence towards Stamen Initials.

A   Continuous Fluorescent Light.

Age Days	Node Number of Spikelet				Inflorescence Status
	8	10	15	20	
9	1.0	0.9	—	—	2.0
11	1.0	2.7	1.1	—	3.0
12	1.0	3.5	3.0	0.6	3.7
13	1.0	5.0	4.2	2.2	5.2
14	2.9	6.2	5.5	4.0	6.2
15	5.0	x	6.8	5.7	7.0
16	5.3	x	x	6.1	7.5
19	6.5	x	x	x	8
Rate of Development towards stamen initials (a) from first appearance	0.5	7.06	1.33	1.37	
(b) from double ridges	0.7	1.17	1.27	1.30	

B   Continuous Fluorescent + Incandescent Light.

Age Days	Node Number of Spikelet				Inflorescence Status
	8	10	15	20	
8	1.0	0.7	—	—	1.7
9	1.0	1.1	—	—	2.2
10	1.0	2.0	1.0	—	3.0
11	1.6	3.5	2.1	0.1	3.4
12	3.5	5.0	3.7	1.6	5.2
13	5.1	6.5	5.6	3.7	6.5
14	6.0	x	6.4	5.4	7.1
15	6.8	x	x	6.1	7.9
Rate of Development towards stamen initials (a) from first appearance	0.85	1.06	1.55	1.50	
(b) from double ridges	1.10	1.50	1.43	1.50	

x = Stamen initials on all spikelets at that node on the inflorescences measured.

Fig. 3.3.3. Appearance of Stage I and Stage II Leaves

with Time for Continuous Fluorescent Light (O)

and Continuous Fluorescent + Incandescent Light (X).

Fig. 3.3.4. Rate of Development of Individual Leaves and

Spikelets for Continuous Fluorescent Light (O)

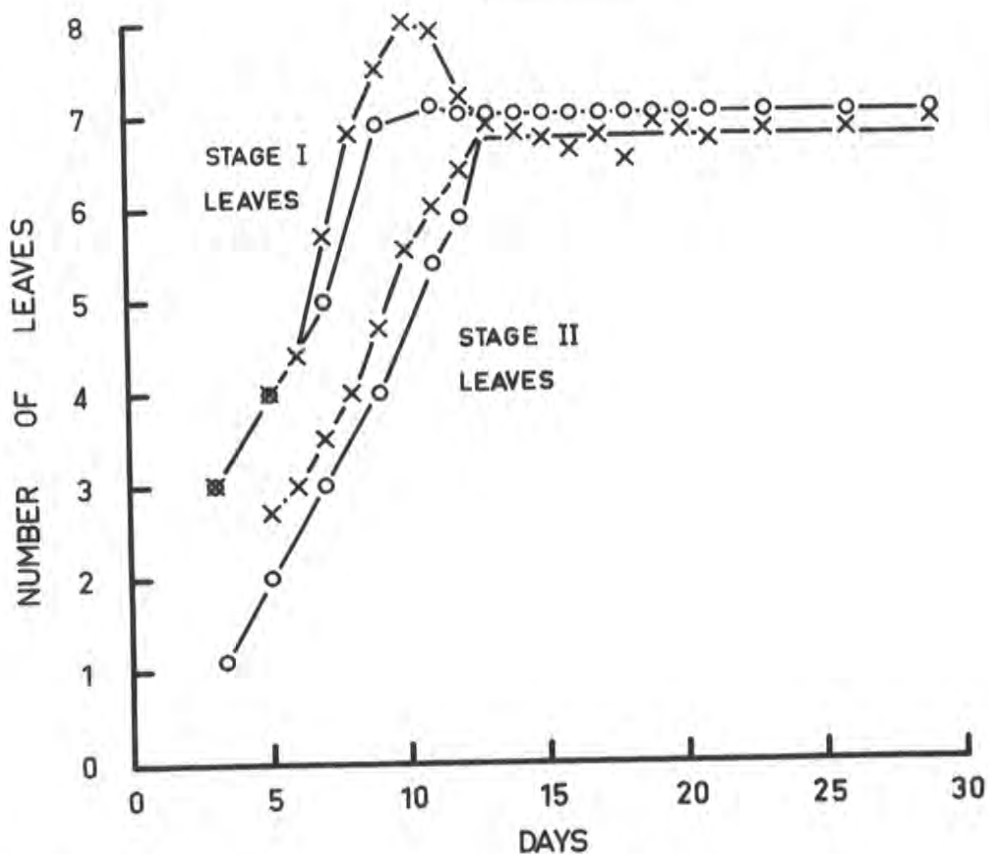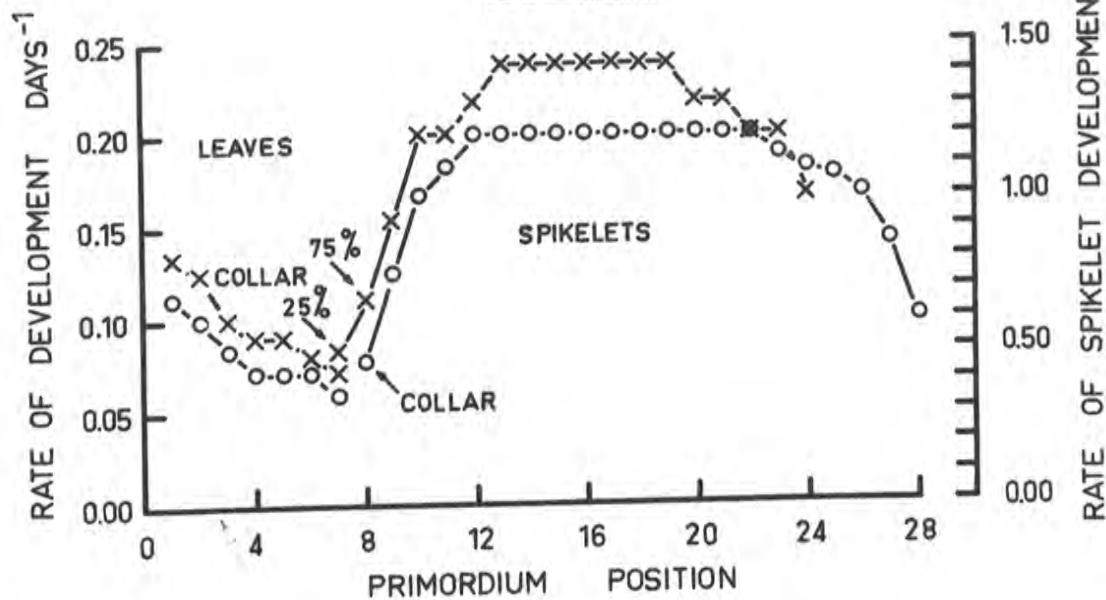and Continuous Fluorescent + Incandescent Light (X).

Fig. 3.3.3.

Fig. 3.3.4.

5.3.2.5.(cont.).

remains as a simple ridge (stage 1) is in contrast to the later
formed spikelets. This early slow development, which is apparent
to a lesser degree in primordium 10, is shown by the lower rate
of development towards stamen initials calculated from first
appearance as a simple ridge compared with that calculated from the
double ridge stage (Table 5.3.2.1.).

5.3.2.4. Leaf Development.

In Fig. 5.3.5. the total number of leaves from all stages of
development and the number of leaves from stage II onwards are shown.
It is apparent that for fluorescent lighting, the chosen criteria
(Section 2.3.2) were adequate for determining whether a primordium
would be a leaf or otherwise. However, the shape of the curve
indicated that there is an inadequacy in the criteria for fluorescent
plus incandescent lighting. Those primordia which at one time were
scored as stage I leaves were scored at a later date as floral
primordia, hence the fall in the curve. Although the lower ridge
had started to grow to form a cup shaped stage I leaf at the time of
scoring its further development was halted and the upper ridge, which
was hidden by the growth of the lower ridge, later became visible
as an incipient triple mound (stage 3I) floral primordium.

The difference (0.25) in final leaf numbers between the
continuous fluorescent light and the continuous fluorescent plus
incandescent light in fig. 5.3.5. is highly significant ($t = 6.83$ at
10 df. P 0.001).

In fig 5.3.4 the rate of leaf development towards the fully

- 59 -

5.5.2.4. (cont.).

expanded leaf (days^{-1} from appearance as stage 1 to fully expanded)
for each leaf is plotted against leaf position. It is to be observed
that the rate of development progressively decreases from the first
leaf to the flag leaf. A similar finding for wheat leaves was
observed by Williams (1960).

5.4. Cell Division and Cell Elongation in the Inflorescence Axis.

5.4.0. Introduction.

As mentioned before the shape of the apex length curve against time fig. 5.5.2.) suggests two phases of growth. The growth of the apex in the first phase was attributed to the addition of new primordia, and in the second to the extension of the internodes. The apices from the continuous fluorescent light experiment were prepared for examination by the histological techniques described previously (sub chapter 2.4).

The following measurements were made:-

(i) the distance between the base of insertion of two consecutive primordia on the same side of the shoot axis; this measurement is defined as the segment length and it comprises two nodes plus two internodes of the axis. The segment is designated by the number of the primordium whose insertion is entirely contained within the segment (Fig. 5.4.4.).

(ii) the number of cells (Cell Number) in the central region of the pith (corpus) along the shoot axis within the segment.

These measurements were made to provide more detail about the nature of the increase in length of the shoot axis, focusing attention on the events surrounding the change-over from one phase to the second.

5.4.1. Results.

Apices from three of the ten replicates of the experiment were sectioned. It was observed that the chosen apices from some of the harvests differed widely from the mean developmental stage and hence
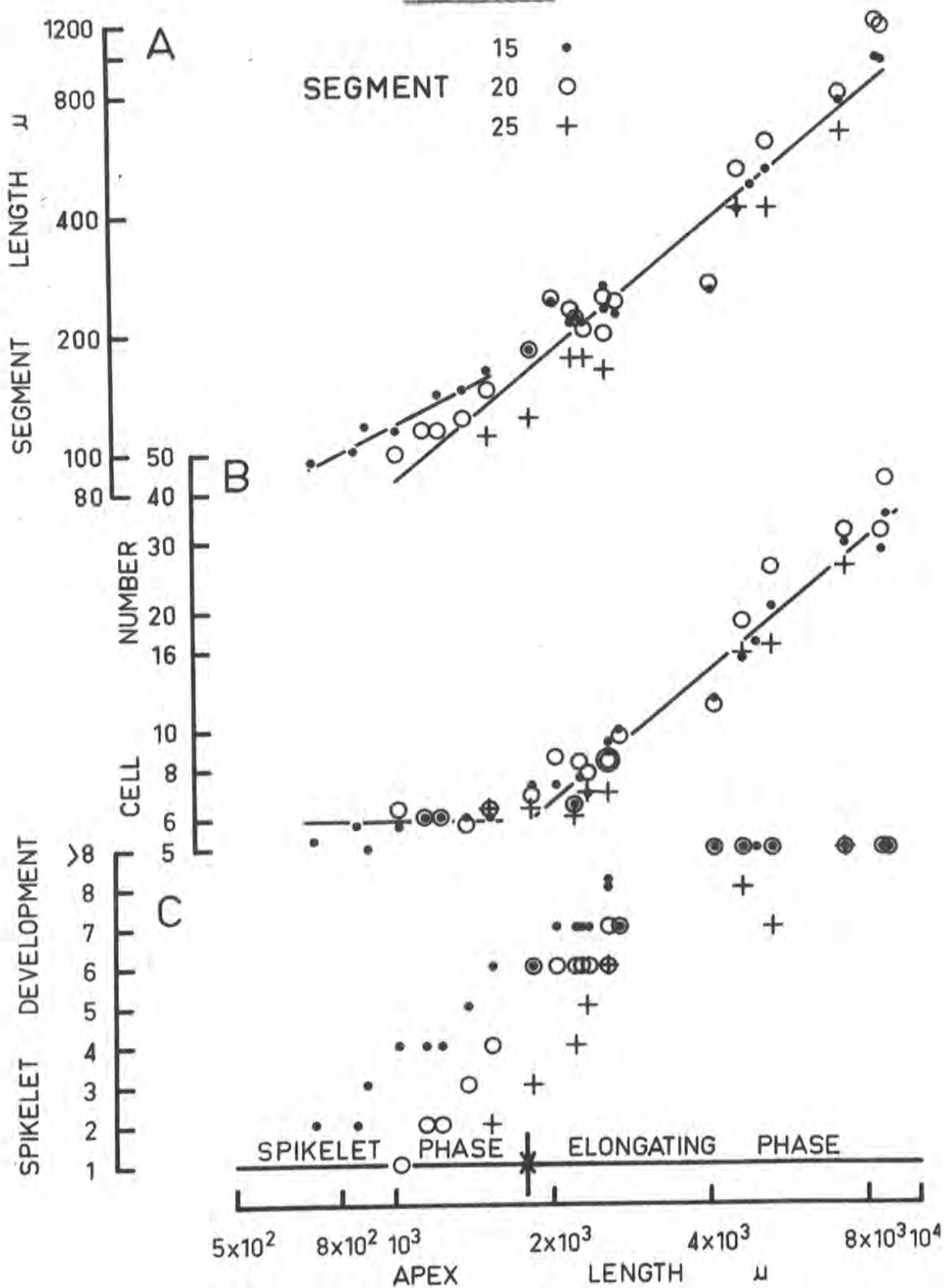
5.4.1. (cont.).

to remove this variability associated with chronological time, the
material was analysed using the apex length, a measure of physiological
time, for reference. The end of the spikelet phase occurred at the
mean apex length of 1,780 μ when stamen initials appeared on primordia
11 and 12 (fig. 3.3.2). At this stage, segments 10, 15 and 20
(Figs. 3.41. and 3.4.2.) are approximately 160 μ long and as they
comprise two potential nodes plus internodes, this value is in very
close agreement with the observed 80 μ for the inter-primordial
distance (Fig. 3.3.2 and appendix table 5.3.3.3). The regression
lines of segment length against apex length (appendix table 5.3.4.1)
for the individual phases of growth within each segment were tested
for heterogeneity and in the spikelet phase the slopes for segments
10, 15 and 20 were significantly different. The lines for segments
15, 20 and 25 in the elongating phase were found to be not significantly
different at the 5% level and the data were pooled, to determine a
common line. It is difficult to find an explanation for the difference
in slopes between segment 10 and the others in the elongating phase.
The spikelet development scale does not cover development in the
elongating phase, however, spikelet 10 was observed to be the most
proximal spikelet to anthese on the spike. If the usual time sequence
of anthesis on a barley spike was followed, it would have been one
of the last spikelets to reach anthesis. Thus it is inferred that
its general rate of development is slower and this is correlated with
the slower rate of increase in segment length.

The cell number per segment along the segment axis is constant
during the spikelet phase, thus the increase in segment length during
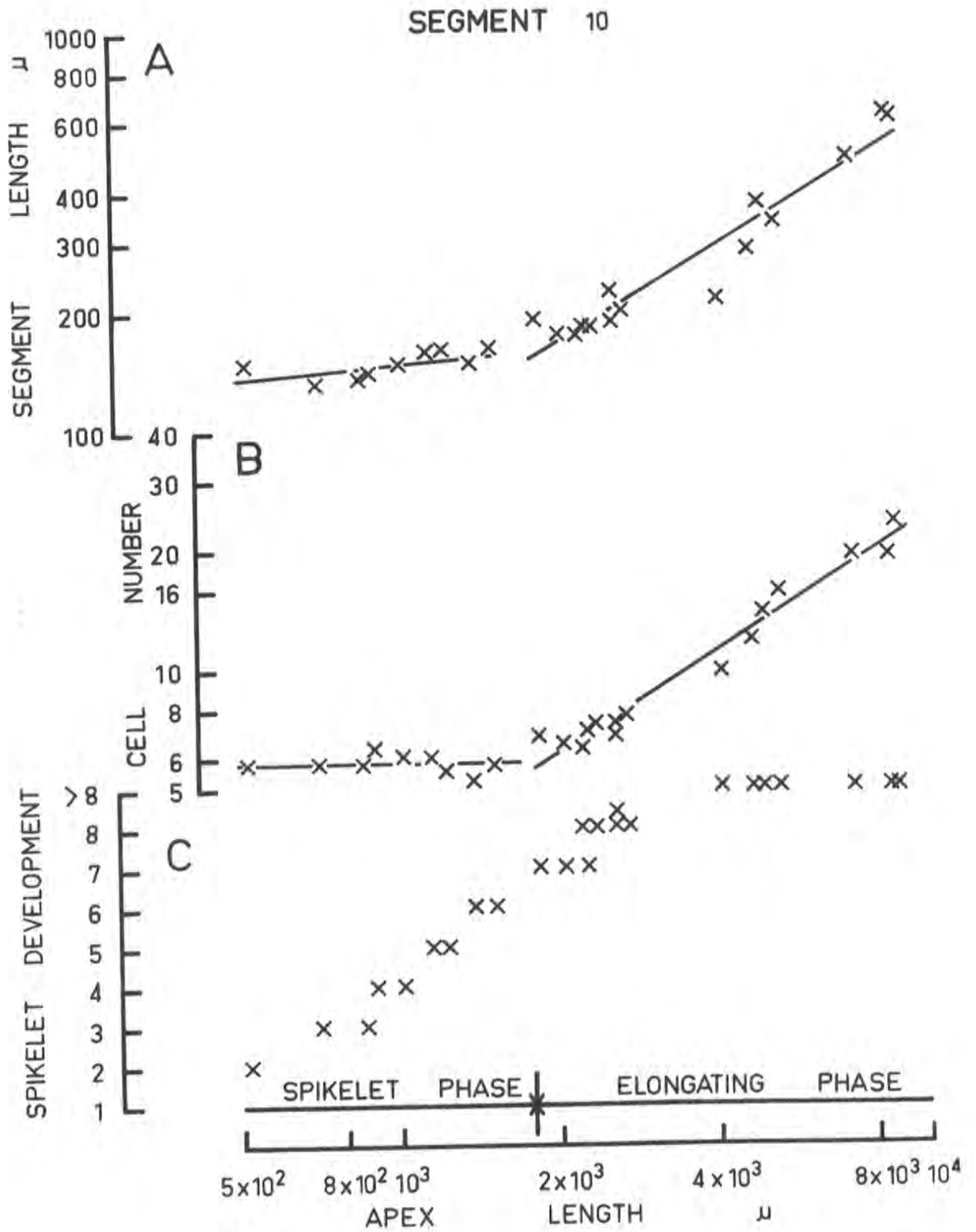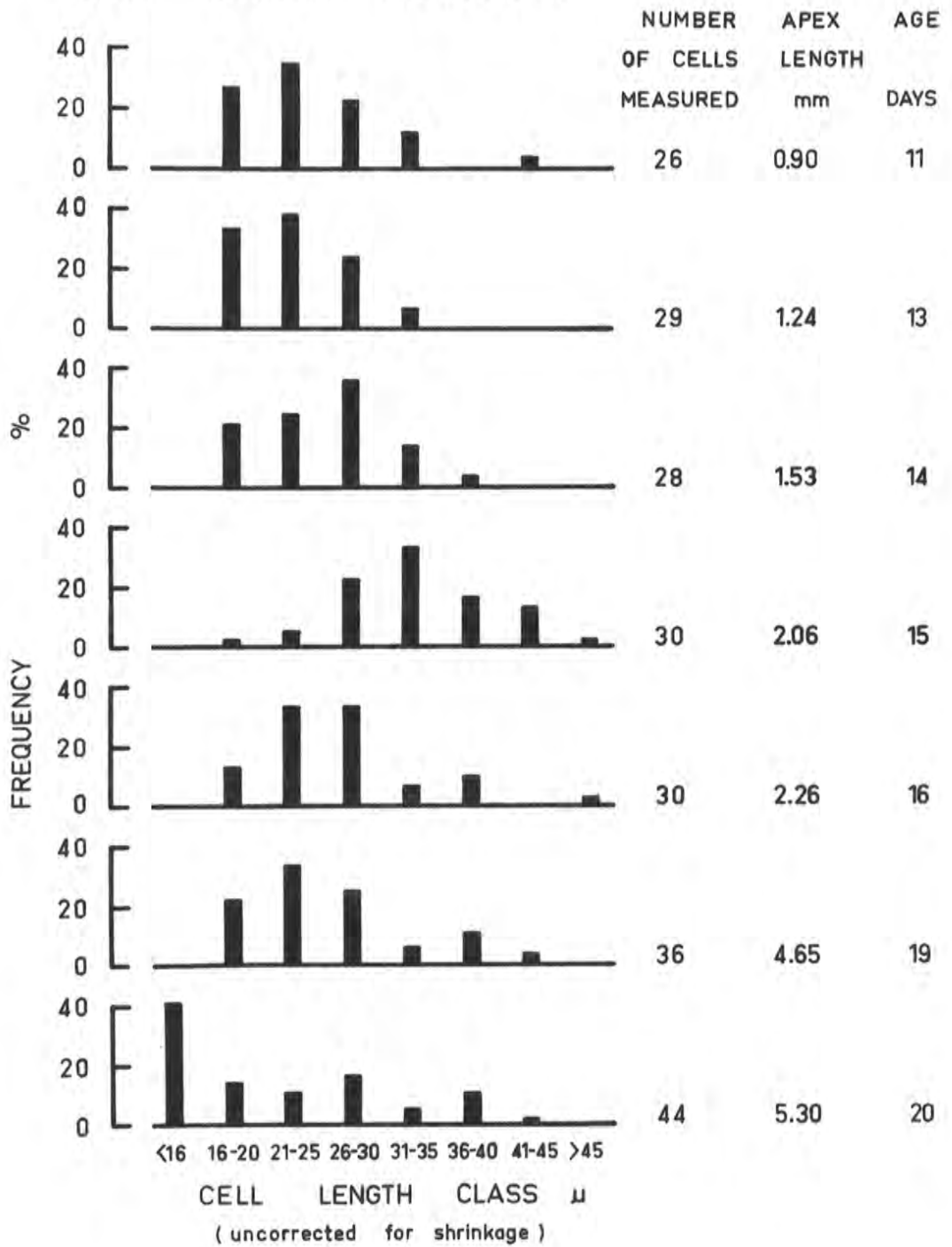
Relationship of Segment Length, Cell Number,
and Spikelet Development with Apex Length.

Fig. 3.4.2.

Relationship of Segment Length, Cell Number,
and Spikelet Development with Apex Length.

CELL LENGTH HISTOGRAM

3.4.1. (cont.).

the spikelet phase is due to cell expansion. Fig. 3.4.5 presents
direct evidence on this point and also indicates that the mean cell
length reaches a maximum shortly after the end of the spikelet phase
(Fig. 3.4.5 and Fig. 3.4.5 15 day old apex) although cell division
has just been initiated. Median sections of 11, 15 and 20 day old
apices are shown in figs. 3.4.4, 3.4.5 and 3.4.6 and the cell length
data presented in fig. 3.4.5 for 11, 15 and 20 day old apices were
obtained from these sections. The last apices between days 16 and
20 on fig. 3.4.5 show a small persistent group of large cells (greater
than 50 μ long), 6 - 8 in number which are found associated with the
nodes (cf. fig. 3.4.6). The large group of very small cells ($\leqslant 15 μ$)
in the 20 day old apex were all in the lower internode of the segment
(fig. 3.4.6).

In the elongating phase, the slopes of the cell number lines and
the segment length lines are almost identical (figs 3.4.1, 3.4.2 and
appendix table 5.3.4.1.). This indicates that the mean cell length
is constant . It is noted that the majority of the cells in the
internodes are approximately of the same length (fig 3.4.3 16 and
19 day old apices). In the 20 day old apex (fig. 3.4.5 and 3.4.6)
the large group of small cells, already mentioned above, are
approximately half the length and twice the number of the corres-
ponding group of cells in the internode immediately above. Such
features are found in groups of cells which are dividing synchronously
and synchronous cell division was found by Sachs and Lang (1961) to
occur in GA$_5$ stimulated stem elongation in Samolus parviflorus, a
dicotyledonous rosette plant.

Fig. 3.4.4.

Youngest
Primordium (19)
Simple Ridge

Segment 15
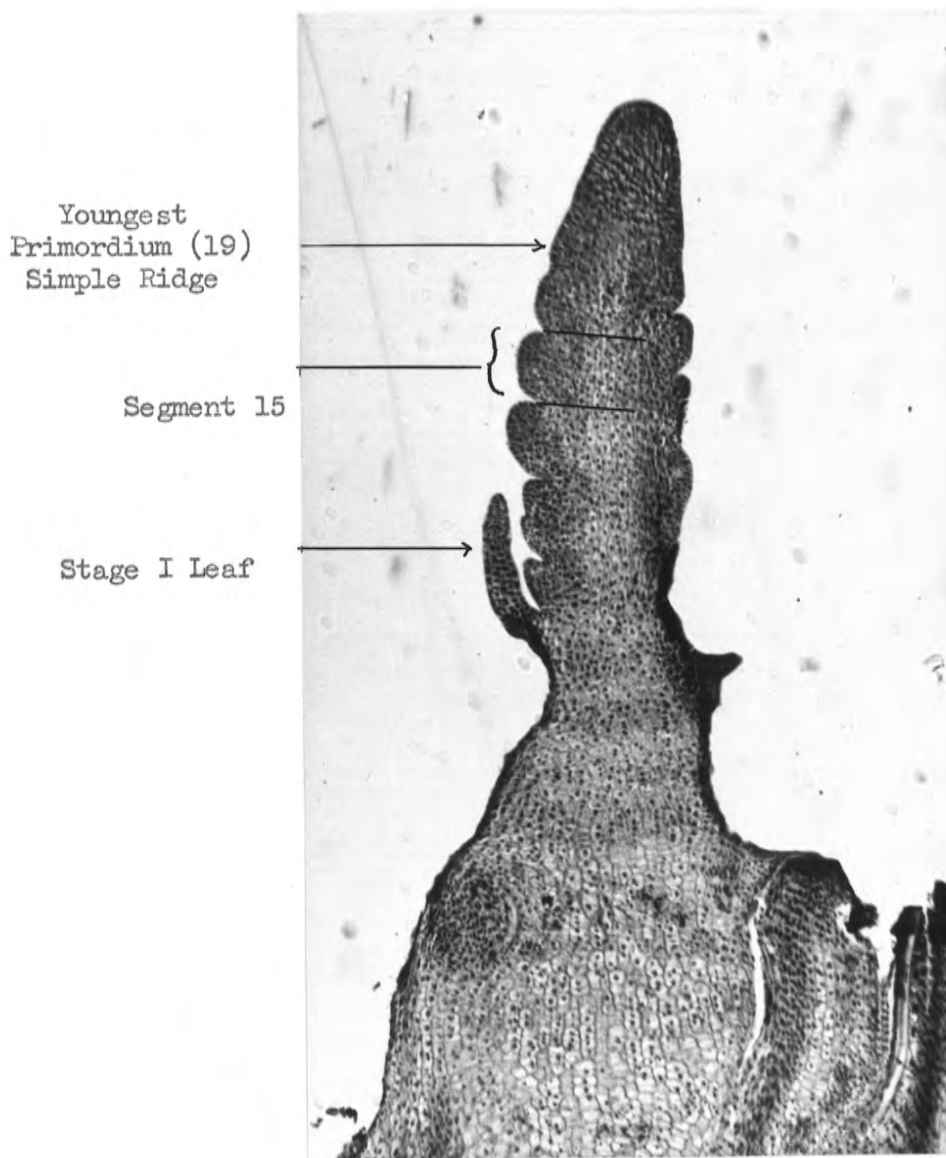
Stage I Leaf

A Median Longitudinal Section of 11 day old Apex.
Length (0.90 mm) from immediately above
Stage I Leaf to top of Dome.

Fig. 3.4.5.

Pith Region

Provascular
Trace

Peduncle
Intercalary
Meristem
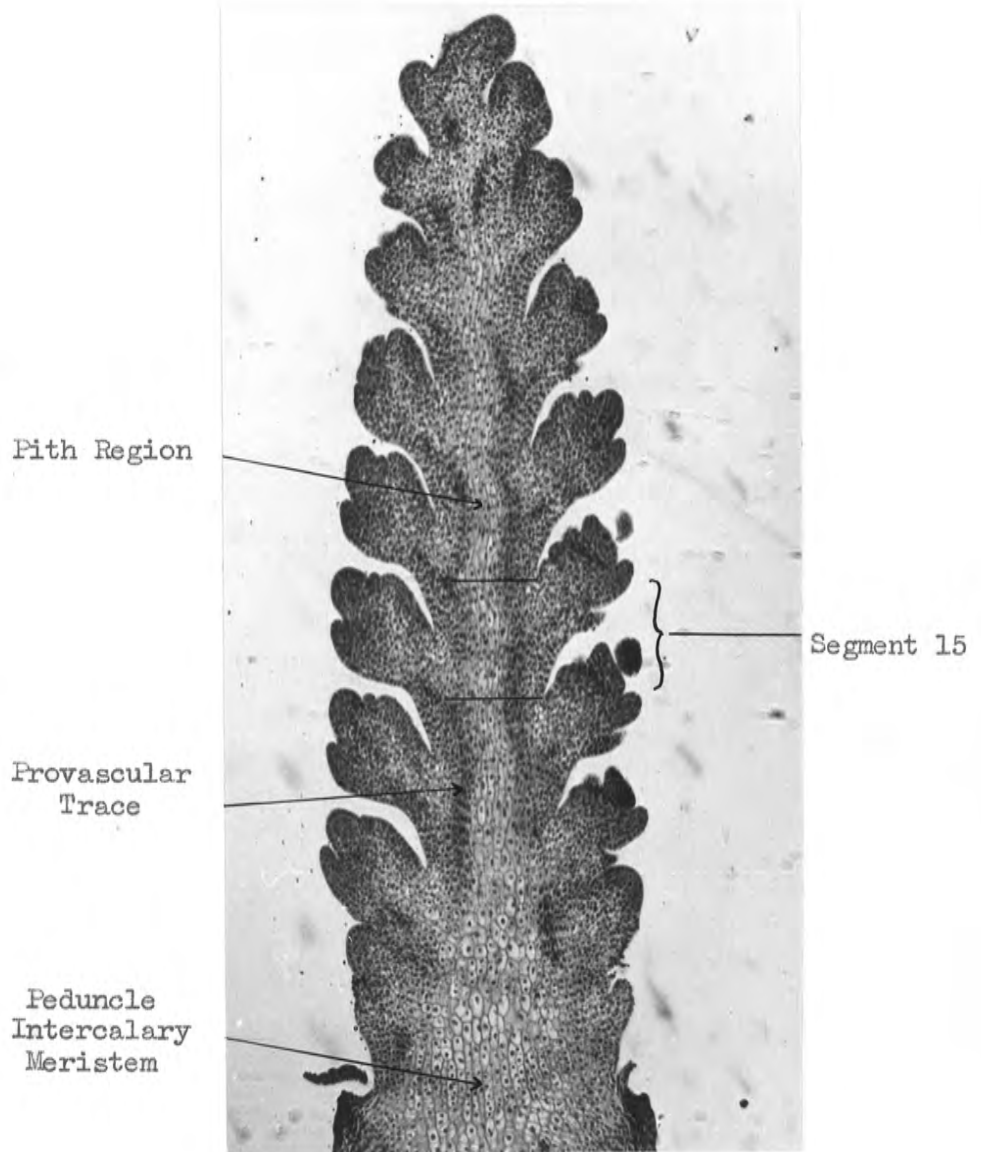
Segment 15

A Median Longitudinal Section of 15 day old Apex.
Length 2.06 mm.

Fig. 3.4.6.
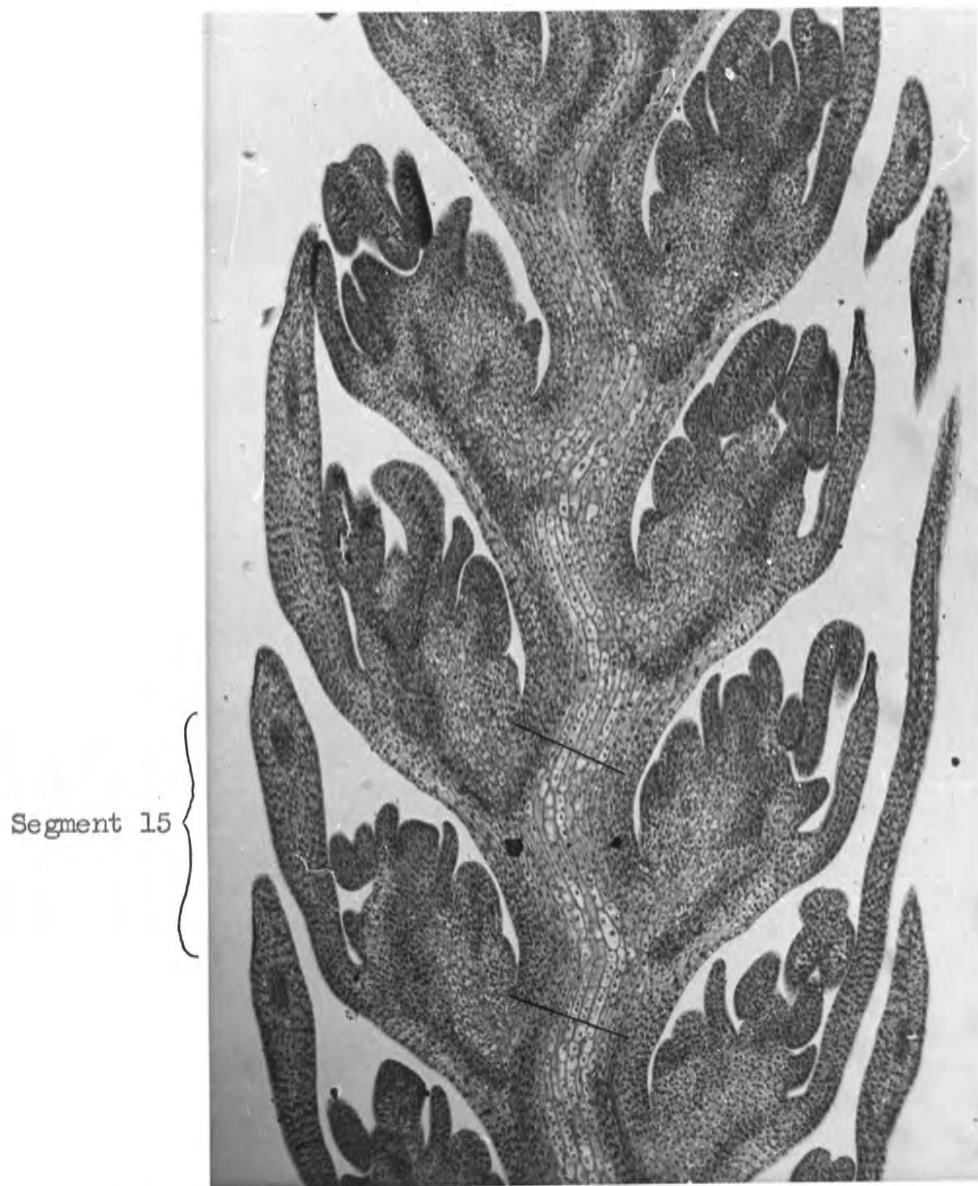
Segment 15

A Median Longitudinal Section of a 20 day old Apex.
Length 5.30 mm, Segment Length 513 µ.

5.4.1. (cont.).

It is also observed in the sections that no pro-vascular strands were differentiated in the inflorescence until the elongating phase was initiated (cf. figs. 3.4.5 and 3.4.6); xylem vessels are visible in apices greater than 5,000 u in length (fig. 3.4.6).

It may appear from fig 3.4.2 that the rates of spikelet development for spikelets 15 and 20 are different whereas previously (Table 3.3.2.1.) they appear to be the same. This apparent discrepancy arises from the method of presentation of the data in terms of log apex length instead of chronological time. Similarly the difference between the slopes of the segment length-apex length curves for segments 15 and 20 may also have arisen from the method of presentation of the data as there is a highly significant correlation between segment length and stage of spikelet development for all segments $(r = 0.804$, at 22 df P 0.001).

The sequence of events may be summarised thus:- (i) after the formation of the segment, cell extension takes place at a rate which is correlated with the rate of spikelet development. (ii) Cell extension continues after the end of the spikelet phase, hence the increase in length of the apex immediately following the cessation of primordia formation in long days is largely correlated with the continued cell extension in the segments, (iii) cell division is initiated in the rachis (and pro-vascular strands are differentiated) at the at the beginning of the elongating phase in long days. Active cell division may still continue in the apical meristem for an undetermined period after the end of the spikelet phase and in short days 3 to 4 more primordia may be formed at a greatly reduced rate.

3.5.   Activities of Gibberellin - like Substances in Barley Apices.

3.5.0.   Introduction.

Earlier (section 1.2.3.) passing reference was made to recent reports on the influence of GA_3 on the growth of cereals. Other work suggests that changes in both quality and quantity of natural gibberellins accompany the initiation of flowering in long days and cold requring plants eg. rudbeckia chrysanthemum and henbane (Harada and Nitsch 1959)(Lang 1960). More recently these changes have been considered to be correlated with stem elongation rather than flower initiation (Reinhard and Lang 1961).

Applied GA_3 stimulates stem elongation in some rosette species (eg. Samolus parviflorus and Hyoscyamus niger) and the initial increase in length is correlated with an increased number of cells along the shoot axis (Sachs et. al. 1959). A similar correlation between the initial increase in internode length and cell number is presented in the previous section of results (subchapter 3.4).

In the light of the above findings the following experiments were made to obtain data on the activities of gibberellin-like substances in barley apices. Plants were grown under either continuous fluorescent light or an eight hour daylength fluorescent light. Harvests were made at the following stages of development:-
(i) vegetative, (ii) double ridges, (iii) stamen initials and (iv) anther lobes to awns elongating (cf. 2.3.2). These stages were designated A,B,C and D respectively. Each assay is designated by a number-letter code, where the number corresponds to the daylength and the letter to the stage of development as mentioned above; eg. sample 8C was the sample taken from plants grown under an 8 hour daylength at the appearance of stamen initials.

3.5.1.   Methods.

In the first harvest series apices were dissected out from the plants at the specified times from planting and quick frozen. The vegetative samples, taken at 7 days from germination, from the 8 hour and the 24 hour daylengths (samples 8A and 24A) were quick frozen directly on dry ice in a wide mouthed Thermos flask. An oily, non volatile substance was found contaminating these samples after subsequent attempts had been made to freeze dry the plant material, and there was evidence that this had come from the dry ice these samples were not included in the assays. Subsequent samples after dissection were quick frozen in a 25 ml. glass stoppered flask buried in the dry ice in a Thermos flask, and no oily contamination was observed.

In the second harvest series, more material was obtained at the 8 hour vegetative, 8 and 24 hour double ridge stages. These inflorescences were collected with the youngest stage II leaf which was 0.65 - 1.00 mm. long. The leaf was slightly larger in the plants grown in 24 hours than in an 8 hour daylength. The inclusion of the leaf facilitated handling of the material and to distinguish this from the previous harvest series the samples were designated as fol- lows:- 8A +L and 8B +L and 24B +L (cf. section 3.5.0).

The assays were performed as indicated in section 2.5. Two batches of gibberellic acid standard serial dilutions were made up; one each fortnight and stored in a refrigerator. The standard dosage-response lines were consistent within each batch of standard dilutions, however the slopes of the two standard lines were significantly different (cf. fig. 2.5.1. and table 2.5.11.) The

3.5.1. (cont.).

No reason for this variation was apparent. The agreement between the serial dilutions of the samples was better when the activities of the gibberellin-like substances were estimated from corresponding standard dosage response lines than from the line obtained by pooling the gibberellic acid dosage-response data from both batches of standard dilutions.

The standard error of estimation of activity given in table 3.5.2.2. for each sample was estimated according to Bliss (1952).

Two subsamples were taken from samples 8D and 24D. The activities of the first subsample were estimated from line 3 and those of the second subsample from line 4 (table 2.5.1.1.). The second subsamples in both cases contained 15 and 8 times more material than the respective first subsamples; good agreement between estimations was obtained for each pair of subsamples.

A mean reducing sugar response which was significantly greater than water control (P = 0.05), for a triplicated measurement of activity, was equal to 27 ug glucose/mg endosperm dry weight (log 1.43). In table 3.5.2.2. samples 8B, 8D subsample 1 and 24D both subsamples failed to reach the level of significance above water control. Hence the activities quoted for 8B and 24D are the maximum that these samples could contain. The activity quoted for sample 8D is based on the estimation made from subsample 2 (67 mg) since it contained the larger amount of plant material.

The units of activity and specific activity were defined in section 2.5.1., a further unit is now defined, and is that of apex activity. Unit apex activity (unit activity / apex) is calculated

5.5.1. (cont.).

by dividing the number of apices used in the extract into the total
extract activity.   This unit is useful when making comparisons
between activities of inflorescences at different stages of
development and between apices with and without the youngest stage
II leaf.

5.5.2.   Results.

The primary data; apex length, weight, number taken at each
harvest and age, are presented in table 5.5.2.1.

In the first harvest series (fig. 5.5.1) there is a marked peak
in the specific activity at the stamen initial stage of development
(stage C) for both daylength treatments.   This finding supports the
observations made on long day dicotyledonous plants, viz. a peak
in activity of gibberellin-like substances occurs at the initiation
of stem elongation.

At double ridges (stage B), the specific activity of gibberellin-
like substances observed in the 24 hour daylength compared with that
in the 8 hour daylength suggests that gibberellins might influence
the rate of primordia formation (table 5.5.2.2. and fig 5.5.1.).

The specific activity of the gibberellin-like substances is
higher in the 24 hour daylength than in the 8 hour daylength at
stage C(stamen initials).   However, at the next harvest (stage D),
there is a large drop in specific activity,   which is more marked
in the apices from plants in continuous light than in those in an 8
hour daylength.   When comparisons between stages C and D are made on
an apex basis (table 5.5.2.2), there is only a slight reduction in

Table 3.5.2.1.

Primary Measures on the Samples of Apices used in Assays

Sample Code	Sample Age (Days)	Total Primordia Number	Number of Primordia on Apex	Spike Length (mm)	Length per Primordium (u)	Dry Weight (mg)	Number of Apices	Dry Weight per Apex (ug)
8A	7	6.6	3.55	0.24	68	-	-	-
8B	23	22.1	14.3	0.85	60	3.1	222	14.0
8C	46	45.75	32.7	2.07	63	6.5	98	66.3
8D	78	49.0	34.05	4.81	142	72.3	63	1148
24A	7	6.95	3.75	0.26	69	-	-	-
24B	11	14.75	9.55	0.73	76	2.4	186	12.9
24C	15	26.15	19.15	1.63	85	4.1	94	43.6
24D	19	28.4	22.4	3.47	155	21.0	86	244.2
8A+L	7	No measurements were taken in this series.				10.0	712	14.0
8B+L	23	Visual checks were made to ensure that the				6.4	284	22.5
24B+L	11	correct stage of development had been reached.			9.4		290	32.4

Table 3.5.2.2.

Activity of Gibberellin-like Substances

Sample Code	Standard Line used to estimate Activity	Log Specific Activity	Specific Activity	Log Activity per Apex	Activity per Apex
8B	1	$\overline{3}.254 \pm 0.204$	1.8×10^{-3}	$\overline{2}.399 \pm 0.204$	2.5×10^{-2}
8C	1	0.420 ± 0.136	2.6	2.242 ± 0.136	170
8D	2	$\overline{2}.741 \pm 0.193$	5.5×10^{-2}	1.789 ± 0.193	62
24B	2	0.280 ± 0.193	1.9	1.391 ± 0.193	25
24C	1	1.678 ± 0.137	48	3.318 ± 0.137	2100
24D	1 and 2	$\overline{3}.758 \pm 0.245$	5.7×10^{-3}	0.146 ± 0.245	1.4
8A+L	1	1.080 ± 0.133	12	2.228 ± 0.133	170
8B+L	2	1.753 ± 0.221	57	3.106 ± 0.221	1300
24B+L	1	0.782 ± 0.148	6.0	2.292 ± 0.148	200

Fig. 3.5.1.

STAGE OF DEVELOPMENT

DOUBLE RIDGE   STAMEN INITIAL   ANTHER LOBE
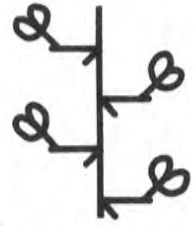
MERISTEMATIC   ACTIVITY

APICAL

INTERCALARY

GIBBERELLIN   CONCENTRATION
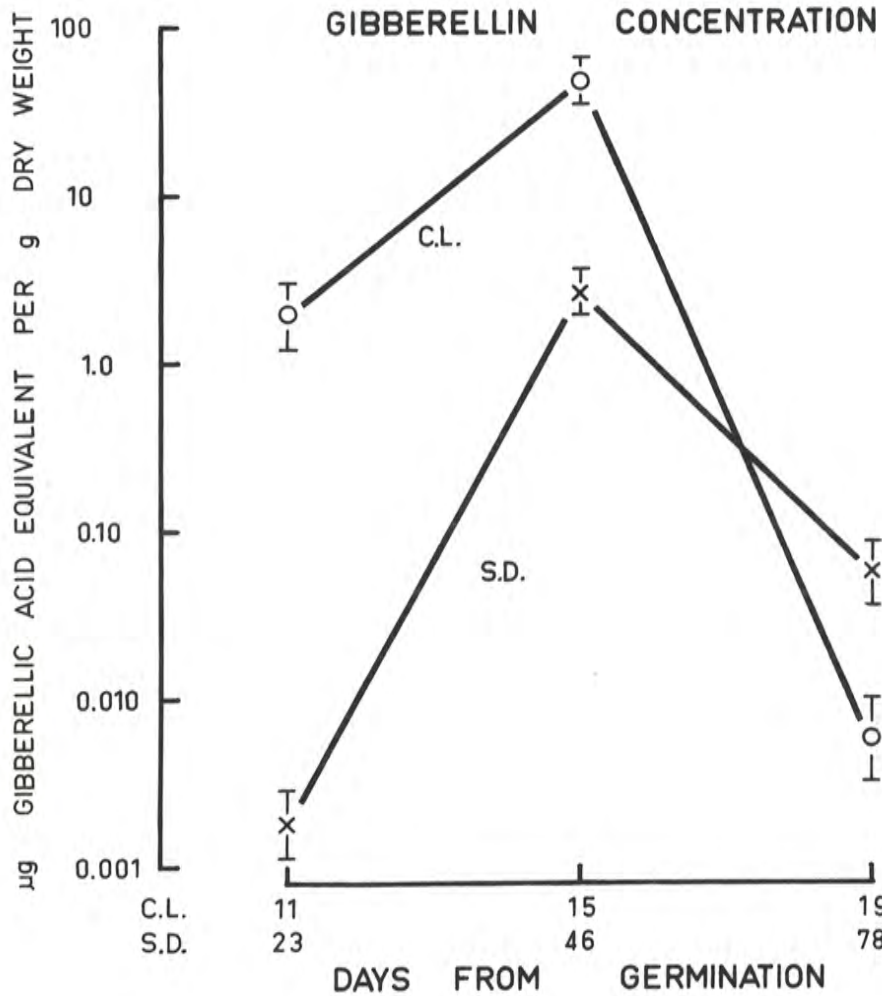
Specific Activity of Gibberellin-like Substances
in Relation to the Development of the Apex.

3.5.2. (cont.).

apex activity observed in the 8 hour daylength while there is again a large decrease in the other treatment.

In the second harvest series (fig. 3.5.2) , in which the youngest expanding leaf was included, high specific activities were found. It is to be noted that there is a very large difference between the two harvest series for the stage B apices from plants grown in an 8 hour daylength, whereas in contrast the specific activities for both harvest series of stage B apices in the continuous light treatment are similar. It is proposed that most of this activity is attributable to the presence of the expanding leaf on the portion of the apex taken.

The expanding leaf included in the second harvest series was at a similar stage of development to leaves in the wheat plant which were found to be undergoing an increase in their relative growth rate (Williams 1960).

Fig. 3.5.2.

STAGE   OF   DEVELOPMENT

VEGETATIVE   DOUBLE RIDGE   DOUBLE RIDGE

MERISTEMATIC   ACTIVITY

APICAL

GIBBERELLIN   CONCENTRATION

C.L.

S.D.

C.L.
S.D.

μg   GIBBERELLIC ACID EQUIVALENT PER g DRY WEIGHT

100

10

1·0

0·10

0·010

0·001

11

23

11

23

7

DAYS   FROM   GERMINATION
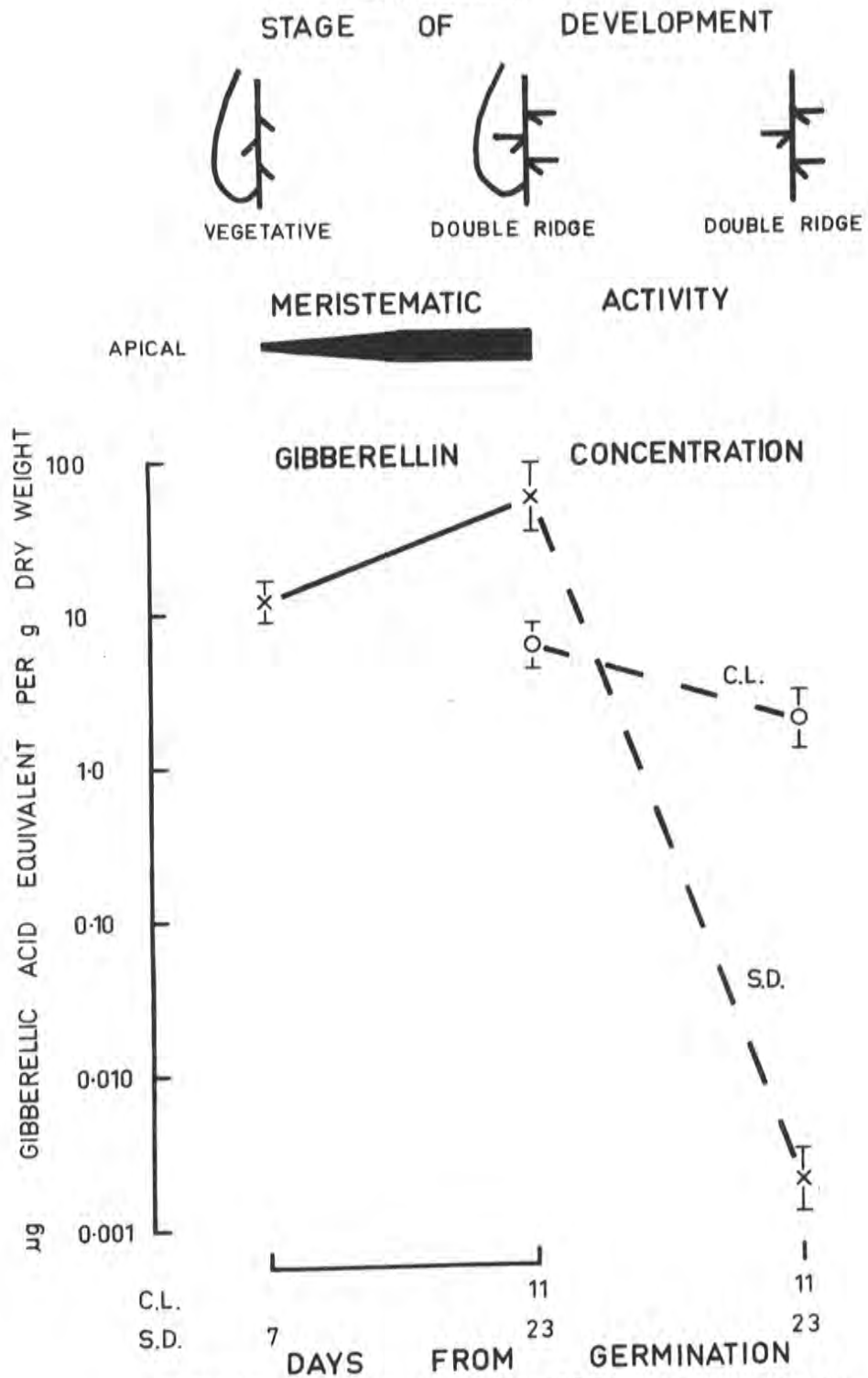
Specific Activity of Gibberellin-like Substances
from Apices containing the Youngest Stage II Leaf
in contrast to those without it.

CHAPTER 4   DISCUSSION.

4.1.   Discussion.

The results will be discussed under three headings, (i) "Morphology", (ii) "Physiology" and (iii) "Mechanisms".

4.1.1. "Morphology"

4.1.1.1.   Relation of Apex Length to the Appearance of Double Ridges.

The results relating to apex length to the appearance of double ridges (sections 3.2.2, 3.3.2) support the suggestion made in the introduction (section 1.2.1) that there is a specific requirement for space on the apex for the appearance of double ridges. Thus, irrespective of the daylength and soil moisture regime, double ridges were visible at a constant apex length (0.45 mm) which was measured from the base of the oldest simple ridge primordium to the top of the apical dome. As stated elsewhere a simple ridge primordium was judged to have changed to a stage I leaf when the angle between the adaxial surface and the longitudinal axis of the shoot became an acute angle. This change in angle stems from the initiation of meristematic activity at the potential apex of the leaf (Sharman 1945).

It is noted in the introduction that increasing numbers of young leaf primordia were observed or apices at double ridge initiation on plants (Lolium temulentum) grown in decreasing day-lengths (Evans 1960 b). If the same criteria for measurement of apex length are used on barley as used by Evans (1960 b) and Cooper (1956) then apex length at double ridges is longer in plants grown in short days than in the longer daylengths, because there are more stage I leaves on the portion of the apex measured (cf. Table 4.1.1.1. below).

4.1.1.1. (cont.).

Table 4.1.1.1.

Number of Stage I Leaves on Apex at Double Ridges.

Daylength	Prior	C.I.5611
24	2.0	2.7
16	2.0	5.5
8	4.3	7.7

However, further work will be needed to extend these observations to
other species in the gramineae and to clarify further the limitations
of the hypothesis that double ridges become visible on apices of a
specified length.

4.1.1.2.   Relation of Internode Elongation to Primordia Formation.

In strongly inductive conditions (fig. 5.3.1) primordia forma-
tion in barley ceases at the same time as the initiation of cell
division in the pith of the inflorescence axis, ie. the initiation
of internode elongation. This observation supports those already
mentioned in the introduction (section 1.2.1) for wheat, rye and
Lolium temulentum. In weakly inductive conditions, there is not
an immediate cessation of primordia formation, but a reduction in the
rate of primordia formation from 1.15 primordia / day to 4 primordia
in 25 days (table 5.5.2.1.). A similar observation has been made by
Aspinall (pte. comm.). In the other genera, which, in contrast to
barley, have a terminal spikelet, cessation of primordia formation
appears to occur, irrespective of the daylength in which the plants
are grown, at the same time as internode elongation is initiated.

4.1.1.5.   Relation of Internode Elongation to Floral Development.

The basic pattern of morphological development of the barley apex was described by Bonnett (1935), in which he observed two phases of growth (i) vegetative and (ii) generative . The phases were separated by the appearance of double ridges and they were associated with short or expanding stem internodes repectively. However, stem internode elongation was not observed until the appearance of stamen initials in the present studies, a finding also recorded by Borthwick et al. (1948). Again, the appearance of expanding stem internodes of 0.5 cm. or longer at or just prior to the appearance of stamen initials has been observed by Aspinall and Paleg (pte. comm.). But, in the absence of correlated developmental anatomical studies, it is difficult to determine precisely when the initiation of stem internode elongation takes place in relation to the floral development of the spike.

In the spike, there is continued cell extension (section 3.4.1) in the pith during the spikelet phase, but the initiation of cell division in the potential intercalary meristems does not occur until the appearance of stamen initials. Thus, in the barley plant, marked shoot growth does not occur until after considerable floral development of the apex, whereas in some long day rosette dicotyledonous plants stem elongation (bolting) occurs at the same time as, or prior to, floral initiation (Sachs et al. 1959).

It should be noted that internode elongation occurred at the same stage of floral development ie. stamen initials and this was correlated with a marked change in the rate of primordia formation

4.1.1.3. (cont.).

irrespective of the environment imposed upon the barley plants in these studies.

4.1.1.4.   Abnormalities induced by Soil Moisture Stress.

In one experiment (Prior 8 hour daylength) in which the soil moisture stress treatments were completed early in the spikelet phase and primordia formation continued for some time after watering up, some abnormalities were observed.   In other variety - daylength combinations comparable soil moisture tensions occurred either at a much later stage in floral development (Prior 16 and 24 hour daylengths and C.I.5611 24 hour daylength) or before flower initiation (C.I.5611 8 and 16 hour daylengths).

The observed abnormalities are listed in table 4.1.1.2 and it appears that there were two types, either one or both occurring on the apex at the same time.   The first was failure of a simple ridge to appear, and as these apices were floral the associated upper ridge failed to develop.   This failure of primordium development, is considered to be caused by the influence of the increasing soil moisture tension for the following reasons.

(i) Plants in both soil moisture treatments had the same number of primordia (31) at Harvest 2 and the number of primordia observed in treatment W_2 at Harvest 3 (32) is not significantly different from that at Harvest 2, ie. primordia formation had almost ceased at a soil moisture stress of pF 3.3 - 3.4.

(ii) The mean position of the failure of the primordum to develop was the same as that which primordia formation stopped (viz 32.2)

4.1.1.4 (cont.).

Table 4.1.1.2.

Abnormalities on Apices.

Harvest	Treatment	Replicate	Primordium	Portion of Apex Rotated
3	W_1	1	-	-
		2	30	-
		3	32	-
4	W_1	1	-	primordia 35 - 42
		2	-	primordia 32 - 40
		3	-	-
4	W_2		no abnormalities observed.	
5	W_1	1	35	primordia 36 - 41
		2	-	-
		3	-	primordia 34 - 44
5	W_2	1	-	primordia 34 - 37
		2	32	primordia 33 - 38
		3	32	primordia 33 - 34
Mean Primordium position of first occurrence of abnormality			32.2	35.86

The second type of abnormality was the rotation about the
longitudinal axis of the plane of insertion of the primordia formed
after watering up. The amount of rotation varied from about 30° to
90° from the original plane of insertion. This second type of
abnormality may also be the result of the dislocation of the normal
metabolism of the apex in the treated plants during the period of soil
moisture stress in that it is the inception of the primordium which
has been affected, and not the subsequent development of the
potential primordium into a visible simple ridge primordium. It is
apparent from the data of Abbe et al. (1951) that the inception of
primordium $n + 1$ occurs after the visible appearance of primordium n

4.1.1.4. (cont.).

for maize in the vegetative state. In Agropyron repens the inception
of primordia n+2 and possibly n+3 occurs after the visible appearance
of primordium n (Sharman 1945). On this basis the increase of
one to two nodes of the first occurrence of this abnormality compared
with the node number of the missing primordium is readily explained,
and it should be noted that where both types of abnormalities occur
on the same apex, the missing primordium is the one immediately below
the first one of the rotated part of the apex.

Of the theories on the determination of the site of leaf formation
which have been put forward in recent years, as reviewed by Clowes
(1961 Chapter 8), it appears that the "Repulsion" theory of Richards
(1948, 1951) provides the most reasonable basis for understanding the
possible mechanism of the formation of these abnormalities. The
evidence here is in favour of Richard's theory since once the position
of the site of the first primordium formed after watering up is
determined, then the sites of insertion of all the subsequent primordia
are determined. However there is not enough known about the sequence
of events between the determination of the site of the potential
simple primordium and its appearance. Probably the stage of the
plastochrone when the dislocation of the metabolism of the apex,
brought about by the increasing soil moisture tension, became critical,
would determine the type of abnormality observed. It should be
noted that similar changes in the siting of potential simple ridges
can be obtained by the surgical destruction of the youngest simple
primordium which is visible in monocotyledons (Snow 1951). Thus it
is possible by physiological means to cause the same perturbations

4.1.1.4. (cont.).

in the determinations of the sites of potential simple ridges as
can be done surgically, and hence further evidence is provided in
support of a dynamic control of differentiation in the apex rather
than one based on geometrical considerations alone.

4.1.2.   "Physiology"

4.1.2.1.   Assimilate Supply or Hormones as Regulators of the Rate
             of Morphological Development in the Shoot Apex.

It is well established that assimilate supply alone cannot
account for all the observed results on the photoperiodic behaviour
of Long Day Plants (Liverman 1955, Lang 1958, Lockhart 1961 and
Salisbury 1961). But a sufficient supply of assimilates is neccessary
and indeed some Long Day Plants have been induced to flower in total
darkness given a sufficient supply of sucrose.

The linear relationship of dry weight to total light energy (fig.
3.1.1.) during the vegetative and spkelet phases of growth indicates
that light energy may be taken as a measure of assimilate supply.
However the change in apex length in Prior (fig. 3.1.2) per unit of
light energy increases with the increase in daylength from 8 to 16
hours. The 16 and 24 hour daylengths data are indistinguishable, but
if incandescent light is added to the 24 hour treatment without
increasing the total light energy by more than 1% a futher increased
rate of growth of the apex is observed. These results clearly
indicate that assimilate supply was not limiting the rate of growth
of the apex in Prior plants, particularly growth following the
appearance of double ridges. It is noted here that for C.I.5611

4.1.2.1. (cont.).

plants a different system appears to be operating as the rate of
growth of the apex for all daylengths is linearly related to the
light energy received by the plants.

Since the other paramenters of growth of the Prior apex (viz.
rate of primordia formation in the spikelet phase and the rate of
spikelet development) are intimately correlated with the rate of
increase in apex length, it follows that these paramenters are also
not limited by assimilate supply. These observations suggest that
a hormone from the leaves, whose production is regulated by the day-
length and the spectral composition of the incoming radiation, is
controlling the rate of the morphological processes occurring in the
apex. It is further suggested that a gibberellin may be this hormone
for the following reasons (i) the synthesis of gibberellins has been
postulated to be daylength controlled (Brian 1959), (ii) higher act-
ivities of gibberellin-like substances are found in the apices from
gr plants grown in continuous light compared with those grown in an
8 hour daylength (fig. 3.5.1), (iii) similar changes in activity have
been found in other long day plants grown in inductive conditions
(Lang 1960, Reinhard and Lang 1961) and (iv) gibberellins increase
the rate of growth of stem internodes without changing the time
sequence of cellular differentiation (Brian 1959).

4.1.2.2.   Influence of the Environment on the Progress to
                     Double Ridge Initiation.

It has been shown earlier in the discussion (4.1.1.1.) that
spikelet initiation occurs at a constant apex length irrespective of

4.1.2.2. (cont.).

the environment imposed upon the plant, hence the apex length-time
curves, which measure the difference between the rates of primordia
formation and development of simple ridges into stage I leaves, are
perhaps the best measure of the progress of the apex towards double
ridges.

The effect of imposition of a soil moisture tension on plants
has been consistently to reduce the slope of the apex length - time
curves in all daylengths (figs. 5.2.1, 5.2.2, 5.2.5) and this
difference between the controls and treated plants is significant at
a soil moisture pF as small as 3.0.

The effect of increasing daylengths above 8 hours has been
consistently to increase the slope of the apex length - time curves.
This increase is primarily due to the increase in the rate of
primordia formation (fig. 5.2.1, 5.2.2, 5.2.5) as the rate of Stage
I leaf appearance is only increased by the change in daylength from
16 to 24 hours (tables 5.2.1.5 and fig. 5.5.5). Hence in examining
the influence of the environment on the progress of the apex towards
spikelet initiation there are two opposing influences to consider
(i) the uniform reduction in rates of the morphological processes
by soil moisture stress and (ii) the selective increase in these rates
by increasing daylength.

The former of these influences, it is proposed, arises from the
effect of drought on the translocation pattern within the tomato
plants as suggested by Gates (1955 b, 1957). The selective effect
of daylength on the morphological processes, it is proposed, arises
from the influence of daylength on hormones which control the processes

4.1.2.2. (cont.).

of cell division and enlargement in particular regions of the apex
as mentioned in section 4.1.2.1.

4.1.2.3.   Determination of Final Leaf Number.

It is shown in the results (sections 3.2.2, 3.3.2) that the
final leaf number is determined by (i) the number of stage I leaves
formed up to the appearance of double ridges and (ii) the subsequent
interaction of the basipetallous appearance of double ridges from the
node of their first appearance and the rate of appearance of stage I
leaves in an acropetallous direction. The influence of the environ-
ment on the number of leaves at double ridges is discussed in the
previous subsection (4.1.2.2). At double ridges initiation there are
at least two simple ridges between it and the youngest stage I leaf.
These nodes may develop either into spikelets or into leaves and the
outcome depends on the balance between the basipetallous development
of the spike and the acropetallous development of the vegetative
portion of the shoot. The trend of basipetallous development of the
spike increases as the daylength increases, for the first observed
double ridge is the collar node in an 8 hour daylength while in
continuous light it is two to three nodes above the eventual collar
node (table 3.3.2.1). In daylengths where this development may be
observed, ie. 16 and 24 hour daylengths, it appears that soil moisture
stress may reduce this basipetallous development of the spike. Thus,
it was deduced in Prior grown in a 16 hour daylength that double ridges
occurred at the same primordia number (fig. 3.2.2) and the same
total leaf number (table 3.2.1.3) in both control and treated plants,

4.1.2.5. (cont.).

but the final leaf number in the treated plants was significantly higher (table 5.2.1.5).

4.1.2.4.   The Influence of the Environment on the Progress towards Stamen Initials.

The increase in length of the apex during the spikelet phase was considered to be due solely to the formation of new primordia (sections 3.2.2, 5.3.2) but the data obtained from microscopic examination of median longitudinal sections of apices suggested two components, viz (i) the formation of primordia at approximately 50μ intervals and (ii) the subsequent expansion of the node plus internode to 80 μ in continuous light. In an 8 hour daylength the mean inter-primordial distance was 60 μ (fig. 3.2.4 and appendix table 5.3.2.1); it is proposed that the primordia are laid down at the same intervals (50 μ) as in long days but that the second component is much smaller ie. the node plus internode only expands slightly to about 60 μ.

Soil moisture stress had no effect on this cell expansion in all cases, but in the Prior plants _after watering up_, in the 8 hour day-length, the interprimordial distance of those apices in the spikelet phase was significantly higher (65 - 70 μ) than the controls. It is also noted that the rate of spikelet development (table 4.1.2.1) is higher in the same plants after watering up. These observations suggest that an increased rate of spikelet development may increase the rate of cell expansion in the node plus internode. It has also been observed (figs 5.4.1, 5.4.2) that the rate of spikelet development of the individuals on a spike is correlated with the rate of increase in the length of the corresponding nodes plus internodes. However, further work will be necessary to decide whether these correlated

Table 4.1.2.1.

Rates of Spikelet Development and Primordia Formation

Daylength	Variety	Treatment	Rate of Spikelet Development	Treated Control	Rate of Primordia Formation	Treated Control
24	Prior	W_0	1.20		3.60	
		$W_1 + W_2$	1.11	0.91	2.40	0.67
16	Prior	W_0	1.00		3.40	
		$W_1 + W_2$	0.45	0.45	1.35	0.40
8	Prior	W_0	0.19		1.15	
		During Stress				
		$W_1 + W_2$ (upto H_2)	0.095	0.50	0.76	0.66
		W_2 (H_2 to H_3)	0.095	0.50	0.12	0.10
		Post Stress				
		W_1	0.36	1.84	1.18	1.00
		W_2	0.43	2.24	0.50	0.43
24	C.I.5611	W_0	0.71		1.95	
		W_1	0.50	0.70	1.60	0.82
		W_2	0.45	0.63	1.45	0.74
16	C.I.5611	W_0	0.31		1.43	

4.1.2.4. (cont.).

changes are related to the activities of gibberellin-like substances in apices from different environments.

The rates of spikelet development and primordia formation are shown in table 4.1.2.1., for all variety, daylength, soil moisture stress combinations, in which plants had reached stamen initials by the end of the experiments. The method of calculation of the rates is given in the appendix (subchapter 5.4.). It is apparent that there is a large increases in the rates for the Prior controls between 8 and 16 hour daylengths, while there is a smaller increase between 16 and 24 hour daylengths. In 16 and 24 hour daylengths stamen initials were reached before Harvest 2 was made (soil moisture pF 3.3 - 5.6), during which time treatments W_1 and W_2 were identical. If comparisons are made between the daylengths for the $(W_1 + W_2)$ soil moisture treatments of Prior it can be seen that the soil moisture treatment has had a relatively greater effect in the 16 hour daylength experiment than in either the 8 or 24 hour daylength. In both the 16 and 24 hour day-lengths the rates of spikelet development were relatively less affected than the rates of primordia formation and hence fewer primordia were formed during the spikelet stage in the treated plants (table 5.2.1.1.). The lower values for the final primordia numbers of the treated plants in the 8 hour daylength experiments arise primarily from the greater post stress ratios of treated to control rates of spikelet development relative to the corresponding ratios for the rates of primordia formation (table 4.1.3.1)

In the 24 hour daylength experiment a comparison between the varieties indicates that in Prior the rate of primordia formation was

4.1.2.4. (cont.).

relatively more sensitive than the rate of spikelet development to
the increasing soil moisture tension, while in C.I.5611 the reverse
situation is found. Thus in every case for Prior where the rate of
spikelet development is less affected by the soil moisture stress than
the rate of primordia formation a lower final primordia number is
observed, in the treated plants. In the converse case, as observed
in C.I.5611, the final primordia number of the treated plants is
higher than in the controls(table 5.2.1.1.).

It should be noted also that the rate of spikelet development
can be further increased in continuous light by adding an incandescent
light source to the fluorescent light source, ie. increasing the
far-red wavelength component relative to the red wavelength component,
while the rate of primordia formation is not increased (fig. 5.3.1).

These results show that the processes of primordia and spikelet
development are independent of each other, they respond differently
to both daylength and soil moisture stress, and differences also
exist in varietal response. However the inception of stamen initials
consistently heralds an alteration in the subsequent pattern of
growth of the shoot apex.

4.1.5.   "Mechanisms."

4.1.5.1.   The Effect of Soil Moisture Tension.

There are two relevant observations on the influence of soil
moisture stress viz; (i) it does not affect the morphological
correlations in the apex occurring at either the appearance of
double ridges or the initiation of internode elongation (sections

<u>4.1.3.1. (cont.).</u>

4.1.1.1, 4.1.1.2 and 4.1.1.3), and (ii) it results in significantly reduced values of all growth estimates made in the treated plants even at <u>low</u> soil moisture pFs (2.7 - 2.9).

In more detail, it is noted that the rates of spikelet development and leaf development (from simple ridges to Stage II leaves) are reduced by an increasing soil moisture stress. Although primordia formation may have been temporarily halted (cf section 4.1.1.4) it is not irrevocably halted until the appearance of stamen initials ie. primordia formation will continue if the soil moisture stress has been relieved before this event. Similarly, double ridges are not visible until the necessary spatial separation between the apical meristem and the Stage I leaves has been attained. These observations suggest that there exists in the spring barley apex the necessary factors for the control of the pattern of differentiation Similar conclusions have been drawn for the apex of <u>Lupinus albus</u> L from a different experimental approach by Ball (1948). If this is so, then the various environments, to which the barley plants have been exposed, have exerted their influence solely on the rate of one or more of the morphological processes occurring in the apex through the rate of supply of water and of assimilate and hormones (both promoting and inhibiting) synthesised in the leaves. This conclusion has an important bearing on the discussion of the mechanism of the initiation of spikelets and internode elongation.

It has been reported by a number of workers, reviewed by Stocker (1960), that growth is slowed down at the onset of a slight soil moisture deficit, although increases in dry weight (Gates 1955 a)

4.1.5.1. (cont.).

and in stem length of tomato plants (Slatyer 1957) will occur even
at quite high intensities of soil moisture tension. As noted
above the rates of all processes here studied are slowed down by
relatively low levels of soil moisture tension (pF 2.7 - 2.9) at
which tension the rates may be as low as half those of the controls.
In the recent reviews on the physiology of plant water deficits (Stocker
1960, Vaadia et al. 1961, Wangermann 1961) no mention is made of
the influence of relatively slight moisture tensions on the develop-
mental processes in plants, in contrast to rates of growth mentioned
above, other than their postulated role in determining the
characteristics of sun and shade grown leaves .

Attention has already been drawn (section 4.1.2.2.) to the
situation in tomato plants (Gates 1955 b and 1957) where there is
an alteration in the pattern of translocation of both carbohydrates
(changes in dry weight) and the mineral elements, nitrogen and phos-
phorous, during a period of increasing soil moisture tension.
Translocation from older leaves to the stem continued, but the
translocation of materials to the younger leaves was depressed.
However on watering up there was a resurgence of translocation to the
younger leaves. Hormones which move in the translocation stream
from the leaves to the apex would be similarly influenced eg. the
postulated florigen and the gibberellins, and competition for these
materials could occur between the apical meristem and the developing
spikelets or leaves as the case may be. However in the absence of
knowledge on the rates of arrival at the apex of the various
materials in different environments it is impossible to make any more

4.1.3.1. (cont.).

specific formulations of the mechanisms of apical growth under conditions of soil moisture tension.

4.1.3.2.   The Initiation of Double Ridges.

Before proposing a mechanism, a brief restatement of events during the formation of spikelet primordia is given below.

It has been noted before that double ridges (spikelet primordia) were first visible about halfway between the apical meristem and the youngest stage I leaf and that in long days there is a subsequent basipetallous development of the spike (subchapter 3.3). The axillary bud primordia (upper ridges) arise from periclinal cell divisions in the hypodermis and subhypodermal cell layers of the apex (fig 3.4.4), a finding which confirms those of Sharman (1945) and Barnard (1955). In Agropyron repens the first cytological signs of axillary bud formation occur very early (Sharman 1945) but in wheat they are not evident at a comparable stage of development (Barnard 1955). No material at an earlier stage of development than double ridges was examined in this work, hence no decision can be reached on the question whether the axillary bud primordia are formed very early in barley as is the case for Agropyron or at a later stage as in wheat. However it can be seen from fig. 3.4.4 that a certain amount of development of the lower ridge takes place before the axillary bud primordium is initiated.

In all apices which were just floral there were two to three simple ridges between the lowest visible double ridge primordium and the youngest stage I leaf. It is now speculated that the stage I leaves exert an inhibitory effect on the initiation of axillary bud

4.1.5.2. (cont.).

primordia at the next two to three nodes above them. This is supported
by the lack of development of floral spikelets in these two to three
nodes in an 8 hour daylength; it is only under highly favourable
conditions (24 hour daylength) that spikelets appear at these nodes
in a basipetallous sequence from the site of first appearance of
double ridges. This situation conforms with the concept put forward
by Purvis and Gregory (1937) that the failure of a spikelet to
develop at a node results in the formation of a leaf. However, the
situation seems not as simple nor as passive in Spring Barley
varieties as suggested by these authors. The trend of decreasing
growth rates of the structures borne at nodes approaching the collar
node from both sides, which in turn are characterised by a minimum
of both leaf and floral development (fig 3.3.4), suggests that the
rate of growth of either leaf or spikelet is the result of a balance
of spikelet promoting and leaf promoting hormones. The leaf
promoting hormones, which determine the rate of development of the
simple ridged primordium into a stage II leaf, could be inhibitors
of flower initiation. If so, such a balanced system is similar to
that which is considered to be operative in Lolium temulentum by
Evans (1960 c).

Thus for the initiation of double ridges at least the following
conditions have to be met: there must be a favourable balance of
spikelet promoting to leaf promoting hormones at a simple ridge
primordium which is at least one plastochron old. It is further
speculated that both spikelet promoting and leaf promoting hormones
are under photoperiodic control and the postulated site of synthesis

4.1.3.2. (cont.).

of the spikelet promoting hormone is the apical meristem. The influence of daylength here is considered to be indirect in that the translocated hormones (eg. gibberellins) from the leaves act on the apical meristem and the developing leaf primordia. These sites in turn produce their respective promoting hormones at rates which are correlated with their own rates of growth, eg. higher rates of primordia formation are correlated with higher rates of production of the spikelet promoting hormones.

This scheme differs from that of Purvis and Gregory (1957) in that there is no partitioning of a substance from the apex into either a spikelet or leaf promoting hormones but rather an interaction of these hormones which are produced from different sites. In this respect it is similar to the "Repulsion" theory of Richards (1948, 1957) in which it was proposed that the site of a leaf primordium is determined by the region of lowest concentration of inhibitory hormones emanating from the already formed leaf primordia and the apical meristem.

The presence of a further inhibitory hormone which is (i) under photoperiodic control and (ii) translocated from the leaves, eg. that postulated in _Lolium temulentum_ (Evans 1960 c, 1962), may distinguish a qualitative Long Day Plant from a quantitative Long Day Plant (spring barley).

4.1.3.3.   The Initiation of Internode Elongation.

It was noted in the introduction (section 1.2.1.) that the initiation of internode elongation was closely correlated with the appearance of floret primordia in wheat, rye and _Lolium temulentum_.

4.1.5.5. (cont.).

The floret primordium is the next organ of cauline origin to be formed in these species following the appearance of double ridges, and represents the formation of secondary branches in the inflorescence which are terminated by a reproductive organ (section 1.1.2). A comparable situation occurs in barley in that the formation of stamen initials in this plant may be equated with the formation of floret primordia in wheat (section 1.1.2).

The shoot apex during the spikelet phase is probably a more complex unit than the shoot apex before double ridge initiation for, as each spikelet is a branch in its own right, each spikelet meristem may control the organisation of the structures formed on its in a manner similar to the shoot apex. Furthermore, the initiation of stamen initials on individual spikelets in barley probably requires comparable conditions to those required for double ridge initiation. Similarly it was considered by Evans (1960 b) that comparable stimuli were required for the initiation of floret and stamen primordia as those required for the initiation of double ridges in some long day grasses.

As a further speculation it is suggested that a favourable balance of hormones produced within the spikelet is required for the inception of stamen primordia; the rates of production of these hormones being correlated with the daylength controlled growth rates of the spikelet. The hormones are also postulated to interact with other substances both in the internodes (inducing cell division) and in the apical meristem of the shoot (inhibiting cell division). It is known that GA5 applied to the plants before the inception of

4.1.5.5. (cont.).

secondary branches cannot initiate internode elongation although it
can promote this process (Purvis 1960, Aspinall pte. comm). But the
possibility that gibberellins are a necessary component for the
initiation of internode elongation must still remain open since they
are observed in high concentrations at this stage of development
of the inflorescence (section 5.5.2).

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5.1. Methodology of Bioassay Technique.

5.1.0. Introduction.

The bioassay procedure described in section 2.5.1. was developed from the results of a sries of experiments described below. The starting point for these experiments was the method described by Paleg (1961) for investigating the mode of action of GA_3 on barley endosperm. At the outset the following modifications were made, (i) a naked variety of barley (Hordeum distichon L. c.v. Nepal) was used to avoid the need to peel the husk off the seed during the sterilization process (ii) only 4 endosperm fragments, obtained in the manner described by Paleg(1961), were used instead of the usual 8. Unless otherwise stated (i) all results tabulated are expressed as ug glucose equivalents per mg endosperm initial dry weight, (ii) the values are the means of duplicate readings, and (iii) the concentration of GA_3 solution in the treated incubates was 2×10^{-6} Molar (2 ug GA_3 per Petrie dish).

5.1.1. Experiment I Time of Soaking.

The time of soaking the seeds in a 5% calcium hypochlorite solution was investigated. Over the range of soaking times from 1 to 4 hours, no significant change in the amounts of sugar released was observed in either the water control or the GA_3 treated incubates. The water control values for a 30 minute soak were significantly higher than those obtained after a longer soak. These results were obtained for both temperatures ($20^{\circ}C$ and $30^{\circ}C$) at which the endosperm fragments were incubated.

5.1.1. (cont.)

Table 5.1.1.

Soaking Time	Incubation Temperature.			
	30°C		20°C	
	+GA₃	-GA₃	+GA₃	-GA₃
30 mins.	60.10	45.30	6.65	6.25
75 mins.	60.10	31.45	8.85	3.30
120 mins.	47.75	28.70	8.10	4.25
240 mins.	54.85	24.35	9.40	3.30

In the following experiments a 2 hour soaking period was used.

5.1.2.   Experiment 2   Size of Endosperm Fragment.

Two endosperm fragment sizes were used, the distal 2mm or the distal 4mm, and these fragments (4 per petrie dish) were incubated for 24 hours at 30°C. The amounts of glucose equivalents per petrie dish were not significantly different between the two sizes. Hence the differences obtained when the results are expressed on a weight basis are solely due to the difference in weight of material.

Table 5.1.2.1.

	Length of Fragment.			
	2mm		4mm	
	+GA₃	-GA₃	+GA₃	-GA₃
ug Glucose per Petrie	2958	1461	2988	1618
ug Glucose per mg D.W.	85.75	39.30	28.05	13.70

5.1.2. (cont.)

An intermediate size (3mm) was chosen for future experiments, and this has proven to yield higher amounts of glucose equivalents per fragment over a number of experiments. The mean values for all observations of sugar release from Nepal seed which had been soaked for 2 hours and incubated at 30°C for 24 hours.

Table 5.1.2.2.

ug Glucose	$+GA_3$	$-GA_3$
per petrie for 3mm fragments.	3956.0	2550.5

5.1.3.   Experiment 3   Number of Endosperm Fragments per Petrie.

In this experiment the number of (3mm) endosperm fragments used per petrie were 2,4,6,8 and 10. The petries were incubated at 24°C for 24 hours. The different amounts of sugar released between various numbers of endosperm fragments employed were only just significant at the 5% level (Table 5.1.3). The distribution of the corrected values for GA_3 induced sugar release (difference between $+GA_3$ and $-GA_3$ values) appeared to be random. Hence it was concluded that changes in number of endosperm fragments over the range 2-10 per petrie did not have a marked influence on the response.

Table 5.1.3.

No. of endosperm fragments/petrie	$+GA_3$	$-GA_3$	Diff.
2	10.90	5.45	5.45
4	14.65	7.35	7.30
6	12.60	7.25	5.35
8	11.00	3.85	7.15
10	10.10	3.65	6.45

5.1.4.   Experiment 4.   Requirement for Buffer.

In all the experiments reported by Paleg (1960, 1961) 0.001M
acetate buffer pH.5.0. had been used, while in experiments 1-5 described
here no buffer was used. The experiment was designed on a 2x2 factorial
scheme and carried out using 4 3mm endosperm fragments per petrie,
which were incubated at 30°C. The results, Table **5.1.4**, indicated that
the presence of the acetate buffer had no effect on the rate of sugar
release from either water control or GA_5 treated endosperm fragments.

Table 5.1.4.

	$+GA_5$	$-GA_5$
+acetate	53.65	35.05
-acetate	64.05	31.55

(mean of duplicate readings).

Analysis of variance indicated that ~~the~~ only the addition of GA_5 had
a significant effect.

5.1.5.   Effect of Temperature

Included in experiments 1, and 4 were observations at 20°C and in
experiment 5 observations at both 20°C and 30°C. The observations at
both temperatures in experiment 4 were only obtained from petries containing
acetate buffer. In all these experiments the naked variety Nepal was
used.

The results are tabulated in the following table (Table 5.1.4)
together with Q 10 values.

5.1.5. (cont.)

Table 5.1.5.

Experiment		1	3	4	Mean
Treatment Temperatures					
+GA$_5$	50°C	47.75	52.55	53.65	51.32
	20°C	8.85	7.55	8.70	8.30
	Q 10	5.4	7.1	6.2	6.2
−GA$_5$	50°C	28.20	27.00	35.05	30.25
	20°C	3.30	3.40	3.60	3.43
	Q 10	8.7	7.9	9.7	8.8
Diff.	50°C	19.05	25.55	18.60	21.07
	20°C	5.55	3.95	5.10	4.87
	Q10	3.4	6.5	3.6	4.4

Results obtained by Paleg (1961) for Prior endosperm after an incubation of 22 hours show a striking difference from the above as the Q 10 for the water controls in Prior is 1.5, whereas here the mean value is 8.8 . However the Q 10 for the GA$_5$ induced sugar release is of the same magnitude for both varieties. The simplest explanation that can be put forward is that Nepal contains some endogenous GA$_5$ like substance. Accordingly a number of different naked varieties were examined for the amount of sugar release in both water controls and GA$_5$ treated endosperm fragments.

5.1.6.   Experiment 5.   Variety Trial.

Five varieties were investigated for their sugar release responses to both temperature and GA$_5$. The time of incubation was 27 hours. Two samples of Arabian blue were used, one from the 1959 harvest and

5.1.6. (cont.)

the second from the 1960 harvest. From the results presented in the &
table below Triple Awned Lemma (T.A.L.) was chosen for further investigation
as it showed a high degree of responsiveness to GA_3 treatment combined
with a relatively low water control sugar release.

Table 5.1.6.

Treatment	25.5°C			30°C		
Variety	$+GA_3$	$-GA_3$	Diff.	$+GA_3$	$-GA_3$	Diff.
Sel B181	15.05	5.55	9.70	74.4	17.5	58.1
Pearl	28.40	7.80	20.60	85.9	25.7	58.2
T.A.L.	39.35	8.95	30.40	115.3	34.3	81.0
Arabian Blue 1959	29.00	10.15	18.85	91.5	39.0	52.5
Arabian Blue 1960	28.15	11.60	16.55	72.3	46.6	25.7
Red Rachis	33.60	15.70	17.90	135.4	54.7	80.7

5.1.7.   Experiment 6   Concentration Series.

The effect of various concentrations of GA_3 on the GA_3 induced sugar
release from T.A.L. fragments was investigated. Four 5mm endosperm
fragments were incubated in the following GA_3 concentrations, 0, $2x10^{-10}$ M
(Molar), $2x10^{-8}$M, $2x10^{-6}$M (2ug GA_3 per petrie), $2x10^{-4}$ M at either 25.5°C
or 30°C for 24 hours. From the results presented in the following
table it was decided to use a temperature of 30°C for incubation in
further investigations.

5.1.7. (cont.)

Table 5.1.7.

Concentration	Temperature	
	23.5°C	30°C
0	5.05	11.85
2x10^{-10}M	5.30	19.25
2x10^{-8}M	12.00	46.25
2x10^{-6}M	20.50	79.60
2x10^{-4}M	22.55	78.65

Since in a bioassay it is desirable to be able to assay for small
quantities of the particular substance, an investigation of the volume
of the solution bathing the endosperm fragments was made. The depth of
the solution was kept constant and the volume was changed by using either
the 5cm. petrie dishes(5ml. of solution) or a 2" by 1" specimen tube
(1ml. of solution). It was found that the change in volume of the
solution did not significantly alter the GA$_5$ induced sugar release.
Therefore in the following experiments the ~~incubateds~~ incubations were
carried out in the 2" by 1" specimen tubes.

5.1.8. Experiment 7.   Reinvestigation of Time of Soaking.

In this experiment the seeds were soaked for 24 hours in a 2%
calcium hypochlorite solution and then treated in the normal manner
and the GA$_5$ induced sugar release measured after either a 24 hour incu-
bation period or a 48 hour incubation period at 30°C. In the results
presented below, the increased time of incubation has increased the
response ~~at~~ in the 2x10^{-10} Molar ~~concentration~~ solution. The other difference was
the reduction in the water controls sugar release values after a 24 hour

5.1.8.(cont.).

period of soaking compared with that of 2 hour soaking in Experiment 8,
for a 24 hour incubation period.

Table 5.1.8

Incubation Time Concentration	24 hour	48 hour
θ	7.8	40.7
$2x10^{-11}M$	8.5	42.2
$2x10^{-10}M$	9.2	80.4
$2x10^{-9}M$	28.8	247.8
$2x10^{-8}M$	71.4	357.8

1ml of a $2x10^{-11}$ Molar GA_3 solution contains $6.7x10^{-12}$ µg GA_3.

5.1.9.   Experiment 8   Gibberellin Specificity

The induced sugar release of response of a number of different
gibberellins (GA_1, GA_3, GA_4, GA_5, GA_6, GA_8, GA_9) was investigated over
a wide range of concentrations ($10^{-11}M$, $10^{-10}M$, $10^{-8}M$, $10^{-6}M$ and $10^{-4}M$)
using T.A.L. endosperm fragments as the biological material. The highest
concentration of GA_9 used was $10^{-5}M$ as difficulty was experienced in
dissolving it in water. The technique followed was that of Paleg 1961
except that (i) no peeling was required (ii) the temperature of incubation
was $30^{\circ}C$. The time of incubation was 22 hours. This experiment was
carried out in partnership with L.G.Paleg.

The slopes of the dosage-response curves for all the gibberellins
were similar. There was, however, a considerable range in relative activity
as indicated below.

$$GA_1 = GA_3 > GA_4 > GA_5 = GA_6 > GA_8 = GA_9$$
$$100 \qquad 66 \qquad 1 \qquad 0.1$$

Thus in assays of extracts the most easily detectable gibberellins are GA_1, GA_5, GA_4. GA_7 may be as easily dectectable as GA_4 as the structural difference between GA_4 and GA_7 is identical with the structural difference between GA_1 and GA_5. Also the structural difference between GA_1 and GA_4 is identical with that between GA_5 and GA_7 (Brian et al 1962).

5.2.   Primary Data.

5.2.1.   Plant Growth   Total Plant Dry Weight.

Table 5.2.1.1.

A   24 Hour Daylength.

Variety	Harvest		1	2	3	4	5
	Treatment	Time 14	26	32	36	41	
Prior	W_O		0.286	1.76	3.59	8.92	11.58
	W_1		0.297	0.72	1.32	2.24	4.10
	W_2		0.220	1.01	0.93	1.00	1.42
C.I.5611	W_O		0.186	1.63	2.39	2.87	4.01
	W_1		0.177	0.46	0.86	1.17	3.48
	W_2		0.158	0.57	0.81	0.63	1.20

B   16 Hour Daylength.

Variety	Harvest		0	1	2	3	4	5
	Treatment	Time 12	21	32	40	44	48	
Prior	W_O	0.064	0.391	2.47	5.95	10.19	14.28	
	W_1		0.173	0.58	0.92	1.64	3.14	
	W_2		0.190	0.47	0.45	0.48	0.80	
C.I.5611	W_O	0.075	0.290	2.66	6.17	8.66	13.12	
	W_1		0.156	0.28	0.54	1.15	1.35	
	W_2		0.111	0.36	0.27	0.60	0.75	

C   8 Hour Daylength.

Variety	Harvest		0	1	2	3	4	5
	Treatment	Time 11	26	40	48	53	57	
Prior	W_O	0.061	0.475	1.12	2.07	3.11	4.86	
	W_1		0.251	0.61	0.87	1.23	1.99	
	W_2		0.360	0.62	0.64	0.81	1.15	
		Time 11	26	43	51	56	60	
C.I.5611	W_O	0.051	0.159	0.95	1.17	2.25	4.59	
	W_1		0.080	0.20	0.43	0.59	1.12	
	W_2		0.145	0.30	0.32	0.45	0.60	

5.3. Regression Lines and Analysis of Variance.

5.3.1. GA_3 Dosage - Response Curves.

Table 5.3.1.1.

Dosage-Response curves for four different standard lines.

Number	Equation	Experiment
1	$Y = 0.282X + 1.17$	From Bioassay of Glasshouse grown apices.
2	$Y = 0.303X + 0.93$	Repeat of the above.
3	$Y = 0.261X + 1.29$	From Bioassay of Controlled
4	$Y = 0.368X + 0.87$	Environment Cabinet grown apices.

Table 5.3.1.2.

Analysis of Variance of Data

from bioassay of glasshouse grown apices, assayed between 12-19/12/61 (Table 2.5.1.).

Item	S.S.	df	M.S.	F.	P.
Between mean Responses for extracts and standard	0.3566	2	0.1783	14.50	$< .001$
Effect of Combined Slope	1.7983	1	1.7983	146.20	$< .001$
Divergence in Slopes	0.0104	2	0.0052	2.37	N.S.
Error	0.2463	20	0.01232		
Total	2.4086	25			

5.3.2. Daylength - Water Stress Experiment.

Table 5.3.2.1.

Regression Lines for the Relationship of the Number of

Primordia in the Apex to Apex Length.

Daylength	Equation	n	s_b	s_y
16 and 24 Hour	$Y = 0.0769X - 0.043$	72	0.0016	0.0974
8 Hour	$Y = 0.0602X + 0.012$	77	0.0012	0.0885

s_b = standard error of the slope

s_y = standard error of the points about the line.

Table 5.3.2.2.

"t" test between slopes of the above line.

$(b_1 - b_2)$	Combined Standard Error	t	df	P
0.0167	0.00198	8.43	145	$<.001$

5.3.3. Continuous Light Experiments.

Table 5.3.3.1.

First order Regression Lines of Apex Length on Time.

Light	Equation	n	s_b	s_y
Fluorescent	$Y = 0.1238X + 0.359$	151	0.0013	0.090
Fluorescent+ Incandescent	$Y = 0.1410X + 0.272$	162	0.0014	0.088

5.3.3. (cont.).

<p style="text-align:center">Table 5.3.3.2.</p>

Analysis of Variance for scatter of points about the line
(Since each point in time is the mean of 8-10 values, analysis can
be made for deviation of points about a straight line.)

<p style="text-align:center"><u>A</u>   Fluorescent Light Source.</p>

Item	S.S.	df	M.S.	F	P
Linear Regression	75.6243	1	75.6243	1062.14	<.001
Deviation about Line	1.0680	15	0.07120	8.88	<.001
Error	1.0748	134	0.008021		
Total	77.7671	151			

<p style="text-align:center"><u>B</u>   Fluorescent + Incandescent Light Source.</p>

Item	S.S.	df	M.S.	F	P
Linear Regression	77.3680	1	77.3680	2157.40	<.001
Deviation about Line	0.5738	16	0.03586	4.67	<.001
Error	1.1054	144	0.007676		
Total	79.0472	161			

In both the above analyses the F ratio for Linear Regression is
obtained by dividing the Linear Regression Mean Square by the
Deviation about Line Mean Square.

<p style="text-align:center">Table 5.3.3.3.</p>

Regression Lines for the Relationship of Number of Primordia
in the Apex to Apex Length.

Light Source	Equation	n	s_b	s_y
Fluorescent	Y = 0.0797X - 0.0179	71	0.0014	0.0818
Fluorescent+ Incandescent	Y = 0.0817X + 0.0045	90	0.0012	0.0641
Pooled	Y = 0.0801X - 0.0012	161	0.0009	0.0747

5.3.4. Cell Division and Cell Elongation.

Table 5.3.4.1.

Regression Lines for Segment Length and Cell Number on Apex Length.

A Spikelet Phase.

SEGMENT LENGTH				
Segment	Equation	n	s_b	s_y
10	Y = 0.1291X + 1.782	9	0.06267	0.02632
15	Y = 0.6671X + 0.0700	8	0.08301	0.02473

B Elongating Phase

SEGMENT LENGTH				
Segment	Equation	n	s_b	s_y
10	Y = 0.8112X - 0.462	15	0.06551	0.05725
15,20,25	Y = 1.0907X - 1.347	42	0.04718	0.07876
CELL NUMBER				
Segment	Equation	n	s_b	s_y
10	Y = 0.8415X - 1.989	15	0.04409	0.03852
15,20,25	Y = 1.1166X - 2.868	36	0.04417	0.05963

Note. (1) The segment length lines for segments 20 and 25 in the elongating and spikelet phases have been pooled since they were not significantly different.

(2) In the elongating phase the data for both segment length and cell number respectively from segments 15, 20 and 25 were pooled as the individual lines were not significantly different.

5.4.   Calculation of the Rate of Spikelet Development during the
                            Spikelet Phase.

5.4.0.   Introduction.

In this thesis two scales of measurement of the progress of the

apex from flower initiation towards anthesis have been used. The first

is based on the development of the most advanced primordium on the apex

and the second is based on the rate of development of each of the

individual spikelets. The values arbitrarily given to the scales are

common from stage 4 (Triple Mound or the appearance of Lateral Spikelet

Initials) onwards (subchapter 2.3). Experimental observations indicate

(i) the node number of the most advanced primordium increases during

the time interval from the appearance of double ridges to the appear-

ance of triple mounds (stage 4),(ii) the position of the most advanced

primordium on the apex does not significantly change during the spike-

let phase from stage 4 onwards, (iii) the rate of development of the

primordia immediately above and including the most advanced primordium

is the same (fig.5.3.4), while for those below, it is slower.   From

these observations it appears that the two scales are strictly comp-

arable from stage 4 to stage 7 (appearance of stamen initials).   Hence

the rate of spikelet development and the rate of inflorescence develop-

ment are identical between these stages. Further, it is observed that

the rate of spikelet development during the spikelet phase for Prior

in a 24 hour daylength is a linear function of time (Table 3.3.2.4),

whereas the rate of inflorescence development is not. The interval

between stages 3(double ridges) and 4(triple mounds) on the inflorescence

scale compounds two intervals, stages 2(double ridges) to 4(triple

mounds) on the spikelet development scale (subchapter 2.3).

The question to be resolved here is whether, by a modification

5.4.0. (cont.).

of the inflorescence scale, the rate of inflorescence development
can be made linear and thus be used as an estimate of the rate of
spikelet development of the most advanced primordium. The proposed
modification of the inflorescence scale would give the change in
development from double ridges to triple mounds a value of 2 units,
ie. equal to the corresponding change on the spikelet development
scale. To test whether this modification of the inflorescence scale
makes it a reasonable expression of the rate of spikelet development
of the most advanced primordium, a rate of inflorescence development
can be derived from the time interval of the spikelet phase. This
rate can be used in turn to calculate the stage of development of
the most advanced primordium at a particular time. The agreement
between this calculated value and that value actually observed at the
same time, then tests the validity of the original modification.

5.4.1.   Calculation of Rates.

The time interval of the spikelet phase is the difference
between the times of appearance of double ridges and stamen initials.
The former is obtained by entering the apex length-time curve at the
ordinate of 0.45 mm, eg. fig. 3.2.2., since double ridges appear at
this length under all environments investigated. The latter time is
obtained in two steps; (i) the mean maximum number of primordia on
the apex is first attained at stamen initials and hence, by multiply-
ing the interprimordial distance by the mean maximum number of
primordia on the apex, the apex length at this stage can be determined,
(ii) on entering this apex length on the apex length-time curve, the
time of appearance of stamen initials can be estimated. (The mean

5.4.1. (cont.).

maximum number of primordia on the apex is equal to the mean final number of primordia minus the mean final number of leaves on the shoot.) Now since there are five stages (units) of development in the spikelet phase a mean rate is readily obtained.

5.4.2.   Results.

The calculations as just described were made (Table 5.4.2.1.) and the agreement between the observed and calculated stages of development is to be noted. On this evidence it is suggested that the hypothesis, that the rate of inflorescence development can be used to estimate the rate of spikelet development of the most advanced primordium and that it is linear, is confirmed, for Prior in 24, 16 and 8 hour daylengths and C.I.5611 in a 24 hour daylength. An extension of this hypothesis is that the rate of inflorescence development also provides an estimate of the rate of development of those primordia above the most advanced one, since their rate of development is the same as the most advanced primordium. However, it is not possible to estimate the rate of the more slowly developing primordia between the most advanced primordium and the collar in the same way.

<div align="center">

Table 5.4.2.1.

Rate of Spikelet Development

</div>

Daylength	Variety	Treatment	Appearance of Double Ridges and Stamen Initials in days		Rate of Spikelet Development	Calculated Stage	Observed Stage	Time Days
24	Prior	W_0	10.5	14.5	1.20	6.2	6.0	14
		$W_1 + W_2$	10.5	15.0	1.11	5.9	5.8	14
24	C.I.5611	W_0	15.0	22.0	0.71			
		W_1	18.0	28.0	0.50	6.0	5.5	26
		W_2	18.0	29.0	0.45	5.6	6.0	26
16	Prior	W_0	14.5	19.5	1.00			
		$W_1 + W_2$	16.5	27.5	0.45	4.0	4.5	21
8	Prior	W_0 During Stress	22.0	48.0	0.192	5.5	5.3	40
		W_1	27.0	40.0 End of	0.095			
		W_2	27.0	48.0 Stress	0.095	3.2	3.0	40
		Post Stress W_1	40.0	51.0	0.363	5.9	6.0	48
		W_2	48.0	55.0	0.43	6.15	6.0	53

The result of a "t" test on the mean difference between observed and calculated stages indicates that there is no real difference.   (t = 0.107, P is greater than 0.9 at 8 df.)