GROWTH PHYSIOLOGY OF HELIANTHUS ANNUUS (LINN.)
DURING WATER STRESS AND RECOVERY

by

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ADDENDUM

1. The term 'field capacity' is used throughout this thesis in preference to 'available water content' (expressed in absolute units), because the former allows the comparison between experiments using different pot size and different soil volumes.

2. The terms 'leaf area' and 'lamina area' are synonymous.

3. The correct term for the seed or 'fruit' of a member of the Compositae is 'cypsela' but the more common agricultural term 'achene' is used throughout the thesis. Read cypsela for achene.

4. The authority for the species name Helianthus annuus is Linnaeus. The correct abbreviation for the citation of Linnaeus is "L." and not "Linn."
DECLARATION

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

T.M. Yegappan
I wish to record my sincere appreciation and gratitude to my supervisors - Dr. D.M.Paton (Reader, Botany Department, Australian National University) and Mr. C.T.Gates (Principal Research Scientist, CSIRO in Canberra) for their unfailing support, advice and encouragement throughout the course of the project.

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SUMMARY

The thesis attempts to evaluate the relative roles of a reduced photosynthetic source (the leaf area) caused by water stress and of the interactions between the developmental stage of an organ and the water stress, in the final size responses of leaves and seeds in the sunflower-Helianthus annuus Linn. The experimental approach to the problem involved the application of brief periods of water stress at specific developmental stages of the shoot and inflorescence.

Water stress reduced the growth of leaves and achenes but after rewatering, they achieved growth rates comparable to the controls. However, the final area and dry weight of some of the leaves and the number and sizes of palisade cells in them were reduced. The magnitude of the reduction was dependent on the stage of the development of the leaf or achene when water stress affected it.

The leaves that were in a folded state during water stress (young leaves) eventually recovered to control leaf sizes. The largest reduction in final leaf area and dry weight of a leaf occurred to leaves that were at the unfolding or early expansion stages (between 3% and 20% of the maximum area of the leaf - $A_{\text{max}}$). Studies on the palisade cell production in relation to the growth of a leaf showed that rapid cell division took place between the unfolding of a leaf and its attainment of $20\% A_{\text{max}}$, division ceasing at about $35\% A_{\text{max}}$. Although cell division was sensitive to water stress, the reduction in the area of a leaf could not be explained solely in terms of a reduction in cell number as cell size reductions also were involved in the leaf area decrease, especially in leaves positioned on the lower part of the shoot. In contrast to this, the reduction in the area of leaves positioned higher up on the shoot (example - leaves 12 and above) were, however, largely associated with the cell number reductions in them because cell sizes in these higher positioned leaves approached a minimum and were
insensitive to water stress. The leaves that were at various stages of expansion were affected to varying degrees by water stress, depending on their stage of expansion and the severity of the water stress.

The full recovery of the folded leaves (young leaves) from water stress was in contrast to the incomplete recovery of young disc florets (disc florets at 20 days before anthesis) and young achenes (disc florets that recently completed anthesis). Another contrasting situation was the large reduction in the final size of leaves that were embarking on a phase of rapid growth (unfolding leaves) and the full recovery from water stress of achenes that were embarking on a phase of rapid growth (grain filling). Leaves and achenes of the sunflower thus differed in these respects in their response to water stress.

The effect of reduced total plant leaf area (arising from effects of water stress on leaf expansion or from accelerated senescence of existing leaves) on the development of the achenes is evaluated together with other possible factors that may influence seed size, such as the interaction between the water stress and the developmental stage of the achene at stress and also such effects as may be mediated by developmental changes in the components of the achene.

The relevance of the results of the controlled environment to a more generalized situation, such as a field environment, is discussed.
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GENERAL INTRODUCTION

Although droughts are common during the summer in Eastern Australia and limit the productivity of herbaceous dicotyledonous plants that are grown under non-irrigated conditions, little attention has been given to the physiological responses that lead to reduced productivity in these plants. This thesis examines physiological responses in a herbaceous plant during water stress and during recovery, with the aim to delineate some of the factors that may be involved in the yield response. Such a study is difficult to perform in the field because water is an environmental variable that cannot be easily controlled. Furthermore, water stress effects in the field can be confounded with temperature, radiation and humidity effects. The analysis of water stress effects per se should be attempted by controlling these confounding influences; understanding under controlled environment should precede the investigation in the field.

Since the examination of a range of herbaceous dicotyledons would inevitably limit the detail of the analysis, a single plant species was selected; this was done with the view to generate spheres of more general interest that can be tested in future on other herbaceous dicotyledonous species. An initial trial (Chapter 2) that involved the examination of six plant species, besides facilitating a study of comparative responses
of the species to water stress, also allowed the choice of a suitable test plant for the main parts of the three year study. The sunflower, *Helianthus annuus* Linn., had several favourable growth and morphological features in comparison to the other species in that trial; the high growth rate, the easily manipulated vegetative and floral apex, the easy identification of leaves and seeds in terms of chronological and physiological age (which would allow comparative studies of tissues of differing age), were all seen as advantageous features for the main study.

The main aim of this thesis is to evaluate the importance of the size reductions of the assimilatory surface (the leaf area) caused by water stress, on the growth of dependent leaves and achenes at various stages of their development and to compare this relationship with other possible direct effects of water stress on the growth of leaves and achenes. **While there have only been a few attempts to study the dependence of 'leaf sinks' on the available leaf source (Wardlaw 1969, Milthorpe and Moorby 1974), the dependence of the growth of grains on the size and efficiency of the leaf assimilatory surface, has received a great deal of attention.**

Watson (1956) interpreted the dependence of the growth of grains on the leaf area as a 'source' limited 'source-sink' relationship; the leaf area was assumed to contribute largely to the 'source' and the developing grains were the 'sink'. Similarly, Fischer and Kohn (1966) have shown that in a determinate crop like wheat, yield under dryland conditions was inversely related to the rate of leaf senescence after flowering. As this resulted in a decrease to plant leaf area, they suggested that this would have effects on yield through a 'source-limited' mechanism.

However, there are several lines of evidence which indicate that contribution of assimilates from other plant sources, beside current photosynthate, may influence yield (Yoshida 1972, Passiora 1976). Also,
there is evidence to show that the plant normally assimilates more carbon than is required for growth and respiration; a reduction to leaf area or to its efficiency in photosynthate production would not necessarily reduce grain yield, once the grain number has been set (Wardlaw 1971, Bremnar 1973). These investigations were mostly performed on wheat and therefore a parallel cannot be drawn for the sunflower. To determine the importance of leaf area on the growth of dependent leaves and achenes, an independent analysis for the sunflower should be made.

The 'source' size of individual leaves in terms of the assimilatory surface area and photosynthetic rate unit area\(^{-1}\) in the sunflower, varies with leaf ontogenetic position (McWilliam et al. 1974). If the 'source' size is important in a general 'source-sink' relationship, the effects of water stress on the 'source' in the sunflower, must essentially be studied in relation to individual leaves. The factors that contribute to a decrease in individual leaf area would consequently become important variables to study.

The two main variables which contribute to leaf size are cell number and cell size (Sunderland 1960, Milthorpe and Newton 1963), although intercellular spaces and space occupied by veins are also important. Most studies which have examined the effects of water stress on leaf area, have tried to relate epidermal and/or stomatal cell number and size responses to leaf area responses (McCree and Davis 1974, Quarrie and Jones 1977, Bunce 1977). Apart from the fact that only isolated leaf systems were studied, the responses of epidermal and stomatal cells are not directly related to the 'source' size, although stomatal efficiency can control photosynthate production. As the palisade cells contain the basic photosynthetic units in the leaf, a study of palisade cell responses would provide information to complement the numerous studies which have
measured leaf area or photosynthate production by gas exchange techniques as indicators of 'source' size.

Apart from the possible importance of a 'reduced source size' on the growth of dependent tissues, there is a need to clarify the response to water deficits at all stages of development of a tissue; the response may vary with the stage of development and the stage of growth (Iljin 1957, Wardlaw 1967, 1969). In leaves, as well as in seeds, phases of predominant cell division and enlargement have been described (Sunderland 1960, Milthorpe and Newton 1963, Clough and Milthorpe 1975) and the effects of water stress on the two processes have been evaluated (e.g. McCree and Davis 1974, Clough and Milthorpe 1975). Whilst recent emphasis on the reduction of leaf area has been placed on the inhibition of cell enlargement (Boyer 1968, 1970, Acevedo et al. 1971), earlier literature contains references to inhibition of cell division as an important or even sole cause of the reduction in leaf area (Ashby 1948). The timing of the two processes in relation to leaf area increase and their sensitivity to water stress may be useful in accounting for leaf area responses. Thus, a knowledge of the developmental stage of the tissue at the time of stress is important.

The importance of the developmental stage of a tissue in determining its response to water stress is also emphasized by Gates (1955a,b, 1957) in findings on leaves of tomato. He found that whilst young leaves suffered the greatest absolute check in growth during water stress, they recovered most rapidly on rewatering, and exhibited a higher relative growth rate than the corresponding controls. Older leaves were checked less during water stress, but recovered from it much more slowly. The apparently juvenile growth characteristics of the rewatered young leaf was ascribed to the lower proteolysis which occurred during water stress in young leaves as compared to old leaves. Unfortunately, the recovery time allowed for the leaves in Gates (1955) experiment was insufficient
to gauge the final responses of the different leaves, including those which were in a primordial form during water stress. Long term recovery may have allowed a more complete assessment of the leaf morphological response in terms of reductions to cell number and size and perhaps also an evaluation of the possible influence of leaf area reduction on the response of primordial leaves.

Subsequent comments on the observed rapid recovery rates of leaves rewatered after brief periods of stress (Slatyer 1973), suggested that there was a mere expansion requirement of those cells which divided during water stress. As Slatyer gave no final values of leaf area or dry weight nor of cell number or size, it is not possible to indicate what effects reduction to the rate of cell division or to the number and size of cells during water stress, would have had on final leaf size.

To fully consider all these possibilities—(effects of 'source' limitation and the interaction between developmental stage of tissue and water stress), a timing of events was obtained for both the vegetative and floral stages of the sunflower apex so that stress effects could be conveniently interpreted in relation to the initiation and subsequent development of the organs. The timing of events included (a) the scoring of time interval for leaf initiation at the apex and their subsequent unfolding from the bud, (b) the phasing of cell division and cell enlargement in a leaf in relation to its initiation, unfolding and subsequent expansion growth, (c) the time interval between the initiation of disc florets and (d) developmental events within disc florets before and after anthesis. Most of these data are presented in Chapter 3, which also includes a description of a growth system which is suited for growing sunflower cultivars in the available controlled environment cabinets.

Initially, the timing of cell division (monitored in terms of the mitotic index) in relation to leaf growth, was done at 1100 hours; this was
later found not to be a suitable time for making mitotic counts because there was a diurnal rhythm for the process. Thus, from time to time, it has been necessary to refine and reassess many of the basic techniques throughout the study. Nevertheless, the calibration graphs for leaf growth and floret growth, has allowed the accurate imposition of water stresses at specific developmental stages of individual organs including meristematic parts.

The experimental approach in this thesis has adopted a move from the examination of water stress effects on plant growth in a generalized glasshouse situation that involved an examination of 6 representative herbaceous dicotyledonous plants to a detailed examination of one of them (the sunflower) under controlled environment. The results obtained with the sunflower under controlled environment were checked for reproducibility in the glasshouse (experiment 7 and 14) and in the field (experiment 16).

The initial experiments on leaves included a short term recovery phase (experiments 6 and 7) but these were followed by experiments in which recovery periods lasted several weeks. This was done with a view to assessing the recovery capacities of individual organs which were at various stages of development during water stress. This approach was also necessary to evaluate if stress responses and stage of development were closely related. Such long term recovery experiments on both leaves and achenes have allowed a comparison of their respective responses, especially with regard to the interaction between the developmental stage and water stress effects.

The main results obtained from leaf responses to water stress have been presented in Chapter 4 (consisting of 4 sub-chapters). Because the results presented in Chapter 4 were derived from similar types of leaf experiments, common results from each of these experiments have been
pooled to constitute a sub-chapter (for example, Chapter 4(1) contains the water relations data obtained from all the leaf experiments). The tables, figures and plates throughout the thesis have been numbered according to the respective chapters that they appear in. The main results obtained from the study of achene responses to water stress are presented in Chapter 5. The general discussion in this thesis performs the function of relating the main results obtained with leaves and achenes, and essentially connects all the individual experimental chapters.

This general introduction does not include an exhaustive survey of the literature nor is there a literature review section in the thesis. Because it was considered that a reasonable evaluation of the present results of the thesis can be obtained by a direct comparison with published information of the sunflower and other crop plants, a consideration of the literature on water stress and plant morphogenesis has been undertaken in the chapter discussions and in the general discussion, where the main results of the thesis are also discussed.
CHAPTER 2
RESPONSES OF STOMATA, TRANSPERSION, NUTRIENT UPTAKE AND GROWTH OF SIX DICOTYLEDONOUS SPECIES DURING WATER STRESS AND RECOVERY

2.1 INTRODUCTION

This experiment was done with two objectives - (1) to study some broad responses of herbaceous plants to water stress and (2) to test the feasability of some basic experimental techniques on which future experimentation would be based. The investigation involved the stressing of six plant species viz: soyabean, tomato, cotton, sunflower, buckwheat and mungbean by briefly withholding the supply of water to them. These species are herbaceous dicotyledons and are important summer crops in Australia. As these crops commonly experience water stress under non-irrigated conditions, a study of their responses to water stress is important.

It is of wider relevance to consider the growth responses of a representative species of plants rather than in one single species. However, it is difficult to examine detailed responses in all the six plant species. Moreover, the large differences in morphology and
flowering behaviour among the species will complicate any investigation that is aimed at studying comparative responses. The tomato and cotton are strongly indeterminate plants in that inflorescence production is phased through a long period in time during which considerable vegetative growth also occurs. In the soyabean, buckwheat and mungbean, this is less so. More determinate soyabean varieties can however be found. The sunflower is probably the most determinate in that sense; there is only a single terminal inflorescence in modern cultivars. The vegetative and floral phases in the sunflower are well demarcated, although some growth of last formed leaves occurs in the first 20 days of inflorescence growth.

Although the sunflower offers itself as an easily manipulable plant, its suitability as an experimental plant has to be assessed. If suitable, it can be used to delineate spheres of more general interest which can then be tested on the other dicotyledonous species. Such a test is not the objective of this thesis and will have to be conducted later. However, to facilitate any future test of this nature, it is necessary to compare some basic responses of the sunflower to water stress with the responses of the other five species. This would reveal any major or particular differences that might exist between the sunflower and the other crops, at least in terms of basic growth and physiological patterns during water stress and subsequent recovery. Future work may then take into account such differences that might exist.

It is also the aim of this experiment to ensure that the droughting cycle which is used does not cause long-term impairment to stomatal physiology. This will eliminate any residual influences of an impaired stomatal mechanism on further growth of the plant. In short, the droughting cycle has been designed to create predominantly direct influences on plant growth.
2.2. MATERIALS AND METHODS

The experiment was conducted in a glasshouse. The plant species used were grown in pots. The species were:

(a) Soyabean - Beeson group 2
(b) Tomato - Grosse Lisse
(c) Cotton - D.P.L. 16
(d) Sunflower - Hysun 30
(e) Mungbean - Berken
(f) Buckwheat

2.2.1. Site of the experiment and prevailing conditions

The investigation was conducted in a lightly whitewashed glasshouse. The whitewash reduced the light intensity within the glasshouse and helped to slow down the rate of soil moisture depletion that occurred through leaf transpiration. Prevailing temperatures and humidity during the experiment were measured with a thermohygrometer. The data for temperature and humidity are shown in figure 2.1.a and b.

2.2.2. Preparation of pots and the soil

6 inch diameter pots (each weighing 82 g) were dressed with plastic liners. They were filled with 1473 g oven dry weight of Nelango loam (sieved) soil obtained from a site at Ginninderra Experimental Station, CSIRO, Canberra. The field capacity of this soil was determined to be in the vicinity of 30% oven dry weight (by sticky point determination - see Appendix B.2). 120.5 mg superphosphate as \( \text{NH}_4\text{H}_2\text{PO}_4 \) and 60 ppm Nitrogen, 50.2 ppm Nitrogen as \( \text{NH}_4\text{NO}_3 \) (the rest in the phosphate salt)
Figure 2.1 a. shows the average temperature in the glasshouse over 24 hour cycles, throughout the experiment.

Figure 2.1.b. shows the average humidity over 24 hour cycles. Note the increase in humidity from day 36 onwards.

\[\downarrow\] = start of germination trial \hspace{1cm} \[\downarrow\] = start of main experiment
A

![Graph A]

B

![Graph B]
were added to each pot. The pots were brought up to field capacity by pouring the requisite amount of distilled water down an inverted glass funnel.

2.2.3. Attainment of similar germination dates for the species and plant selection

An initial germination test was conducted and the pattern of germination was recorded for all the species. In the main experiment, the sowing dates were staggered so that germination dates of the six species roughly coincided. 10 to 15 seeds were sown in each pot and covered with 250 g of gravel. This was done before raising the pots to field capacity. The plants were thinned by removing the smallest and largest plants in turn until a desired single healthy plant remained. Size and positional differences in the glasshouse were further controlled by categorizing the plants into size groups and randomizing plants within a group as often as possible.

2.2.4. Glasshouse procedure

The windows and roof vents of the glasshouse were opened at 0830 hours and closed at 1750 hours. When water stress treatments were commenced, the glasshouse floor was kept wet all day by hosing it with water.
2.2.5. Commencement of water stress cycles and plant analysis

When the plants were 22 days old (from emergence), the water stress treatments were started. At 22 days from emergence, all the plant species had reached a sufficient size and were transpiring a significant volume of water. However, there were some differences in both the physiological age and morphological size between the plant species. The experiment had 108 plants arising from 2 treatments, 3 replicates, 3 harvests and 6 species. The first harvest was made on day 22 (from emergence) and it coincided with the start of the water stress treatments. The second harvest varied for the species because of the different times taken to reach the wilted state. The plants that were to recover from the wilted state were re-watered at the second harvest. The third harvest was made 6 days after rewatering.

A daily record of water consumption for the controls and water stressed plants was kept. Three pots without plants gave a rough estimate of the evaporation from the soil. Harvested plants were separated into stems, leaves and floral parts. Petioles were included with stems. Fresh and dry weights of plant components were determined in the usual way; dry weights were obtained after drying fresh samples at 80°C for 24 hours. The replicates of each treatment within a harvest were bulked for further analysis of nitrogen and phosphorus. The leaf samples were ground separately from the stem. Nitrogen and Phosphorus were determined by micro-kjeldahl digestion using selenium as catalyst and followed by colorimetric determination in the Technicon autoanalyser.

2.2.6. Measurement of stomatal resistance

The response of the adaxial and abaxial stomata before, during and after the water stress treatment, was monitored with a ventilated
diffusion porometer (Byrne et al'70) equipped with a self-timer that recorded the response time of the needle between 2 and 6 µA on the humidity scale of the porometer. The measurement had to be made on a suitable leaf. An initial study demonstrated that the second or third youngest almost fully expanded leaf was suitable. Measurements were made three times a day (in the morning, noon and late noon) on all 36 plants that were destined for the last harvest. (For details of resistance measurements, please see Appendix B.21). The resistance has been expressed as conductance ($C = \frac{1}{R}$).

2.3. RESULTS

The responses during water stress and during recovery are described together for each of the variables that was studied. The primary data (2.3.1 to 2.3.8) are described first and then the derived data (2.3.9 to 2.3.11) are described.

2.3.1. Days to harvest and depletion of soil water content

The number of days to each harvest for the different species is shown in table 2.1. This table also shows the total moisture depleted by the different species at the end of the water stress cycle (harvest 2). The depletion of water from the soil to the wilting point varied from 2 days for the sunflower to 7 days for the mungbean. The plants were kept at the wilted stage for 1 day before rewatering. The course of soil water depletion is shown in figure 2.2. (a-f). Figure 2.2 (a-f) also shows the transpiration of the recovering water-stressed plants. The transpiration of the recovering plants has been expressed
Figures 2.2 a–f. show the amount of water (in ml.) transpired by the different species (for controls □—□ (C) and water stressed —– (W) plants) before during and after the drying cycle. The plants were 22 days old when the drying cycle began (WC). The plants were rewatered (RW) when the stressed plants stopped transpiring significant amounts of water. The low transpiration values for the controls on some days (during the recovery phase of the water stressed plants) coincided with low atmospheric demand, also with overcast conditions in the environment. The data points are means of three replicates.
as a percentage of the controls in figure 2.3. Figure 2.3. does not include data for days when the evaporative demand was low.

Table 2.1. Days to harvest (from emergence) and total soil moisture depleted

<table>
<thead>
<tr>
<th>Plant</th>
<th>Days</th>
<th>HARVEST</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Soyabean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
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<td>27</td>
<td>33</td>
</tr>
<tr>
<td></td>
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<td>0</td>
<td>0</td>
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<td></td>
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<td></td>
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<td>299</td>
<td>0</td>
</tr>
<tr>
<td>Mungbean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>30</td>
<td>36</td>
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<td>0</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>0</td>
<td>324</td>
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</tr>
<tr>
<td>Buckwheat</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>33</td>
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<td></td>
<td></td>
<td>0</td>
<td>297</td>
<td>0</td>
</tr>
</tbody>
</table>

SWD(C) = Soil water deficit of CONTROL POTS
SWD(W) = Soil water deficit of WATER STRESSED POTS
Figure 2.3. The transpiration values of the plant species that were previously water stressed have been expressed as a percentage of the transpiration values of their respective controls. The data for days when the evaporative demand was low are not included in the figure. For example, in tomato, the transpiration values on the 2nd and 4th days after rewatering (↓) are not included. Data points are means of three replicates.
100 = REWATERED

Days from start of drying cycle

ml. of water transpired (% of control) during recovery

Sunflower
Buckwheat
Tomato
Cotton
Soyabean
Mungbean
2.3.2. Effects on leaf turgidity during the drying cycle and recovery

No measurements of turgor potential are given here because neither \( \psi \) (water potential) nor \( \pi \) (osmotic potential) were measured. Leaf turgidity was observed visually. The older leaves in sunflower lost their turgid condition sooner than younger developing leaves. This was also seen in buckwheat, tomato and cotton. This response was less marked in the soyabean and mungbean. Upon rewatering, the younger developing leaves of all species, except soyabean, regained turgidity within two hours. In the soyabean, the young leaves were slow to respond to the rewatering; there was also some evidence of permanent death to leaf tissue.

2.3.3. Effects on leaf and stem fresh weight

During the drying cycle, there was a significant increase in the fresh weight of tomato leaves. In contrast, there was a decrease for soyabean and sunflower leaves. For buckwheat, mungbean and cotton, there was some increase but this was not significant. In comparison to the controls, however, there was a significant reduction in leaf fresh weight for all the species at the end of the drying cycle. 6 days after rewatering, leaf fresh weight in sunflower and buckwheat recovered fully but not in the other species. The stem fresh weight at this time, however, was reduced significantly in all the species. These data are presented in table 2.2.

2.3.4. Effects on leaf and stem dry weight

During the drying cycle, all species except sunflower had a
Table 2.2. Response of leaf and stem fresh weight in the six species to water stress and recovery.

<table>
<thead>
<tr>
<th>HARVEST</th>
<th>Soyabean</th>
<th>Tomato</th>
<th>Cotton</th>
<th>Sunflower</th>
<th>Mungbean</th>
<th>Buckwheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1* C</td>
<td>3.82</td>
<td>3.83</td>
<td>4.70</td>
<td>6.69</td>
<td>2.54</td>
<td>3.77</td>
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<tr>
<td>W</td>
<td>4.49</td>
<td>3.65</td>
<td>4.92</td>
<td>7.00</td>
<td>2.24</td>
<td>4.02</td>
</tr>
<tr>
<td>2** C</td>
<td>7.88</td>
<td>16.26</td>
<td>10.94</td>
<td>8.76</td>
<td>7.47</td>
<td>8.85</td>
</tr>
<tr>
<td>W</td>
<td>3.38</td>
<td>5.29</td>
<td>4.27</td>
<td>4.99</td>
<td>3.04</td>
<td>4.09</td>
</tr>
<tr>
<td>3 C</td>
<td>13.36</td>
<td>27.34</td>
<td>15.65</td>
<td>12.16</td>
<td>10.84</td>
<td>10.73</td>
</tr>
<tr>
<td>W</td>
<td>7.06</td>
<td>16.34</td>
<td>10.25</td>
<td>11.38</td>
<td>6.25</td>
<td>10.17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HARVEST</th>
<th>Soyabean</th>
<th>Tomato</th>
<th>Cotton</th>
<th>Sunflower</th>
<th>Mungbean</th>
<th>Buckwheat</th>
</tr>
</thead>
<tbody>
<tr>
<td>1* C</td>
<td>0.53</td>
<td>0.48</td>
<td>0.38</td>
<td>1.06</td>
<td>0.26</td>
<td>0.79</td>
</tr>
<tr>
<td>W</td>
<td>0.58</td>
<td>0.46</td>
<td>0.38</td>
<td>1.09</td>
<td>0.25</td>
<td>0.81</td>
</tr>
<tr>
<td>2** C</td>
<td>0.86</td>
<td>1.14</td>
<td>0.81</td>
<td>1.22</td>
<td>0.75</td>
<td>1.32</td>
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<tr>
<td>W</td>
<td>0.47</td>
<td>0.67</td>
<td>0.40</td>
<td>0.95</td>
<td>0.35</td>
<td>0.86</td>
</tr>
<tr>
<td>3 C</td>
<td>1.14</td>
<td>1.36</td>
<td>1.00</td>
<td>1.46</td>
<td>0.93</td>
<td>1.42</td>
</tr>
<tr>
<td>W</td>
<td>0.83</td>
<td>1.11</td>
<td>0.68</td>
<td>1.32</td>
<td>0.66</td>
<td>1.29</td>
</tr>
</tbody>
</table>


Standard errors of differences of means

A

leaf fresh weight 0.520
stem fresh weight 0.036

B

leaf fresh weight 0.500
stem fresh weight 0.035

A: s.e. for comparing 2 species at the same level of harvest and treatment or 2 treatments at the same level of harvest and species

B: s.e. for comparing 2 harvests at the same level of treatment and species
Figures 2.4. a-f. show the response of the dry weight of the stem (S) leaf (L) and total dry weight of leaf and stem (TDW) of controls (C) and water stressed (W) plants of the six species. For example, (CS) stands for the stem dry weight of the control. The plants were 22 days old when the drying cycle commenced (WC) and were rewatered (RW) when all the available water had been transpired. Least significant values (P=0.05) for some of the data points are shown below. Data points are means of three replicates.

<table>
<thead>
<tr>
<th>Leaf dry weight:</th>
<th>LSD 5%</th>
<th>A</th>
<th>LSD 1%</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.1699</td>
<td>0.1620</td>
<td></td>
</tr>
<tr>
<td>Stem dry weight:</td>
<td>LSD 5%</td>
<td>0.0725</td>
<td>0.0765</td>
<td></td>
</tr>
<tr>
<td></td>
<td>LSD 1%</td>
<td>0.0967</td>
<td>0.0901</td>
<td></td>
</tr>
</tbody>
</table>

A = LSD value for comparing (i) 2 species at the same level of harvest and treatment
(ii) 2 treatments at the same level of harvest and species

B = LSD value for comparing 2 harvest at the same level of treatment and species.
SOYABEAN

MUNGBEAN

SUNFLOWER

BUCKWHEAT

TOMATO

COTTON
significant increase in the dry weight of leaves. In comparison to the controls, however, there was a significant reduction in dry weight of leaf and stem for all species at the end of the drying cycle. Like the recovery in leaf fresh weight, buckwheat and sunflower showed a similar recovery in dry weight of leaves, 6 days after rewatering. Stem dry weight was reduced in all species at this time. These data are presented in figure 2.4. (a-f).

2.3.5. Effects on stomatal conductance

With the decline in soil water content, the conductance of both abaxial and adaxial stomata were reduced to the point of zero conductance (figure 2.5. a-f). In all species, the adaxial stomata closed sooner than the abaxial stomata. This was particularly pronounced in tomato leaves in which adaxial stomata closed 5 days before the abaxial stomata did. Large differences also existed between the conductances of abaxial and adaxial stomata within the control plants of some of the species. The stomatal conductance of abaxial and adaxial surfaces was almost similar in sunflower and mungbean but differed markedly for the two surfaces in cotton, buckwheat, tomato and soyabean. In the latter named species, the adaxial stomatal conductance was less than half of the abaxial stomatal conductance.

After rewatering, stomatal conductance of both leaf surfaces began to be re-established. However, the re-establishment of conductance occurred more quickly for the abaxial stomata than for adaxial ones. Generally, stomatal conductance of abaxial stomata was immediately re-established after rewatering. This response was delayed by 2 or 3 days for adaxial stomata, especially in soyabean. In most cases, there was evidence for
Figure 2.5. a-f. show the response of the stomatal conductance of both the abaxial and adaxial leaf surfaces before, during and after the water stress in the six species. Plants were 22 days old when the drying cycle commenced (WC) and they were rewatered (RW) when all the available water in the soil had been transpired. The stomatal response was measured as a resistance but has been expressed as conductance (reciprocal of resistance). Data points are an average of three replicates.

Cab = Control abaxial stomata
Cad = Control adaxial stomata
Wab = Water stressed abaxial stomata
Wad = Water stressed adaxial stomata
MUNGBEAN

COTTON

SUNFLOWER

SOYABEAN

BUCKWHEAT

TOMATO

STOMATAL CONDUCTANCE (cm sec⁻¹)

DAYS
complete recovery of the stomatal mechanism, except perhaps in soyabean. In the soyabean, stomatal recovery was slow and not complete.

2.3.6. Relationship between stomatal conductance and soil water content

To arrive at a common basis for comparing stomatal conductances of the six species, the stomatal data was plotted against the declining field capacity which occurred with soil water depletion. Figure 2.6. a shows the response of the abaxial stomata and figure 2.6.b shows that of the adaxial. Complete stomatal closure occurred on both leaf surfaces at between 30% and 40% field capacity. This is equivalent to 11% oven dry weight of the soil and is in agreement with the wilting point of this soil. Mungbean and cotton maintained higher conductances over a wide range of field capacity. In contrast, below 50% field capacity, conductances in tomato, soyabean, sunflower and buckwheat were drastically reduced.

2.3.7. Effects on the uptake of Nitrogen and Phosphorus

Water stress reduced the uptake of nitrogen and phosphorus (figure 2.7.a-f). The net amount of nitrogen and phosphorus in the recovering plants was less than the control plants. However, the amount of nitrogen and phosphorus per unit dry weight of leaf, stem or whole plant was not affected by water stress, except perhaps the phosphorus content in water stressed cotton plants (table 2.3).
Figure 2.6. a. shows the response of the abaxial stomata of the water stressed plant species, during the drying cycle (expressed as a percentage of the stomatal conductance of the abaxial stomata of their respective controls), in relation to the field capacity of the soil at that time.

Figure 2.6. b. shows the response of the adaxial stomata of the water stressed plant species, during the drying cycle (expressed as a percentage of the stomatal conductance of the adaxial stomata of their respective controls), in relation to the field capacity of the soil at that time.

Soil field capacity has been calculated as a percentage i.e. (amount of soil water left / total water at field capacity) X 100.
Figure 2.7. a-f. show the absolute (abs) amount of nitrogen (N) and phosphorus (P) in the plant (leaf and stem) at the start of the drying cycle (WC), at the end of the drying cycle when the plants were rewatered (RW) and six days after rewatering. Plants were 22 days old when the drying cycle began. Each data was obtained from a bulked sample of three replicates. Wilt stands for water stressed plants.
Table 2.3. Nitrogen and phosphorus content per unit dry weight of plant at the end of recovery (g nutrient/g dry weight)

<table>
<thead>
<tr>
<th>Plant</th>
<th>Nitrogen</th>
<th>Phosphorus</th>
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</thead>
<tbody>
<tr>
<td>Soyabean</td>
<td>.042</td>
<td>.0033</td>
</tr>
<tr>
<td>W</td>
<td>.043</td>
<td>.0032</td>
</tr>
<tr>
<td>Tomato</td>
<td>.039</td>
<td>.0031</td>
</tr>
<tr>
<td>W</td>
<td>.044</td>
<td>.0033</td>
</tr>
<tr>
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<td>Mungbean</td>
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<td>W</td>
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<td>.0027</td>
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<tr>
<td>Buckwheat</td>
<td>.032</td>
<td>.0054</td>
</tr>
<tr>
<td>W</td>
<td>.039</td>
<td>.0044</td>
</tr>
</tbody>
</table>

2.3.8. Effects on number of flowers and on their dry weight

The number of flowers was reduced by water stress in most of the species; this was not investigated in buckwheat and sunflower. In soyabean, tomato and mungbean, the number of flowers in the water-stressed plants was reduced by over 50%. In the sunflower, the size of the inflorescence was reduced but no measure of this was taken. Dry weights of the flowers was also reduced markedly. These data are presented in table 2.4.
Table 2.4 Effects of water stress on floral parts

<table>
<thead>
<tr>
<th></th>
<th>Dry weight of floral parts (g)</th>
<th>Number of flowers</th>
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<tr>
<td></td>
<td>Harvest 1*</td>
<td>Harvest 2**</td>
</tr>
<tr>
<td>Soyabean</td>
<td>C -</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>W -</td>
<td>-</td>
</tr>
<tr>
<td>Tomato</td>
<td>C -</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>W -</td>
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</tr>
<tr>
<td>Cotton</td>
<td>C -</td>
<td>-</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>Sunflower</td>
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<td>-</td>
</tr>
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<td>Mungbean</td>
<td>C -</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>W -</td>
<td>-</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>C .0321</td>
<td>.1491</td>
</tr>
<tr>
<td></td>
<td>W .0400</td>
<td>.0930</td>
</tr>
</tbody>
</table>

* water stress commenced

** plants rewatered

*** ray florets and disc florets of the sunflower and the numerous flowers of the buckwheat were not counted.

† No flowers in the first 2 harvests for all species except buckwheat.

2.3.9. Effects on transpiration/unit dry weight of leaf

The amount of water transpired on the last day of recovery (the third harvest) was divided by the leaf dry weight of the plant on that day. The transpiration rate, calculated in this way was unaffected in
all the species except soyabean and to some extent in cotton. In soyabean and cotton, the transpiration/unit weight of leaf was reduced (table 2.5).

Table 2.5. Transpiration unit weight\(^{-1}\) of leaf on the last day of recovery (harvest 3)

<table>
<thead>
<tr>
<th>CONTROL</th>
<th>WATER STRESSED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf dry weight</td>
<td>ml. water transpired</td>
</tr>
<tr>
<td>Soyabean</td>
<td>2.197</td>
</tr>
<tr>
<td>Tomato</td>
<td>2.803</td>
</tr>
<tr>
<td>Cotton</td>
<td>2.119</td>
</tr>
<tr>
<td>Sunflower</td>
<td>1.465</td>
</tr>
<tr>
<td>Mungbean</td>
<td>1.910</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>1.326</td>
</tr>
</tbody>
</table>

2.3.10. Effects on relative growth rate of leaves and stem

The relative leaf growth rate (RLGR), relative stem growth rate (RSGR) and the relative growth rate of both stem and leaves (RGR) are presented in table 2.6. The rates are presented as g g\(^{-1}\) day\(^{-1}\) and includes responses of the rates during the phase of water stress and during the phase of recovery. RLGR was less than RSGR during water stress for all the species. During recovery, RSGR was less than RLGR; marginal exceptions to this are sunflower and buckwheat.
Table 2.6. Effects of water stress on the relative growth rates of leaves, stem, leaves + stem (g g\textsuperscript{-1} day\textsuperscript{-1})

<table>
<thead>
<tr>
<th>Species</th>
<th>During water stress</th>
<th>During recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RLGR</td>
<td>RSGR</td>
</tr>
<tr>
<td>Soyabean</td>
<td>C</td>
<td>0.134</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>0.046</td>
</tr>
<tr>
<td>Tomato</td>
<td>C</td>
<td>0.215</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>0.112</td>
</tr>
<tr>
<td>Cotton</td>
<td>C</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>0.049</td>
</tr>
<tr>
<td>Sunflower</td>
<td>C</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>0.041</td>
</tr>
<tr>
<td>Mungbean</td>
<td>C</td>
<td>0.135</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>0.084</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>C</td>
<td>0.181</td>
</tr>
<tr>
<td></td>
<td>W</td>
<td>0.063</td>
</tr>
</tbody>
</table>

RLGR = Relative leaf growth
RSGR = Relative stem growth rate
RGR = Relative growth rate (leaves + stem)
C = Control
W = Water-stressed

2.3.11. Effects on water content of leaves and stem

The water status of the plant in all species was reduced by water stress. This was manifested by a lowering of tissue water content of
### Table 2.7a. Effect on water content of leaf in water stressed plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>At the end of water stress (% control)</th>
<th>At the end of recovery (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soyabean</td>
<td>51.42</td>
<td>80.06</td>
</tr>
<tr>
<td>Tomato</td>
<td>65.17</td>
<td>11.95</td>
</tr>
<tr>
<td>Cotton</td>
<td>53.74</td>
<td>98.89</td>
</tr>
<tr>
<td>Sunflower</td>
<td>64.48</td>
<td>104.50</td>
</tr>
<tr>
<td>Mungbean</td>
<td>63.26</td>
<td>112.22</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>76.99</td>
<td>95.65</td>
</tr>
</tbody>
</table>

### Table 2.7b. Effect on water content of stem in water stressed plants

<table>
<thead>
<tr>
<th>Plant</th>
<th>At the end of water stress (% control)</th>
<th>At the end of recovery (% control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soyabean</td>
<td>44.62</td>
<td>108.42</td>
</tr>
<tr>
<td>Tomato</td>
<td>48.98</td>
<td>126.11</td>
</tr>
<tr>
<td>Cotton</td>
<td>42.11</td>
<td>75.15</td>
</tr>
<tr>
<td>Sunflower</td>
<td>61.63</td>
<td>93.23</td>
</tr>
<tr>
<td>Mungbean</td>
<td>45.55</td>
<td>95.24</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>65.79</td>
<td>125.22</td>
</tr>
</tbody>
</table>
both leaves as well as stems. Rewatering re-established the water status of both leaves and stems; the exception to this were leaves of soyabean. The differences between species and the extent to which desiccation and recovery occurred (expressed as a percentage of controls) is shown in table 2.7a and 2.7b.

2.4. DISCUSSION

The discussion first considers some general aspects of the results. It then appraises some of the more specific aspects of plant behavior.

As the result of differences in leaf and root morphology of the species and the different degrees of stomatal conductance, the rate at which water was transpired differed for the species. The time taken to reach the wilting point consequently differed for the species. Although this may have caused some differences in the total amount of stress suffered by each species, the differences may have been only marginal. This is in part supported by the stomatal data (figure 2.5 a-f); for species that depleted water slowly, stomatal conductance was maintained for at least 3 days before any response to water stress occurred. Therefore, the periods during which the stomata were closed in species that either depleted water quickly or slowly from the soil, were only marginally different. The preceding argument will be valid if the period of stomatal closure is taken as an indicator of water stress. However, the valid basis for comparison under these circumstances becomes a response to one day of actual wilting (the plants were kept in a wilted state for one complete day) irrespective of the course taken to reach the wilting point. Wilting describes a physiological state in the plant and at this state, the physiological responses of the species can be compared.
The physiological responses of the six species were not markedly different. Between most species, there were only small differences in the magnitude of the response but not in the pattern. The only species that did exhibit marked differences from the other species in the magnitude of the responses was soyabean. Stomatal closure during water stress and stomatal opening after rewatering were common features among the species. Other responses that the species had in common included (1) the general depression in the growth of leaves and the stem during water stress and a resumption of growth when water was available again. During this time, the leaves were affected more during water stress but recovered in preference to the stem when water was supplied again to the plant, (2) the uptake of nitrogen and phosphorus were limited by water stress but resumed during recovery, (3) floral production and growth was reduced by stress. The more specific aspects of the results will now be discussed. The specific aspects are best discussed under headings.

2.4.1. Stomatal responses

The reasons for the quicker response of adaxial stomata to water stress are not very clear. However, the recent work of Brown, Jordan and Thomas (1976) with cotton implicates a lack of osmotic adjustment in the guard cells of adaxial stomata as being the cause. Delays in the opening of the stomata and recovery of photosynthesis have been reported by Sanchez Diaz and Kramer (1971) and by Levoys and Kriedemann (1973). The slower response of adaxial stomata to rewatering may be due to their remaining closed for longer, hence suffering a higher total stress. Indeed, the delay of stomatal opening in tobacco and bean leaf discs
placed on water to recover from water stress was shown to be dependent on the degree and duration of the stress treatment (Fischer et al. 1970). In another study, the major after effect of water stress was shown to be due to the persistence of abscisic acid, which had a direct effect on guard cells (Allaway and Mansfield 1970).

Recovery of stomatal conductance of both leaf surfaces occurred in all species except soyabean. This may be due to the poor functioning of stomatal cells in soyabean, even after rewatering; given more time, they may have recovered although this is quite unlikely. Alternatively, many of the stomatal cells may have incurred permanent injury. A similar reference to permanent injury of guard cells arising from water stress was reported by Iljin (1957). The apparently greater effect of water stress on soyabean stomata is not because the plant had suffered a greater duration or magnitude of stress. Apart from the sunflower, the other species had been without water for as long if not for longer than the soyabean; yet recovery of stomatal conductances was better in the other species compared to soyabean.

2.4.2. Leaf and stem responses

The dry weight increase for all species, except sunflower, between the start of water stress (harvest 1) and visible wilting (harvest 2) can be attributed to a continuation of photosynthesis during that time; the drying cycle was slower in the other species as compared to sunflower, allowing stomatal conductance to be maintained for some time. Continuation of photosynthesis is thus possible in these species, especially in cotton and mungbean in which stomatal conductance is still quite persistent at soil field capacities approaching as low as 16%. In
contrast, the sunflower depleted the available soil water quickly and caused rapid stomatal closure; as such no significant amount of photosynthate could be added during the drying cycle.

The rapid closure of the adaxial stomata in tomato may be a characteristic of this plant to prevent rapid loss of water. Indeed, this may explain the significant increase in leaf fresh weight for tomato between the start of water stress and visible wilting.

The recovery of leaf fresh and dry weights in the sunflower and the buckwheat may be attributed to the following. In the buckwheat, a flush of new leaves was produced soon after rewatering. Recovery of the sunflower leaf fresh and dry weight may be due to the short duration of water stress in this plant species. Alternatively, the recovery of the leaf component in the sunflower may be explained in terms of specific stages of sensitivity to water stress in leaf development. If there is any merit in this postulate, then only certain leaves in the sunflower would be affected by water stress, because at any time the leaves in the sunflower are various different stages of development. This postulate may also explain the greater reduction in leaf fresh and dry weights in the other species. Leaf production in the soyabean, mungbean and cotton is such that several leaves would be of the same close order in chronological and physiological age. It is not unreasonable to expect a greater number of leaves to be affected in these species.

2.4.3. Transpiration

The similarity between controls and water-stressed plants in the transpiration/unit leaf dry weight (table 2.5) and the recovery of stomatal conductance (figure 2.5.a-f), indicate a reduction in plant
leaf area, although no actual leaf area measurements were made.

Similarly, Gates (1955a) for tomato leaves, Loustalot (1945) for pecan leaves, Schneider and Childers (1941) for apple leaves found that the rates of transpiration of selected leaves approached the control rate after rewatering from the wilted condition. It can be concluded that the period of water stress in this experiment, did not effect any economy in subsequent water usage per unit leaf weight. This is contrary to the commonly held view that wilting 'adapts' plants to be economical in the subsequent use of water when they are rewatered.

The persistence in the transpiration recovery of sunflower and buckwheat is probably due to the small effect of water stress on leaf growth and the rapid recovery of stomatal conductance. Low evaporative demands on several days during the recovery period of tomato, mungbean and cotton reduced the transpiration capacity. There is also one case where the recovery in transpiration may be brought about by other means. For example, the reduction of leaf area differences between the controls and water-stressed plants in the buckwheat arose partly because the control plants of the buckwheat came into flower early, and therefore probably tended to use less assimilate for leaf growth. The recovering water-stressed plants which were still using assimilates for active leaf growth, were able to reach near control values of transpiration.

2.4.4. Floral initiation and development

The reduction in the number of flowers of water stressed plants is an important result so far as the productivity of the crop is concerned. In species where the majority of flowers is initiated at
about the same time (determinate flowering), the effects of water stress on the number of flowers would be to cause a large reduction. In non-determinate flowering species, such an effect may be less severe, but the results of the present experiment are too limited to confirm this. However, it may be interesting to test if the initiation and development of new flowers is affected by previous stress history in indeterminate flowering species. Long term experiments are needed to gauge the final responses of reproductive parts to water stress.

2.4.5. Nutrient uptake and distribution

The data indicates that during water stress, the uptake of both nitrogen and phosphorus is reduced. As no analysis of the nutrient content of the roots was made in this experiment, there is no data to confirm or discard the possibility that uptake of nutrient into the root was unaffected but that translocation to the aerial parts of the plant was. The percentage distribution of nitrogen and phosphorus generally in preference to the stem during water stress and in preference to the leaf after rewatering, is similar to the findings of Gates (1957) on the movement of nitrogen and phosphorus from laminar to non-laminar portions of the leaf during water stress in tomato leaves (see table C.5. in Appendix C).

For these nutrients to move preferentially into the stem during water stress suggests that the normal tendency for leaves to synthesize soluble protein and phosphorus into more highly organized compounds may have been checked, especially in the older tissues, thus giving an increasing proportion of hydrolytic breakdown products. Maximov (1941) has claimed that water stress leads to an enzymatically produced
intensification of hydrolytic decomposition dependent on the physical state of the cell enzymes and Petrie and Wood (1933) have shown that the net rate of protein hydrolysis increases with the reduction of leaf water content. Such hydrolytic changes in the individual leaves would be a reversal of their normal metabolic state, reminiscent of a senescent change.

2.4.6. Conclusions

Since the broad responses exhibited by these dicotyledons are quite similar, it is logical to select one species among them for more precise and detailed evaluation of the morphological responses to water stress. It is also evident from this experiment that innumerable difficulties will be encountered if a large comparative experiment involving several species, is attempted. The differences in plant organization and structure, together with the rate of stress incurrence will make it a very difficult if not an impossible experiment to conduct. For instance, it will be a forbidding task to ensure that the chronological and physiological ages are comparable among the species that are used in any such comparative experiment. Therefore, the examination of specific responses in one species should precede similar examinations on other species of the group.

For this purpose, the sunflower is a suitable plant. Apart from being an important summer crop, the growth physiology of the sunflower makes it a suitable plant on which to measure precise and detailed effects of water stress. The sunflower has distinct vegetative and floral phases; leaves are initiated first, followed by involucre bracts and then by disc florets. Because this allows the identification of
age of leaves and disc florets, either in terms of chronological or physiological age, the interpretation of water stress effects on leaf and disc floret development in those terms is facilitated.

The drying cycle that was employed in this experiment is quite suitable for further use. The internal plant water stresses caused by the soil drying cycle are not severe enough to cause permanent injury to the stomatal mechanism or to plant tissue in general. The soyabean responded to the stress differently and has been an exception in this regard. Establishment of stomata functions and the observed high relative growth rates during recovery indicate that no long term impairment to plant functions was caused by the magnitude of the water stress. The drying cycle is thus suitable for investigations on the direct influences of water stress, and minimises the secondary and tertiary effects that can occur. For instance, nutrient deficiency will not be a cause of the morphological responses because nutrient content/unit weight of tissue is not affected. In another glasshouse experiment (which has not been reported in this thesis), the responses of cotton and sunflower to a water stress imposed at a later developmental stage showed the same general trends. Some hastened senescence of the lower leaves was noticed in both cotton and sunflower in that experiment, and as this could have effects on inflorescence development, it must be investigated in future.

The main inadequacy of this experiment with the six species is the inability to state the exact internal plant stresses that were suffered by each plant species. The use of soil moisture deficit measurements takes no account of plant features such as root depth and density and therefore its relevance to plant performance is inevitably empirical. It will be necessary at some future stage to monitor changes
in plant water status either by the measurement of water saturation deficits, water potential ($\psi$) and osmotic potential ($\pi$). This would help to apply comparable stresses in future investigations on other dicotyledons.

For the evaluation of precise responses to water stress, the study has to be conducted under controlled environment, where light intensity, temperature and daylength can be controlled. This necessarily means that the sunflower plant has to be grown in the available 1.5 M high controlled environment cabinets. Chapter 3 considers this proposition.
The cultivar Hysun 30 was chosen for the study because it is fast replacing the traditional open-pollinated cultivars in many of the sunflower cropping areas of Australia. Studies under controlled environment could be of practical use in the cultivation of this cultivar.
CHAPTER 3

THE GROWTH RESPONSE AND TIMING OF EVENTS AT THE PLANT APEX AND IN LEAVES OF SUNFLOWER UNDER CONTROLLED CONDITIONS

3.1. INTRODUCTION

The study of the development of the inflorescence in the Giant Russian Sunflower cv. Hysun 30, may be complicated by the large size to which the plant normally grows. This often precludes the use of controlled environment cabinets and compels recourse to the field or glasshouse, with a consequent loss of reproducibility of response.

In the field, the pattern of maturation varies with the changing environment, the most important factor being temperature (Hanna 1924; Robinson 1971). The variability of anther dehiscence may be particularly high (Anderson 1975). Temperature and longer days have been shown to affect both phenological development and oil quality in the field (Doyle 1975; Keefer et al. 1976).

It was the aim of the present work to enhance plant uniformity so that environmental responses could be measured on a few plants at any desired level of detail. Controlled environment lends itself to this purpose better than does the field, but most controlled environment
work with light and temperature (Rajan et al. 1971, 1973; Rajan and Blackman 1975) and with temperature alone (Blackman et al. 1955; Hodgson 1967) has been restricted to the vegetative development of Helianthus annuus. This may be because of the large size normally attained by sunflowers at maturity. The present work extends these studies to the mature plant so it has been necessary to develop a method for growing mature plants that would develop normal inflorescences in the available 1.5 metre high cabinets. Previous experience in the field and glasshouse had suggested that this might be achieved by a correct choice of environmental factors. Such a method of approach had been found successful on a previous occasion when the development of cuttings of the Washington navel orange was studied through to fruit set by Gates, Bouma and Grovenewegan (1961a, b, c). Nutrition was found to be an important factor in the quality of mature fruit in their studies. Accordingly in this case, the interaction of nitrogen, phosphorus and potassium on the development of the inflorescence of the sunflower was also again investigated. The present study then, considers factors that are important for growing uniform mature sunflower plants of any stage of maturity in cabinets.

The effect of pot size on plant growth was also assessed so as to arrive at an optimum for pot size and the number of plants that can fit in a cabinet. Because the next phase of the studies was aimed at evaluating the effects of water stress on plant growth and development, accordingly a timing of events at the plant apex during both the vegetative and floral phases, and the timing of cell division and enlargement in a leaf from its initiation to full expansion were also obtained, so that water stress effects could be interpreted in terms of these events.

* Even with controlled work on the maize, (Voldeng and Blackman 1973), it was restricted to the vegetative development of the plant.
3.2. MATERIALS AND METHODS

Seeds of the Giant Russian Sunflower cv. Hysun 30 from Pacific Seed Company, Toowoomba, Queensland, Australia were sown in controlled environment cabinets into plastic lined pots containing 2.2 Kg oven dry Nelango loam soil from the A horizon of Association E, Ginninderra Research Station, Canberra. The moisture characteristics of this soil are: a field capacity of 43% and a permanent wilting percentage of 11.5% oven dry weight. The pots were maintained at field capacity by frequent watering and the seedlings were thinned to one plant per pot. The plants were lit with fluorescent lamps supplemented with tungsten lamps providing a quantum flux density of 650 microeinstein m\(^{-2}\) sec\(^{-1}\), 140 cm. below the light source. Cross fertilization of disc florets was aided by frequent brush transfer of pollen.

3.2.1. Experiment 1

Two environmental regimes were contrasted: one which was considered would suit growth to maturity in cabinets (Treatment A) and its direct opposite (Treatment B). Plants of Treatment A received a 14 hour photoperiod at 27°C constant temperature for 32 days, followed by a 10 hour photoperiod at 21°C/9°C, day/night temperature. The temperature change occurred immediately after disc floret initiation. In treatment B, the order of these combinations was reversed.

There were 4 replicates. Total leaf area, height, the number of unfolded leaves (for definition of an unfolded leaf - see below (3.2.2. and appendix B.7) and branch number were recorded at 14 day intervals until maturity.
Nutrient was applied at rates per pot comparable with those of the field: 380 mg \( \text{K}_2\text{SO}_4 \), 600 mg \( \text{NH}_4\text{NO}_3 \) in solution at sowing; 140 mg \( \text{CaH}_2\text{PO}_4 \) and 1.16 g \text{CaCO}_3, thoroughly mixed into the dry soil prior to sowing.

3.2.2. Experiment 2

The photoperiod-temperature regime was as in Treatment A of experiment 1, except that the temperature change at day 32 occurred gradually to ensure adjustment, over 3 days: 21/22°C, 21/16°C and 21/9°C. Plants were sampled on 27 occasions (see Fig. 3.1a and 3.1b) and events at the apex followed by dissection. Counts were made of the number of primordial, folded and unfolded leaves during the vegetative phase, and of bracts and disc florets during the floral phase.

The leaves were categorized as primordial \( (N_p) \), folded \( (N_f) \) and unfolded \( (N_{uf}) \), following the method of Paton (1969) for distinguishing between foliage and total leaves. Primordial and folded leaves refer to leaves in the bud. A leaf that has half its lamina detached from the bud is an unfolded leaf.

3.2.3. Experiment 3

Plant development to maturity was assessed in response to the interaction of phosphorus, nitrogen and potassium. The statistical design was an unreplicated factorial with 3 levels of nitrogen (0, 300 and 600 mg/pot \( \text{NH}_4\text{NO}_3 \)), 3 of phosphorus (0, 70 and 140 mg/pot \( \text{CaH}_2\text{PO}_4 \)), 2 of potassium (0 and 380 mg/pot \( \text{K}_2\text{SO}_4 \)) and a basal dressing of calcium (1.16 g \text{CaCO}_3/pot). Temperature and daylength regimes were as in Experiment 2.
During development, plant height (from the gravel surface to the base of the capitulum) and inflorescence diameter were measured at approximately 3 day intervals. At final harvest on day 105 from sowing, dry weight of the leaves, stem, inflorescence and seeds were determined after 24 hour drying under forced drought at 80°C. Seeds were sampled along a spiral of 4 rows across the capitulum (see Appendix B.9 for description of sampling).

3.2.4. Experiment 4

Plant height and total leaf area in response to the soil volume and nutrient status was assessed. The statistical design was an unreplicated factorial with 3 levels of soil volume (9 litres, 5 litres, 2.4 litres) and 3 levels of nutrient status (No nutrient, nutrient of the amount added in experiment 1, nutrient supplemented for the extra amount of soil above 2200 g). Leaf areas and height were measured non-destructively on 2 occasions (day 15 and day 25 from sowing). A destructive harvest was done on day 43 from sowing. Macroscopic appearance of the inflorescence at the plant apex was recorded. Leaf number was also recorded.

3.2.5 Experiment 5

A population of plants was grown to determine most of the following. Some of the data were however obtained from control plants of other experiments (these are indicated where appropriate).

(a) A timing of palisade, stomatal and epidermal cell production and their enlargement in relation to leaf area increase was obtained.
Plate 3.1. shows 2 plants obtained from the use of the growth conditions of Treatment A (Expt.1) (on the left of the plate) and 2 plants obtained from the use of the growth conditions of Treatment B (Expt.1) (on the right of the plate). Note that there are side shoots from the cotyledonary axils of plants arising from Treatment B. Each side shoot also bears a miniature inflorescence. Height of plants in both treatments was about 100 cm.
The atheric activity of the cell population in the initiation of a leaf at various stages of its development and at various times during a diurnal cycle was determined.

(a) The atheric activity of the cell population in the atherous tip (particularly of nature limits) at various stages of its development was calculated.

(b) The antholytic pattern of wide-apart were studied (data obtained from experiment 2).

(c) The antholytic pattern of closely-related were studied (data obtained from experiment 1).

(d) The antholytic pattern of closely-related were studied (data obtained from experiment 1).
(b) The mitotic activity of the cell population in the lamina of a leaf at various stages of its development and at various times during a diurnal cycle was determined.

(c) The mitotic activity of the cell population in the ovary wall (pericarp of mature fruit) at various stages of its development was determined.

(d) The anthesis pattern of disc florets was studied (data obtained from experiment 1).

(e) The growth pattern of individual leaves on the plant (data obtained from several leaf experiments eg. experiments 6-12) was studied.

(f) The growth of disc florets (data obtained from experiments 14,16) was studied.

3.3. RESULTS

A large portion of the results, especially from experiment 2 and experiment 5(a-f)) will appear as control plant responses in the various stress experiments of chapters 4 and 5. However, they are being described here for convenience so that the chapter discussion may emphasize the responses to water stress.

3.3.1. Responses in experiment 1

Truncation to 1 metre high (Plate 3.1) was achieved in both treatments. This was at least 60 cm. shorter than glasshouse or field
Figure 3.1. a. shows the relationship between the total number of leaves at any time ($N_t$), the number of unfolding leaves ($N_{uf}$), the number of primordial and folded leaves ($N_p + N_f$), the number of involucre bracts and the number of disc florets and the chronological age of the plant (in days). There were two primordial leaves (that could be vaguely seen in cut sections) in the freshly imbibed seed.
Figure 3.1. b. shows the relationship between the total leaf number (○), the number of involucre bracts (△) and the number of disc florets (□) in relation to the number of unfolded leaves on the plant. The unfolded leaf number as an indicator of the events at the apex was tested by growing a different lot of plants; the events were predicted by counting the number of unfolded leaves and then confirmed by dissection. There was a close fit. This is indicated by ○ (total leaf number),△ (involucre bracts) and □ (number of disc florets). It was particularly difficult to estimate the unfolded leaf number at the early stages of plant growth.
Figure 3.2. shows the response of plant height to various environmental conditions. In the case of the irrigated field crop (1977) - and the glasshouse crop (planted Oct. 75.)- only final height data was collected (data of Begg and Turner). The controlled environment plants were much shorter than the plants grown in the other environments. For a comparison of other plant characters, see Table 3.1.
Change in temperature from 27°C to 21°C/16°C

GLASSHOUSE (30°C/25°C)
PLANTED OCT. 75

GLASSHOUSE (27°C/22°C)
PLANTED JUNE 76

IRRIGATED FIELD CROP (1977)
PLANTED 17 NOV. 76

CONTROLLED ENVIRONMENT CABINET
(27°C for 32 days) (21°C/9°C till maturity)
grown plants of the same cultivar (figure 3.2). Such comparisons are also shown for leaf number, leaf area, inflorescence diameter and average achene weight in table 3.1. The truncated plants had a lower leaf area and seed weight than was found in the field, but the field grown plants grew at longer photoperiods initially than did plants of treatment A. They also grew for a longer total period before setting seed. Glasshouse plants were either larger or smaller than those of treatment A, depending on the time of the year at which they were grown.

Table 3.1. Comparison of leaf number, leaf area, inflorescence diameter and average achene weight between plants grown in different environments

<table>
<thead>
<tr>
<th>Environment</th>
<th>Month planted</th>
<th>Average leaf number</th>
<th>average leaf area (cm²)</th>
<th>Inflorescence diameter (cm)</th>
<th>Average achene weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigated field</td>
<td>November</td>
<td>32</td>
<td>5500</td>
<td>21.8</td>
<td>72.4</td>
</tr>
<tr>
<td>Glasshouse (21°C/16°C)*</td>
<td>October</td>
<td>40</td>
<td>10200</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glasshouse (30°C/25°C)*</td>
<td>October</td>
<td>49</td>
<td>11000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Glasshouse (27°C/22°C)</td>
<td>June</td>
<td>29</td>
<td>2800</td>
<td>9.0</td>
<td>37.0</td>
</tr>
<tr>
<td>Controlled** environment</td>
<td></td>
<td>-</td>
<td>40</td>
<td>3700</td>
<td>13.0</td>
</tr>
<tr>
<td>cabinet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>40.0</td>
</tr>
</tbody>
</table>

* Day and night temperature; ** Treatment A of experiment 1.

Treatment B plants were more variable than those of treatment A, for they branched to different sized shoots which each bore an inflorescence (Plate 3.1). Unfolded leaf number and leaf area were also more variable at all stages of growth (table 3.2). Variation in leaf area
among treatment A plants was also far less than in field grown plants (compare table 3.2 with table 3.3).

Table 3.2. Comparison of some plant characteristics between treatment A and treatment B plants of experiment 1 at various plant ages

<table>
<thead>
<tr>
<th>Plant age (days)</th>
<th>Unfolded leaf number</th>
<th>Leaf area</th>
<th>Inflorescence number</th>
<th>Branch number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>14</td>
<td>9.00 (0)</td>
<td>4.00 (0)</td>
<td>115 (1)</td>
<td>24 (2)</td>
</tr>
<tr>
<td>28</td>
<td>24.00 (0.58)</td>
<td>14.25 (1.43)</td>
<td>1355 (79)</td>
<td>482 (51)</td>
</tr>
<tr>
<td>42</td>
<td>35.50 (0.29)</td>
<td>34.25 (1.65)</td>
<td>2781 (56)</td>
<td>2734 (73)</td>
</tr>
<tr>
<td>56</td>
<td>36.00 (0.71)</td>
<td>45.25 (5.36)</td>
<td>3272 (38)</td>
<td>3752 (158)</td>
</tr>
<tr>
<td>70</td>
<td>37.50 (0.96)</td>
<td>42.25 (4.62)</td>
<td>2683 (69)</td>
<td>2709 (108)</td>
</tr>
</tbody>
</table>

A = Treatment A, B = Treatment B. Figures in brackets ( ) are standard errors.

Table 3.3. Variation in leaf area and leaf number of field grown plants at various ages (Planted 26.11.74)

<table>
<thead>
<tr>
<th>Plant age (days)</th>
<th>Leaf area (cm²)</th>
<th>Leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>103 ± 25</td>
<td>8.00 ± 0.7</td>
</tr>
<tr>
<td>43</td>
<td>646 ± 130</td>
<td>16.80 ± 1.0</td>
</tr>
<tr>
<td>52</td>
<td>1315 ± 79</td>
<td>26.60 ± 1.0</td>
</tr>
<tr>
<td>64</td>
<td>2711 ± 246</td>
<td>32.00 ± 1.0</td>
</tr>
<tr>
<td>80</td>
<td>1763 ± 282</td>
<td>21.60 ± 0.6</td>
</tr>
<tr>
<td>90</td>
<td>2156 ± 304</td>
<td>19.60 ± 0.8</td>
</tr>
<tr>
<td>101</td>
<td>3272 ± 446</td>
<td>27.00 ± 1.0</td>
</tr>
<tr>
<td>112</td>
<td>7072 ± 657</td>
<td>27.36 ± 0.5</td>
</tr>
</tbody>
</table>
Inflorescence development was faster in treatment B than A, but the stems were thinner and broke more readily than in A during grain filling. Development following disc floret initiation was therefore not as sturdy at high temperatures and shorter photoperiods as it was at low temperatures and longer daylengths. This is to be expected.

3.3.2. Responses in experiment 2

The results of experiment 2, for convenience, are presented in three sections. They are (a) leaf initiation and unfolding, (b) Transition of the apex from the vegetative to the floral state and (c) Relationship between events at the apex, the chronological age and the number of unfolded leaves.

(a) leaf initiation and unfolding

Figure 3.1a shows the relationship between leaf initiation, leaf unfolding, other events at the plant apex and the chronological age of the plant. After the third day from sowing, leaves were initiated at a plastochron interval of 11.9 hours. The first two leaves unfolded between the 4th and 5th day from sowing. Further unfolding of leaves took place after the 7th day at a plastochron interval of 22 hours. The difference of 10 hours in the plastochron for leaf initiation and leaf unfolding caused an accumulation of leaves at the apex. Leaf unfolding continued unabated by the transition of the vegetative apex to a floral one.
(b) Transition of the apex

The first signs, of the transition to a floral apex from a vegetative one, were apparent on the 22nd day from sowing. This concurred with the initiation of the inflorescence which originally appeared as a rounded raised dome at the centre of the plant apex. This raised structure was quite in contrast to the flat centre when the apex was vegetative. Soon after, about 75 bracts were initiated over 4 days. Disc florets were then initiated. Commencing from the periphery of the inflorescence, disc floret initials were laid down in regular phyllotaxy to the centre. About 1200 disc florets were initiated in 4 days. The macroscopic appearance of the inflorescence at the apex was evident on day 32. Full emergence of the inflorescence out of the apical region coincided with the completion of leaf unfolding.

(c) Relationship between events at the apex, the plant chronological age and the number of unfolded leaves

Greater variability in the total leaf number was present among plants of a similar chronological age as compared to plants having a similar number of unfolded leaves. Variability in the floral events among plants of similar chronological age was, however, only slightly greater than among plants having a similar number of unfolded leaves (compare figure 3.1a with figure 3.1b). A test of reliability of the unfolded leaf number as an indicator of events at the apex showed high reliability (closed data points in figure 3.1b).
The endogenous levels of N, P and K was not determined for the soil, since all soil samples were assumed to have a low nutrient status following absence of nutrient application on the collection site for several years.
3.3.3. Responses in experiment 3

Potassium significantly ($P < 0.05$) increased the fresh and dry weight of the inflorescence (table 3.4). The plants developed more slowly up to anthesis but the fresh weights of the leaves and the stem of these plants were significantly higher ($P < 0.05$) than in plants that did not receive potassium. Final seed weights between plants receiving potassium and those not receiving it were not different however. Nitrogen and Phosphorus had little influence on the plant's development, in this soil.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Without potassium</th>
<th>With potassium</th>
<th>Standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Leaf fresh weight</td>
<td>15.05 g</td>
<td>17.81 g</td>
<td>1.18</td>
</tr>
<tr>
<td>(b) Leaf dry weight</td>
<td>10.68 g</td>
<td>12.41 g</td>
<td>0.85</td>
</tr>
<tr>
<td>(c) Stem fresh weight</td>
<td>71.70 g</td>
<td>82.25 g</td>
<td>4.37</td>
</tr>
<tr>
<td>(d) Stem dry weight</td>
<td>17.37 g</td>
<td>20.56 g</td>
<td>0.80</td>
</tr>
<tr>
<td>(e) Capitulum fresh weight</td>
<td>141.40 g</td>
<td>169.40 g</td>
<td>10.16</td>
</tr>
<tr>
<td>(f) Capitulum dry weight</td>
<td>31.78 g</td>
<td>35.71 g</td>
<td>1.58</td>
</tr>
<tr>
<td>(g) % moisture in the capitulum</td>
<td>344.90%</td>
<td>374.37%</td>
<td></td>
</tr>
</tbody>
</table>

The interval between commencement of anthesis and completion of anthesis was unaffected by nutrient. The transition temperatures hastened inflorescence development for all nutrient regimes in comparison to treatment A of experiment 1. While plants of treatment A in experiment 1 took 66 days to reach 'first anthesis' (anthesis of the first disc floret), plants in experiment 3 took an average of only 57 days (table 3.5).
Table 3.5. Effects of various treatments on the number of days to anthesis and the interval between commencement and completion of anthesis in the capitulum

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days to first anthesis (days)</th>
<th>Days to last anthesis (days)</th>
<th>Days between first and last anthesis (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment A</td>
<td>66</td>
<td>78</td>
<td>12</td>
</tr>
<tr>
<td>(experiment 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment B</td>
<td>57</td>
<td>64</td>
<td>7</td>
</tr>
<tr>
<td>(experiment 1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment 3</td>
<td>57*</td>
<td>69*</td>
<td>12</td>
</tr>
<tr>
<td>K.N.P.</td>
<td>58</td>
<td>70</td>
<td>12</td>
</tr>
<tr>
<td>(experiment 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.N.P.</td>
<td>60</td>
<td>73</td>
<td>13</td>
</tr>
<tr>
<td>0.2.2 (experiment 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.N.P.</td>
<td>57</td>
<td>70</td>
<td>13</td>
</tr>
<tr>
<td>1.0.0 (experiment 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.N.P.</td>
<td>63</td>
<td>75</td>
<td>12</td>
</tr>
<tr>
<td>2.2 (experiment 3)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Average value for plants in experiment three (average of 18 plants)

3.3.4. Responses in experiment 4

At 15 days from sowing, there were no apparent differences in leaf area nor height between the plants grown under the different combinations of pot size and soil nutrient level. At 25 days from sowing, differences in leaf area between the plants of the different treatment combinations became apparent; the heights of the plants were, however, not different. At 43 days from sowing, there was a linear relationship between soil volume and the leaf area of the plants; the heights of plants were not different. The addition of nutrient to the soil increased total leaf
Figure 3.3. shows the response of the total leaf area of plants to increases in the soil volume. The response was studied at three plant ages viz. 15, 25 and 43 days from sowing. 

Δ = (No nutrient, other than what was residual in the soil). ○ = (Nutrient added to the 2.4 litre soil volume level but not adjusted for increases in soil volume)

O = (Nutrient added to account for the increase in the soil volume). At 43 days, the uppermost leaves, especially in the larger soil volume treatments had not completed expansion but the experiment was terminated because the plants were beginning to shade each other.
LEAF AREA (cm²)

SOIL VOLUME (LITRES)

(AT 43 DAYS)

(AT 25 DAYS)

(AT 15 DAYS)
area by about 20%. However, the addition of extra nutrient did not result in large increases in plant leaf area. The plants grown in 9 litres and 5 litres of soil had between 3 to 6 more leaves than plants grown in 2.4 litres of soil. However, inflorescence development was more rapid in the plants grown in 2.4 litres compared to plants grown in the other two soil volumes; the inflorescence also emerged earlier out of the plant apex in the plants grown in 2.4 litres of soil. These results are presented in table 3.6 and figure 3.3.

Table 3.6. Effects of soil volume and nutrient level on the leaf number and the emergence of the inflorescence at the apex

<table>
<thead>
<tr>
<th>Soil Volume (litres)</th>
<th>Nutrient</th>
<th>Days to macroscopic appearance of the inflorescence of the apex</th>
<th>Total leaf number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>Nil</td>
<td>31</td>
<td>39</td>
</tr>
<tr>
<td>5.0</td>
<td>Nil</td>
<td>33</td>
<td>40</td>
</tr>
<tr>
<td>9.0</td>
<td>Nil</td>
<td>36</td>
<td>45</td>
</tr>
<tr>
<td>2.4</td>
<td>Normal</td>
<td>32</td>
<td>40</td>
</tr>
<tr>
<td>5.0</td>
<td>Not adjusted</td>
<td>34</td>
<td>44</td>
</tr>
<tr>
<td>9.0</td>
<td>Not adjusted</td>
<td>36</td>
<td>46</td>
</tr>
<tr>
<td>5.0</td>
<td>Adjusted</td>
<td>34</td>
<td>43</td>
</tr>
<tr>
<td>9.0</td>
<td>Adjusted</td>
<td>37</td>
<td>44</td>
</tr>
</tbody>
</table>

Normal = amount of nutrient as in treatment A, experiment 1.
Not adjusted = Normal nutrient added but not adjusted for the increase in soil volume.
Adjusted = the nutrient added was adjusted for the increase in soil volume.
Figure 3.4. shows the relationship between the increase in palisade cell number (○—○) and the leaf area (Δ—Δ) of leaves 2, 4 and 6 (numbered from the base of the plant, the cotyledons not being regarded as leaves), and also in relation to plant age. ↓ denotes cessation of palisade cell division. ---- = denotes that cell number was not determined at those stages of growth.
3.3.5. Responses in experiment 5

(a) timing of palisade stomatal and epidermal cell production and their enlargement in relation to leaf area increase

The palisade and stomatal cells appeared to cease dividing slightly later than the epidermal cells, although the confirmation of this was made difficult by the periodical sampling of leaves on different plants. In general, about 90% of the cells in a leaf were formed after the leaf had unfolded from the bud. In leaves 2, 4 and 6 on which a detailed count of palisade cell production (see Appendix B.11 for details) was made, cessation of palisade cell division occurred at around 35% of maximum leaf area ($35\% A_{\text{max}}$) except in laminae 1 and 2 (figure 3.4). This was also generally true for stomatal cells but epidermal cells ceased division slightly earlier. In laminae 1 and 2, cell division ceased at around 50% $A_{\text{max}}$. In most cases, the grand period of cell division was between 3% and 25% $A_{\text{max}}$. Cell enlargement was small during the active periods of cell division. Rate of cell enlargement increased soon after cessation of cell division and then declined up to the last stages of leaf expansion (figure 3.5). With increasing leaf position on the shoot, cells would have doubled for a longer time in leaves that were folded because leaves higher up on the shoot took extra time to unfold (see figure 3.1a).

(b) The mitotic activity of the cell population in the lamina of a leaf at various stages of its development and at various times during a diurnal cycle

The mitotic index (for details of method, see Appendix B.14), determined at 1100 hours, was 0.5% for leaves that had recently unfolded and
Figure 3.5. shows the relationship between the enlargement of the palisade cell (▲—▲), expressed as cross-sectional area, and the leaf area increase of leaf 3 (numbered from the base, not regarding the cotyledons as leaves. Data obtained from experiment 11.
Figure 3.6. a. shows a diagramatic representation of the mitotic index (% of cells that are dividing in a population of cells) of leaves at various positions on the shoot, when determined at between 1900 hours and 2100 hours. The unfolding leaf was leaf 7 and there were about 24 leaves on the plant. Data obtained from experiment 11 (controlled environment). Single rep.

Figure 3.6. b. shows a diagramatic representation of the mitotic index of leaves at various positions on the shoot, when determined at between 1000 hours and 1100 hours. The data was obtained in experiment 7 (glasshouse). Each data point is a mean of three replicates.
Mitotic Index (%) vs. Leaf position on shoot

Graph A:
- Peak Mitotic Index at leaf position 8
- Apex indicated

Graph B:
- Peak Mitotic Index at leaf position 8
- Apex indicated

Legend:
- UNFOLDED
- FOLDED
was about 3.5% for leaves which were 6 plastochrons away from the apex. The leaves between the stage of unfolding and 6 plastochrons from the apex had mitotic indices between 0.5% and 3.5% (figure 3.6b). However, the mitotic index of 0.5% for recently unfolded leaves was too low to account for the rapid cell production as described in 3.3.5 (a) in the recently unfolded leaves.

A mitotic index determination of a folded, about to unfold, a recently unfolded leaf and a 30% expanded leaf (of a 11 day old plant) on a diurnal basis indicated that the mitotic index was highest in the early part of the dark period. The mitotic index increased from 0.5% in the light to almost 5% in the dark period for the recently unfolded leaves. This increase was also quite large for a leaf that was about to unfold. The mitotic index of a folded leaf was largely the same during the light and the dark period. The leaf that had achieved 30% of its maximum area had a mitotic index approaching zero at all times of sampling (see figure 3.6a and figure 3.7). The response of the leaf that had reached 30% $A_{\text{max}}$ is not shown in the figure.

(c) The mitotic index in the fruit

The mitotic index (determined at 1100 hours) was studied in relation to the increment in fruit length. The increment in fruit length was slow up to the point when the mitotic index declined; the mitotic index was about 2% at most stages of fruit growth but declined to about 0.3% shortly after the anthesis of the disc floret. The increment in fruit length became rapid just shortly before anthesis. Observations indicated that marked elongation of cells occurred with the decline in the mitotic index and the rapid increase of fruit length.
Figure 3.7. shows the mitotic index of leaves 6 (▲▲), 9 (▲▲) and 12 (■■) on a diurnal basis. The mitotic index was not determined in the latter part of the dark period (indicated by ------). The unfolded leaf was leaf 7. Data obtained from experiment 11. Data points are from single replicates.
Mitotic index (%) vs. sampling time (during a diurnal cycle)

- Lights on
- Lights off

Sampling times:
- 600
- 1200
- 1800
- 2400
- 0600
- 1200
Figure 3.8 shows the attainment of area by leaves 2, 5, 10 and 15 in terms of the percentage of the individual maximum area ($A_{max}$) at various unfolded leaf numbers. It is a generalized diagram obtained from growth data of control plant leaves in experiment 6.
(d) Growth patterns of individual leaves on the plant

The final lamina area of individual leaves increased progressively up to around leaf position 17 and then declined. The last few laminae (e.g. laminae above position 35) had areas lower than laminae 1 and 2. The unfolding of leaf 17 from the bud, coincided with the initiation of the inflorescence. The unfolding of all leaves above leaf 17 took place during the growth of the inflorescence.

When the temperature change took place (from 27°C constant to 21°C/9°C day/night), the first 10 leaves had attained their maximum areas. The time from unfolding of a leaf to its attainment of maximum area, increased slightly with leaf position; presumably it increased for leaves up to position 15. Detailed monitoring of area increase versus time was not done for the leaves at the top of the plant.

The percentage of maximum area (∑_max) attained by individual leaves at various stages of shoot development (in terms of unfolded leaves) is shown in figure 3.8. This data has been derived from the control plants of various leaf experiments and is actually a generalized picture. The rate at which the leaves attained 35% A_max (the point of cessation of cell division) was much slower than the unfolding rate. For instance, when about 8 leaves (leaves 1 to 8) had attained 35% of their individual maximum area, there were 17 unfolded leaves.

(e) Final cell number, cell size and intercellular space size in the palisade layer and in the epidermis of the different leaves

The final cell number (obtained by multiplying cell number unit area⁻¹ by leaf area) of palisade, stomatal and epidermal cell types increases up to leaf position 20 and then declines (figure 3.9. a,b,c).
Figure 3.9. a-c. (The three diagrams on the upper half of the page) show the palisade cell number, epidermal cell number of the upper epidermis and stomatal cell numbers of the upper epidermis in relation to the leaf position on the shoot, respectively. Data from experiment 11.

Figure 3.10. a-c. (The three diagrams on the lower half of the page) show the palisade cross-sectional area, the epidermal cell area and the stomatal cell size (measured as length X breadth) in leaves at different positions on the shoot. Data from experiment 11. Note the asymptotic decrease in cell size in the three cell types, especially in the palisade cells.
Figure 3.11. shows the increase in fruit length of the fruit in the first floret and in the thirteenth floret, in relation to time.

Figure 3.12. shows the fruit length of florets at different positions on the capitulum on days 43, 48, 53, 59 and 62 from sowing. Length is shown in mm.

Figure 3.13. shows the increase in corolla length (floral tube length) for the florets at various positions on the capitulum on days 38, 43, 48, 53 and 59 from sowing.
\[= \text{anthesis of 1st disc floret}\]

\[\text{FLORET 1}\]

\[\text{FLORET 13}\]

\[\text{PLANT AGE (DAYS)}\]

\[\text{LENGTH OF FRUIT (mm)}\]

\[\text{LENGTH OF COROLLA (mm)}\]

\[\text{FLORET POSITION ON CAPITULUM}\]
The cross-sectional area of palisade cells, the approximate area of epidermal cells and the lengths of stomatal cells decrease from leaf 1 in an asymptotic manner, such that the decrease approaches a minimum after a certain leaf position (figure 3.10,a,b,c). There is a large decrease in the cross-sectional area of palisade cells from leaf position 1 to 6. Variations in the inter-cellular space/unit area in the palisade layer is described in the general discussion.

The product of palisade cell number and palisade cross-sectional area accounted for approximately 50% of $A_{\text{max}}$. The remaining 50% was area contributed by the intercellular space in the palisade and space occupied by vascular tissue. As the proportion of vascular tissue respective of leaf area, appeared to increase with leaf position on the shoot, the estimates of palisade cell number and the contribution of space occupied by palisade cells towards the leaf area, are only approximate.

(f) Anthesis of disc florets

The disc florets at the periphery of the capitulum reached anthesis before those in the centre (fig.3.12.). The time taken for all the disc florets to reach anthesis was about 12 days (fig.3.14). Anthesis of the 1st disc floret was usually 55 days from sowing the seeds, but this varied with changes to the environment (eg. experiment 14). Higher temperatures during anthesis hastened its completion (figure 3.14). Figure 3.11 also shows the difference in the anthesis date and in the length increase of two differently positioned florets.

(g) Growth of disc floret components

Among the components of the disc floret, the floral tube was the first to attain maximum length (fig.3.13). Floral tubes of the peripheral
Figure 3.14. shows the pattern of anthesis of the disc florets (expressed as the number of concentric rows on the capitulum that reach anthesis on a particular day). Treatment A data was obtained in experiment 1. The field data was obtained from experiment 16; it took about 7 days for all the disc florets on the capitulum to reach anthesis but day to day records of the anthesis pattern were not made—hence the dotted(----) line for the pattern in the field plants.
Figure 3.15. a. shows the length increments of the individual pericarps (P) in relation to plant age. The number after P denotes the position of that achene on the capitulum, from which the data of the pericarp was obtained.

Figure 3.15. b. shows the length increments of the integuments (I) in relation to plant age. The number after I denotes the position of the seed (achene) on the capitulum from which the integument data was obtained.

Figure 3.15. c. shows the length increments of the embryo (E) in relation to plant age. The number after E denotes the position of the achene from which the embryo data was obtained.
disc florets attained maximum lengths before the inner ones did. The attainment of maximum length by the pericarp (originally the ovary wall), followed by the integuments and finally by the embryo, was the pattern of development. As for the floral tube, the maximum length for each of these components was first attained by the peripheral disc florets (see figures 3.11, 3.12, and 3.15 a-c).

The dry weight gain by the pericarp of the achene was completed before the embryo dry weight gain was over. The embryo continued to gain dry weight until the disc of the capitulum became yellow. As for the attainment of maximum lengths, the maximum dry weights of the pericarp and the embryo were first attained by the peripheral florets of the capitulum. There was a gradient in length as well as in dry weight of pericarps and embryos, for the achenes from the peripheral to central positions. The embryos of outermost peripheral achenes were about 20% higher than those in the centre.

3.4. DISCUSSION

The discussion in this chapter has been divided into two parts. The first part of the discussion (Part A) considers results from sections 4.1 to 4.4. Part B of the discussion evaluates the timing of events at the plant apex and within individual leaves. While part A evaluates the use of controlled environment to truncate the sunflower plant and reduce variability among plants, part B discusses some detailed aspects of the growth of the sunflower.

3.4.1. PART A

All combinations of temperature and daylength regimes resulted in
the truncation of this cultivar. Although the truncation of the tall stature of this cultivar was quite spectacular and of immediate experimental use, there is insufficient evidence to fully explain the response. However, it is possible that the short daylength and high night temperatures during the first 32 days of growth allowed a preferential utilization of the available assimilates by the leaves such that stem growth and elongation were reduced. Truncation did not affect the normal physiological pattern of growth and development of the plant, although these controlled environment plants differed, mainly in size, from their field counterparts.

Variability can be reduced to a very large extent by controlled environment and this is best achieved by the growing conditions of treatment A (experiment 1) incorporating the stepwise change in temperature. Further reduction in variability can be achieved by selecting for uniformity in apical and vegetative development. Some small differences exist in leaf area among these controlled environment plants but visual selection at an early stage of growth can reduce this. The selection for uniformity in apical development, without dissecting the plant, is favoured by predictions based on the relationship between apical events and the unfolded leaf number. The main advantage of this is that the study of environmental constraints at specific developmental stages of the apex becomes more exact. Damptey and Aspinall (1976) have described similar events for Zea mays under glasshouse conditions which facilitated the imposition of water stress at precise developmental stages of that plant. Their method of following plant development by dissection is not feasible for the present purposes, because the population of maturing plants in a cabinet is small for this.

The use of the unfolded leaf number or some version of it, for purposes of reducing variability, is not new. Generally, its use has been advantageous in the study of normal plant development. It has been
used to reduce variability that was present in plants as a response to environmental fluctuations. Erickson and Michelini (1957) originally formulated the plastochron index (the number of leaves above 10 mm. in length in their study) and used it to advantage in the study of vegetative development of Xanthium. Similarly, its use is seen in the study of the development of the shoot apex of Zea mays (Abbe and Phinney 1951), in the study of leaf development in Xanthium (Maksymowych 1956) and in tomatoes (Coleman and Grayson 1976). Higgins (1952) used the number of "fully developed nodes" in peas as an index of development.

The major objectives of using the unfolded leaf number in Helianthus annuus are (1) to accurately impose environmental constraints at desired stages of apical development and (2) to choose uniform sets of plants for studies at later growth stages.

The large variation noticed in treatment B (experiment 1) plants, especially the production of shoots of different sizes at the cotyledonary axils, may have resulted from the cool nights in the early part of vegetative growth. This may be related to a suppression of shoot apical dominance during the early growth stages of the main shoot. Similarly, the delay in inflorescence development of treatment A plants (in experiment 1) may be due to the sudden change from continuous high temperature to cool nights. The introduction of step-wise changes in temperature hastened inflorescence development (experiment 3). The transition temperatures may have reduced any shock the plants would have otherwise experienced. The development of the inflorescence, as evidenced by fresh and dry weights, is further improved by potassium nutrition.

In comparison to field grown plants, these controlled environment plants have a lower total leaf area and seed weight. It was earlier suggested (see 3.3.1, experiment 1) that this may be due to the fact
that field grown plants grow for longer and under longer initial photo-periods. While this may be true, experiment 4 emphasizes that it is the restricted root volume that reduces leaf area. It appears that neither the quantum flux density nor nutrition levels are as important a limitation here, as is soil volume (see figure 3.3). This simple trial was not continued to maturity because mutual shading of plants began to occur around day 43. For this reason, the experiment was terminated. It would be worthwhile to study the responses further; unfortunately that is outside the interests of this thesis. However, it is apparent that the use of larger pots will result in a very large plant. This will limit the number of experimental plants that can be placed in a cabinet without introducing mutual shading among plants. Of immediate importance is the need to have sturdy, healthy plants with well developed leaves and inflorescence. These requirements are manifest in plants grown in 7 inch pots, although they are smaller than their field counterparts. Thus, the need to arrive at a compromise cannot be avoided.

The stable relationship between the unfolding leaves and events at the apex (see figure 3.1b) and the ability to grow sunflowers in this manner, places this plant in an advantageous position for observation and experimentation. But even more important as an experimental method for future work is that timing of environmental stresses can be precisely related to the stage of development, especially in the study of the initiation and early growth of leaf and disc floret primordia. This method is used in the next phase of the experimental work to study the effects of water stress on the initiation and growth of leaf and disc floret primordia.
3.4.2. PART B

The phasing of cell division in the lamina, in relation to the attainment of lamina area (see figure 3.4) follows a pattern exhibited by dicotyledonous leaves which have been examined by other workers; in cucumber (Milthorpe and Newton 1963), subterranean clover (Williams and Bouma 1970) and tobacco (Clough and Milthorpe 1975), cell division in leaves was completed before the leaves reached 50% $A_{\text{max}}$. In most cases, the grand period of cell division was between leaf unfolding and its attainment of 30% $A_{\text{max}}$. This is true for sunflower leaves in this system. However, the contrasting results of this finding for sunflower leaves and that of Sunderland (1960) may be reconciled with the contrasting growth conditions between these experiments and his experiment.

Sunderland showed that in the 10th leaf of sunflower, cell division did not cease until 90% $A_{\text{max}}$ was achieved. Moreover, there was little indication of a grand period of cell division. The 10th leaf in his experiment was initiated on the 30th day, unfolded on the 52nd day and was fully expanded on the 120th day from sowing. In contrast, the 10th leaf in these experiments was initiated on the 6th day, unfolded on the 15th day and was fully expanded on the 32nd day from sowing. This difference may be due to the high temperature and high light used during the growth of leaf 10 in my experiments ($27^\circ\text{C temp. and 650 m.e. M}^{-2}\text{sec}^{-1}$ of light) and the low temperature and winter light conditions employed in Sunderland's experiment. Evidence from cucumber leaves (Milthorpe and Newton 1962) indicated that the percentage of maximum area at which cell division ceased, increased with a lowering of light intensity. The low winter light during Sunderland's experiment may be one reason why cell division continued for a much longer time than was the case in the present experiments. One other reason for the difference, though perhaps remote, could be due to the cultivars that were used. Nevertheless, the common
feature in all these experiments, is the confinement of the majority of cell divisions to stages after the unfolding of the leaf from the bud.

It was this last observation that raised doubts about the low mitotic indices in recently unfolded laminae, when the mitotic index was determined at 1100 hours (figure 3.6b). As the mitotic index at that sampling time was too low to explain the large increment in absolute palisade, stomatal and epidermal cells, it was suspected that the mitotic index could fluctuate diurnally, within a leaf. As the diurnal sampling of leaves for a determination of the mitotic index showed that rapid divisional activity was confined to the early and middle parts of the dark period in 11 day old seedlings, the increase in the mitotic index during the night and the decrease during the day, in unfolded leaves (figure 3.7) suggested that several factors may be involved in the response. Furthermore, it was also interesting to note that the folded leaf did not show a similar fluctuation in the mitotic index.

Among the factors, the diurnal fluctuation in leaf water potential (Slatyer 1968) could be important. Although leaf water potential was not monitored on a diurnal basis, experiments with the sunflower (Boyer 1970) have shown that leaf water potentials are least negative during the dark period. Boyer (1970) also showed that cell enlargement in sunflower leaves ceased at a water potential of $-0.4\, \text{MPa}$ and even when leaf turgor was as high as $0.6\, \text{MPa}$. Direct determinations of leaf water potential and calculated values of leaf turgor in well-watered plants of the present experiments, during the day, showed that leaf water potential was $-0.7\, \text{MPa}$ and leaf turgor potential was $0.2\, \text{MPa}$ (see results of Chapter 4.1). Although diurnal measurements of leaf and of cell growth were not made, visual observations indicated that large discernible increases in leaf area could only be seen after a dark cycle. Presumably, the leaf water potential becomes less negative and the leaf turgor more positive during the dark, in these experiments. As cell division basically depends on a
certain amount of cell expansion (Hsiao 1973), the large increases in the mitotic activity in the dark, may be associated with the less negative water and higher turgor potentials. The absence of fluctuations in the mitotic index of folded leaves suggests that fluctuations in leaf water potential may not exist for these folded leaves; it is generally agreed that the apical parts maintain a high water potential.

However, the low mitotic index of unfolded leaves at 0800 hours (15 minutes before the lights came on after the dark period) (see figure 3.7) is inconsistent with the earlier explanation that cells would divide rapidly at a less negative water potential, and leaf water potentials at that hour would presumably be still favourable for cell division to take place. The low mitotic index at that hour, however, could also be due to the majority of already divided cells entering a quiescent state. The postulate that the low mitotic index at that hour may be related to diminished supply of photosynthate also cannot be effectively confirmed or denied with the data at hand. However, several experiments can be planned to evaluate the importance of photosynthate supply. Nevertheless, the rapid cell divisions in the dark appear to be related to the less negative leaf water potentials and higher turgor in leaves.

An accurate estimate of total palisade, stomatal or epidermal cells in a leaf had some problems. The attainment of accuracy was hampered by the space occupied by vascular tissue and by the increase in space occupied by vascular tissue with increasing leaf position on the shoot. The tendency to overestimate the palisade cell population thus increased with leaf position. The space occupied by vascular tissue was only assessed visually. The estimates of cell number, especially to show the effects of water stress treatment in chapter 4.3, do not contain corrections for space occupied by vascular tissue.

The cell size decreases for all cell types with increasing leaf position, the increase in cell number and leaf area up to leaf position
20 followed by a decline by both of them raise some interesting points for discussion. Consistent decreases in epidermal and stomatal cell sizes with increasing leaf position have been noted in *Nicotiana rustica* and *Dactylis glomerata* (Zalenski 1904) and in *Bryonia dioica* and *Ipomoea purpurea* (Alexandrov, Alexandrov and Timofeev 1921). Generally, the decrease in cell size with leaf position has been interpreted as a xeromorphic character induced by a competition for water (Zalenski 1904). This concept has not remained unchallenged. Ashby (1948) provided evidence that gradients in leaf area, cell number and cell sizes of leaves are related to the influence of immature leaves upon still younger leaves developing above them.

In the present studies, there was no evidence for cells of folded leaves at higher ontogenetic leaf positions to divide at increasingly small sizes which would have an influence on the final cell size. As there were no noticeable differences between cell sizes in differently positioned leaves at the unfolding stage, the ontogenetic gradient in final cell size is probably the result of events occurring in the plant after individual leaves had unfolded from the bud. Further discussion of this point is included in the general discussion where the effects of water stress on cell size and cell number are also discussed.

The leaf area responses with ontogenetic position on the shoot may be closely related to the timing of events at the plant apex. As the inflorescence was initiated when the 17th leaf unfolded from the bud, all leaves unfolding after leaf 17 did so at a time when the inflorescence began to differentiate and grow. Thus, the decline in leaf area of leaves from leaf 17 up to the last leaf may be associated with the competitive growth of the developing inflorescence.

Since cell division ceased at about 35% of *A* max, the latter part of leaf growth was mainly due to the enlargement of cells. Because the temperature and photoperiod regimes were changed on the 32nd day from
seed sowing, the effect of this change on the ontogenetic decrease in cell size, the initial increase in area of laminae up to leaf position 17 and then followed by a decline becomes important. As the 10th leaf was fully expanded before the temperature and photoperiod change, the observed decrease in cell cross-sectional area (cell size) of palisade, epidermal and stomatal cells from lamina 1 to lamina 10, was not due to the change in the environment. Parallel studies on leaf area of individual leaves in an environment that was maintained under constant temperature and photoperiod was not done. However, when sunflower plants cv. Sunfola (personal communication - J. Marc) were grown at a constant 28°C from germination to stages approaching anthesis, a similar increase in leaf area followed by a decrease in the leaf area was observed; J. Marc also observed an ontogenetic decrease in the sizes of cells. Thus, the ontogenetic decrease in cell size in these sunflower plants is not due to the change in environmental conditions, although the rate of the ontogenetic decrease could have been influenced by the temperature and photoperiod change.

The gradients in cell size and cell numbers in leaves were not investigated to a similar extent in the pericarp and embryo of seeds because of the many practical difficulties that were involved. The gradient in achene weight from peripheral to central positions in the normal well-watered plant, however, suggested that a competition for assimilates, a competition for space or the differences in the distribution of conducting vessels to various seed positions could account for the existing gradient. As detailed investigations along these lines were not done, it is difficult to make any firm comments. However, the responses of individual achenes to water stress (Chapter 5) raise some points for discussion with regard to the above mentioned reasons for the existing gradient in achene weight.
It was unfortunate that the mitotic index for the fruit wall (the pericarp) was not determined on a diurnal basis as was done for the leaves. In the one study where the mitotic index of the fruit wall was determined at 1100 hours, the mitotic index approached zero shortly after the anthesis of the disc floret; furthermore, the cells were beginning to elongate quickly around this time. Thus, the growth of the fruit after the anthesis of the disc floret, would mainly be due to cell enlargement.

The timing of events in the growth and development of the leaves and achenes will lend itself to advantage in the interpretation of water stress effects on their growth. This, together with the presence of the ontogenetic trend in the variables that contribute to leaf area are evaluated in the general discussion (Chapter 6).
CHAPTER 4
EFFECTS OF WATER STRESS ON SUNFLOWERS DURING THE VEGETATIVE PHASE

4.1. INTRODUCTION

The successful truncation of the sunflower cultivar Hysun 30 (Chapter 3) enabled the use of the available 1.5 metre high LB cabinets (Morse and Evans 1962) for investigating the effects of water stress on the growth and development of this sunflower cultivar up to mature stages under controlled environment. But the initial experiments investigated mainly the growth physiology of the vegetative parts, especially the leaf laminae, during water stress and subsequent recovery. The results are presented in 4 sub-chapters, the reasons for which have been raised in the general introduction.

The most relevant study which provides some basic information on leaf laminae responses to water stress is the work on tomato plants (Gates 1955b). Gates found a general depression in growth of the first eight laminae during water stress. The depression was much greater for those laminae positioned near the apex. During recovery, the dry weights of the lower laminae (1, 2, 3 and 4) appeared to have recovered to control values. The dry weight gain of the 5th, 6th and 7th laminae was, however, not sufficient to represent complete recovery; lamina 7 especially, showed inflections in its growth curve which indicated that growth would
cease soon and prevent recovery to control values.

The apparently higher than control growth rate during recovery, of the previously water stressed laminae, led Gates to conclude that there was a trend towards senescence during water stress and a return to a more juvenile condition upon recovery. The observed higher relative growth rates during recovery were attributed to sustained cell division in the laminae during water stress. A similar interpretation was given by Slatyer (1973) who attributed the observed rapid expansion of leaves recovering from water stress, to a plain requirement for expansion of cells that divided during water stress. Even recently, Ludlow and Ng (1977) have attributed the increased expansion rates of previously water stressed Panicum leaves to the expansion of cells which were formed during water stress and were only partially expanded.

Although many of the laminae on the plant in Gates experiment were still growing when the experiment was terminated, some had reached full growth and had lower dry weights than their corresponding controls. Therefore, although cell division can continue during water stress, there is the possibility that cell number in the leaf could have been reduced. It is difficult to confirm this on either Gates' or Ludlow and Ng's data because there are no associated data on cell numbers and sizes. There is clearly a need to follow responses of cell number and size together with leaf area and dry weight responses. For reasons already given in Chapter 1, palisade cell responses have been followed in the present experiments.

The possible involvement of a reduced photosynthate supply in the leaf responses during water stress has also been evaluated by some workers. Boyer (1970) examined leaf enlargement in the 4th and 6th sunflower leaves (numbered from the base of the plant) during a period of moderate leaf dessication and then during subsequent recovery. It was found that while leaf enlargement ceased at a leaf water potential of -0.4 MPa during the drying cycle, photosynthesis continued until a leaf water potential of
-1.6 MPa was reached. After rewatering, enlargement of the leaves continued but was insufficient to represent complete recovery to the control rate. It was inevitably concluded that in spite of the high availability of photosynthate, some other factor limited growth after rewatering.

Another investigation on sunflowers showed that the order of wilting started from the lower leaves, the younger expanding leaves and finally the leaves located near the apex (Baer 1956). Since leaf turgor largely determines stomatal opening (Zelitch 1969) and as photosynthetic inhibition in Boyer's 1970 study could have been a stomata mediated response, the leaves located nearer the apex should carry on photosynthesizing for longer than the lower leaves. Thus, if the availability of photosynthate is not seen to be a limitation for the growth of the lower leaves after rewatering (as in Boyer's 1970 study), it is unlikely to be a limitation in the growth of leaves positioned nearer the apex. The reductions in the growth of the leaves positioned nearer the apex may be controlled by other factors, especially in response to mild water stresses.

In contrast to the investigations which examined only a few leaves for their response to water stress (McCree and Davis 1974, Clough and Milthorpe 1975, Bunce 1977), the present experiments have been designed to investigate the responses of all the leaves on the plant. Water stress was applied at various stages of shoot development and has been timed to affect the growth and development of individual leaves at various stages of growth. The application of water stress has relied largely on the calibration graphs of Chapter 3 (figure 3.1b) and the interpretation of stress effects has taken into account the timing of cell division and cell enlargement in leaves.

The effects of water stress on cell division was initially assessed in terms of its effect on the mitotic index of a leaf. Although the assessment of the mitotic index (at 1100 hours) provided valuable
information on the sensitivity of cell division to water stress, the data was insufficient to explain the morphological responses that were observed. A diurnal determination of the mitotic index indicated that most of the cells in a leaf went into division at the early part of the dark cycle. Fortunately, there was time to investigate the effects of water stress on the mitotic index at this time (experiment 11). However, despite the data showing very large effects of water stress on cell division of unfolding leaves, it was realized that the mitotic index had several limitations for accurately explaining morphological responses. Hence, most of the leaf area response have been accounted for largely by responses of the absolute palisade cell population. The calculation of the absolute palisade cell population has not been corrected for vascular tissue space and therefore is only approximate. Despite this, the absolute populations have been calculated to explain some of the morphological responses of the stressed plants. However, the general discussion (Chapter 6) emphasizes the precautions needed in calculating absolute cell populations.

This chapter has been divided into 4 sub-chapters, mainly for the purpose of clearly presenting all the results. The first sub-chapter describes the water relations data, the second describes leaf morphological and dry weight responses, the third describes the responses of the cell while the fourth sub-chapter describes some responses of the initiation and early development of the inflorescence to water stress. Results of each sub-chapter are discussed separately.

4.2. MATERIALS AND METHODS

While essential details of the methods and measurements are provided in tables 4.1 and 4.2, a full description of experiments 6 to
12 and details of the experimental techniques are contained in Appendices A and B respectively.

Experiments 6, 7 and 12 were relatively short term experiments because they did not investigate the full recovery of primordial leaves. Other leaf experiments were terminated only when all the leaves on the plant had attained their maximum expanded state; the last few leaves reached maximum expansion a few days before anthesis of the 1st disc floret. All comparisons between leaves to assess recovery from water stress were done at this stage. The comparisons between leaves during the recovery process were at times inevitably conducted on physiologically non-similar leaves.

4.2.1. Generation of the water stress in the plant

Water stress was generated in the plants by allowing them to transpire all the 'available' water in the soil medium. The 'available' water in the soil was calculated from previous determinations of the field capacity and the permanent wilting point of a large amount of soil (see appendix B.2). When a quick drying cycle was desired for young plants, a small quantity of soil was used. In such cases (example - experiments 9 and 12), the plants were repotted into the standard 7 inch pots (see materials and methods of Chapter 3) after the completion of the drying cycle; the remainder of soil, water and nutrient was also added. In all other experiments, the drying cycles were done in 7 inch pots containing 2200 g oven dry weight of soil.

The drying cycle was commenced at a particular developmental stage of the shoot apices of the experimental plants. The identification of particular developmental stages of the apex was achieved by the use of the calibration graph between the unfolded leaf number and the total leaf number (see figure 3.1b). Depending on the size of the plant at the commencement of the drying cycle and the volume of 'available' water in
Plants received only one drying cycle. Where two drying cycles were incorporated in an experiment, $W_1$ refers to the drying cycle on the 1st batch of plants and $W_2$ refers to the drying cycle on the second batch of plants. This procedure allowed the efficient use of cabinet space.
the soil, the length of the drying cycle varied from one experiment to another. When the calculated amount of water had been transpired (determined by the method of pot weighing) and no further amount of water was being transpired, the plants were rewatered. Details of the duration of the drying cycles and the precise developmental stages of the experimental plants at the start of the drying cycle in the various experiments are provided in table 4.1.

Table 4.1 Some details of experimental conditions and magnitude of water stress in experiments 6 to 12.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Unfolding leaf at the start of the drying cycle</th>
<th>Environment</th>
<th>Total number of leaves at start of the drying cycle</th>
<th>Days to completion of the drying cycle</th>
<th>Amount of soil used in the drying cycle (g)</th>
<th>Available water in the soil (g)</th>
<th>Internal plant water stress at the end of a drying cycle (MPa)</th>
<th>Light intensity (E m⁻² s⁻¹)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>15</td>
<td>LB</td>
<td>39</td>
<td>4</td>
<td>2200</td>
<td>700</td>
<td>*</td>
<td>500</td>
<td>32%</td>
</tr>
<tr>
<td>7</td>
<td>7</td>
<td>GH</td>
<td>20</td>
<td>5</td>
<td>1200</td>
<td>350</td>
<td>WSD = 32%</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>W₁ = 7, W₂ = 9</td>
<td>LB</td>
<td>W₁ = 20, W₂ = 26</td>
<td>W₁ = 7</td>
<td>2200</td>
<td>700</td>
<td>ψ = -16.5</td>
<td>650</td>
<td>**</td>
</tr>
<tr>
<td>9</td>
<td>W₁ = 7, W₂ = 9</td>
<td>LB</td>
<td>W₁ = 20, W₂ = 25</td>
<td>W₁ = 6</td>
<td>1200</td>
<td>350</td>
<td>ψ = -17.5</td>
<td>650</td>
<td>**</td>
</tr>
<tr>
<td>10</td>
<td>17</td>
<td>LB</td>
<td>41</td>
<td>4</td>
<td>2200</td>
<td>700</td>
<td>ψ = -22.5</td>
<td>650</td>
<td>**</td>
</tr>
<tr>
<td>11</td>
<td>7</td>
<td>LB</td>
<td>20</td>
<td>7</td>
<td>2200</td>
<td>700</td>
<td>ψ = 17.5</td>
<td>650</td>
<td>**</td>
</tr>
<tr>
<td>12</td>
<td>W₁ = 3, W₂ = 5</td>
<td>LB</td>
<td>W₁ = 10, W₂ = 14</td>
<td>W₁ = 5</td>
<td>280</td>
<td>100</td>
<td>*</td>
<td>650</td>
<td>**</td>
</tr>
</tbody>
</table>

LB = Controlled environment (Light and temperature)
GH = Glasshouse
* stress not measured
** temperature conditions followed Experiment 3
+ Average net radiation (approximately similar to experiment 1)
+ Daily minimum temperature 25°C, maximum about 31°C.

The plants in experiments 9 and 12 were repotted after the drying cycles into 7 inch pots containing the remainder of soil and nutrient and water.
4.2.2. The measurements made in experiments 6 to 12

Measurements relating to the water status in the plant and in the soil, morphological responses of leaves, cell responses in leaves and inflorescence initiation responses were made in experiments 6 to 12. The specific measurements that were taken in each experiment are indicated by a plus (+) in table 4.2. The details of how the measurements were obtained, the concepts and definitions of some of the parameters that were measured are provided in Appendix B; the measurements in the table are followed by indices that refer to the relevant sections in appendix B.

The measurement of some parameters (for example leaf water potential and stomatal resistance) depended on the availability of the instruments. However, the water status of either the soil or the plant tissue was always measured.
Table 4.2. Measurements that were made in experiments 6 to 12. Numbers in brackets refer to relevant sections in Appendix B where details are provided.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Experiment No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Leaf water potential (B.22)</td>
<td>+</td>
</tr>
<tr>
<td>Leaf osmotic potential (B.23)</td>
<td>+</td>
</tr>
<tr>
<td>Water saturation deficit (B.25)</td>
<td>+</td>
</tr>
<tr>
<td>Stomatal resistance (B.21)</td>
<td>+</td>
</tr>
<tr>
<td>Soil water content (B.20)</td>
<td>+</td>
</tr>
<tr>
<td>Leaf initiation (B.7)</td>
<td>+</td>
</tr>
<tr>
<td>Leaf unfolding (B.7)</td>
<td>+</td>
</tr>
<tr>
<td>Lamina area (B.17)</td>
<td>+</td>
</tr>
<tr>
<td>Lamina dry weight (B.18)</td>
<td>+</td>
</tr>
<tr>
<td>Petiole length (B.17)</td>
<td>+</td>
</tr>
<tr>
<td>Petiole dry weight (B.18)</td>
<td>+</td>
</tr>
<tr>
<td>Palisade cell number (B.11)</td>
<td></td>
</tr>
<tr>
<td>Palisade X-sectional diameter (B.12)</td>
<td></td>
</tr>
<tr>
<td>Intercellular space in the mesophyll (B.13)</td>
<td></td>
</tr>
<tr>
<td>Stomatal cell number (upper epidermis) (B.11)</td>
<td></td>
</tr>
<tr>
<td>Epidermal cell number (upper epidermis) (B.11)</td>
<td></td>
</tr>
<tr>
<td>Epidermal cell size (upper epidermis) (B.12)</td>
<td></td>
</tr>
<tr>
<td>Inflorescence initiation and development (B.7)</td>
<td></td>
</tr>
<tr>
<td>Mitotic index (B.14)</td>
<td></td>
</tr>
</tbody>
</table>
**Sub-Chapter 4[1]**

**Water relations in the Sunflower during water stress and recovery**

**4[1].1. Results**

The responses of the leaf osmotic potential ($\pi$), leaf water potential ($\psi$), leaf turgor potential ($P$), leaf water saturation deficit (LWSD), stomatal resistance ($R_s$) of abaxial and adaxial leaf surfaces and transpiration during water stress and recovery are reported in this sub-chapter. In each case, the responses during water stress are described first followed by the responses during recovery. The terms wilt$_1$ and wilt$_2$ (for the first and second batches of water-stressed plants) are used in experiments that incorporated 2 drying cycles.

**4[1].1.1. Decline in the soil water content during water stress**

The rate at which the water in the soil was transpired depended on the environmental conditions and on the size of the plant when the drying cycle was started. As the soil was maintained at field capacity before the drying cycle, the amount of water that could be transpired by the plant was approximately 32% of the oven dry weight of the soil contained in each pot.

In experiments 6 and 10, the drying cycle was rapid. In these experiments, a large part of the available water in the soil was transpired.
Figure 4[1].1. shows the nature of the drying cycles in the experiments 6 to 12. The available water in the soil has been calculated from a previous knowledge of the field capacity and the wilting point (as determined by the sunflower test). WC = commencement of the drying cycle while RW = rewatered. The subscripts 1 and 2 denote the first and second drying cycles. The data points are means of two replicates.
Figure 4[1].2. a. shows the transpiration of the plants in expt. 6 for a control (■—■) and for a water stressed (○—○) treatment, during and after the drying cycle. WC = commencement of the drying cycle, RW = rewatered, C = control, W = water stressed. Data points are means of two replicates. The plants were unfolding the 16th leaf at the start of water stress.

Figure 4[1].2. b. shows the transpiration of plants in expt. 8 for a control (■—■), for the first batch of stressed plants (△—△) and the second batch of stressed plants (○—○). WC₁ = drying cycle was started on first batch of plants, WC₂ = drying cycle was started on second batch of plants, RW₁ = first batch of plants were rewatered, RW₂ = second batch of plants were rewatered. The data points are means of two replicates. The symbols C = control, W₁= first batch of water stressed plants and W₂= second batch of water stressed plants.

Figure 4[1].2. c. shows the transpiration of the plants in expt. 9 for a control (■—■), for the first batch of water stressed plants (△—△) and the second batch of water stressed plants (○—○). The symbols WC₁, WC₂, RW₁ and RW₂ follow that shown in figure 4[1].2. b. The data points are means of 2 replicates.
within 2 days; the remainder of the available water was transpired in a further 2 days. The soil water depletion curve was asymptotic. In contrast, the drying cycles in experiments 7, 8, 9, 11 and 12 were less rapid. The rate of soil water depletion in these experiments was much more constant in comparison to experiments 6 and 10. The nature of the drying cycles for all the experiments is illustrated in figure 4[1].1.

4[1].1.2. Transpiration responses during the drying cycle

While the transpiration of the control plants increased steadily during the experimental period, the transpiration of the water stressed plants in experiments 6 and 10 (rapid drying cycles) declined consistently throughout the drying cycle. In experiments where the drying cycle was less rapid, the transpiration of the water stressed plants either increased slightly at first or was maintained at a steady rate for 3 to 4 days before a decrease occurred. Such a contrast in the transpiration of stressed plants between a slow and a rapid drying cycle is shown in figure 4[1].2.a,b and c.

4[1].1.3. Transpiration responses during recovery

One day after rewatering, the transpiration of the previously water stressed plants increased but was still less than the transpiration of control plants. During this period, the transpiration of the control plants also continued to increase but there were indications of it becoming less rapid in comparison to transpiration of control plants at earlier growth stages. The transpiration of the previously water-stressed plants was insufficient to reach control values (see figure 4[1].2.a,b and c).
4[1].1.4. Responses of the transpiration rate (ml.cm\(^{-2}\)) during the drying cycle

The transpiration rate of the water stressed plants declined progressively throughout the drying cycle, from approximately 1 ml. cm\(^{-2}\) at the start of the drying cycle to 0.1 ml. cm\(^{-2}\) at the end of it. The transpiration rate of the controls also declined during this period but at a very slow rate; it declined from 1 ml. cm\(^{-2}\) at the start of the drying cycle to about 0.65 ml. cm\(^{-2}\) at the point when the drying cycle was completed for the water-stressed plants. From that point, the transpiration rate of the control plants appeared to become stabilized. These responses in experiments 8 and 9 are illustrated in figures 4[1].3f and 4[1].4f.

4[1].1.5. Responses of the transpiration rate (ml. cm\(^{-2}\)) during recovery

One day after rewatering, the transpiration rate of the previously water stressed plants was less than control plant values but recovered completely 2 days later. Thereafter, the transpiration rate of the previously water stressed plants followed the trends of the control plants (also see figure 4[1].3f and 4[1].4f).

4[1].1.6. Adaxial and abaxial stomatal responses during the drying cycle

The resistance of the adaxial and abaxial stomata of the control plants in experiments 6, 8, 9 was about 0.5 sec.cm\(^{-1}\) generally. In experiment 6, the stomatal resistance of the abaxial and adaxial leaf surfaces in a stressed expanding leaf increased 30 hours after the start of the drying cycle and reached about 20 sec. cm\(^{-1}\) at the end of the drying cycle. In experiment 8, the stomatal resistance of wilt\(_1\) plants
In all the figures, (O---O) = CONTROL, (▲--▲) = WATER STRESS 1 and (■--■) = WATER STRESS 2

Figure 4[1].3.a-f. show the relationship between the leaf water potential, leaf turgor potential, stomatal resistance, transpiration and the decline in soil water content. The data is from expt. 8. In all cases, WC₁ = start of 1st. drying cycle, and WC₂ = start of second drying cycle.

Figure 4[1].3.a. shows the response of the leaf turgor potential of a leaf near the apex, in relation to time and the soil water content (compare with Figure 4[1].3.c.). The leaf that was used for the determination of leaf turgor potential changed slightly at the later stages of the experiment. Data points are means of two replicates.

Figure 4[1].3.b. shows the response of leaf water potential of a leaf near the apex (that was in expansion), in relation to time and the soil water content (compare with Figure 4[1].3.c.)

Figure 4[1].3.c. shows the decline in soil water content in the two drying cycles. Compare the responses in the other figures in relation to this. Also compare the responses in the other figures when the soil water content is restored to field capacity by rewatering (RW₁ and RW₂).

Figure 4[1].3.d. shows the response of the abaxial stomata in relation to time and the decline in soil water content.

Figure 4[1].3.e. shows the response of the adaxial stomata in relation to time and the decline in soil water content.

Figure 4[1].3.f. shows the response of the transpiration /cm² in relation to time and the decline in soil water content.
The unit for leaf water potential and leaf turgor potential has been expressed as 'bars'.

The unit that is currently being used is 'Mpa'. 1 bar = 0.1 Mpa.
In all the figures, (●-●) = CONTROL, (△-△) = WATER STRESS 1 and (■-■) = WATER STRESS 2.

Figures 4(1).4.a-f. show the relationship between the leaf water potential, leaf turgor potential, stomatal resistance, transpiration and the decline in soil water content. The data is from expt. 9. In all cases, WC₁ = start of first drying cycle, and WC₂ = start of second drying cycle. Compare the responses in all figures in relation to the Figure 4(1).4.c.

Figure 4(1).4.a. shows the response of leaf turgor potential of a leaf near the apex (that was in expansion), in relation to time and the decline in soil water content. The leaf that was used for the determination of leaf turgor potential changed slightly at the later stages of the experiment. Data points are means of two replicates.

Figure 4(1).4.b. shows the response of leaf water potential of a leaf near the apex (a leaf that was in expansion) in relation to time and the decline in soil water content.

Figure 4(1).4.c. shows the decline in soil water content in the two drying cycles. Compare the responses in the other figures in relation to this. Also compare the responses in the figures when the soil water content is restored to field capacity by rewatering (RW₁ and RW₂).

Figure 4(1).4.d. shows the response of the abaxial stomata in relation to time and the decline in soil water content.

Figure 4(1).4.e. shows the response of the adaxial stomata in relation to the time and the decline in the soil water content.

Figure 4(1).4.f. shows the response of the transpiration/cm² in relation to time and the decline in the soil water content.
The unit for leaf water potential and leaf turgor potential has been expressed as 'bars'. The unit that is currently being used is 'MPa'. 1 bar = 0.1 MPa.
In order to prevent plants from becoming very desiccated and subsequently evoking other secondary responses during the recovery phase, the water stress imposed in these experiments has been mild. With larger stresses, more negative leaf water potentials can be created under controlled environment (e.g. see Marc and Palmer 1976).
began to increase 5 days from the start of the drying cycle and reached a resistance of 11 sec. cm$^{-1}$ at the end of the drying cycle. In plants of wilt$_2$, the stomatal resistance increased 3 days after the start of the drying cycle and reached a resistance of 12 sec. cm$^{-1}$ at the end of the drying cycle. In experiment 9, plants of wilt$_1$ and wilt$_2$ both reached stomatal resistances of 10 sec. cm$^{-1}$ at the end of the drying cycle. These responses are illustrated in figures 4(1).5, 4(1).3e and f, 4(1).4e and f respectively.

4(1).1.7. Adaxial and abaxial stomatal responses during recovery

Within one day of rewatering, the resistance of abaxial and adaxial stomata in experiments 6, 8 and 9 was reduced to control levels. This was similar to the response for sunflower in Chapter 2. Subsequent measurements of the stomatal resistance on several days after rewatering showed that the stomatal resistance of the recovering plants was maintained at the same level of the control plants (also see figures 4(1).3d and e, 4(1).4d and e).

4(1).1.8. Response of leaf water potential during the drying cycle

In experiments 8, 9 and 10, the leaf water potential of the control plants (determined between 1000 and 1100 hours) was between -0.6 and -0.7 MPa.

In experiment 8, the leaf water potential of wilt$_1$ plants became increasingly negative 4 days from the start of the drying cycle to reach -1.7 MPa at the end of the drying cycle. In plants of wilt$_2$, the leaf water potential became more negative within 1 day from the start of the drying cycle to reach -1.75 MPa. In experiment 9, the leaf water potential of wilt$_1$ plants reached -1.7 MPa and that of wilt$_2$ plants
Figure 4[1].5. shows the stomatal response of the abaxial stomata in experiment 6. WC = water stress commenced and RW = rewatered. •—• = Control

The measurements in Figure 4[1].6.b-d were made on leaf 12.

Figures 4[1].6.a-d. show the relationships between the leaf water potential, the leaf turgor potential, the water saturation deficit in the leaf and the decline in the soil water content in experiment 10. Compare the responses in all the figures in relation to figure 4[1].6.a.

Figure 4[1].6.a. shows the decline in the soil water content during the drying cycle. C = control while W = water stressed. Data points are means of two replicates.

Figure 4[1].6.b. shows the response of leaf water potential in relation to time and the decline in the soil water content. C = control, W = water stressed.

Figure 4[1].6.c. shows the response of the leaf turgor potential in relation to the time and the decline in the soil water content. C = control, W = water stressed.

Figure 4[1].6.d. shows the response of the leaf water saturation deficit in relation to time and the decline in the soil water content. C = control, W = water stressed.
The unit for leaf water potential and leaf turgor potential has been expressed as 'bars'.

The unit that is currently being used is 'MPa'. 1 bar = 0.1 MPa.
reached -1.8 MPa. The development of negative water potentials was relatively faster in experiment 9 as compared to experiment 8. The development of negative leaf water potentials was the fastest in experiment 10. Leaf water potential decreased within 30 hours of the start of the drying cycle in experiment 10 and in 3 days reached -2.2 MPa (see figures 4[1].3b, 4[1].4b and 4[1].6b).

4[1].1.9. Response of leaf water potential during recovery

In experiments 8, 9 and 10 the leaf water potential of the recovering plants was established to control plant levels within 2 days of rewatering (see figures 4[1].3b, 4[1].4b.

4[1].1.10. Response of leaf osmotic potential during the drying cycle

The decrease in leaf osmotic potential over the drying cycle was less rapid than the decline in the leaf water potential in experiments 8, 9 and 10. Although initially leaf osmotic potential was more negative than the leaf water potential, with progressive drying of the soil the leaf water potential became more negative than the leaf osmotic potential. In all these experiments, the leaf osmotic potential reached about -1.45 MPa at the end of the drying cycle. The sequential response of the leaf osmotic potential to water stress has not been presented for any of these experiments because the data has been used to calculate the leaf turgor potential response which will be presented in a following section.
The high values of turgor recorded in the stressed plants is probably a result of underestimating the leaf osmotic potential due to addition of water from the vascular system to water in the leaf tissue, after the thawing process and plunger injury.
4(1).1.11. Response of leaf osmotic potential during recovery

Leaf osmotic potential recovered from -1.45 MPa to less negative values on rewatering. But it was always more negative than the leaf water potential at all stages during recovery.

4(1).1.12. Response of leaf turgor potential during the drying cycle and during recovery

Leaf turgor potential essentially depended on the responses of leaf water potential and leaf osmotic potential because P was calculated from the difference between \( \psi \) and \( \pi \). The calculation assumed that matric potential was zero. At the point where leaf water potential became more negative than leaf osmotic potential, leaf turgor potential also became negative and caused the leaves to become flaccid. In the control plants the leaf turgor potential was maintained at between 0.2 MPa and 0.3 MPa during the day. The leaf turgor potential in experiment 10 reached more negative values than in experiments 8 and 9, at the end of the drying cycle.

After rewatering, the leaf turgor potential was re-established to that of control plants within one day. In experiments in which the leaf turgor potential response was not calculated (eg. experiment 6), visual observations indicated that leaf turgidity was quickly re-established. The responses of the leaf turgor potential during water stress and recovery are presented in figures 4(1).3a, 4(1).4a.

4(1).1.13. Response of leaf water saturation deficit during the drying cycle and during recovery

In experiment 7, the leaf water saturation deficit at the end of the
of the drying cycle was 32%. In experiment 10 in which the leaf water saturation deficit was measured daily during the drying cycle in leaves 2, 6 and 12, LWSD became greater in leaf 12 first. But the final leaf water saturation deficit of leaf 2 was much greater than in leaf 6 or 12; for instance, leaf 2 has a WSD of 50% compared to 42% in leaf 12 (compare the responses in tab.4[1].1). After rewatering, the leaf water saturation deficit in control plant leaves and previously water stressed leaves was the same.

Table 4[1].1. Effects of water stress* on the leaf water saturation deficit (%) of leaves at different positions on the main shoot

<table>
<thead>
<tr>
<th>Leaf position on shoot</th>
<th>Treatment</th>
<th>Days from start of drying cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>9.61</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>9.10</td>
</tr>
<tr>
<td>6</td>
<td>Control</td>
<td>12.92</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>11.70</td>
</tr>
<tr>
<td>12</td>
<td>Control</td>
<td>14.70</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>16.46</td>
</tr>
</tbody>
</table>

* Plant age at start of the drying cycle was 22 days and the 16th leaf was unfolding. Leaf 2 was fully expanded before stress and leaves 6 and 12 were expanding.

4[1].1.14. Relationships between the soil water content, R \_s, P, \psi, \pi and WSD during the drying cycle

In the controlled environment experiments, large changes to leaf water potential, leaf turgor potential and stomatal resistance took place only when more than half the available water in the soil had been transpired by the plants. Time course trends of these responses in experiments
8, 9 and 10 are shown in figures 4[1].3a-f, 4[1].4a-f and 4[1].6a-d.

Among the notable features in these figures are the relationships between the leaf turgor potential, the leaf water potential and the stomatal resistance of the leaves. Leaf turgor potential approached negative values when the leaf water potential became -1.1 MPa and at this point, the stomatal resistance began to increase.

The decrease in the transpiration of the water stressed plants started just before the stomatal resistance of the leaf near the apex began to increase.

4[1].2. DISCUSSION

The rapid recovery of the stomata, leaf water potential and leaf turgor potential after rewatering the previously water-stressed plants raised two expectations. Firstly, the drying cycle would not cause long-term complications due to residual water stress effects such as leaf dessication or poorly functioning stomata. Secondly, the effects of water stress on the plant would be mediated directly during the drying cycle or through changes to the morphology of the plant after its relief. These expectations and the relationships that existed between the soil water content, leaf turgor, stomatal resistance, leaf water potential and transpiration shall be discussed here.

The closure of stomata during water deficits in the plant, an observation that has been made by many workers (e.g. Dale 1961, Boyer 1970, Sánchez-Díaz and Kramer 1971, Turner 1974) on different plants, was also observed in these studies. The observation that the stomatal resistance increased only after the incurrence of a critical leaf water potential of -1.1 MPa or a leaf water saturation deficit of 22%, is in general agreement to other findings (Boyer 1970, Turner 1974) where the stomata
did not respond to changes in the leaf water potential or water saturation
deficit until a critical threshold level of these parameters was reached.
However, numerous studies (eg. Frank, Power and Willis 1973) have shown
that there is not a unique value for the critical leaf water potential
of a particular species or cultivar, but the value will depend on previous
treatment, age and position in the canopy. The present threshold value of
-1.1 MPa leaf water potential for the start of stomatal closure in sun-
flowers agrees with Boyer (1970) and Turner (1973). However, Turner also
found that in a field study with sunflowers (unpublished data) the stomata
were still open at -2.0 MPa leaf water potential. This difference between
plants grown in small pots (as in these experiments) and field grown plants
has been explained in terms of the osmotic adjustments that occur in leaves
of field grown plants. First, plants in the field are subject to higher
irradiances relative to those in the growth room and secondly, they have an
unrestricted soil volume into which the roots may grow. Both these factors
can permit the osmotic potential of the leaf to adjust to values lower than
those in leaves of potted plants in the growth room as the soil water
potential decreases (Kreeb 1963, Turner and Begg 1973) thus leading to
different leaf water potentials at the same turgor potential.

The regulation of evaporation by stomatal closure resulting from low
leaf water potential is well recognised. When the evaporation of maize
plants growing in a pair of weighing lysimeters was compared under
identical weather conditions (Turner 1974), the stomatal conductance and
evaporation of the stressed plants were only one-third of those in the
unstressed plants. The regulation of evaporation is sometimes considered
as a means of controlling or preventing the development of further stress.
However, further decreases to the leaf water potential continued to occur
despite the start of stomatal closure, within the same leaf. This finding
is similar to that of Turner (1975) who also found that stomatal closure
did not prevent the development of further stress in maize plants that
were grown in the field.

Another important effect of stomatal closure is the reduction in photosynthesis that occurs concomitantly. Although stomatal resistance is only one among the several resistances encountered in the transfer of carbon dioxide from the atmosphere to the site of carboxylation in the cell, it nevertheless is a large component of the total resistance. The conclusive evidence for stomatal closure being the primary cause of depressed photosynthesis under water limiting conditions was provided by Troughton (1969). However, Boyer (1971) showed that in sunflower nonstomatal effects on photosynthesis were apparent at the same leaf water potential at which the stomata closed. Inhibition of chloroplast activity by low water potential in sunflower (Boyer and Bowen 1970) tends to confirm that nonstomatal effects on CO$_2$ exchange may be equally as sensitive as stomata in this species. It can be concluded that photosynthesis declines initially as a result of stomatal closure, but prolonged severe water stress can lead to depression of chloroplast and enzyme activity and to nonstomatal effects on photosynthesis. The stomatal closure in the present experiments would inevitably have reduced photosynthesis but as the stress was not prolonged, it is unlikely that any major effects on chloroplast and enzyme activity may have taken place. The rapid re-establishment of stomatal functions after rewatering ensured that any limitation to CO$_2$ exchange would at least not be due to partial stomatal closure. It is unlikely that any permanent injury due to water stress was inflicted on chloroplast activity because visual observations of the leaf greeness showed no apparent differences between controls and stressed plants, and leaf growth of the rewatered plants (as reported in sub-chapter 4.2) continued at the same rate as in control plants.

Studies involving general plant responses to water stress in some of the older literature have tended to use the soil water content as an
indicator of plant water stress. Even in the comparative experiment
(Chap. 2), only the decline in soil water content was monitored but the
discussion of that experiment emphasized the need to recognize that
plant growth is controlled directly by plant water deficits. This is
generally borne out by the relationships between the soil water content,
plant stress parameters (leaf water potential, leaf turgor potential) and
the plant growth responses in the present experiments. Although the
growth responses to water stress are reported in sub-chapter 4.2., some
selected responses are included in this discussion to illustrate the
last point.

The general trend in all experiments was that even with a depletion
of half the available water in the soil, no effects on plant growth or
stomatal physiology were observed. Over this range of soil water
depletion, there were only very small changes to the leaf water potential
and the leaf turgor potential. As effects of water stress on stomatal
resistance, leaf enlargement, leaf initiation and unfolding started at a
leaf water potential of -1.1 MPa, it is probable that the plant begins
to experience internal stress only after this point. Thus, for large
changes in the soil water content at the beginning of the drying cycle,
no effects on plant growth were observed. Further evidence of the soil
water content being an inaccurate indicator of plant water stress is
provided by the contrasting levels of internal plant water stress that
were developed by water stressed plants in experiments 8 and 10. A
comparison of the decrease in leaf water potential between these
experiments in relation to soil water depletion shows that for a similar
decrease in soil water content in the earlier parts of the drying cycle,
approximately the same rate of decrease in leaf water potential occurred
in both experiments, despite the differences in the rate of soil water
deposition. However, towards the end of the drying cycle large
differences in the rate of decrease in leaf water potential occurred
between leaves of experiment 8 and 10. For the same amount of soil water
depletion, leaves in experiment 10 also developed more negative water
potentials than leaves in experiment 8. Similarly, water saturation
deficits in leaves of experiment 10 were larger than in leaves of
experiment 7. These differences may be reconciled with the different
sizes of the plants between experiments 8 and 10 and between experiments
7 and 10 at the beginning of the drying cycle. While plants in
experiment 8 (wilt\textsubscript{1}) were 12 days old having a total leaf area of 90 cm\textsuperscript{2},
plants in experiment 10 were 22 days old and had a total leaf area of
650 cm\textsuperscript{2}. Plants in experiment 7 were 14 days old and had a total leaf
area of about 150 cm\textsuperscript{2}.

The differences in the stress severity between experiments 8 and
10 was also observed in other terms. Even during the latter part of the
drying cycle in experiment 8, leaf turgidity was somewhat regained during
the dark period probably as a result of equilibration between the soil
water potential and the leaf water potential. Such equilibration was
not possible in the larger plants of experiment 10 because of the quick
drying cycle. Consequently, plants in experiment 10 appeared wilted
even during the dark period. These differences in the severity of the
stress between experiments 8 and 9 would probably have been more apparent
if measurements of leaf water potential and leaf turgor potential were
made towards the end of the dark period. The measurements were not made
for three reasons. Firstly, water potential was measured so that future
water stress experiments could be planned to impose comparable stresses;
it therefore did not matter when the measurements were made so long as
the time was standardized. Secondly, it was of interest to compare the
responses of stomatal resistance and leaf water potential; as the
stomatal resistance had to be measured in the light, leaf water potential
measurements had also to be measured at the same time. Thirdly, there
were no spare plants on which leaf water potential measurements could
be done at the end of the dark period. As mentioned in Chapter 3, the process of cell division, especially in an unfolding leaf, showed a diurnal rhythm. The possible influence of leaf water potential on such a rhythm can only be evaluated if diurnal measurements of leaf water potential are made. Thus, future experimentation should take this into account.

The slow but consistent decline in the transpiration rate of the control plants may be related to several factors. As the control plants increased in size, self shading and mutual shading between plants became greater. Although mutual shading between plants was minimised by carefully arranging the plants at proper distances, the reduction of incident light on the lower leaves by self shading could not be avoided. As stomatal opening is partly dependent on light activation, it is understandable that the stomatal efficiency of the lower leaves will be reduced. Besides this, stomatal efficiency also declines with leaf age and senescence. However, for the sunflower, the stomatal efficiency has been shown not to decrease for a long time, provided light was not limiting (Rawson and Woodward 1976). The plants in these leaf experiments were quite young and it is unlikely that leaf ageing of the lower leaves is a factor in any decline of their stomatal efficiency. The overall reduction in the transpiration rate with increasing plant age probably results from a reduction to the transpiration of the lower leaves which arises from shading.

The re-establishment of the transpiration rate of the previously water-stressed plants is mainly due to the quick recovery of the stomatal mechanism. As the stomatal resistance in the previously stressed plants was unaffected by water stress, the stomatal cell number per unit area and the individual stomatal pore size would have been affected in a such a way to maintain normal conductance. The reduced transpiration of the recovering plants is mainly due to the decrease in transpiring leaf area. These effects of water stress on leaf area are described and
discussed in sub-chapter 4.2.

Iljin (1957) has reported that up to 50% of stomata are rendered inactive by water stress. The drying cycles that have been employed in these experiments do not cause permanent injury to the stomatal mechanism. The lag in transpiration between the control and the previously stressed plants is more or less equal to the difference in leaf area. Thus, all responses to water stress are directly manifested during the drying cycle or by changes to the plant size and form after water stress is relieved.
LEAF RESPONSES TO WATER STRESS

4[2].1. RESULTS

The leaf responses to water stress in experiments 6 to 12 and that in experiment 14 are reported in this sub-chapter. The responses during water stress are reported first followed by the responses during recovery. Wilt 1 refers to the first batch of stressed plants.

LEAF RESPONSES DURING WATER STRESS

4[2].1.1. Leaf initiation

Leaf initiation was affected by water stress in plants that were still initiating leaf primordia. Initiation of leaf primordia either stopped completely as a result of water stress or it proceeded at rates below that of control plants.

In experiment 7, leaf initiation was affected about 3 days after the start of the drying cycle (fig.4[2].1.a.). At the end of the drying cycle, the stressed plants had about five leaves less than the control plants.

In experiment 8, leaf initiation was affected by water stress five days after the start of the drying cycle in wilt 1 (water stress 1) and three days after the start of the drying cycle in wilt 2. Leaf primordia were initiated in the plants during water stress but at a much slower rate than the control plants. The leaves 22 to 29 were
Figures 4(2).1.a-d. show the responses of leaf initiation and leaf unfolding in experiments 7, 8, 9 and 12 respectively. WC = water stress cycle commenced, RW = rewatered. 1 and 2 denote the first and second drying cycles in an experiment. (●—●) = control, (△—△) = the responses of the first water stress cycle and (○—○) = the responses of the second water stress cycle. The dotted line (------) is used to denote the expected trend in situations where it was difficult to distinguish between leaves and bracts, but in experiment 12 it is used because there were no extra plants to obtain data on those days.

In expt. 7, the 8th leaf was unfolding at the start of water stress and there were 22 leaves on the plant. In expt. 8, the 7th leaf was unfolding from the bud at the start of water stress_1 and there were 22 leaves on the plant; a similar situation was true of expt. 9. In expt. 12, the third leaf of water stress_1 and the fifth leaf of water stress_2 were unfolding at the start of the stresses.
initiated during the drying cycle of wilt\textsubscript{1} and leaves 26 to 32 were initiated during the drying cycle of wilt\textsubscript{2}. There was a reduction of 6 leaves in wilt\textsubscript{1} plants and 4 leaves in wilt\textsubscript{2} plants at the end of their respective drying cycles (see fig. 4[2].1.b).

In experiment 9, the leaf initiation responses to water stress were essentially the same as in experiment 8 (see fig. 4[2].1.c). The effects of water stress on leaf initiation in experiment 9 were however manifested earlier than in experiment 8. Leaves 20 to 25 were initiated in wilt\textsubscript{1} plants and leaves 24 to 28 were initiated in wilt\textsubscript{2} plants during the drying cycle.

In experiment 12, leaf initiation in wilt\textsubscript{1} was affected 2 days after the start of the drying cycle while in wilt\textsubscript{2} leaf initiation was affected 1 day after the start of the drying cycle. Leaves 10 to 14 were initiated during the drying cycle of wilt\textsubscript{1} and leaves 14 to 18 were initiated during the drying cycle of wilt\textsubscript{2}. At the end of the drying cycle, wilt\textsubscript{1} plants had 5 leaves less than controls and wilt\textsubscript{2} plants had 3 leaves less than controls (see fig. 4[2].1.d).

4[2].1.2. Leaf unfolding (see figs. 4[2].1.a-d)

Leaf unfolding from the bud was affected by water stress in all the experiments. Leaf unfolding was affected at about the same time that leaf initiation was. Like leaf initiation, unfolding continued during water stress but at a slower rate than control plants. In most experiments, there was a difference of between 1.5 and 2.5 unfolded leaves between control and stressed plants at the end of a drying cycle. Because it was necessary for an overall appraisal of the effects of water stress on the events at the plant apex, the leaf unfolding responses have been plotted within the same axes used for leaf initiation responses.
Figure 4[2].3. shows the response of total plant leaf area to water stress. The data was derived from expt.6. WC = start of water stress cycle, RW = rewatered. the experiment was of a short term nature and the response on day 32 do not represent the final recovery response. Data points are means of two replicates.

Figure 4[2].2. shows the responses of total lamina fresh weight and total lamina dry weight to water stress in experiment 6. WC = start of drying cycle while RW = rewatered. Data points are means of two replicates. Note that while lamina fresh weight gain is arrested, the increase in the dry weight of the water stressed laminae continues, but at a slower rate than in the control.
### 4[2].1.3. Total lamina dry and fresh weight

Total lamina dry and fresh weight were reduced by water stress. Since lamina fresh weight was measured in only experiment 6, the comparative responses of lamina dry weight and lamina fresh weight are based solely on data from that experiment. At the end of the drying cycle in experiment 6, the lamina fresh weight of the stressed plants was reduced more than the lamina dry weight. While lamina fresh weight increase was stopped completely by water stress, lamina dry weight continued to increase slowly (see fig. 4[2].2). Total lamina dry weight was also reduced in the other leaf experiments but as in experiment 6, water stress did not completely arrest dry weight increase.

### 4[2].1.4. Total lamina area

In all the experiments, the total lamina area was reduced by about 50% at the end of the drying cycle. An example of this is shown in fig. 4[2].3. In most experiments, effects on total lamina area were apparent at about the same time when leaf initiation was affected. This comparison can be drawn by reference to the leaf initiation responses in experiment 8 (fig.4[2].1.b) and the total lamina area responses in the same experiment (fig.4[2].11).

### 4[2].1.5. Individual lamina area

Lamina area increase of all the unfolded leaves was affected by water stress. The increase in area of the lower laminae was affected sooner than in leaves that had just unfolded. In all experiments, the first signs of leaf wilting were observed on leaves that were positioned lowest on the plant. The time course trends of area responses to water
Figure 4[2].4.a-k. show the responses of individual laminae area (of laminae 1-10, 15 and 17) to water stress in experiment 6. WC = start of the water stress cycle, RW = rewatered. (■■■■) = control while (○○○○) = water stressed. Laminae 1 to 10 were fully expanded while laminae 15 and 17 were still in expansion. The data for laminae 15 and 17 were included to provide a comparison of the responses of very young leaves to the stress. The long term recovery responses of these young leaves were studied in expt.8, 9 and 10. Also note that laminae 1 and 2 senesced as a result of the water stress. The leaves have been numbered from the base of the plant, the cotyledons not being regarded as leaves.
Figure 4[2].5.a-d. show the lamina area responses of leaves 6 to 9 to water stress in experiment 7. WC = water stress cycle started, RW = rewatered, (●—●) = control (○—○) = water stressed. The 8th leaf was unfolding at the start of the water stress cycle. Data points are means of five replicates.

Figure 4[2].5.e. shows the responses of individual laminae area to water stress in experiment 7, expressed as a percentage of their respective control leaves. Note that the largest depression to the lamina area occurred to leaf 7, which had just unfolded before the start of the water stress cycle.

Figure 4[2].5.f-i. show the dry weight responses of leaves 6 to 9 to water stress in experiment 7. WC = water stress cycle started, RW = rewatered, (●—●) = control (○—○) = water stressed. The 8th leaf was unfolding at the start of the water stress cycle. Data points are means of five replicates.

Figure 4[2].5.j. shows the responses of individual laminae dry weight in experiment 7, expressed as a percentage of their respective control leaves. Note that the largest reduction to the dry weight of the lamina occurred to leaf 7 which had just unfolded before the start of the water stress cycle. Also note that extent of the reduction in the dry weight of the lamina of leaf 7 is about the same as the reduction in its area.
Figure 4[2].6.a-g. show the responses of individual laminae area to water stress in experiment 8. WC\textsubscript{1} = start of the first water stress cycle on a batch of plants, WC\textsubscript{2} = start of the second water stress cycle on a batch of plants. RW\textsubscript{1} = first batch of stressed plants were rewatered, RW\textsubscript{2} = second batch of stressed plants were rewatered. The 7th leaf was unfolding at the start of the first water stress cycle (WC\textsubscript{1}) and the 9th leaf was unfolding at the start of the second water stress cycle (WC\textsubscript{2}). (●●●) = control, (△△△) = response of first batch of water stressed plants, (○○○) = response of second batch of water stressed plants.
stress for some of the laminae in experiment 6 (fig. 4[2] 4 a-k), in experiment 7 (fig. 4[2].5.a-d) and in experiment 8 (fig. 4[2].6.a-g) will illustrate the response much more clearly.

At the end of the drying cycle however, the younger laminae experienced a greater reduction in leaf area than the older laminae that were positioned further down the leaf profile. In experiments 6 and 10, laminae 1 and 2 showed prominent signs of senescence. These two laminae also were shrunk, resulting in a very large decrease in their area. The areas of individual laminae in experiment 6 have been expressed as a percentage of their corresponding controls, at the end of the drying cycle (see fig. 4[2].7). The reduction in lamina area ranged from 80% in young laminae to about 30% reduction in older laminae.

4[2].1.6. Individual lamina dry weight

In experiment 6, dry weight increase in individual laminae was less sensitive to water stress than was lamina area increase. Dry weight increase continued in all the laminae during water stress, but at a slower rate than in control plants. This was in contrast to an abrupt stop to lamina area increase in some of the same leaves - compare the responses of individual lamina area (fig. 4[2].4 a-k) with the responses of individual lamina dry weight (fig. 4[2].8 a-j). The dry weight responses of the individual laminae to water stress in experiment 7 are shown in fig. 4[2].5 f-i. The responses of individual laminae, at the end of the drying cycle, have been expressed as a percentage of their respective controls in fig. 4[2].7. for leaves of experiment 6. The figure also shows that the laminae dry weight was affected to a smaller degree in comparison to the laminae area. Also, laminae 1 and 2 had senesced heavily but incurred only small reductions in their dry weight.
Figure 4.7 shows the responses of individual lamina area, individual lamina dry weight; individual petiole length and individual petiole dry weight at the end of the drying cycle in experiment 6. Leaves 1 and 2 had senesced by this time and were shrunk. The responses have been expressed as a percentage of their respective controls. Data points are means of two replicates. Note that the lamina area is much more reduced than the lamina dry weight, and similarly the petiole length is reduced more than the petiole dry weight.
LEAF POSITION ON THE SHOOT

% of CONTROL
AT THE END OF THE DRYING CYCLE

100
90
80
70
60
50
40
30
20

△ Lamina area
▲ Lamina dry weight
○ Petiole length
• Petiole dry weight
Figure 4[2].8.a-j. show the responses of individual laminae dry weight to water stress in experiment 6. WC = start of the water stress cycle, RW = rewatered, (■■■) = control, (○○○) = water stressed. The unfolding leaf at the start of the water stress cycle was leaf 16. Data points are means of two replicates.
4[2].1.7. Leaf senescence

Accelerated leaf senescence was observed in leaves 1 and 2 of experiments 6 and 10, the lower leaves of experiments 13, 14 and 15 and also the lower leaves of the 'dry plot' plants of experiment 16 (field experiment). Water stress did not induce accelerated senescence in any of the other leaves in experiments 6 and 10 or of the leaves positioned at the top of the plants in experiments 13, 14, 15 and 16. As more interesting patterns of senescence occurred during recovery, especially in experiment 14, a full description of senescence responses has been left till a later section of the results in this chapter.

4[2].1.8. Specific lamina weight

Table 4[2].1. has been derived from the raw data of the responses of dry weight and leaf area of individual leaves to water stress (on the last day of the drying cycle in experiment 6 - see fig. 4[2].4.f-i and fig. 4[2].8.f-i). It shows the responses of the specific lamina weight to water stress in laminae 6 to 9, at the end of the drying cycle.

Table 4[2].1. Specific lamina weight responses of leaves 6 to 9 in experiment 6, to water stress (response on the last day of the drying cycle)

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>specific lamina weight of leaf 6</th>
<th>leaf 7</th>
<th>leaf 8</th>
<th>leaf 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>2.769</td>
<td>2.777</td>
<td>2.894</td>
<td>2.720</td>
</tr>
<tr>
<td>WATER STRESSED</td>
<td>3.450</td>
<td>3.708</td>
<td>3.605</td>
<td>4.258</td>
</tr>
</tbody>
</table>

It can be seen that the specific lamina weight increased in the stressed leaves.
Figure 4[2].9. a-j. show the responses of individual petiole lengths to water stress in experiment 6. WC = start of the water stress cycle, RW = rewatered, (●●●●) = control, (O—O) = water stressed. The unfolding leaf at the start of the water stress cycle was leaf 16. Note that petiole 7 and above have not stopped elongation. Compare this with the expansion responses of the lamina (shown in figure 4[2].4.a-k.) Data points are means of two replicates.
4[2].1.9. Petiole length

Petiole length was affected by water stress (see fig. 4[2].9.a-j). In the same leaf, petiole length was affected sooner than was the area of the lamina subtended by the petiole; this is illustrated by data of experiment 6 (compare fig. 4[2].4.a-k with fig. 4[2].9.a-j). However, for leaves near the bud (unfolding leaves), petiole length and lamina area of a leaf were affected at about the same time by water stress. At the end of the drying cycle, the magnitude of the depression in petiole length was greatest in young petioles. However, in comparison to lamina area responses of individual leaves, petiole length was less affected in the younger leaves (see fig. 4[2].7).

4[2].1.10. Petiole dry weight

Dry weight increase in petioles was affected by water stress (see fig. 4[2].10.a-j) but later than the effect on petiole length (compare with fig. 4[2].9.a-j). The dry weight of petioles of lower leaves was affected only very slightly; in contrast, the dry weight of petioles of the upper leaves was greatly affected. At the end of the drying cycle, the magnitude of the depression was greatest for petioles of the upper leaves. The magnitude of the dry weight reductions in petioles were less than the reductions in petiole length (see fig. 4[2].7).

LEAF RESPONSES DURING AND AT THE END OF RECOVERY

4[2].1.11. Leaf initiation

When the stressed plants were rewatered, leaf initiation continued at rates comparable to that in control plants. There was no evidence in
Figure 4[2].10. a-j. show the responses of individual petiole dry weight to water stress in experiment 6. WC = start of the water stress cycle, RW = rewatered, (■■■) = control, (□□□) = water stressed. The unfolding leaf at the start of the water stress cycle was leaf 16. Note that, like the petiole elongation pattern, the dry weight of petioles 7 and above continue to increase at the end of the experimental time. Compare this with the lamina dry weight increase pattern shown in figure 4[2].8.a-j; the increase in dry weight of lamina 8 had come to a stop.
any of the experiments for leaf initiation to attain rates above that of control plants. However, there was a decrease in the total leaf number for the water stressed plants.

In experiment 8, wilt$_1$ had 5 leaves less than the controls which had 47 leaves. In the same experiment, wilt$_2$ plants had 4 leaves less than controls. In experiment 9, a smaller reduction in the leaf number of water stressed plants was noticed. In experiments where extended recovery was not studied, it was difficult to distinguish between the last formed leaves and the first formed involucre bracts; as such, no conclusive statements on the final leaf number can be made for the water stressed plants of those experiments.

In experiment 8, leaves 30 to 42 of wilt$_1$ plants were initiated after water was resupplied to the stressed plants. In the same experiment, leaves 33 to 42 of wilt$_2$ plants were initiated after water was resupplied. In experiment 9, leaves 36 to 41 of wilt$_1$ plants and leaves 29 to 41 of wilt$_2$ plants were initiated after water was resupplied to the stressed plants. In experiment 12, leaf 15 and above of wilt$_1$ and leaf 18 and above of wilt$_2$ plants were initiated after water was supplied again to the plants (see fig. 4[2].1.a-d).

4[2].1.12. Leaf unfolding

There was little evidence for leaves of stressed plants to unfold at rates greater than in control plants, when water was supplied again. In most cases, leaf unfolding rates were comparable to that of control plants during recovery.

In experiment 8, leaf 11 and above of wilt$_1$ and leaf 12 and above of wilt$_2$ unfolded after the stressed plants were rewatered. In experiment 9, leaf 10 and above of wilt$_1$ and leaf 11 and above of wilt$_2$ unfolded after the
Figure 4[2].11. shows the response of the total leaf area on the plant to water stress in experiment 8. $\text{WC}_1 = \text{start of the first water stress cycle}$, $\text{WC}_2 = \text{start of the second water stress cycle}$, $\text{RW}_1 = \text{the first batch of water stressed plants were rewatered}$, $\text{RW}_2 = \text{the second batch of water stressed plants were rewatered}$. $\pm$ = non-destructive measurements of leaf area were made on these days because there were insufficient plants for destructive analysis. (see Appendix B 17 for details of methods to obtain leaf area non-destructively).
First anthesis
stressed plants were rewatered. In experiment 10, leaf 20 and above unfolded after the stressed plants were rewatered (see fig. 4[2].1.a-d).

4[2].1.13. Total lamina dry and fresh weight

In the short term recovery experiment (experiment 6), total lamina fresh weight of stressed plants gained increment rates comparable to the control plants when water was supplied again. Total lamina dry weight of the stressed plants also exhibited similar responses (see fig. 4[2].2.). Since the recovery period was only short term, it was not possible to state if recovery would be complete.

In experiments where extended recovery was followed, there was an eventual reduction in the total lamina dry weight.

4[2].1.14. Total lamina area

In experiment 6, when water was supplied again, the increase in lamina area of stressed plants followed rates comparable to control plants (see fig. 4[2].3). In experiment 8, the final total lamina area was reduced by 10% (see fig. 4[2].11) while in experiment 10, there was a 25% reduction (see fig. 4[2].14.a).

4[2].1.15. Individual lamina area

These responses are best described separately for unfolded leaves and leaves already present before water stress, leaves that were initiated during water stress and leaves that were initiated during recovery.

Among all the leaves that were present on the plant before water stress, the leaves that were eventually most reduced in area were
Figure 4[2].12.a. shows the responses of the area of individual leaves on plants in experiment 8 to water stress. The responses were measured when all the leaves had completed expansion; this was just prior to the anthesis of the first disc floret on the capitulum. \((O--O) = \text{control, } (\uparrow\uparrow) = \text{responses of leaves resulting from the first water stress cycle; the 7th leaf was unfolding at the start of stress, } (\bullet\bullet) = \text{responses of leaves resulting from the second water stress cycle; the 9th leaf was unfolding at the start of the stress cycle. Note that the leaf number in the stressed plants has been reduced.}

Figure 4[2].12.b. shows the responses of the area of individual leaves to water stress in experiment 8, expressed as a percentage of the leaf area of their respective control leaves. The unfolding leaf at the start of water stress\(_1\) was leaf 7 while that in water stress\(_2\) was leaf 9. Note that the 8th leaf in water stress\(_1\) (\(\uparrow\uparrow\)) and the 11th leaf in water stress\(_2\) (\(\bullet\bullet\)) were most markedly reduced in area by the water stress. Leaves 22 to 29 were initiated during the drying cycle of water stress\(_1\) and leaves 26 to 32 were initiated during the drying cycle of water stress\(_2\). Leaves 30 and above of water stress\(_1\) and 33 and above of water stress\(_2\) were initiated after rewatering. Leaves 11 and above of water stress\(_1\) and leaves 12 and above of water stress\(_2\) unfolded after rewatering. There are one or two anomalous points on the response trend; for instance, the response of leaves 13 and 14 are not consistent with the general trend. Also note the apparent compensatory responses, especially in water stress\(_1\), of leaves 25 to 32 and the marked depression in area of leaves 35 and above. AW = apex of water stressed plants, AC = apex of control.
unfolding when water stress became severe. With reference to fig. 4[2].5.a-g, in most cases, the leaves that showed marked depression in area were those which were between 3% and 20% $A_{\text{max}}$ when water stress became very severe. The leaves that were markedly depressed in the different experiments and the extent of their depression are shown in table 4[2].2.

The other unfolded leaves were also reduced in area but to a lesser extent. In experiments 7, 8 and 9, laminae that had attained 50% $A_{\text{max}}$ or more before the water stress, recovered almost completely in most cases when water was supplied again. The response of such unfolded leaves in experiment 8 are shown in fig. 4[2].6.a-g and in figs. 4[2].12 a and b. In experiment 10, however, even the leaves that had achieved 50% $A_{\text{max}}$ before the stress started, were quite markedly reduced in area eventually. Laminae that were between 20% and 50% $A_{\text{max}}$ before stress were reduced in area to various levels (figs. 4[2].14 a and b). The magnitude of the area reduction in these leaves was much greater in experiment 10 as compared to the other experiments. The responses of laminae area in experiments 7 and 9 are shown in fig. 4[2].5e and figs. 4[2].13 a and b respectively.

The eventual reduction in the area of leaves that were in a folded state before the water stress, was very small and in some cases there was no reduction but showed some apparent compensation growth (see fig. 4[2].12., 4[2].13 and 4[2].14). This was in contrast to the eventual reduction in the area of leaves that were unfolded or unfolding before the start of the stress. In experiment 8, leaves 11 to 22 which were in a folded state at stress were eventually reduced from between 15% to 0% in area; this was in contrast to the 32% reduction in area or thereabouts of some of the leaves which were already unfolded before stress (e.g. leaves 7 to 10). However, there are one or two anomalous
Figure 4[2]. 13.a. shows the response of the area of individual leaves on plants in experiment 9 to water stress. The responses were measured when all the leaves had completed expansion; this was just prior to the anthesis of the first disc floret on the capitulum. (○—○) = control, (▲▲) = responses of leaves resulting from the first water stress cycle; the 7th leaf was unfolding at the start of the stress cycle. (■■■) = responses of leaves resulting from the second water stress cycle; the 9th leaf was unfolding at the start of the stress cycle. Note that the number of leaves on the stressed plants has been reduced by only one.

Figure 4[2]. 13.b. shows the responses of the area of individual leaves to water stress in experiment 9, expressed as a percentage of the leaf area of their respective control leaves. The unfolding leaf at the start of water stress₁ was leaf 7 while that in water stress₂ was leaf 9. Note that the 7th leaf in water stress₁ (▲▲) and the 8th leaf in water stress₂(■■■) were most markedly reduced in area by the water stress. Leaves 20 to 25 in water stress₁ and leaves 24 to 28 in water stress₂ were initiated during the drying cycle. Leaves 26 and above in water stress₁ and leaves 29 and above in water stress₂ were initiated after rewatering. Leaf 10 and above of water stress₁ and leaf 11 and above of water stress₂ unfolded after rewatering. The compensation responses of some of the leaves, e.g. 17 to 30 are not as large as in experiment 8. But note the large depression in the area of leaves above leaf position 30. AW = apex of water stressed plant, AC = apex of control.
LEAF AREA ($cm^2$)

LEAF POSITION ON SHOOT

LEAF AREA (% of control)

CONTROL

LEAF POSITION ON SHOOT
Figure 4[2].14.a. shows the responses of the area of individual leaves in experiment 10 to water stress. (–O–) = control, (▲▲▲) = water stressed. The 16th leaf was unfolding at the start of the stress cycle. There was no reduction in the leaf number because the water stress started after leaf initiation in the plants had been completed. Leaves 1 and 2 senesced as a result of the water stress.

Figure 4[2].14.b. shows the responses of the area of individual leaves in experiment 10, expressed as a percentage of the area of their respective control leaves. The unfolding leaf at the start of the water stress was leaf 16. Note that the largest reduction in leaf area occurred to leaf 14. Also note that compensatory growth in the leaves at the higher ontogenetic positions (eg. leaves 33 and above) are not as large as in experiments 8 and 9. Also note that the last few leaves on the plant are not reduced in area, and contrast this with leaves at the same position in experiments 8 and 9. Leaves 1 and 2 had senesced as a result of water stress and are shown by the dotted (-----) line. AW = apex of water stressed plants, AC = apex of control.
Table 4[2].2. Relationship between the unfolding leaf, water stress and the magnitude of reduction in the leaf area of the unfolding leaf in the various experiments.

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Unfolding leaf at the start of drying</th>
<th>Leaf that was most markedly reduced</th>
<th>Magnitude of reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>8th leaf</td>
<td>7th leaf</td>
<td>25%</td>
</tr>
<tr>
<td>8, wilt₁</td>
<td>7th leaf</td>
<td>8th leaf</td>
<td>27%</td>
</tr>
<tr>
<td>8, wilt₂</td>
<td>9th leaf</td>
<td>11th leaf</td>
<td>32%</td>
</tr>
<tr>
<td>9, wilt₁</td>
<td>7th leaf</td>
<td>7th leaf</td>
<td>30%</td>
</tr>
<tr>
<td>9, wilt₂</td>
<td>9th leaf</td>
<td>8th leaf</td>
<td>22%</td>
</tr>
<tr>
<td>10</td>
<td>16th leaf</td>
<td>14th leaf</td>
<td>43%</td>
</tr>
<tr>
<td>12, wilt₁</td>
<td>3rd leaf</td>
<td>3rd leaf</td>
<td>27%</td>
</tr>
<tr>
<td>12, wilt₂</td>
<td>5th leaf</td>
<td>5th leaf</td>
<td>40%</td>
</tr>
</tbody>
</table>

Data points in the response pattern of leaves of wilt₁ plants in experiment 8 (see fig. 4[2].12). For example, the responses of leaves 12, 13 and 14 of wilt₁ were inconsistent with the expected trend. In experiment 10, the leaves that were in a folded state before stress, eventually recovered fully (leaves 20 onwards, fig. 4[2].14 a and b).

However, in contrast to the full or near to full recovery of the folded leaves in experiments 8, 9 and 10, the leaves that were in a folded state before stress in experiment 12 were eventually reduced drastically in leaf area (see fig. 4[2].15). For example, leaves 4 onwards of wilt₁ and 6 onwards of wilt₂ in experiment 12 were in a folded state before the start of the drying cycle; despite this, they were eventually reduced in leaf area.

The leaves that were initiated during water stress in experiment 8 eventually showed some evidence of compensatory growth (e.g. leaves 26 to 32 in fig. 4[2].12, which show up to 35% increase in leaf area). This type of compensatory growth was less clear in experiments 9 and 10.
Figure 4(2).15. shows the responses of the area of individual leaves in experiment 12 to water stress, expressed as a percentage of the area of their respective control leaves. Leaves 1 to 11 had reached their maximum expansion at the time this leaf area measurement was made, while those above leaf 11 were still expanding. Leaf 3 of water stress$_1$ (Δ-Δ) and leaf 5 of water stress$_2$ (O-O) were unfolding at the start of the first and second stress cycles respectively. Note that the 3rd leaf of water stress$_1$ and the 5th leaf of water stress$_2$ are most markedly reduced in area. Also note that the leaves which were in a folded state before the start of water stress (e.g., leaves 6 and above) were also quite drastically reduced in area; contrast this with the folded leaves in the expts. 8, 9 and 10.

Figure 4(2).16. shows the specific lamina weight responses of the individual leaves in experiment 12 to water stress. (●-●) = control, (Δ-Δ) = specific lamina weight response of plants from water stress$_1$, (O-O) = specific lamina weight response of plants from water stress$_2$. Note that the largest effect of water stress is on the leaves positioned lower on the plant (e.g., leaves 1 to 6). Leaves that are higher up on the shoot, especially in water stress$_1$, approach full recovery. There is an interaction between the stress effect and the position of the leaf on the shoot, in this case.
LEAF POSITION ON SHOOT

LAMINA AREA (% CONTROL) AT END OF RECOVERY

SPECIFIC LAMINA WEIGHT (mg/cm²)

LEAF POSITION ON SHOOT
The leaves that were initiated after water stress was relieved became markedly reduced in both the experiments 8 and 9. These were the last formed leaves on the plant, just below the inflorescence (e.g. leaves above position 33 in fig. 4[2].12.b and fig. 4[2].13.b). In contrast, the last formed leaves in experiment 10 were not reduced in area (fig. 4[2].15.b).

4[2].1.16. Individual lamina dry weight

In most cases, the lamina dry weight reduction at final recovery was the same as the lamina area reduction (compare fig. 4[2].5e with 4[2].5j., see fig. 4[2].17a), except in experiment 12. The dry weight and area of laminae were depressed by about equal proportions in most experiments, such that there was no change to the specific leaf weight; in experiment 12, the dry weight of a lamina was reduced more than the area of the lamina. This was the case with laminae 1 to 13 of both wilt₁ and wilt₂ plants of experiment 12. However, besides the stress-induced lowering of the specific weight of leaves in experiment 12, there was also an ontogenetic effect (see fig. 4[2].16).

4[2].1.17. Leaf senescence

Accelerated senescence of leaves was observed in experiments 6, 10, 13, 14, 15 and 16. In experiments 6 and 10, laminae 1 and 2 senesced very quickly and dried up in the stressed plants even after the supply of water. There was no evidence for accelerated senescence of the other laminae on the plant. Laminae 1 and 2 had attained their maximum areas at the time when stress was imposed.

The senescence pattern in the other experiments may be illustrated with detailed data obtained from experiment 14. In experiment 14, accelerated senescence began to occur for most of the leaves in plants
which experienced water stress at stages of grain filling. A water stress applied at the completion of anthesis resulted in the accelerated senescence and death of half the leaves. When water stresses were applied between stages of inflorescence initiation and just prior to first anthesis, accelerated senescence and death of the first 4 or 5 leaves was noticed. In all cases, accelerated senescence occurred first in the lower leaves. The accelerated senescence and death of leaves, especially in plants that were at stages of grain filling, reduced the total photosynthesizing leaf area (see table 4[2].3).

Table 4[2].3. Effects of water stress at various stages of plant development on the number of viable leaves and the total plant leaf area (data from experiment 14).

<table>
<thead>
<tr>
<th>Time of application of water stress</th>
<th>Number of viable leaves on plants at last harvest</th>
<th>Total leaf area (cm²) at the last harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>24.3</td>
<td>2456</td>
</tr>
<tr>
<td>just before initiation of disc florets</td>
<td>21.3</td>
<td>1985</td>
</tr>
<tr>
<td>at macroscopic appearance of inflorescence</td>
<td>21.3</td>
<td>2077</td>
</tr>
<tr>
<td>3 days before 1st anthesis</td>
<td>20.6</td>
<td>2346</td>
</tr>
<tr>
<td>5 days after 1st anthesis</td>
<td>19.3</td>
<td>1965</td>
</tr>
<tr>
<td>8 days after 1st anthesis</td>
<td>14.3</td>
<td>1319</td>
</tr>
<tr>
<td>13 days after 1st anthesis</td>
<td>12.0</td>
<td>968</td>
</tr>
</tbody>
</table>

4[2].1.18. Petiole dry weight and length

It was difficult to assess petiole length responses on mature plants because there was shrinking and curvature of the petioles due to the growth conditions at maturity. The responses could also be
Figure 4[2].17.a. shows the response of area and dry weight of individual leaves in experiment 6 to water stress, expressed as a percentage of the area and dry weight of their respective control leaves at the end of the 6 day recovery period. Only the responses of laminae 1 to 10 are presented because the other leaves had not completed expansion at that time. Note that the reduction in the area and the dry weight of leaves is of the same order in the individual leaves. Also note that leaves 1 and 2 had senesced at the end of the experiment (denoted by the dotted line).

Figure 4[2].17.b. shows the responses of the length and dry weight of individual petioles in experiment 6 to water stress, expressed as a percentage of the length and dry weight of the respective control petioles at the end of the 6 day recovery period. Although the responses of petioles 1 to 10 are presented, only petioles 1 to 6 had completed their growth by this time. Compare the responses of the first six leaves and the first six petioles. Dry weight was more affected than length in petioles while dry weight & area were equally affected in laminae.
LEAF POSITION ON THE SHOOT

% of CONTROL
(at the end of recovery)

A  Lamina area
Δ Lamina dry weight

B  ○ Petiole length
    ● Petiole dry weight
confounded with migration of dry matter into the petioles from the lamina. However, the assessment of petiole responses in experiment 6 were free from these interferences because the experiment was short term. At the end of the 6 day recovery period in experiment 6, only the first six petioles were fully grown. The reduction to the dry weight was greater than the reduction to length in the six petioles. Petioles 7 to 10 were not fully grown at the time of the assessment. However, in petioles 7 to 10 the dry weight reduction was about twice the reduction in length. The petiole length and dry weight responses were in direct contrast to that exhibited by the laminae (compare fig. 4[2].17a with 4[2].17b).

4[2].2. DISCUSSION

The differences in total leaf area between well-watered control plants in the different experiments arose not only from differences in leaf number but also from differences in the sizes of individual leaves, although principally because of the latter. The differences between experiments in the rates of leaf initiation and leaf unfolding, together with the differences in total leaf area and total leaf number possibly involve two cabinet factors—light intensity and air circulation.

While light intensity in experiment 6 was 500 microeinsteins $M^{-2}$ sec.$^{-1}$, in all other leaf experiments (experiments 8 to 12) it was 650 microeinsteins $M^{-2}$ sec.$^{-1}$. The differences in plant growth between experiment 6 and the other experiments may be related to the effects of light intensity. Such a relationship between leaf area increase, leaf initiation rates, total leaf number and the light intensity level has been shown to exist in Cucumis sativus (Milthorpe and Newton 1963). But, despite the adjustment of light intensity to 650 microeinsteins $M^{-2}$ sec.$^{-1}$ at the beginning of experiments 8 to 12, differences in
plant growth between experiments still occurred. This may be due either to the differences in the decay of the power of lamps during the experiments or due to the differences in the reflective capacities of the aluminium walls in the different cabinets. The latter complication could have been avoided if the different experiments were grown in the same cabinet. This was not possible because the same cabinet was not always available and the use of a single cabinet would have restricted experiments from being run concurrently. Possible differences in the air circulation of the cabinets may also have had an influence on the composition and physical properties of air within them. This is a difficult factor to control but it may have been a significant component in the observed differences in plant growth.

Although it is difficult to attribute the observed differences in plant growth wholly to any of these factors, it is apparent that even with the greatest care, precisely reproducible plants will rarely be obtained. Although increased reproducibility in plant growth will increase the flexibility in experimentation, the effort and care needed to achieve such reproducibility often appears to outweigh the possible advantages. In an earlier chapter (Chapter 3), it was suggested that reproducibility in apical events can be obtained. Despite differences in total leaf area and individual leaf area between plants, the reproducibility in the timing of events between the unfolded leaf number and apical events during the vegetative phase can still be obtained and put to advantageous use.

The timing of events, which has been used as an important basis for selecting uniform plants (uniformity in apical events) was not altered in one sense by differences in the light intensity. The relationship between leaf initiation and leaf unfolding was not affected because light intensity differences affected leaf initiation.
rate in the same way that it affected the leaf unfolding rate. Thus, the number of folded and primordial leaves in the bud \((N_f + N_p)\) remained the same over the ranges of light intensity that occurred in these experiments (see fig. 4[2].1.a-d). However, in experiment 6, floral initiation took place when the 14th leaf was unfolding, in contrast to the occurrence of floral initiation when the 17th leaf was unfolding in the other experiments. While the unfolded leaf number as an indicator of apical events during the vegetative stage is not influenced by the range of light intensities, the relationship between the unfolded leaf number and the transition from a vegetative to floral phase is affected by light intensity.

The decision to follow leaf initiation and leaf unfolding in every experiment allowed the identification of leaves which were unfolded at the start of the drying cycle, leaves that were folded at the start of the drying cycle, leaves that were initiated during the drying cycle and leaves that were initiated after rewatering. This has allowed an interpretation of the growth physiology of a leaf during water stress and recovery in relation to whether it was unfolded, folded or initiated before, during or after water stress. Thus, despite differences in leaf number and leaf area of the controls in the different experiments, the leaf responses can be effectively interpreted in terms of their developmental stage and time of initiation.

One remaining factor whose contribution to the observed differences in plant growth that has not been discussed is that of pot size. It was explained in the materials and methods of Chapter 4, that to obtain quicker drying cycles in experiments 9 and 12, the plants were initially grown in small pots containing small volumes of soil. To prevent any restriction to root growth from occurring, these plants were repotted in larger pots immediately after the completion of the drying cycles. As there were no signs of temporary wilting or of a reduction
to the recovery of transpiration after repotting, it was unlikely that there had been any transplantation shock. An earlier experiment on the effects of pot size (Chapter 3) indicated that restrictions to root growth are unlikely to occur at the early stages of growth. Visual observations of root growth also indicated that the root system begins to completely occupy the soil space only at growth stages after the macroscopic appearance of the inflorescence. In experiments 9 and 12 however, repotting was done at very early growth stages. There was also no evidence at that stage that the roots already present completely occupied the available space. Thus the initial small pot sizes and the process of transplanting are unlikely to be involved in the final shoot responses. Having explained some of these possible important complications, some specific aspects of leaf responses during water stress and subsequent recovery can now be considered. Responses during water stress are discussed first.

The greater sensitivity of lamina area increase to water stress compared with dry weight accumulation in the lamina was also generally true for petioles. Because the petioles are not generally regarded as actively photosynthesizing organs, their dry weight increases during water stress may have been due to translocated matter.

The differential responses of leaf enlargement and dry weight increase to water stress suggests that different plant processes may have different critical water potentials below which they cease to perform well. It is possible that while cell enlargement is affected by a small drop in water potential, photosynthesis can continue relatively unaffected. Direct evidence for this to happen in sunflower was provided by Boyer (1970). He showed that a small decline in leaf water potential was sufficient to prevent enlargement but photosynthesis could continue to the point of visible wilting of the plant.
The dry weight increase of a petiole during water stress could have been due to transportation of dry matter from the lamina it subtended. Such transportation of dry matter and nutrient from laminar to non-laminar portions of the leaf during water stress was demonstrated in tomato leaves (Gates 1955b, 1957) and in sunflower leaves (Wilson and McKell 1961). In sugar beet (Zholkevich et al 1958) photosynthate was translocated, although slowly, from the leaves to the roots during prolonged and severe wilting. Thus, as the 'in situ' synthesis of photosynthate in the petioles may be so small to account for the large increments in its dry weight, it is possible that the dry weight increase of the petioles during water stress was largely caused by movement of dry matter from the laminae to the petioles.

The responses of individual laminae and petioles during water stress may be considered in several ways. In experiment 6, the gradients in the responses of laminae and petioles in relation to their position on the plant at the end of the drying cycle (see fig. 4[2].7) raised the possibility that the reduction in photosynthesis of mature leaves and a general reduction in translocation could account for the large reduction in the growth of young leaves. According to Milthorpe and Moorby (1974), until the leaf is about 20% A\text{max}, it continues to import photosynthate from other parts of the plant. As such, the leaves nearer the apex would depend on continuing transport of photosynthate during water stress for their growth. Owing to a possible reduction in translocation of photosynthate to the apex during water stress, as was found by Gates (1955b) in tomatoes, dependent leaves would be affected.

However, the larger reduction in petiole dry weight compared to lamina dry weight up to leaf position 10 followed by larger reductions in lamina dry weight from leaf position 11 (see fig. 4[2].7) suggested that the little assimilate that may have been transported to the younger leaves (above leaf position 11) is probably deposited in the petiole.
in preference to the lamina. This would enhance the weight of the young petioles at the expense of the lamina which they subtend. There is, however, another possible explanation for the differential laminae and petiole responses.

Water stress affected the growth of young leaves at a time when the corresponding controls were growing rapidly. In contrast, the older leaves were reaching or had reached maximum growth when they faced water deficits; the corresponding controls were also growing slowly at that time. Accordingly, the large differences in the responses of young and old leaves at the end of a drying cycle arose from the large differences in the growth patterns of the corresponding controls. If this same reasoning is applied separately for petiole and laminae responses, some important conclusions are possible. As petiole growth lags behind lamina growth, both in terms of extension growth as well as dry weight increase, the magnitude of the lag will increase with leaf position on the plant. Therefore, in very small leaves, petiole growth in the controls will be much slower than lamina growth. The laminae above position 11 would be at growth stages very sensitive to water stress. This may explain why the petioles in leaves above position 11 are affected by water stress less than laminae are affected.

The more or less equal sensitivity of leaf initiation and leaf area increment during water stress raised the possibility that the processes of cell division and cell enlargement would be equally sensitive to water stress. This is because leaf initiation is basically a function of cell division and leaf area increase is largely dependent on cell enlargement. While these points will be discussed more fully in sub-chapter 4[3], it is apt to refer to some similar work on leaf responses to water stress. The responses of
tomato leaves during the drying (Gates 1955b) were largely the same as what is now being reported for sunflower leaves. However, the observed increase in the relative growth rates (over and above the controls) of individual tomato leaves recovering from water stress were not apparent in the present experiments. Also, the observed rapid expansion rate of recovering leaves was interpreted by Slatyer (1973) as being due to rapid enlargement of cells that divided during water stress and which were only partially enlarged. However, neither in Gates nor Slatyers work are final laminae recovery responses provided because their experiments were short term. This precludes any comparison of the long term recovery responses of leaves in experiments 8, 9 and 10 of this thesis and their results.

An extended recovery time will allow final responses of all leaves to be interpreted in terms of (1) the physiological stage of development of the leaf during water stress or (2) the altered physiology of the whole plant. Although experiments 6 and 7 were short term experiments, they provided the many interesting individual petiole and lamina responses which prompted experiments that incorporated long-term recovery for all the leaves on the plant. The short term recovery experiments showed that the laminae which suffered the greatest check to growth during water stress were also the laminae which exhibited a very poor recovery. This occurred despite the time given for these laminae to recover. The failure to monitor the recovery responses of the folded leaves (that were present before the water stress) fully in the short term experiments was unfortunate. The discussion of the recovery responses of the leaves are best considered separately for unfolded and folded leaves that were present before the drying cycle, leaves that were initiated during the drying cycle and finally leaves that were initiated during recovery (after rewatering).
The largest decrease that occurred to the lamina area and dry weight of the recently unfolded leaf, even after extended recovery, was a consistent feature in all the experiments (see figs. 4[2].5.e, 4[2].12b, 4[2].13b and 4[2].14b). The differences in the magnitude of the reduction in these unfolding laminae in the different experiments indicated that the response may be related to the differences in stress severity that occurred in the different experiments. For instance, the reduction in leaf area of the unfolding leaf in experiment 10 (fig. 4[2].14b) was 43% (stress severity was about -2.25MPa at the end of the drying cycle) while the reduction in leaf area of the unfolding leaf in experiment 8 (fig. 4[2].12b) was about 28% (stress severity was about -1.75 MPa at the end of the drying cycle). Such differences in the magnitude of the reduction of leaf was also observed in the other leaves which were at various stages of expansion. Such a large difference in the magnitude of the reduction can be seen by a comparison between leaf responses between experiment 10 and experiments 7, 8 and 9. The almost full recovery of leaves which were past 50% A_max during water stress in experiments 7, 8 and 9 was directly in contrast to a 20% and 30% reduction for similar leaves in experiments 6 and 10. The rapid senescence of laminae 1 and 2 of experiments 6 and 10 were also not observed in experiments 7, 8 or 9. These findings suggest that the magnitude of the reduction of the newly unfolded leaves in experiment 10 may have been related to the severity of stress. However, the leaves which experienced marked reductions in their areas and dry weights in experiments 7, 8 and 9 were at lower leaf positions on the plant in comparison to the leaf which experienced marked reduction in experiment 10. This raised the possibility that the differences in the magnitude of the depression may not be due to stress severity but may be related to the different leaf positions. Firmer conclusions in
this regard can be made if cell number and cell size responses are also considered. Therefore, this aspect of the leaf response is evaluated in the general discussion, where cell responses are related to morphological responses.

The contrasting responses of the folded leaves in experiment 12 and the experiments 8, 9 and 10 (compare fig. 4[2].15 with fig. 4[2].12b, 4[2].13b and 4[2].14b) were interesting. As the water stress in experiment 12 was applied when the early primary sources (leaves 1 and 2) were expanding, it appears that photosynthate may have been generally limiting even after the plants were rewatered, such that the folded leaves (which are dependent on expanding leaves) are affected both in area and dry weight, but more so in their weight. The decreasing effect of the stress on the area of leaves at higher ontogenetic positions (e.g. leaf 13 in fig. 4[2].15) and also on the specific weight of leaves at higher ontogenetic positions (e.g. leaves 12 and above in fig. 4[2].16) suggest that the available photosynthate in the stressed plants may not have been limiting at the time of the rapid development of leaf 12 and above. The full recovery of folded leaves in experiments 8, 9 and 10 may also be due to the sufficient available photosynthate despite some decrease to the total plant leaf area. However, in the absence of conclusive evidence, it is difficult to confirm the postulate. However, data on cell numbers and sizes (presented in chapter 4[3]) allow some interpretation of the full recovery of folded leaves in experiments 8, 9 and 10. As there is the possibility that other factors may also be involved, this is best left to be discussed in the general discussion (Chapter 6). The responses of some of the other leaves, especially those which were initiated during water stress and those that were initiated after water stress was relieved, will now be discussed.
The compensation type of response exhibited by the leaves which were initiated during water stress (leaves 20 to 32) in experiments 8 and 9 (figs. 4[2].1.b and c) and the marked depression to the growth of the leaves which were initiated during recovery (leaves 33 to 42) also in the same experiments suggested that several factors could be involved. Among the factors that were possibly involved were (a) co-relative growth between leaves (b) competition for some essential factors between the inflorescence and the last formed leaves and (c) different leaves were being compared, especially from leaf 26 onwards because leaf number in the stressed plants was reduced.

Leaf 20 to 32 had either been initiated and were growing during water stress (as in experiment 10) or were initiated during water stress (as in experiments 8 and 9). Although water stress would have affected their growth temporarily, at final recovery these leaves were larger than the corresponding controls. In experiment 10, the comparisons were based on identical leaves, but in experiments 8 and 9, the comparison may not have been on identical leaves. The reduction to the final leaf number of the water-stressed plants suggests that different ranked leaves are being compared. This difficulty does not arise for laminae comparisons between leaf positions 1 to 26, but it does for leaf positions 27 to 42. Reductions to leaf initiation rate began only after the 26th leaf had been initiated. If the suggestion that different leaves between positions 27 to 42 are being compared is upheld, it supposes about 5 leaves are missing either between positions 26 and 31 or between positions 32 and 42 or between positions 26 and 42. This is a difficult problem but suggestions as to how it may be approached are considered in sub-chapter 4[3]. However, as water stress does not delay the transition of the vegetative apex to a floral one (sub-chapter 4[4]) in time, the transition appears to be determined more by the
chronological age of the plant than by ontogenetic age. A similar conclusion was arrived at by Marc and Palmer (1976). The effect of water stress on leaf initiation may just delay initiation of leaves without affecting the inductive process leading to floral initiation. If this were true, then the comparison between leaves 27 to 42 would be on identical leaves. It is difficult to confirm or deny either possibility. The compensation type response is thus difficult to confirm as true in experiments 8 and 9. Confirmation for a very small amount of compensatory growth to occur was, however, obtained in experiment 10.

In experiment 10, the small amount of compensation growth that was observed for leaves 22 to 40 (fig. 4[2].14) was of a lesser magnitude than the supposed compensatory growth in experiments 8 and 9. However, the compensatory growth is real in experiment 10 because identical leaves were being compared. The benefit of this small compensatory growth to the plant in experiment 10 is rather obscure. It is quite apparent that the large reduction in area incurred by laminae at positions 1 to 18 in experiment 10 is permanent. This reduction cannot be effectively counteracted by the small amounts of compensatory growth of laminae 22 to 40.

The difficulty experienced earlier in effectively confirming that identical leaves were being compared in experiments 8 and 9 to demonstrate compensatory growth, is also a problem in explaining the responses of leaves 33 to 42 in those experiments. The large reduction in growth of laminae between positions 33 to 42 in experiments 8 and 9 may be related to comparisons based on non-identical leaves. However, one other important factor may be involved. The displacement of leaf initiation by about 4 days and the lack of effect on inflorescence
initiation by water stress, may have disadvantaged the last formed leaves in their competition with the inflorescence for some essential substance. Even in the normally watered control plants, there was the suggestion of possible competition for some essential substance between the last formed leaves and the inflorescence; since the largest leaf is around position 17 on the shoot and leaves after this position taper towards very small sizes, the growth and development of leaves 18 onwards in the normally well-watered plants probably coincided with a time when the initiated inflorescence was also dependent on essential substrates for its development. As the initiation of the inflorescence coincides with the unfolding of leaf 17, leaves 18 onwards unfold when the inflorescence begins to differentiate and develop rapidly. Thus the normal competition that exists between these leaves and the inflorescence may be accentuated for the last formed leaves in experiments 8 and 9. The absence of a similar reduction for the last formed leaves in experiment 10, may be due to direct effects of water stress on the developing inflorescence such that the competitive potential of the inflorescence is reduced. The compensatory growth of the last formed leaves in experiment 10 may be a result of this. Thus the final responses of a leaf to water stress depends on a number of factors.

The responses of the fully expanded leaf differs from that of an unfolded expanding leaf. These in turn differ in their response from folded leaves. It is uncertain whether a single mechanism alone can explain the responses of all the leaves. Three possible mechanisms can however be suggested. The first mechanism which considers competition between the leaves and the inflorescence, especially concerning the last formed leaves, for a limited supply of an essential substance has already been discussed at some length. The second mechanism considers the availability of photosynthate for the growth
needs of dependent leaves. The possible role of photosynthate in the responses of younger leaves during the stress cycle have already been evaluated in the earlier part of this discussion. The possible importance of photosynthate availability in the recovery responses has to be evaluated. This is evaluated in the general discussion (Chapter 6) where the third mechanism which considers the direct effects of water stress on cell division and cell enlargement in a leaf is discussed.
SUB-CHAPTER 4[3]

CELL NUMBER AND CELL SIZE RESPONSES IN THE LEAF TO WATER STRESS

4[3].1. RESULTS

The effects of water stress on the mitotic index, the numbers and sizes of palisade, epidermal and stomatal cells are described. In experiments 9, 10 and 12, cell number and size responses were assessed only at the end of the recovery period.

RESPONSES DURING WATER STRESS

4[3].1.1. The mitotic index in primordial, folded and unfolded leaves

With reference to fig. 4[3].1.a-c., there were no noticeable differences in the mitotic index between leaves of the control and the stressed plants that were at various stages of development, from after initiation to unfolding, at 2 days after the commencement of the drying cycle. Four days after the commencement of the drying cycle, the mitotic index was reduced in all the primordial leaves and in some of the unfolding leaves of the stressed plants; this reduction was greater in the primordial leaves than in the unfolding leaves. The unfolded leaves did not suffer any reduction in their mitotic index on this day. However, five days after the commencement of the drying cycle (which was the last day of the drying cycle), the mitotic index of even the unfolded leaves was reduced by water stress. But the mitotic index of the primordial...
Figure 4[3].1. a-e. show the response of the mitotic index in the individual leaves of experiment 7 to water stress at 2, 4 and 5 days after the start of the water stress cycle and at 2 and 7 days after rewatering the stressed plants. The mitotic index was determined at between 1000 hours and 1100 hours. ca = apex of the control plant, wa = apex of the water stressed plant. cuf = unfolded leaves of the control plant, wuf = unfolded leaves of the water stressed plant. • = control, △ = water stressed. Each data point is a mean of three replicates. Note that the mitotic index of unfolded leaves even in the control plants was less than 0.5% (see chapter 3 discussion, part B for the possible reasons).
leaves and some of the leaves approaching unfolding was about half that of the controls at the end of the drying cycle; the effects of water stress on the mitotic index of unfolded leaves was also greater than its effects on primordial leaves, at the end of the drying cycle. A similar pattern of response in the mitotic index of leaves in the folded and unfolded state of the plants in experiment 9 was noticed.

Since it was found at a later stage in the thesis work that there was a diurnal rhythm in the mitotic activity of cells, especially in the unfolded leaves, an evaluation of water stress on the mitotic index of leaves in experiment 11 was made at the time of day when the index was high in the leaves of the control plants (between 1900 and 2100 hours). Such a determination, made on the last day of the drying cycle, revealed that while the mitotic index was about 5% in the recently unfolded leaves of the control plants, it was reduced to less than 0.5% in the unfolded leaves of the water stressed plant. The mitotic index of the primordial leaves and leaves approaching unfolding was also reduced but to a lesser degree than in the unfolded leaves (see fig. 4 [3].2) Thus, at both sampling times (1100 hours in experiments 7 and 9, 2100 hours in experiment 11) the mitotic index in unfolded leaves was much more reduced than in primordial leaves and leaves which were approaching unfolding.

4 [3].1.2. Palisade, epidermal and stomatal cell numbers

Water stress reduced the number of these cell types in laminae that were below 35% \( A_{\text{max}} \). However, in laminae 1 and 2, cell numbers were reduced up to stages of 50% \( A_{\text{max}} \). In experiment 11, discernible differences in the numbers of palisade, epidermal and stomatal cells were seen about 4 days after the commencement
Figure 4[3].2. shows the responses of the mitotic index of individual leaves on the shoot in experiment 11 to water stress. The analysis was made on the last day of the water stress cycle. The samples were taken at 2100 hours. (●-●) = control, (▲-▲) = water stressed. C-unfolded = unfolded leaves of the control plant, W-unfolded = unfolded leaves of water stressed plants. C-apex = apex of control, W-apex = apex of water stressed plant. Note the large effect of water stress on the mitotic index of the newly unfolded leaves of the water stressed plants and the small effect on the mitotic index of the folded leaves. Contrast the response of the unfolded leaves in this figure with that in figure 4[3].1.
of the drying cycle. The total numbers of palisade cells, epidermal
cells (upper epidermis) and stomatal cells (upper epidermis) at the
end of the drying cycle in experiment 11 for a control and a water
stressed plant are shown in table 4 [3].1.

Table 4 [3].1. Effects of water stress on palisade, epidermal
and stomatal cell numbers in different leaves of experiment 11.
The drying cycle started when the 7th leaf was unfolding.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>WATER STRESSED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(at the end of the drying cycle)</td>
<td>(at the end of the drying cycle)</td>
</tr>
<tr>
<td>Palisade cell number ( x10^6 )</td>
<td>Epidermal cell number ( x10^6 )</td>
<td>Stomatal cell number ( x10^6 )</td>
</tr>
<tr>
<td>Leaf 1*</td>
<td>3.36</td>
<td>1.13</td>
</tr>
<tr>
<td>Leaf 2*</td>
<td>3.13</td>
<td>1.65</td>
</tr>
<tr>
<td>Leaf 3</td>
<td>4.92</td>
<td>2.23</td>
</tr>
<tr>
<td>Leaf 4</td>
<td>10.40</td>
<td>2.91</td>
</tr>
<tr>
<td>Leaf 5</td>
<td>13.83</td>
<td>4.28</td>
</tr>
<tr>
<td>Leaf 6</td>
<td>10.68</td>
<td>3.05</td>
</tr>
<tr>
<td>Leaf 7</td>
<td>10.15</td>
<td>4.29</td>
</tr>
</tbody>
</table>

* Leaves 1 and 2 almost fully expanded while other leaves had not
  attained maximum expansion.

The responses of the cell types in relation to the drying
cycle for some of the leaves in experiment 11 indicated that cell number
in the younger leaves was reduced much sooner than in the older laminae.

4 [3].1.3. Palisade cross-sectional area and epidermal cell area

Cell areas were affected about 1 day earlier than cell
numbers. Effects of water stress on cell area was observed in all
the unfolded laminae. The cross-sectional areas of the palisade cells and the epidermal cell areas of the unfolded leaves at the end of the drying cycle in experiment 11 are shown in table 4 [3].2. Stomatal cell sizes are shown as length x breadth.

Table 4 [3].2. The effects of water stress on palisade cross-sectional area, epidermal cell area and stomatal cell size. Data obtained from the last day of the drying cycle in experiment 11.

<table>
<thead>
<tr>
<th>CONTROL</th>
<th>WATER STRESSED</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palisade cross-sectional area x 10^{-6} cm^2</td>
<td>Palisade cross-sectional area x 10^{-6} cm^2</td>
</tr>
<tr>
<td>Epidermal cell area x 10^{-6} cm</td>
<td>Epidermal cell area x 10^{-6} cm</td>
</tr>
<tr>
<td>Stomatal cell size (length x breadth)</td>
<td>Stomatal cell size (length x breadth)</td>
</tr>
<tr>
<td>Leaf 1*</td>
<td>3.73</td>
</tr>
<tr>
<td>Leaf 2*</td>
<td>4.86</td>
</tr>
<tr>
<td>Leaf 3</td>
<td>3.36</td>
</tr>
<tr>
<td>Leaf 4</td>
<td>3.48</td>
</tr>
<tr>
<td>Leaf 5</td>
<td>1.43</td>
</tr>
<tr>
<td>Leaf 6</td>
<td>0.99</td>
</tr>
<tr>
<td>Leaf 7</td>
<td>0.42</td>
</tr>
</tbody>
</table>

* Leaves 1 and 2 almost fully expanded while other leaves had not attained maximum expansion.

4 [3].1.4. Density of palisade and epidermal cells

At the end of the drying cycle in experiment 11, there were more palisade cells unit area^{-1} in the water stressed leaves than in the control leaves. In the same way, there was a larger number of epidermal cells unit area^{-1} in the stressed leaves compared to the control leaves.
4[3].1.5. Intercellular space between palisade cells.

As the palisade cell cross-sectional diameter was reduced relatively more than the increase in the density of the palisade cells, the intercellular space between palisade cells in water-stressed leaves was greater than in control leaves. This difference was greater between young unfolded laminae of control and water-stressed leaves than between older control and stressed laminae.

RESPONSES DURING AND AT THE END OF RECOVERY

4[3].1.6. The mitotic index in primordial, folded and unfolded leaves.

With reference to fig. 4[3].1.d-e., the mitotic index of most leaves in experiment 7 was re-established when the index was determined 2 days after rewatering. In some of the primordial leaves that were near the apex, the mitotic index was re-established to levels higher than in control leaves. However, the total leaf number in water stressed plants was about 6 less than in the control plants. This difference in leaf number was also present in the comparisons during the drying cycle. This aspect of the differences in leaf number is evaluated to some detail in the discussion of this sub-chapter.

There was no evidence for the previously stressed leaves to continue cell division for a longer time than the controls. The mitotic index of the recovering leaves, like that of control leaves, declined to very low levels (0.5%) shortly after unfolding (see fig. 4[3].3.a-c).

4[3].1.7. Palisade, epidermal and stomatal cell numbers

After rewatering, palisade, epidermal and stomatal cell production continued in leaves that had not attained $35\% A_{\text{max}}$ at
Figure 4[3].3.a-c. show the response of the mitotic index in laminae 15, 18 and 26 during water stress and recovery in experiment 7. The data is re-representation of the data in figure 4[3].1. WC = water stress cycle started, RW = rewatered. (■■■) = control, (○○○) = water stressed. At the start of the water stress (WC), lamina 15, 18 and 26 were still in a folded state. Note that after rewatering, the mitotic index of the stressed lamina reaches values slightly higher than the control laminae but soon decline in a pattern similar to that of the control laminae.

** in situations where a leaf mitotic index value was missing (eg. the data for lamina 18 on the 7th day after rewatering) the mitotic index of the next leaf was used to plot the trend. This missing data occurred due to the poor quality of some of the squashes in experiment 7.
Mitotic index (%) vs. days from start of drying cycle for Laminae 15, 18, and 26.
the end of the drying cycle. In expt. 11, there was no evidence for the duration of cell division in a water stressed leaf to be increased during the recovery phase. This resulted in a net loss in the number of palisade, epidermal and stomatal cells at the end of the recovery period. Table 4[3].3. shows palisade cell responses in expt. 11.

In experiment 9, palisade cell number was not markedly reduced at final recovery in the leaves that had just unfolded when water stress affected them. Despite the rapid cell division that occurred in these leaves shortly after unfolding, only small reductions to their final cell number were noted; very small reductions in palisade cell number were noticed in laminae 4 to 14 of wilt1. In wilt2, palisade cell number of stressed leaves were usually higher than in corresponding control leaves (see table 4[3].4.)

In experiment 10, palisade cell number was reduced in laminae that had not reached 35% $A_{max}$ at the time when water stress was intense. This reduction was highest in laminae that had just unfolded from the bud and were about 10% $A_{max}$ during water stress (eg. laminae 12, 14 and 16). Laminae that were above 35% $A_{max}$ did not experience any reduction to their palisade cell numbers. (see table 4[3].5.).

In experiment 12, palisade cell number was reduced in laminae 1 to 4; these laminae had unfolded or were unfolding during the drying cycle. The palisade cell number of lamina 5 up to lamina 14 was also reduced; this occurred despite the fact that they were still folded leaves when the drying cycle was completed. (see table 4[3].6.).

4[3].1.8. Palisade cross-sectional area and epidermal cell area

The ontogenetic decrease in the cross-sectional area of palisade cells and in the area of epidermal cells in leaves of control plants was also present in the leaves of water-stressed
Table 4[3]. Responses of palisade cell number and palisade cross-sectional area in leaves at different ontogenetic positions, to water stress. The responses presented below refer to that at final recovery (after several days from rewatering) in the experiment 11.

<table>
<thead>
<tr>
<th>Leaf position on the shoot</th>
<th>CONTROL</th>
<th>WATER STRESSED</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf area (cm²)</td>
<td>Cell number per unit area</td>
</tr>
<tr>
<td>1</td>
<td>30.20</td>
<td>38</td>
</tr>
<tr>
<td>2</td>
<td>33.42</td>
<td>42</td>
</tr>
<tr>
<td>3</td>
<td>63.62</td>
<td>54</td>
</tr>
<tr>
<td>4</td>
<td>69.07</td>
<td>62</td>
</tr>
<tr>
<td>5</td>
<td>125.04</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>150.36</td>
<td>84</td>
</tr>
<tr>
<td>7</td>
<td>164.75</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>180.16</td>
<td>110</td>
</tr>
<tr>
<td>9</td>
<td>202.14</td>
<td>122</td>
</tr>
<tr>
<td>10</td>
<td>220.16</td>
<td>152</td>
</tr>
<tr>
<td>16</td>
<td>280.60</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Responses of palisade cell number and palisade cross-sectional area in leaves at different ontogenetic positions, to water stress. Responses presented below refer to that at final recovery - several days after rewatering stressed plants (experiment 9).

<table>
<thead>
<tr>
<th>Leaf position on the shoot</th>
<th>CONTROL</th>
<th>WATER STRESS 1*</th>
<th>WATER STRESS 2**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf area (cm²)</td>
<td>Absolute cell number (x10^6)</td>
<td>Cell cross-sectional area (x10⁻⁶ cm²)</td>
</tr>
<tr>
<td>2</td>
<td>31.81</td>
<td>43</td>
<td>2.375</td>
</tr>
<tr>
<td>4</td>
<td>53.73</td>
<td>65</td>
<td>6.064</td>
</tr>
<tr>
<td>6</td>
<td>89.32</td>
<td>73</td>
<td>11.321</td>
</tr>
<tr>
<td>8</td>
<td>130.60</td>
<td>112</td>
<td>25.395</td>
</tr>
<tr>
<td>10</td>
<td>153.34</td>
<td>142</td>
<td>37.803</td>
</tr>
<tr>
<td>12</td>
<td>185.00</td>
<td>172</td>
<td>55.244</td>
</tr>
<tr>
<td>14</td>
<td>203.55</td>
<td>261</td>
<td>92.237</td>
</tr>
<tr>
<td>16</td>
<td>202.84</td>
<td>348</td>
<td>122.553</td>
</tr>
<tr>
<td>18</td>
<td>227.14</td>
<td>389</td>
<td>153.404</td>
</tr>
<tr>
<td>20</td>
<td>211.18</td>
<td>405</td>
<td>148.562</td>
</tr>
</tbody>
</table>

* 7th leaf was unfolding at the start of the drying cycle.

** 9th leaf was unfolding at the start of the drying cycle.

† Unit area = 0.000578 cm².
Table 4 [3].5. Responses of palisade cell number and palisade cross-sectional area in leaves at different ontogenetic positions, to water stress. Responses presented below refer to that at final recovery (experiment 10).

<table>
<thead>
<tr>
<th>Leaf Position on the shoot</th>
<th>CONTROL</th>
<th>WATER STRESSED*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf area (cm²)</td>
<td>Cell number per unit area</td>
</tr>
<tr>
<td>2</td>
<td>29.61</td>
<td>48</td>
</tr>
<tr>
<td>4</td>
<td>65.07</td>
<td>77</td>
</tr>
<tr>
<td>6</td>
<td>120.38</td>
<td>83</td>
</tr>
<tr>
<td>8</td>
<td>188.42</td>
<td>91</td>
</tr>
<tr>
<td>10</td>
<td>203.32</td>
<td>155</td>
</tr>
<tr>
<td>12</td>
<td>246.98</td>
<td>174</td>
</tr>
<tr>
<td>14</td>
<td>263.25</td>
<td>141</td>
</tr>
<tr>
<td>16</td>
<td>260.61</td>
<td>184</td>
</tr>
</tbody>
</table>

* 16th leaf was unfolding at the start of the drying cycle.
+ Unit area = 0.000368 cm².
Table 4 [3]. Responses of palisade cell number and palisade cross sectional area in leaves at different ontogenetic position, to water stress. Responses presented below refer to that at final recovery (experiment 12).

<table>
<thead>
<tr>
<th>Leaf Position on the Shoot</th>
<th>CONTROL</th>
<th>WATER STRESS 1*</th>
<th>WATER STRESS 2**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Leaf area (cm²)</td>
<td>Cell number per unit area (x10^6)</td>
<td>Absolute cell number (x10^6 cm²)</td>
</tr>
<tr>
<td>2</td>
<td>33.76</td>
<td>46</td>
<td>4.220</td>
</tr>
<tr>
<td>4</td>
<td>62.76</td>
<td>61</td>
<td>10.403</td>
</tr>
<tr>
<td>6</td>
<td>115.36</td>
<td>67</td>
<td>21.003</td>
</tr>
<tr>
<td>8</td>
<td>169.53</td>
<td>84</td>
<td>38.697</td>
</tr>
<tr>
<td>10</td>
<td>211.71</td>
<td>95</td>
<td>54.653</td>
</tr>
<tr>
<td>12</td>
<td>211.81</td>
<td>138</td>
<td>79.428</td>
</tr>
<tr>
<td>14</td>
<td>178.99</td>
<td>175</td>
<td>85.117</td>
</tr>
</tbody>
</table>

* 3rd leaf was unfolding at the start of the drying cycle.
** 5th leaf was unfolding at the start of the drying cycle.
† Unit area = 0.000368 cm².
plants, only that the rate of ontogenetic decrease was greater in
the water stressed plants.

In experiment 9, the cross-sectional area of palisade cells
in laminae 4 to 12 of wilt1 and laminae 6 to 10 of wilt2 were reduced
by water stress. The reduction was about 20% to 30% in laminae of wilt1
and in wilt2 it ranged from a 30% reduction in lamina 6 to virtually
no reduction in lamina 14 (see table 4 [3].4.).

In experiment 10, the palisade cross sectional area of
laminae 1 and 2 were not measured because they had senesced and
dried up. In laminae 4 to 8, the cross sectional areas of palisade
cells was reduced substantially (see table 4 [3].5.) but not so in
laminae 10 to 16 which were greatly reduced in leaf area (see sub-
chapter 4 [2]) and which also experienced a reduction in palisade cell
number (see 4 [3].1.7.).

In experiment 11, the responses of palisade cross-sectional
area were similar to that of wilt1 in experiment 9.

In experiment 12, the cross-sectional area of palisade
cells in laminae 2 to 4 of both wilt1 and wilt2 were reduced by water
stress. The magnitude of the reduction was higher in wilt1 than in
wilt2 (see table 4 [3].6.).

4 [3].1.9. Density of palisade and epidermal cells

Generally, water stress increased the number of palisade
cells per unit area of leaf mesophyll tissue, in leaves that had
already unfolded before or during water stress. The exceptions to
this were laminae 12 to 15 of experiment 10 (which were unfolded
before water stress) which did not experience a change to their
palisade cell density.

In experiment 9, laminae 6 to 10 of wilt1 and laminae
8 to 12 of wilt2 experienced an increase in the density of palisade
Table 4[3]. 7. The responses of the inter-cellular space per unit area of mesophyll to water stresses in experiment 9 (wilt$_1$) and experiment 11.

<table>
<thead>
<tr>
<th>Leaf position on the shoot</th>
<th>Experiment 9</th>
<th></th>
<th>Experiment 11</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Water stressed</td>
<td>Control</td>
<td>Water stressed</td>
</tr>
<tr>
<td>1</td>
<td>343</td>
<td>331</td>
<td>405</td>
<td>392</td>
</tr>
<tr>
<td>2</td>
<td>385</td>
<td>304</td>
<td>406</td>
<td>372</td>
</tr>
<tr>
<td>3</td>
<td>388</td>
<td>388</td>
<td>421</td>
<td>431</td>
</tr>
<tr>
<td>4</td>
<td>237</td>
<td>412</td>
<td>395</td>
<td>457</td>
</tr>
<tr>
<td>5</td>
<td>320</td>
<td>398</td>
<td>412</td>
<td>456</td>
</tr>
<tr>
<td>6</td>
<td>320</td>
<td>403</td>
<td>412</td>
<td>448</td>
</tr>
<tr>
<td>7</td>
<td>278</td>
<td>412</td>
<td>384</td>
<td>429</td>
</tr>
<tr>
<td>8</td>
<td>274</td>
<td>403</td>
<td>384</td>
<td>447</td>
</tr>
<tr>
<td>9</td>
<td>274</td>
<td>412</td>
<td>384</td>
<td>429</td>
</tr>
<tr>
<td>10</td>
<td>356</td>
<td>384</td>
<td>381</td>
<td></td>
</tr>
</tbody>
</table>
cells. There was little evidence of an effect of water stress on the
density in leaves which were folded during the water stress (eg.
laminae 14, 16, 18) or in leaves that had attained a large part of
their maximum area. These trends in experiment 9 were generally
true in the other experiments (see tables 4[3].5. and 4[3].6.).

4[3].1.10. Intercellular space between palisade cells

The ontogenetic decrease in the intercellular space between
palisade cells in the control plants was also present in the water
stressed plants. However, some specific effects of water stress
were recorded.

Generally, the intercellular space between the palisade
cells was increased by water stress, especially in leaves at low
ontogenetic positions (eg. leaves 4 to 8) (see table 4[3].7.).

4[3].2. DISCUSSION

Although water stress affected cell division slightly later
than cell enlargement, the observation that marked reduction in the
cell number of a lamina at the end of a drying cycle was not always
fully reinstated at final recovery, suggests that reductions in cell
numbers together with reductions in cell sizes are involved in the
responses of the leaf to water stress. As such, while cell division
may not react to water stress as quickly as cell enlargement, it is
still sensitive in the context of a direct reduction in cell numbers.

Clough and Milthorpe (1975) recognized these differences in the
rapidity of the response and interpreted the sensitivity of the
processes of cell division and enlargement to water stress along
those terms. In contrast, the earlier investigators interpreted
the sensitivity of cell division and enlargement to water stress in

terms of the relative reductions in numbers and sizes of cells in an
organ. However, the finding that water stress affects both cell
division and enlargement is discussed to highlight their importance
in the leaf morphological responses, although the actual relationship
between leaf morphological responses and cell number/size responses
are evaluated in the general discussion (chapter 6).

The responses at final recovery indicated that in laminae
which were above 35% $A_{\text{max}}$ (except for laminae 1 and 2) during the
drying cycle, there was no reduction in their palisade cell number;
this was consistent with an earlier observation that palisade cell
production is completed at about 35% $A_{\text{max}}$. The reduction in the
palisade cell number in leaves that were about to unfold and up to
those which were below 35% $A_{\text{max}}$ at the peak of water stress, indicated
that a major proportion of cells would be formed after stages of
unfolding and this too was consistent with the previous observation
that rapid palisade cell production occurred between unfolding of a
leaf and its attainment of 35% $A_{\text{max}}$. This raised the possibility
that cell number reductions in the smaller folded leaves would be
insignificant and as there was no transient effect of water stress
on the mitotic activity in these leaves, the final reductions in the
cell numbers of these primordial and folded leaves would be very
small.

While palisade, epidermal and stomatal cell numbers were not
reduced in laminae that were above 35% $A_{\text{max}}$ at stress, the data showed
that palisade cell cross-sectional area and epidermal cell area could
be affected in leaves between the stages of unfolding and the stages
of more than 50% $A_{\text{max}}$. This raises questions as to the relative
sensitivities of cell enlargement at various stages of cell growth.

In experiment 9, the laminae which were unfolding or had
recently unfolded during the drying cycle eventually exhibited a
large reduction in palisade cross-sectional area but only a small reduction in palisade cell number. This was perhaps unusual but nevertheless it could be reconciled with the nature of the stress in experiment 9, if it is assumed that cell division in an unfolded leaf can continue for the major part of the drying cycle provided the stress was incurred slowly and did not result in a very low leaf water status. In experiment 7, in which the drying cycle was also slow and caused a leaf water saturation deficit of 32%, cell division in the unfolded leaves continued at the same rate until the last day of the drying cycle. Similarly in experiment 9, the drying cycle took 6 days and generated a leaf water potential of -1.7 MPa; the mitotic index also continued unabated until the last day of the drying cycle. As there were no signs of accelerated senescence or death of leaf tissue in either experiment 7 or 9 and as there were prominent signs of overnight recovery in the turgidity of leaves until the last stages of the drying cycle, cell division may have continued in the dark. Since the peak cell divisional period is in the early and middle parts of the dark period (see chapter 3), the overnight recovery in the turgidity of leaves may have allowed the continuation of normal rates of cell division in the water stressed plants. It is unfortunate, however, that water potential and turgor potential of leaves during the dark period was not obtained. Nevertheless, in tobacco (Clough and Milthorpe 1975) cell division in unfolded leaves has been shown to occur unabated during water shortage until very severe pre-dawn water deficits were incurred. In leaves of tomatoes (Gates and Bonner 1958), RNA synthesis continued during water stress but because of accelerated breakdown, the net gain in RNA was low and this was only noticed towards the end of a drying cycle. While the present results and those of the workers may explain why palisade cell numbers are not markedly reduced during a slowly induced water stress, the reduction in the area of the palisade cells in experiment 9 may be interpreted in
Firstly, the reduction to the area of palisade cells may be interpreted as a long term effect of a slow stress on the expansion mechanism of a cell. Cell enlargement is a plastic phenomenon, closely linked with the deposition of fairly rigid cell wall materials (Plaut and Ordin 1964). In consequence, slower rates of enlargement are generally associated with a smaller final cell size. On this basis, the cells of already rapidly expanding leaves would not suffer from a long term effect because most of the cell wall materials would probably have been laid down. The full recovery of the cross-sectional area of palisade cells was observed in laminae 1 to 3 in experiment 9 (these laminae were in rapid expansion). This would assume that the cells of the unfolding and newly unfolded laminae in experiment 9 would experience a reduction in their final sizes because of a reduction in the amount of cell wall materials that are deposited. However, if this is correct, it is hard to reconcile this result with the observed responses of the area of palisade cells of unfolding and newly unfolded laminae in experiment 10. The slow and mild stress (as in experiment 9) had a long term effect on the final area of the cells in the unfolding/new unfolded laminae, while cell area was not reduced in the unfolding/new unfolded laminae of experiment 10 (in which the stress was severe). As the unfolding laminae were laminae 7 to 10 in experiment 9 and laminae 14 to 16 in experiment 10, the differential responses may be related to the ontogenetic differences in the palisade cell area rather than to the differences in stress severity that were experienced. It is possible that cell sizes in higher positioned leaves are less sensitive than cell sizes in the lower positioned leaves to water stress.

The second interpretation of the cell area responses considers the requirement of a small amount of enlargement in the division of
the cell. Since enlargement is generally the first process to be
affected by water stress (Ordin 1961), Clough and Milthorpe 1975)
and cells can continue to divide unabated in an unfolded leaf up
to almost the last stages of a slow drying cycle (expt.7), there is a
possibility that cells which continue to divide during water stress
may do so at smaller sizes. There are difficulties involved in
attempts that may wish to demonstrate this. While the leaf is still
within the apex (folded leaf), the cells in it are regular in shape
but when the leaf unfolds and begins to expand, the rapid associated
differentiation results in irregular cell shapes. There is also an
increasing amount of cell content in the palisade cells at this
stage. Both these factors make it difficult to study the sizes of
the dividing cells. Furthermore, as the mitotic index was determined
in experiment 9 at a time when cell divisional activity was not at its
peak in the unfolded leaves, it was difficult to make an evaluation
and base any conclusion on a small population of dividing cells. There­
fore, there is little evidence either way relating to the possibilities
that cells dividing at smaller sizes eventually cause small palisade
cells or that the slower rate of enlargement may be the cause of the
smaller cell sizes.

Although the stressed unfolding laminae of experiment 9
(laminae 7 to 10) had reduced palisade cell areas as a result of water
stress and this was in contrast to the absence of an effect of water
stress on cells of unfolding laminae of experiment 10, the responses
of palisade cell areas in comparable laminae in the two experiments
(eg. laminae 4 to 6) show that the magnitude of the reduction in
cell size was not markedly different. In addition to the contrast
in cell area responses between the unfolding laminae of experiments 9
and 10, the large reduction in the palisade cell number of unfolding
laminae of experiment 10 was not noted in the unfolding laminae of
experiment 9. As the affected laminae in experiment 10 had a larger
number of palisade cells unit area$^{-1}$ than the affected laminae of experiment 9, it is tempting to explain the response along those lines. However, palisade cell production in relation to lamina area increase of the affected laminae in the two experiments was not different; peak cell division in the affected laminae occurred after the stage of unfolding and water stress in both experiments coincided with a peak period of cell division. The different population of palisade cells per se in the affected laminae of experiment 9 and 10 is therefore unlikely to be an important factor in the responses. Of importance is the earlier observation that cell division is sensitive to water stress and would be very much more sensitive to a severe water stress such as the stress generated in experiment 10.

Permanent reductions to the palisade cell number, especially in experiment 10, and that of palisade, epidermal and stomatal cells in experiment 11 raise questions on the duration of palisade cell division and that of epidermal and stomatal cells as well. Where cell division in primordial, folded and unfolded leaves were followed during water stress and during recovery (experiment 7, fig. 4(3) 1.a-e), cell division in the leaves recovered to control values after rewatering and thereafter followed the trends of the control unfolded leaves. As the mitotic index is only an approximate measure of the cell divisional capacity in a lamina, it may not be sensitive enough to be able to detect any extended duration of cell division in the re-watered recovering leaf. However, the final reduction in the absolute cell number of a leaf indicates that the duration of division in a leaf is most probably fixed, or if it can be extended, the extra duration and the rate would be insufficient to compensate for the cells that were not divided during water stress.

An extension of the preceding conclusion to the responses of cell division in primordial leaves during water stress and
during recovery yields quite interesting conclusions. Although reductions to the mitotic index during water stress were first observed in the primordial leaves, the actual reduction of cells per se would be so small because the bulk of the cells in a leaf are formed after it unfolds from the bud. However, the cumulative effects of such a small reduction can cause enormous reductions in the cell number at leaf maturity. Since there appeared to be some evidence of compensation in the mitotic index of primordial leaves or at least re-establishment to control levels 2 days after rewatering and this was sustained at even 7 days after rewatering, the possible cumulative effects of a reduction to the mitotic index of cells may be negated.

One other observation, however, does complicate this last interpretation. This is related to the observation that the water stressed plants had fewer leaves than the controls and therefore the comparison of the mitotic index between control leaves and recovering leaves may not have been made on comparable leaves. As such, the evidence in support of compensation has to be weighed against the possibility that the comparison of the mitotic index was made on non-comparable leaves. The original comparison involved the numbering of leaves from the base of the plant to the apex. As the rate of leaf production was reduced by water stress, the apices of the control and the water stressed plants were separated by about 5 leaves at the time of the comparison. The replotting of the data involved numbering of leaves from the apex downwards. These numbering systems are illustrated in the diagrams below. Figure 4 shows a numbering system from the base of the plant to the apex while fig. 4 shows a numbering system from the apex downwards.
The compensation type effect with the first numbering system (Fig. 4[3].4.) was absent in the second numbering system (fig. 4[3].5.). This raised the possibility that the mitotic index of a leaf primordium depends on its distance from the apex and that the apex may have regulatory control on the mitotic activity in the newly formed leaves. If this were true, compensation will not be real.

In experiment 7, in which the mitotic indices were obtained, the final leaf number in the controls and stressed plants were not accurately estimated because the experiment was terminated at a stage where it was difficult to distinguish between the last formed primordial leaves and the first formed involucre bracts of the inflorescence. In later experiments, in which plants were grown to
near the mature stages, such a separation between leaves and involucre bracts was possible. It was evident, then, that leaves which were 'lost' as a result of water stress were never regained. There is no reason to believe that this would not be the case in Experiment 7 as well. The difference in the total leaf number between the control and water stressed plants may, however, occur in 2 ways.

Firstly, the leaves that were to be formed below the inflorescence may have not been initiated before the vegetative apex slowly transformed into a floral one. The transition was dependent on the chronological age and was thus unchanged by water stress. As the transition of the apex to a floral state marks the cessation of leaf initiation, fewer leaves are present in the water stressed plants. In the second case, leaf initiation immediately below the floral apex may have been unhindered but the initiation of leaves in the middle of the leaf profile, which should have been initiated, may not have occurred; the lower leaf number may result from leaves being 'missed out' during the initiation process. It would be hard to prove this second suggestion experimentally. I can only offer a suggestion as to how it may be done in a plant that has prominent ontogenetic change in leaf shape i.e. heteroblastic leaf development. In a plant that shows heteroblastic leaf development, one can calibrate the change in leaf shape and look for such deviations as may occur during water stress. In the sunflower, marked changes to leaf shape are not apparent other than the transition from an ovate shape for laminae 1 and 2 to chordate shapes for leaves at higher positions on the shoot. This would make any experimental approach, to resolve the problem, a very difficult one because of the small changes in leaf shape over a large number of leaf positions. However, there is no evidence in the literature where such a phenomenon of leaves being 'missed out' has been recorded nor has there been an attempt to explain the phenomenon in those terms. The suspension or reduction in the
rate of leaf initiation has been observed in many plants (Gates 1968, Marc and Palmer 1976, Clough and Milthorpe 1975) and it is difficult, in the absence of other concrete and meaningful interpretations, to interpret the leaf initiation responses here in any concrete terms. It is difficult, therefore, to conclude if the mitotic index of similar leaves was being compared in experiment 7.

The sequential reduction of the mitotic index first in the primordial leaves, followed by reductions in the folded leaves and finally by reductions in the index of the unfolded leaves, is an aspect that has not been reported before. It is consistent with some observations made by Clough and Milthorpe (1975) in tobacco. They found primordial initiation to be affected first and then followed by a reduction in cell numbers in the unfolded leaves. They expressed surprise that the response in the apex (a region of predominant cell division) should differ so much from that of cell division in the expanding leaf, particularly if the apex maintains its water potential at a very high level, as Hussain and Aspinall (1970) contend. Hussain and Aspinall suggested that the supply of essential substrates to the apex is curtailed by water stress, possibly through the failure of the vascular elements to differentiate and expand. But Clough and Milthorpe (1975) contend that in the absence of detailed observations on the responses of the various elements of the apical bud and estimates of their water status, Hussain and Aspinall's interpretation would encroach the realms of pure conjecture. However, the data of Munns et al. (1977) show that in droughted plants of Triticum aestivum cv. Heron, the water potential and osmotic potential of developing floral apices and leaves decreased at similar rates. Early in the stress period, decreases in the water content relative to the dry matter were similar in apices and leaves but later, the water content of the leaves fell rapidly, while that of the apices remained at about
300 per cent. The apices survived and grew after experiencing water potentials of -6.0 MPa; exposed leaves on these plants did not survive. The respiratory debt in the stressed apices was 7 per cent of dry matter and 29 per cent of the soluble carbohydrate pool per day. They concluded that continuing translocation to the apex would seem necessary for the extended survival of the apex that was observed:

The rapid reduction in the mitotic index of the primordial leaves may perhaps be associated with an initial drop in the apex water potential. The continuation of cell division in the primordial leaves during water stress may be associated with the sustained, although reduced, water status of the apex. In the absence of information of respiratory debts of stressed sunflower apices, it is not possible to effectively differentiate between the roles that the water status or the reduction of translocation would play in the cell division responses of primordial leaves.

However, the reduction in leaf initiation rate (primarily a function of cell division) which coincides with the rise in stomatal resistance (compare fig.4[2].1.b with 4[1].3.d) raises questions about the possible role of the growth regulator - abscisic acid (ABA) in the leaf initiation response. As the rise in stomatal resistance coincides with ABA increases in the leaf, and ABA may be stimulated by the loss in cellular turgor, appears to be responsible (Milborrow 1974). Milborrow (1974) suggested that a major site of increased ABA level in water-stressed plants appeared to be in the mature leaves. In further studies with sunflowers (Hoad 1975), the rise in ABA levels in the xylem sap was shown to be due to prior movement from the leaves. In contrast, Walton, Harrison and Cote (1976) showed large increases in ABA levels in stressed roots of mungbean, peas and sunflower and they indicated that there was no transport requirement from the leaves for the increase in the root levels to occur. However, in either case, ABA levels in the plant are increased by water stress
and may be transported. Although applications of ABA have caused a reduction in leaf primordium production in some tree seedlings, there is no evidence for a direct role of ABA on cell division. If ABA had a direct effect on cell division, it is hard to understand why cell division was affected first at the apex and only later in the unfolded leaves, unless of course if ABA production was first started at the apex or the ABA produced in the flaccid leaves was quickly transported to the apex. The normally postulated role of ABA as being responsible for the closure of stomata has been questioned (Hsiao 1973) because stomatal closure occurs much more quickly than the rise in ABA levels. These are interesting aspects of the physiology of the sunflower plant and need further study.

Perhaps a more important role in the cell division and leaf initiation responses may be played by the group of growth regulators collectively known as the kinins. Itai and Vaadia (1965) reported that cytokinin concentration of the root exudate from sunflower plants was markedly reduced after a twenty-four hour period of water stress. It has been shown by others (Kende 1964, 1965, Weiss and Vaadia 1965, Kende and Sutton 1967) that the roots of sunflower plants were the sites of cytokinin synthesis and that three such factors are translocated to the shoot through the transpiration stream. Removal of the root caused a decrease in the protein content and enhances the senescence in the leaves (Mothes 1960, Parthier 1964) and either of these processes is delayed by the application of a cytokinin (Richmond and Land 1957). It appears possible that, in the intact plant, cytokinins formed in the root and translocated with the transpiration stream to the shoot have a similar effect. In other words, they function in the endogenous regulation of protein metabolism in the leaves. This assumption is supported by two lines of evidence. Kulaeva (1962) assumed that attached leaves
contained optimal amounts of root-supplied cytokinins; removal of the roots caused a deficiency of these factors and rendered the leaves sensitive to applied kinetin (6-furfurylaminopurine). Shah and Loomis (1965) and Itai and Vaadia (1965) provided more direct evidence. Shah and Loomis found a decline in RNA and protein content of sugar beet leaves in plants subjected to water stress. A spray of benzyladenine (6-benzylaminopurine) prevented these symptoms of senescence from occurring. It was concluded that water stress very likely causes leaf senescence by reducing the production of cytokinins in the roots and consequently reducing the cytokinin supply to the shoot.

In the experiments of this thesis, accelerated senescence was only observed in plants that had already achieved a large amount of vegetative growth before the water stress. In young seedlings, accelerated senescence of leaves was not observed. It may be possible that in a young seedling, the production of cytokinin in the roots is efficient or the requirement for it in the shoot is low. It has been reported that at a time when the cytokinin supply from the root declines and when the leaves become deficient in cytokinins, a new center of cytokinin synthesis is formed in the growing fruits (Miller 1964). He found that sunflower seeds are rich in a cytokinin which was identical with the 'maize factor' now known to be zeatin (Miller 1965). It has been postulated that the high cytokinin content of developing fruits may direct the flow of assimilates from the leaves to the fruits, thus enhancing ageing of leaves (Leopold and Kawase 1964). Furthermore, added kinins increase mitosis in roots (Guttman 1956) and encourage mitosis in cultured flower anthers (Vasil 1957, Walker and Dietrich 1961). Kinins also stimulate leaf enlargement (Scott and Livermann 1956). These interesting aspects of cytokinin function in the plant, including the role it is postulated to play in cell proliferation, should be pursued further.
This difference between a C\textsubscript{3} and a C\textsubscript{4} plant may not apply with regard to internal resistance.
in the context of relating cell responses and leaf morphological responses to water stress.

These were not followed up because there were other urgent aspects of the 3 year study which were of greater importance. This had to do with the role of cell numbers and cell sizes in the overall leaf response, an aspect that is discussed in part 1 of the general discussion (chapter 6).

Changes in the density of palisade cells and concurrent changes in the cross-sectional area of individual palisade cells due to water stress affected the area occupied by them. This was reflected in changes in the intercellular space between palisade cells. Although the intercellular space between palisade cells increased in the water-stressed laminae, the increase is not a direct effect of water stress but occurs due to changes in the area occupied by palisade cells. The increase in the intercellular space is an interesting phenomenon for the following reasons.

In C₄ plants, the stomatal resistance is high (Slatyer 1970, Downes 1971, Ludlow 1971). An increase in the intercellular spaces in the mesophyll tissue of a C₄ plant may be an advantage in that it might lower the net resistance to CO₂ exchange between the atmosphere and the mesophyll. For a C₃ plant, like the sunflower, stomatal resistance is not inherently high and CO₂ exchange meets stomatal with less resistance than in a C₄ plant. In these experiments, there was no evidence for stomatal resistances to remain high after the relief of water stress (see chapter 4 [1]). The small but consistent increase in the intercellular space between palisade cells may not be of any significance in lowering the net resistance to CO₂ exchange in a C₃ plant while it may be important in a C₄ plant. However, the response may be viewed as a step in the direction of compensating for other reductions to leaf growth that are caused by water stress.
It is evident that both cell number and cell sizes in a sunflower lamina can be reduced by water stress, although there are ontogenetic influences on the responses. These are discussed to some length in the general discussion.

The speculation included in this discussion of the chapter results will suggest possible lines of investigation.
EFFECTS OF WATER STRESS ON INFLORESCENCE INITIATION AND DEVELOPMENT

4.1. RESULTS

The responses of the inflorescence that are reported in this subchapter concern the first stage in reproductive development i.e. the stage of floral initiation and inflorescence development when the potential fruit number is determined. The responses of the second and third stages of inflorescence development to water stress are reported in Chapter 5.

4.1.1. Effects of a water stress before the stage of initiation of the inflorescence on its initiation

In experiments 7, 8, 9 and 12 in which the drying cycles were started before and also completed before the initiation of the inflorescence, the number of days from sowing to floral initiation was not markedly affected. In most cases, the initiation date was not changed by the brief period of water stress (see table 4.1.)
Table 4[4].1. Effects of water stress on the number of days to inflorescence initiation, when stress was applied before initiation

<table>
<thead>
<tr>
<th>TREATMENT</th>
<th>Expt. number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Expt. 7</td>
</tr>
<tr>
<td>CONTROL</td>
<td>between 21 -26</td>
</tr>
<tr>
<td>STRESSED</td>
<td>between 21 -26</td>
</tr>
</tbody>
</table>

In all experiments, the water stress cycle commenced and terminated before the initiation of the inflorescence.

Subsequent development of the inflorescence was also unaffected by the previous water stress history; the initiation of disc floret primordia in the previously stressed plants occurred at the same time as, if not sooner, than the control plants (see tables 4[4].2., 4[4].3. and 4[4].4.)

In experiment 7, it was difficult to determine if the inflorescence had been initiated at a lower leaf number because at the time of the completion of the experiment, it was difficult to distinguish between primordial leaves and the involucre bracts of the inflorescence. In the experiments 8 and 9, however, the plants were allowed to grow to more mature stages where it was possible to distinguish between leaves and involucre bracts. The inflorescences in these two experiments were initiated at a lower leaf number.

In experiment 12, the emergence of the inflorescence (macroscopic appearance at the plant apex) in the wilt₂ plants (plants of the second water stress cycle) occurred much sooner than in the control or the wilt₁ (plants of the first water stress cycle) plants. There were probably also fewer leaves in the wilt₂ plants (see fig. 4[2].1.d.
Table 4[4].2. Effects of rewatering previously water-stressed* plants on the development of the inflorescence (experiment 7).

<table>
<thead>
<tr>
<th>Plant age (days)</th>
<th>Treatment</th>
<th>Initiation of inflorescence</th>
<th>Number of involucre bracts</th>
<th>Appearance of disc</th>
<th>Number of disc florets</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Control</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>26</td>
<td>Control</td>
<td>Appd.</td>
<td>7</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>Appd.</td>
<td>28</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>32</td>
<td>Control</td>
<td>Appd.</td>
<td>42</td>
<td>Appd.</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Stressed</td>
<td>Appd.</td>
<td>55</td>
<td>Appd.</td>
<td>Appd.</td>
</tr>
</tbody>
</table>

* Stress applied 8 days before inflorescence initiation and also rewatered before initiation.

Table 4[4].3. Effects of rewatering previously water-stressed* plants on the development of the inflorescence (experiment 8).

<table>
<thead>
<tr>
<th>Plant age (days)</th>
<th>Treatment</th>
<th>Initiation of inflorescence</th>
<th>Number of involucre bracts</th>
<th>Appearance of disc</th>
<th>Number of disc florets</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Control</td>
<td>Appg</td>
<td>2</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>wilt&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Appg</td>
<td></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>wilt&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Nil</td>
<td></td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>23</td>
<td>Control</td>
<td>Appd</td>
<td>6</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>wilt&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Appd</td>
<td></td>
<td>21</td>
<td>DEV</td>
<td>Nil</td>
</tr>
<tr>
<td>wilt&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Appd</td>
<td></td>
<td>5</td>
<td>Appd</td>
<td>Nil</td>
</tr>
<tr>
<td>25</td>
<td>Control</td>
<td>Appd</td>
<td>15</td>
<td>Appg</td>
<td>Nil</td>
</tr>
<tr>
<td>wilt&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Appd</td>
<td></td>
<td>15</td>
<td>Appg</td>
<td>Nil</td>
</tr>
<tr>
<td>wilt&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Appd</td>
<td></td>
<td>11</td>
<td>Appg</td>
<td>Nil</td>
</tr>
<tr>
<td>28</td>
<td>Control</td>
<td>Appd</td>
<td>57</td>
<td>DEV</td>
<td>S</td>
</tr>
<tr>
<td>wilt&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Appd</td>
<td></td>
<td>54</td>
<td>DEV</td>
<td>S</td>
</tr>
<tr>
<td>wilt&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Appd</td>
<td></td>
<td>61</td>
<td>DEV</td>
<td>S</td>
</tr>
</tbody>
</table>

* Stress applied 10 days and 7 days before inflorescence initiation in wilt<sub>1</sub> and wilt<sub>2</sub> respectively and also rewatered before initiation.

Appg = appearing; Appd = appeared; DEV = developing; C = concentric circles of disc floret initials. S = starting
Table 4[4].4. Effects of rewatering previously water stressed* plants on the development of the inflorescence (experiment 9)

<table>
<thead>
<tr>
<th>Plant age (days)</th>
<th>Treatment</th>
<th>Initiation of inflorescence</th>
<th>Number of involucre bracts</th>
<th>Appearance of disc florets</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>Control</td>
<td>Appg</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>wilt&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>wilt&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>22</td>
<td>Control</td>
<td>Appd</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>wilt&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Appd</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>wilt&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Appg</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>24</td>
<td>Control</td>
<td>Appd</td>
<td>7</td>
<td>Appg</td>
</tr>
<tr>
<td></td>
<td>wilt&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Appd</td>
<td>11</td>
<td>DEV</td>
</tr>
<tr>
<td></td>
<td>wilt&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Appd</td>
<td>9</td>
<td>Appg</td>
</tr>
<tr>
<td>28</td>
<td>Control</td>
<td>Appd</td>
<td>51</td>
<td>DEV</td>
</tr>
<tr>
<td></td>
<td>wilt&lt;sub&gt;1&lt;/sub&gt;</td>
<td>Appd</td>
<td>61</td>
<td>DEV</td>
</tr>
<tr>
<td></td>
<td>wilt&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Appd</td>
<td>53</td>
<td>DEV</td>
</tr>
</tbody>
</table>

* Stress applied 10 days and 7 days before inflorescence initiation in wilt<sub>1</sub> and wilt<sub>2</sub> respectively and also rewatered before initiation.

Table 4[4].5. Effects of water stress on involucre bract formation when stress is applied at the time of inflorescence initiation* (Expt.6)

<table>
<thead>
<tr>
<th>Days from start of drying</th>
<th>Control</th>
<th>Stressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>nil</td>
<td>nil</td>
</tr>
<tr>
<td>2</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>4**</td>
<td>30</td>
<td>8</td>
</tr>
<tr>
<td>6</td>
<td>56</td>
<td>38</td>
</tr>
<tr>
<td>8</td>
<td>66</td>
<td>62</td>
</tr>
</tbody>
</table>

*plant age at initiation was 22 days; ** rewatered. The count of the involucre bracts in all these tables is only approximate.

Appg = appearing; Appd = appeared; DEV = developing; C = concentric circles of disc floret initials
Figure 4[4].1. shows the response of the inflorescence diameter in experiment 6 to water stress. The inflorescence had been just initiated when the water stress cycle was started. WC = start of water stress cycle, RW = re-watered. (●●) = control, (O—O) = water stressed. The inflorescence diameter was obtained from serial sections of the plant apex. Each data point is a mean of two replicates.
Plate 4[4].1. shows a reconstructed model of an inflorescence of a control plant and a water stressed plant in expt. 6. The models were reconstructed from serial sections of the shoot apices of a control and a water stressed inflorescence on the last day of the drying cycle i.e. day 26. The method used was that of Williams (1970). Note that the diameter and height of the inflorescence in the water stressed (wilt) plant is about half that of the control inflorescence.
RECONSTRUCTED MODELS FROM TRANSVERSE SECTIONS

DAY 26 INFLORESCENCE - CONTROL

leaf 36
dome
bract

DAY 26 INFLORESCENCE - WILT

leaf 36
dome
bract
4[4].1.2. Effects of a water stress at the stage of inflorescence initiation on the subsequent development of the inflorescence

The diameter of the inflorescence disc in experiment 6 was reduced by water stress to about half at the end of the drying cycle. After rewatering, the diameter of the disc increased in the previously stressed plants, at a rate comparable to that of the disc of the control plant see figure 4[4].1). As most of this data was obtained from serial sections of the apex, the responses of disc floret initiation was not obtained. However, the rate of initiation of involucre bracts was reduced by water stress (see table 4[4].5).

The height of the inflorescence disc was also reduced by water stress. While the control inflorescences had an average height (at the centre of the inflorescence) of about 50µ, the water stressed inflorescences had a height of 20µ. This effect and the effects of water stress on the size of the inflorescence (at the end of the drying cycle) are illustrated by three dimensional reconstructed models of a control and a water stressed inflorescence (see plate 4[4].1).

4[4].2. DISCUSSION

Since the total leaf area of a plant was reduced to about half the control plant leaf area at the end of a water stress cycle (see Chapter 4[2]) and as there was no delay to the initiation of the inflorescence in the stressed plants, it seems clear that inflorescence initiation is not affected by the reduced leaf area or by the lower leaf number.

In contrast to this, Whiteman and Wilson (1965) found in sorghum that leaf number at which floral initiation commenced depended on the interaction between the amount of vegetative development necessary to allow
the formation of a floral stimulus and the extent to which the stress may suspend development. With stress imposed well ahead of the normal time of floral initiation, there was insufficient vegetative development so that, on rewatering, leaf formation was resumed and the number of vegetative primordia formed was unaffected. By comparison, when stress was imposed closer to the normal time of floral initiation, there was sufficient vegetative development to permit formation and translocation of the floral stimulus and, upon rewatering floral initiation occurred with the first primordia appearing at a lower leaf number. In the sunflower, water stresses imposed 14, 10 and 7 days before the normal time of floral initiation did not affect the initiation date but reduced the leaf number at which initiation occurred. It is thus possible that the floral stimulus is formed and translocated at a very early stage in the sunflower such that neither the reduced leaf number nor the reduced leaf area resulting from water stress can delay initiation. Alternatively, it is possible that the floral inductive process in sunflower is not sensitive to water stress although Aspinall and Hussain (1970) have shown its sensitivity in Pharbitis nil. It has also been pointed out by Slatyer (1973) that variable degrees of water stress may affect the formation and translocation of the floral stimulus differently than primordial initiation. If primordial initiation is the more sensitive, flowering can be expected to occur at a lower node number; if less sensitive, flowering can be expected to occur at a higher node number. Following this argument, in either case an associated effect of water stress will almost always be a delay in the date of flowering, regardless of whether it occurs at a younger ontogenetic age. This has not been the case with sunflower. However, Nicholls and May (1963) found that water stress did not affect the number of vegetative primordia formed in barley, but the appearance of the first double ridges was delayed several days by even mild stress. Also, Hopkinson (1968)
showed that water stress in tobacco could lead to the laying down of additional vegetative primordia before floral initiation occurred, but the maximum number observed did not exceed five; this could lead to a delay in the initiation of the inflorescence.

The absence of an effect on inflorescence development by water stresses imposed and completed before the initiation, suggested that the potential number of fruits would be realized. As the disc floret number was not counted in the experiment in which stress was imposed at floral initiation, it is not possible to make any definite estimates on the fruit number that might have been reduced. However, as there was an effect of water stress on the size of the disc (see 4.1.2.) it is possible that the number of disc florets that can be laid down will be reduced. As the growth of the inflorescence disc after rewatering was comparable to the control (see figure 4.1.), it is also possible that the final size of the stressed inflorescence may be no different from that of the control. Whiteman and Wilson (1965) found in sorghum that development of the inflorescence could be suspended during water stress yet could be resumed on rewatering and result in a flowering head not significantly different from that of control plants. It seems quite clear that such conclusive statements for the sunflower can only be made if the recovery periods of the inflorescences are extended to more mature stages. This aspect is covered in Chapter 5.

The occurrence of floral initiation at a lower leaf number in the previously water stressed plants has other important implications in this thesis. For instance, the presence of different leaf numbers in control and water stressed plants made it difficult to conclude if comparisons of leaf responses were being done on identical leaves. This difficulty was particularly relevant for leaf primordia that were formed during water stress. Whether the missing leaves in the stressed
plants were from positions just below the inflorescence or were missed from positions within the leaf profile during leaf initiation, is a question that is difficult to resolve. As most of this has already been discussed in Sub-Chapter 4[2] and 4[3], the remainder of this discussion will be restricted to emphasizing the importance of assuring the proper early development of the inflorescence in the sunflower.

Modern cultivars of sunflower are determinate plants and they differ from their wild ancestors in having only a single terminal inflorescence as compared to several inflorescences of the wild ancestors. For this reason, the yield potential in a hybrid cultivar such as Hysun 30 depends on the proper growth and development of its only inflorescence. Some detailed analysis of the effects of water stress on the yield of sunflower is thus warranted. The data collected in this regard are presented in Chapter 5.
CHAPTER 5

ACHENE RESPONSES TO WATER STRESS

5.1. INTRODUCTION

The study of the responses of individual leaves to water stress (sub-chapter 4[2]) revealed some interesting patterns of response between the water stress and the developmental stage of the leaf when it experienced the stress. It was apparent that the extent of recovery of a leaf which had experienced water stress depended on its developmental stage during water stress. Such a study on the responses of the sunflower achenes would be particularly relevant for two reasons.

Firstly, the responses of individual sunflower achenes to water stress have not been reported before. As there is a definite difference in the developmental patterns between achenes at different positions in a spiral row of the capitulum (see Chapter 3), there could be interactions between the achenes and the water stress such that some achenes would be more drastically affected than others. This result would be of importance in formulating an irrigation strategy for a sunflower crop. Secondly, the study of sunflower achene responses to water stress will allow a direct comparison between the responses of vegetative and reproductive tissue to water stress in the same plant. This aspect, too,
Embryo in this chapter includes the cotyledons and the embryonic axis.
has not been considered to any detail in any other plant. The study of individual achene responses necessitated the development of a sampling system; this is described in Appendix B.9.

The response of sunflower achenes to water stress was first investigated in a controlled environment cabinet. This was followed by a large glasshouse experiment that imposed water stress at successive stages of inflorescence development; the glasshouse environment was chosen to impose a more severe stress at grain filling stages than could be provided by the cabinet environment. The responses that were observed in the glasshouse experiment were checked for reproducibility in a controlled environment cabinet by altering the normal growth conditions (treatment A, Chapter 3) to provide an environment that would cause internal plant stresses comparable to that of the glasshouse. At a later stage of the three year course, the achene responses in an unirrigated field crop of Hysun 30 were compared with that in an irrigated crop; this part of the study was superimposed on a field experiment (Turner and Begg unpublished) that was originally designed for other purposes.

In all experiments, the responses of the pericarp and the embryo (the principal components by weight, of the achene) were studied separately. The analysis of variance for the pericarp, embryo and the whole achene responses were done separately for the glasshouse experiment. The discussion uses these separate analyses to formulate a general pattern of response.

5.2. MATERIALS AND METHODS

The plants were always grown in 7 inch pots. The method of inducing plant water stress consisted of withholding the supply of water
at a particular developmental stage of the inflorescence. In the
glasshouse experiment (experiment 14), the early stages of inflorescence
development were gauged by dissecting a few spare plants. The internal
plant water stress was not measured in most of these experiments, but
the decline in the soil water content was recorded. In the field
experiment (experiment 16), leaf water potential of the unirrigated
plants, measured with a pressure bomb, reached about -30 bars.

5.2.1. Experiment 13

The experimental plants were grown in the same way as in previous
leaf experiments (see materials and methods of Chapter 2, treatment A
of Chapter 3). Water stress was started by withholding the supply of
water to (1) a batch of plants when the inflorescence had just been
initiated and disc florets were about to be formed and (2) a batch of
plants in which all the disc florets had reached anthesis. For (1),
the plants were rewatered 3 days from the start of the drying cycle and
only a single harvest was made when the plants were mature. For (2)
the plants were rewatered 6 days after the start of the drying cycle.
Three harvests were made. The first harvest coincided with the end
of the drying cycle, the second at 5 days after rewatering and the
third at maturity, at 15 days after rewatering.

5.2.2. Experiment 14

The plants were grown in 7 inch pots containing 2200 g oven dry
weight with the appropriate amount of nutrient and water. The experi-
iment was conducted in a glasshouse of the CERES phytotron in Canberra.
The glasshouse temperature was maintained at 27°C during the day and 22°C at night. Although light intensity was not controlled, the natural daylength was extended by 2 hours with incandescent lamps.

Water stresses were imposed at six different stages of inflorescence development; three of them were imposed before the anthesis of the 1st disc floret and the other three were imposed after the anthesis of the first disc floret (see table 5.1). Each water stress was imposed on a separate batch of 3 plants. Non-destructive sampling of the inflorescence twice during grain filling was performed before the final destructive harvest. The number of viable leaves and total leaf area at the last harvest were recorded. This has been described in the results section of sub-chapter 4(2). The number of achenes, filled and unfilled, total achene weight and the inflorescence diameter were also recorded.

5.2.3. Experiment 15

The plants were initially grown in the glasshouse under a temperature regime of 27°C during the day and 22°C during the night. When the plants had macroscopically visible inflorescences, they were transferred to a growth cabinet that was maintained at 27°C during the day and the night. The light intensity was 650 microeinsteins m$^{-2}$ sec.$^{-1}$. Water stresses were applied at three stages of inflorescence development viz. (1) just before anthesis of the 1st disc floret (2) 5 days after the anthesis of the 1st disc floret and (3) 8 days after the anthesis of the 1st disc floret. Each treatment was performed on 3 plants. Only one single destructive harvest at maturity was made.
Table 5.1. Application of water stress A to F in experiment 14

<table>
<thead>
<tr>
<th>PLANT AGE (days)</th>
<th>Bract Disc floret formation</th>
<th>Macroscopic appearance of inflorescence</th>
<th>Ray florets yellowing and unfolding</th>
<th>Anthesis of 1st disc floret</th>
<th>Anthesis of last disc floret</th>
<th>Maturity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER STRESS BEGAN ON day</td>
<td>22</td>
<td>24 to 27</td>
<td>32</td>
<td>50</td>
<td>51</td>
<td>63</td>
</tr>
<tr>
<td>WATER STRESS ENDED ON day</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td></td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>34</td>
<td>47</td>
<td>56</td>
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<td>64</td>
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<td></td>
<td>28</td>
<td>38</td>
<td>51</td>
<td>60</td>
<td>63</td>
<td>68</td>
</tr>
</tbody>
</table>

Developmental stage of the inflorescence
There were 3 distinct drying cycles in the dry plot and the lowest water potential reached was - 2.6 Mpa.
5.2.4. Experiment 16

Hysun30 seeds were sown on half acre block of land at Ginninderra experimental station. One half of the crop was irrigated frequently while the other half received only one irrigation at sowing (this is referred to as the dry plot in the rest of this chapter). Inflorescences were sampled after the completion of anthesis. Sampling was done on days 69, 74, 79, 84 and 90 from sowing. A sample at each of the harvests consisted of three inflorescences from the dry and three from the irrigated plots. Achenes were sampled from the inflorescence by the method that has been described in Appendix B. 9.

5.3. RESULTS

The responses in the different experiments are presented together. However, the responses of achene components that resulted from pre-anthesis water stresses are described separately from the responses that resulted from post-anthesis water stresses.

5.3.1. Effects of pre-anthesis water stress on pericarp dry weight

The pericarp dry weight of achenes at all sampling positions on the capitulum was reduced by water stress. In experiment 14, pericarp dry weight reduction by water stresses A and B was about 35% for all achene positions. The reduction by water stress C ranged from 15% for achene position 1 to about 30% for achene position 8. However the raw data (see figure 5.1.a.) indicates that there was an equal reduction in the dry weight of pericarps at all achene positions in the three pre-anthesis water stresses. A similar response was observed in the pre-
Figure 5.1. a. shows the responses of the dry weight of pericarps to water stresses applied before the anthesis of the disc florets in experiment 14. Wilts A, B and C refer to water stresses applied at various stages of the development of the inflorescence, the details of which are provided in table 5.1. The data points are means of three replicates. It was difficult to follow the spiral rows further than position 8 in the water stressed capitula. The pericarps were analysed for their weight from achenes at various positions on a spiral row (from the periphery of the capitulum to its center).

Figure 5.1. b. shows the responses of the dry weight of pericarps to water stresses applied after the anthesis of the first disc floret in experiment 14. Wilts D, E and F refer to water stresses applied at various stages of development of the inflorescence after anthesis of the first disc floret, the details of which are shown in table 5.1. (\(\downarrow\downarrow\)) = wilt D, (\(\downarrow\uparrow\)) = wilt E, (\(\uparrow\uparrow\)) = wilt F. Wilt refers to water stress. Least significant figures are provided.
(a) Pre-anthesis wilts
LSD (P=0.05) = 1.96 mg

(b) Post-anthesis wilts
LSD (P=0.05) = 1.96 mg
anthesis wilt treatment of experiment 13 (see figure 5.4.b). In the field experiment (experiment 16), such a response was also seen but the magnitude of the reduction in the pericarp dry weights of the dry plot was much greater than the reductions that were recorded in the glasshouse or the controlled environment (see figure 5.5.b).

An analysis of variance on the pericarp dry weight responses in experiment 14 of both the pre- and post-anthesis water stress treatments (table 5.2.a) and a separate unpooled analysis for pre-anthesis water stress alone (table 5.2.b) showed that the treatment and position effects were highly significant ($P < 0.01$) at the 3rd harvest. However, the interaction between the treatment and the position in both the pooled and unpooled analyses was significant at the first two harvests ($P < 0.01$) but insignificant at the 3rd harvest (see appendix C for analyses of variance of the first 2 harvests). Tables C.1.a and b (harvest 1) while tables C.2.a and b (harvest 2).

5.3.2. Effects of pre-anthesis water stress on embryo dry weight

The embryo dry weight was reduced by water stress. This occurred to embryos at all achene positions. In experiment 14, the magnitude of the reduction was greatest for water stresses that were imposed at stages of disc floret initiation or at stages shortly after disc floret initiation - i.e. water stresses A and B. For a water stress imposed just before the anthesis of the 1st disc floret, embryos were only slightly reduced in weight (see figure 5.2.a). In experiment 13, the reduction in the dry weight of the embryo was also large (see figure 5.4.a).

An analysis of variance on the embryo dry weight responses of both pre- and post-anthesis water stresses (table 5.3.a) and an unpooled analysis for pre-anthesis water stresses (table 5.3.b) showed that the
Figure 5.2. a. shows the responses of the dry weight of the embryo of the achenes at various positions on the capitulum, to water stress in experiment 14. Wilts A, B and C refer to water stresses applied before the anthesis of the first disc floret on the capitulum, the details of which are provided in table 5.1. It was difficult to follow the spiral rows further than position 8 in the water stressed capitula. The data points are means of three replicates.

Figure 5.2. b. shows the responses of the dry weight of the embryo of the achenes at various positions on the capitulum, to water stresses in experiment 14. Wilts D, E and F refer to water stresses applied after the anthesis of the first disc floret on the capitulum, the details of which are provided in table 5.1. The data points are means of three replicates. Note the interaction between the water stress treatment and the position of sampling, especially in wilt F. The embryos of achenes near the periphery of the capitulum have dry weights comparable to the controls in wilt F. Least significant values are provided.
(a) Pre-anthesis wilts
LSD (P=0.05) = 10.66 mg

(b) Post-anthesis wilts
LSD (P=0.05) = 10.66 mg
treatment and the position effects were highly significant ($P < 0.01$) for both the pooled and the unpooled analyses at the 3rd harvest. The interaction between the treatment and the sampling position was also significant ($P < 0.01$) at all three harvests, but in the unpooled analysis, the interaction between the treatment and the sampling position in the pre-anthesis water stresses was only marginally close to being significant at $P < 0.05$ level (see appendix C for analyses of variance of the first two harvests). Tables C.3.a and b (harvest 1) while tables C.4.a and b (harvest 2).

5.3.3. Effects of post-anthesis water stress on pericarp dry weight

In experiments 14 and 15, the pericarp dry weight at all sampling positions was reduced by water stress. In experiment 14, the reduction in pericarp dry weight for positions near the periphery was less than corresponding positions in the pre-anthesis water stresses (compare figure 5.1.b. with 5.1.a.). For positions nearer the center, the reduction in the pericarp dry weight was as much if not more than in corresponding positions in the pre-anthesis water stress treatments. The large reductions to the pericarp dry weight for achene sampling positions near the centre was also observed in experiment 15 (figure 5.6.b.) and in the field experiment (figure 5.5.b.).

An unpooled analysis of variance of the pericarp dry weight responses in the post-anthesis water stresses of experiment 14 (table 5.2.c.) showed the interaction between the treatment and the sampling position was significant at the 3rd harvests. (See appendix C for analyses of variance of the first two harvests). Table C.1.c. contains analysis of data from harvest 1 while table C.2.c. contains data from harvest 2.
Figure 5.3. a. shows the response of the whole achene dry weight (pericarp + embryo) of achenes at various positions on the capitulum, to water stresses applied at various stages before the anthesis of the first disc floret in experiment 14. Wilts A, B and C refer to water stresses applied at various stages of the development of the inflorescence, the details of which are provided in table 5.1. Note that there are no interactions between the water stress and the position of sampling. The data points are means of three replicates.

Figure 5.3. b. shows the response of the whole achene dry weight (pericarp + embryo) of achenes at various positions on the capitulum, to water stresses applied after the anthesis of the first disc floret in experiment 14. Wilts D, E and F refer to water stresses applied at various stages of anthesis, the details of which are provided in table 5.1. Note that there are interactions between the water stress treatment and the position of sampling the achene. The data points are means of three replicates.
(a) Pre-anthesis wilts
LSD (P=0.05) = 12.88mg

(b) Post-anthesis wilts
LSD (P=0.05) = 12.88mg
Figure 5.4. a. shows the responses of the embryo dry weight of individual achenes at various positions on the capitulum, to water stress in experiment 13. The water stress was applied when the inflorescence had been initiated and disc florets were about to be initiated. The plants were 27 days old at the start of water stress. It was a severe stress; the plants took a longer time than usual to regain turgidity after rewatering.

Figure 5.4. b. shows the responses of the pericarp dry weight of individual achenes at various positions on the capitulum, to water stress in experiment 13.
Figure 5.5. a. shows the responses of the embryo dry weight of achenes at various positions on the capitulum, to water stress in experiment 16. The experiment was done in the field. The water stress treatment refers to the dry plot of the experiment; it received one irrigation at sowing only and the rest of plant growth was largely dependent on rainfall. This response was measured at crop maturity, 90 days from sowing the crop. The data points are means of three replicates.

Figure 5.5. b. shows the responses of the pericarp dry weight of achenes at various positions on the capitulum, to water stress in experiment 16. The data points are means of three replicates.
Figure 5.6. a. shows the response of the embryo dry weight of achenes at various sampling positions on the capitulum, to water stresses applied just prior to the anthesis of the first disc floret (A), 5 days after the anthesis of the first disc floret (B) and 8 days after the anthesis of the first disc floret (C). The responses represent those at the final harvest, after several days of recovery. The data points are means of three replicates. Note the interaction between the water stress (C) and the response of the achenes at the different sampling points. Data from experiment 15.

Figure 5.6. b. shows the response of the pericarp dry weight of achenes at various sampling positions on the capitulum in experiment 15. Note that there is no interaction between the water stress and the position of sampling, even in the case of water stress (C). This is similar to the response in experiment 14.
5.3.4. Effects of post anthesis water stress on embryo dry weight

Generally, the dry weight of the embryos of achenes positioned near the periphery of the capitulum was not reduced by the water stress. In experiment 14, the dry weight of embryos at achene positions 1 and 2 of water stress D, 1 to 5 of water stress E and 1 to 4 of water stress F were not reduced. Embryo dry weight at other positions were reduced to varying degrees (see figure 5.2.b.). Thus, the reduction in embryo dry weight was greater in the pre-anthesis water stresses than in the post-anthesis water stresses for achene positions near the periphery but for positions near the centre, the reductions were greater in the post-anthesis water stresses. A similar response pattern was exhibited by the embryos in experiment 15 (figure 5.6.a.). In contrast, there were large reductions in the embryo dry weight at all achene positions in experiment 16 (figure 5.5.a.).

An unpooled analysis of variance on the responses of embryo dry weights of post-anthesis water stresses in experiment 14 showed high significance ($P < 0.01$) at 3rd harvest (see table 5.3.c.). (Analyses of variance for first two harvests are shown in Appendix C). Table C.3.c. contains data from harvest 1 while table C.4.c. contains data from harvest 2.

5.3.5. Effects of pre- and post anthesis water stresses on achene dry weight

The achene dry weight responses (a combined response of pericarp and embryo) to water stress in experiment 14 are illustrated in figure 5.3.a and b. Responses resulting from the post-anthesis water stress of experiment 13 (in which 3 harvests were made) are illustrated in figure 5.7. In experiment 13, embryo dry weight at all sampling positions was unaffected by water stress, at final recovery.
Figure 5.7. shows the responses of the embryo dry weight of achenes at various positions on the capitulum, to water stress in experiment 13. The water stress was started when all the disc florets had completed anthesis and this coincided with day 69 of the plants age. The first harvest was made on day 75 (at the end of the drying cycle), the second harvest was made on day 80 (5 days after rewatering the stressed plants) and the last harvest was made on day 90 (15 days after rewatering the stressed plants). (●—●) = control, (○—○) = water stressed. There was little evidence of accelerated senescence of the lower leaves in this experiment, as a result of water stress. Compare the responses of the embryos at the central positions of the capitulum in this experiment with the responses of embryos at the central positions in experiment 14.
EMBRYO DRY WEIGHT (mg)

---

day 75

day 80

day 90

periphery  Achene position on capitulum  center
Table 5.2.a. Pooled analysis of variance (pre-anthesis and post-anthesis water stresses) on pericarp dry weight responses in experiment 14. 3rd harvest.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>6</td>
<td>0.000623183</td>
<td>0.000103864</td>
<td>5.977***</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>14</td>
<td>0.000243261</td>
<td>0.000017376</td>
<td>11.868</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20</td>
<td>0.000866445</td>
<td>0.000043322</td>
<td>29.590</td>
</tr>
<tr>
<td>POSITION</td>
<td>7</td>
<td>0.000389988</td>
<td>0.000055713</td>
<td>38.053***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>41(1)</td>
<td>0.000084735</td>
<td>0.000002067</td>
<td>1.412 N.S.</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>92(6)</td>
<td>0.000134696</td>
<td>0.000001464</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>140</td>
<td>0.000609419</td>
<td>0.000004353</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>160</td>
<td>0.001475864</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF = degrees of freedom; SS = sum of squares; MS = mean of squares; VR = variance ratio.

N.S. = non-significant  * = 5% significance  ** = 1% significance  *** = .1% significance

Table 5.2.b. Unpooled analysis of variance (pre-anthesis water stress only) on pericarp dry weight responses in experiment 14. 3rd harvest.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
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<td>0.000515760</td>
<td>0.000171920</td>
<td>7.016***</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>8</td>
<td>0.000196038</td>
<td>0.000024505</td>
<td>14.666</td>
</tr>
<tr>
<td>TOTAL</td>
<td>11</td>
<td>0.000711798</td>
<td>0.000064709</td>
<td>38.727</td>
</tr>
<tr>
<td>POSITION</td>
<td>7</td>
<td>0.000123286</td>
<td>0.000017612</td>
<td>10.541**</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>21</td>
<td>0.000015161</td>
<td>0.000000722</td>
<td>0.432 N.S.</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>54(2)</td>
<td>0.000090227</td>
<td>0.000001671</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>82</td>
<td>0.000228675</td>
<td>0.000002789</td>
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</tr>
<tr>
<td>GRAND TOTAL</td>
<td>93</td>
<td>0.000940473</td>
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<td></td>
</tr>
</tbody>
</table>
Table 5.2.c. Unpooled analysis of variance (post-anthesis water stresses only) on pericarp dry weight responses in experiment 14. 3rd harvest.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
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<td>0.000485676</td>
<td>0.000161892</td>
<td>6.851***</td>
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<tr>
<td>RESIDUAL</td>
<td>8</td>
<td>0.000189049</td>
<td>0.000023631</td>
<td>22.151</td>
</tr>
<tr>
<td>TOTAL</td>
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<td>0.000674725</td>
<td>0.000061339</td>
<td>57.497</td>
</tr>
<tr>
<td>POSITION</td>
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<td>0.000321294</td>
<td>0.000045899</td>
<td>43.025***</td>
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<tr>
<td>TREATMENT x POSITION</td>
<td>20(1)</td>
<td>0.0000516181</td>
<td>0.000002584</td>
<td>2.422**</td>
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<tr>
<td>RESIDUAL</td>
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<td>0.000055474</td>
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<tr>
<td>TOTAL</td>
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<td>0.000428449</td>
<td>0.000005423</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>90</td>
<td>0.001103174</td>
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</table>

DF = degrees of freedom; SS = sum of squares; MS = mean square; VR = variance ratio.
Table 5.3.a. Pooled analysis of variance (both pre- and post anthesis water stresses) on embryo dry weight responses in experiment 14. 3rd harvest.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
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<td>0.00499804</td>
<td>0.00083301</td>
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<tr>
<td>RESIDUAL</td>
<td>14</td>
<td>0.00456188</td>
<td>0.00032585</td>
<td>7.526</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20</td>
<td>0.00955992</td>
<td>0.00047800</td>
<td>11.041</td>
</tr>
<tr>
<td>POSITION</td>
<td>7</td>
<td>0.00929652</td>
<td>0.00132807</td>
<td>30.675***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>41(1)</td>
<td>0.00321570</td>
<td>0.00007843</td>
<td>1.812**</td>
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<td>RESIDUAL</td>
<td>92(6)</td>
<td>0.00398309</td>
<td>0.00004329</td>
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</tr>
<tr>
<td>TOTAL</td>
<td>140</td>
<td>0.01649532</td>
<td>0.00011782</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>160</td>
<td>0.02605524</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF = degrees of freedom; SS = sum of squares; MS = mean of squares; VR = variance ratio.

Table 5.3.b. Unpooled analysis of variance (only pre-anthesis water stresses) on embryo dry weight responses in experiment 14. 3rd harvest.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>3</td>
<td>0.00361965</td>
<td>0.00120655</td>
<td>6.147**</td>
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<tr>
<td>RESIDUAL</td>
<td>8</td>
<td>0.00157015</td>
<td>0.00019627</td>
<td>17.769</td>
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<tr>
<td>TOTAL</td>
<td>11</td>
<td>0.00518980</td>
<td>0.00047180</td>
<td>42.714</td>
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<tr>
<td>POSITION</td>
<td>7</td>
<td>0.00279021</td>
<td>0.00039860</td>
<td>36.087***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>21</td>
<td>0.00041285</td>
<td>0.00001966</td>
<td>1.780 N.S.</td>
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<tr>
<td>RESIDUAL</td>
<td>54(2)</td>
<td>0.00059646</td>
<td>0.00001105</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>82</td>
<td>0.00379952</td>
<td>0.00004634</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>93</td>
<td>0.00898932</td>
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<td></td>
</tr>
</tbody>
</table>

DF = degrees of freedom; SS = sum of squares; MS = mean of squares; VR = variance ratio.
Table 5.3.c. Unpooled analysis of variance (post-anthesis water stresses only) on embryo dry weight responses in experiment 14.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
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<td>0.00268065</td>
<td>0.00089355</td>
<td>2.088*</td>
</tr>
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<td>RESIDUAL</td>
<td>8</td>
<td>0.00342373</td>
<td>0.00042797</td>
<td>6.337</td>
</tr>
<tr>
<td>TOTAL</td>
<td>11</td>
<td>0.00610438</td>
<td>0.00055494</td>
<td>8.217</td>
</tr>
<tr>
<td>POSITION</td>
<td>7</td>
<td>0.00749982</td>
<td>0.00107140</td>
<td>15.865***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>20(1)</td>
<td>0.00244876</td>
<td>0.00012244</td>
<td>1.813*</td>
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<td>RESIDUAL</td>
<td>52(4)</td>
<td>0.00351178</td>
<td>0.00006753</td>
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</tr>
<tr>
<td>TOTAL</td>
<td>79</td>
<td>0.01346035</td>
<td>0.00017038</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>90</td>
<td>0.01956474</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF = degrees of freedom; SS = sum of squares; MS = mean of squares; VR = variance ratio.

Effects of water stress on embryo dry weight responses in experiment 14. The analyses of variance were performed on the unpooled data. The interaction of position and treatment was significant (P < 0.01) in all the analyses. The interaction between the treatments and position in a pooled analysis was also significant. The interaction of treatment and water stress was significant in the post-anthesis water stress treatments. This interaction was not significant in water stress A, which was the least stressful in the experiment. The number of unfilled schemes was lowest in the experiment. The majority of unfilled schemes were right at the central part of the capitulum.

The total scheme number was generally unaffected in all the water stress treatments, except in water stress treatment A. The diameter of the inflorescences in treatment A was also smaller than that of the other treatments. In the field experiment, the diameter of the inflorescences
A pooled and an unpooled analysis of variance on the responses of achene dry weight at the 3rd harvest in expt. 14 (tables 5.4a,b,c) showed that treatment and position effects were significant \((P < 0.01)\) in all the analyses. The interaction between the treatment and position in a pooled analysis was also significant \((P < 0.01)\). However, in the unpooled analyses, this interaction in the pre-anthesis water stresses was insignificant at the final harvest while it was highly significant \((P < 0.01)\) in the post-anthesis water stresses.

5.3.6. Effects of water stress on achene number, unfilled achenes, total achene weight and the diameter of the inflorescence

In experiment 14, the total achene weight was reduced in all the water stress treatments. This reduction was large in water stresses A, B and E. The least reduction in the total achene weight was in the pre-anthesis water stress treatment C (see table 5.5.).

A major contributor to the reduced total achene weight in water stress A of experiment 14, was the low total achene number. The number of unfilled achenes ranged from 30% to 35% of the total achene population in most water stress treatments, including the controls. In water stress treatments E and F, however, the percentage of unfilled achenes was closer to 50%. A large part of the reduction in seed weight in water stress E was due to the high number of unfilled achenes. The majority of unfilled achenes were right at the central parts of the capitulum.

The total achene number was generally unaffected in all the water stresses, except in water stress treatment A. The diameter of the inflorescence in treatment A was also smaller than that of the other treatments. In the field experiment, the diameter of the inflorescence
Table 5.4.a. Pooled analysis of variance (both pre- and post-anthesis water stresses) on achene dry weight responses in experiment 14. 3rd harvest.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>6</td>
<td>0.00813703</td>
<td>0.00135617</td>
<td>2.924***</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>14</td>
<td>0.00649343</td>
<td>0.00046382</td>
<td>7.353</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20</td>
<td>0.01463046</td>
<td>0.00073152</td>
<td>11.597</td>
</tr>
<tr>
<td>POSITION</td>
<td>7</td>
<td>0.01625732</td>
<td>0.00232247</td>
<td>36.820***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>41(1)</td>
<td>0.00672797</td>
<td>0.00016410</td>
<td>2.602**</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>94(4)</td>
<td>0.00592921</td>
<td>0.00006308</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>142</td>
<td>0.02891450</td>
<td>0.00020362</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>162</td>
<td>0.04354496</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.4.b. Unpooled analysis of variance (only pre-anthesis water stresses) on achene dry weight responses in experiment 14. 3rd harvest.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>3</td>
<td>0.00659413</td>
<td>0.00219804</td>
<td>7.260***</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>8</td>
<td>0.00242214</td>
<td>0.00030277</td>
<td>19.116</td>
</tr>
<tr>
<td>TOTAL</td>
<td>11</td>
<td>0.00901627</td>
<td>0.00081966</td>
<td>51.751</td>
</tr>
<tr>
<td>POSITION</td>
<td>7</td>
<td>0.00403950</td>
<td>0.00057707</td>
<td>36.434***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>21</td>
<td>0.00050142</td>
<td>0.00002388</td>
<td>1.508 N.S.</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>54(2)</td>
<td>0.00085529</td>
<td>0.00001584</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>82</td>
<td>0.00539620</td>
<td>0.00006581</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>93</td>
<td>0.01441247</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 5.4.c. Unpooled analysis of variance (only post-anthesis water stresses) on achene dry weight responses in experiment 14. 3rd harvest.

<table>
<thead>
<tr>
<th>Source of Variance</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>3</td>
<td>0.00497950</td>
<td>0.00165983</td>
<td>2.580**</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>8</td>
<td>0.00514732</td>
<td>0.00064341</td>
<td>6.601</td>
</tr>
<tr>
<td>TOTAL</td>
<td>11</td>
<td>0.01012682</td>
<td>0.00092062</td>
<td>9.445</td>
</tr>
<tr>
<td>POSITION</td>
<td>7</td>
<td>0.01426484</td>
<td>0.00203783</td>
<td>20.906***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>20(1)</td>
<td>0.00514687</td>
<td>0.00025734</td>
<td>2.640**</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>54(2)</td>
<td>0.00526371</td>
<td>0.00009748</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>81</td>
<td>0.02467542</td>
<td>0.00030463</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>92</td>
<td>0.03480224</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DF = degrees of freedom; SS = sum of squares; MS = mean of squares; VR = variance ratio.
Table 5.5. Response of achene numbers, achene weight and inflorescence diameter to water stresses applied at various stages of inflorescence development.

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>Filled Achenes</th>
<th>Unfilled Achenes</th>
<th>Total Number of Achenes</th>
<th>Total Achene Weight (g)</th>
<th>Inflorescence Diameter (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water stress A</td>
<td>280</td>
<td>168</td>
<td>448</td>
<td>7.96</td>
<td>8.2</td>
</tr>
<tr>
<td>Water stress B</td>
<td>371</td>
<td>186</td>
<td>557</td>
<td>9.57</td>
<td>9.0</td>
</tr>
<tr>
<td>Water stress C</td>
<td>401</td>
<td>246</td>
<td>647</td>
<td>12.32</td>
<td>9.4</td>
</tr>
<tr>
<td>Water stress D</td>
<td>433</td>
<td>194</td>
<td>627</td>
<td>10.54</td>
<td>9.3</td>
</tr>
<tr>
<td>Water stress E</td>
<td>272</td>
<td>275</td>
<td>547</td>
<td>7.82</td>
<td>9.4</td>
</tr>
<tr>
<td>Water stress F</td>
<td>362</td>
<td>241</td>
<td>603</td>
<td>9.48</td>
<td>9.2</td>
</tr>
<tr>
<td>CONTROL</td>
<td>435</td>
<td>185</td>
<td>620</td>
<td>13.99</td>
<td>9.5</td>
</tr>
</tbody>
</table>

* For further details of the treatments see table 5.
of the dry plot plants was only about half that of irrigated plants; the number of achenes was also reduced by half in the dry plot plants. The inflorescences of the irrigated plants had only a few unfilled achenes but the achenes at the centre of the inflorescence in the dry plot plants were not properly filled and often lacked pigmentation in the pericarp.

5.4. DISCUSSION

The contrasting patterns in the response of the whole achene to water stresses before and after anthesis are conveniently described as Response type 1 and Response type 2. This generality is based on data from experiment 14. The responses in the other experiments may then be examined to see if they fit this generality. The response types are as follows:

Response type 1: involving little or no interaction between the water stress treatment and the position of the achene on achene weight. The responses of the achenes in water stress treatments A, B and C in experiment 14 would fall into this category. As the achene size in these treatments is decreased consistently at all positions of sampling, there is no interaction between the treatment and the sampling position.

Response type 2: involving an interaction between the water stress treatment and achene position. The responses of the achenes in water stress treatments D, E and F in experiment 14 would fall into this category. As the achene sizes near the periphery of the capitulum are unaffected by water stress while achenes nearer the center of the capitulum are affected, there is interaction between the stress treatment and the achene position.

The response types can be generally illustrated by the diagram.
The general decrease in achene size from the peripheral positions to the central positions of the capitulum in the well watered controls may be due to a number of reasons. It may be assumed that the rapid growth of the outer achenes imposes a constraint on available space for the centrally positioned achenes. An alternative explanation for the gradient in achene size in the capitulum may be related to a competition for a limited supply of assimilates. If a shortage of assimilates is the cause of the gradient in achene size, it is hard to understand why there are still quite a few viable and actively photosynthesizing leaves on the plant even at very late stages of maturity. Perhaps a more plausible explanation would be that the conducting vessels to the peripheral achenes are better developed than those leading up to the central achenes. The distribution of conducting vessels can be easily observed by cutting longitudinally through a sunflower inflorescence. The pith below the central achenes is loosely packed and the conducting vessels are less well developed than those leading up to
the peripheral achenes. This visual evidence is preliminary and it would be difficult to attach unequivocal significance to the observed differences in the distribution of conducting vessels.

If physical constraint between the peripheral achenes and the central achenes is a factor, then some compensatory growth of the central achenes should have occurred in wilt Response type 1. This is expected because when the peripheral achenes are reduced in size, they would remove some of the constraint on central achenes. Response type 1 should give rise to the supposed response as indicated by the broken line in the diagram below.

![Diagram](image)

Such compensatory growth of the central achenes should result in a significant interaction between the stress treatment and the achene position. However, the analysis of variance that was performed on the achene responses of the pre-anthesis water stress treatment showed no significant interaction between stress treatment and achene position. Thus, compensatory growth of the central achenes did not occur despite the reduction in the size of the peripheral achenes. It may be
postulated, however, that the water stress was not severe enough to reduce the sizes of peripheral achenes sufficiently to cause any significant change to the constraint. This is worthy of further consideration but it is important also to acknowledge that larger stresses may evoke other responses. The evidence so far provided by these experiments does not suggest that peripheral achenes impose a constraint for space on the central achenes.

In the wilt Response type 2, the full recovery of peripheral achenes and the marked reduction in the dry weight of the central achenes may be due to the following reasons.

(a) That some of the embryos recovered fully suggests that there may be a critical growth stage in the embryo after which water stress can only delay the achievement but cannot reduce the final weight of the embryo. The critical growth stage in the embryo may be associated with the completion of cell division, such that growth thereafter would depend only on the enlargement of the cells or it may depend on the attainment of a minimum critical osmotic potential such that the embryo can withstand water stress. In *Pisum sativum*, Dure 111 (1975) showed that cell divisions in an embryo are completed when the embryo has attained \( \frac{1}{4} \) of its final dry weight. If this were a general pattern for most dicotyledonous embryos, it is likely that cell divisions are completed in the embryos of peripheral achenes in the sunflower before the embryos in the centre and also before water stress became severe. The osmotic potential of the peripheral embryos would also be higher than the central embryos such that they would withstand the stress.

(b) The observed accelerated senescence of leaves in water stresses E and F (see Chapter 4[2]) raised the possibility that assimilate supply would be reduced. It was shown by Rawson and Woodward (1976) that the high demands for photosynthate supply imposed by the developing sunflower inflorescence on leaves caused leaves to maintain a steady rate of
photosynthesis for a long time before declining. The accelerated senescence would shorten the period at which photosynthesis can be maintained at a steady rate and thus deprive the inflorescence of some assimilate. However it was shown by McWilliam, English and McDougall (1974) that the leaves in the mid-position and the top position of the leaf canopy contributed most of the assimilate requirements of the inflorescence such that accelerated senescence of the lower leaves should have no effect on inflorescence growth. This is discussed further in Chapter 6.

(c) If it is assumed that the central achenes have an influence on the growth of the peripheral achenes, in terms of competing for space to grow, the wilt Response type 2 may yet be interpreted in a third way. In this, it is assumed that the large reduction in the size of central achenes will create more space for the peripheral achenes to grow; the peripheral achenes could then grow to control sizes. This possibility assumes that the wilt Response type 2 is a modification of a wilt Response type 1 such as illustrated in the diagram below.

![Diagram showing the relationship between achene position and size for Response Types 1 and 2.](image-url)

If the assumption that central achenes have an influence on
peripheral achenes in terms of space is correct, then the peripheral achenes in the pre-anthesis water stresses A and B should be as shown below.

That this result was not observed suggests that the wilt Response type 2 is distinct from a Response type 1. It is unlikely that the growth of the peripheral achenes are dependent on space limitations controlled by the central achenes.

It was perhaps surprising that the wilt Response type 2 that was observed in experiment 14 was not observed in experiment 13. The full recovery of achenes at all positions on the capitulum in experiment 13, in contrast to experiment 14, may be related to the following. The drastic decline in the number of viable leaves and in the total leaf area observed in experiment 14 was not observed in experiment 13. Furthermore, the drying cycle in experiment 14 took only 3 days and presumably caused a much larger stress than the slow drying cycle (6 days) in experiment 13. The differences in the time taken to transpire the available water in experiments 14 and 13 is probably related to the different environmental
conditions. While the temperature was 21°C/9°C day/night with a light intensity of 650 microeinstein\( \text{m}^{-2} \text{sec}^{-1} \) during the day in experiment 13, it was 27°C/22°C day/night with normal daylight (about 900 microeinstein\( \text{m}^{-2} \text{sec}^{-1} \) at that time of the year) in experiment 14. The stress developed quickly in experiment 14 in comparison to experiment 13. As the wilt Response type 2 was once again observed in experiment 15 (in which the temperature was high during the drying cycle), it may be postulated that wilt Response type 2 was not observed in experiment 13 because of the mild nature of the stress that was generated.

The responses of the achenes in the field experiment (experiment 16) differed slightly from those in experiments 13, 14 and 15. As the plants on the dry plot received only one irrigation at sowing, their subsequent growth depended largely on rainfall. As severe water deficits were incurred at several stages of plant growth, a mixture of the two achene Response types could be expected. Because water deficits occurred from very early stages of inflorescence development, the achene responses followed a Response type 1 only to be modified by water deficits at later stages of inflorescence development to also cause a Response type 2 among the central achenes. The Response type 1 of the peripheral achenes and the Response type 2 of the central achenes remain unchanged despite the heavy rainfall that occurred in the latter part of grain filling. There was also a number of viable leaves on the dry plot plants at that time. Thus, the permanency of the Response type 1 may be due to some developmental limitations within the achene.

To consider aspects of developmental limitations, it may be necessary to examine the possible effects that pericarp development can have on embryo development.

As large increases to embryo dry weight occurs after anthesis, pollination and fertilization, reductions in embryo dry weight resulting
from pre-anthesis water stresses may be related to reductions in pericarp development. It was unfortunate that volumes of achenes were not measured; since the pericarp attains its maximum dimensions before the embryo does, reductions to the volume enclosed by the pericarp (which may result from effects of water stress during its early development or otherwise) could possibly affect embryo size. This aspect of a possible role of the pericarp in determining embryo size is evaluated in part 2 of the general discussion.

As achene growth was studied towards the end of the experimental part of the thesis work, there was no time to examine the effects of water on cell division and enlargement in the various components of the achene. Such data quantifying cell number responses would have required techniques different from that used to determine cell number responses in leaves. Essentially, this would have become a major experimental work and could not have been conducted in the time that was available. However, the sequential imposition of water stress on the growth of the achene has allowed meaningful interpretations of the responses of embryonic and non-embryonic tissues of the achenes to water stress and possible lines of future research. These responses of the achene are compared with the responses of the leaf, in the general discussion.
CHAPTER 6
GENERAL DISCUSSION

6.1. Introduction

Since final leaf area is among the principal factors affecting the 'source' size in a general 'source-sink' relationship between production and accumulation of photosynthates, the several component cell variables which together determine leaf area are of critical importance in evaluating their possible roles in such a relationship.

Variations in the component variables of leaf area together with final leaf area are summarized for well-watered plants in figure 6.1 while figure 6.2 (a-d) and figure 6.3 (a-d) summarize the effects of stresses that were imposed early and late in vegetative development. A comparison of the trends in the final leaf size and the final achene size that are associated with early, late and very late water stresses in vegetative and inflorescence development are illustrated in figure 6.4 a and b. These diagrams are used throughout this general discussion of the effects of water stress on leaf and achene growth; the first part considers leaf responses to water stress, the second evaluates the relationship between the leaf and achene responses to water stress while the third assesses the relevance of the main results in the first two parts to a field situation.
Figure 6.1. shows the relationship between the position of the leaf on the shoot and some of the components that contribute to the area of the leaf. Also, the area of individual leaves has been presented. The data has been derived from experiment 11 (from the well watered controls). The data has been expressed as a percentage of a maximum for that particular component. For example, the largest leaf on the plant is leaf 16 and accordingly, the areas of the other leaves have been expressed as a percentage of the area of leaf 16. As the largest palisade cells are in leaf 1, the sizes of the palisade cells in the other leaves have been expressed as a percentage of the palisade cell size in leaf 1.
6.2. Part 1: leaf responses to water stress

The trends in figure 6.1 suggest that the palisade cell frequency and the final leaf area are directly related. This relationship contrasts sharply with the inverse relationship between palisade cell area and leaf area. While the intercellular space remains constant up to about leaf 8, the increase in palisade cell frequency is apparently more than enough to balance the decrease that occurs in palisade cell area. Between leaves 8 and 17, the balance is maintained towards an increase in leaf area despite decreases in both palisade cell area and intercellular space. The balance is reversed, however, above leaf 17 when final leaf area decreases are without associated decreases in palisade cell frequency. In these higher ranking leaves, not only are the palisade cell areas and intercellular spaces very small, but the increase in veinal tissue causes further decreases to the effective photosynthetic area of the whole leaf. Assuming that the area occupied by the palisade cells in the leaf determines 'source' size, the source of photosynthates thus appears to increase up to leaf 17 but thereafter, the marked decreases in leaf area, while cell frequency and cell area remain constant, indicate that the 'source' size is decreased in direct proportion to leaf size.

One complication introduced by the ontogenetic increase in veinal tissue is that the palisade cell frequency cannot be used as an index to provide an accurate estimate of the total number of palisade cells by multiplying leaf area and cell frequency. Accordingly, the leaf area responses to water stress should be considered in terms of directly measured variables (cell frequency and cell area) or in terms of the derived intercellular space which is accurate because it is derived from the other two measured variables. This precaution contrasts with previous attempts to calculate the total palisade cell number, which have disregarded the correction needed to take care of the space occupied by veinal tissue (see Chapter 3; Clough and Milthorpe 1975).
Figures 6.2. a-d. show the effects of water stress on leaf area, palisade cell area (cross-sectional area), palisade cell number/unit area and intercellular space/unit area in experiment 11. The diagrams are drawn from primary data of this experiment, the values of individual leaves have been expressed as a percentage of the maximum for that particular component, as in figure 6.1.

Figure 6.2. a. shows the response of the area of individual leaves on the shoot to water stress. The areas of control water stressed leaves have been expressed as a percentage of the largest control leaf i.e. leaf 16. The unfolding leaf at the start of the water stress cycle was leaf 7.

Figure 6.2. b. shows the response of palisade cell area of the individual leaves of water stressed and control leaves. Note that the palisade cell area is not affected in leaves above position 10.

Figure 6.2. c. shows the response of the palisade cell number/unit area in individual leaves of water stressed and control leaves to the stress applied when the 7th leaf was unfolding on the plant.

Figure 6.2. d. shows the response of the derived variable - the intercellular space/unit area of individual leaves to the stress applied when the 7th leaf was unfolding. The intercellular space in this case refers to the space in the mesophyll, between palisade cells and does not include vascular tissue occupied space.
Figure 6.3. a-d. show the effects of water stress on leaf area, palisade cell area (cross-sectional area), palisade cell number/unit area and intercellular space/unit area in experiment 10. The diagrams are drawn from primary data of this experiment. The values of the individual leaves have been expressed as a percentage of the maximum for that particular component, as figures 6.1 and 6.2 a-d. The unfolding leaf at the start of the water stress was leaf 16.

Figure 6.3. a. shows the response of the area of individual leaves on the shoot to water stress. The areas of the control and water stressed leaves have been expressed as a percentage of the largest control leaf i.e. leaf 16. Note that leaves 1 and 2 had senesced by this time and are represented by the dotted (----) line.

Figure 6.3. b. shows the response of palisade cell area of the individual leaves to water stress. Note that the palisade cell area is not affected by water stress in leaves above position 11 although leaves 11 to 16 had unfolded before the stress and were in expansion.

Figure 6.3. c. shows the response of the palisade number/unit area in individual leaves of water stressed plants.

Figure 6.3 d. shows the response of the derived variable - the intercellular space/unit area of the individual leaves to the stress applied. The definition of the intercellular space follows that of figure 6.2. d.
LEAF AREA

PALISADE CELL AREA

PALISADE CELL NUMBER /UNIT AREA

INTERCELLULAR SPACE UNIT AREA

LEAF POSITION ON THE SHOOT
As the trends in figure 6.2 (a-d) and figure 6.3 (a-d) indicate that water stress modifies but does not reverse the main trends in figure 6.1, it is possible to interpret the reduced leaf area associated with water stress in terms of the trends in the component variables of leaf area. These trends suggest that leaf area decreases for low leaf positions in both early and late water stress treatments, are directly related to the decrease in palisade cell area. In contrast to this, water-stress induced changes in the palisade cell frequency and the derived intercellular space are both inversely related to reduced leaf area. Although it should be re-emphasized that precise estimates of the magnitude of each of these components in determining final leaf area are not possible, it is reasonable to assume that the water-stress induced increase in palisade cell frequency and intercellular space is more than counterbalanced by the water stress induced decrease in palisade cell area.

In the late water stress treatment, however, the marked decreases in leaf area of leaves above node 10 (figure 6.3 a), are related to marked changes neither in palisade cell area nor palisade cell frequency. However, the marked decreases in veinal tissue in these higher leaves again precludes interpretation of the reduced leaf area as a reduction in total number of cells alone. Nevertheless, the different roles that palisade cell area and palisade cell frequency appear to have in reducing leaf area in lower and in higher leaves raise some interesting questions about possible adaptive mechanisms that may operate during and subsequent to water stress in leaves at different ontogenetic positions in this sunflower cultivar.

The asymptotic decrease in palisade cell area together with its stability above node 10, which remains at approximately 25% of the maximum palisade cell area irrespective of large changes in either node position or water stress, suggests that cell area approaches a minimum in the higher leaves. The ontogenetic stability of this minimum cell area
together with its stability during water stress implies that the character is under direct genotypic control. Such a fixed minimum in palisade cell area for the upper leaves is in contrast to the metastable or phenotypic expression of palisade cell area in the lower leaves as affected by water stress.

As Leopold (1964) states, "metastable changes are supremely characteristic of biological organisms" and it would be surprising if such plasticity was not involved in adaptive responses to water stress involving a balance between water loss through transpiration and the 'source' size for photosynthate production. Stomatal closure in response to water stress has been interpreted as involving such a balance (Boyer 1971; Turner 1973) but the importance of an increase in the internal surface area of mesophyll cells in increasing transpiration during periods of adequate watering as stated by Turrell (1965) may be equally important. If Turrell's conclusions are valid for sunflower, the decrease in the palisade cell area in the lower leaves of the sunflower as a result of water stress may constitute an adaptive response. Viewed in this light, the smaller palisade cells and the increased cell frequency (with increasing ontogenetic position of leaves - up to leaf 17) which together will provide a larger evaporative surface, may seem to be associated with an increasing trend to transpire more. Nevertheless, Tanton and Crowdy (1972) provide evidence that the majority of evaporation from a leaf occurs from the walls of guard cells and other epidermal cells such that evaporative surface would only be slightly influenced by differences in the mesophyll cell surface area. Although it is difficult to reconcile these two views on the available evidence, it seems clear that if the stress is brief and mild, any reduced size of palisade cells which may lower surface area for CO₂ absorption and presumably also lower the photosynthetic capacity, would recover when water stress is alleviated. Such a form of recovery of leaf area as was recorded by Acevedo et al. (1971) which presumably involved
recovery of palisade cell area, is also perhaps involved in the recovery of lower leaves in experiment 7 of this thesis. However, in a more severe stress such as in experiment 10 (figure 6.3 b), the 'plastic' responses of the palisade cells in the lower leaves are permanent and may therefore be associated with a change between transpiration losses and photosynthesis so as to reduce the former, despite concommitant decreases in the latter. This change in balance which may be effected in individual leaves is more marked on a whole plant basis. The large decline in leaf area that results from water stress appears to be primarily responsible for the observed total reduction in transpiration thus suggesting that the reduction in leaf area could be an adaptive response.

A similar kind of adaptation may also be involved in the ontogenetic decrease in palisade cell area in the well-watered plants. As water stress can be assumed to develop towards the top of a large plant, the presumed genotypic decrease towards a minimum in the palisade cell area of upper leaves, could have evolved in response to these stresses in the sunflower. The 'plasticity' of the palisade cell area in the lower leaves may then be related to the assumed absence of severe water stress in small plants at early stages of growth, but giving the lower leaves the potential to respond by decreasing palisade cell area. At one extreme it is possible perhaps that stress, if it is applied in a plant continually from germination, could accelerate the ontogenetic decrease in palisade cell area so much that there would be very little difference in the palisade cell area between the lower leaves and the upper leaves. Such a situation may be approached in a sunflower plant grown under dry non-irrigated field conditions during summer in areas of New South Wales, Australia.

However, there are alternative explanations for the ontogenetic decrease in cell size along with other increasingly xeromorphic characters such as smaller leaf areas (Zalenski 1904; Alexandrov, Alexandrov and Timofeev 1921; Maximov 1929; Ashby 1950). These references provide a
useful basis to examine the effects of water stress on cell sizes and leaf area but it is surprising that the approach used in these early investigations has not been followed up in the numerous recent investigations of water stress on plant behavior. Two views set out by Maximov (1929) state that some xeromorphic characters, especially those depending on cell size in leaves can be induced by subjecting plants to water shortage and that the normal structure of individual leaves on one and the same shoot is a function of their distance from the root system. This second view follows the work of Zalenski (1904) who found on shoots of Nicotiana rustica, Dactylis glomerata and other plants a consistent decrease in the size of epidermal cells from the lower to the upper leaves, accompanied by other signs of increasing xeromorphy. Zalenski favoured the interpretation that the upper leaves may be deprived of water during their growth by the lower leaves. More evidence in this regard was provided by Alexandrov et al. (1921) who defoliated plants of Bryonia dioica and Ipomoea purpurea and by examination of remaining leaves showed that cell sizes in those leaves were no smaller than the lower leaves which had been defoliated. But not only in this early work were there no controls to compare with treated plants but the stomatal frequency was used as an indicator of cell size. Ashby (1948) re-examined this kind of relationship between cell size in leaves and level of insertion on a shoot. He found that while there were significant gradients in leaf area and in epidermal cell size and number from leaf to leaf up the shoot, only the leaf area and cell number were significantly affected by drought while cell size was not affected. By further observations of the response of cell sizes to various leaf removal treatments, Ashby concluded that the ontogenetic decrease in cell size was not the result of a competition for water but was related to the influence of immature leaves upon still younger leaves developing above them. While he concedes that it was a surprise not to find cell sizes responding to the experimental water stress, he suggests
that this may be an exclusive response of the species that was used. In a single recent later study, Terry, Waldron and Ulrich (1975) found cell volume to be unaffected by water stress in sugar beet leaves although large reductions in cell number occurred. In this study, leaf $\psi$ was not measured and since the root media $\psi$ was lowered by only 0.03 MPa, it is uncertain whether the changes in the leaf were due to water deficits or to the polyethylene glycol that was used to induce stress in their experiments.

The present results from the sunflower are not fully comparable with the conclusions arrived at by Ashby or by the workers before him, because their studies were on different plants and were confined to epidermal cells while the present study is concerned mainly with palisade cells. Furthermore, the method of inducing drought in Ashby's experiment is different from the present method, and as he provides no water deficit measurements, again the comparison cannot be done fully.

A comparison of the ontogenetic decrease in cell size in the sunflower and that in Ipomoea caerulea (Ashby and Wangermann 1950) may however be made. They comment that the smaller cell sizes with increasing leaf ontogenetic position in Ipomoea caerulea was a result of an increasing duration of cell division after unfolding. There is also the evidence of Sunderland (1960) that in sunflowers, the duration of division in the 10th leaf continues up to 90% $A_{\text{max}}$ while in the second leaf cell division continues to only 50% $A_{\text{max}}$. The present experiments have indicated that the higher leaves spend more time in a folded state before unfolding (Chapter 3) and as this will increase the relative population of cells in these leaves at the time of unfolding, the maximum number of cells in a higher positioned leaf would be formed in about the same percentage leaf area as the cell population in a lower leaf. An examination of leaves 2, 4 and 6 (Chapter 3) also showed little differences in the duration of division in the palisade cell layer or the $A_{\text{max}}$ at which
division ceased, although the palisade cell area in leaf 6 at maturity was about half that of leaf 2 (Fig. 6.1). The differences between these results and that of Sunderland's (1960) have been fully considered in the discussion of Chapter 3. Thus the ontogenetic decrease in cell size of either palisade or epidermal cells in the sunflower may not be associated with an increasing duration of cell division in the upper leaves, as was found by Ashby and Wangermann (1950) in Ipomoea.

An important aspect of the reduction to the palisade cell area and consequently a decrease in the area of leaves is that a reduction to the 'source' will occur. This reduction would be significantly greater if decreases to the higher ranking leaves (e.g. 10 to 22) occur. It is apparent that a reduction to the 'source' can occur at any stage of vegetative development provided the water stress is sufficiently severe. The reduction becomes much more severe with late water stresses which either affect the growth of potentially large leaf sources (e.g. leaves 10 to 22) or by accelerating the senescence of already fully developed leaf sources. While the reduced leaf area and the accelerated senescence will reduce transpiration, the decrease to the 'source' that is associated with it poses one question: What does a reduction of the 'source' do to the development of dependent leaf sinks?

This question has to be evaluated from the viewpoint of what happens during water stress and during subsequent recovery. The dependent leaves during water stress would be the folded and the newly unfolded leaves because they would not be completely independent in providing photosynthate for their growth needs, if it is assumed that sunflower leaves import assimilate until they are 20% $A_{max}$ as was found in some plants (Milthorpe and Moorby 1974). The reduction to the growth of the leaf 'sources' at that time (e.g. the rapidly expanding and fully expanded leaves) would presumably curtail the supply of assimilate to these dependent leaves. As the unfolding leaves at that time eventually become
markedly reduced in leaf area and dry weight and these responses are associated with cell number and size changes in them, it becomes important to know if the changes were directly afflicted by water stress or by the reduction of assimilate supply from the reduced 'source'. The evidence in this thesis does not permit such a precise separation of the mechanisms. However, Wardlaw (1969) showed that in the case of darnel (Lolium temulentum) the 'sink' for assimilates (the expanding leaf) was more sensitive to water stress than photosynthesis of the 'source' leaf and the inhibition of leaf growth was considered to be the major factor reducing translocation. Also, the movement of assimilate from the stems to roots and buds in desiccated, dormant Phalaris tuberosa (McWilliam 1968) suggests that the transport system is highly resistant to dessication. Although water stress displaced the growth events of folded leaves, the resultant reduced 'source' either from an early or late water stress did not prevent the folded leaves from achieving the size of comparable control leaves. While the exceptions to this generalized statement are considered in great detail in Chapters 4.[2]and 4.[3] the importance of the result is discussed here.

The non-effect of the reduced 'source' on the growth of folded leaves raises three possibilities. In the first place, the 'source' may be insufficiently reduced to affect the growth of the folded leaves; after water is supplied again, the rate of photosynthesis, as reflected by the re-establishment of stomatal conductance and transpiration (Chapter 4.[1]), is apparently re-established. Secondly, the leaves at the top of the shoot are potentially small 'sinks' and do not suffer in spite of a reduction to the source. The former possibility is perhaps true of an early water stress while the latter possibility is perhaps true of a late water stress in vegetative development. Alternative to these possibilities is the role of the cell number and size variables in the realization of leaf area. Folded leaves which experience water stress suffer a small
reduction in the cell number and size variables which either do not express themselves significantly in the final area responses or are compensated for the loss when water is supplied again. Following this interpretation, the unfolding leaves would experience the largest reduction to their cell number and sizes because the stage of unfolding coincides with a period of rapid cell division (Chapter 3) and cell division and enlargement are sensitive to water stress (Chapter 4). Although it is difficult to effectively separate the relative role of the possible reduced photosynthate supply during water stress from the direct effects of water stress, especially on unfolding leaves, the final leaf responses are closely related to the changes in palisade cell variables in the leaves.

6.3. Part 2: Relationship between leaf and achene responses

As the achenes do not have photosynthetic tissue, they depend on expanded leaves for photosynthate supply in the same way as folded leaves depend on expanded leaves. On this basis, the relationship between achene development and 'source' leaves is comparable with that between folded leaves and 'source' leaves previously considered in this discussion.

Final size is reduced if water stress is applied after the start of rapid expansion of leaf lamina (unfolding stages of a leaf) or before a critical stage in embryo development (at floret initiation or shortly after floret anthesis). These important differences in reductions in final leaf size and final achene size are most obvious with a late water stress occurring near the end of vegetative development but at the beginning of inflorescence development. Under these conditions, all achenes are consistently reduced in size whereas leaves that are folded at the time of the stress remain unaffected. Very late water stresses which are applied during the rapid growth stage of achenes do not reduce achene size, however. Thus, the trends in figure 6.4 a and b may be related to
Figure 6.4. a. shows the response of individual leaf sizes of leaves on plants that occurred as a result of an early water stress (experiment 11 - the total leaf area reduced by the stress is shown on the figure), of a late water stress (experiment 10 - total area reduction is also shown on the figure) and of a very late water stress (experiment 14, post anthesis water stress - area reduction also shown). Experiments 10 and 11 were conducted in controlled environment cabinets while experiment 14 was conducted in a glasshouse. The plants grown in the glasshouse were smaller than the controlled environment plants and also had fewer leaves; thus the responses shown for experiment 14 are only approximate in relation to the other two experiments and therefore is represented by dotted (-----) line.

Figure 6.4. b. shows the responses of the individual achenes to a late and a very late water stress (data of experiment 14).

NOTE: The responses in figures 6.4. a and b are expressed as a percentage of the maximum value of the component, as in the previous figures of this chapter. Also, the late water stress responses of leaves in figure 6.4. a were obtained in a different experiment from that of the achene responses in the late water stress in figure 6.4. b. This has been done because detailed leaf responses were not studied in the experiment where detailed achene responses were studied.
LEAF POSITION ON THE SHOOT

- leaf area reduced by early water stress (6th leaf unfolded at start of water stress)
- leaf area reduced by late water stress (16th leaf unfolded at start of water stress)
- leaf area reduced by very late water stress (all leaves expanded at start of water stress)

ACHENE POSITION ON THE CAPITULUM

- CONTROL
- LATE WATER STRESS
- VERY LATE WATER STRESS
differences in the timing of inception of rapid growth in leaves and achenes. Accordingly, developmental stages distinct from source size appear to be among the main variables determining final size in achenes as well as in leaves.

The contrasting responses of leaves and achenes are consistent with the separate findings of Wardlaw (1967, 1969) for wheat and darnel. In wheat, he found that no reduction in grain growth resulted from water stress imposed after anthesis when rapid growth was occurring whilst in darnel (Lolium temulentum), growth reductions of newly expanding leaves occurred as a result of water stress. The difference now appears equally valid, at least for the sunflower.

Although the leaf responses to water stress have been evaluated in terms of cell numbers and sizes, the practical difficulties of obtaining precise information on cells in very small embryos precludes any attempt to relate the achene reduction in terms of reduced cell numbers or sizes. However, the associated changes in final total achene weight and final leaf area for both early and late water stresses suggest that the relationship is not a linear one (see fig.6.5 and fig.6.6). The regression line calculated and shown in fig.6.6 is not significant and therefore suggests that any 'leaf source - achene sink' relationship is not clear-cut and may be influenced by other factors such as the developmental stage of the achenes at stress. An example of this is provided by the absence of an effect of water stress on the development of peripheral achenes in the very late water stress treatment (see fig.6.4 b). This is the critical evidence that suggests that developmental stages are involved as well as any source-sink relationship.

Apart from the compounding effects of achene development in the very late water stress on the source-sink relationship, the evidence for a 'source-limited' control of achene growth has to be weighed against the other available evidence in this thesis which seem to suggest that source effects do not have an over-riding role. Among these are the possible effects of
Figure 6.5. shows the response trends of total seed (achene) weight and total leaf area on the plant at maturity, to water stresses applied at various times before and after anthesis. The data is from experiment 14. Note that the smallest reduction to the total seed weight and the total leaf area occur for the water stress applied just before anthesis; also the trends of the total seed weight follow the trends of the total leaf area except in stresses that are applied several days after the anthesis of the first disc floret. The large reduction to leaf area of plants experiencing post-anthesis water stress was due to the accelerated senescence of the lower leaves while the reduction in area of plants experiencing early pre-anthesis water stresses is due to the effect of stress on the area of the large leaf sources eg. leaves 14 to 20. The data points are means of three replicates. The leaf area responses are contained in table 4(2).2, while the seed weight responses are contained in table 5.2.
Control leaf area

Control total seed weight

Anthesis begins

Final seed weight (g) at maturity vs. water stress applied on day

Final leaf area (cm²) at maturity
Figure 6.6. shows the relationship between the total achene (seed) weight and the total leaf area on the plant at maturity. The data has been plotted from the data points in figure 6.5. C = control, E = pre-anthesis water stresses. The number following E denotes the time of application of the stress before anthesis, eg. E₁ = stress at disc floret initiation. L = post-anthesis water stresses. The number following L denotes the time of application of water stress after the anthesis of the first disc floret, eg. L₁ = stress at 5 days after the anthesis of the first disc floret. The regression between the two parameters is given by the equation $Y = 5.62 + 0.0016X$ in which $Y$ = total achene weight and $X$ = total plant leaf area in the post anthesis period. The regression is however not significant.
the pericarp in controlling embryo growth and the importance of the
timing of events in achene development.

The direct relationship between the pericarp dry weight and the
embryo dry weight in the pre-anthesis water stress treatments suggests
that final embryo size may be dependent on the pericarp but since this
relationship does not hold for very late water stress treatments, the
embryo dry weight is not wholly controlled by pericarp size. Nonetheless,
the reduced pericarp size could impose a physical constraint on embryo
growth but volume measurements are needed to distinguish between this
possibility and the possibility that the pericarp responses themselves be
related to the reduction in the leaf 'source'.

A critical stage in embryo development after which water stress has
no effect implies that timing of water stresses and the developmental
event may be involved in the magnitude and type of response to water stress.
The timing of the main developmental stages in the sunflower are listed in
table 6.1 to allow a comparison to be made between leaf and achene
responses and the timing of water stress.

In contrast to a time interval or plastochron of nearly a day between
unfolding of successive leaves, only minutes separate the initiation of
disc florets in the whole inflorescence or in a spiral row. Whilst only
a few leaves become markedly reduced by water stress because the drying
cycles are short and the unfolding stage is sensitive to water stress, the
effects of a water stress during disc floret initiation can reduce the
disc floret population causing a 'sink-limited' situation as was the case
with treatment A (experiment 14) and also in the field experiment
(experiment 16). Furthermore, as there would presumably be only small
differences in the developmental stages of the disc floret, water stress
would affect all the florets more or less equally. However, marked
differences in the developmental pattern of disc florets take place at
stages after anthesis. For example, the anthesis between disc florets
in a spiral row takes 13 hours (table 6.1 f); as such, while the time
### TABLE 6.1

<table>
<thead>
<tr>
<th>PROCESS OR EVENT</th>
<th>Time interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) LEAF INITIATION</td>
<td>11.9 hours</td>
</tr>
<tr>
<td>(b) LEAF UNFOLDING</td>
<td>22.0 hours</td>
</tr>
<tr>
<td>(c) DISC FLORET INITIATION</td>
<td>4 minutes</td>
</tr>
<tr>
<td>(d) DISC FLORET ANTHESIS</td>
<td>14.4 minutes</td>
</tr>
<tr>
<td>(e) Initiation between disc florets in a spiral row</td>
<td>3 hours</td>
</tr>
<tr>
<td>(f) Anthesis between disc florets in a spiral row</td>
<td>13 hours</td>
</tr>
<tr>
<td>(g) Between leaf initiation and unfolding</td>
<td>11 days</td>
</tr>
<tr>
<td>(h) Between initiation of 1st disc floret &amp; its anthesis</td>
<td>28 days</td>
</tr>
<tr>
<td>(i) Between initiation of last disc floret &amp; its anthesis</td>
<td>38 days</td>
</tr>
</tbody>
</table>

interval between the initiation of the first and the last disc floret is about 3 days, at anthesis the interval between the anthesis of the first and the last disc floret is 13 hours. An additional 9 days had come into play between the initiation and anthesis of a flower. However, the flower would cause significant interference with the critical side before water stress. To achieve control levels after rowing, may be related to the high sucrose potential of seeds at that time, if it is assumed that embryos maintain a high sucrose potential because they are the storage points of assimilates at grain filling. This may allow the maintenance of a positive balance of water despite the dehydration that may be occurring in other parts of the seed. In senescence or vegetative tissue, Warrilow (1971) also noted that during water stress at grain filling in wheat, despite a reduction in water content of the leaves, stem and to a smaller extent in the root structure, there was little or no effect on the water content of the grain. Such an absence of an effect on the water content of the grain is also
interval between the initiation of the first and the last disc floret is about 3 days, at anthesis the interval between the anthesis of the first and the last disc floret is 12 days. As an additional 9 days had come into the developmental pattern of the first and last disc floret at anthesis, a water stress at this time would cause significant interactions between achene position and the water stress treatment. The effect of a reduced 'source' may be superimposed on the rapidly changing developmental sequences in the achene population. On the available evidence, it is not possible to precisely separate the respective roles of the reduced 'source' of pericarp reduction and of developmental effects in the expression of the achene responses.

As the embryos which had started exponential growth were unaffected by the reduced 'source', it is possible that a water stress applied later in grain filling would be without any effect on achene size whilst causing very large reductions to the leaf source by accelerating leaf senescence. While this is a worthwhile point to pursue in future, the ability of embryos which have attained the critical amount of growth to escape reduction in final weight is an interesting point.

The capacity of the embryos which had already attained the minimum critical size before water stress, to achieve control levels after rewatering, may be related to the high osmotic potential of embryos at that time, if it is supposed that embryos maintain a high osmotic potential because they are the storage points of assimilates at grain filling. This may allow the maintenance of a positive balance of water despite the dehydration that may be occurring in other parts of the seed, inflorescence or vegetative tissue. Wardlaw (1971) also noted that during water stress at grain filling in wheat, despite a reduction to water content of the leaves, stem and to a smaller extent in the ear structure, there was little or no effect on the water content of the grain. Such an absence of an effect on the water content of the grain is also
similar to an observation obtained during the period of rapid starch
deposition in the grain 15-20 days after anthesis (Wardlaw 1967). Similar
observations were made by other workers (Konovalov 1959; Aspinall 1965;
Kydrev 1969).

However, Wardlaw (1971) found an initial increase in grain size under
water stress conditions which was associated with a greater rate of cell
division in the endosperm; although the increase in weight per grain
resulting from water stress initially acted as a compensation for reduced
seed set, the advantage was lost during the latter stages of development
and grain growth ceased prematurely in the stressed plants. In the
present study, an increase was not observed even in achenes that had reached
anthesis 10 days before the onset of water stress. In achenes which had
reached anthesis closer to the day of stress, an initial decrease rather
than an increase was observed. The initial decrease for some achenes was
just a displacement of events, in the same way that leaves were displaced.
As the sunflower is a dicotyledonous plant whose achenes have a rather
large embryo and negligible endosperm in comparison to the small embryo
and large endosperm of monocotyledonous seeds like in the wheat, the
contrasting responses of an initial increase and decrease in embryo
weight cannot be realistically compared.

The apparent 'source-limited' responses observed in the sunflower are
rather similar to that in wheat (Fischer and Kohn 1966) in which decreases
in yield were related to the accelerated senescence of leaves which was
interpreted by them to be a yield reduction caused by a limitation to
the 'source'. However, there are several lines of evidence which indicate
that the contribution of assimilates from other plant sources, besides
current photosynthate production may influence yield.

Yoshida (1972) cited several studies in rice where 40% of the grain
yield was translocated from the stem. Gallagher et al. (1975) showed that
up to 70% of the final grain yield in barley was translocated from the
stem under extreme conditions. Furthermore, experiments conducted by Bremnar (1973) involving shading and defoliation of wheat plants indicated that in that wheat variety, the supply of assimilates exceeded the normal requirement of the plant. Similarly, Wardlaw (1971) found that although water stress accelerated senescence and reduced photosynthesis in wheat, the supply of assimilates from the uppermost parts of the plant were always in excess of grain requirements. Even in the sunflower, McWilliam et al. (1974) showed that the leaves in the upper part of the stem contributed the major part of the achene requirements, a conclusion also arrived at by Vasilevskaya and Ermolaeva (1970) working with potted plants of sunflower. Accordingly, the reduced area of the lower leaves or the accelerated senescence of the lower leaves caused by water stress may not be of prime importance in the achene responses, especially at stages of grain filling. Furthermore, the various suggestions of stem reserves contributing towards grain requirements is consistent with an earlier speculation that a water stress applied late in grain filling will be without effect on achene growth whilst reducing leaf area through accelerated senescence. Talha and Osman (1975) also found very little effect of a late water stress during grain filling but because they did not provide leaf area data, it is not possible to evaluate the role of the leaf 'source' in their experiment. However the carbon economy of this plant under water stress conditions needs further elucidation to evaluate the role of the 'source' in the achene responses.

The findings of Bingham (1969) and Gifford (1970) which suggest that limitations to grain yield in graminaceous crops may be due concurrently to both assimilate supply (source limitation) and to factors within the grain itself (sink limitation) and also the finding of a role for involucre bracts in achene growth in sunflower (Dhopte and Upadhyay 1974) will also need to be considered in any study of the carbon economy of the sunflower plant.
6.4. Part 3: Relevance of the main results to a field situation

Since the sunflower is an important summer crop in Eastern Australia where rainfall is unpredictable, internal plant water stress can occur frequently in non-irrigated crops. Not only does a moderately severe water stress occurring during the early stages of inflorescence development reduce the total number of achenes but also the size of individual achenes is reduced. Lovett et al. (1976) also found that moisture stress during the reproductive phase leads to a reduction in head size, in number of achenes and achene weight. Water stress at different stages may have different effects but irrespective of whether achenes are affected directly (very late water stress) or indirectly (reduced leaf area), total yield is reduced. Thus, some of the main results obtained in the present study may be related to those variables that may be important under field conditions. Extreme caution is needed in this approach, however, since there are many differences in the nature and the magnitude of the water stresses between those that can be imposed in controlled environments and those that occur in a field situation.

Apart from the contrast between the commencement of closure of both adaxial and abaxial stomata at -1.1 MPa in the present experiments and around -2.0 MPa in the field (Turner and Begg 1974) which suggests photosynthesis continues at a much lower water potential in field grown plants than in phytotron grown plants, there is equally good evidence that reduced water potential stops leaf expansion in sunflower and maize plants at -0.4 MPa in controlled environment (Boyer 1970) but at -0.8 or -1.0 MPa in the field (Watts 1974). There are many examples of this kind where field grown plants can withstand water stress conditions better than phytotron grown plants. For this reason, the particular values of leaf water potential at which stomata close in growth cabinet studies cannot be directly applied in the field. In an extreme case, cotton plants grown in
a growth room had a critical leaf water potential of -1.6 MPa whereas similar plants in the field had open stomata at a leaf water potential of -27 bars (Jordan and Ritchie 1971). Similarly, onion stomata closed at a leaf water potential of -0.5 MPa in a growth chamber but were open at -0.7 MPa in the field (Millar, Gardner and Goltz 1971). Comparisons between maize (Boyer 1970; Sánchez-Diaz and Kramer 1971; Turner 1974) and sorghum (Sánchez-Diaz and Kramer 1971; Turner 1974) have also yielded similar results, but in these cases differences in cultivars may have also confounded the comparisons. The possible reasons for these differences between plants grown in the field and ones grown in a growth cabinet can be suggested. Firstly, plants in the field are subject to higher irradiances relative to those in a growth cabinet and secondly, they have an unrestricted soil volume into which the roots may grow. Both these factors can permit the osmotic potential of the leaf to adjust to values lower than those in leaves of potted plants in the growth room as the soil water potential decreases (Kreeb 1963; Turner and Begg 1973) thus leading to different leaf water potentials at the same turgor potential. Despite this superiority of field grown plants, both high net radiation and low atmospheric humidities in the summer create evaporative demands in the field which appear sufficiently high to subject the plants to water stress and thus cause reductions to leaf area and inflorescence growth. The rainless periods of the summer in Eastern Australia can evoke internal plant water stresses up to -3.0 MPa (Turner and Begg - unpublished data) and even in summers of less serious drought, large decreases to leaf area and achene yield of non-irrigated sunflower have been observed in field trials at Ginninderra (Canberra - CSIRO field station) (Turner and Begg - unpublished data). Furthermore, Downes and Davidson (1976) also concluded from work with the sunflower cv. Peredovik that it was not suited for cropping, in the Eastern plains of Australia because of the
The leaf responses of plants subjected to water stress in the cabinets (low night temperature) was essentially the same as those observed for plants which were stressed in the glasshouse (warm night temperature). The first 10 leaves on cabinet grown plants attained maximum area by the 32nd day of sowing while the change to low night temperature regimes was introduced on day 33. These two lines of evidence suggest that the area responses of the first 10 leaves to water stress and their subsequent recovery were completed before the plants were exposed to low night temperature. The significant effects of water stress on leaf area and dry weight of leaves between positions 1 and 10 (see results of experiments 8,9 and 12) were evident before day 32 and did not change appreciably by day 50. Thus, the low night temperature during the latter stages of the plant growth does not influence the sensitivity of the lower leaves to water stress.

There is no evidence on the effects of a water stress applied to leaves during the exposure to low night temperature. There is also no evidence either way to indicate whether the findings on leaf area responses have general applicability. There is however firm evidence for cypsela size that the responses to water stress under cabinet and glasshouse conditions are similar to those observed under field conditions. This is shown by experiment 16, Turner and Begg (unpublished data), Downes and Davidson 1976. Thus, at least in part, the results in this thesis may help explain some of the environmentally induced variation in crop yield of Hysun 30.
moisture stress that occurs. Thus, while the magnitude of the response to water stress between a controlled environment trial and a field trial may be different, the responses observed in a controlled environment trial can be reproduced in the field.

Modern sunflower cultivars form a single terminal inflorescence and have no effective compensation mechanisms for loss of yield either from an early or late water stress. This is in contrast to compensation mechanisms observed in sorghum and wheat. In sorghum, when the number of panicles was reduced by water stress, the grain weight per panicle could be increased either by an increase in the number of grains per panicle (Blum 1973) or by larger grains (Bagga et al. 1973). In wheat, Turner (1966) found compensation for the reduction in grain number through larger and heavier grains. The potential for compensation by increasing grain size or weight is however limited and this is indicated by the studies of Fischer (1973) and Gallagher et al. (1975) who provide evidence that a genotype has a maximum grain size. Comparable data to the present experiments for the sunflower is provided by Talha and Osman (1975) who showed that effective compensation in the inflorescence to overcome water stress effects does not occur in the sunflower. Marc and Palmer (1976) showed a decrease in seed number for water stressed sunflower but as they did not provide any information on sizes and weights, their data and mine are not fully comparable.

The single most important feature of the sunflower is that all the disc florets within the inflorescence are initiated in about 4 days. As the early development of the inflorescence disc takes place prior to the initiation of disc florets, the early size of the disc may determine the number of disc florets that can be laid down. Evidence for the role of disc size in the control of disc floret number is provided in part by the early water stress treatment in experiment 14, the smaller disc sizes in
the field experiment (experiment 16) and by the effect of water stress on early disc size in experiment 6. As the development of the disc and the initiation of the disc florets take place over a period of 10 days, this can either be a liability or an asset to the plant. If internal plant water stress is prevented during the 10 days, the potential 'sink' size will not be affected. On the other hand, the incurrence of stress during that period will reduce the potential size of the sink which cannot be reversed by the provision of suitable growth conditions later on.

As the critical period of disc and disc floret formation begins around the 22nd day from sowing, water stresses that occur before this day will not have direct effects on their formation. Even the indirect effects through reduced leaf area may only be small because vegetative development is slow up to day 22 and reductions to leaf area may only be small. Furthermore, as sowing is timed to coincide with rain, the amount of water may be sufficient to maintain proper plant growth in the early vegetative stages. The plant water stresses that occur at early vegetative stages are small because the transpiring surface of the plants is small. As exponential growth of the shoot begins during the critical period of disc and disc floret initiation, the water stresses that can occur is high because the transpiring surface is quite large at this time. Evidence for this difference in stress development to occur is shown by the contrasting patterns of internal plant water stress development between experiment 8 and experiment 10. A similar observation was made by Fischer (1973) who stressed wheat at various stages of development. The ability of small plants to minimise the development of very large internal plant water stress may be due to their capacity to adjust osmotically. As larger plants are capable of developing high stresses, especially at the critical stage of disc floret initiation, the only way to avoid this would be the provision of water.
In a field study with sunflowers (Talha and Osman 1975), plants that were deprived of irrigation water at the 'slow elongation stages' of the stem eventually had only half the number of seeds present in the control plants. Although their description 'slow elongation stage' is empirical, the present results suggest that the 'slow elongation stage' coincides with the initiation stage of disc florets. While adequate water supply at these early stages of plant growth will ensure the full achene complement, the achenes can only attain their maximum size, if water is available at the later stages of achene development as well.

Indeed, the flowering stage (beginning with the anthesis of the first disc floret) is sensitive to water stress and results in yield reduction. A similar conclusion was arrived at by Talha and Osman (1975) in sunflowers. Furthermore, the anthesis period is particularly sensitive to environmental stress, especially moisture stress in many crops (Bielorai et al. 1964; Ikonnikov 1972; Hiler et al. 1975). In other studies, Henckel (1964) and Bingham (1966) have shown that anther development and meiosis are particularly sensitive to water stress. However, this has not apparently been the case in the present studies; although anthesis was delayed slightly, there were no drastic effects on anther development or meiosis because fertilization was not affected by water stress.

As pronounced effects on the development of the embryo occur if water stress is imposed soon after the anthesis of the disc floret, the spread of anthesis in a field crop of sunflower becomes an important variable. The exact nature of the relationship between water stress and the embryo development under field conditions is complicated by the spread of anthesis in an individual capitulum from its periphery to its centre, and in a crop by the spread from the anthesis of the first disc floret in the first capitulum to the anthesis of the last disc floret in the last capitulum. Anderson (1975) has recognized that some demarcation of the
spread of anthesis is required before the effects of environmental stresses on achene development can be accurately studied and has actually suggested an estimate of the mid-point of anthesis in a capitulum or crop. The present study has planned the timing of water stresses to coincide with specific stages during the anthesis of disc florets in the individual capitula. However, some estimate of the mid-point of anthesis, along the lines suggested by Anderson (1975) will be necessary for a similar appraisal of water stress effects in a field crop of sunflower.

In Australia, there is an increasing trend towards the sowing of commercial hybrid sunflower cultivars. While these cultivars have a shorter flowering span and consequently a shorter spread of anthesis in the crop, the commercial open-pollinated cultivars have variable flowering periods which result in a longer spread of anthesis. As the development of the embryo shortly after anthesis is sensitive to water stress, and the attainment of a critical embryo size enables the embryo to tolerate stress, the yield response to a short term water stress at flowering in a crop of an open-pollinated sunflower cultivar may be different from that of a hybrid cultivar such as Hysun 30. If the two crop types are grown under non-irrigated conditions, as is half the sunflower crop in Australia (state reports in the 'Sunflower' - official publication of the Australian Sunflower Association), water stress that coincides with flowering will cause large yield reductions in the hybrid crop. If water stress can be avoided at flowering, by timely rain, the hybrid crop may yield its full potential. On the other hand, the open-pollinated crop minimises the risk of large yield losses at flowering because of the variation in flowering, but will be unable to take full advantage of a short rain. As the Australian summer, especially in New South Wales, is characterized by intermittent spells of drought, the decisions to grow an open-pollinated or hybrid cultivar under non-irrigated conditions will need to consider the relative risks involved.
However, if irrigation is provided, the choice of growing either an open-pollinated or hybrid crop will depend on the amount of irrigation water available. With a limited amount of irrigation water, the attainment of maximum yields from a hybrid crop can be assured because water can be supplied at critical stages of growth. As the timing of critical stages in individual plants of an open-pollinated crop is spread out, the application of irrigation will be less effective than it would be in the hybrid crop that has less variation. This can result in a wasteful use of irrigation water by the open-pollinated cultivar. As water is becoming a scarce commodity because of the demands imposed by both agriculture and industry, there is a need to conserve water. The conservation of water in agriculture involves a reconsideration of irrigation practices and the development of cultivars that are efficient users of water. This would be particularly true for growing sunflowers in Australia, where water management may be the key to success in sunflower growing (McWilliam 1977). Based on this, there is the possibility that the cultivation of hybrid cultivars of sunflower, while having attendant risks under non-irrigated conditions, may be superior to open-pollinated varieties in situations where irrigation is provided. Such superiority is over and above the genetic potential of hybrid cultivars to outyield open-pollinated cultivars and also to resist rust better. Currently, U.S. hybrids in State trials have out-yielded Peredovik by 23%, added resistance to rust (verticillium wilt) and have maintained a similar oil content (Johnson 1977). This calls for larger and more rigorous trials in the field using hybrid and open-pollinated cultivars, under both non-irrigated as well as irrigated cultivation systems.

This study of the responses of the various plant parts and their pattern of development during water stress and recovery has allowed a specific appraisal of the response in a representative dicotyledonous plant.
While this appraisal under controlled environment can now be profitably used in further field experimentation, a similar appraisal of the responses to water stress may be conducted on other dicotyledonous plants. The presence of a critical embryo stage in the sunflower, above which short term water stress has no effect, and the extreme sensitivity of unfolding leaves become particularly relevant in the study of water stress effects on determinate and non-determinate flowering species. Although there is a need to examine the effects of various levels and durations of water stress, an initial systematic examination of dicotyledonous plant species along the lines adopted for the sunflower, would be appropriate.
APPENDIX A

A.1. Experiment 6

The source of the soil, its preparation, the level of nutrient and water, temperature and daylength regimes followed treatment A of experiment 2 (see Chapter 3), with a step wise change in temperature on day 32.

To obtain data on the early growth of this cultivar, 3 plants were raised in each of 20 pots. These were equally spaced around the pot. Harvests were made on alternate days from the 4th day after sowing. The harvests up to day 22 involved the sampling of 4 plants each time. The remaining plants after this day (1 in each pot) were randomly divided into 2 groups. The first group received frequent watering to the field capacity of the soil, while the second group was subjected to a drying cycle beginning on day 22. The commencement of the drying cycle coincided with the completion of leaf initiation and at a time when the apex was in transition from a vegetative to a floral one.

Harvests during the drying cycle were made at 2 day intervals. 2 control and 2 stressed plants were sampled at each harvest. The stressed plants were rewatered 4 days after the commencement of the drying cycle. Three more harvests, at 2 day intervals, were made after rewatering. Plant analysis at each harvest consisted of measuring the length, fresh and dry weights of petioles, laminae and the stem. Lamina area was also recorded. Structures at the apex were left for description by microscopy. Three dimensional models of a control inflorescence and associated structures and of a stressed inflorescence were constructed from sections of the apices for the last harvest of the drying cycle.
A.2. Experiment 7

The plants were grown in 6 inch pots, each pot containing 1200 g of oven dry soil. The daily minimum temperature of the glasshouse was set at 25°C and the maximum was in the vicinity of 30°C. A control and a water stress treatment, each replicated five times were included. The drying cycle began when the 7th leaf was unfolding from the bud. Harvests were made at the start of the drying cycle and then on the 2nd, 4th and 5th days from the start of drying. Plants that were to recover from the stress were rewatered on the 5th day from the start of drying. Three more harvests, on the 2nd, 7th and 12th day from rewatering were made. The leaf water saturation deficit was measured at the end of the drying cycle.

A.3. Experiment 8

This experiment was conducted in a controlled environment cabinet. The plant culture conditions followed that of treatment A in experiment 2, with a step-wise change in temperature on day 32. There were 2 water stress treatments besides the control lot of plants that were frequently watered. The first water stress treatment consisted of a drying cycle which began when the 7th leaf of the allocated batch of plants was unfolding. The second water stress treatment began when the 9th leaf was unfolding in the allocated plants. Each treatment was replicated twice.

Harvests were made daily during the drying cycle at 1000 hours approximately. The decline in soil water content, the resistance of the stomata (both abaxial and adaxial ones) and the leaf water potential were determined within minutes of each other. All measurements of $\psi$, $R_g$ and $\pi$ were made on the same leaf, the $\pi$ being measured on stored leaf material. Events at the apex were also followed by dissection.

Further harvests were made after rewatering the plants. Non-destructive measurements of leaf area (based on the product of the length and the breadth of the leaf) were made at certain points during the recovery phase. The final harvest was made when the 1st disc floret in the capitulum had reached anthesis.
A.4. Experiment 9

The plants in this experiment were initially grown in 1200 g of oven dry soil that was contained in 6 inch pots, so that the drying cycle would be quicker than in 2200 g of soil. Appropriate amounts of nutrient and water for 1200 g of soil was added. As in experiment 8, there were 2 drying cycles which were started off at the same unfolded leaf numbers (i.e. leaf 6 and leaf 9 respectively). Each treatment was replicated twice.

Harvests were made daily during the drying cycle. All measurements of water potential, stomatal resistance, osmotic potential followed experiment 8. In addition, the mitotic index of leaves that were in expansion as well as those which were in an unfolded state was determined during the drying cycle and also during recovery. Plants were repotted into 2200 g of soil with appropriate amounts of nutrient and water, at the end of the drying cycle.

At the final harvest of the recovery phase, leaf discs were punched from alternate leaves up to leaf position 20. These discs were fixed in formalin-acetic acid and alcohol (90:5:5 mixture). Estimates of palisade cell number, palisade cross-sectional diameter were made on these discs.

A.5. Experiment 10

To assess the long-term recovery responses of a plant that had already initiated all the leaves (the short term responses were studied in experiment 6) experiment 10 was done. The plant culture conditions followed Treatment A of experiment 2 with a step wise change in temperature on day 32. Besides a set of control plants which was watered frequently to field capacity, a set of plants was taken through a drying cycle when the 16th leaf was unfolding in them. Each treatment was replicated twice. This coincided with the transition of the vegetative apex to a floral one. Harvests were made daily during the drying cycle. 2 more harvests were made at intervals after rewatering with a final harvest at the anthesis of the first disc floret.
Measurements of internal water stress were made on leaves 2, 6 and 12 during the drying cycle and during recovery. Plant analysis and measurement of stress parameters followed previous experiments. Cell numbers and sizes of palisade cells were estimated from leaf discs.

A.6. Experiment 11

The plant culture conditions followed treatment A of experiment 2 with a step wise change in temperature on day 32. Besides a control lot of plants, one lot of plants were taken through a drying cycle when the 7th leaf was unfolding in them. Harvests were made daily during the drying cycle; the treatments were unreplicated during the drying cycle. The final harvest during recovery was, however, replicated twice.

Each leaf was measured for its area. A leaf disc was punched from each leaf and stored in FAA fixative. From the same leaf, an epidermal strip was taken. This was done at each harvest during the drying cycle and during recovery. Palisade cell number and size were estimated from the leaf discs while epidermal and stomatal cell numbers and sizes were estimated from the epidermal strips. The plants were rewatered 6 days after the commencement of the drying cycle. Harvests were made 2, 7, 12, 17, 22 and 30 days after rewatering. Leaf water potential was measured at 1000 hours at the end of the drying cycle.

A.7. Experiment 12

To assess the effects of a reduced primary source (eg. laminae 1 to 4) on the growth of dependent sinks (eg. leaves that were folded), experiment 12 was done. Plants were raised in 280 g oven dry weight soil containing appropriate amount of nutrient and water. Besides a control lot of plants, two drying treatments were imposed. One lot of plants was taken through a drying cycle when the 3rd leaf was unfolding while the second lot of plants were taken through the drying cycle when the 5th leaf was unfolding.
During the drying cycle, harvests were made daily, except on the day immediately after the commencement of the drying cycle. The treatments were replicated twice. After rewatering, all plants were repotted into 7 inch pots containing appropriate amounts of soil, water and nutrient. A destructive harvest was made after rewatering, followed by two other non-destructive harvests. A final destructive harvest was made 22 days from rewatering.

Leaf discs were punched from alternate laminae from plants of the final harvest. Palisade cell number and cross-sectional diameter were determined. Analyses of plants at all harvests and the study of apical responses followed methods that were used in the previous leaf experiments.
B.1. Source of seed and cultivar.

Sunflower seeds cv. Hysun 30 were obtained from the Pacific seed Company, Toowoomba, Queensland. They were stored in a cool room (10°C) in well aerated cloth bags.

B.2. Source of soil and its characteristics.

Soil was scraped from the top 6 inches with the use of a grader on an open fallow paddock at Ginninderra Experiment Station, CSIRO, Canberra. It was a Nelango loam (Association E, Sleeman 1972). For the experiment with 6 species (Chapter 2), the soil was sieved through a fine mesh. The field capacity of the sieved soil (as determined by the sticky point determination) was 32% oven dry weight of soil. The permanent wilting point, determined by the sunflower test, was 11% oven dry weight soil. For all the other experiments, the soil was not sieved and the field capacity and permanent wilting point were 43% and 14% oven dry weight respectively. Other than in the experiment of Chapter 2 and in experiment 7, the soil was steam-sterilized and later fumigated with methyl bromide. This was a basic requirement for the use of soil within the controlled environment of the CERES phytotron in Canberra. After fumigation, the soil was spread out for 2 days and turned over regularly to ensure that residual methyl bromide would be eliminated. The soil used in all these experiments was from the one initial collection; this was placed in the open and covered well with plastic sheeting to prevent rain from soaking the soil.
B 3. Source of nutrient

Nutrient was always added into the soil in one initial application only. Nitrogen, Potassium, Calcium and Phosphorus were obtained from appropriate salt compounds manufactured by 'Analar'.

DETAILS OF METHODS

B 4. Growth environments

Experiments 1, 7 and 14 were conducted in glasshouses. Experiments 1 and 7 were conducted in the same glasshouse. The night temperature was controlled in experiment 7. Experiment 14 was conducted in a glasshouse under controlled temperature in the CERES phytotron.

Experiment 16 was a superimposed trial on a field experiment (Begg and Turner) planted in November 1976 on an experimental site at Ginninderra Experimental Station, CSIRO, Canberra.

All other experiments were conducted in LB cabinets (Morse and Evans 1962) under controlled light and temperature in the CERES phytotron.

B 5. Plant culture

Pots were lined with plastic liners. Appropriate amount of soil (usually 2200 g oven dry weight soil) was weighed out and calcium carbonate and calcium dihydrogen phosphate were added as powders and mixed thoroughly into the soil. The amount was calculated from field application rates (personal communication, C.T. Gates) and converted to equivalent rates for the amount of oven dry soil that was used. The mixture of soil and nutrient was put into the plastic lined pot and appropriate amounts of Nitrogen and Potassium were pipetted on in the
form of solutions. The seeds (about 10) were sown under a very light
cover of soil. The soil was then covered with a 1/2" thick layer of
coarse gravel. The soil was brought up to field capacity by pouring
distilled water down an inverted glass funnel and the final amounts of
water were added after placing the pot on a balance slowly. All the
pots in controlled and glasshouse experiments were randomized as often
as possible to reduce positional variations. Seedlings were thinned
regularly until a representative, healthy seedling remained in each pot.

B 6. Source of light and its control

In experiment 1, 7 and 16, the source of light was natural daylight.
For experiment 1, the glasshouse was whitewashed to reduce the light
intensity. In experiment 14, natural daylight was supplemented with
2 hours of light from incandescent lamps. Experiments that were
conducted in LB cabinets received 650 microeinsteins cm$^{-2}$ of light
during the day which was provided by a bank of 28 fluorescent lamps
and 4 incandescent bulbs. The decay of lamps, variations between
cabinets, and differences in the reflection off walls were the main
factors that caused difficulty in maintaining a constant light intensity
on plants in the different experiments.

B 7. Definitions and standards

The leaves were categorized as primordial (N$_p$), folded (N$_f$),
unfolded (N$_{uf}$) and expanded (N$_{exp}$) leaves, following the method of
Paton (1969) for distinguishing between foliage and total leaves.
(a) Primordial and folded leaves refer to those leaves which are part of the bud. A very strict distinction between primordial and folded leaves cannot be made but references to primordial leaves in the text are generally concerned with those leaves within about 8 plastochrons from the apex. An unfolded leaf is one that has half its lamina detached from the bud. An expanded leaf is one that has reached its maximum expanded state (see figure B.1.)

(b) References to disc florets include the stages of development of the floret from initiation to anthesis. References to developing seeds or achenes include stages of development of the floret from anthesis to maturity.

(c) The terms fruit wall and pericarp are terms which describe a similar component in the fruit only that the fruit wall refers to the structure in a disc floret before anthesis and the pericarp refers to the same structure in a mature seed (achene). The embryo in the present context refers to the embryo and cotyledon.

(d) Inflorescence initiation was considered to have occurred when the first sign of a transition from a flat apex to a rounded, raised one was observed. This coincided with the rapid initiation of involucre bracts which was quite in contrast to the slower initiation of leaf primordia (see below).
Figure B.1. shows a diagramatic sunflower plant for the purpose of distinguishing between folded, unfolded, expanding and expanded leaves. Leaves 5 and 6 have been "cut away" from the plant. Note that leaves 1 and 2 become fully expanded when the 15th leaf is unfolding from the bud. The cotyledons are not regarded as leaves. The plant is 20 days old.
APICAL BUD folded leaves

LEAF 15 unfolding

LEAF 14 unfolded

LEAF 11 expanding

LEAF 10

LEAF 3

LEAF 4

LEAF 1 expanded

COTYLEDON
B 8. Pollination of inflorescences

To ensure good seed set, the pollen from disc florets in anthesis was collected once daily in the morning in a petri-dish. The pollen was then re-distributed to receptive stigma using a soft paint brush.

B 9. Sampling the inflorescence for achenes

Achene responses to water stress were assessed in relation to their position on the capitulum. There are 2 types of spiral rows which transect the capitulum from the periphery to the centre. The type which transects the capitulum much more quickly was chosen for this purpose. In some capitula, this spiral was from right to left and in others it was from left to right. Achenes were numbered according to their position on the spiral row. In one experiment, the spiral row was followed on to its corresponding spiral row on the other half of the capitulum. More often, spiral rows on only one half of the capitulum was sampled. If the capitulum was small (example expt. 14) and non-destructive analysis of the inflorescence was required only 2 spiral rows on one half of the capitulum was removed by the use of forceps, at each harvest. The spiral rows for successive harvests were done at different parts of the capitulum, separated by several spirals from the first sampling position. Identical positions on the individual spiral rows of a harvest were pooled.

B 10. Decision to base comparisons between leaves in a plant and comparisons between plants on their physiological age rather than on chronological age

Because leaf primordia were initiated at plastochron intervals of 11 hours and unfolded from the bud at plastochron intervals of 22 hours,
comparisons between leaves at the end of a water-stress cycle were not free of differences in chronological and physiological age. Leaves that were in expansion at this time were chronologically as well as physiologically more advanced than unfolding, folded or primordial leaves. Therefore comparisons between responses of leaves on the plant to water stress had to take account of these factors. Much more important was the fact that comparisons between leaves at the time of final recovery were also not free of physiological and chronological age differences. Comparisons between leaves of identical chronological ages could have been conducted but were avoided for good reasons. Such comparisons would have involved taking measurements on a prescribed day from the day of initiation of the leaf. The measurement would have had to be limited to a non-destructive one because removal of the leaf from the plant for destructive measurement would have caused other disturbances which would then become difficult to interpret. There would also be the danger of comparing leaves which were at different expansion stages. Chronological age was thus regarded as a difficult variable to account for but because it was presumed not to markedly upset comparisons, it was eventually not considered. The comparisons were then made on the physiological age of the leaves. Each leaf was measured for lamina area and dry weight at $A_{\text{max}}$ and this was done for all the leaves at the time when the first disc floret on the capitulum anthesced. In most experiments, there were prominent signs of senescence in laminae 1 and 2 at this time but only weak signs, if at all, were observed in the other lower leaves.
Estimation of epidermal, stomatal and palisade cell numbers in leaves

Epidermal and stomatal cell numbers were estimated from epidermal strips. Epidermis of leaves were painted with a silicon rubber coagulant and left to dry for about 30 minutes. The leaf was excised and the leaf area was measured. A glass slide previously attached with a double face sticky tape was pressed on to the surface of the leaf that had the coat of coagulant. The leaf epidermis was thus transferred on to the slide. The cells were counted in a fixed field area and later to obtain absolute cell populations, was corrected for the area of the leaf. Corrections for space occupied by leaf hairs and veins were not made. The two guard cells surrounding the stomatal pore were counted together to give 1 stomatal cell (also generally referred to as the 'stoma' - Esau). Companion cells around the stomata were hard to differentiate from epidermal cells at early stages of growth and consequently were grouped into the epidermal cell category.

Palisade cell numbers were estimated from leaf discs that were punched off the leaf and later stored in FAA (a fixative containing 90 ml 50% alcohol, 5 ml 40% w/v Formaldehyde and 5 ml of acetic acid). The discs were placed on a glass slide and placed on the stage of a calibrated microscope. A strong light was focused through the epidermis and palisade cells were counted in a known field area. Corrections for leaf area were later done to get absolute populations. No correction for vascular tissue occupied spaces were made.
B 12. Estimation of palisade cross-sectional diameter, epidermal and stomatal cell areas

Ten palisade cells were selected at random in a field. The random selection was assured by choosing the palisade cell that fell closest to 0.50 on the linear scale of the micrometer eyepiece, after the stage of the microscope was moved. The diameter of the palisade cell was then measured accurately with the eyepiece.

Epidermal cell areas were calculated by dividing the field area by the number of the cells in the field. Stomatal cell sizes were measured in terms of length and breadth.

B 13. Estimation of inter-palisade cell space sizes

Areas in the mesophyll, free from vascular tissue interference were chosen for this estimation. The palisade cell number in that field was counted and this was multiplied by the average cross-sectional area of a single palisade cell (the cross-sectional area was calculated from diameter measurements in B 12). The inter-palisade cell space was obtained by subtracting the product of cell number in the field and average cell area from the actual area of the field.

B 14. Estimation of the mitotic index in laminae and the ovary wall

The apex and the attached leaves were fixed in FAA fixative for several minutes. Individual whole leaf primordia were then transferred on to a slide containing 1 drop of 1N HCl. In the case of larger laminae, small samples were used. The hydrolysis by the acid took
between 5 and 10 minutes and this duration depended very much on the
softness of the leaf. The acid was blotted away and one drop of 2%
lactopropionic orcein stain was introduced on to the tissue. The
staining took approximately 30 minutes. The excess of stain was
blotted. One drop of water mounting medium was placed on the tissue
and a cover slip placed over it. The tissue was squashed. This
procedure was also used for making squash preparations of ovary walls.

A statistical procedure was devised to score the squashes for
mitosis. Only cells at clearly visible prophases, metaphases and
telophases (Plates B1, B2, B3) were regarded as mitotic. The approx-
imate length and breadth of a squash were determined by the grid on the
microscope. A range of such length and breadth measurements for the
smallest to the largest squash was obtained. Using a set of random
numbers prepared on the basis of the measurements of length and breadth,
3 points on the squash were chosen at random by moving the stage as
stipulated by the random numbers. These points were referred to as
boxes. Within each box, 4 counts were made in adjacent areas. A
mitotic count consisted of counting the number of mitotic cells and
expressing the number as a percentage of the cell population in that
field. The boxes were meaned individually and then an average for the
three boxes was calculated. For fields containing a large population
of cells, total cell number was estimated by counting a sector in the
field and then correcting this for the whole field area. The mitotic
index for primordial leaves very near the apex was not always obtained.
This was because of the difficulties involved in transferring the
small primordia on to slides and working with them; frequently, they
were lost during the process.
Plate B.1. shows the nucleus of a cell in a sunflower leaf, at the first stage of mitosis - the prophase stage. Cells that did not show the chromosomes as clearly as can be seen in this plate, were not regarded as being mitotic.

Plate B.2. shows the chromosomes of a cell in a sunflower leaf at the equator - at the metaphase stage of the mitosis process.

Plate B.3. shows the chromosomes in a cell of a sunflower leaf in migration to the polar ends of the cell - the stage of prophase. Cells that were in stages of division more advanced than this were omitted from the mitotic count.
The dissection of plant apices was done under high powered dissection binoculars. Leaf primordia were easy to identify until the stage when the inflorescence was initiated. After inflorescence initiation, the bracts had begun to form and it was difficult to differentiate between the last formed leaf primordia and the first formed involucre bracts. Such differentiation was only possible when the plants were at a more mature stage.

For purposes of sectioning and staining, the shoot apex was removed from the plant and immediately fixed in FAA fixative (made up of 90 ml 50% alcohol, 5 ml acetic acid, 5 ml 40% formalin). The shoot apex was later observed under the dissection binoculars and length measurements on some of the attached leaves were made; these leaves were then trimmed away with a scalpel. The shoot apex then underwent a dehydration procedure which consisted of the following.

1. immersed in 50% alcohol for 2 to 4 hours
2. " 70% " " " " " " " "
3. " 85% " " " " " " " "
4. " 95% " " " " " " " "
5. "100% " " " " " " " "
6. immersed in tertiary butyl alcohol - 3 changes of 8 hrs. each.

After the final dehydration, the apices were kept immersed in liquid paraffin wax for 2 days in an oven maintained at 60°C. The final embedding was done by pouring the liquid paraffin wax and the apex into a paper boat. Rapid cooling of the wax was achieved by floating the boat on water. The wax was trimmed to leave the apex well aligned. The wax block was mounted on to the stage of a microtome, equipped with a diamond edged knife. 10µ thick sections were cut.
Between 20 to 40 of these sections were mounted in serial order on slides that had previously been coated with 2% gelatin. 1 ml of distilled water was placed on the slide before mounting the sections. These slides were placed on a slide warmer for quick fastening and stretching of the sections. Each slide was labelled and numbered accordingly. The slides were stained as follows:

- Xylene - 5 mins
- Xylene/Alcohol - 5 mins
- 100% Alcohol - 5 mins
- 95% Alcohol - 5 mins
- 70% Alcohol - 3 mins
- 50% Alcohol - 3 mins
- Distilled water - 10 dips
- Toluidine blue - 1½ mins
- 100% Alcohol - 13 dips
- 100% Ethanol - 3 dips
- Xylene - 5 mins

The sections were permanently mounted with MERCK gel.

B 16. Reconstructing 3-dimensional models of apical structures

Reconstruction of models of structures (the inflorescence and associated structures of a control and wilted apex at the end of the drying period) follows the method of Williams (1970).

B 17. Leaf area measurement

Leaf areas were measured on an automatic leaf area meter (Hayashi Denko). In some experiments, when there was a shortage of plants to allow destructive harvests, leaf area was calculated by
multiplying the length and breadth of a leaf and correcting the product with a factor of 0.71. This correction factor was previously determined from a regression analysis between actual leaf area and calculated leaf area.

\[ y = 0.64 + 0.71x \]

B 18. Dry weight measurements for leaves and achenes

The dry weight of leaves and achenes was obtained after forced air drying of samples in an oven at 80°C for 24 hours. The petioles were measured separately from the laminae, the pericarps separately from embryos.

B 19. Analysis of nitrogen and phosphorus

The replicates of each treatment within a harvest were bulked for analysis of nitrogen and phosphorus. The leaf samples were ground separately from the stem samples. Nitrogen and phosphorus
were determined by micro-Kjeldahl digestion using selenium as a catalyst and followed by colorimetric determination in the Technicon autoanalyser. Nitrogen and Phosphorus were determined only in experiment 1.

B 20. Measurement of transpiration

Transpiration of plants was monitored by weighing the pots regularly. The small daily increase in plant fresh weight was ignored in the calculation of transpiration. At early stages of growth, plant fresh weight increase was too small to affect transpiration recordings. At later stages of growth, especially after the initiation of the inflorescence, fresh weight increases were proportionately larger but the transpiration values were also very high and the small errors involved would not have seriously upset the transpiration values. Evaporation values were obtained from three pots without plants.

However, the fresh weight increase of plants was taken into account for the calculation of the total plant + soil + water + pot weight so that the soil would be always at field capacity in the controls. This was largely achieved by increasing the total weight by an amount equivalent to the increase in plant fresh weight.

Transpiration has been expressed as ml H₂O/day. The rate of transpiration in experiment 1 has been based on the amount transpired per unit dry weight of leaves. In other experiments, the rate has been based on unit area of leaf.
B 21. Measurement of stomatal resistance

In experiment 1, stomatal resistance was monitored with a ventilated diffusion porometer (Byrne et al'70) equipped with a self timer that recorded the response time of the needle between 2 to 6μA on the humidity scale. In all other experiments, where stomatal resistance was monitored, a ventilated diffusion porometer (Turner and Parlange) was used.

The measurements had to be done on a suitable leaf. An initial study demonstrated that the second or third youngest expanded leaf was suitable. The measuring site on the leaf was always between the leaf edge and mid-rib and half way between the tip and base of the leaf blade. The measurement of resistances for abaxial and adaxial stomata were made on adjacent sites of the leaf. In experiment 1, the resistance readings were converted to their reciprocal (conductance).

B 22. Measurement of water potential (ψ)

Water potential was always measured between 1000 hours and 1100 hours, about 2 hours after the lights had come on. Leaf water potential was measured by a pressure bomb (Turner 1971); the gas used was nitrogen. The measurement was always made on the same leaf on which stomatal resistance was monitored. The end point was determined by examining the exudation of sap from the cut end of the petiole. In experiment 10, the measurement was made on leaves 2, 6 and 12.
B 23. Measurement of osmotic potential ($\pi$)

The main veins of the leaf that was used for water potential measurement was stripped off and the remaining leaf tissue was frozen immediately in liquid nitrogen for 5 minutes and then stored in a deep freeze (-10°C). Osmotic potential on these leaf samples was determined after thawing them for 30 minutes and estimating $\pi$ in a vapour pressure osmometer. The osmometer was calibrated by standard NaCl osmolarity solutions. At regular intervals of 6 samples, the osmometer was recalibrated and if the sensor was dirty, it was cleaned with alcohol and then air-dried with a fine nozzle aspirator attached to a gas tank. Every batch of 6 samples was read off from the calibration curve made previous to the measurements.

B 24. Calculation of turgor potential ($P$)

Turgor potential was calculated from the difference between the water potential and the osmotic potential i.e.

$$P_L = \psi_L - \pi_L$$

B 25. Measurement of water saturation deficits

Leaf discs were punched from the leaf and quickly weighed to four decimal places. They were then floated on distilled water in petri dishes for 4 hours at a light intensity of about 100 micro-einsteins/m$^2$/sec. The discs were removed, blotted to remove water on their surfaces and weighed again. After drying the discs at 80°C for
24 hours, the dry weight was determined. Water saturation deficit was calculated from the formula

$$WSD = 100 - \left[ \frac{\text{Fresh wt.} - \text{Dry wt.}}{\text{Saturated wt.} - \text{Dry wt.}} \right] \times 100$$
### Table C.1.a. Pooled analysis of variance (both pre- and post-anthesis water stresses) on pericarp dry weight. 1st harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS (0.000487365)</th>
<th>MS (0.000081228)</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>6</td>
<td>0.000487365</td>
<td>0.000081228</td>
<td>3.456*</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>14</td>
<td>0.000329094</td>
<td>0.000023507</td>
<td>17.228</td>
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<tr>
<td>TOTAL</td>
<td>20</td>
<td>0.000816459</td>
<td>0.000040823</td>
<td>29.920</td>
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</table>

<table>
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<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS (0.0001034784)</th>
<th>MS (0.000147826)</th>
<th>VR</th>
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<td>0.000136625</td>
<td>0.000003332</td>
<td>2.442***</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>93</td>
<td>0.000126891</td>
<td>0.000001364</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>141</td>
<td>0.001298300</td>
<td>0.000009208</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>161</td>
<td>0.002114759</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table C.1.b. Unpooled analysis of variance (pre-anthesis water stresses only) on pericarp dry weight. 1st harvest.

<table>
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<th>Source of variation</th>
<th>DF</th>
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<th>MS (0.000151454)</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
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<td>0.000454362</td>
<td>0.000151454</td>
<td>5.139*</td>
</tr>
<tr>
<td>RESIDUAL</td>
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<td>0.000235772</td>
<td>0.000029471</td>
<td>21.086</td>
</tr>
<tr>
<td>TOTAL</td>
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<td>0.000690133</td>
<td>0.000062739</td>
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</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS (0.000478386)</th>
<th>MS (0.00068341)</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.000478386</td>
<td>0.00068341</td>
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<tr>
<td>TREATMENT x POSITION</td>
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<td>53</td>
<td>0.000074077</td>
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<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>80</td>
<td>0.000629086</td>
<td>0.000007864</td>
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</tr>
<tr>
<td>GRAND TOTAL</td>
<td>91</td>
<td>0.001319219</td>
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<td></td>
</tr>
</tbody>
</table>
Table C.1.c. Unpooled analysis of variance (post-anthesis water stress only) on pericarp dry weight. 1st harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
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<td>0.000080722</td>
<td>2.803 N.S.</td>
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<td>0.000028803</td>
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<td>TOTAL</td>
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<tr>
<td>POSITION</td>
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<td>0.000084439</td>
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</tr>
<tr>
<td>TREATMENT x POSITION</td>
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</tr>
<tr>
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<td>0.000001169</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
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<td>0.000008765</td>
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</tr>
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<td>GRAND TOTAL</td>
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</table>

Table C.2.a. Pooled analysis of variance (pre- and post-anthesis water stresses) on pericarp dry weight. 2nd harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>6</td>
<td>0.000514673</td>
<td>0.000085779</td>
<td>3.393 *</td>
</tr>
<tr>
<td>RESIDUAL</td>
<td>14</td>
<td>0.000353924</td>
<td>0.000025280</td>
<td>20.529</td>
</tr>
<tr>
<td>TOTAL</td>
<td>20</td>
<td>0.000868597</td>
<td>0.000043430</td>
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</tr>
<tr>
<td>POSITION</td>
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<td>0.000709311</td>
<td>0.000101330</td>
<td>82.286 ***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>41</td>
<td>0.000092688</td>
<td>0.000002261</td>
<td>1.836 *</td>
</tr>
<tr>
<td>RESIDUAL</td>
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<td>0.000110830</td>
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<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>138</td>
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<td>0.000006615</td>
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<tr>
<td>GRAND TOTAL</td>
<td>158</td>
<td>0.001781427</td>
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</table>
Table C.2.b. Unpooled analysis of variance (pre-anthesis water stress only) on pericarp dry weight. 2nd harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>3</td>
<td>0.00037990</td>
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<td>3.333 N.S</td>
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<tr>
<td>RESIDUAL</td>
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<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
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<th>MS</th>
<th>VR</th>
</tr>
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<tbody>
<tr>
<td>POSITION</td>
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<td>0.00004661</td>
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</tr>
<tr>
<td>TREATMENT x POSITION</td>
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<td>3.053</td>
</tr>
<tr>
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<td>53</td>
<td>0.00004229</td>
<td>0.00000079</td>
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</tr>
<tr>
<td>TOTAL</td>
<td>81</td>
<td>0.00041970</td>
<td>0.00000518</td>
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</tr>
</tbody>
</table>

GRAND TOTAL          | 92  | 0.00114000   |              |            |

Table C.2.c. Unpooled analysis of variance (post-anthesis water stress only) on pericarp dry weight. 2nd harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
<td>3</td>
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<td>0.000124847</td>
<td>4.563 *</td>
</tr>
<tr>
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</tr>
<tr>
<td>TOTAL</td>
<td>11</td>
<td>0.000593447</td>
<td>0.000053950</td>
<td>36.451</td>
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</tbody>
</table>

<table>
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<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
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<td>0.000058996</td>
<td>39.861 ***</td>
</tr>
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</tr>
<tr>
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<td>0.000001480</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>81</td>
<td>0.00041970</td>
<td>0.00000518</td>
<td></td>
</tr>
</tbody>
</table>

GRAND TOTAL          | 89  | 0.001128958  |              |            |
Table C.3.a. Pooled analysis of variance (both pre- and post-anthesis water stresses) on embryo dry weight. 1st harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
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<td>TREATMENT</td>
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<td>0.00062973</td>
<td>3.599*</td>
</tr>
<tr>
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<td>0.00017497</td>
<td>5.072</td>
</tr>
<tr>
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<td>20</td>
<td>0.00622798</td>
<td>0.00031140</td>
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</tr>
<tr>
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<td>7</td>
<td>0.01534490</td>
<td>0.00219213</td>
<td>63.546***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
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<td>0.00007600</td>
<td>2.203**</td>
</tr>
<tr>
<td>RESIDUAL</td>
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<td>0.00320821</td>
<td>0.00003450</td>
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</tr>
<tr>
<td>TOTAL</td>
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<tr>
<td>GRAND TOTAL</td>
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<td>0.02789701</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table C.3.b. Unpooled analysis of variance (pre-anthesis water stress only) on embryo dry weight. 1st harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
<tr>
<td>TREATMENT</td>
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<td>0.00316753</td>
<td>0.00105584</td>
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<tr>
<td>RESIDUAL</td>
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<td>0.00014796</td>
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<tr>
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<td>0.00067594</td>
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</tr>
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<td>0.00002448</td>
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</table>
Table C.3.c. Unpooled analysis of variance (post-anthesis water stresses only) on embryo dry weight. 1st harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
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<td>0.00044584</td>
<td>2.410 N.S.</td>
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<td>0.00018500</td>
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<td>0.00237158</td>
<td>0.00004392</td>
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</tr>
<tr>
<td>TOTAL</td>
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<td>0.01586218</td>
<td>0.00019344</td>
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</tr>
<tr>
<td>GRAND TOTAL</td>
<td>93</td>
<td>0.01867967</td>
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<td></td>
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</table>

Table C.4.a. Pooled analysis of variance (pre- and post-anthesis water stresses) on embryo dry weight. 2nd harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
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<td>0.00008960</td>
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</tr>
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<td>0.00396296</td>
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<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>138</td>
<td>0.02513026</td>
<td>0.00018210</td>
<td></td>
</tr>
<tr>
<td>GRAND TOTAL</td>
<td>158</td>
<td>0.03365690</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table C.4.b. Unpooled analysis of variance (pre-anthesis water stresses only) on embryo dry weight. 2nd harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
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<td>TREATMENT</td>
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<td>0.00095845</td>
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</tr>
<tr>
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<td>0.00001860</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
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<td>0.00852871</td>
<td>0.00010529</td>
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</tr>
<tr>
<td>GRAND TOTAL</td>
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<td>0.01366453</td>
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</tr>
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</table>

Table C.4.c. Unpooled analysis of variance (post-anthesis water stresses only) on embryo dry weight. 2nd harvest.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>VR</th>
</tr>
</thead>
<tbody>
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<td>0.00083062</td>
<td>2.286N.S</td>
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<tr>
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<td>8</td>
<td>0.00290631</td>
<td>0.00036329</td>
<td>6.099</td>
</tr>
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<td>TOTAL</td>
<td>11</td>
<td>0.00539818</td>
<td>0.00049074</td>
<td>8.239</td>
</tr>
<tr>
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<td>7</td>
<td>0.01291636</td>
<td>0.00184519</td>
<td>30.979***</td>
</tr>
<tr>
<td>TREATMENT x POSITION</td>
<td>20</td>
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<td>0.00010934</td>
<td>1.836*</td>
</tr>
<tr>
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<td>51</td>
<td>0.00303773</td>
<td>0.00005956</td>
<td></td>
</tr>
<tr>
<td>TOTAL</td>
<td>78</td>
<td>0.01814081</td>
<td>0.00023257</td>
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<tr>
<td>GRAND TOTAL</td>
<td>89</td>
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</table>
Table C.5. Distribution of Nitrogen (N) and Phosphorus (P) in the leaf and in the stem at the beginning of the drying cycle ($H_1$), at the end of the drying cycle ($H_2$) and at the end of recovery ($H_3$).

<table>
<thead>
<tr>
<th>Plant</th>
<th>%N in the leaf</th>
<th>%N in the stem</th>
<th>%P in the leaf</th>
<th>%P in the stem</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$H_1$</td>
<td>$H_2$</td>
<td>$H_3$</td>
<td>$H_1$</td>
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<tr>
<td>Soyabean</td>
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<td></td>
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</tr>
<tr>
<td>C</td>
<td>74.27</td>
<td>79.29</td>
<td>70.90</td>
<td>25.73</td>
</tr>
<tr>
<td>W</td>
<td>77.35</td>
<td>73.21</td>
<td>79.62</td>
<td>22.65</td>
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<td>Tomato</td>
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<tr>
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<td>76.52</td>
<td>68.18</td>
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<tr>
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<td>80.72</td>
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<td>78.76</td>
<td>19.28</td>
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<tr>
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<td>82.13</td>
<td>81.64</td>
<td>80.54</td>
<td>17.87</td>
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<tr>
<td>W</td>
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<td>81.18</td>
<td>21.86</td>
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<tr>
<td>C</td>
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<td>69.14</td>
<td>64.10</td>
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<tr>
<td>W</td>
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<td>66.15</td>
<td>66.45</td>
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<td>82.58</td>
<td>78.15</td>
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<tr>
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<tr>
<td>Buckwheat</td>
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</tr>
<tr>
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<td>73.28</td>
<td>66.52</td>
<td>63.25</td>
<td>26.72</td>
</tr>
<tr>
<td>W</td>
<td>71.82</td>
<td>69.51</td>
<td>67.78</td>
<td>28.18</td>
</tr>
</tbody>
</table>

C = CONTROL  W = WATER STRESSED
BIBLIOGRAPHY


Robinson, R.G. 1971. Sunflower phenology - year, variety and date of planting effects on day and growing degree day summations. Crop Science. 11, 635


