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SOME EFFECTS OF WATER STRESS AND ENVIRONMENT
ON SOYBEAN PLANTS

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ABSTRACT

Although a great deal of research has been carried out on the effects of water stress on plant processes, the influence of environmental conditions on plant response to water stress has received comparatively little attention. In this study the rates of CO₂ exchange and transpiration and the leaf water status of whole soybean plants (Glycine max (L.) Merr. cv. Merit) were measured under contrasting sets of environmental conditions when

- (a) the plants were maintained under conditions of adequate soil water supply.
- (b) water stress was imposed by withholding water and,
- (c) when water stress was imposed and then relieved by rewatering.

Light intensity and quality, atmospheric CO₂ concentration, wind speed and daylength were all constant; the between-treatment variables were air temperature and vapour pressure deficit (VPD). Plants were grown under one of four environmental treatments in a growth cabinet and the experiments carried out under very similar conditions in a plant chamber with facilities for measuring CO₂ exchange and transpiration. Details of this equipment are given.

Under conditions of adequate soil water supply rates of photosynthesis were lower under low VPD than under high VPD conditions at the same temperature. The effect was particularly marked at low temperature (22.5°C). Between-treatment differences in photosynthetic rate appeared to be mainly attributable to differences in the magnitude of the mesophyll resistance to CO_2 transfer. Transpiration rates were largely determined by the VPD, plants under high VPD treatments having the higher rates. At low VPD temperature had little effect on the rate of transpiration, but at high VPD plants under low temperature had lower rates of transpiration than plants under high temperature (27.5°C). Possible mechanisms whereby low temperatures may reduce transpiration under conditions of high VPD are discussed.

When water stress was imposed the rates of photosynthesis and transpiration declined in parallel under all treatments at soil moisture tensions in excess of 0.2 atm. This suggested that both plant processes were subject to a common controlling mechanism, probably stomatal diffusion resistance. At soil moisture tensions below 0.2 atm. the rates of photosynthesis and transpiration were independent of the soil moisture status. Between 0.2 and 0.4 atm. tension they appeared to be determined by plant, soil and atmospheric factors. The relative rates of photosynthesis and transpiration were reduced to a

greater extent at any tension between 0.2 and 0.4 atm. under high VPD than under low VPD conditions. Above 0.4 atm. soil moisture tension the rates of photosynthesis and transpiration became independent of the atmospheric conditions and it is suggested that (transpiration was limited chiefly by the rate of movement of water into the root zone from the surrounding soil.) (Photosynthesis may have been limited by direct effects of dehydration on the biochemical components of the process at these severe stress levels.) It was thus possible to distinguish three stages in the development of water stress, the significance and possible general application of which are discussed.

Under high temperature/high VPD conditions the rates of photosynthesis and transpiration recovered simultaneously and to a very similar extent when stress was relieved by rewatering, the degree of recovery being inversely proportional to the soil moisture tension at the time of rewatering. Possible causes of the failure of the rates of photosynthesis and transpiration to recover to their original prestressed values are discussed.

These results are discussed in relation to the findings of other workers, and suggestions for further research in this field made.

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TABLE OF CONTENTS

	Page
Acknowledgements	v.
List of Figures	viii.
 CHAPTER 1 INTRODUCTION	
Introduction to the present study ..	1.
Review of the literature	4.
 CHAPTER 2 MATERIALS AND METHODS	
I. Outline of experimental design ..	20.
II. Conditions in the growth cabinet and plant chamber	21.
III. Preparation of plants immediately prior to experiment	23.
IV. Equipment used for measuring CO ₂ exchange and transpiration .. ² ..	24.
V. Experimental procedures	33.
VI. Calculation of results	37.
 CHAPTER 3 EFFECTS OF TEMPERATURE AND VAPOUR PRESSURE DEFICIT ON THE CO ₂ EXCHANGE AND TRANSPIRATION RATES OF ² SOYBEAN	
Introduction	45.
Methods	52.
Results	54.
Discussion	70.
 CHAPTER 4 THE EFFECT OF WATER ON THE CO ₂ EXCHANGE AND TRANSPIRATION RATES OF SOYBEAN PLANTS UNDER CONTRASTING ENVIRONMENTAL CONDITIONS	
Introduction	76.
Methods	77.
Results	79.
Discussion	116.

CHAPTER 5	RESPONSE OF SOYBEAN PLANTS TO REWATERING FOLLOWING WATER STRESS UNDER TWO CONTRASTING SETS OF ENVIRONMENTAL CONDITIONS	
Introduction		134.
Methods		137.
Results		138.
Discussion		150.
CHAPTER 6	SOME ASPECTS OF LEAF STRUCTURE WHICH MAY AFFECT RATES OF GASEOUS EXCHANGE	
Introduction		156.
Methods		158.
Results		161.
Discussion		170.
CHAPTER 7	CONCLUSIONS	173.
APPENDICES		
Appendix 1.	Preliminary tests with various potting mixtures	190.
Appendix 2.	Evolution of CO ₂ and evaporation of water from pots	193.
Appendix 3.	Measurement of plant water stress	196.
Appendix 4.	Measurement of leaf area and determination of the leaf area- leaf dry weight relationships of plants grown under the four treatments	205.
Appendix 5.	Calibration of the CO ₂ -addition system	212.
Appendix 6.	Distribution of dry matter in the plant	214.
Appendix 7.	Detail plots of Figures 4-4, 4-10 and 4-11	216.
REFERENCES	223.

LIST OF FIGURES

Figure		Page
2-1	Gas flow circuit diagram of the plant CO ₂ exchange and transpiration measuring equipment	28.
3-1	Mean maximum rates of photosynthesis (mg CO ₂ /dm ² /hr) and transpiration (gm water/dm ² /hr) under the four treatments	56.
3-2	Effect of atmospheric VPD on the rate of transpiration (gm water/dm ² /hr) ..	58.
3-3	Relationship between the mean maximum rates of photosynthesis (mg CO ₂ /dm ² /hr) and transpiration (gm water/dm ² /hr)	62.
4-1	Representative time courses of photosynthesis under the four treatments	81.
4-2	Representative time courses of transpiration under the four treatments	83.
4-3	Changes in RWC (%), transpiration rate (gm water/dm ² /hr) and soil moisture tension (atm.) during the course of a three day experiment under HT/LH conditions	86.
4-4	Relationships between rates of photosynthesis (mg CO ₂ /dm ² /hr) and transpiration (gm water/dm ² /hr) between 0 and 0.4 atm. soil moisture tension of plants grown under the four treatments	89.
4-5	Effect of soil moisture tension on the rate of transpiration (gm water/dm ² /hr) of plants under the four treatments	93.
4-6	Effect of soil moisture tension on the rate of photosynthesis (mg CO ₂ /dm ² /hr) of plants under the four treatments	95.
4-7	Effect of soil moisture tension on the relative transpiration rates of plants under the four treatments.. .. .	99.

Figure		Page
4-8	Effect of soil moisture tension on the relative rates of photosynthesis of plants under the four treatments ..	101.
4-9	Effect of soil moisture tension on the RWC of plants under the four treatments	104.
4-10	Relationships between RWC and rate of transpiration (gm water/dm ² /hr) of plants under the four treatments	107.
4-11	Relationships between RWC ₂ and rate of photosynthesis (mg CO ₂ /dm ² /hr) of plants under the four treatments	109.
4-12	Relationship between relative rates of photosynthesis and transpiration of plants under the four treatments ..	113.
5-1	Time courses of rewatering experiments under HT/LH conditions	141.
5-2	Time courses of rewatering experiments under LT/HH conditions	143.
5-3	Dependence of the recovery of photosynthetic and transpiration rates following rewatering on the soil moisture tension (SMT) at the time of rewatering under HT/LH conditions	148.
A1-1	Soil moisture characteristic curve for the 1:1 pumice-peat mixture	192.
A3-1	Change in fresh weight with time of floating discs of soybean leaflets	199.
A4-1	Relationship between true leaf area and the sum of leaflet length and maximum width for the whole plant (ΣLW)	207.
A4-2	Relationships between leaf area and leaf dry weight of plants grown under the four treatments	210.
A7-1	Detail plots of the relationships given in Fig. 4-4	218.
A7-2	Detail plots of the relationships given in Fig. 4-10	220.
A7-3	Detail plots of the relationships given in Fig. 4-11	222.

CHAPTER 1

INTRODUCTION

Introduction to the present study

Since the publication in 1727 of Stephen Hales' classic Vegetable Staticks, literally hundreds of papers have been published on the subject of plant water relations and the effects of drought on plant processes. Until comparatively recently, almost all such experiments were carried out in the field or in glasshouses where environmental factors such as light, temperature and atmospheric water vapour concentration were uncontrolled or uncontrollable, and subject to rapid changes. The effects of these factors on the response of plants to drought (water stress) were thus unknown, the only parameters to which the plant's response could be related being the water status of the soil and, in a few cases, the plant.

The post-Second World War advent of controlled climate facilities, ranging in size from the "phytotron" to single leaf chambers, provided the opportunity to determine the effects of environmental conditions on plant response to water stress. This opportunity has not been taken:

examination of the published literature reveals very few studies in which water stress has been imposed under more than one set of environmental conditions, although the influence of soil temperature has been more frequently examined (e.g. Kuiper, 1964; Cox and Boersma, 1967; Babalola, Boersma and Youngberg, 1968). The influence of environmental factors on plant response to water stress remains largely unknown.

In the few cases where more than one set of environmental conditions has been employed (Gavande and Taylor, 1967; Pallas, Michel and Harris, 1967), the plants have been grown under one treatment prior to experiment under different environmental regimes. The conditions under which plants are grown may affect their photosynthetic capacity under other conditions (Hesketh, 1968; Rook, 1969) and there is reason to believe that the response to water stress may be affected by change in a single environmental factor, such as light intensity (Ashton, 1956). Results from such experiments may reflect only the response of plants grown under the original treatment to stress under the experimental conditions. If grown and water stressed under a single set of conditions the results might well be different.

Few investigators have attempted continuous measurement of photosynthesis and transpiration throughout their experiments. Transpiration is easily measured by weighing,

provided that evaporational losses are known and appropriate allowances made, but measurement of photosynthesis is more difficult. Most present day investigators use infra-red gas analysers to measure photosynthesis and are faced with the choice of measuring the response of a single plant over long periods, or the responses of several plants in sequence, each plant being measured for a short period. In the two investigations involving more than one set of environmental conditions cited above, photosynthesis was either not measured (Gavande and Taylor, 1967) or measured for short periods on a series of plants (Pallas, et al, 1967). In the latter only one plant per treatment was measured, each measurement being of two minutes duration.

Such short-period measurements may provide misleading results, for even under constant environmental conditions cyclic changes in transpiration and photosynthesis may occur (Ehrler, Nakayama and van Bavel, 1965; Cox, 1968; Kriedemann, 1968), and Barrs and Klepper (1968) showed that water stress could initiate cycling in some species. With a periodic sampling system it is conceivable that measurements could be made consistently at a particular phase of the cycle, thus leading to over or under estimation of the true average rate. Continuous measurement avoids this pitfall.

In the present investigation the photosynthetic and transpiration rates of single plants were measured continuously and simultaneously over periods of several days. As the plants were grown from an early stage under conditions as close as

possible to those under which the measurements were ultimately made, there is no question of the plants not being fully acclimatized to the environmental conditions. Further, disturbance to the plant's environment between removal from the growth cabinet and the commencement of the experimental period was minimal.

It is believed that no previous study on plant responses to water stress has incorporated as many as four environmental treatments (the number used in this investigation), or involved the continuous and simultaneous measurement of CO_2 exchange and transpiration over several days.

The aims of this investigation were to determine the differences, if any, in the response of soybean to water stress imposed under contrasting environmental conditions, and to account for these differences. Additionally, the CO_2 exchange and transpiration responses of soybean to these conditions were determined when the plants were not subject to water stress, and experiments were also carried out to determine the immediate responses of photosynthesis and transpiration to the relief of stress by rewatering.

Review of the literature

Many reviews, of varying degrees of scope, have been written concerning the effects of water deficits on plant processes. The earlier work in this field is largely covered in the books by Crafts, Currier and Stocking (1949) and

Kramer (1949) and more recent work has been reviewed by among others, Stocker (1960), Vaadia, Raney and Hagan (1961), Gates (1964), Henckel (1964), Jarvis (1967) and Slatyer (1967).

This is by no means an exhaustive list and does not include the many symposium proceedings and articles in encyclopaedias published during the same period. It is thus felt that little point would be served by the compilation of an exhaustive review of the subject. Instead only the more interesting and significant results will be discussed in an attempt to outline the approaches that have been used in research on plant water stress.

Results from previous work relevant to other aspects of this investigation (effects of temperature and vapour pressure deficit on rates of photosynthesis and transpiration in the absence of water stress, effects of rewatering following stress, and the relationships between plant anatomy and physiological processes), are reviewed in the introductions to the appropriate chapters.

At a symposium in St. Louis in 1961, Kramer (1963) stated:

"Many of the results from research on the relationships between plant growth, crop yields, and soil moisture have been inconclusive or even contradictory. This probably is because attention has been centred on one

part of the soil-plant system. Too much emphasis has been placed on soil water stress and too little on plant water stress and on the reasons why water stress reduces plant growth."

Arising from the results produced during the first half of this century, two schools of thought concerning the effects of water stress on plant processes emerged. One maintained that between soil field capacity and the permanent wilting point (corresponding to c. 15 atm. soil moisture tension), water was readily available for uptake by the plant, and plant processes (photosynthesis, transpiration) would not be reduced until the soil moisture tension exceeded 15 atm. (Veihmeyer and Hendrickson, 1927, 1950, 1955). The opposing group led by Richards and Wadleigh, held that as the soil dried water became less available, water deficits would ensue, and photosynthesis and transpiration would be reduced. (Richards and Wadleigh, 1952). Evidence in favour of the former view was provided by Allmendinger, Kenworthy and Overholser (1943) who found that glasshouse-grown apple trees showed no reduction in apparent photosynthesis until the soil moisture content was almost at the permanent wilting point. Similarly, Upchurch, Peterson and Hagan (1955) and Ashton (1956) found no reduction in the photosynthetic rate of ladino clover and sugar cane respectively, until the permanent wilting

point was reached. Veihmeyer and Hendrickson (1955) also cited the results of Burns (1926) showing that photosynthesis was not reduced by decreasing soil moisture content until death of the plants.

Contrary evidence indicating a gradual reduction in photosynthesis and transpiration with increasing soil moisture stress was reported by Heinicke and Childers (1936), and Schneider and Childers (1941), apple trees being used in both investigations, and a marked reduction in the photosynthesis and transpiration of pecan leaves, some two to three days before the soil reached the permanent wilting point, was found by Loustalot (1945). (Surprisingly the last paper was cited by Upchurch et al (1955) as providing corroborative evidence for their own work). Schneider and Childers reported that the stomata appeared to be closed before wilting was apparent.

Kramer (1963) has criticised the results of Upchurch et al (1955) and Ashton (1956) on the grounds that the chambers in which the tops of the plants in these experiments were enclosed greatly reduced transpiration. The plants were thus not subjected to severe water stress until the soil had dried to near the permanent wilting point, and would therefore not be expected to exhibit any reduction in photosynthesis before this stage. This criticism is fully justified, for plant water balance depends basically

upon the relative rates of water loss and water absorption. If loss exceeds absorption the plant will become subject to a water deficit, but there are two ways in which this situation may arise. If transpiration is rapid because of the atmospheric conditions (e.g. high vapour pressure deficit), the rate of absorption of water may be less than the water loss, even with a high soil moisture content or with plants grown in water culture. (The mid-day wilting of plants in the field is frequently attributable to this effect). Alternatively, with a dry soil and a low rate of transpiration the rate of absorption may be sufficient to maintain a positive water balance in the plant, wilting will not occur, and photosynthesis and transpiration will proceed at normal rates. It is this situation that was produced in the experiments of Upchurch et al (1955) and Ashton (1956), prior to the permanent wilting point being reached.

Water deficits may also occur if the quantity of water in the soil is such that, regardless of the rate of water loss, the rate of absorption of water is lower. At this stage the plant will only recover from the water deficit if water is added to the soil. (In the older literature this point is referred to as the permanent wilting point,

and was believed to be a soil characteristic (Veihmeyer and Hendrickson, 1950). It is currently believed that it corresponds to the point of zero turgor pressure in the leaves and is basically determined by leaf osmotic potential (Slatyer, 1957, 1967)). At this stage, as reported by Upchurch et al (1955) and Ashton (1956), wilting occurs and plant processes are reduced.

Kramer (in the discussion following Veihmeyer and Hendrickson, 1955) put forward as a further possible explanation for the results showing no reduction in plant processes above the permanent wilting point, the nature of the soil moisture characteristic curve for the soils used in these experiments. This curve relates the soil moisture content to the soil moisture tension, and in the case of the Yolo clay loam used in much of Veihmeyer and Hendrickson's work shows that soil moisture tension rises very rapidly for small reductions in soil moisture content when the latter falls below 20%. In such a soil, having much of its available water held at low tensions, there would be a very rapid transition from a non-stress to a water stress situation, and no reduction in transpiration or photosynthesis would be apparent until just before the wilting point. In soils in which much of the water is held at tensions greater than 1.0 atm. a gradual reduction in transpiration and photosynthesis might be expected.

An analysis of all experiments in which the soil had been allowed to dry to some measured point and then rewatered, was made by Stanhill (1957). He found that in over 80% of these experiments, growth had been reduced by the reduction in soil water content before rewatering took place. The view that plant response is unaffected before the wilting point is reached is no longer generally accepted, it being realised that there is no simple relationship between soil moisture tension and plant processes, but rather that the relationship is affected by soil conductivity, environmental factors, root distribution and the stage of development of the plant (Kozlowski, 1964). The effects of water stress at different stages of growth of various crop species have been comprehensively reviewed by Salter and Goode (1967).

Kramer's comments, quoted earlier, can be applied to all the investigations cited above, for in none of these were measurements of plant water stress made. One difficulty that confronted workers in this field was the lack of acceptable, and accepted, methods for the expression of plant water stress.

Within the period during which soil water stress was the main item of interest in assessing plant response to water deficits, certain work was done on plant water status.

Brilliant (1924) found that photosynthesis ceased when leaf water content fell to 50% of its maximum value, and Dastur (1925) showed a linear relationship between photosynthesis and leaf water content, the exact nature of which was species dependent.

Clements and Kubota (1942) related the moisture level of the leaf sheaths to the moisture level of the remainder of the tops of sugar cane plants. Their original intention to use this 'moisture index' as an indicator for irrigation scheduling was frustrated, as it was found that by the time the sheath moisture level dropped significantly, a drought reaction had already been triggered (H.F. Clements, pers. comm. 1968).

Weatherley (1950, 1951) studied the leaf water relations of field grown cotton in Uganda, and found that above a certain critical soil moisture content, leaf relative turgidity (see Chapter 2, section II (iii)) was determined by atmospheric factors only, whilst below the critical level it was controlled by both atmospheric conditions and soil moisture. Werner (1954) subsequently used Weatherley's relative turgidity technique with a potato crop, and found that when atmospheric factors led to reduced transpiration there was a rapid rise in the relative turgidity of the leaves. Slatyer (1955) also used the technique on a range of crops in Northern Territory, Australia, in a study of plant internal control over transpiration. Effective internal control over transpiration was reflected in the maintenance

of high relative turgidity and a slow rate of decrease in relative turgidity under arid conditions. The relative turgidity technique has, with certain modifications (Barrs and Weatherley, 1962) become a standard expression of plant water status, although the term relative turgidity is being replaced by relative water content (RWC).

A major problem in relating plant response to soil moisture tension lies in the difficulty of measuring soil moisture tension in the root zone. Because plants may remove water from the soil surrounding the roots more rapidly than water can move into this zone, the moisture content and moisture tension of the soil mass not permeated by the roots may not be an accurate reflection of the moisture status in the root-permeated soil (or rhizosphere). No method of measuring rhizosphere soil moisture has yet been developed. In an attempt to circumvent this problem many workers (e.g. Slatyer, 1961; Brouwer, 1963; Jarvis and Jarvis, 1963a; Janes, 1968) have used osmotic solutions of known concentration in place of soil, the assumption being that plant response to osmotic stress is the same as to an equivalent soil moisture tension, and that plant roots function as an ideal osmometer, i.e. there is no uptake of the solute constituting the osmotic solution. There are however, several objections to these assumptions. Gingrich and Russell (1957) compared the effects of osmotic stress and soil moisture tension on the growth of corn roots, and

found that the effects were not the same. Root growth was greater in osmotic media than in soil at equivalent stress levels, and increase in stress in osmotic solutions had no effect on root dry weight, whereas in soil the root dry weight decreased with increasing stress. Those growth characteristics that were influenced by stress responded linearly to osmotic stress throughout the 0.3 to 12.0 atm. range, but showed marked deviations from linearity under soil moisture stress. Gingrich and Russell concluded that the water transmission characteristics of the soil were responsible for these differences.

Plant roots do not in fact function as ideal osmometers; this is not surprising as mineral nutrients are absorbed by the roots. Consequently some of the substances used as osmotic agents have been shown to be absorbed from the solution, e.g. mannitol (Slatyer, 1961). Jarvis and Jarvis (1963a) briefly discuss the objections to other osmotic agents, including polyethylene glycol (PEG) of various molecular weights. PEG 1500 may not only be absorbed by the plant, but may produce toxic effects (Macklon and Weatherley, 1965).

The most serious objection to equating osmotic and soil water stress remains the effect of soil water conductivity on stress development. Macklon and Weatherley (1965) found

that the leaf water potential of transpiring castor bean plants depended on the nature of the root medium; osmotic solution, water or soil. Their conclusions were that water deficits in plants originate not from increases in root resistance to water uptake, but from the soil water conductivity being too low to permit rapid rewetting of soil in the rhizosphere during periods of rapid transpiration. Such rewetting occurs when the rate of transpiration falls, subject to the availability of water in the soil outside the rhizosphere. Thus osmotic solutions cannot be regarded as an adequate substitute for a drying soil.

The improved techniques of the last few years have enabled new approaches to the problems of water stress to be made. Water potential (previously known as diffusion pressure deficit) measurements of both plant tissue and soil have become routine in many laboratories with the introduction of the thermocouple psychrometer, originally developed by Spanner (1951) and subsequently refined by a number of other workers (e.g. Box, 1965; Merrill, 1968). Understanding of the effects of soil water stress on plant processes and the movement of water through the plant appears to have been facilitated by the growing use of the water potential terminology introduced by Slatyer and Taylor (1960).

Weatherley and Slatyer (1957) showed that there was a species dependent relationship between leaf water potential

and RWC. The relationship is analogous to the soil moisture characteristic curve which relates soil moisture tension (or water potential) to soil moisture content. It was originally thought that the relationship might be constant for any given species, and Slatyer (1960) found that environmental conditions had no effect on it in Acacia aneura. However, later evidence has shown that the relationship is not necessarily constant within a species, but may be affected by leaf age (Knipling, 1967; Millar, Duysen and Wilkinson, 1968), level of insertion of the leaf (Millar et al., 1968) and environment (Knipling, 1967; Hoffman and Splinter, 1968; Millar et al., 1968). The leaf water potential - RWC relationship must therefore be used with care when attempting to determine water potential from the more easily measured RWC, as was done by Ehlig and Gardner (1964). Nevertheless the relationship can be of considerable value, especially in comparing the response of various species to water stress. From their studies on a range of tree species Jarvis and Jarvis (1963b) suggested that a leaf moisture characteristic which involved a large fall in water potential for a small decrease in RWC conferred greater drought resistance than a leaf moisture characteristic of opposite effect. However, such drought resistance tended to be correlated with poor growth at low levels of moisture stress.

As soil moisture content and soil water potential decrease, the leaf water potential must also decrease to

maintain the water potential gradient between leaf and soil necessary for water movement through the plant to take place. A decrease in leaf water potential is associated with a fall in leaf turgor; loss of turgor in the guard cells leads to stomatal closure (Heath, 1938) and to reduction in transpiration and photosynthesis. The leaf water potential at which this occurs depends not only on the relationship between stomatal aperture and guard cell turgidity, but also on the leaf turgidity - water potential relationship. (Jarvis, 1963).

Brix (1962) showed that under the influence of water stress the photosynthetic and transpiration rates of loblolly pine and tomato decreased in phase, indicating a change in the diffusion resistance to CO_2 and water vapour. Whilst transpiration is normally regarded as being basically controlled by stomatal resistance, except under conditions of still air (Bange, 1953), CO_2 diffusion is also limited by a mesophyll resistance (Gaastra, 1959) associated with the liquid phase diffusion of CO_2 from the mesophyll cell wall surfaces to the chloroplasts. Bierhuizen and Slatyer (1964) considered that under non water stressed conditions the mesophyll resistance was the most dominant factor in CO_2 transfer in cotton leaves, but later reports from Slatyer's laboratory (Troughton, 1969; Troughton and Slatyer, 1969) show that the stomata are of primary significance in controlling CO_2 exchange in water stressed cotton plants and suggest that mesophyll resistance is not affected by water stress until the leaf RWC has fallen to 75%. The contribution of the mesophyll resistance to reduction of photosynthetic rate under stress is therefore

uncertain.

Non-stomatal control of transpiration, proposed by Livingston and Brown (1912) and supported by Knight (1917), now appears to be unlikely. Subsequent repeat of the experiments (Gregory et al., 1950) failed to confirm the crucial aspects of the original findings. Slatyer (1966) made an analysis in physical terms of the mechanisms proposed for non-stomatal control of transpiration and found them untenable, except under conditions of extreme leaf dessication when the stomata would be almost certainly completely closed.

A recent re-examination of some of the plant water stress literature has led Idso (1968) to propound a new theory of water stress effects. Proceeding from the observation that photosynthesis is a chemical process and transpiration a physical movement, he suggests that photosynthesis is largely determined by the water potential in the vicinity of the chloroplasts, and is thus largely independent of the atmospheric water content, whereas transpiration is controlled by the water potential difference between the evaporating sites in the leaf and the atmosphere, and is thus largely independent of soil moisture. He thus regards water stress as a double entity, the two components being essentially independent in origin and action. However the theory is limited in its application to a comparatively

narrow range of conditions. Denmead and Shaw (1962) showed that transpiration rate falls below the potential rate at lower and lower soil moisture stress levels as the atmospheric moisture stress increases. Idso's theory can be applied only to conditions of low atmospheric stress, when as discussed earlier, the movement of soil water into the rhizosphere is sufficient to maintain the rate of absorption by the roots. Again, the theory does not apply to plants with low root density which show a reduction in transpiration that is closely related to the soil water potential (Cowan, 1965). This theory cannot, therefore, be regarded as a definitive statement of the nature of plant water stress, especially in the absence of information on the effects of water stress under various environmental conditions.

CHAPTER 2

MATERIALS AND METHODS

Three series of experiments were carried out in which the CO₂ exchange and transpiration responses of soybean plants, grown under one of four environmental treatments, were determined. The three series were briefly:

- (i) in which the plants were supplied with adequate soil moisture throughout the experiment,
- (ii) in which water stress was allowed to develop by withholding water,
- and (iii) in which water stress was allowed to develop to various levels, and was then relieved by rewatering.

This chapter contains details of procedures common to all the above experiments. Techniques or procedures used in individual cases are described in the chapter dealing with the particular experimental series.

I. OUTLINE OF EXPERIMENTAL DESIGN

Soybean (Glycine max (L.) Merr. cv. Merit) plants were grown from seed in 5" plastic pots. One seed was sown in each pot in a 1:1 pumice-peat mixture which had been selected as a suitable potting medium after preliminary experiments (see Appendix 1). Germination and initial growth of the seedlings took place in a heated glasshouse. Water and Hoagland's nutrient solution were given occasionally.

Before the first true leaf was fully developed the plants were transferred to a growth cabinet where they were grown under one of four sets of environmental conditions (see section II). Whilst in the growth cabinet each plant received 50mls of Hoagland's nutrient solution twice daily. The plants were examined frequently and those showing excessive, reduced or abnormal growth were discarded. Plants having at least three fully expanded leaves were selected for the experiments.

The experiments were carried out in the plant chamber of the CO₂ exchange-transpiration measuring equipment (see section IV) which was separate from the growth cabinet and possessed its own environmental control facilities. In the plant chamber the plant was subjected to conditions very similar to those experienced in the growth cabinet. Measurements of CO₂ exchange, transpiration and water stress were made on single plants which were harvested after the experiment (see section V (vi)). There were at least four replicates of each experiment.

II. CONDITIONS IN THE GROWTH CABINET AND PLANT CHAMBER

For each treatment conditions in the growth cabinet and in the plant chamber were as similar as possible (Table 2-I).

Table 2-I. Conditions in the growth cabinet and plant chamber for each of the four sets of environmental treatments.

<u>Treat- ment</u>	<u>Cabinet & Chamber</u>		<u>Cabinet</u>	<u>Chamber</u>	<u>Cabinet & Chamber</u>	
	<u>Day temp. °C</u>	<u>Night temp. °C</u>	<u>Day RH%</u>	<u>Day RH%</u>	<u>Night RH%</u>	
HT/LH*	27.5	22.5	60	68	90	
HT/HH	27.5	22.5	80	85	90	
LT/LH	22.5	17.5	50	55	90	
LT/HH	22.5	17.5	80	80	90	

* High (day) temperature : low (day) relative humidity, etc.

Daylength was 12 hours in both growth cabinet and plant chamber for all treatments. The day time relative humidities in the plant chamber correspond to equal values of vapour pressure deficit (saturated vapour pressure of the air minus the actual vapour pressure at the same temperature) for the two LH and the two HH treatments. The actual vapour pressure deficit (VPD) for each treatment was:

HT/LH	11.7 mb	(8.78 mm Hg)
LT/LH	12.2 mb	(9.15 mm Hg)
HT/HH	5.5 mb	(4.13 mm Hg)
LT/HH	5.4 mb	(4.05 mm Hg)

The VPD's for the two LH treatments and for the two HH treatments were not identical because very fine adjustments

to the appropriate controls could not be made. Nevertheless, the discrepancies are small and the conditions obtained are regarded as satisfactory. Air temperature was always within 0.1°C of the required temperature.

Illumination in the growth cabinet was the same for all treatments and was provided by four 700 w mercury vapour lamps (Philips HPLR), two 300 w tungsten lamps (Mazda reflector floods), one 150 w tungsten lamp (Atlas E 27/27 reflector flood) and seven 80 w blue fluorescent lamps (Philips, type 126221). These were separated from the growth space by a flowing water screen and gave a total irradiance of 224.5 w m^{-2} (as measured with an Eppley pyrannometer) of which approximately 173 w m^{-2} was in the photosynthetic range (400-700 nm) at plant level. Illumination in the plant chamber was provided by a 700 w mercury vapour lamp (Philips HPLR) and two 100 w tungsten lamps (Philips Comptalux). The total irradiance was 194 w m^{-2} within the plant chamber, of which approximately 154 w m^{-2} was in the photosynthetic range.

Thus a factorial-type experiment was set up in which the effect of change in either temperature or VPD on CO_2 exchange and transpiration, could be determined at high or low VPD or high or low temperature respectively.

III. PREPARATION OF PLANTS IMMEDIATELY PRIOR TO EXPERIMENT

At least 24 hours before the start of an experiment the cotyledons (if still attached) and the primary leaves were removed from the test plant with a razor blade. These leaves are much thicker than the mature trifoliate leaves and the primary leaves are a much darker green, suggesting a higher chlorophyll content. It was considered that these leaves might have photosynthetic and transpiration rates different from the mature leaves, and that their retention might, therefore, affect the results obtained.

At 0800 hours on the first day of each experiment the plant was given 50 mls of Hoagland's nutrient solution to ensure that an adequate soil moisture supply was present initially. Excess nutrient solution was allowed to drain from the pot during the next hour, during which time the top of the pot was covered with a sheet of wax-backed aluminium foil to prevent evaporation from the soil surface. This sheet was cut to fit closely round the stem of the plant and to overlap the pot edges by at least $1\frac{1}{2}$ inches. The tops of the pots had an outward-protruding horizontal lip and the sheet was folded down and under this lip and secured with paper clips. The effect of this covering was to reduce evaporation to a negligible amount (see Appendix 2), thus allowing all weight loss from the pot to be attributed to transpiration.

Before the plant was transferred from the growth cabinet to the plant chamber the environmental conditions required in the chamber were set up and checked. Plants were transferred from the growth cabinet to the chamber at 0900 hours, the time at which the cabinet photoperiod started. Thus the transfer from the growth cabinet to the chamber was similar in its effect on the plant to the normal dark-light changeover in the growth cabinet, and involved minimum exposure of the plant to non-experimental environmental conditions. Subsequent light periods in the plant chamber commenced at 0815 hours.

IV. EQUIPMENT USED FOR MEASURING CO₂ EXCHANGE AND TRANSPIRATION

The equipment used in the measurement of CO₂ exchange and transpiration rates provided, automatically, a continuous record of these processes. In its basic concept the machine is similar to that described by Koller and Samish (1964), but in detail represents a significant improvement, for adjustments to the CO₂ concentration are made automatically and do not require the intervention of the operator. Once the required conditions have been set up, the machine will operate with minimal attention for several days. CO₂ concentration, maintained within a pre-set range by the automatic addition or extraction of CO₂, is monitored by a Grubb-Parsons

SB 2 infra-red gas analyser (IRGA). Transpiration is measured directly, as gm water lost from the plant, by a strain gauge transducer coupled to a modified beam balance located in the plant chamber. The plant pot rests on the pan of the balance (hereafter referred to as the transpiration balance). Thus, whilst plant CO_2 exchange is measured by a compensation system, transpiration is not. It is this feature, plus the automatic regulation of CO_2 concentration that marks the major difference between this model and that of Koller and Samish.

Basically the machine consists of two main sections, (i) the plant chamber and transpiration balance, associated air conditioning unit and the lamps, and (ii) the control units for temperature, relative humidity and CO_2 concentration, the IRGA and a chart recorder, (Esterline-Angus, model E1124E, 24 channels).

The plant chamber, 12" diameter and 12" deep is surrounded by a water jacket and covered with a glass lid. The lamps are situated 3 ft. above the chamber top and a flowing-water screen, to remove excess heat, lies immediately below it. (Details of the lamps used, and their outputs, are given in Section II of this chapter). Light from the lamps is concentrated into the chamber by a funnel lined

with aluminium foil. Extensive tests were performed to determine the type of funnel required to give even illumination in the chamber. Circular cones were found to concentrate the light in ring patterns, regardless of how the angles of the lamps were adjusted. A cone of rectangular section however, gave even illumination across the chamber top and was adopted for use in the experiments. Lamp angle was not critical with this funnel, but a constant setting was maintained.

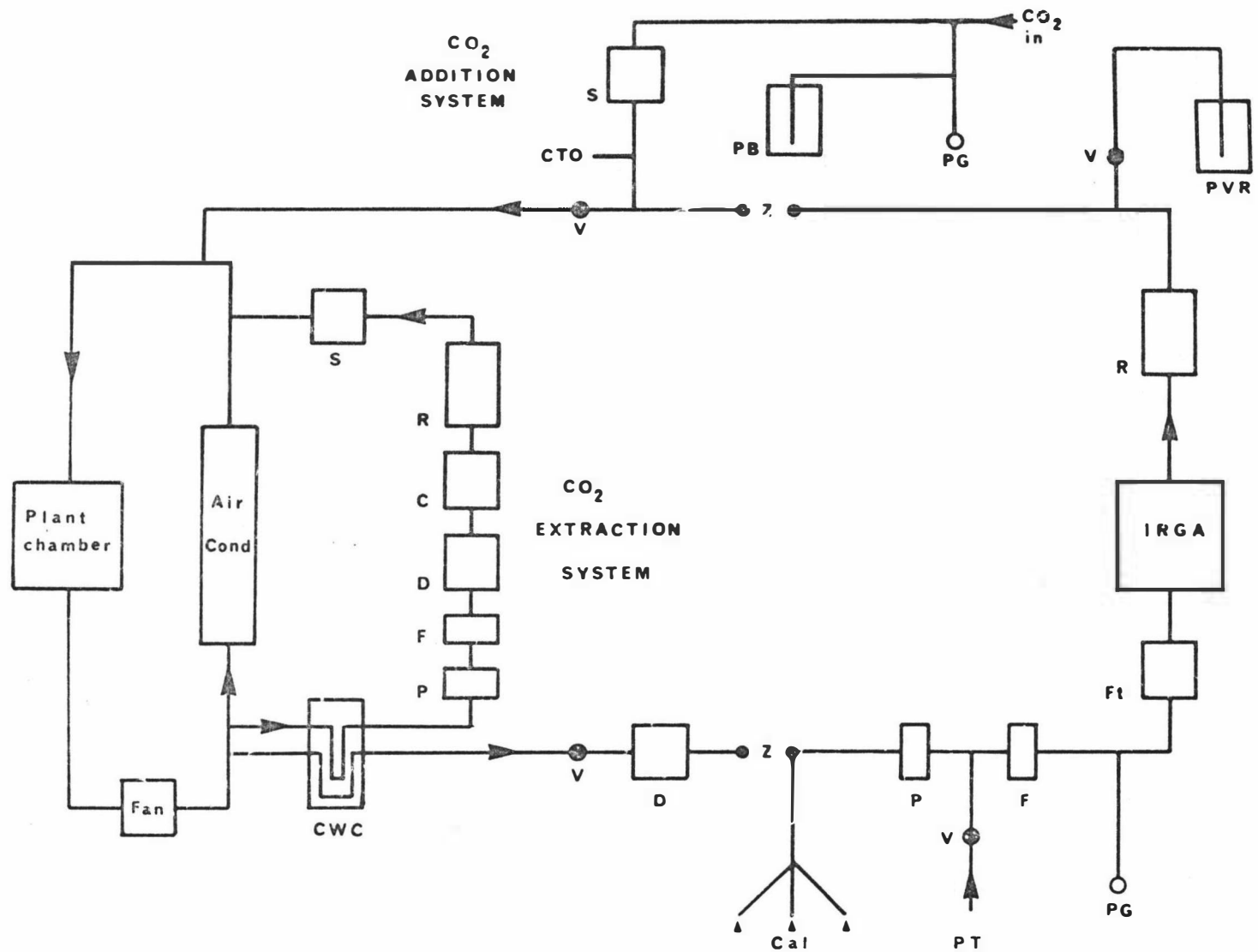
Air is circulated continuously through the plant chamber and air conditioning system (see Fig. 2-1), where temperature and humidity are regulated. The air stream is circulated by a centrifugal fan: the air speed across the chamber is c. 0.6 m/sec. In the air conditioner the air passes over a series of cooling coils which are maintained at the required dew point temperature for relative humidity (RH) control. After being cooled to the dew point temperature the air is reheated to the required dry bulb temperature by a one kilowatt heater directly in the path of the air stream. Relative humidity is sensed for recording by a wet and dry bulb thermocouple system immediately downstream of the plant chamber. Thermocouples, referenced to a melting ice bank, are located at the inlet and outlet ports of the plant chamber for sensing air temperature.

The plant chamber is surrounded by a jacket through which water is continuously circulated. Chilled water is automatically

Figure 2-1

Gas flow circuit diagram of the plant CO₂ exchange
and transpiration measuring equipment.

Air cond.	: Air conditioning unit.	PB	: Pressure bleed.
C	: CO ₂ scrubbing tower ("Carbosorb").	PG	: Pressure guage.
Cal	: Entry ports for calibration gases.	PT	: Pressure test entry port (Used for leak detection).
CTO	: CO ₂ -addition pulse take-off point for calibrating pulses.	PVR	: Pressure and vacuum relief.
CWC	: Cold water condenser.	R	: Rotameter flow gauge.
D	: Air drying tower (calcium chloride).	S	: Solenoid valve.
F	: "Flo-stat" pressure regulator.	V	: Valve. (Used to isolate various parts of system during leak detection process).
Ft	: Dust filter	Z	: 4 way, 2-bank switch. Selects gas stream from plant chamber or calibration gases for passage to IRGA. The two points marked Z are coincident.
P	: Pump.		



added if cooling is required.

The control units for relative humidity and air and water jacket temperature are slaves to a central programme controller. The required conditions for light and dark periods are set up on the programme controller which then provides the necessary signals to the individual control units to maintain these conditions. Change-over from light to dark and dark to light conditions is effected automatically at the required times, the control source being a time clock and relay system.

CO₂ concentration is maintained by an electronic control unit which receives its signal from the IRGA. A continuous bleed of air is taken from the plant chamber air conditioning circuit, and after passing through a cold water condenser and calcium chloride drying towers to remove all water vapour, is passed through the IRGA. The IRGA thus monitors the CO₂ concentration in the system at any given time with a time lag of about 40 secs. required for the passage of the air stream through the drying towers to the IRGA. The sample stream is then returned to the plant chamber circuit. CO₂ concentration is printed out on the chart record.

The CO₂ concentration range within which it is desired to operate is set up on the control unit, and whilst the CO₂ concentration in the system remains within these limits, no action is taken by the control unit. When the CO₂ concentration falls below the lower point of the set range, CO₂ is added in the form of pulses of CO₂ in nitrogen. (The calibration of

these pulses is described in Appendix 5). Addition continues until the CO_2 concentration is once again within the operating range. The number of pulses added is printed out by the chart recorder.

Extraction of CO_2 , which occurs automatically when the concentration rises above the upper limit of the set range, is effected by passing a part of the gas stream from the plant chamber system through a tower packed with "Carbosorb" or "Ascarite" which removes the CO_2 . Prior to passing through the tower the gas stream is dried by passage through a cold water condenser and a calcium chloride drying tower. These precautions are taken mainly to prolong the life of the "Carbosorb". (Self-indicating granules were used and changed regularly and frequently).

Preliminary tests were made on the effectiveness of carbosorb in removing CO_2 from air passed through it. Ambient air (c. 300 ppm CO_2) was passed continuously through a tower containing 20 gm of carbosorb and then through the IRGA. It was then discharged into the atmosphere. After 9 hours the IRGA still gave a zero CO_2 concentration reading. The carbosorb tower in the CO_2 extraction circuit contained approximately 200 gms of the material, and there is therefore no doubt that all CO_2 in the air stream passing through it was removed. A rotameter flow gauge was installed in the extraction system to provide flow rate information for the calculation of

respiration rate (see section VI). The air stream through the extraction system was driven by a pump at approximately 800 ml/min. The exact rate of flow varied from one experiment to the next, but was recorded in each case. The time for which the extraction system had been in operation during any given period was recorded on the chart as counts, each of 10 seconds duration.

The transpiration balance in the plant chamber provided a continuous record of weight loss (transpiration) from the plant. It was constructed from an Ohaus triple-beam balance and a Kyowa strain-gauge transducer. The balance beams were shortened to allow the unit to fit into the confines of the plant chamber, and the strain-gauge transducer was fixed to the base of the balance with its probe attached to the truncated beam. The signal from the strain-gauge was amplified and fed to the chart recorder where it was printed out. Adjustment of the amplified signal was made so that the change in signal caused by a weight change of 1 gm corresponded to one division of the chart paper. Total transpiration could thus be read directly from the chart.

The chart recorder, a 24 channel continuous operation model, was used to record not only the information on CO₂ addition, extraction and concentration, but also leaf temperature, air temperature, wet bulb depression, water jacket temperature and light intensity. (This last factor was measured by a small silicon cell inside the plant chamber.

The chart print-out from it was in arbitrary units, its function being to warn of reduced output from the lamps).

The time required for the chart recorder to print out all 24 channels was 173 seconds. As there were fewer than 24 items of information to be recorded, a number of the items were recorded on more than one channel, thus reducing the sampling interval for these items. CO₂-addition pulses, CO₂ extraction counts, CO₂ concentration and the mini-lysimeter output signal were each allotted four channels. They were thus sampled and recorded four times per cycle. Air and leaf temperatures were sampled twice per cycle.

Accuracy of control of CO₂ concentration, air temperature and relative humidity

CO₂ concentration was controlled within the range 285-330 ppm., i.e. addition of CO₂ occurred when the CO₂ concentration fell below 285 ppm; the extraction system came into operation just above, and switched off just below, 330 ppm. The hysteresis loop in the extraction system was necessary to prevent very short bursts of CO₂ extraction. Unlike the Hartmann-Braun IRGA's, the Grubb-Parsons model has a distinct and continuous tremor in its output signal. Without the hysteresis effect in the extraction system, very short bursts of extraction could have been generated. Attempts to narrow the working range of CO₂ concentration led to "hunting" and the range used was the narrowest that could be obtained. The normal atmospheric CO₂

concentration is between 300 and 315 ppm. (Bolin and Keeling, 1963), and it is thought that no significant effects on photosynthetic rates would be brought about by the small deviations from this level in these experiments. No corrections for changes in CO₂ concentration in different parts of the system were necessary as the whole system was designed to operate close to ambient atmospheric pressure. The maximum deviation observed during the experiments was 0.15 cm Hg.

Air temperature was always within 0.1°C of the required temperature.

Although relative humidity was controlled by dew point depression, the chart read-out was in terms of wet bulb depression. Control of relative humidity was rather better at low levels than at high. Under the HT/LH and LT/LH treatments the wet bulb depression was within 0.1°C of the required level; under the HT/HH and LT/HH treatments it was within 0.25°C.

V. EXPERIMENTAL PROCEDURES

(i) General course of experiments.

After removal from the growth cabinet the plant was placed on the pan of the transpiration balance in the plant chamber and thermocouples, for the measurement of leaf temperature, attached to the leaves (see section V (ii)). The chamber lid was then replaced and secured, and measurement of CO₂ exchange, transpiration and leaf temperature proceeded automatically and continuously. The light period in the plant

chamber was of 12 hours duration, from 0815 to 2015 hours. At chamber midday (1415 hours) and at the end of the light period the chamber was opened and the plant temporarily removed to take samples for the measurement of plant and soil water stress. As the time required for this process was not more than a minute, disturbance to the plant was minimal. After sampling the plant was replaced in the chamber, the thermocouples reattached, and the chamber lid secured. The sampling at the end of the light period was actually carried out slightly before 2015 hours as initial experiments showed that opening the chamber tended to raise the CO_2 concentration in the system, thus bringing into action the CO_2 extraction unit. If this occurred after the change to dark conditions, the combination of respiring plant and additional CO_2 produced too great a load for the CO_2 extraction system, and return to the 285-330 ppm CO_2 range was delayed. Returning the plant to the chamber before the change over to dark conditions enabled the plant and the extraction system to combine in reducing the CO_2 level.

These procedures were continued for from two to five days. Experiments run under HT/LH and LT/LH conditions were of shorter duration than those under the other two treatments because plants subjected to these conditions transpired more rapidly and so became subject to water stress sooner.

(ii) Measurement of leaf temperature

In earlier experiments fine-wire thermocouples were attached to the underside of leaflets with cellotape, but later concentric

type thermocouples, having the junction at the tip of a single wire, were used. These were sufficiently rigid to retain contact with the leaf undersurface without cello tape attachment. The thermocouples were referenced to a melting ice bath contained in a thermos flask, and leaf temperature was printed out directly on the chart record.

(iii) Measurement of plant water stress

Plant water stress was measured by the relative water content (RWC) method of Barrs and Weatherley (1962). At each sampling five leaf discs, each of 1.3 cm diameter, were punched at random from the leaves with a sharpened cork borer, placed in a tared, stoppered weighing bottle and weighed. The discs were then floated on distilled water in a covered petri dish for 1 hour, removed, blotted dry with filter paper, and reweighed in the same bottle. Dry weight was determined after drying the discs overnight in an oven at 95°C.

Relative water content is given by

$$\frac{FW - DW}{TW - DW} \times 100$$

Where FW = weight of discs at sampling

TW = weight of discs after floating on water

DW = dry weight of discs.

Relative water content is synonymous with relative turgidity, but is preferred to the latter term because the technique measures not turgidity, but water content. Details of the preliminary evaluation of the technique are given in Appendix 3 a.

It was originally intended to measure leaf water potential as well as RWC. Attempts to do this were unsuccessful (Appendix 3 b).

(iv) Measurement of soil water stress

Although the plants were grown in a mixture of pumice and peat, and not in a soil, it is convenient to refer to this mixture as 'soil'.

Soil moisture content was determined by removing a small sample of the soil from below the surface, weighing it in a tared, stoppered weighing bottle and then oven-drying the sample overnight at 95°C.

Soil moisture content (%) is given by:

$$\frac{\text{Loss in weight on drying}}{\text{Weight of dry soil}} \times 100$$

Soil moisture tension was then obtained by reference to the soil moisture characteristic curve. (Appendix 1, Fig. A1-1).

(v) Measurement of respiration rates

To enable calculations of respiration rates to be made, the following factors, which were not printed out on the chart record, were written on the chart at 2015 hours and 0815 hours the following morning; room temperature, atmospheric pressure and the flow rate through the CO₂ extraction system. The means of the morning and the previous night's readings were used in the calculations. (See section VI).

(vi) Harvesting of plants at the end of experiments

Experiments were normally terminated at 2015 hours (the end of the chamber light period), except when breakdown of equipment or power-cuts dictated otherwise.

At harvest the usual measurements of RWC and soil moisture content were made and the plant stem was then cut flush with the soil surface. The plant was separated into two fractions, leaves, and stems and petioles, and the dry weights of these parts determined by drying overnight in an oven at 95°C and weighing following a 10 minute cooling period at room temperature.

CO₂ exchange and transpiration rates were calculated on a leaf area basis, which required the conversion of leaf dry weight to leaf area*. The relationship of the two characters was determined for plants grown under each of the four treatments and was as follows:

$$\text{HT/LH} : \text{Leaf area} = 269.87 \times D + 21.0 \text{ cm}^2$$

$$\text{HT/HH} : \text{ " " } = 300.06 \times D + 45.8 \text{ cm}^2$$

$$\text{LT/LH} : \text{ " " } = 192.55 \times D + 41.7 \text{ cm}^2$$

$$\text{LT/HH} : \text{ " " } = 213.40 \times D - 34.7 \text{ cm}^2$$

where D is leaf dry weight in gms.

The determination of these relationships is detailed in Appendix 4b.

* i.e. area of the surface enclosed by the drawn outline of the leaves.

VI. CALCULATION OF RESULTS

Most of the information necessary to calculate the rates of CO₂ exchange and transpiration was printed out on the chart

record, and was read off accordingly. This was combined with other necessary data (plant leaf dry weight, atmospheric pressure etc.) to complete the calculations.

Photosynthesis was recorded as the number of pulses of a CO_2/N_2 gas mixture injected into the system in unit time. Each pulse was recorded on the chart record. (The calibration of the CO_2/N_2 addition system is described in Appendix 5).

Respiration was measured in terms of the running time, during any given time interval, of the CO_2 extraction system. Counts corresponding to 10 seconds running time were printed on the chart record, and were converted to respiration rate as described later in this section. Transpiration was recorded directly as gm water lost from the pot and plant system.

The light and dark periods of each experiment were divided into 12 one hour periods (0815-0915, 0915-1015 hours etc.), and the CO_2 -addition pulses, water loss and CO_2 -extraction time counts for each period read off. CO_2 -addition pulses per hour were converted to mg CO_2 added per hour by multiplying by the weight of CO_2 per pulse (see Appendix 5).

Because the pot surface was not separated from the plant chamber, CO_2 evolved from the pot was available for uptake by the plant in photosynthesis, and was additional to CO_2 evolved in respiration. A correction for this quantity of CO_2 was made on the basis of measurements of CO_2 evolution from the pot surface when the leaves and stems of the plants had been removed.

A mean CO_2 evolution rate of 5 mg/hour was found for such pots (see Appendix 2). Consequently this figure was added to the

weight of CO₂ added each hour by the CO₂-addition system to give total photosynthesis per hour. In respiration rate calculations, 5 mg was subtracted from each calculated hourly respiration rate to give total hourly respiration rate.

Hourly respiration rates were calculated from the number of extraction counts per hour as follows. The number of extraction counts in each hour was read off the chart and recorded. Each count corresponded to 10 seconds of running time of the extraction system, hence dividing by 6 and multiplying by the flow rate (litres per minute) through the extraction system gives the volume (litres) of air passed in the hour. The weight of CO₂ (mg) in this volume of air is given by multiplying by

$$5.9136 \times P/T$$

Where P = atmospheric pressure in inches of Hg

T = room temperature (°K)

(This relationship is derived as follows:

Molecular weight of CO₂ = 44.005

At NTP 44.005 gm CO₂ occupies 22.4 litres

1 ppm CO₂ therefore weighs $\frac{44.005 \times 10^{-6}}{22.4}$ gm/l

$$= 1.965 \times 10^{-6} \text{ gm/l}$$

At given P and T (for P in inches Hg)

$$1 \text{ ppm CO}_2 = 1.965 \times 10^{-6} \times 9.12 \times \frac{P}{T} \text{ gm/l}$$

$$= 1.792 \times 10^{-5} \times \frac{P}{T} \text{ gm/l}$$

$$\text{and } 330 \text{ ppm CO}_2 = 1.792 \times 10^{-5} \times 330 \times \frac{P}{T} \text{ gm/l}$$

$$= 591.36 \times 10^{-5} \times \frac{P}{T} \text{ gm/l}$$

$$= 5.9136 \times \frac{P}{T} \text{ mg/l}$$

The final calculation is made for 330 ppm CO₂ because this was the mean concentration at which CO₂ extraction took place. The CO₂ control system was such that setting extraction to occur at lower CO₂ concentrations led to "hunting" (i.e. alternate addition and extraction of CO₂), which made the calculation of CO₂ exchange rates impossible).

Because mean values of flow rate, atmospheric pressure and room temperature were applied to the whole 12 hour night period, a single correction factor derived from

$$\frac{\text{Flow rate}}{6} \times \frac{5.9136}{1} \times \frac{P}{T}$$

was applied to each hourly extraction count. This gave total CO₂ absorbed by the extraction system per hour. Subtraction of the 5 mg/hour pot respiration correction gave total plant respiration per hour.

Because of the length of time that plants remained in the plant chamber, it was necessary to correct for the changes in dry weight and leaf area that took place. The corrections were based on the total amounts of CO₂ assimilated or respired during the various light and dark periods of the experiments. Each light period was divided into two six-hour periods (hours ending 0915 to 1415 and 1515 to 2015), and the total CO₂ assimilated during these periods determined. Each gram of CO₂ is converted in the plant into 0.682 gm of carbohydrate (as a working approximation), so that multiplying the total CO₂ assimilated in any given period by 0.682 gives the dry weight increase in the plant over that period. However, this increase will not be confined to the leaves,

but will be distributed between all parts of the plant. To obtain an approximation of the distribution pattern, plants were grown under HT and LT conditions and the dry weights of leaves, stems and roots determined. These were then expressed as a percentage of the total dry weight of the plant. Because plant chamber conditions were very close to the growth cabinet conditions for any given plant, and plants were grown in the cabinets for at least two weeks prior to experiment, it was assumed that the plants would be fully acclimatized to their environment and that the dry weight distribution would closely reflect the distribution of new photosynthate within the plant. Moreover the distribution of such photosynthate would be the same in both cabinet and plant chamber. The dry weight distribution trials showed that under HT treatments 50% of the total dry matter was present in the leaf; under LT treatments 53%. These results were accordingly interpreted as indicating that 50% of photosynthate would remain in the leaf under HT conditions; 53% under LT conditions. (Details of these experiments will be found in Appendix 6). Thus multiplying the total dry weight increase for each six-hour light period by 0.50 or 0.53, as appropriate, gave the leaf dry weight change during that period.

Total leaf dry weight at the time of harvest was known, and by subtracting the leaf dry weight increase for the previous six hours, the total leaf dry weight at 1415 hours was

determined. Subtracting from this the dry weight increase for the 0815-1415 period gave the dry weight at 0815 hours. Allowance was made in these calculations for the weight of tissue removed in sampling for RWC determinations, the dry weight of the material removed being determined in each case.

For each six hour light period the mean leaf dry weight was determined as

$$(\text{leaf dry weight at } t_1 + \text{leaf dry weight at } t_2/2)$$

where t_1 and t_2 are 0815 hours and 1415 hours for the first half of the light period, 1415 hours and 2015 hours for the latter half.

Overnight weight change due to conversion of carbohydrate to CO_2 was allowed for in a similar manner. Each 1 gm of CO_2 liberated from the plant represents 0.682 gm carbohydrate broken down. It was assumed that the distribution of respiratory activity in the plant was also proportional to the distribution of photosynthate, an assumption that is supported by the results of Weigl, Warrington and Calvin (1951). The possible errors resulting from this assumption are small. Taking, for example, a plant under HT/LH conditions that is not suffering from water stress, with a leaf area of 500 cm^2 and a total CO_2 loss during the dark period of 0.18 gm (these figures being typical for the HT/LH treatment), the overnight dry matter weight change is $(0.18 \times 0.682 \times 0.5) = 0.0615 \text{ gm}$.

This corresponds to a leaf area correction of 39.3 cm^2 (Appendix 4 B). In the unlikely case of all the respiration

taking place in the leaves the leaf dry weight change during the dark period would be $(0.18 \times 0.682) = 0.123$ gm, which is equivalent to a leaf area of 57.2 cm^2 . The difference between the two leaf area corrections, 17.9 cm^2 , amounts to 3.6% of the total leaf area. The error involved in the assumption that 50% of the total respiration occurs in the leaves is certainly smaller than this.

Thus 50% of the respiration taking place in a plant under HT treatments was assumed to occur in the leaves, 53% under LT treatments. Because respiration rates were much lower, in terms of CO_2 exchange per unit area, than photosynthetic rates, and the total leaf dry weight change was correspondingly small, the twelve hour night period was treated as a single time unit and not as two six-hour periods. Thus the dry weight (and hence leaf area) used in calculating the respiration rate for any given night period was derived from the mean of the leaf dry weights at 2015 hours and 0815 hours on the following day.

In this way a dry weight change 'balance sheet' for the duration of the experiment was drawn up, working backwards in time from the time of harvest. Thus changes in dry weight due to photosynthate accumulation were subtracted from, and changes due to respiratory losses were added to, the previous leaf dry weight. Mean leaf dry weight for each period was then converted to leaf area (as described previously), and the total hourly rates of photosynthesis or respiration divided by the

appropriate leaf area to give mg CO₂ exchanged/dm² leaf area/hour.

The total hourly transpiration rates were similarly divided by the appropriate leaf area to give transpiration rate as gm water lost/dm² leaf area/hour. The aluminium foil covers on the pots proved highly effective in preventing evaporative water loss, and no correction for evaporation was necessary. All weight loss from the pot and plant was attributed to transpiration (Appendix 2).

In all the results presented the leaf area shown is that of one surface of the leaf only.

CHAPTER 3

EFFECTS OF TEMPERATURE AND VAPOUR PRESSURE DEFICIT ON THE CO₂ EXCHANGE AND TRANSPIRATION RATES OF SOYBEAN

INTRODUCTION

Effect of temperature on photosynthesis (intake of CO₂)

In general an increase in temperature results in an increase in the rate of photosynthesis, provided that other factors are not limiting. At temperatures above about 45°C thermal inactivation of the biochemical processes of photosynthesis may occur and the rate of photosynthesis then falls rapidly, and ultimately reaches zero.

Within the temperature range normally encountered by plants, the response of photosynthesis to increase in temperature appears to be species dependent. El-Sharkawy and Hesketh (1964) found that the photosynthetic rates of Sorghum vulgare, Helianthus

annuus (sunflower) and cotton increased as the temperature increased from 20° to 35°C. The photosynthetic rates of sunflower and cotton leaves decreased above 35°C, but the rate for Sorghum continued to increase up to 45°C. The photosynthetic rate of Thespesia populnea started to decline at 30°C. However Hew, Krotkov and Calvin (1969) found that the photosynthetic rate of sunflower leaves declined rapidly above 15°C at light intensities of 300 and 1800 ft-c. Other species studied by these authors showed more or less constant rates of photosynthesis between 20 and 25°C followed by a decline above 25°C. Soybean was among these species. The decline was attributed to increasing evolution of CO₂ with increase in temperature.

Whiteman and Koller (1964) showed that above 22°C the photosynthetic rate of Pinus halepensis declined. They could find no evidence of thermal inactivation, and indeed it seems unlikely that this would commence at such a low temperature. Increasing the CO₂ concentration at different temperatures showed that photosynthesis was most affected by CO₂ concentration at high temperature (32°C). It is therefore possible that high temperature depresses photosynthesis by increasing the carboxylation resistance.

It would appear therefore, that photosynthesis increases with increasing temperature until some other factor(s) become

limiting. The temperature at which the maximum rate is realised when other factors are not limiting appears to be species dependent.

Effect of vapour pressure deficit (VPD) on photosynthesis

There is no general agreement on the effects of VPD on photosynthesis. Mitchell (1936) found that increased VPD had no effect on the photosynthetic rates of Cineraria, tomato, Primula and Pelargonium, except when wilting occurred at very high VPD's. His experiments however, involved measuring the photosynthetic response to changes in VPD over a short time period (2-3 hours) and any effects caused by long term exposure would therefore not be observed. Additionally, absence of preconditioning of the plants could have affected their response. Nevins and Loomis (1970) reported that changes in VPD between 4.0 and 13.5 mm Hg at 23°C had no effect on the photosynthetic rate of Beta vulgaris leaves.

Kriedemann(1968) found that the photosynthetic rate of single attached leaves of orange and lemon increased up to approximately 20°C, and then levelled off or fell depending on the humidity of the air. In dry air both species showed a marked optimum, followed by a sharp decline in photosynthesis.

In humid air (>80% RH) there was no such well-defined optimum and the maximum rates were slightly higher for orange, slightly lower for lemon.

Bierhuizen and Slatyer (1964) found that the photosynthesis of individual cotton leaves decreased approximately 11% when the VPD was increased from 10 to 40 mm Hg at temperatures between 30 and 40°C. Baker (1965) also with cotton, found a linear reduction in photosynthesis when the VPD was increased from 7.5 to 30 mm Hg at 40°C under a range of light intensities.

Bierhuizen and Slatyer (1964) ascribed the reduction in photosynthesis with increasing VPD to high transpiration rates giving a partial drying of the mesophyll cell surfaces, and predicted that the effect would be more pronounced if the whole plant, rather than a single leaf, were exposed to the conditions described. Whiteman and Koller (1964), who found a decrease in photosynthesis of whole plants of Pinus halepensis when the VPD increased from 5 to 20 mm Hg at 26.5°C, pointed out that the partial drying of the mesophyll cell surfaces might be expected to give an increase, rather than a decrease, in photosynthesis, because CO₂ diffuses more rapidly in the gaseous phase than in the liquid phase and mesophyll drying would shorten the liquid phase pathway. Instead they suggest that cytoplasmic dehydration, and the

consequent reduced enzyme activity may be the cause of the decrease in photosynthesis. Their results confirm the prediction of Bierhuizen and Slatyer (1964), that the response of a whole plant to high VPD is greater than that of a single leaf.

Results for cotton contrary to those of Bierhuizen and Slatyer (1964) have been reported by Pallas, Michel and Harris (1967). Under light intensities similar to those used by Baker (1965) they found that photosynthesis increased with increasing VPD at 25°C, and suggested that the lowering of leaf temperature at high VPD (presumably due to increased transpiration) may have increased photosynthesis. Increase in photosynthesis with increase in VPD has also been reported for Pinus silvestris by Hodges (1967). His results came from field experiments, and his data were simplified to mean temperatures, VPD's and photosynthetic rates. They may therefore not be relevant to the present discussion, but it is of interest to note that under the same field conditions he found a decrease in photosynthesis with increasing VPD in Pseudotsuga menziesii and Abies procera.

It is not therefore possible to draw any general conclusions from the information available on the effects of VPD on photosynthesis. Whilst the photosynthetic response to VPD may be species dependent, the possibility of water stress having developed during the course of some of the

experiments cited above cannot be ruled out. The development of water stress in the leaves would lead to reduced rates of photosynthesis (Slatyer, 1967).

Effect of temperature and VPD on transpiration

The rate of transpiration, E , may be described by the equation,

$$E = \frac{D (e_1 - e_a)}{r}$$

where D is the diffusion coefficient of water vapour in air, $(e_1 - e_a)$ is the vapour pressure difference between the evaporating sites in the leaf and the ambient air, and r is the total resistance to water vapour diffusion from the evaporating sites in the leaf to the air (Milthorpe, 1959).

Any increase in temperature will increase the vapour pressure difference between the evaporating sites in the leaf and the air and so increase the rate of transpiration, assuming that the leaf evaporating sites remain saturated. If the rise in temperature were accompanied by an increase in e_a so that $(e_1 - e_a)$ were the same at both temperatures then no change in transpiration rate would be expected, provided that r remained constant.

The effect of vapour pressure difference between leaf and air on transpiration rate is well documented (e.g. Rufelt, Jarvis and Jarvis, 1963; Skidmore and Stone, 1964; Whiteman and Koller, 1964; Ehrler, van Bavel and Nakayama, 1966; Pallas et al, 1967), and is not in question.

Whiteman and Koller showed that the transpiration rate of Pinus halepensis increased linearly at 26.5°C as the vapour pressure gradient increased from 5 to 12.5 mm Hg. Thereafter it remained more or less constant. Skidmore and Stone however, reported that the transpiration rate of cotton increased linearly between 5 and 18 mm Hg VPD, when measured at mid-day. Within these limits there was no indication of any levelling-off in the rate. The temperature in this case was cited as varying between 20° and 36°C . Rufelt et al (1963) concluded that when the stomata are open transpiration is proportional to leaf temperature and depends to a large extent on the vapour pressure difference between the air at the evaporating surface in the leaf and the ambient air.

It may be concluded therefore, that within the temperature range normally encountered by plants, that increase in temperature generally results in an increase in photosynthetic rate, but that at some species-dependent temperature the rate will start to decline. Transpiration also increases with increase in temperature, the effect being mediated via the resulting increase in vapour pressure difference between the evaporating sites in the leaf and the ambient air. Increased VPD has been variously reported as increasing,

decreasing, and having no effect on, the photosynthetic rate of various species, and of increasing (Pallas et al, 1967), and decreasing (Bierhuizen and Slatyer, 1964; Baker, 1965) the photosynthetic rate of cotton. The effect of VPD on photosynthesis is therefore uncertain.

This chapter concerns a study of the effects of VPD and temperature on the rate of photosynthesis of one species, soybean, under conditions of adequate soil moisture supply and normal atmospheric CO₂ concentration. Plants were grown, and measurements made, under each of the four selected sets of environmental conditions (see Chapter 2, section II). Measurements were made only under the conditions under which the plants had been grown. Simultaneous measurements of transpiration rate were also made to assist in the interpretation of the data obtained from the water stress experiments (Chapter 4).

METHODS

Plants grown under the conditions described in Chapter 2, section II, were placed in the plant chamber of the CO₂ exchange-transpiration measuring equipment and continuous and simultaneous measurements of CO₂ exchange and transpiration made over the following two to three days. At the end

of each light period (2015 hrs) a quantity of water equal in volume to that lost by the plant since the previous watering was added to the pot to restore the soil moisture status to its original level. The volume of water required to make good these transpiration losses was determined from the transpiration trace on the chart record.

Leaf and soil samples for the determination of RWC and soil moisture content were taken periodically (see Chapter 2, section V) to ensure that water stress had not developed.

Mean rates of photosynthesis and transpiration were determined for the plants grown under each treatment from the rates obtaining during the hour previous to each RWC sampling. As these measurements apply to plants not subject to water stress they are referred to hereafter as "mean maximum rates".

The mean hourly dark respiration rate was calculated for every dark period to which each plant was subjected and from these results the mean maximum dark respiration rate was calculated for each treatment. The night-time transpiration rates were similarly determined.

RESULTSPhotosynthesis and transpiration

The mean maximum rates of photosynthesis and transpiration of plants grown under the four treatments are shown in Fig. 3-1. The highest photosynthetic rates were found in plants grown under the two high temperature treatments, and at both temperatures the rates were higher at high VPD (low relative humidity) than at low VPD.

Rates of transpiration were related to the VPD (Fig. 3-2), the higher rates being found in plants grown under the high VPD treatments. Under the two HH (low VPD) treatments the rates of transpiration were very similar, but under the LH treatments the rate of transpiration was significantly lower at the lower temperature (Fig. 3-2 and Table 3-II).

The difference between treatments, and the least significant differences, are given for photosynthesis in Table 3-I and for transpiration in Table 3-II.

Table 3-I Differences in photosynthetic rate ($\text{mg CO}_2/\text{dm}^2$ leaf area/hr) between the four treatments. Least significant differences (5% level) in brackets.

<u>Treatment</u>	<u>HT/LH</u>	<u>HT/HH</u>	<u>LT/LH</u>
HT/LH	-	-	-
HT/HH	3.0 (1.98)	-	-
LT/LH	4.9 (2.04)	1.9 (1.62)	-
LT/HH	11.2 (2.15)	8.2 (1.76)	6.3 (1.82)

Figure 3-1

Mean maximum rates of photosynthesis
(mg CO₂/dm²/hr) and transpiration
(gm water./dm²/hr) under the four treatments.

Standard errors:

	<u>Photosynthesis</u>	<u>Transpiration</u>
HT/LH	0.73	0.08
HT/HH	0.49	0.04
LT/LH	0.64	0.07
LT/HH	0.79	0.05

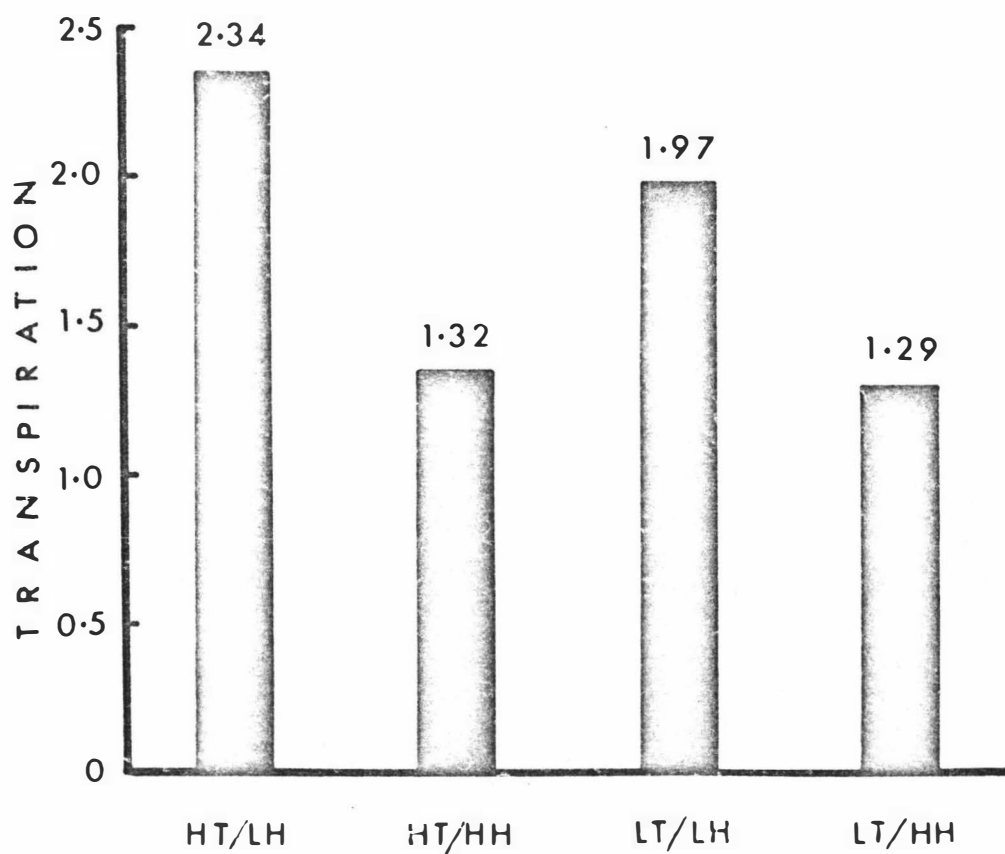
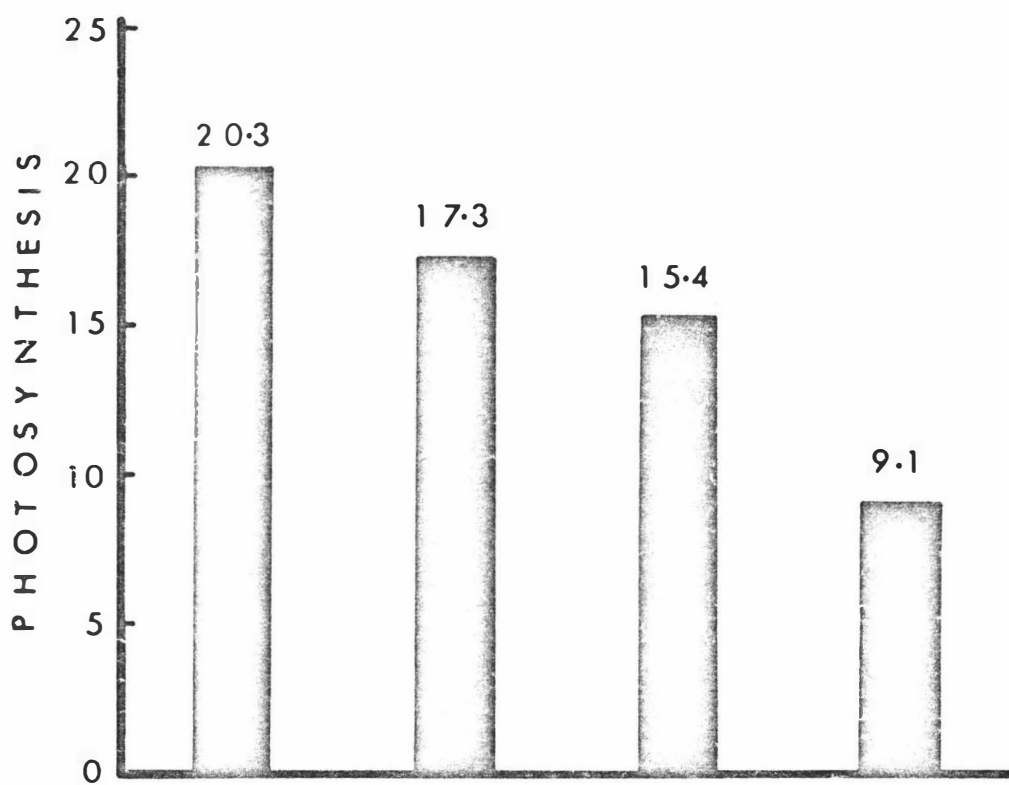
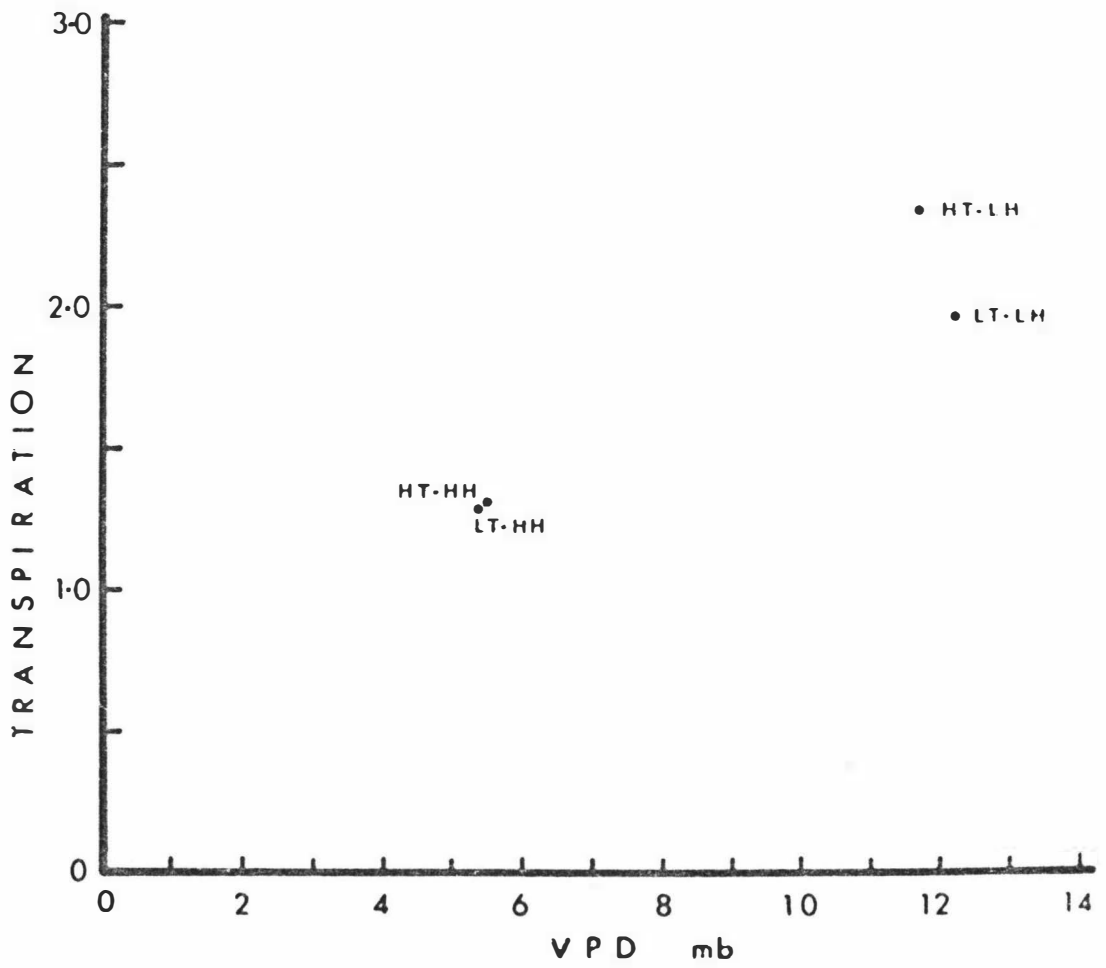


Figure 3-2

Effect of atmospheric VPD on the
rate of transpiration (gm water/dm²/hr).



All between-treatment differences in photosynthetic rate are significant at the 5% level.

The LSD is different for each treatment comparison because the number of observations in each treatment was different.

Table 3-II Differences in transpiration rate (gm/dm^2 leaf area/hr) between the four treatments. Least significant differences (5% level) in brackets.

<u>Treatment</u>	<u>HT/LH</u>	<u>HT/HH</u>	<u>LT/LH</u>
HT/LH	-	-	-
HT/HH	1.02 (0.18)	-	-
LT/LH	0.37 (0.18)	0.65 (0.15)	-
LT/HH	1.05 (0.19)	0.03 (0.16)	0.68 (0.16)

Except for the difference between treatments HT/HH and LT/HH, all the differences are significant at the 5% level.

Four of these treatment differences were brought about by a difference in one environmental factor only, either temperature or VPD. These differences are brought out most clearly when expressed as the percentage reduction in photosynthesis or transpiration brought about by the difference in temperature or VPD (Table 3-III).

Table 3-III Percentage reductions in photosynthesis and transpiration brought about by a reduction in temperature or VPD.

<u>Environmental change</u>	Percentage reduction in	
	<u>Photosynthesis</u>	<u>Transpiration</u>
Reduced VPD at high temperature	15	44
Reduced VPD at low temperature	41	35
Reduced temperature at high VPD	24	16
Reduced temperature at low VPD	47	2(a)

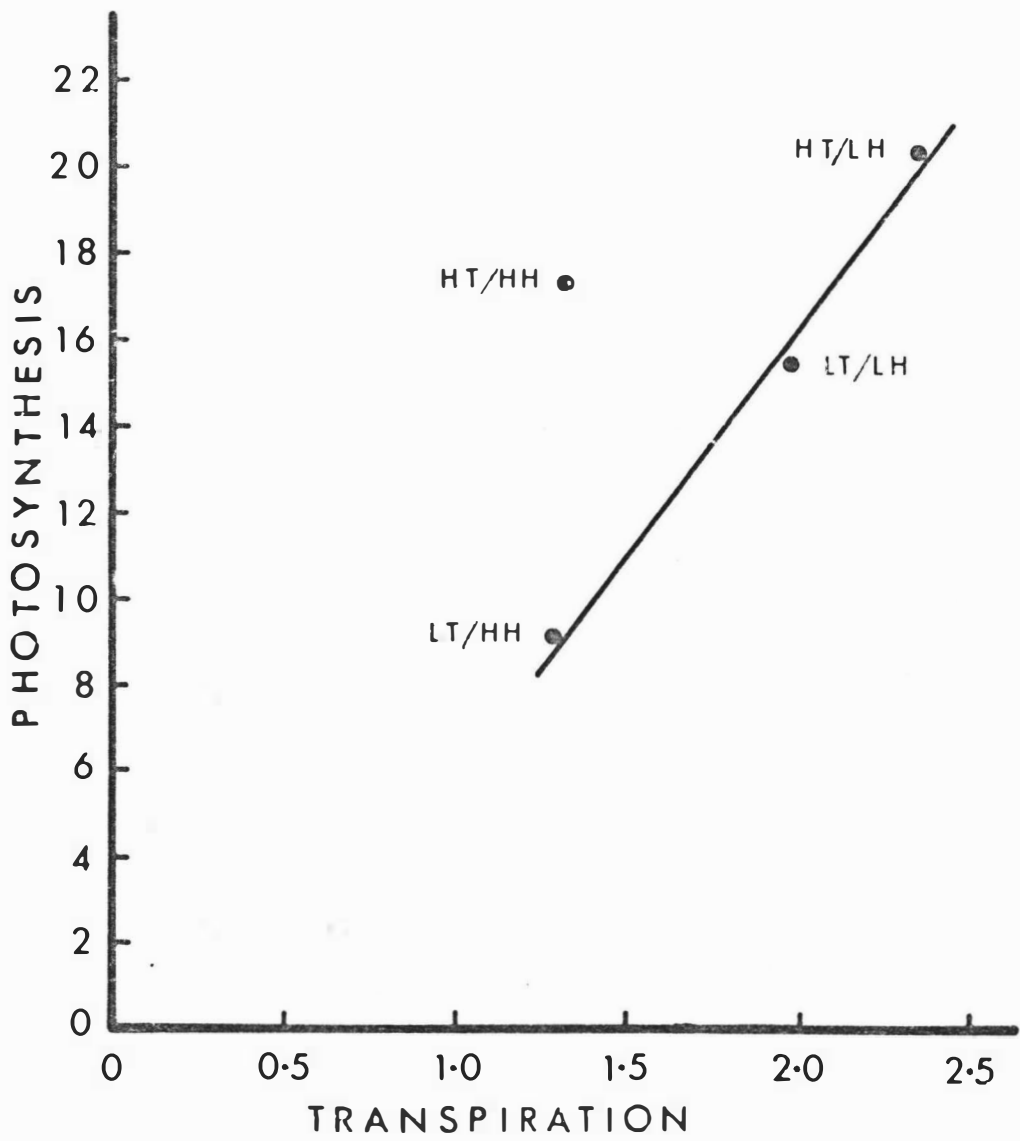
(a) Not significant at the 5% level.

Transpiration was less affected by difference in temperature than photosynthesis (2-16% reduction compared with 24-47%), but was strongly affected by reduced VPD. At high temperature photosynthesis was only slightly affected by reduced VPD, but at low temperature it was reduced by 41%, a change comparable with that brought about by reduction in temperature at low VPD.

When the mean maximum rates of photosynthesis and transpiration are plotted against each other (Fig. 3-3) the points for three of the treatments (HT/LH, LT/LH and LT/HH) fall close to a straight line, but that for the HT/HH treatment is well removed from this relationship. Thus whilst the ratio between the photosynthetic and transpiration rates is very similar for the three treatments yielding results that lie close to the line, that for the HT/HH plants is higher.

Figure 3-3

Relationship between the mean maximum rates of photosynthesis ($\text{mg CO}_2/\text{dm}^2/\text{hr}$) and transpiration ($\text{gm water}/\text{dm}^2/\text{hr}$).



Resistances to water vapour and CO₂ transfer

Resistances to water vapour and CO₂ diffusion out of and into the leaf are normally calculated from results obtained from single-leaf experiments where the measured rates of transpiration and photosynthesis can be related to a known area of leaf held under accurately known conditions. With whole plant experiments, such as those described here, the rates of transpiration and photosynthesis are averages for all the leaves on each plant. Resistances calculated from such data, whilst not quantitatively reliable, should be qualitatively valid and indicate the relative magnitude of the resistances operating under each set of environmental conditions.

Mean resistances to water vapour and CO₂ diffusion were therefore calculated for each treatment as described below: in all cases rates of photosynthesis and transpiration were related to both leaf surfaces (i.e. leaf area x 2).

The sum of the stomatal and boundary layer resistances to water vapour diffusion ($r_a + r_s$) is given by

$$\frac{M \quad H \quad (e_1 - e_a)}{C \quad L \quad E} \quad \text{sec/cm}$$

where M = density of moist air (gm/cm³)

H = specific heat of moist air (cal/gm/°K)

($e_1 - e_a$) = vapour pressure difference between the evaporating sites in the leaf and the air

C = the psychrometric constant (mb/°K)

L = latent heat of vapourisation of water

E = transpiration rate (gm/cm²/sec)

This is a rearrangement of equation VI of Kanemasu, Thurtell and Tanner (1969). It was assumed that the vapour pressure at the evaporating sites in the leaf (e_1) was equal to the saturation vapour pressure at leaf temperature. As leaf temperature was always very close, or equal, to air temperature (see section 'Leaf temperature' below), ($e_1 - e_a$) was set equal to the VPD for each treatment. Values of ($r_a + r_s$) for each treatment were thus obtained by substitution of the appropriate values of E and are given in Table 3-IV.

The analagous resistances to CO₂ diffusion ($r'_a + r'_s$) are related to the resistances for water vapour diffusion by the ratio of the coefficients for water vapour (D) and CO₂ (D') diffusion in air (Gaastra, 1959), i.e.

$$(r'_a + r'_s) = (r_a + r_s)(D/D') \text{ sec/cm}$$

Following Gale and Poljakoff-Mayber (1968) D and D' were taken as 0.258 and 0.165 cm²/sec respectively, so that

$$(r'_a + r'_s) = 1.56 (r_a + r_s) \text{ sec/cm}$$

Values of ($r'_a + r'_s$) so determined are given in Table 3-IV.

The total resistance to the diffusion of CO₂ from the

atmosphere to the chloroplast (r_t') is given by

$$\frac{(C_a - C_i)}{P} \quad \text{sec/cm}$$

where $(C_a - C_i)$ is the difference in CO_2 concentration between the atmosphere (C_a) and the chloroplast (C_i) in units of $\text{mg CO}_2/\text{cm}^3$ air, and P is the rate of photosynthesis ($\text{mg CO}_2/\text{cm}^2$ leaf surface/sec), (Holmgren, Jarvis and Jarvis, 1965).

Although some authors (e.g. Gaastra, 1959; Gale and Poljakoff-Mayber, 1968) have assumed C_i to be equal to zero, net CO_2 assimilation ceases in plants possessing the Calvin-type photosynthetic pathway (of which soybean is one) at atmospheric CO_2 concentrations considerably above zero. Setting C_i equal to the CO_2 compensation point is felt to be a preferable procedure and has also been adopted by, among others, Holmgren et al. (1965) and Whiteman and Koller (1967). In a previous investigation the CO_2 compensation point of soybean plants under HT/LH conditions had been found to be 100 ppm CO_2 , and in the absence of further data this value was used in the calculations for all treatments. $C_a - C_i$ was therefore set constant at $0.00036 \text{ mg CO}_2/\text{cm}^3$ for all treatments.

The values of r_t' thus determined (Table 3-IV) include, in addition to the boundary and stomatal resistances to CO_2 diffusion ($r_a' + r_s'$), a residual resistance which includes the resistance to diffusion in the intercellular spaces of

the leaf, the resistance to the movement of CO_2 into and in the liquid phase, and the chemical resistances associated with the biochemical component of the photosynthetic process. This residual resistance consisting of a variety of resistances, the magnitude of the contribution of each to the total being unknown, is here designated the mesophyll resistance (r'_m).

Thus

$$r'_t = (r'_a + r'_s) + r'_m \quad \text{sec/cm}$$

and therefore

$$r'_m = r'_t - (r'_a + r'_s) \quad \text{sec/cm}$$

The value of $(r'_a + r'_s)$ for each treatment is known, having been determined by conversion of the $(r_a + r_s)$ values for water vapour diffusion to the analogous CO_2 resistances. The calculated values of r'_m are given for each treatment in Table 3-IV.

Table 3-IV Mean resistances (sec/cm) to transfer of water vapour and CO_2 through the boundary layer and stomata ($r_a + r_s$) and $(r'_a + r'_s)$, total resistance to CO_2 transfer from the air to the chloroplasts (r'_t) and mesophyll resistance to CO_2 transfer within the leaf (r'_m) for plants grown under the four treatments. Mean maximum rates of transpiration (E) and photosynthesis (P) in units of gm water/dm²/hr and mg CO_2 /dm²/hr respectively.

Treatment	Water Vapour		CO_2			
	$(r_a + r_s)$	<u>E</u>	<u>r'_t</u>	<u>$(r'_a + r'_s)$</u>	<u>r'_m</u>	<u>P</u>
HT/LH	2.9	2.34	12.9	4.5	8.4	20.3
HT/HH	2.4	1.32	15.0	3.7	11.3	17.3
LT/LH	3.6	1.97	17.1	5.6	11.5	15.4
LT/HH	2.4	1.29	27.7	3.7	24.0	9.1

The boundary layer resistances (r_a and r'_a) were probably small as the windspeed through the plant chamber was 60cm/sec and sufficient to cause constant slight leaf flutter. It is therefore probable that between-treatment differences in the values of $(r_a + r_s)$ and $(r'_a + r'_s)$ are mainly attributable to differences in the stomatal component of this combined resistance.

For water vapour diffusion the value of $(r_a + r_s)$ is the same for both HH treatments, the plants under which had similar transpiration rates, whereas $(r_a + r_s)$ is higher for the LT/LH than for the HT/LH plants. Plants under the LT/LH treatment had a lower rate of transpiration than those under the HT/LH treatment.

It is apparent that for all treatments the mesophyll resistance (r'_m) is the largest component of the total resistance to CO_2 transfer (r'_t), and that it differs much more between treatments than does the combined boundary layer and stomatal resistance ($r'_a + r'_s$). At each temperature r'_m is greater in plants grown under the HH treatment. The difference in the value of r'_m between the two LT treatments is especially marked, as is the difference in photosynthetic rate between plants grown under these treatments.

Dark respiration

The mean maximum dark respiration rates for plants under each treatment are given in Table 3-V.

Table 3-V Mean maximum dark respiration rates (mg CO₂/dm²/hr) and standard errors for plants grown under the four treatments.

<u>Treatment</u>	<u>Respiration rate</u>	<u>Standard error</u>
HT/LH	2.90	0.19
HT/HH	2.47	0.19
LT/LH	1.83	0.35
LT/HH	1.88	0.16

At the 5% level only the differences between HT/LH and LT/LH and HT/LH and LT/HH plant rates are significant, although the differences between the rates for the two LT treatments and the HT/HH treatment fall just short of significance at this level. Plants at the higher temperature had the higher respiration rates.

Differences between rates cannot be attributed directly to VPD differences as these were virtually the same for all treatments during the dark period.

Night-time transpiration

The mean night-time transpiration rates are given in Table 3-VI.

Table 3-VI Mean night-time transpiration rates ($\text{gm}/\text{dm}^2/\text{hr}$) and standard errors of plants grown under the four treatments.

<u>Treatment</u>	<u>Night-time transpiration</u>	<u>Standard error</u>
HT/LH	0.08	0.02
HT/HH	0.13	0.01
LT/LH	0.21	0.03
LT/HH	0.21	0.01

Night-time transpiration rates were higher under LT than under HT conditions. Differences between rates at the same temperature are not significant at the 5% level: all other treatment differences are significant at this level.

Leaf temperature

Throughout these experiments leaf temperature during the light period was very close, or equal, to ambient air temperature. The maximum difference between leaf and air temperature was less than $\pm 0.5^\circ\text{C}$. During the dark period leaf temperature was equal to air temperature.

Morphological effects

Morphological differences between plants grown under the four treatments (see Chapter 6) did not appear to be correlated with the differences in the mean maximum rates of photosynthesis and transpiration.

DISCUSSION

Dark respiration

The higher respiration rates at the higher temperature (Table 3-V) are expected, as respiration is known to increase with temperature (Meyer and Anderson, 1952). Daytime VPD levels appear to have had no effect on the dark respiration rates, the differences between which may be attributed to temperature. The percentage difference between the means of the high and low temperature rates is 44.9% which corresponds to a Q_{10} of 1.8. This is in agreement with the Q_{10} of most plant tissues in the 10-30°C range (Meyer and Anderson, 1952 p. 408).

Night-time transpiration

The differences in night-time transpiration rates between the HT and LT treatments (Table 3-VI) cannot be attributed to differences in VPD as this was very similar for all treatments (2.0-2.5 mb). Although it is possible that the stomata were more completely closed under HT conditions, it is suggested that the differences may be attributable to the nature of the cuticle developed under the different conditions. Skoss (1955) and Hull (1958) have shown with Nicotiana glauca and Prosopis juliflora respectively that the proportion of wax in the cuticle increases with increasing temperature. Removal of the wax fraction of the cuticle has been shown to increase water loss from the leaves (Skoss, 1955; Clark and Levitt, 1956; Hall

and Jones, 1961) and it is therefore possible that the increased wax content of cuticles developed under high temperatures reduces cuticular transpiration.

If the stomata were closed during the dark period, and the plants grown under the HT treatments had a greater proportion of wax in the cuticle than plants grown under the LT treatments, it is possible that the differences in night-time transpiration rates were due to differences in the wax content of the cuticles.

Transpiration

The rates of transpiration of plants under the two HH treatments were very similar, whereas under LH conditions there was a significant effect of temperature, the rate of transpiration of the LT/LH plants being lower than that of the HT/LH plants (Fig. 3-1).

The construction of the plant chamber in which these experiments were carried out is such that the pot is exposed to the same air stream as the leaves and stem, and consequently the soil and root mass will tend to attain the same temperature as the air and the leaves.

Low soil temperature may limit uptake of water by the roots through its effect on the permeability of the roots to water (permeability decreasing with decrease in temperature) and on the viscosity of water, which increases with decrease in temperature (Kramer, 1969).

With a low flux of water through the soil-plant-atmosphere system (as under low VPD conditions), the absorption of water by the roots may not be limited by effects of temperature, and the rate of uptake may be sufficient to satisfy the transpiration demand, but when the VPD is high (thus increasing the transpiration demand) the rate of uptake may be limited at low soil temperatures as described above. At higher soil temperatures the effect is not apparent.

Interactive effects on transpiration between soil temperature and VPD, qualitatively similar to the results given here, have been reported for sunflower (Tew, Taylor and Ashcroft, 1963), bean (Unger and Danielson, 1967) and mesquite (Wendt, Haas and Runkles, 1968). Babalola, Boersma and Youngberg (1968) have shown that the increase in the viscosity of water between 26.7 and 21.1°C can markedly reduce the transpiration rate of Monterey pine seedlings.

It would thus appear that under the HH treatments where the transpiration rate was low, water uptake was sufficiently rapid to satisfy the demand. Temperature had no limiting effect. Under the LH conditions at low temperature the rate of removal of water from the soil by the roots was limited by the effects of temperature on root permeability and the viscosity of water. The consequent failure to satisfy the

transpiration demand probably resulted in a fall in leaf water potential and an increase in stomatal resistance (Cowan, 1965). This possibility is borne out by the results given in Table 3-IV where it is shown that plants under the LT/LH treatment did have a higher ($r_a + r_s$) than plants grown under the HT/LH treatment. A significant fall in leaf water potential need not necessarily be reflected in a decrease in RWC. The RWC-leaf water potential relationship given for barley (Hordeum vulgare) by Millar, Duysen and Wilkinson (1968) shows that at high RWC's a fall in leaf water potential of 15 bars was accompanied by a decrease in RWC of little over 1%. If the RWC - leaf water potential relationship for soybean were similar to this, it would account for the observation that under conditions of adequate water supply there were, with the techniques used, no observable differences in the RWC's of the plants under the various treatments.

Rufelt et al (1963) concluded that with open stomata transpiration depends to a large extent on the vapour pressure difference between leaf and air. The present results (Fig. 3-2) would seem to confirm this view, the lower rate of transpiration under LT/LH than under HT/LH conditions being apparently attributable to an increased stomatal resistance in the plants under the former treatment.

Photosynthesis

Soybean is grown extensively in the United States in areas having high summer temperatures and comparatively low relative humidities. Extensive breeding programmes have been carried out on this species and it would therefore be expected that it would show a high temperature optimum for photosynthesis, possibly as high as 35°C (Hofstra and Hesketh, 1969a). It is therefore not surprising that it should have higher rates of photosynthesis at 27.5°C than at 22.5°C (Fig. 3-1). The mean maximum rates of photosynthesis of plants under the two HT treatments (20.3 and 17.3 mg CO₂/dm²/hr) are in good agreement with reported rates for cv. Merit and other Group O varieties. Dreger, Brun and Cooper (1969) reported an average photosynthetic rate of 23 mg CO₂/dm²/hr for cv. Merit at 27°C, and Curtis, Cgren and Hageman (1969) gave rates of 23 and 18 mg CO₂/dm²/hr for the varieties Grant and Mandarin respectively at 29°C. However, in neither of these reports is any mention made of the atmospheric VPD, or whether or not it was controlled.

At both high and low temperatures photosynthesis was lower under HH conditions (Fig. 3-1), the depression being greater at low temperature (Table 3-III). The suggestion of Pallas et al (1967) that reduced leaf temperatures at high VPD may contribute to increased photosynthetic rates is not applicable to the present results as leaf temperature was virtually the same as air temperature in all cases.

It would appear (Table 3-IV) that the rate of photosynthesis under each treatment was primarily determined by the mesophyll resistance to CO_2 transfer (r'_m). For all treatments ($r'_a + r'_s$) is the minor component of the total resistance to CO_2 transfer (r'_t) and varies little between treatments compared with the between-treatment variation in r'_m . The rates of photosynthesis are inversely proportional to the magnitude of r'_m . The conclusion that r'_m may be the dominant influence on the rate of CO_2 uptake in soybean plants adequately supplied with water is in agreement with the findings of Bierhuizen and Slatyer (1964) for cotton.

Troughton and Slatyer (1969) found that the r'_m of leaves of cotton plants grown under a $30^\circ/25^\circ\text{C}$ day/night temperature regime was independent of temperature between 21.5 and 38.5°C . It would therefore appear possible that a substantial part, at least, of the r'_m is determined by the environmental conditions under which the plants are grown.

CHAPTER 4

THE EFFECT OF WATER STRESS ON THE CO₂ EXCHANGE AND TRANSPIRATION RATES OF SOYBEAN PLANTS UNDER CONTRASTING ENVIRONMENTAL CONDITIONS

INTRODUCTION

In Chapter 1 it was pointed out that little use has been made of controlled climate facilities in the investigation of the influence of environmental factors on the effects of water stress on CO₂ exchange and transpiration. In the few cases where this has been attempted the plants were grown under one set of conditions and then subjected to stress under other environmental regimes. In such circumstances the possibility that the response of the plants to stress was modified by the conditions under which they were originally grown cannot be ruled out.

In the experiments discussed in this chapter the responses of CO_2 exchange and transpiration of soybean plants to water stress were measured under essentially the same conditions as those under which they were grown (see Chapter 2, section II). The mean maximum rates of photosynthesis and transpiration of soybean plants under these conditions when water was non-limiting were discussed in the previous chapter.

METHODS

In general, experiments were carried out as detailed in Chapter 2 (Materials and Methods). Plants were transferred from the growth cabinet to the plant chamber of the CO_2 exchange-transpiration measuring equipment soon after watering, and remained in the chamber until they were severely wilted, no water being given while they were in the chamber. Under the HT/HH and LT/HH treatments total water use by the plants was low, and in order to obtain data on their response to severe levels of stress water was withheld from some of these plants while they were still in the growth cabinet. As growth cabinet and plant chamber conditions were very similar this procedure was consistent with the other experiments.

Samples of leaf and soil were taken periodically for the determination of plant and soil water status (see Chapter 2, section V). This was normally done daily at 1415 hours (chamber midday) and again shortly before 2015

hours (the end of the light period), but in some cases additional samples were taken at other times and in others sampling was foregone in the early stages of the run. This omission of sampling was to conserve plant material so that there would be no lack of leaf available for sampling when the plant was under stress. (Each RWC sampling removed approximately 6.7 cm^2 of leaf which was small compared with total leaf area which averaged c. 500 cm^2 . However, earlier experience had shown that when discs were removed from closely adjacent sites, the intervening tissue often died. To avoid this sampling sites were always separated by at least 1.0 cm. The effect of this precaution was to reduce the area available for sampling).

In the results which follow, RWC and soil moisture tension are related to rates of photosynthesis and transpiration for the hour previous to sampling. As one of the sampling times (2015 hours) corresponded to the end of the light period, there were no data for photosynthesis or transpiration following this sampling. RWC at 1415 hours showed the same relationship to these processes regardless of whether the rates during the preceding or following hours were considered.

It is not possible to relate dark respiration rates to RWC as RWC samples were not taken during the dark period (2015-0815 hours). The RWC at 2015 hours cannot be related to ensuing respiration rates because leaf water content increased during the night (Fig. 4-3).

No data on stomatal aperture could be obtained. Impression techniques using quick-setting silicon rubber (Zelitch, 1961) were found to be unsuitable as the leaf frequently tore when the rubber was removed, possibly because of the leaf hairs becoming embedded in the rubber, and when tearing did not occur necrotic patches appeared at the sampling site within 24 hours. The reliability of the impression technique has recently been queried (Glinka and Meidner, 1968; Leshem and Thaine, 1969), and it appears that results so obtained may be of questionable value. No equipment for the measurement of stomatal diffusion resistance was available.

RESULTS

Development of water stress with time

Representative time courses of photosynthesis and transpiration for each treatment are shown in Figs. 4-1 and 4-2 respectively. Because considerable differences between treatments were found in the actual rates of these processes, the data have been normalised to the rates obtaining during the hour ending 1415 hours on the first day of the experiment for each treatment. The normalised rates are referred to as 'proportional rates', where

$$\text{proportional rate at time } t = \frac{\text{Actual rate at time } t}{\text{Actual rate for hour ending 1415 hrs on day 1.}}$$

Figure 4-1

Representative time courses of photosynthesis under the four treatments.

Rates of photosynthesis are proportional to the rate obtaining during the hour ended 1415 hrs. on the first day of each experiment.

Solid circles - high temperature

Open circles - low temperature

(N.B. The dark period time scale is compressed).

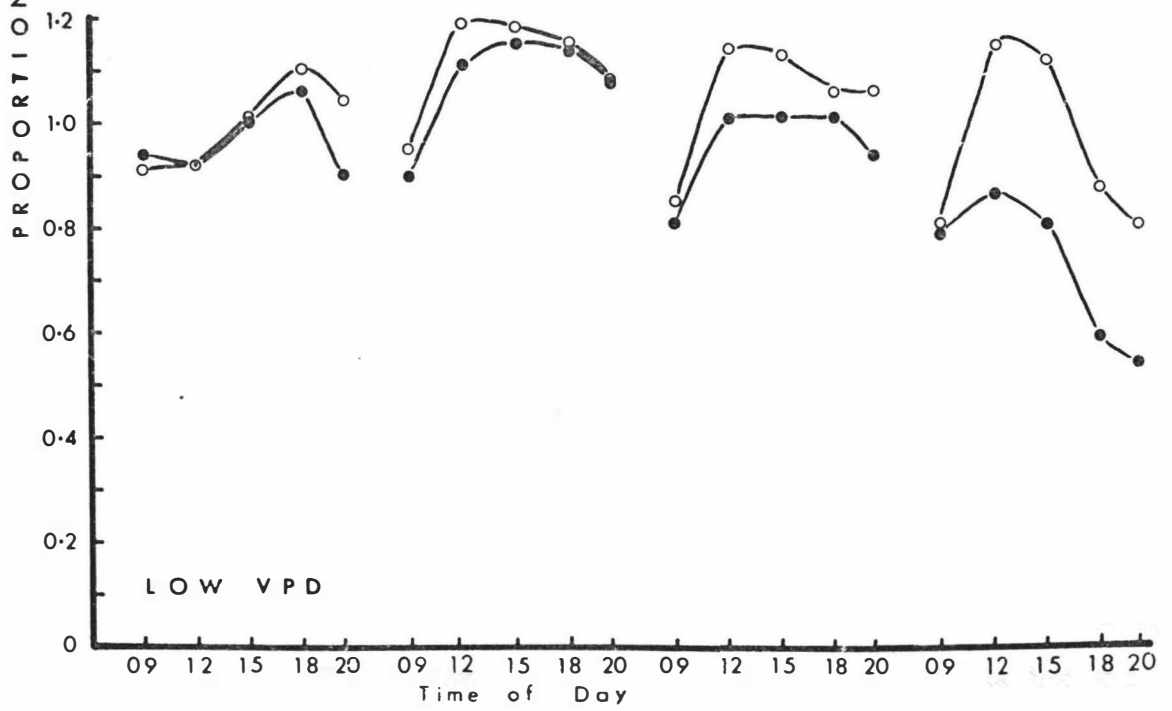
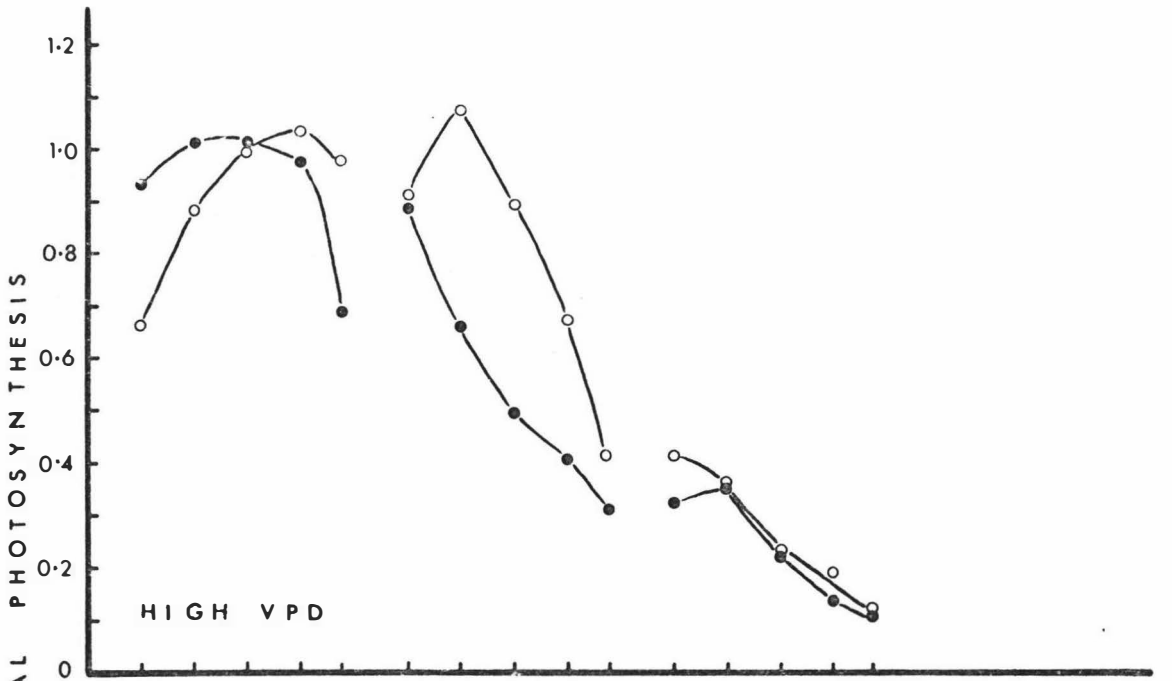


Figure 4-2

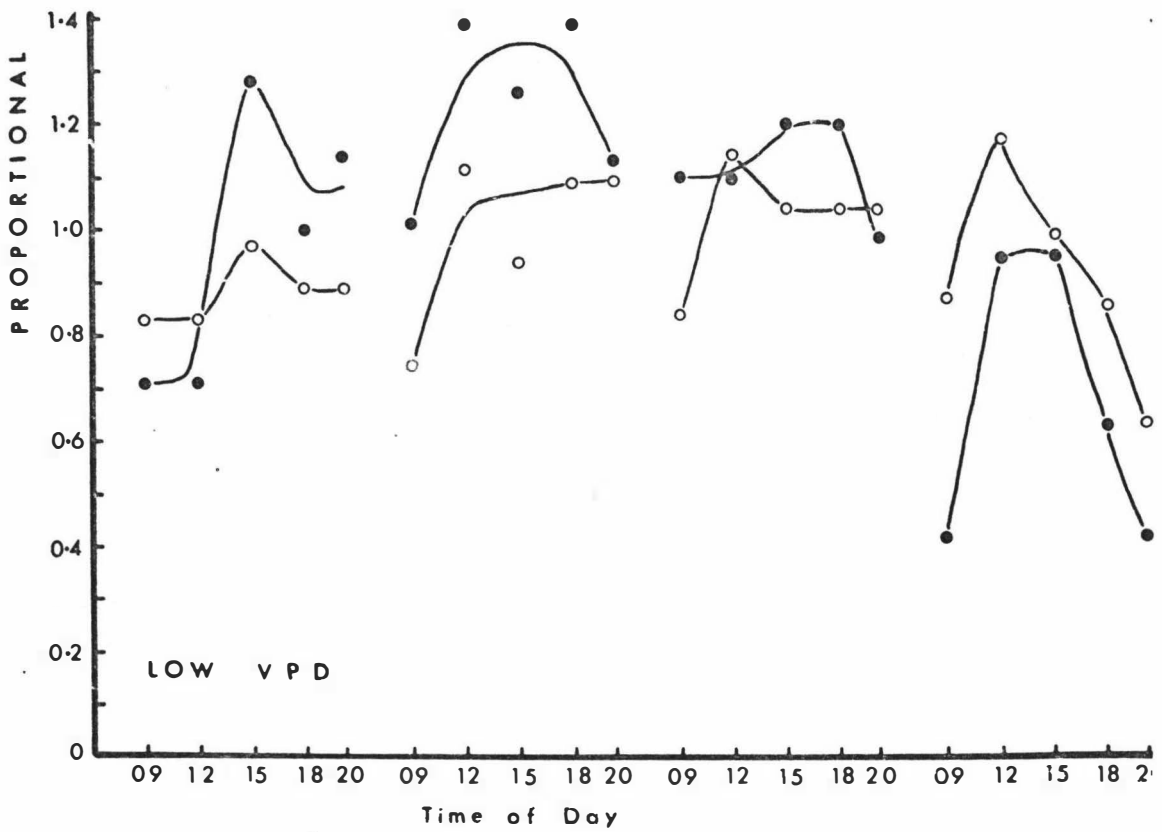
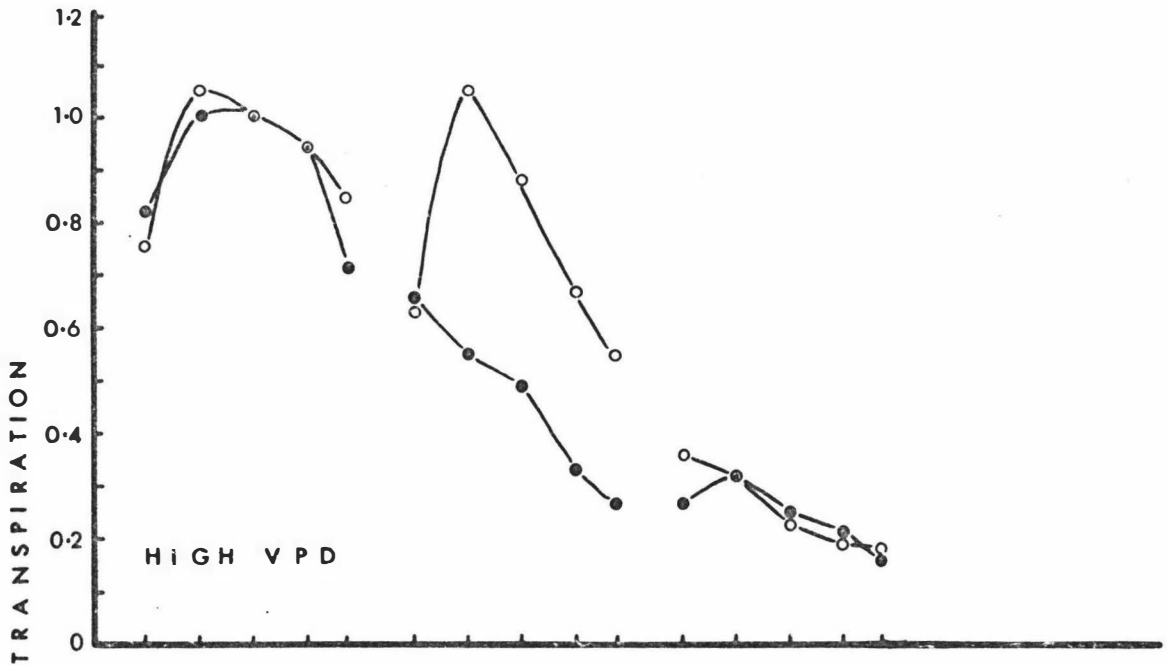
Representative time courses of transpiration under the four treatments.

Rates of transpiration are proportional to the rate obtaining during the hour ended 1415 hrs. on the first day of each experiment.

Solid circles - high temperature

Open circles - low temperature

(N.B. The dark period time scale is compressed).



It is apparent that the time course pattern of each experiment is determined for both photosynthesis and transpiration by the VPD and not by the air temperature. Thus the time courses of photosynthesis and transpiration are similar for the HT/LH and LT/LH treatments and also for the HT/HH and LT/HH treatments. The time courses of the two processes have been shown in separate figures in the interests of clarity, but comparison shows that they are very similar for any one treatment.

Plants under the two LH treatments developed water deficits by the end of the first, or early on the second, day and their rates of photosynthesis and transpiration started to decline. Under the two HH treatments plants did not become stressed until the fourth day, but once this occurred the rate of decline of photosynthetic and transpiration rates, as judged by the slope of the appropriate curves, was similar to that of the LH plants. For all treatments the decline in these processes commenced at soil moisture tensions between 0.20 and 0.25 atm.

The changes in transpiration rate, RWC and soil moisture tension during the course of a three day experiment under HT/LH conditions are shown in Fig. 4-3. In this experiment RWC samples were taken at 0815 hours (the beginning of the light period) rather than at 1415 hours. RWC showed an overnight recovery to values characteristic of unstressed

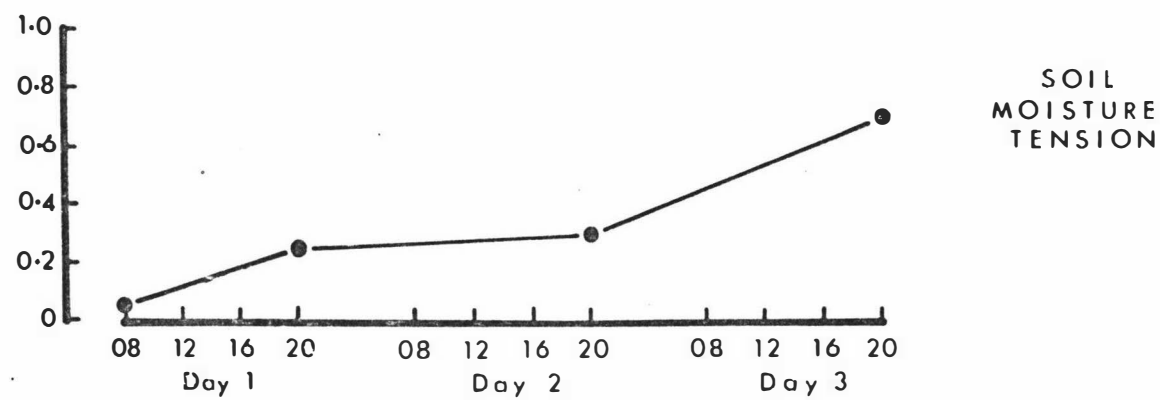
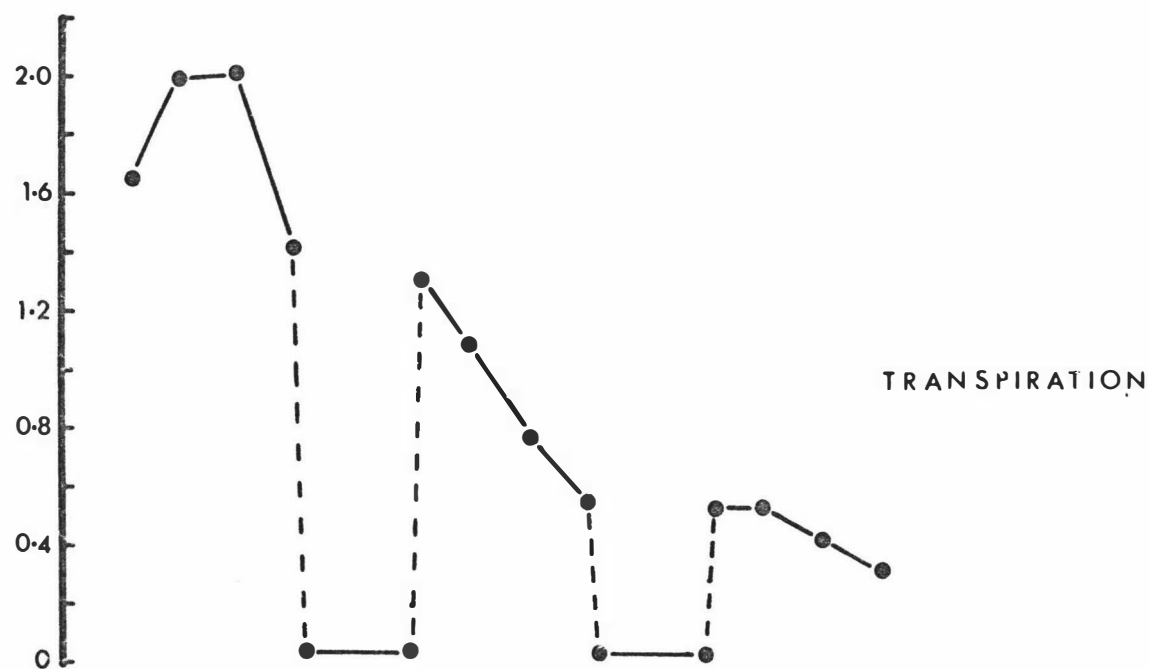
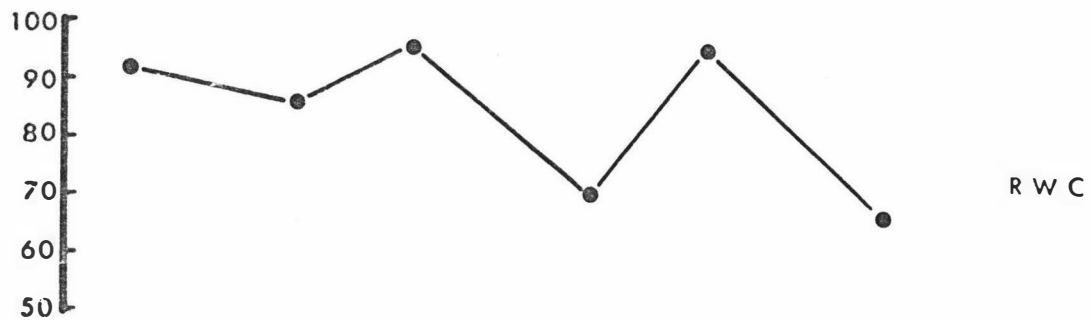
Figure 4-3

Changes in RWC (%), transpiration rate (gm water/dm²/hr) and soil moisture tension (atm.) during the course of a three day experiment under HT/LH conditions.

The time scale for the dark period (2015 - 0815 hrs) is slightly compressed. Light and dark periods were both of 12 hours duration.

Day/night temperatures : 27.5/22.5°C

Day/night VPD : 11.7/2.0 mb.



plants (91% +), but fell rapidly on the second and third days after the start of the light period.

Observation of plants in the plant chamber necessitated the removal of the light funnel above the chamber, with consequent disturbance to the light regime. It is not therefore possible to state exactly when plants started to show signs of wilting, but on a few occasions slight wilting was evident when RWC and soil samples were taken. The highest RWC's measured on such plants were 86% under the HT/LH treatment and 80-82% under the other three treatments.

Relationship between the rates of photosynthesis and transpiration

The similarity of the photosynthesis and transpiration time courses for any one treatment (Figs. 4-1 and 4-2) has been commented on previously, and suggests a close relationship between the rates of these processes. It is shown in Fig. 4-4 that the relationship between photosynthesis and transpiration is linear for any given treatment at soil moisture tensions between 0 and 0.4 atm. The regressions of photosynthesis on transpiration (Fig. 4-4) were calculated from the rates of these processes for the hours ending 1315 to 1615 inclusive, of every day and for every plant used in the experiments. (Figures showing the individual points will be found in Appendix 7). The data for all times outside the 1315 and 1615 period conformed to the same relationship, which is

Figure 4-4

Relationships between rates of photosynthesis ($\text{mg CO}_2/\text{dm}^2/\text{hr}$) and transpiration ($\text{gm water}/\text{dm}^2/\text{hr}$) between 0 and 0.4 atm. soil moisture tension of plants grown under the four treatments.

Regression equations and correlation coefficients (r) were calculated from the rates for hours ending 1315 to 1615 inclusive for every day and every plant used in the experiments.

$$\text{HT/LH} : P = 7.41 T + 1.33 \quad (r = 0.94)$$

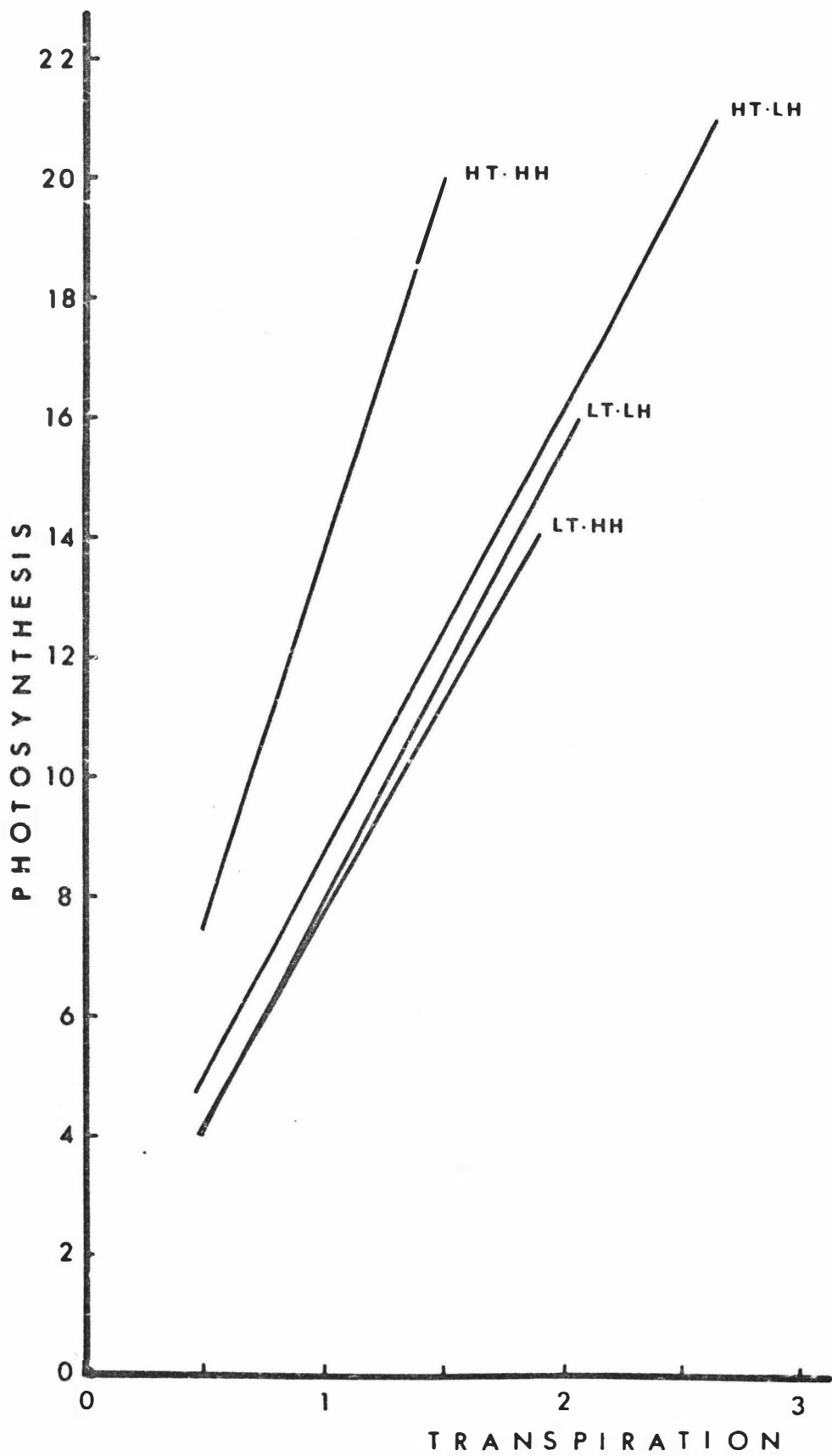
$$\text{HT/HH} : P = 12.45 T + 1.30 \quad (r = 0.95)$$

$$\text{LT/LH} : P = 7.58 T + 0.37 \quad (r = 0.96)$$

$$\text{LT/HH} : P = 7.09 T + 0.60 \quad (r = 0.85)$$

where P and T are the rates of photosynthesis and transpiration respectively.

(The individual points from which these relationships were derived are given in Fig. A7-1, Appendix 7).



therefore valid for all times during the light period.

The photosynthesis-transpiration relationship is similar for plants under all treatments except HT/HH. Under this treatment the plants had a higher rate of photosynthesis per unit rate of transpiration at all levels of stress between 0 and 0.4 atm. soil moisture tension. It will be recalled that under conditions of adequate water supply (Chapter 3) the HT/HH plants also had a higher rate of photosynthesis per unit rate of transpiration than plants under the other treatments, and it would therefore appear that the factors responsible for this difference operated under both stressed and non-stressed conditions. Nevertheless, a linear relationship between the rates of photosynthesis and transpiration as stress increases is a feature common to plants under all four treatments.

Thus associated rates of photosynthesis and transpiration of approximately $12 \text{ mg CO}_2/\text{dm}^2/\text{hr}$ and $1.5 \text{ gm water}/\text{dm}^2/\text{hr}$ respectively are found under all treatments except HT/HH, but whereas such a combination represents the maximum rates achieved under the LT/HH treatment it is associated with a certain degree of water stress under the other two HT/LH and LT/LH treatments.

Effects of soil moisture tension on transpiration and photosynthesis

The effect of soil moisture tension on transpiration rate is shown in Fig. 4-5. At soil moisture tensions below 0.2 atm. the transpiration rate appears to be independent of soil moisture and mainly determined by the VPD, plants under the two LH treatments having higher rates of transpiration than those under the two HH treatments.

Between 0.2 and 0.4 atm. soil moisture tension there is a rapid decline in the transpiration rates of plants under all treatments, but for each treatment there is a considerable variation in rate at any given tension. It appears that other factors, in addition to soil moisture tension, were controlling the rate of transpiration over this range of tensions (0.2 to 0.4 atm.).

At about 0.4 atm. soil moisture tension there is an abrupt change in the pattern of response, the transpiration rates of plants under all four treatments becoming very similar (approximately $0.5 \text{ gm/dm}^2/\text{hr}$). At soil tensions above 0.4 atm. the rate of transpiration declines gradually and appears to be unrelated to the atmospheric conditions but limited by the soil moisture tension.

The response of photosynthesis to soil moisture tension (Fig. 4-6) is generally similar to that of transpiration,

Figure 4-5

Effect of soil moisture tension on the rate of transpiration (gm water/dm²/hr) of plants under the four treatments.

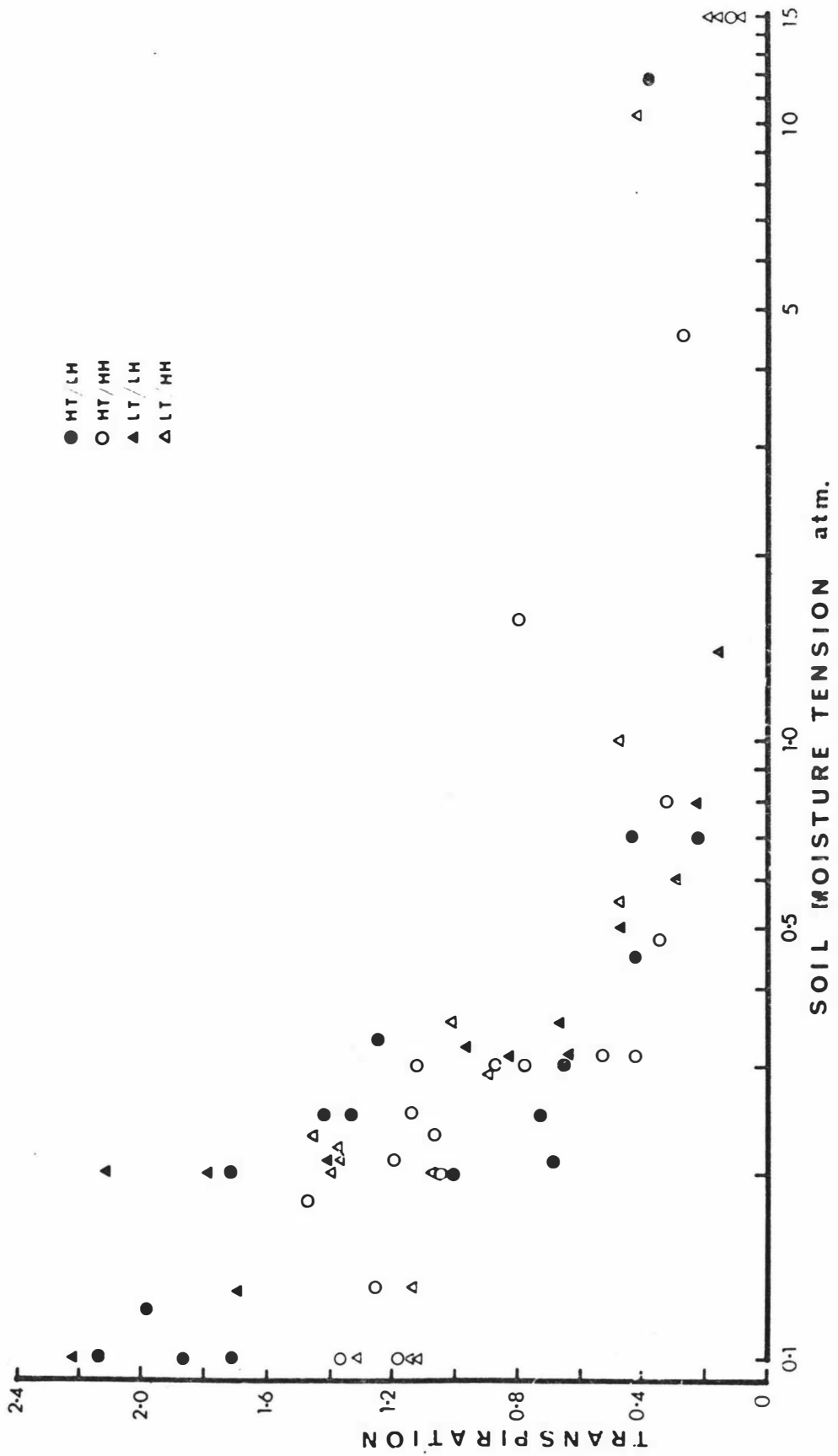
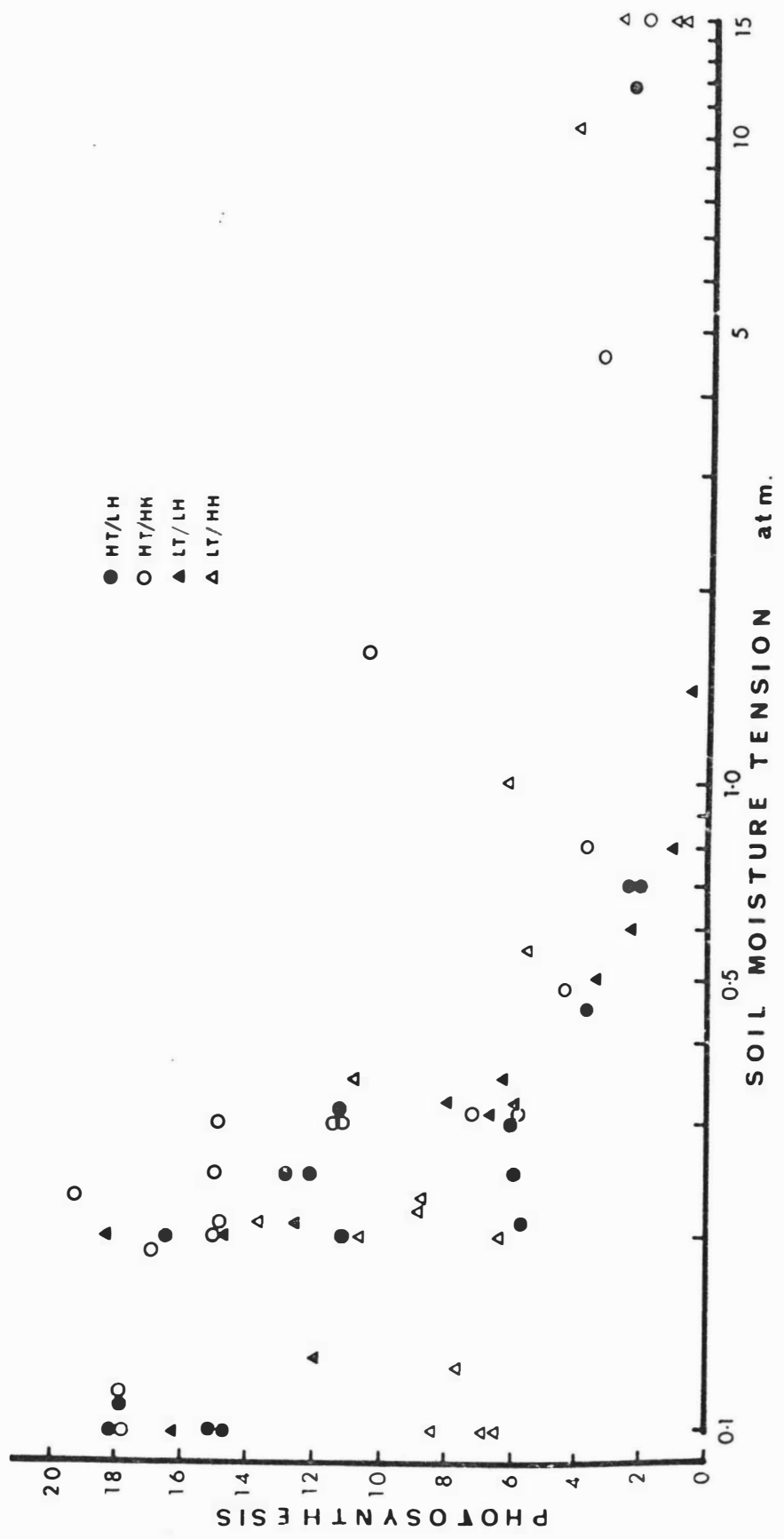


Figure 4-6

Effect of soil moisture tension on the rate of photosynthesis ($\text{mg CO}_2/\text{dm}^2/\text{hr}$) of plants under the four treatments.



the main difference being a rather greater scatter of points at any given tension. At 0.4 atm. soil moisture tension the rate of photosynthesis of plants under all treatments was about $5 \text{ mg CO}_2/\text{dm}^2/\text{hr}$. At tensions above 0.4 atm. the rate of photosynthesis declined slowly, and rates of between 1 and $3 \text{ mg CO}_2/\text{dm}^2/\text{hr}$ were recorded at soil moisture tensions between 11 and 15 atm. In view of the close relationship between the rates of photosynthesis and transpiration (Fig. 4-4) the similarity of the response of these processes to increasing soil moisture tension is not unexpected.

The data of Figs. 4-5 and 4-6 were converted to relative rates by dividing the actual rate of transpiration or photosynthesis by the mean maximum rate of transpiration or photosynthesis for the appropriate treatment. The mean maximum rates for each treatment were discussed in Chapter 3 and are given again in Table 4-I.

Table 4-I Mean maximum rates of photosynthesis and transpiration of plants under the four treatments. Standard errors in brackets.

<u>Treatment</u>	<u>Photosynthesis</u> <u>(mg CO₂/dm²/hr)</u>	<u>Transpiration</u> <u>(gm water/dm²/hr)</u>
HT/LH	20.3 (0.73)	2.34 (0.08)
HT/HH	17.3 (0.49)	1.32 (0.04)
LT/LH	15.4 (0.64)	1.97 (0.07)
LT/HH	9.1 (0.79)	1.29 (0.05)

Thus a photosynthetic rate of $16.5 \text{ mg CO}_2/\text{dm}^2/\text{hr}$ under HT/LH conditions corresponds to a relative photosynthetic rate of 0.81 ($16.5/20.3$) whereas under HT/HH conditions it would represent a relative rate of 0.95 ($16.5/17.3$). Expression of the data as relative rates facilitates direct between-treatment comparisons of the effects of a given degree of stress. Such comparisons based on actual rates are complicated by the large differences in rates between treatments.

Relative rates of transpiration and photosynthesis are shown plotted against soil moisture tension in Figs. 4-7 and 4-8 respectively. At soil moisture tensions below 0.2 atm., the relative rates are independent of soil moisture tension, but start to decline at tensions greater than 0.2 atm. The relative rates of transpiration and photosynthesis at any tension greater than 0.2 atm. tend to be lower under the two LH treatments than under the two HH treatments, although there is comparatively little difference between the responses of the plants under the LT/LH and HT/HH treatments.

Figure 4-7

Effect of soil moisture tension on the relative
transpiration rates of plants under the four
treatments.

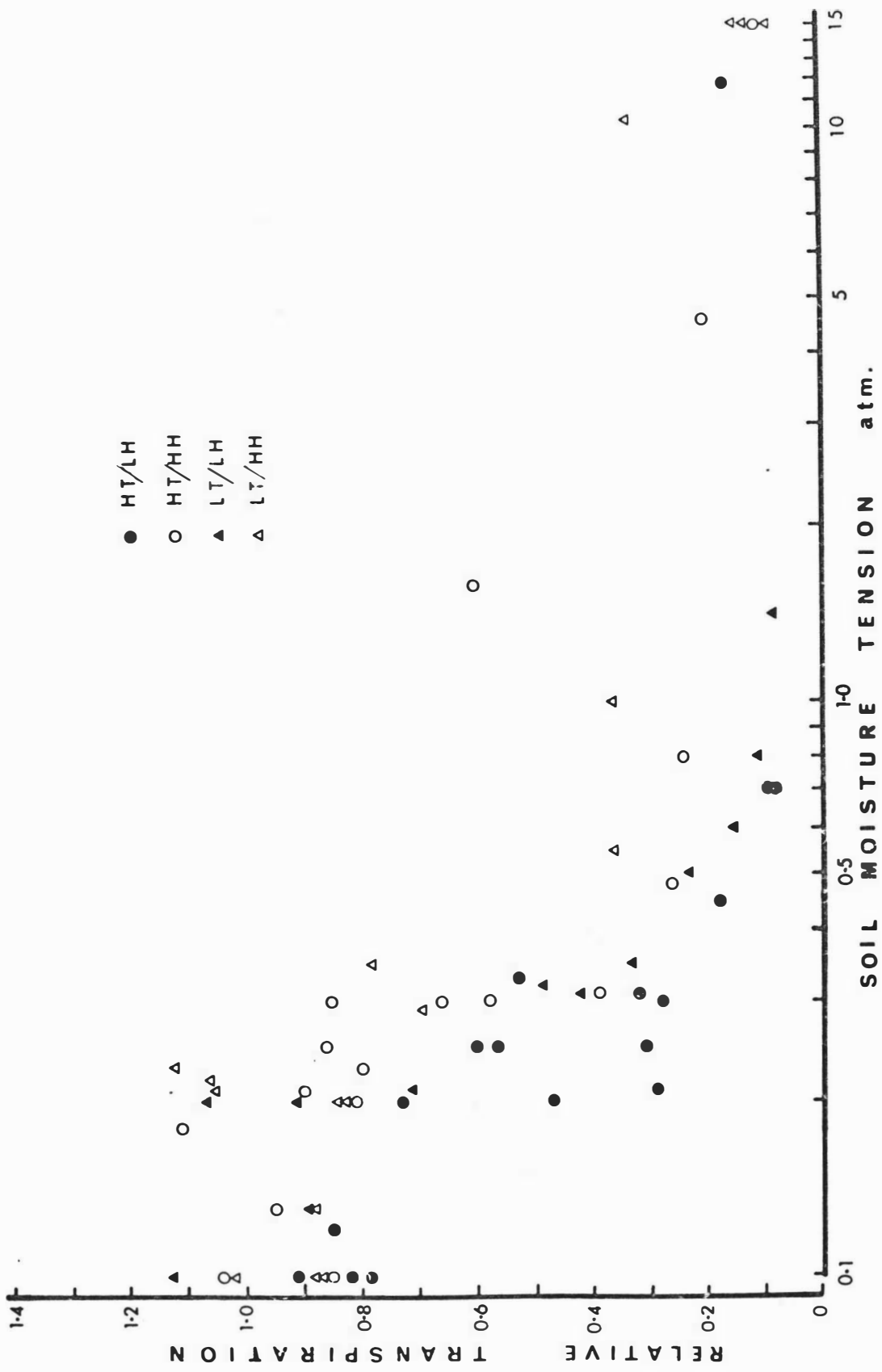
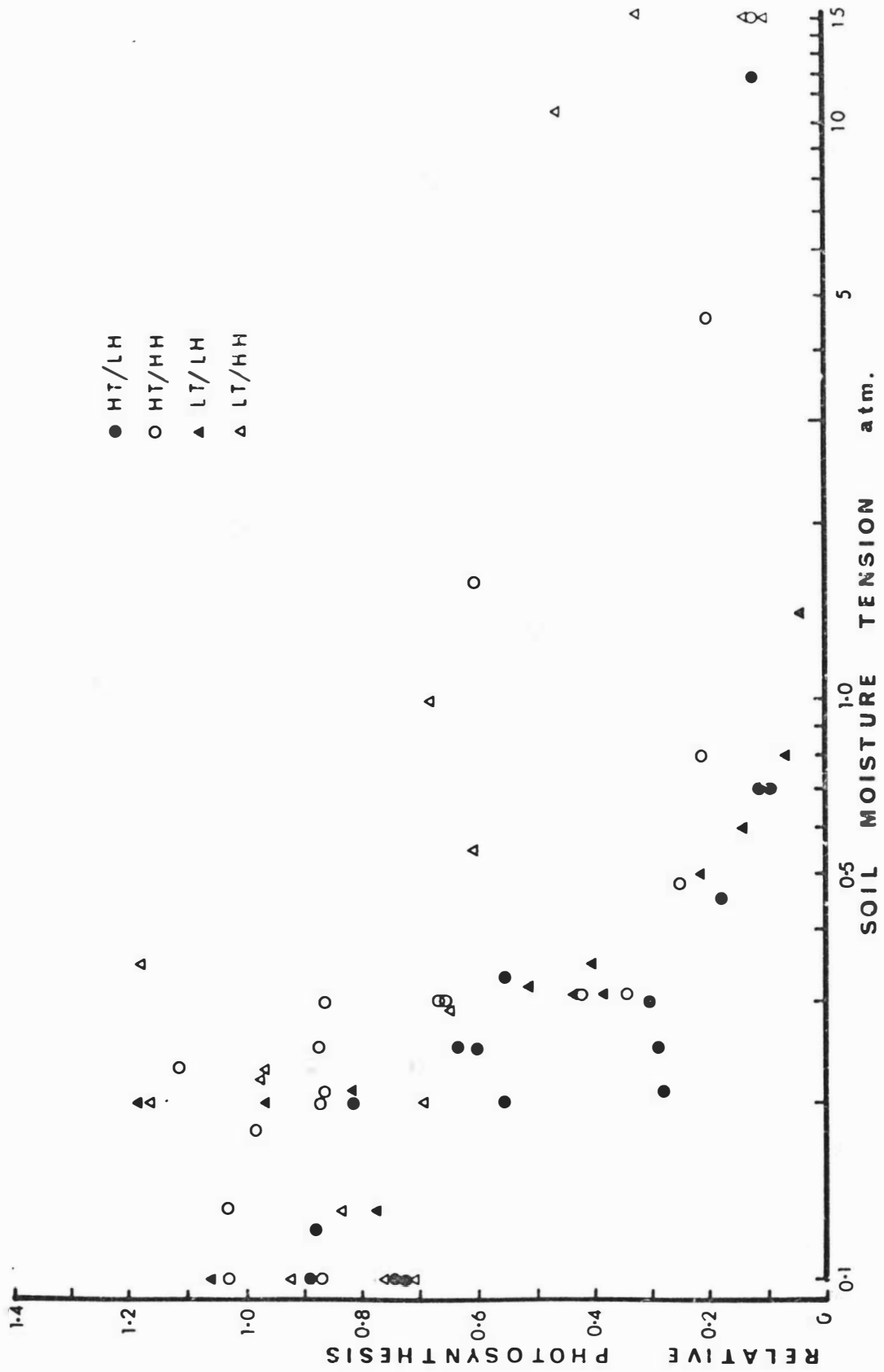


Figure 4-8

Effect of soil moisture tension on the relative rates of photosynthesis of plants under the four treatments.



RWC and soil moisture tension

The relationship between RWC and soil moisture tension (Fig. 4-9) is of a similar nature to the relative rate-soil moisture tension relationships, i.e. the highest values at any given tension above 0.2 atm. are associated with plants under the HH treatments and the lowest with those under the LH treatments. However the RWC-soil moisture tension relationships for the HT/HH and LT/LH treatments are similar.

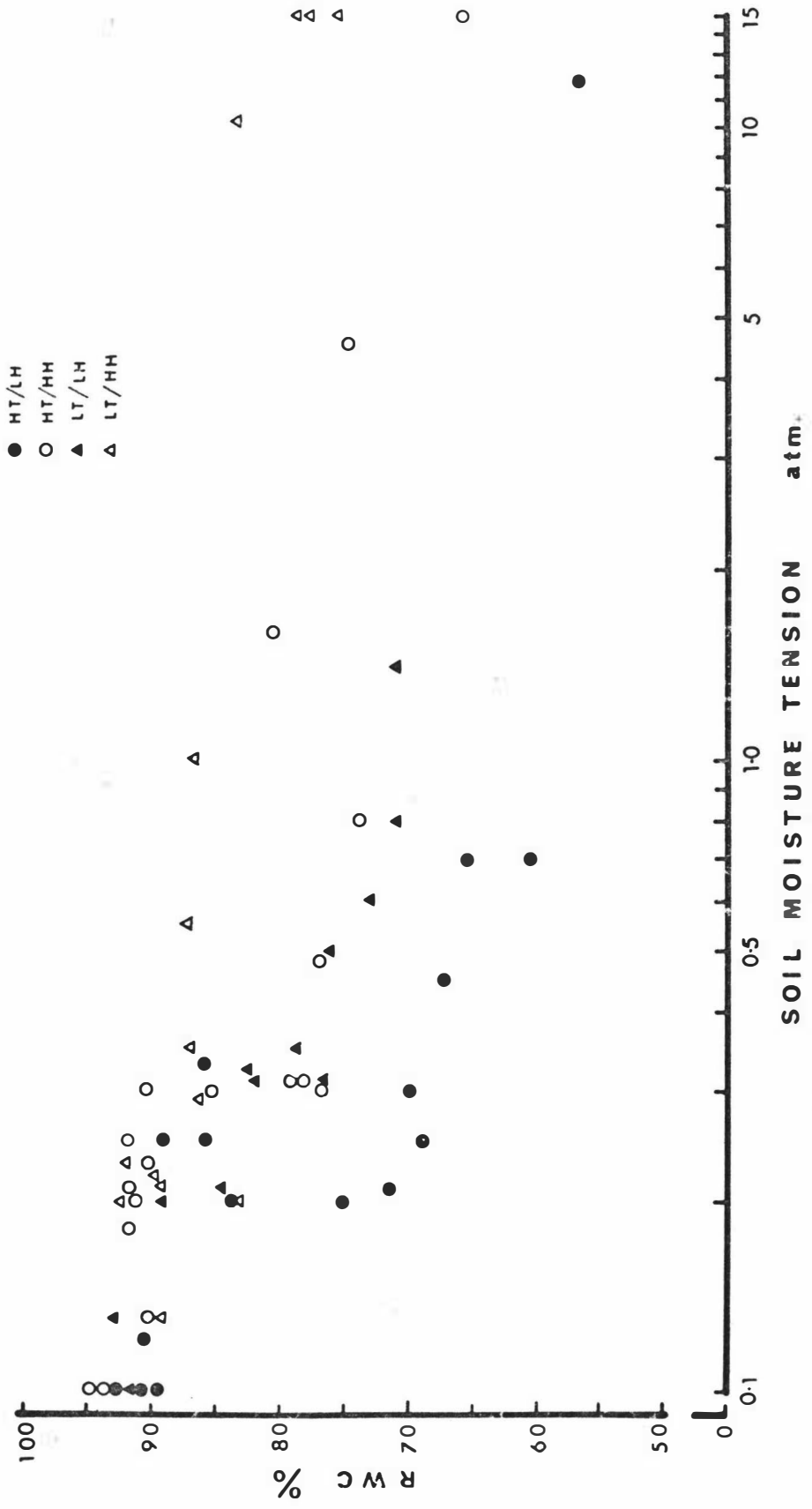
Below about 0.2 atm. soil moisture tension RWC is independent of tension and similar for plants under all four treatments. Between 0.25 and 0.4 atm. tension RWC tends to fall rapidly, except for plants under the LT/HH treatment, and thereafter the rate of decline is less rapid. At 0.4 atm. tension there are considerable differences between the RWC's of plants under the various treatments, a point that is considered in the following section.

Relationships between RWC and transpiration and RWC and photosynthesis

The curves for the RWC-transpiration and RWC-photosynthesis relationships (Figs. 4-10 and 4-11) were fitted by eye, and are presented without the individual points in the interests of clarity and to facilitate comparisons. (Figures showing the individual points will be found in Appendix 7).

Figure 4-9

Effect of soil moisture tension on the RWC
of plants under the four treatments.



The relationships between RWC and transpiration rate for the four treatments are shown in Fig. 4-10. The vertical bars across the curves at the RWC corresponding to a transpiration rate of $0.5 \text{ gm water/dm}^2/\text{hr}$ mark the points at which soil moisture tension appears to limit the transpiration rate, i.e. 0.4 atm. (see Fig. 4-5).

Transpiration rate tends to be unaffected by changes in RWC above 90% except under the HT/LH treatment where a reduction in RWC is associated with a reduction in transpiration rate. Below 90% RWC the rates of reduction of transpiration with decreasing RWC are similar for the two HT treatments and for the two LT treatments, until the point at which soil moisture tension appears to become the limiting influence is reached. Under the HH treatments there is a tendency for the curves to flatten out parallel to the abscissa at RWC's below this point. The rate of reduction of transpiration with decreasing RWC is greater under LT than under HT conditions.

The relationships between RWC and photosynthetic rate for the four treatments are shown in Fig. 4-11 : the vertical bars across the curves mark the RWC at 0.4 atm. soil moisture tension (i.e. the point at which soil moisture tension appears to become the limiting influence on the rate of photosynthesis. See Fig. 4-6). Except under the

Figure 4-10

Relationships between RWC and rate of transpiration ($\text{gm water/dm}^2/\text{hr}$) of plants under the four treatments.

Vertical bars indicate the RWC at 0.4 atm. soil moisture tension.

(The individual points from which these relationships were derived are given in Fig. A7-2, Appendix 7).

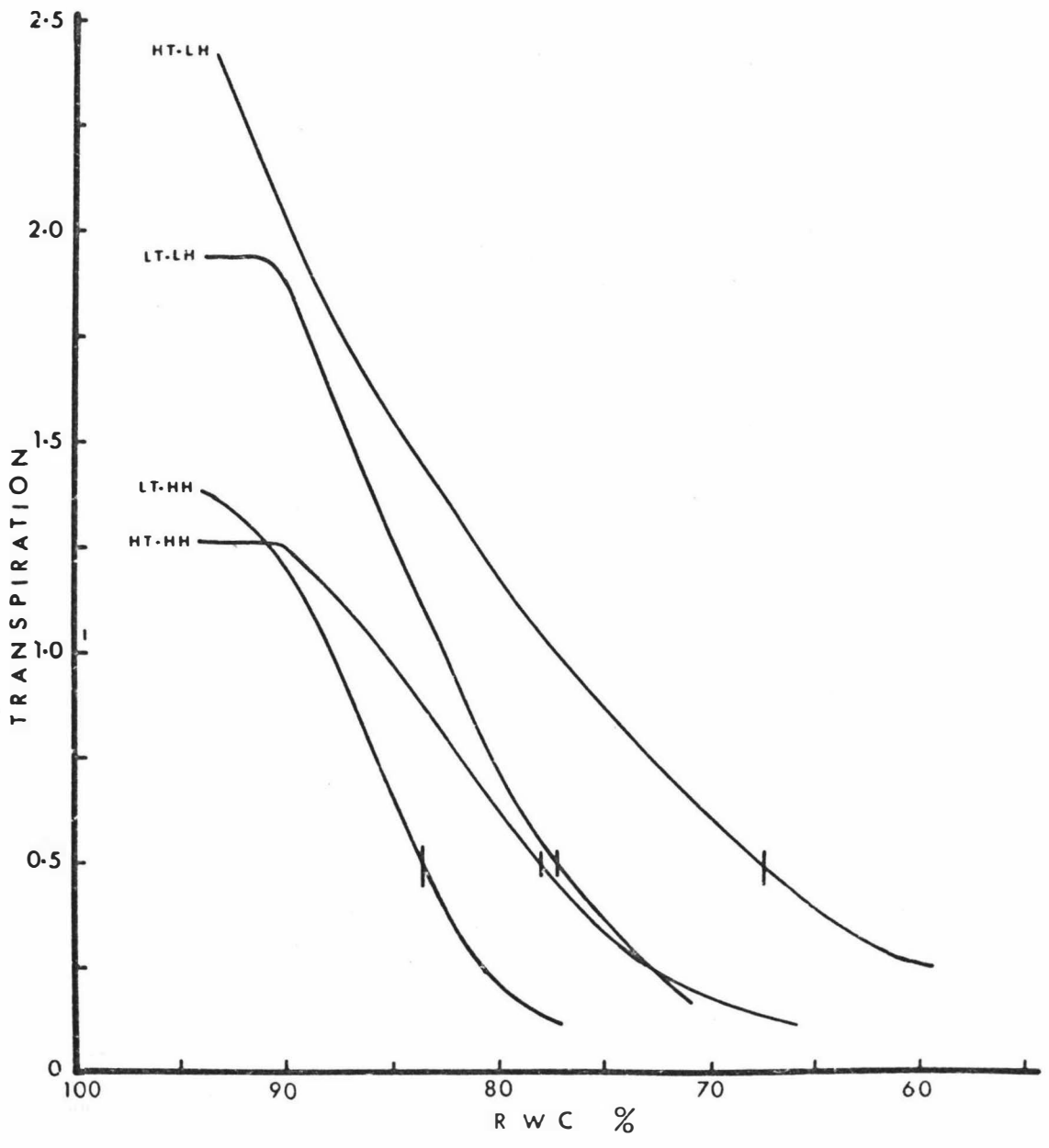
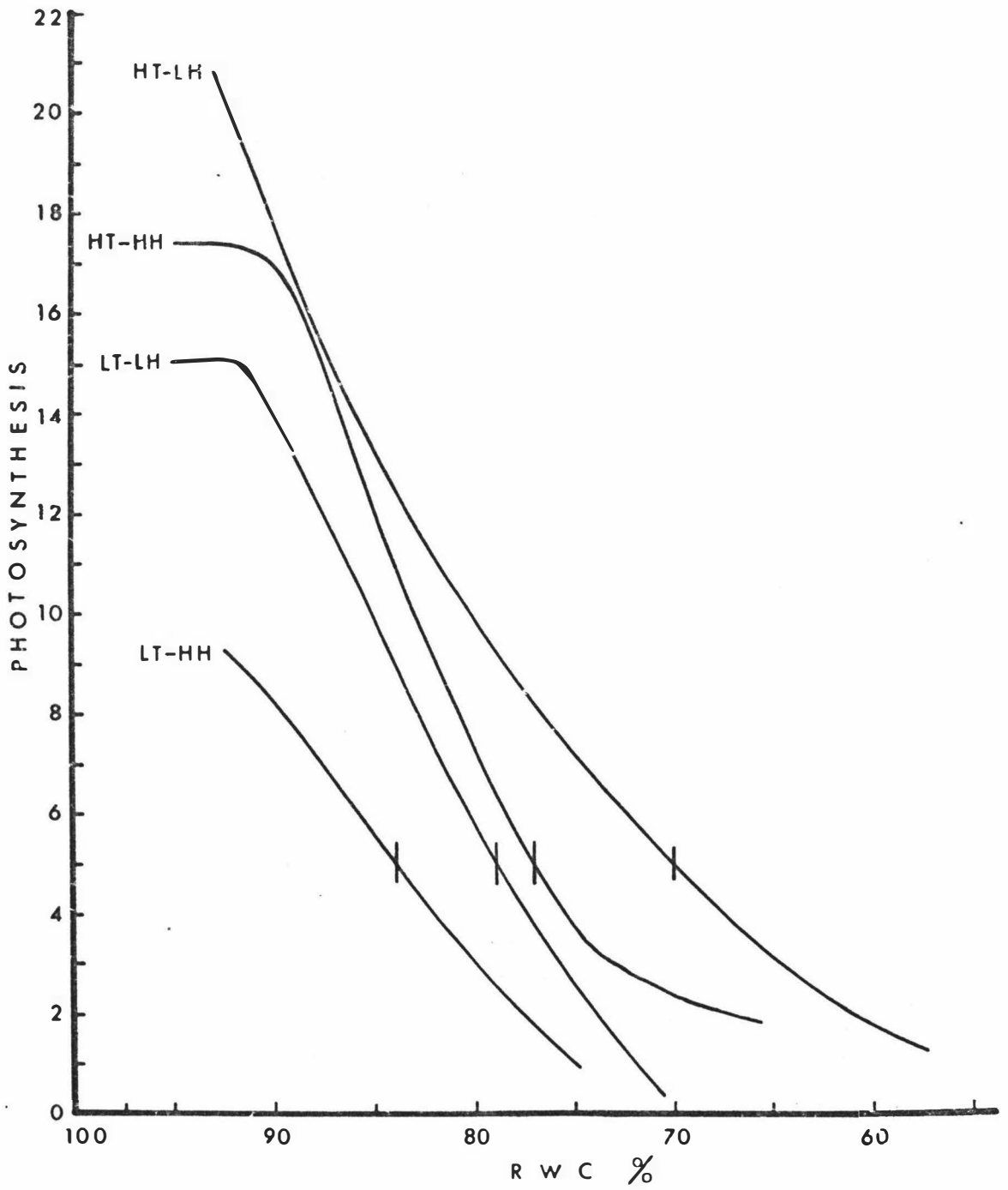


Figure 4-11

Relationships between RWC and rate of photosynthesis ($\text{mg}/\text{CO}_2/\text{dm}^2/\text{hr}$) of plants under the four treatments.

Vertical bars indicate the RWC at 0.4 atm. soil moisture tension.

(The individual points from which these relationships were derived are given in Fig. A7-3, Appendix 7).



HT/HH treatment there is little apparent tendency for the curves to flatten out below this RWC.

The RWC at 0.4 atm. soil moisture tension is different for each treatment, being highest for the LT/HH treatment and lowest for the HT/LH treatment, but for a given treatment is similar for both photosynthesis and transpiration (Table 4-II). To facilitate between-treatment comparisons the rates of transpiration and photosynthesis at various RWC's were taken from Figs. 4-10 and 4-11 respectively, and converted to relative rates as described in an earlier section of this chapter (Effects of soil moisture tension on transpiration and photosynthesis). Table 4-II shows the relative rates of photosynthesis and transpiration at 0.4 atm. soil moisture tension, and also the corresponding RWC's of plants under each treatment.

Table 4-II RWC and relative rates of transpiration and photosynthesis of plants under each treatment at 0.4 atm. soil moisture tension. The actual rates of transpiration and photosynthesis at this tension were taken as 0.5 gm/dm²/hr and 5 mg CO₂/dm²/hr respectively.

Treatment	RWC			Relative rate of	
	from Fig. 4-10	from Fig. 4-11	Mean	Transpiration	Photosynthesis
HT/LH	67.5	70.0	68.8	0.24	0.23
HT/HH	78.0	77.0	77.5	0.35	0.32
LT/LH	77.0	78.7	77.9	0.28	0.28
LT/HH	83.8	84.0	83.9	0.38	0.55

Thus at 0.4 atm. soil moisture tension the physiological state of plants under the two HH treatments was closer to 'optimum' than under the two LH treatments.

For each treatment the RWC at 0.4 atm. tension obtained from Fig. 4-10 agrees well with that obtained from Fig. 4-11 despite possible uncertainties in plotting the curves of these figures, and the fact that the rates of photosynthesis and transpiration at 0.4 atm. tension may not have been exactly $5\text{mg CO}_2/\text{dm}^2/\text{hr}$ and $0.5\text{ gm}/\text{dm}^2/\text{hr}$ respectively.

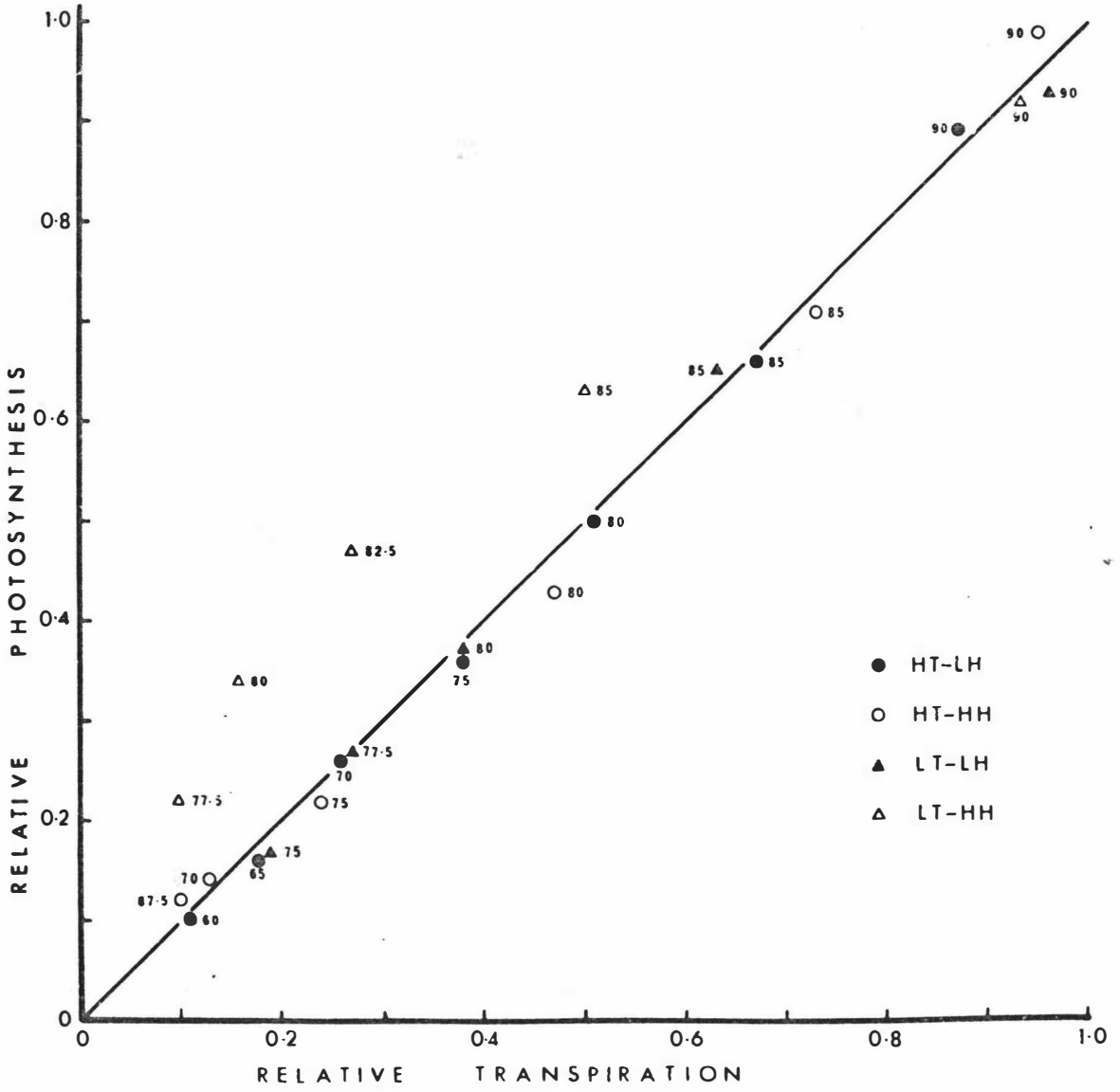
The relative rates of photosynthesis and transpiration for various values of RWC are shown plotted against each other in Fig. 4-12. For three of the treatments all the points fall close to the line of 1:1 correspondence, but the points for the LT/HH treatment deviate from this relationship. This may be due to uncertainties in the plotting of the RWC-transpiration and RWC-photosynthesis curves, for the data from which these curves were constructed (Figs. A7-2 and A7-3) are rather sparse at low levels of RWC for this treatment. The deviation from the 1:1 correspondence shown by these plants may therefore, be more apparent than real.

Figure 4-12

Relationship between relative rates of
photosynthesis and transpiration of plants
under the four treatments.

The RWC for each datum point is shown.

The line represents 1:1 correspondence.



Leaf temperature

With two exceptions leaf temperatures were always within 0.5°C of ambient air temperature. The exceptions, one each under HT/LH and LT/LH conditions, occurred with plants under severe water stress and involved a rise in leaf temperature to 2.5°C above air temperature. This rise in leaf temperature did not bring about any deviation from the normal photosynthesis-transpiration relationship (Fig. 4-4), nor from the RWC-transpiration or RWC-photosynthesis relationships (Figs. 4-10 and 4-11) and the results were therefore not treated separately.

Dark respiration

Rates of dark respiration declined with increasing water stress in plants under all four treatments (Table 4-III), but it is not possible to correlate these rates with RWC because measurements of RWC were not made during the dark period. The rates given in Table 4-III are the means of the hourly rates for each night of the experiment. Respiration rates tended to fluctuate during the dark period and were usually higher during the first and last few hours of the night than during the intervening period.

Table 4-III Respiration rates ($\text{mg CO}_2/\text{dm}^2/\text{hr}$) of plants subjected to increasing water stress under the four treatments.

<u>Treatment</u>	<u>Plant No</u>	<u>Night 1</u>	<u>Night 2</u>	<u>Night 3</u>
HT/LH	1	2.4	1.5	0.6
	2	3.1	1.6	0.7
	3	3.5	2.0	1.0
	4	3.5	2.7	1.2
	5	3.3	2.0	-
HT/HH	1	2.4	2.2	1.3
	2	2.4	2.1	1.9
	3	2.0	2.7	1.9
	4	3.3	2.1	2.1
	5	2.3	1.9	-
LT/LH	1	1.5	1.6	1.3
	2	0.5	0.5	-
	3	2.1	1.8	-
	4	2.4	2.6	-
	5	2.3	2.4	-
LT/HH	1	1.9	1.5	-
	2	2.7	2.3	2.0
	3	2.2	2.5	2.2
	4	1.9	1.4	1.3
	5	2.1	1.8	1.0

Respiration rates tended to be higher under the HT than under the LT treatments. Rates for the third night of plants under the LT/HH treatment were higher than those of plants under the HT/LH treatment because the latter were subject to more severe water stress at this stage (compare with Figs. 4-1 and 4-2).

Night-time transpiration rates

The night-time transpiration rates were similar to those measured under non water-stressed conditions (see Chapter 3). There was no evidence of reduction in rate with increasing water stress under any of the four treatments.

DISCUSSION

Dark respiration

Dark respiration rates generally declined with increase in water stress (Table 4-III) and there was no evidence of significant increases in respiration rate at slight to moderate stress levels, as has been reported for loblolly pine (Brix, 1962), and wheat (Kaul, 1966). Slatyer (1967) considers that the rise in respiration rate at slight water deficits may be due to a rapid imposition of stress, and that when stress is imposed gradually a progressive decline will be observed with no rise in rate. This has been shown to be the case with excised leaves of Pinus nigra (Parker, 1952). The rate of

imposition of stress in the present experiments however, was rapid so that Slatyer's supposition may not be generally true. Under the combined effects of water stress and zero light intensity the stomata may be expected to be closed, and thus constitute a barrier to the outward diffusion of CO_2 , but the mean maximum rates of respiration seldom exceeded $3.5 \text{ mg CO}_2/\text{dm}^2/\text{hr}$ (Table 4-III) and this quantity of CO_2 may have been dissipated partly through the cuticle (Freeland, 1948; Dugger, 1952). Respiration rates tended to be higher early in the dark period and towards the end of the dark period than they were during the middle of the night. This may be correlated with a diurnal rhythm of stomatal opening, the stomata taking some time to close after the onset of darkness, and commencing to open rather before the end of the dark period. Stomatal opening in darkness has been observed in Rumex patientia and Upland cotton (Meidner and Mansfield, 1965). It is also possible that some of the reduction in respiration rate may be due to enzyme inactivation following partial desiccation of the leaf tissue, especially under more severe levels of water stress.

Night-time transpiration

The night-time transpiration rates were unaffected by increasing water stress. Clark and Levitt (1956) showed that the transpiration rates of soybean plants that had been wilted and then rewatered were lower than those of plants that had been maintained under an adequate water supply. They attributed this to an increase in the wax content of the cuticle developed by the wilted plants. They did not state the length of time for which the plants were subjected to drought conditions, but it may have been longer than the few days of stress imposed in the present experiments. This short period may have been insufficient for the development of additional cuticular wax, and hence the night-time transpiration rates were unaffected by increasing water stress.

Development of water stress

The similarity of the time courses of transpiration for plants under the two LH and under the two HH treatments (Fig. 4-2) may be explained on the basis of total water use by the plants under the various treatments. Plants under LH conditions at both high and low temperature transpired at a considerably greater rate per unit area than those under HH conditions (Table 4-I), and the rate of transpiration was largely determined by the VPD (Fig. 3-2). The amount of water available to each

plant was the same regardless of treatment, and plants under LH conditions lost more of the available water during the early part of the experiment than did those under HH conditions, thus becoming subject to water stress earlier.

Under water stress conditions rates of transpiration and photosynthesis are reduced; results from work on a wide variety of plant species have led to general agreement on this (e.g. Schneider and Childers, 1941; Slatyer, 1957; Brix, 1962; Millar, Duysen and Wilkinson, 1968). The decline in photosynthesis closely paralleled the decline in transpiration under all treatments (compare Figs. 4-1 and 4-2) and the two processes were linearly related over the range of water stress levels to which the plants were subjected (Fig. 4-4), until soil moisture tension became the limiting influence. Similar results for loblolly pine and tomato were reported by Brix (1962) who attributed the parallel decline in the rates of photosynthesis and transpiration to an increase in diffusion resistance, but whether of stomatal or mesophyll diffusion resistance (or of both) he was unable to determine.

The parallel between the rates of transpiration and photosynthesis as water stress increases certainly suggests that the two processes are subject to a common controlling mechanism, which by the nature of these processes may well be

stomatal. The mechanism of stomatal opening, extensively and frequently reviewed (e.g. Heath, 1959; Ketellapper, 1963; Zelitch, 1965; Pallas, 1966), is not yet fully understood, but turgor changes are certainly involved, although the mechanism controlling the changes is in doubt (Meidner and Mansfield, 1968). As discussed in the introduction (Chapter 1) water deficits occur when transpiration exceeds absorption, and turgor is then progressively lost. This is reflected in a fall in RWC. In response to the loss of turgor the stomata start to close and the transpiration rate is reduced. Simultaneously the supply of CO_2 for photosynthesis is also reduced, and the rate of photosynthesis falls. Reduction in the rate of photosynthesis may lead to increased CO_2 concentration in the intercellular spaces and this may also contribute to stomatal closure (Heath and Meidner, 1961).

It therefore seems reasonable to suggest that the stomata start to close in response to a slight loss of turgor in the leaf brought about by water loss exceeding water uptake, and that further closure may be in part consequent upon the resulting increase in CO_2 concentration in the intercellular spaces. The initial closing affects transpiration and photosynthesis proportionately as is shown by Fig. 4-4. This conclusion is supported by the work of Brix (1962) and Barrs (1968). The latter, in discussing results for sunflower that

closely quantitatively resemble the photosynthesis-transpiration relationship for HT/HH plants in Fig. 4-4, stated: "It is difficult to escape the conclusion that both gas exchange rates were directly and principally limited by stomatal aperture".

Gaastra (1963) suggested that because CO_2 exchange is regulated by a mesophyll resistance (r'_m) in addition to the boundary layer and stomatal resistances common to both CO_2 and water vapour exchange, photosynthesis should not be affected by stomatal closure until the stomatal resistance exceeds r'_m . Thus as water stress develops, the rate of transpiration should decline before the rate of photosynthesis. In the present study there is no evidence of this effect (Figs. 4-1 and 4-2) and the relationship between the rates of photosynthesis and transpiration (Fig. 4-4) is linear for all treatments until a stress corresponding to a soil moisture tension of 0.4 atm. is reached.

Under conditions of adequate water supply the rates of photosynthesis of plants under all treatments were apparently mainly determined by r'_m , but the conclusion that the stomata are of major importance and that r'_m has comparatively little effect under conditions of water stress is in agreement with the conclusions of Troughton (1969) and Troughton and Slatyer (1969).

Effects of soil moisture tension on transpiration and photosynthesis

Idso (1968) has suggested that Brix's (1962) observations of parallel reduction of photosynthesis and transpiration were due to his plants having a low root density and to the moisture characteristics of the soil (a sandy loam) that he used. The soil moisture characteristic curve for Brix's soil is similar to that for the pumice-peat mixture used in the present experiments (see Appendix 1, Fig. A1-1), i.e. much water held at low tensions and a sharp inflection point beyond which tension rises rapidly for small decreases in water content. Idso's suggestions might thus be regarded as also being pertinent to the present results. Cowan (1965) calculated that plants with low root densities would show a reduction in transpiration at lower soil moisture tensions than plants with high root densities, and Idso considers that in plants with low root densities photosynthesis would behave much like transpiration. Although no measurements of root density were made in the present study all plants had well developed root systems occupying a large part of the soil mass. The pots themselves were small having a volume of approximately 850 ml. Idso further considers that photosynthesis and transpiration would be reduced in phase once the soil had dried to a level corresponding to the inflection point of the soil moisture characteristic curve. In this study the point at which soil moisture tension became

the limiting influence on transpiration and photosynthesis, 0.4 atm., corresponded to the inflection point (Fig. A1-1), and photosynthesis and transpiration were reduced in phase well before this degree of soil dryness was reached, viz. at about 0.2 atm. soil moisture tension. Thus Idso's comments are not applicable to the present results.

As soil moisture tension increases above 0.2 atm. the transpiration rate of plants under all treatments starts to decline (Fig. 4-5). At tensions greater than 0.4 atm. the transpiration rate is very similar for plants under all treatments, and it is at 0.4 atm. tension that the inflection in the soil moisture characteristic curve (Fig. A1-1) occurs. As soil moisture content falls below the equivalent of 0.4 atm. tension, tension increases very rapidly with small reductions in moisture content and removal of water from the soil by the plant becomes increasingly difficult. The maximum rate of absorption would appear to be the equivalent of a transpiration rate of approximately $0.5 \text{ gm/dm}^2/\text{hr}$, and transpiration rate thus becomes determined by soil moisture characteristics rather than by atmospheric conditions. Similar results for a variety of tree species grown in a range of soil types have been reported by Vzduznaev (1968). He found that in all cases a point was reached in the drying of the soil at which transpiration became independent of the environmental conditions

and was related only to soil moisture tension. A transpiration-soil moisture tension relationship for Pinus sylvestris, similar in form to the relationship in Fig. 4-5 has been reported by Jarvis and Jarvis (1963 c).

Photosynthesis is similarly affected by increasing soil moisture tension (Fig. 4-6). As photosynthesis and transpiration are linearly related at RWC's between maximum and that at which soil moisture tension becomes the major influence on the transpiration rate, this is not unexpected. Thus, for each treatment photosynthesis and transpiration become limited by soil moisture tension at very similar RWC's (Table 4-II). The similarity between the relative rates of transpiration and photosynthesis in their response to soil moisture tension is also expected in the light of the relationship between the actual rates of these processes.

The influence of environmental conditions on the response of relative transpiration rate to soil moisture tension is shown in Fig. 4-7, and confirms the findings of Denmead and Shaw (1962). Using container-grown corn plants in the field they found that the relative transpiration rate was reduced to a greater extent at any given soil moisture tension as the atmospheric evaporative demand increased. Their data show this effect more clearly than the present results because the differences in atmospheric evaporative demand between the days on which their results were obtained were much greater than the between-treatment differences

in this investigation. Similar results for orchard grass and tomato have also been reported (Gavande and Taylor, 1967).

Transpiration depends on the maintenance of a water potential gradient between soil and leaf: as soil moisture tension (which can be equated with soil water potential) falls, leaf water potential must also fall to maintain the water potential gradient. High rates of transpiration may lead to a drying of the soil in the root permeated zone, and the conductivity of the soil may be too low to permit of immediate rewetting (Macklon and Weatherley, 1965). When the soil is well supplied with water most of the impedance to water movement through the soil-plant system occurs in the plant, but as the soil dries the impedance in the soil assumes greater importance and may quite suddenly become much greater than the plant impedance. This can occur at low soil moisture tensions. Gardner and Ehlig (1962) report it as occurring at 0.6 bars (0.59 atm.) soil moisture tension for pepper plants growing in a sandy loam soil.

Macklon and Weatherley (1965) stated: "Atmospheric conditions favouring high rates of transpiration do not by themselves induce high values of DPD in the plant. It is only when rapid water flux is coupled with the low water conductivity of the soil that high values of DPD arise". (High DPD is equivalent to low leaf water potential). In the present case the change in the

relative importance of soil and plant impedance to water movement appears to have occurred at about 0.2 atm. soil moisture tension. The higher the transpiration rate, the greater the effect on the relative transpiration rate at this point. The relative transpiration rate of plants under the LT/HH treatment was the least affected because these plants had the lowest transpiration rate and therefore the lowest rate of removal of water from the soil. The soil conductivity was high enough to maintain a supply of water to the roots of these plants, but not to those transpiring more rapidly. As the transpiration rates of LT/HH and HT/HH plants were very similar it would be expected that the latter would show a response similar to the former, rather than to the LT/LH plants which had a higher transpiration rate. It may be that the difference in transpiration rate (and thus of the rate of water absorption) between the HT/HH and LT/HH plants was sufficient to exceed the water supplying capabilities of the soil at these tensions (0.2 to 0.4 atm.).

RWC and soil moisture tension

Under all treatments the RWC was similar at tensions below 0.2 atm. and appeared to be independent of soil moisture tension. At tensions above 0.2 atm. plants under the LH treatments had lower RWC's than plants under the HH treatments, and RWC fell

more rapidly with increasing soil moisture tension under the LH treatments (Fig. 4-9). Similar results for orchardgrass and tomato have been reported by Gavande and Taylor (1967). However, between 0.2 and 0.4 atm. soil moisture tension there is a considerable difference in the RWC's of plants under the various treatments, which suggests that RWC is not determined by soil moisture tension alone. Rather it would seem that both soil and environmental factors are involved. Weatherley (1951) also found, with field-grown cotton plants, that above a certain critical soil moisture tension RWC was determined by soil and environmental factors, whereas at tensions below the critical point RWC was independent of soil moisture tension. Other field experiments have also shown atmospheric and soil moisture conditions to be of prime importance in determining RWC (Namken, 1964).

Denmead and Shaw (1962) referred to the point at which actual transpiration fell below the potential rate (i.e. the rate when the soil was at field capacity) as the "turgor loss point", but made no measurements of plant water status. The turgor loss point occurred at lower soil moisture tensions for plants with a high initial transpiration rate.

Although RWC does not measure leaf turgor, it is clear from Fig. 4-9 that RWC tends to be reduced to a greater extent at any soil moisture tension above 0.2 atm. under LH than under HH conditions. Plants under the HT/LH treatment were observed to show signs of wilting at high^{er} RWC's than plants under

the other treatments. These results would therefore seem to confirm the observations of Denmead and Shaw.

At 0.4 atm. soil moisture tension the mean RWC of plants under the HT/LH treatment was 68.8%, that of plants under the LT/HH treatment 83.9% (Table 4-II). It is of interest to speculate on the possible RWC-leaf water potential relationships of the plants under these treatments. As far as is known there are only two reports in the literature concerning the effects of temperature and VPD on the RWC-leaf water potential relationship. These are for orchardgrass and tomato (Gavande and Taylor, 1967) and for barley (Millar et al, 1968). Both groups of authors compared the RWC-leaf water potential relationship of plants under high temperature/low humidity and low temperature/high humidity conditions, although the actual conditions used were different in each study (Table 4-IV).

Table 4-IV Comparison of atmospheric conditions in the experiments of Gavande and Taylor (1967) and Millar et al (1968).

<u>Authors</u>	High temperature/ low humidity		Low temperature/ high humidity	
	<u>°C</u>	<u>VPD (mb)</u>	<u>°C</u>	<u>VPD (mb)</u>
Gavande and Taylor	30.0	29.6	21.0	9.9
Millar <u>et al</u>	27.0	22.7	17.0	7.7

For all three species involved in these investigations the results were qualitatively the same: under low temperature/high humidity conditions the plants had a lower RWC for any given leaf water potential.

Consider now the situation for soybean plants at 0.4 atm. soil moisture tension. If, at this point plants under all four treatments had the same leaf water potential, then the LT/HH plants had a higher RWC than the HT/LH plants, i.e. the opposite of the findings for orchardgrass, tomato and barley. However, soybean would fit the same pattern as these three species if the leaf water potential under HT/LH conditions fell more rapidly than under LT/HH conditions as soil moisture tension increased, so that at 0.4 atm. tension the soil water potential-leaf water potential gradient was greater for the HT/LH plants. Gavande and Taylor (1967) showed that at a given soil moisture tension, the leaf water potential of orchardgrass and tomato plants under their low temperature/high humidity treatment was higher than under the high temperature/low humidity treatment. The RWC of soybean plants fell more rapidly under the HT/LH than under the LT/HH conditions (Fig. 4-9) and it therefore seems likely that at 0.4 atm. tension the leaf water potential of HT/LH plants was lower than that of LT/HH plants, and that the RWC-leaf water potential relationships of soybean plants under these two treatments were qualitatively similar to those of orchardgrass, tomato and barley plants under the corresponding conditions. It should

also be noted that at 0.4 atm. tension the physiological state of the plants was closer to 'optimum' under the LT/HH treatment than under the HT/LH treatment (Table 4-II).

RWC, photosynthesis and transpiration

Between-treatment comparisons of the RWC-photosynthesis and RWC-transpiration relationships are complicated by the fact that under each treatment the plants at any given RWC below about 90% were subject to different soil moisture tensions (Fig. 4-9), and probably had different leaf water potentials (previous section of this chapter). However certain aspects of these relationships require comment.

The slopes of the RWC-transpiration curves (Fig. 4-10) are similar for the two HT treatments between about 90% RWC and the 0.4 atm. soil moisture tension point, and similarly the curves for the two LT treatments are parallel. This may be due to the effect of temperature on the rate of water movement in the soil and in the plant. Low temperatures reduce water uptake under certain conditions (see Chapter 3) by reducing root permeability and increasing water viscosity. Soil moisture diffusivity is also temperature dependent resulting from the temperature dependence of viscosity (Jackson, 1963); thus soil water moves more rapidly at high than at low temperatures.

Although the rate of water uptake by plants under the two HH treatments was not affected by temperature at low soil

moisture tensions (Chapter 3), it is suggested that as soil moisture tension increases the rate of water movement to the roots may be partly limited by temperature. Thus water moves to the roots more rapidly under HT than under LT conditions, and the rate of decline of transpiration with fall in RWC becomes temperature, rather than VPD, dependent.

Earlier in this chapter the effect of stomatal closure on rates of photosynthesis and transpiration was discussed, and it was noted that as water stress increased the rates of photosynthesis and transpiration declined in parallel. The relationship between these processes was similar for three of the treatments, but was rather different for plants under the HT/HH treatment which had higher rates of photosynthesis per unit rate of transpiration than plants under the other treatments. In Fig. 4-12 the relative rates of photosynthesis and transpiration are shown plotted against each other. For three of the treatments the points all lie along the line of 1:1 correspondence but the points for the LT/HH treatment deviate from this relationship. This deviation was tentatively ascribed in the 'Results' section of this chapter to possible uncertainties in the plotting of the RWC-transpiration and RWC-photosynthesis curves, for it is difficult to find any physiological basis for this result. (Had the deviation been in the opposite direction, i.e. higher relative transpiration than relative photosynthetic rates at

any given RWC, an influence of the mesophyll resistance (r'_m) might have been suspected, for the r'_m of the LT/HH plants was apparently very much higher than that of plants under the other treatments (Table 3-IV), and might have contributed to lower rates of photosynthesis when the plants were subject to water stress. This however was not the case). As water stress increases the relative rates of transpiration and photosynthesis of the plants under the treatments that conform to the 1:1 relationship, and possibly also of the LT/HH plants, decline in phase and proportionately. The 1:1 correspondence is followed regardless of the atmospheric environment, but the extent to which the relative rates are reduced by any given level of stress is dependent on the environmental conditions. Figure 4-12 thus emphasises the close relationship between the rates of photosynthesis and transpiration which is apparently mediated through the influence of transpiration on leaf turgor and the consequent effects on stomatal aperture (and thereby on stomatal resistance). Thus the RWC-photosynthesis relationships (Fig. 4-11), although involving a different set of reactions, are apparently the result of the interactions between transpiration, leaf turgor (which is reflected in the value of leaf RWC) and stomatal resistance, and consequently are extremely difficult to interpret in isolation.

It may be noted that there is a tendency for the RWC-transpiration curves (Fig. 4-10) to flatten out parallel

to the abscissa, suggesting that transpiration may continue down to very low levels of RWC, a tendency that is not so apparent in the RWC-photosynthesis curves (Fig. 4-11). It is also apparent from Fig. A7-1 (which shows the individual points from which the regressions in Fig. 4-4 were calculated), that there is a tendency for the rates of transpiration and photosynthesis below those obtaining at 0.4 atm. soil moisture tension not to be related in the same way as those above 0.4 atm. tension. Above 0.4 atm. tension photosynthesis tends to decrease rather more rapidly than transpiration. Had the experiments been extended in duration it is probable that transpiration would have been measurable after photosynthesis had fallen to zero, indeed this did occur in one instance during the stress phase of a rewatering experiment (see Chapter 5). Slatyer (1967) states that transpiration continues at a low rate until death occurs. It is probable that for a period before death occurs the plant acts mainly as an inert wick between the soil and the atmosphere. At these high soil moisture tensions and low levels of leaf hydration it is possible that disruption of the biochemical processes of photosynthesis occurs. Photosynthesis and transpiration may thus be determined by separate mechanisms after soil moisture characteristics become the dominant limiting influence on the rate of transpiration, but between 0 and 0.4 atm. soil moisture tension it would appear most likely that both processes were subject to the controlling influence of stomatal resistance.

CHAPTER 5

RESPONSE OF SOYBEAN PLANTS TO REWATERING FOLLOWING WATER STRESS UNDER TWO CONTRASTING SETS OF ENVIRONMENTAL CONDITIONS

INTRODUCTION

Results given in the literature for the patterns of recovery of CO₂ exchange and transpiration of plants rewatered following a period of water stress differ on two main points. Firstly, the rate of recovery to the original, unstressed rates is variously reported as two to seven days for apple (Schneider and Childers, 1941), and less than 24 hours for ladino clover (Upchurch, Peterson and Hagan, 1955). Recovery of photosynthetic and respiration rates to 80% of the original rates within one hour has been reported for rye seedlings (Murata, Iyama and Honma, 1966), and Thompson, Stelzy and Taylor (1965), reported a 2½ fold increase in the rate of photosynthesis of rough lemon within 10 minutes of rewatering; prior to rewatering the photosynthetic rate was 16% of the original rate.

The second point of difference concerns the order in which photosynthesis and transpiration respond to the relief of water

stress. Transpiration was observed to recover before, or simultaneously with, photosynthesis in apple (Schneider and Childers, 1941), pecan (Loustalot, 1945) and loblolly pine (Brix, 1962). Photosynthesis has been reported to recover before transpiration in tomato (Brix, 1962), rough lemon (Thompson et al., 1965) and cotton (Pallas, Michel and Harris, 1967). Recovery to the original rates does not appear to be dependent on the re-establishment of full leaf turgor. Schneider and Childers (1941) found that although full turgor was regained three to five hours after rewatering, the rates of CO₂ exchange and transpiration did not fully recover for two to seven days. The tomato plants in Brix's experiments never regained their original rates of photosynthesis and transpiration, although full leaf turgor was regained.

Brix (1962) also showed that the photosynthetic rate of loblolly pine seedlings recovered very much more rapidly when the roots were excised than when the seedlings remained intact. He suggested that during periods of water stress changes affecting the water absorbing and/or conducting capacity of the roots take place. In part, at least, these changes may involve the death of roots and root hairs and increased suberisation of the roots (Slatyer, 1967). Kramer (1950) found that water uptake through the roots of tomato and sunflower plants that had been allowed to wilt was reduced by 50% if the plants were wilted overnight, and 80-90% in plants wilted over four days. Tomato plants showed a high degree of recovery from the latter treatment,

but sunflower did not. He attributed the reduced water uptake mainly to suberisation and cessation of elongation of the roots, and suggested that reduced protoplasmic permeability might be a further contributing factor.

Recent studies suggest that water stress may affect stomatal functioning, and that the effects may persist after the release of the plant from stress. Fischer (1967) showed that stomatal functioning in tobacco was impaired by leaf water deficits of 20% or more, and was proportional to the duration and severity of the water deficit. The impairment persisted for two to three days following rewatering. He also showed that the source of the impairment in bean plants resided in the mesophyll and epidermal cells. Allaway and Mansfield (1970) found that after leaves of Rumex sanguineus had been allowed to wilt the stomata did not open as widely as usual and 2% of the guard cells were killed. They suggested that after wilting an inhibitor of stomatal opening accumulates, or that the level of some promoter of stomatal opening falls.

Little attention appears to have been given to the effects of environmental factors on the rate and degree of recovery of CO₂ exchange and transpiration rates from water stress after rewatering. Caldwell (1913) found that herbaceous plants allowed to wilt slowly in a humid atmosphere required twice as long to recover their original transpiration rates as plants rapidly wilted under conditions conducive to high transpiration.

Under the humid conditions the root hairs were usually killed, but this did not occur with the rapidly wilted plants. Pallas et al (1967) found that cotton plants which were rewatered after prolonged water stress regained their original rates of photosynthesis and transpiration under a light intensity equivalent to full sunlight. Under one half and one quarter full sunlight equivalents they failed to regain their original rates; photosynthesis under one quarter sunlight reached 65% of its original value after rewatering. Stomatal activity also failed to attain the pre-rewatering level at any of the light intensities.

The results which follow were obtained from experiments in which soybean plants were rewatered after being subjected to various levels of water stress under one of two contrasting sets of environmental conditions. The object of the experiments was to follow the immediate post-rewatering response of photosynthesis and transpiration. No attempt was made to determine the 'lethal-limit' of water stress beyond which recovery could not take place, and the experiments constitute only a preliminary investigation of the after effects of water stress on soybean plants.

METHODS

The rewatering experiments were carried out on plants grown under two contrasting sets of environmental conditions,

the HT/LH and LT/HH treatments. Under each treatment five plants were subjected to water stress by withholding water, either in the plant chamber of the CO₂ exchange - transpiration measuring equipment, or in the growth cabinet. (Wilting in the growth cabinet was necessary for some LT/HH plants because of the length of time required for these plants to become subject to water stress. Such plants were transferred to the plant chamber and their photosynthetic and transpiration rates measured before stress was relieved by rewatering). Plants were rewatered after being subjected to various degrees of stress, the water added being sufficient to return the plant and pot to the original weight before stress was imposed. After rewatering, the plant was returned to the plant chamber and further measurements of photosynthetic and transpiration rates made. Rewatering was carried out before the plants became too severely wilted, and passed the 'lethal limit' beyond which recovery was impossible. Consequently the time of day at which rewatering was carried out was not consistent, and plants were rewatered at either the end of, or at some time during, the light period. Measurements of leaf temperature, RWC and soil moisture content were made as usual, although the times at which plant and soil water stress samples were taken were dictated by the state of the plant.

RESULTS

As in all the previous experiments, leaf temperature remained within 0.5°C of ambient air temperature.

The time courses of typical rewatering experiments, Figures 5-1 (HT/LH) and 5-2 (LT/HH), show the effects of rewatering at the end of the light period, and during the light period, for each treatment. The RWC at various times is also shown. When rewatering took place at the end of the light period, the subsequent time courses of photosynthesis and transpiration were similar to those for unstressed plants (Figs. 4-1 and 4-2, day 1), and the two processes were more or less in phase.

When rewatering was carried out during the light period, there was no immediate increase in either photosynthesis or transpiration, which either remained the same as before rewatering (Fig. 5-2 b), or continued to fall (Fig. 5-1 b). Within two hours of rewatering however, both processes showed a rapid increase in rate, photosynthesis tending to increase more rapidly than transpiration and to reach its maximum post-rewatering rate rather earlier. Thus whilst the rate of recovery of transpiration was less than that of photosynthesis, the two processes commenced recovery more or less simultaneously.

The results of the rewatering experiments are summarised in Table 5-I, which shows the maximum rates of photosynthesis and transpiration before and after rewatering, the post rewatering maximum rates as a percentage of the maximum rates

Figure 5-1

Time courses of rewatering experiments under HT/LH conditions.

Rewatering (indicated by the arrow) took place at the end of the light period (A), or during the light period (B). RWC at various times during the experiments is indicated.

Solid circles : photosynthesis ($\text{mg CO}_2/\text{dm}^2/\text{hr}$)

Open circles : transpiration ($\text{gm water}/\text{dm}^2/\text{hr}$)

(Data are for HT/LH replicates 4, (A) and 2, (B)).

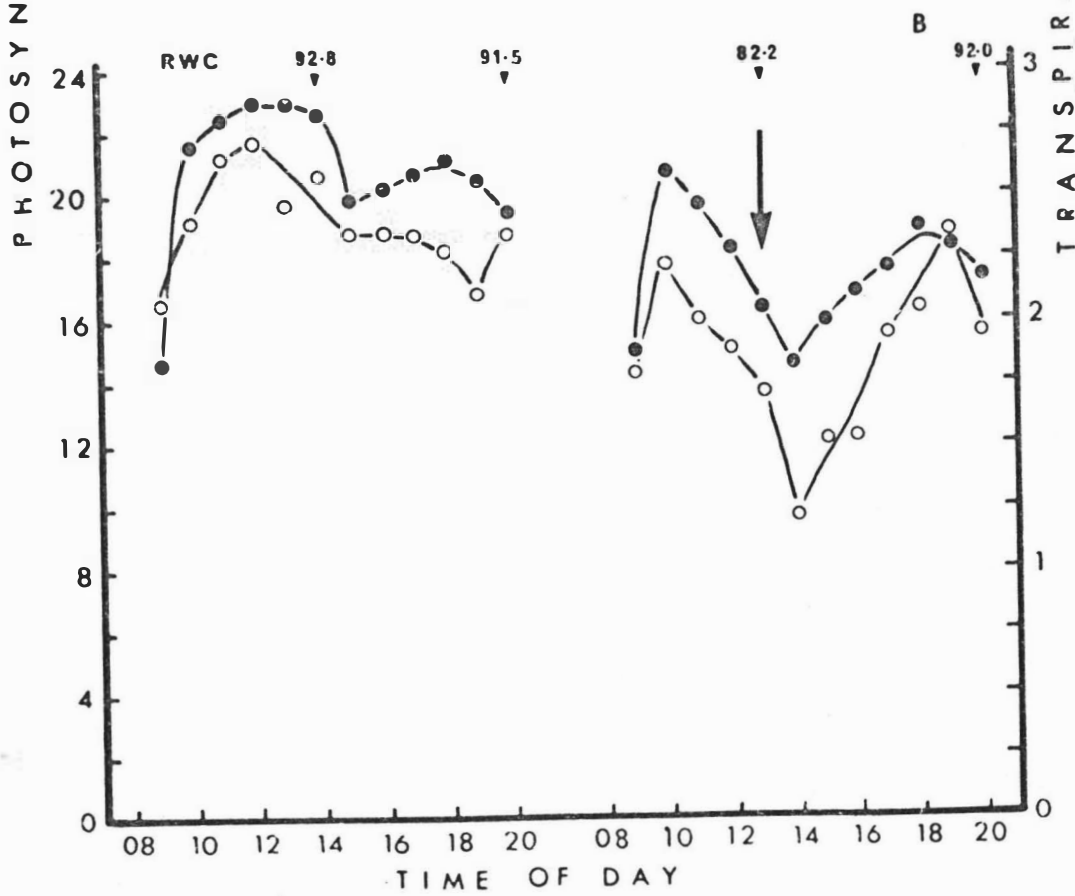
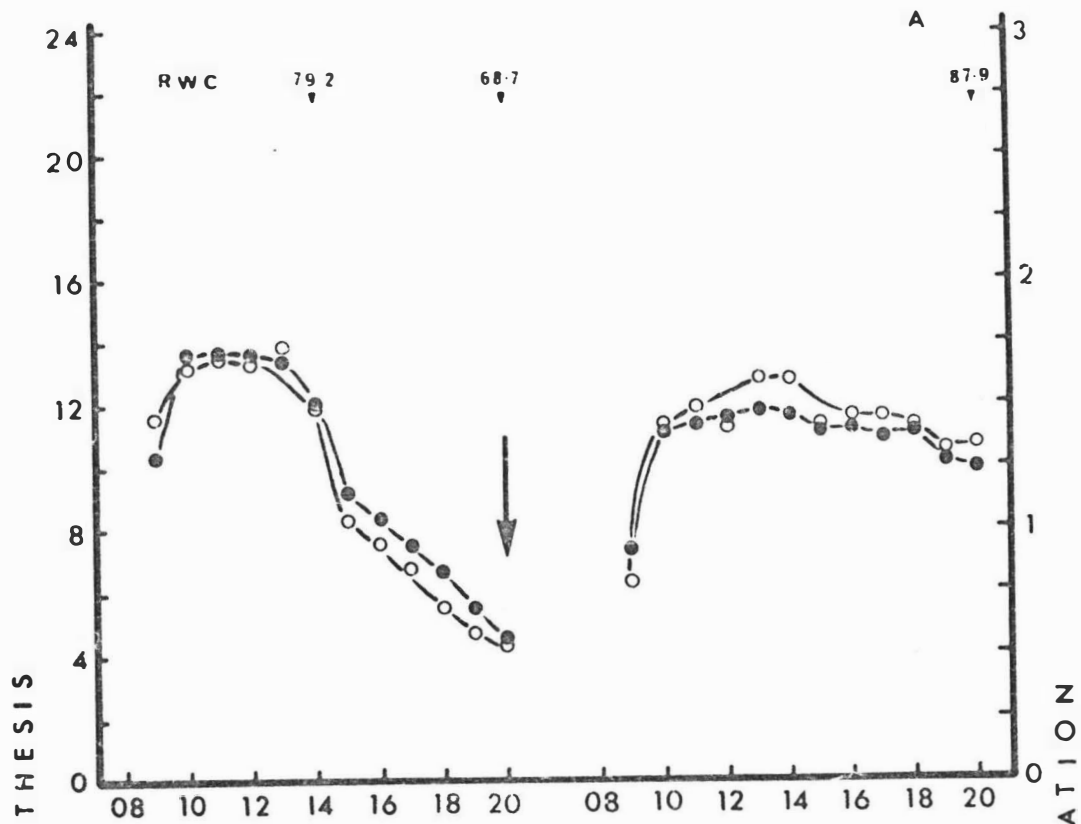


Figure 5-2

Time courses of rewatering experiments under
LT/HH conditions.

Rewatering (indicated by the arrow) took place
at the end of the light period (A), or during the
light period (B).

RWC at various times during the experiments is
indicated.

Solid circles : photosynthesis ($\text{mg CO}_2/\text{dm}^2/\text{hr}$)

Open circles : transpiration ($\text{gm water}/\text{dm}^2/\text{hr}$)

(Data are for LT/HH replicates 1, (A) and 5, (B)).

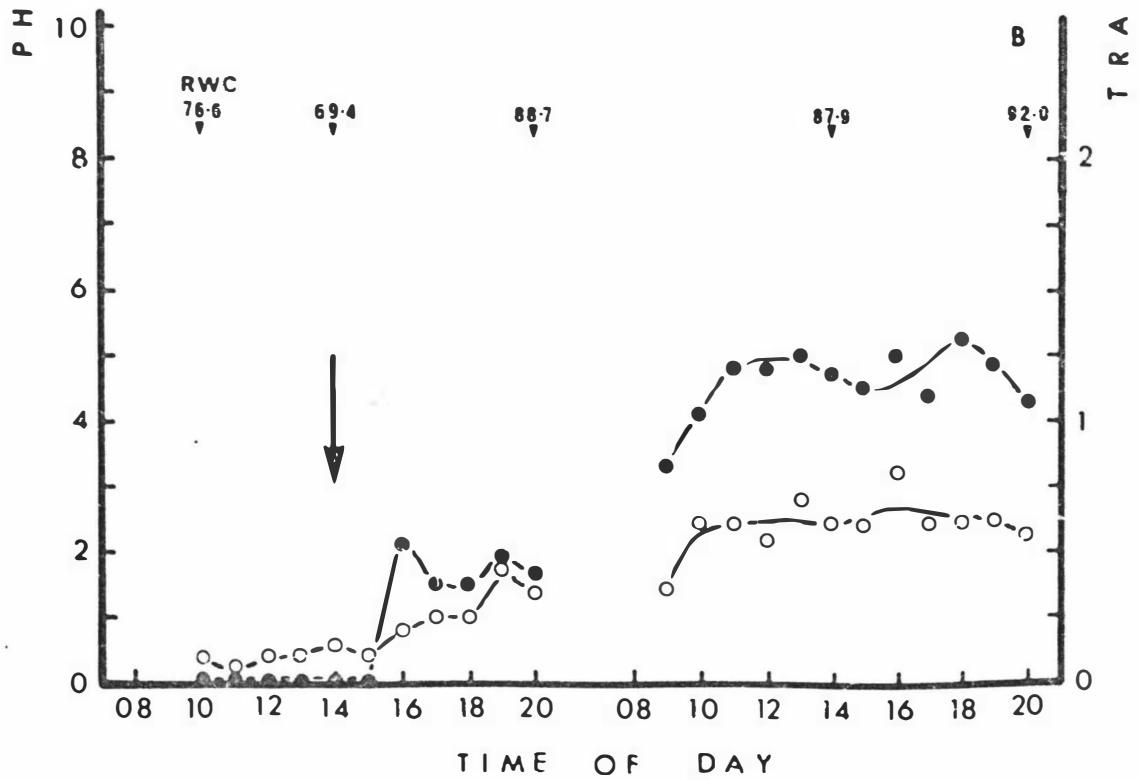
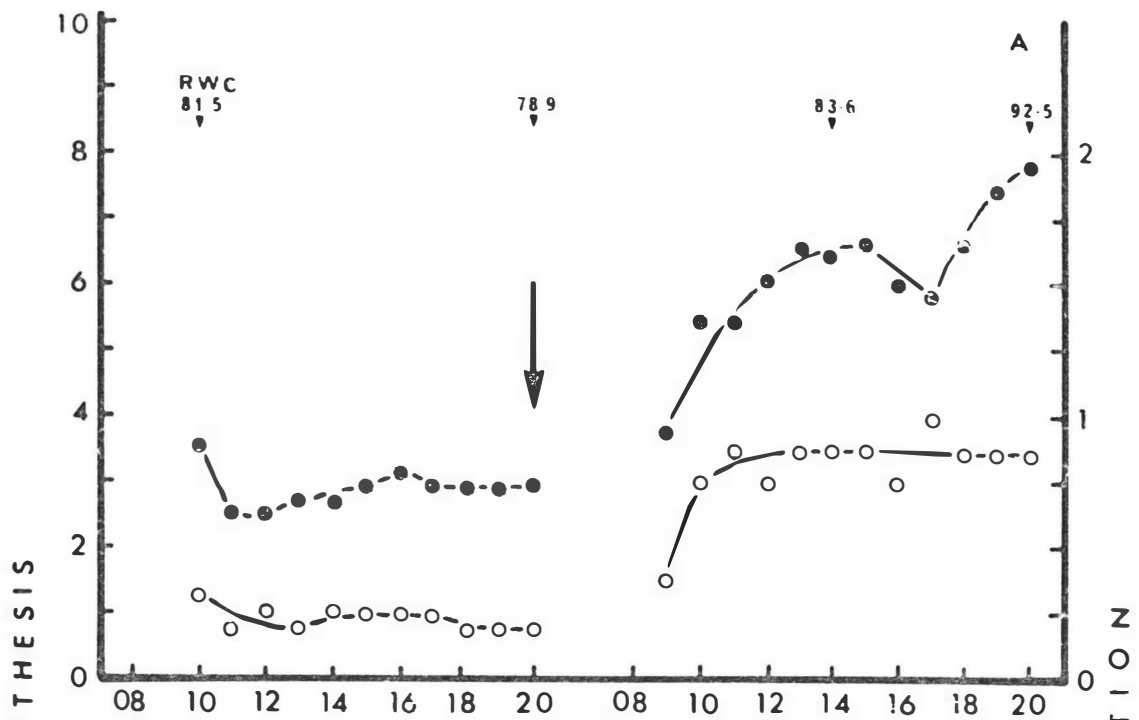


Table 5-I. Summary of rewatering experiments carried out under two sets of environmental conditions. Figures in brackets following maximum post-rewatering rates of photosynthesis and transpiration are these rates expressed as a percentage of the maximum pre-rewatering rate.

Treatment and Replicate	maximum pre-rewatering rate		RWC(%) at		Maximum post-rewatering rate	
	<u>Photosynthesis*</u>	<u>Transpiration+</u>	<u>rewatering</u>	<u>after</u>	<u>Photosynthesis*</u>	<u>Transpiration+</u>
HT/LH						
1	23.7	2.76	76.7	89.4	14.9 (62.9)	1.63 (59.1)
2	23.2	2.80	82.2	92.0	18.9 (81.4)	2.35 (83.9)
3	22.7	2.88	76.8	87.5	16.1 (70.9)	1.89 (65.6)
4	14.5	1.95	68.7	87.9	11.8 (81.4)	1.59 (81.5)
5	22.7	2.54	83.8	91.0	17.5 (77.1)	2.00 (78.7)
LT/HH						
1	Stressed in cabinet		78.9	93.6	7.8	0.98
2	7.7	1.20	91.1	93.1	7.6 (98.7)	1.22 (101.6)
3	Stressed in cabinet		79.3	91.8	7.5	1.11
4	7.0	1.17	86.1	93.1	7.1 (101.4)	1.00 (85.4)
5	Stressed in cabinet		69.4	92.0	5.2	0.81

* mg CO₂/dm²/hr

+ gm H₂O/dm²/hr

before rewatering, and leaf RWC at the time of, and after, rewatering.

Maximum rates prior to rewatering are not given for replicates 1, 3 and 5 of the LT/HH treatment because these plants were stressed in the cabinet to induce low RWC's.

Table 5-II shows the soil moisture tension at the time of rewatering in each experiment, and in conjunction with Table 5-I emphasises the effect of environment on plant-soil water relations. Table 5-I shows that there is no relationship between the RWC at the time of rewatering and the subsequent degree of recovery, as given by the post-rewatering rates as a percentage of the pre-stress maximum rates. Replicates 2 and 4 (HT/LH) showed the same degree of recovery although the RWC's at the time of rewatering were different (82.2% and 68.7% respectively); Table 5-II shows that the soil moisture tensions for these two plants were the same at the time of rewatering.

Table 5-II. Soil moisture tension at the time of rewatering for the plants under the two treatments.

<u>Replicate</u>	Soil moisture tension (atm.) at rewatering	
	<u>Treatment HT/LH</u>	<u>Treatment LT/HH</u>
1	0.50	15 +
2	0.23	0.20
3	0.29	15 +
4	0.23	0.29
5	0.16	15.+

Figure 5-3 provides evidence that for plants grown under the HT/LH treatment the degree of recovery of photosynthesis and transpiration was related to the soil moisture tension at the time of rewatering; the higher the soil moisture tension, the lower the degree of recovery. It is not possible, from the data available, to determine the exact nature of this relationship, nor to plot this relationship for the LT/HH treatment. After rewatering, soil moisture tension did not exceed 0.05 atm. in any instance.

The data in Table 5-I suggest that full leaf RWC (i.e. RWC above 91%) was not regained in some cases (HT/LH, replicates 1, 3 and 4) but it is possible that these plants regained higher RWC's than those shown, prior to sampling. The rate of imposition of water stress under the HT/LH treatment was very rapid (see Chapter 4), and these plants may have again become water stressed after regaining full turgidity. Nevertheless, the maximum rates of photosynthesis and transpiration prior to water stress were not regained in the plants that did have high RWC's at the time of sampling (replicates 2 and 5). With all HT/LH plants, the degree of recovery of photosynthesis and transpiration was essentially the same. The greatest difference between the recovery of photosynthesis and transpiration was 5.3% (HT/LH replicate 3), and there was no evidence of one of these processes consistently recovering to a greater degree than the other.

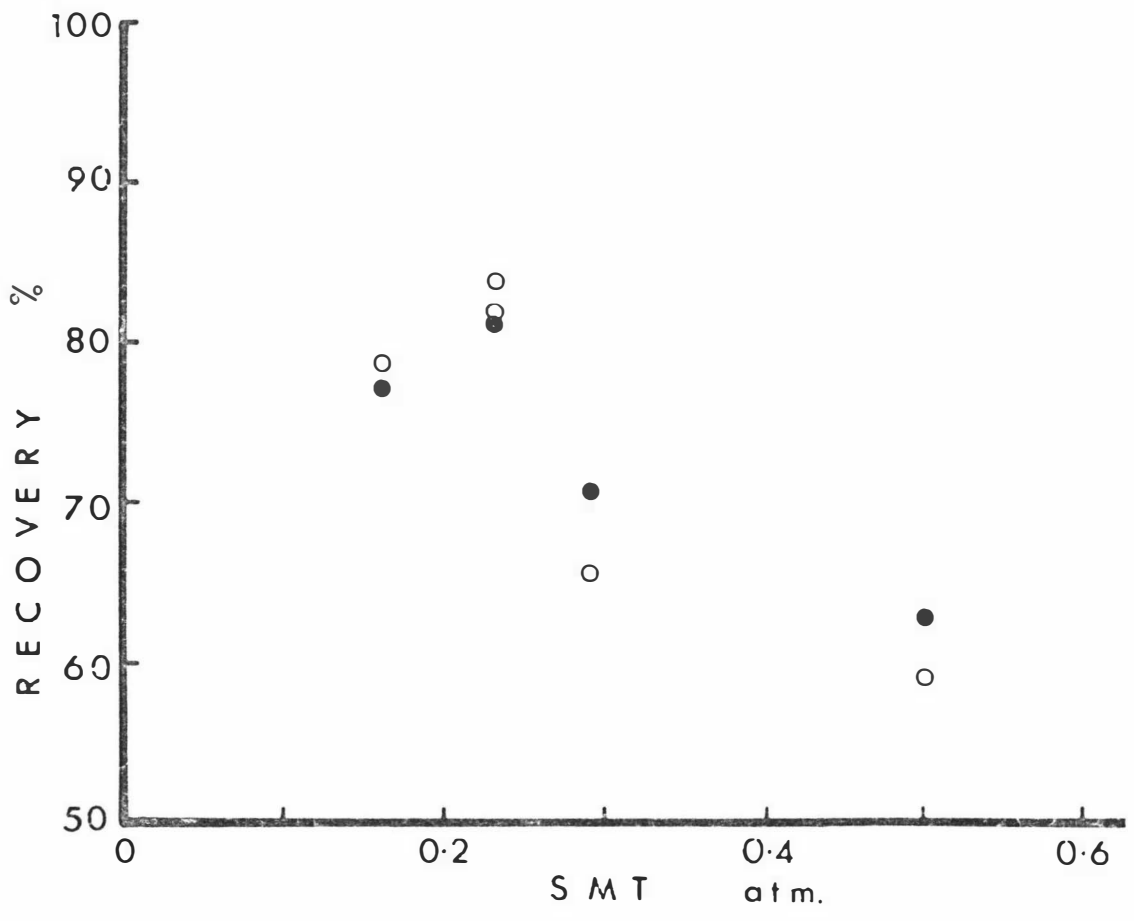
Figure 5-3

Dependence of the recovery of photosynthetic and transpiration rates following rewatering on the soil moisture tension (SMT) at the time of rewatering under HT/LH conditions.

Recovery (Recovery %) is expressed as the maximum post-rewatering rate of photosynthesis or transpiration as a percentage of the maximum rate prior to the imposition of stress.

Solid circles : photosynthesis

Open circles : transpiration



The results from the LT/HH treatment are more difficult to interpret because more than half the plants used underwent the initial period of stress in the growth cabinet, and consequently there are no unstressed maximum rates available on which to base the degree of recovery. The maximum pre-rewatering rates of photosynthesis and transpiration for replicates 2 and 4 were rather lower than the treatment average (Chapter 3), and the post-rewatering maximum rates of photosynthesis of replicates 1 and 3 were similar to those for replicates 2 and 4. It is therefore possible that these two plants (1 and 3) showed full recovery of photosynthesis after rewatering, although their transpiration rates were lower than normal. Replicate 2, which was rewatered at 91.1% RWC, was not visually under water stress at that time. The subsequent rates were little different from those obtaining before rewatering.

Replicate 5 of the LT/HH series, which suffered the greatest degree of water stress before rewatering (RWC 69.4% : soil moisture tension in excess of 15 atm.) clearly did not recover its original rates of photosynthesis and transpiration within the period of the experiment.

Replicate 4, LT/HH, is of interest in providing a marked contrast with the recovery pattern of the HT/LH plants. Although this plant fully regained both leaf RWC and the original photosynthetic rate, transpiration recovery lagged well behind, and showed a maximum of 85.4% of the original rate at the end of the experiment, compared with 101.4% for photosynthesis.

DISCUSSION

The close resemblance of the time course of recovery of plants rewatered at the end of the light period to the normal time course of non-stressed plants (Figs. 4-1 and 4-2, day 1) is attributable to overnight recovery of leaf turgidity. Night-time evaporative demand was low (low VPD) and the stomata almost certainly closed, so that the resulting slight loss of water was exceeded by water absorption. At the start of the following light period the leaf water deficit had been made good, and the plant was no longer subject to water stress. The influence of the time of rewatering on the pattern of recovery is uncertain, but may be non-existent. Replicates 2 and 4 of the HT/LH treatment were rewatered at 1315 and 2015 hrs. respectively; the soil moisture tension at rewatering was the same in both cases, as was the degree of recovery of photosynthesis and transpiration.

Under both treatments the period of water stress appeared to produce after-effects which persisted at least for the duration of the experiment, as evidenced by the failure to regain the original rates of photosynthesis and/or transpiration. This effect was more marked under the HT/LH treatment. There are many possible reasons for this, e.g. root suberisation, death of roots and/or root hairs, impaired

stomatal functioning and enzyme inactivation, but the data do not permit the effect to be definitely ascribed to any particular cause. It may be pointed out that root suberisation does not necessarily prevent, although it may reduce, water absorption. It has been shown, most recently by Pickersgill (1967), that water absorption can occur through the suberised portions of roots of various species.

That photosynthesis did not fully recover to its original level could be due to damage to the photosynthetic mechanism (possibly partial enzyme inactivation) resulting from the period of water stress, but the similarity of the degree of recovery of photosynthesis and transpiration also suggests that stomatal functioning may have been impaired. Impaired stomatal functioning as a consequence of water stress has been reported in a variety of species (Fischer, 1967; Pallas et al, 1967; Allaway and Mansfield, 1970).

It is possible that the maximum rates of photosynthesis and transpiration following rewatering given here do not represent the maxima that the plants could have attained. At the most, experiments were terminated at the end of the next full light period following rewatering, which was possibly too short a period for normal plant functioning to be resumed (Fischer, 1967), but the concern of these experiments was with the immediate post-rewatering response, and not with the longer term effects.

The continued fall in the rates of photosynthesis and transpiration after rewatering during the light period before recovery occurred, may at least in part, have been due to chilling of the root system by the added water. The roots and soil were at approximately plant chamber air temperature (Chapter 3) and the added water was colder than air temperature. Low soil temperature reduces photosynthesis and transpiration, (Babalola, Boersma and Youngberg, 1968), and has been used to induce water stress in the leaves of plants (Troughton, 1969). The delay in recovery of the rates of transpiration and photosynthesis in Brix's (1962) experiments with loblolly pine and tomato may also have been partly due to such temperature effects. Water given in future rewatering experiments should, therefore, be at the same temperature as the roots and soil to which it is to be added.

The recovery of maximum rates of photosynthesis but not of transpiration under the LT/HH treatment (Table 5-I), contrasts with the results from the HT/LH treatment where the two processes recovered to the same extent. Caldwell (1913) found that the transpiration rates of plants stressed under high humidities required twice as long to recover as those of plants stressed under low humidities, and that under the former treatment the root hairs were usually killed. In discussing this paper, Kramer (1950) suggests that under the treatment conducive to a slow imposition of water stress (high humidity), the plants were subjected to a

more severe level of stress than when rapidly wilted. Table 5-II shows that this was the case in the present experiments, the plants under the LT/HH treatment being subjected to higher soil moisture tensions than those under the HT/LH treatment, although in both cases the plant RWC's were similar (Table 5-I). If, as in Caldwell's experiments, the root hairs were killed under the LT/HH treatments, the reduced recovery of transpiration in replicate 4 (LT/HH) would be understandable. It would however, be necessary to postulate that the reduced water uptake was nevertheless adequate for the restoration of the original photosynthetic rate. As only 1-2% of the water absorbed by the roots is used in metabolic processes (Meyer and Anderson, 1952), the remainder being lost by transpiration, this postulate appears reasonable. The resumption of the original photosynthetic rates (except in the case of replicate 5 (LT/HH)) suggests that the enzymes associated with the photosynthetic mechanism were not affected by the period of water stress.

Under the HT/LH treatment plants showed a rapid and simultaneous recovery of both photosynthesis and transpiration. This is similar to the finding of Brix (1962) for loblolly pine. The degree of recovery of these

processes was inversely related to the soil moisture tension at the time of rewatering (Fig 5-3). Because leaf water potential falls with increasing soil moisture tension, it is suggested that this relationship was due to damage to some aspect of guard cell mechanism resulting from the development of low water potentials; the lower the level of water potential, the greater the resultant damage. Fischer (1967) showed that the impairment of stomatal functioning following water stress in tobacco was proportional to the duration and severity of the water deficit. Allaway and Mansfield (1970) suggested that the failure of stomata to open as widely as usual after wilting could be due to the fall in concentration of some promoter of stomatal opening. It is suggested that the degree of impairment of stomatal functioning is related to the water potential developed during the period of water stress, and that low water potentials may inhibit a promoter of stomatal opening. The observations and suggestions of Fischer (1967) and Allaway and Mansfield (1970) are in accordance with this possibility.

The simultaneous recovery of photosynthesis and transpiration is in accordance with the findings of Schneider

and Childers (1941), Loustalot (1945), and Brix (1962) for loblolly pine. The sequential recovery of the two processes reported by some authors (Thompson et al, 1965; Pallas et al, 1967) was not found in the case of soybean, although under the LT/HH conditions transpiration lagged behind photosynthesis in its degree, but not in its commencement, of recovery.

CHAPTER 6

SOME ASPECTS OF LEAF STRUCTURE WHICH MAY AFFECT RATES OF GASEOUS EXCHANGE

INTRODUCTION

Certain structural features of the leaf play a large part in the determination of the resistances to gaseous exchange between the leaf and the surrounding air. The boundary layer resistance, that resistance produced by the layer of non-turbulent air immediately adjacent to the leaf surface, is determined by the size and shape of the leaf as well as by windspeed and leaf orientation (Milthorpe and Penman, 1967; Slatyer, 1967). The depth, and consequently the resistance, of the boundary layer might be expected to be increased by the presence of leaf hairs (Slatyer, 1967), but although they have been shown to significantly decrease wind speed near the surface of soybean leaves, they may, if water-filled, contribute to the non-stomatal component of transpiration (Woolley, 1964).

The presence of leaf hairs may not, therefore, necessarily contribute to a reduction in transpiration: the structure of the hairs would also appear to be important.

Stomatal resistance to CO_2 and water vapour diffusion is partly determined by the dimensions and frequencies of the stomatal pores, and can be determined from measurements of these parameters (Lee and Gates, 1964; Jarvis, Rose and Begg, 1967), but certain aspects of stomatal dimensions, notably pore depth and width are very difficult to measure. The structure of the pore as seen in vertical section is frequently complex and seldom approximates that of a simple cylinder (Lee and Gates, 1964), so that no single measurement adequately characterises depth or width. The width is also subject to rapid changes, and this dimension, as measured on excised material probably bears little relation to the width prior to excision. The validity of measurements made using surface impression techniques (e.g. Zelitch, 1961) has recently been called into question by Glinka and Meidner (1968) and Leshem and Thaine (1969).

Because of the difficulties of making meaningful measurements of stomatal dimensions considerable effort has been put into the development of instruments for in situ measurements of diffusion resistance (e.g. van Bavel, Nakayama and Ehrlner,

1965; Slatyer and Jarvis, 1966; Kanemasu, Thurtell and Tanner, 1969). A knowledge of the stomatal dimensions is not a prerequisite for the interpretation of the results obtained from such instruments. Unfortunately no such equipment was available for use in the present study.

The experiments reported in this thesis were not designed, nor was the resulting data adapted, for the quantitative determination of resistances to gaseous diffusion. However, it was considered worthwhile to calculate such resistances from anatomical data to determine whether these gave any indication of the causes of differences in rates of photosynthesis and transpiration between treatments.

Accordingly stomatal pore and leaf hair lengths and frequencies were determined for the upper and lower leaf surfaces. In order to provide a more complete description of the morphology of the plants grown under the different treatments, leaflet thickness and epidermal cell frequencies were also determined.

METHODS

Selection and preparation of material

Soybean plants were grown under the four environmental treatments in a growth cabinet as described in Chapter 2, section II.

All measurements of leaf structure were made on leaflets of the third fully mature leaf; the lateral leaflets were used for leaf hair measurements, and the central leaflet for all other investigations. Strips approximately 2 cm x 1 cm were cut from the basal half of the centre leaflet with a razor blade. The main leaf vein was avoided. The strips were killed by immersion in boiling water and then transferred successively to boiling 70% ethyl alcohol and hot 88% lactic acid, each for two minutes to clear them (Clarke, 1960). They were then stored in cold 88% lactic acid. Material from seven plants per treatment was prepared in this way. Measurements of leaf hair length and distribution were made on fresh material.

Leaf hair distribution

Lateral leaflets were removed from plants with a razor blade and the number of leaf hairs in each of 10 fields of view (area 12.57 mm^2) on the upper and lower leaf surfaces counted under a Zeiss dissecting microscope. Five leaflets from each treatment were examined. Results for upper and lower surfaces were expressed as number of leaf hairs per cm^2 .

Leaf hair length

Leaf hairs were removed with microforceps from the leaflets used for leaf hair distribution determinations. Care was taken to remove the whole hair, and not to break it off short. The lengths of ten hairs from each surface of five leaflets per treatment were measured on a piece of millimetre graph paper to the nearest 0.1 mm under the microscope.

Stomatal and epidermal cell frequency

The preserved strips were temporarily mounted in 88% lactic acid and the number of stomata in each of ten fields of view (area 0.204 mm^2) counted on each surface. The counts were converted to numbers of stomata per cm^2 for the upper and lower leaf surfaces. Epidermal cell frequency was similarly determined.

Stomatal length

The lengths of ten stomata (pore only) on each surface of each of the seven strips per treatment were measured using an eyepiece micrometer previously calibrated against a standard micrometer slide. Pore length was expressed in microns. (μ).

Leaflet thickness

Leaflet thickness was determined from sections of the preserved material that had been dehydrated, wax-embedded, and cut on a hand microtome into 10μ thick transverse sections.

RESULTS

Plants grown under the two low temperature treatments tended to have rather smaller leaflets than those grown under the high temperature treatments. An index of this difference was obtained from the data used for the determination of leaf area - leaf dry weight relationships. Dividing leaf area by the number of leaflets on the plant gave mean leaflet area. The means for 14 plants from each treatment are given in Table 6-I. The average area of the 5 largest leaflets measured in each treatment is also given in this table.

Table 6-I Mean leaflet areas (cm^2) and standard errors, and mean area (cm^2) of the 5 largest leaflets from plants grown under each treatment.

<u>Treatment</u>	<u>Mean leaflet area</u>	<u>Mean area of 5 largest leaflets</u>
HT/LH	15.7 \pm 0.72	49.1
HT/HH	17.3 \pm 0.75	50.6
LT/LH	14.8 \pm 0.63	39.4
LT/HH	12.7 \pm 0.58	33.6

Leaf hair distribution

The mean frequencies of leaf hairs on the upper and lower surfaces of leaflets from plants grown under the four treatments are shown in Table 6-II. In all cases leaf hairs were at least one and one half times more frequent on the lower

surfaces than on the upper. There were no consistent effects of temperature or VPD on leaf hair number, the plants with the most leaf hairs being those grown under the HT/LH and LT/HH treatments. Plants grown under LT/LH conditions had the least number of leaf hairs on both leaf surfaces, but with the other treatments the order of hirsuteness was not consistent between upper and lower surfaces.

Table 6-II Mean frequencies (number per cm^2) and standard errors of leaf hairs on upper and lower surfaces of leaflets from plants grown under the four treatments.

<u>Treatment</u>	<u>Upper Surface</u>	<u>Lower Surface</u>
HT/LH	150 \pm 3.2	229 \pm 3.3
HT/HH	109 \pm 1.8	241 \pm 2.5
LT/LH	89 \pm 2.2	203 \pm 3.0
LT/HH	142 \pm 3.9	251 \pm 4.0
Least significant difference (LSD) 1%	16.9	13.8

The difference in leaf hair frequencies for the upper surfaces of HT/LH and LT/HH plants was not significant at the 1% level; all other treatment differences were significant at this level. The lower surface leaf hair frequencies were significantly different, at the 1% level, between LT/LH plants and all other treatments, barely significant between

HT/LH and LT/HH plants, and not significant for all other treatment differences.

Leaf hair length

The mean lengths of leaf hairs on the upper and lower surfaces of leaflets from plants grown under the four treatments are shown in Table 6-III. In all treatments the upper surface leaf hairs were longer than those on the lower surface, but there were no consistent differences in length attributable to temperature or VPD. The great majority of the hairs were water-filled.

Table 6-III Mean lengths (mm) and standard errors of leaf hairs on upper and lower surfaces of leaflets from plants grown under the four treatments.

<u>Treatment</u>	<u>Upper Surface</u>	<u>Lower Surface</u>
HT/LH	1.45 ± 0.04	1.37 ± 0.03
HT/HH	1.29 ± 0.02	1.27 ± 0.02
LT/LH	1.25 ± 0.04	1.22 ± 0.04
LT/HH	1.38 ± 0.04	1.25 ± 0.03
LSD 1%	0.14	0.09

Differences in the upper surface leaf hair lengths are significant at the 1% level between HT/LH and LT/LH plants, and barely significant between HT/LH and HT/HH

plants. All other treatment differences are not significant at this level. For the lower surface there are significant (1% level) differences between HT/LH and all other treatments only. Overall, the differences between the mean lengths are small.

Stomatal frequency

Table 6-IV shows the mean numbers of stomata per cm^2 on the upper and lower surfaces of leaflets from plants grown under the four treatments. Plants grown under the two high temperature treatments had proportionately more stomata on the upper surface (28% of total) than plants grown under the low temperature conditions (24-25% of total). Plants grown under the LH treatments had rather fewer stomata per cm^2 on either surface than the HH plants, the differences being significant at the 1% level.

Table 6-IV Mean frequencies (number per cm^2) and standard errors of stomata on upper and lower surfaces of leaflets from plants grown under the four treatments.

<u>Treatment</u>	<u>Upper Surface</u>	<u>Lower Surface</u>
HT/LH	9700 \pm 114	24800 \pm 190
HT/HH	10200 \pm 155	26200 \pm 215
LT/LH	8000 \pm 155	25200 \pm 207
LT/HH	10440 \pm 133	31600 \pm 280
LSD 1%	833	1250

The greater frequency of stomata on the lower surfaces of the leaflets is in accordance with the stomatal distribution pattern of most dicotyledonous plants (Zucker, 1963).

Stomatal length

There were no great differences between the lengths of stomatal pores of plants grown under the four treatments (Table 6-V).

Table 6-V Mean lengths (μ) and standard errors of stomata on the upper and lower surfaces of leaflets from plants grown under the four treatments.

<u>Treatment</u>	<u>Upper Surface</u>	<u>Lower Surface</u>
HT/LH	12.66 \pm 0.16	13.22 \pm 0.14
HT/HH	13.27 \pm 0.14	14.23 \pm 0.15
LT/LH	12.54 \pm 0.15	12.86 \pm 0.17
LT/HH	12.63 \pm 0.10	13.04 \pm 0.13
LSD 1%	0.50	0.50

The stomatal pores of HT/HH plants were significantly longer on both leaf surfaces than those of plants from the other treatments, but the differences involved were small. There are no significant differences (1% level) between the pore lengths of plants from the other treatments. In all cases the pores were slightly longer on the lower leaf surface than on the upper.

Epidermal cell frequency

The mean number of epidermal cells per cm^2 on the upper and lower surfaces of leaflets from plants grown under the four treatments are given in Table 6-VI.

Table 6-VI Mean frequencies (number per cm^2), and standard errors, of epidermal cells on upper and lower surfaces of leaflets from plants grown under the four treatments.

<u>Treatment</u>	<u>Upper Surface</u>	<u>Lower Surface</u>
HT/LH	69500 \pm 2070	36460 \pm 2115
HT/HH	65700 \pm 1985	39900 \pm 1820
LT/LH	66750 \pm 2313	36800 \pm 3115
LT/HH	69830 \pm 2195	34750 \pm 3453

None of the treatment differences were significant at the 1% level.

Differences in epidermal cell size between the treatments were small for either leaf surface. The lower frequency of epidermal cells per cm^2 on the lower surface is due to the greater frequency of stomata on this surface. The subsidiary cells of the stomata were not counted as epidermal cells.

Leaflet thickness

The mean leaflet thicknesses for plants grown under the four treatments are given in Table 6-VII.

Table 6-VII Mean leaflet thickness (μ) and standard errors of plants grown under the four treatments.

<u>Treatment</u>	<u>Mean leaflet thickness</u>	<u>Standard error</u>
HT/LH	175.8	2.9
HT/HH	161.8	2.5
LT/LH	193.2	2.1
LT/HH	183.7	2.7
LSD 1%	16.3	

Leaflets from plants grown under the low temperature conditions were thicker than those from plants grown under high temperature, and at each temperature the leaflets were thicker under LH conditions. The difference in leaflet thickness between the HT/LH and LT/HH plants is not significant at the 1% level.

Summary of leaf morphological features

A summary of the morphological features of leaves from plants grown under the four treatments is given in Table 6-VIII.

Table 6-VIII Morphological features of leaves of plants grown under the four treatments. Rank order (highest to lowest) in brackets.

Morphological feature	Leaf * surface	Treatment			
		HT/LH	HT/HH	LT/LH	LT/HH
Leaf hair frequency (no/cm ²)	u	150 (1)	109 (3)	89 (4)	142 (2)
	l	229 (3)	241 (2)	203 (4)	251 (1)
Leaf hair length (mm)	u	1.45(1)	1.29(3)	1.25(4)	1.38(2)
	l	1.37(1)	1.27(2)	1.22(4)	1.25(3)
Stomatal frequency (no/cm ²)	u	9700 (3)	10200 (2)	8000 (4)	10440 (1)
	l	24800 (4)	26200 (2)	25200 (3)	31600 (1)
Stomatal length (μ)	u	12.66(2)	13.27(1)	12.54(4)	12.63(3)
	l	13.22(2)	14.23(1)	12.86(4)	13.04(3)
Epidermal cell frequency (no/cm ²)	u	69500 (2)	65700 (4)	66750 (3)	69830 (1)
	l	36460 (3)	39900 (1)	36800 (2)	34750 (4)
Mean leaflet area (cm ²)	-	15.7 (2)	17.3 (1)	14.8 (3)	12.7 (4)
Leaflet thickness (μ)	-	175.8 (3)	161.8 (4)	193.2 (1)	183.7 (2)

* u = upper surface. l = lower surface.

Plants grown under the LT/LH treatment had the lowest (or second lowest) frequencies of stomata and leaf hairs and also the shortest stomatal pores and leaf hairs. Otherwise there were no consistent effects of temperature or VPD on the rank order of the morphological features examined.

Stomatal diffusion resistance

Stomatal diffusion resistance to water vapour diffusion per unit leaf area (one surface), r , is given by

$$r = \frac{d}{wlD} \left(\frac{1}{n} \right) \text{ sec/cm} \quad (1)$$

where d , w and l are the mean depth, width, and length respectively of the stomatal pore, D is the diffusion coefficient of water vapour in air and n is the number of stomata per unit area of leaf (Jarvis et al, 1967). The upper and lower surfaces of the leaf constitute parallel pathways for diffusion, the total stomatal resistance (both surfaces), R , being obtained from

$$\frac{1}{R} = \frac{1}{r_u} + \frac{1}{r_l} \text{ sec/cm} \quad (2)$$

where r_u and r_l are the stomatal diffusion resistances per unit leaf area of the upper and lower surfaces respectively, calculated from equation (1).

As no measurements of stomatal pore width or depth were made, pore depth was assumed equal to pore length, and pore widths were taken from the data of Hofstra and Hesketh (1969 b) for soybean. The assumed widths were 4μ and 8μ for the upper and lower surfaces respectively for plants grown under the two HT treatments, and 3μ and 7μ for plants grown under the LT treatments.

Total stomatal diffusion resistance, R , was calculated for water vapour and CO_2 diffusion for plants grown under each

treatment, the appropriate values for l and n (Table 6-VIII) being substituted into equation (1), and the resulting values of r_u and r_l into equation (2). The values of the diffusion coefficients of water vapour and CO_2 in air were taken as 0.258 and 0.165 cm^2/sec respectively after Gale and Poljakoff-Mayber (1968). The values of R obtained are given in Table 6-IX.

Table 6-IX Total stomatal diffusion resistances per cm^2 leaf surface (R) for plants grown under the four treatments.

<u>Treatment</u>	<u>Resistance sec/cm</u>	
	<u>Water vapour</u>	<u>CO_2</u>
HT/LH	0.17	0.27
HT/HH	0.16	0.25
LT/LH	0.19	0.30
LT/HH	0.15	0.23

DISCUSSION

Boundary layer resistance

The leaf boundary layer resistance may be reduced by leaf flutter (Heath, 1969) and becomes negligible at windspeeds in excess of 89.4 cm/sec (Gates, 1968). The windspeed across the plant chamber used in the present experiments was 60 cm/sec and was sufficient to cause a constant fluttering of the leaves. It is therefore probable that the boundary layer resistance was small.

It would appear unlikely that the leaf hairs had any significant effect on the magnitude of the boundary layer resistance, for compared with the stomata their frequency was low. The ratio of the numbers of stomata to numbers of leaf hairs per cm^2 ranged from 65:1 (HT/LH) to 94:1 (HT/HH) for the upper surface, and from 108:1 (HT/LH) to 126:1 (LT/HH) for the lower surface of the leaves. Additionally, as noted by Woolley (1964), the majority of the hairs were water filled and might therefore be expected to contribute to the non-stomatal component of transpiration.

It would therefore appear unlikely that the leaf hairs had any reducing effect on the rate of transpiration.

Stomatal diffusion resistance

Minimum values of stomatal resistance to water vapour diffusion per unit surface (both sides) of the leaf (R) were collected from the literature by Cowan and Milthorpe (1968). For mesophytic plants R ranged from 0.7 sec/cm for sunflower to 2.0 sec/cm for bean. Holmgren, Jarvis and Jarvis (1965) also found sunflower to have an exceptionally low stomatal diffusion resistance.

The values of R calculated for soybean plants grown under the various treatments (Table 6-IX) are all rather similar,

and are so much lower than the value given above for sunflower that it is difficult to regard them as realistic. It is felt that no useful purpose would be served in attempting to relate them to the measured rates of transpiration and photosynthesis of plants grown under the various treatments.

Although there is no indication that the morphological features examined were associated with the differences in rates of photosynthesis and transpiration of the plants under the various treatments, it is unlikely that these processes were totally independent of leaf morphology. That no correlation between the morphological features and the rates of photosynthesis and transpiration can be shown is probably due to the nature of the data involved: physiological data from whole plants and morphological data from a specific leaf.

Nevertheless the morphological data are of value as a partial definition of the structure of the plants produced under the various environmental treatments, and it is primarily from this standpoint that they should be regarded.

CHAPTER 7

CONCLUSIONS

Conditions of adequate soil water supply

From the measured responses of adequately watered soybean plants to the various environmental treatments (Chapter 3), three points of particular interest emerge.

The first and most striking of these, is the reduction in the rate of photosynthesis under conditions of low VPD compared with the rates at high VPD at the same temperatures. This is attributed, on the basis of appropriate calculations, to the plants grown under the low VPD (HH) treatments having a higher mesophyll resistance to CO_2 transfer than those under the high VPD (LH) treatments. It would appear that the magnitude of the mesophyll resistance is, at least in part, a product of the environmental conditions under which the plants are grown, but the mechanisms by which environment affects the mesophyll resistance are not known.

Previous reports, in which cotton has been the subject of investigation (Bierhuizen and Slatyer, 1964; Baker, 1965; Pailas, Michel and Harris, 1967), disagree as to whether high VPD increases or decreases the rate of photosynthesis, but none of the above authors showed any large change in the rate of photosynthesis for a VPD difference of 6.2 to 6.8 mb (the between-treatment differences in the present investigation). The present report is believed to be the first to show effects of VPD on the rate of photosynthesis comparable in magnitude with those normally associated with fairly substantial changes in temperature.

Secondly, whilst temperature had no significant effect on the rates of transpiration of plants under the two HH treatments, there was a significant difference between the transpiration rates of plants under the HT/LH and LT/LH treatments, the latter having the lower rates. This is attributed to the effects of temperature and the viscosity of water on the rate of water uptake by the roots. Low temperature reduces the permeability of the root to water and increases water viscosity (Kramer, 1969), and it is suggested that under LT/LH conditions water uptake was limited by these effects. This probably resulted in a lowering of leaf water potential and a certain degree of stomatal

closure (Cowan, 1965), as these plants were unable to meet the transpiration demand. Under HH conditions the effect of temperature was not apparent as the flux of water through the soil-plant-atmosphere system was lower than under LH conditions, and water uptake was probably sufficient to satisfy the transpiration demand. It would therefore appear that before the limiting effects of temperature are manifested the flux of water must exceed a certain value. The values of boundary layer plus stomatal resistances to water vapour diffusion ($r_a + r_s$) were calculated for plants under each treatment, and showed that plants under the LT/LH treatment did have a higher ($r_a + r_s$) than the HT/LH plants. Because the boundary layer resistance (r_a) was probably small and similar in magnitude for plants under all four treatments, it is thought that between-treatment differences in ($r_a + r_s$) are mainly attributable to differences in r_s . Apart from this effect of temperature at high VPD, the rates of transpiration of plants under the various treatments appeared to be mainly determined by VPD. This is basically in agreement with the conclusions of Rufelt, Jarvis and Jarvis (1963).

The third point of interest arising from the responses of adequately watered plants to their atmospheric environment concerns the relationship between the mean maximum rates of

photosynthesis and transpiration. When plotted against each other (Fig. 3-3) the points for three of the treatments fall close to a straight line, but the point for the HT/HH treatment lies well away from the line in the direction of a higher photosynthesis:transpiration ratio. The plants under the other three treatments had similar photosynthesis:transpiration ratios.

Water stress conditions

When water stress was imposed (Chapter 4) the decline in transpiration paralleled the decline in photosynthesis under all the environmental conditions used, thereby suggesting that the two processes were subject to a common controlling mechanism. From considerations of the nature of the processes of photosynthesis and transpiration it is suggested that this common mechanism was stomatal aperture and its corollary, stomatal diffusion resistance. Plotting the rates of photosynthesis and transpiration at various levels of water stress against each other (Fig. 4-4), showed that the HT/HH plants maintained a higher rate of photosynthesis per unit rate of transpiration whilst under water stress. Thus the factors responsible for the higher photosynthesis:transpiration ratio in these plants under conditions of adequate water supply continued to be effective under conditions of water stress.

Under all treatments the rates of photosynthesis and transpiration started to decline rapidly at a soil moisture tension of 0.2 atm. It is suggested that this decline was brought about by the onset of stomatal closure: as the rates declined RWC was observed to fall also. Shaw and Laing (1965) reported that the stomata of field-grown soybeans started to close in response to water stress at a leaf RWC of 89%, and there is mounting evidence that under water stress conditions the stomata are of prime importance in regulating the rates of gaseous exchange (e.g. Brix, 1962; Barrs, 1968; Troughton, 1969). When the relative rates of transpiration and photosynthesis between 0.2 and 0.4 atm. soil moisture tension are considered (Figs. 4-7 and 4-8) it is apparent that these were reduced less under the HH treatments than under the LH treatments at any given tension in this range. Denmead and Shaw (1962) reported qualitatively similar results for corn plants under field conditions. This effect is probably due to the rate of water movement into the root zone being sufficiently rapid to satisfy the demand made by plants transpiring at a low rate but not of plants transpiring more rapidly. Macklon and Weatherley (1965) also found that plant water deficits may originate in the failure of the root zone to become sufficiently rapidly re-wetted by water moving in from the surrounding soil mass.

Thus between 0.2 and 0.4 atm. soil moisture tension the rates of transpiration and photosynthesis appear to have been

determined by environmental and plant factors and also by soil moisture characteristics.

At about 0.4 atm. soil moisture tension the actual rates of photosynthesis and transpiration of plants under all four treatments were reduced to a very similar level and thus became independent of the atmospheric environment. It is suggested that this was the result of the rate of water uptake being limited by the rate of water movement into the root zone from the surrounding soil, and thus the maximum rate of transpiration was limited. This upper limit to the rate of transpiration corresponded to a rate of 0.5 gm water/dm²/hr. Increase in soil moisture tension above 0.4 atm. resulted in a further decline in the rates of photosynthesis and transpiration and in the level of leaf RWC, but the rate of decline was much less than between 0.2 and 0.4 atm. soil moisture tension.

It may be thought that the similarity of the transpiration rates of plants under all four treatments at soil moisture tensions above 0.4 atm. could be due to cuticular transpiration alone, the stomata being closed, rather than to a limiting effect of soil moisture conductivity on the rate of water uptake. This is not considered to be a likely explanation, for the mean night-time transpiration rates (Chapter 3) were considerably higher for plants under the two LT treatments

($0.21 \text{ gm/dm}^2/\text{hr}$ for both LH and HH treatments) than for plants under the HT/LH ($0.08 \text{ gm/dm}^2/\text{hr}$) and HT/HH ($0.13 \text{ gm/dm}^2/\text{hr}$) treatments, the VPD in all cases being very similar (2.0 to 2.5 mb). Assuming that the stomata were completely closed during the dark period these results imply that the cuticular resistance to water vapour diffusion was higher in the HT plants than in the LT plants. If the stomata were completely closed during the light period as a result of water stress, higher rates of cuticular transpiration would be expected from the LH than from the HH plants at each temperature, and this was not the case. It would therefore appear that transpiration rate was limited by the rate of water uptake from the soil.

It is thus possible to divide the response of soybean plants to increasing water stress under the conditions used in this investigation into three stages which may be characterised as follows:

Stage I: High soil moisture content and soil moisture tension below 0.2 atm. Rates of transpiration and photosynthesis are independent of soil moisture tension and determined by plant and atmospheric factors. Leaf RWC generally remains above 90%.

Stage II: Soil moisture tension between 0.2 and 0.4 atm.

Water uptake from the soil is reduced and water loss exceeds water absorption. Water deficits thus develop (RWC falls) and the stomata start to close, thus reducing, in parallel, the rates of transpiration and photosynthesis. Atmospheric, plant and soil moisture characteristics all influence the rates of photosynthesis and transpiration, and the level of RWC.

Stage III: Soil moisture tension above 0.4 atm. The rate of water uptake is limited chiefly by the rate at which water moves into the root zone. This imposes an upper limit on the rate at which plants may transpire, and transpiration and photosynthetic rates become independent of the atmospheric conditions.

During 'Stage II' of stress development the stomata would appear to be of considerable importance in regulating the rates of photosynthesis and transpiration. It is suggested that stomatal aperture is at least partially regulated by leaf turgor and RWC and that turgor and RWC are directly influenced by the balance between transpiration rate and the rate of supply of water to the leaf. Stomatal closure, brought about by loss of turgor (Meidner and Mansfield, 1968), affects the rates of

photosynthesis and transpiration proportionately as evidenced by the linear relationship between these processes at soil moisture tensions between 0 and 0.4 atm. (Fig. 4-4) and by the 1:1 correspondence between the relative rates at various levels of RWC (Fig. 4-12).

At soil moisture tensions above 0.4 atm. the rates of photosynthesis and transpiration may well be determined by separate mechanisms. Photosynthesis tended to decline more rapidly than transpiration (Fig. A7-1), and there is some evidence to suggest that transpiration might continue after the rate of photosynthesis had fallen to zero (compare Figs. 4-10 and 4-11). It is possible that at low levels of leaf hydration (low RWC) there is a direct effect of dehydration on the biochemical components of the photosynthetic mechanism.

Relief of water stress

When stress was relieved by rewatering photosynthesis and transpiration recovered simultaneously and, under HT/LH conditions, to a very similar extent (Chapter 5). The degree of recovery was inversely proportional to the soil moisture tension at the time of rewatering, and as leaf water potential falls in response to increase in soil moisture tension it is suggested that this relationship could be due to an effect of leaf water potential on some aspect of guard cell mechanism. Thus the lower the leaf water potential the

greater the resulting impairment of stomatal functioning and the lower the degree of recovery of the rates of photosynthesis and transpiration. It must be emphasised that this is hypothesis, for there was no direct evidence of an effect of leaf water potential on guard cell functioning. The hypothesis does however, complement the suggestions and observations of Fischer (1967) and Allaway and Mansfield (1970).

The 'three stages of stress' as a general phenomenon

Although the soil moisture tensions at which one 'stage' of the process of water stress development succeeds the former are specific to the combinations of soil type, plant species and environmental conditions used in this study, there are grounds for thinking that the three stages identified above may be of general application.

'Stage I' is the situation in which soil moisture content is high, soil moisture tension low, and the rates of transpiration and photosynthesis not limited by soil water availability. Under these conditions the rates of transpiration and photosynthesis are determined primarily by plant and environmental factors.

'Stage II' commences when the rate of transpiration exceeds the rate of water uptake due to the failure of the soil around the roots to become rewetted sufficiently rapidly to allow the original rate of absorption to be maintained. Plant water deficits develop, the stomata start to close and the rates of photosynthesis and transpiration are reduced. This stage of

the process is frequently reported (e.g. Weatherley, 1951; Brix, 1962; Cowan, 1965).

As the soil moisture content decreases still further soil moisture conductivity also decreases (Gardner, 1965), and it is suggested that ultimately a point is reached at which the conductivity of the soil is so low that transpiration is limited almost entirely by the rate of supply of water to the roots ('Stage III'). At this point transpiration ceases to be related to the atmospheric conditions (cf. Vznuzdaev, 1968). Even if the stomata are completely closed at this stage it is conceivable that under certain conditions (e.g. high VPD) non-stomatal transpiration could be similarly limited by the rate of supply of water to the roots.

Critique of Idso's water stress theory

Idso (1968), in a theoretical analysis of the effects of water stress on plants, proposed that "two essentially independently induced and independently acting water stresses operate upon the plant processes of photosynthesis and transpiration", and based this on the supposition that whereas transpiration is determined by the difference in water potential between the water in the leaves and the atmosphere, photosynthesis is dependent on the water potential in the vicinity of the chloroplasts and is therefore almost totally

dependent on the soil moisture status. His analysis however, does not consider the effect of stomatal resistance on gaseous exchange and implicitly assumes that no changes take place in stomatal aperture as the soil moisture tension increases. With increase in soil moisture tension leaf water potential falls, thus maintaining the water potential gradient between soil and leaf which is essential to the continued movement of water through the soil-plant system. As water potential decreases leaf turgor falls, and turgor changes are fundamentally involved in the regulation of stomatal aperture (Meidner and Mansfield, 1968). As turgor falls the stomata tend to close, and it is not therefore possible to conclude with any justification (as did Idso) that transpiration is 90% independent of the soil moisture status. Because stomatal resistance is a major determining factor in the rates of photosynthesis and transpiration (e.g. Holmgren, Jarvis and Jarvis, 1965; Whiteman and Koller, 1967; Troughton, 1969), and is affected by leaf turgor, the evidence would appear to point to a single stress rather than to two essentially different stresses, at least until the point is reached at which transpiration becomes independent of the atmospheric environment. As suggested above, photosynthesis may at this point be directly affected by the level of leaf hydration, and thereafter two independent stresses may indeed be operating. Under conditions of low evaporative demand the

rate of transpiration may be unaffected until quite high values of soil moisture tension are reached (Denmead and Shaw, 1962), but it is arguable if, under these circumstances, the plant can be regarded as being subject to water stress until the rate of transpiration starts to decline, and Idso's thesis is concerned with water stress situations.

Further considerations

It will be apparent to the reader that certain aspects of the results obtained in this investigation require further study if a full understanding of the interrelationships between plant, environment and water stress (both soil and atmospheric) is to be achieved. So far as the environmental conditions used in this investigation are concerned, it is of interest to note that the HT/LH treatment approximated average midsummer conditions of temperature and VPD in Illinois (U.S. Department of Commerce Weather Bureau, 1959), one of the world's major centres of soybean production, and that the LT/LH treatment similarly approximated conditions in the north-east of the North Island of New Zealand (N.Z. Meteorological Service, 1966), an area considered for soybean production.

The possible combinations of environmental factors (e.g. air and soil temperature, VPD, light intensity and soil type) under which plants may become subject to water stress are almost infinite, and the range of conditions employed in this study covers only a very small proportion of these combinations.

Within this limited environmental framework a number of plant responses were confined to one treatment only, for example the higher photosynthesis:transpiration ratio of the HT/HH plants and the effect of temperature on the transpiration rate of the LT/LH plants when soil water was plentiful. The 'threshold' conditions for the manifestation of these effects are at present unknown. There is consequently little justification for attempting to predict, on the basis of the results obtained, the responses of soybean plants to other combinations of environmental factors. In conclusion therefore, the following of many possible suggestions for further work in this field are offered.

Differences in the rates of photosynthesis of soybean plants under the various treatments appeared to be largely attributable to differences in the mesophyll resistance (r'_m). What are the mechanisms by which the environment affects r'_m ? How constant is the value of r'_m in the face of changing environmental conditions? (Troughton and Slatyer (1969) found that the r'_m of leaves of cotton plants was independent of temperature over a 20°C range; Bierhuizen and Slatyer (1964) that r'_m decreased with increasing light intensity). How is r'_m affected by water stress, in particular, is there a sudden increase in its value at the point at which water uptake becomes limited by soil moisture conductivity? (Troughton (1969) found that the r'_m was unaffected by decrease in RWC in cotton leaves until RWC fell to 75%. Thereafter further decrease in RWC

was accompanied by an increase in r'_m).

To what extent are RWC-leaf water potential relationships modified by environmental conditions, how sensitive is the relationship to small differences of temperature and VPD, and do differences in the relationship affect the sensitivity of the stomata to water stress? (Results of Jarvis and Jarvis (1963b) indicate that the answer to the last question may be in the affirmative).

The results obtained in this investigation suggest that under some circumstances damage to the root system may result from periods of water stress and that subsequently the ability of the roots to take up water from the soil is reduced. Information on the state (both physiological and anatomical) of roots and root hairs before and after stress would be of interest. Further information is also needed on the post-rewatering responses of stomata, and the effects of water deficits on guard cell metabolism.

Again some of the results suggest that there may be 'threshold' values of some factors which must be exceeded before certain effects are apparent. (Reference was made to these earlier in this chapter). What are these threshold values (if any) and what aspects of plant functioning do they affect?

In order to resolve these problems more fully, facilities for the measurement of leaf water potential and stomatal diffusion resistances are required. The former can be measured with the

thermocouple psychrometer and stomatal resistances with diffusion porometers (e.g. Kanemasu, Thurtell and Tanner, 1969). Measurement of CO_2 exchange and transpiration rates, in association with measurements of leaf water potential, RWC and stomatal resistances, are probably best made in single-leaf assimilation chambers. Rates can then be related to a precisely known area of leaf surface and the problem of averaging rates over the whole plant is avoided. Additionally such chambers permit evaluation of the CO_2 compensation point which must be known if accurate determinations of the resistances to CO_2 transfer are to be achieved.

Regarding the conditions under which such experiments should be carried out, the following recommendations are made. Firstly, it would be preferable to use a soil or potting mixture with a less inflected soil moisture characteristic curve than was used in this study. This would result in a rather more gradual imposition of plant water stress which would allow closer definition of the processes involved in stress development. In the experiments reported here, stress developed very rapidly after the soil moisture tension reached 0.2 atm. Secondly, it is desirable that as wide a range of environmental conditions as possible be employed. In this connection it would seem profitable to start with a range of VPD's at temperatures similar to those used in this investigation, for VPD appears to have a considerable influence on the mesophyll resistance to CO_2 transfer as well as on the rate of transpiration.

It is not suggested that the successful completion of the work outlined above will complete our knowledge of the processes involved in the effects and development of water stress in plants: it will merely bring that state a little closer. In the process it will be surprising if, as in this investigation, the number of new questions raised does not equal, or exceed, the number of questions answered.

APPENDIX 1

PRELIMINARY TESTS WITH VARIOUS POTTING MIXTURES

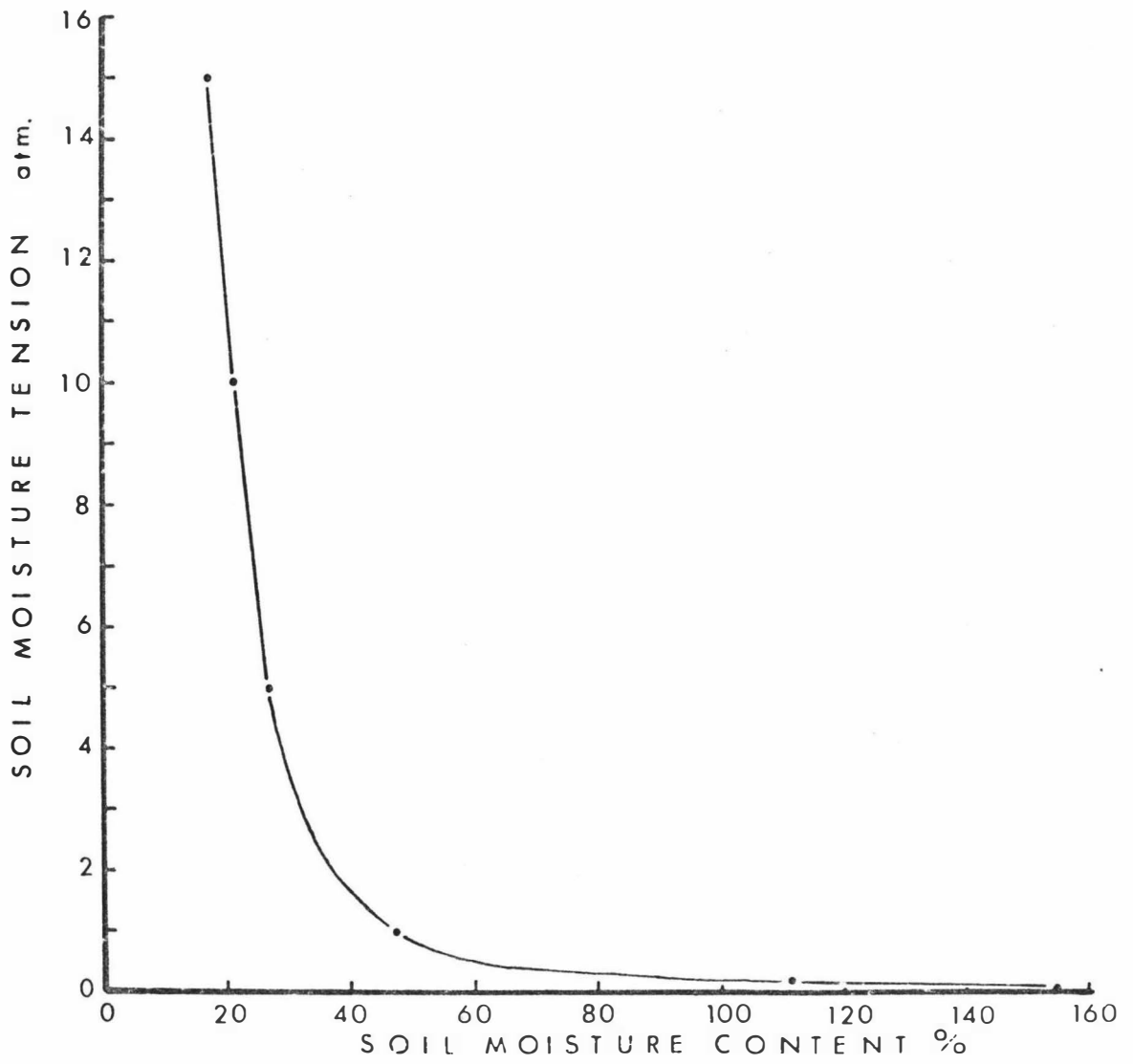
A number of preliminary trials were carried out to determine a suitable potting medium for the experiments on water stress and its effect on CO_2 exchange and transpiration. In the course of these trials (in which perennial ryegrass, white clover and tomato plants were used) it was found that a hard surface crust formed when the potting medium was soil or any combination of soil and pumice or soil and peat. The crust formed three or four days after transplanting the seedlings into the pots, and subsequently the plants made very poor growth, tomato being particularly severely affected. Such effects did not occur when a 1:1 (by volume) pumice-peat mixture was used and all species grew vigorously in this medium.

The continued availability of the soil (Manawatu silt loam) could not be guaranteed, and its initial nutrient status was an unknown, and possibly variable, quantity. Hence it was decided that the 1:1 pumice-peat mixture, of very low initial nutrient status, and in which plants had shown good growth with added nutrient solution, would be satisfactory.

The soil moisture characteristic curve for this mixture (Fig. A1-1) was determined by Mr. M.W. Gradwell (Soil Bureau, D.S.I.R., Lower Hutt) to whom I make grateful acknowledgement. Each point on the curve is the mean of three or four determinations. The samples used in these determinations were of the same density as the mixture in the pots used in the experiments.

Figure A1-1

Soil moisture characteristic curve for
the 1:1 pumice-peat mixture.



APPENDIX 2

EVOLUTION OF CO₂ AND EVAPORATION OF WATER
FROM POTS

The plant chamber of the CO₂ exchange-transpiration measuring equipment (Chapter 2, section IV) could be separated into root and shoot compartments with a metal plate, a hole in which accommodated the plant stem. The space between the plate and the stem could be sealed with silicone rubber to effect a gas-tight seal between the two compartments. Measurement of transpiration with the transpiration balance requires that the pot be free to move vertically. Complete separation of the pot and the stem and leaves of the plant was therefore impossible in the present experiments, and it was necessary to determine the quantity of CO₂ evolved, and the amount of water that evaporated, from the pot in unit time under various atmospheric and soil water stress conditions.

Water was withheld from soybean plants in the growth cabinet for periods ranging from one to ten days. The plant stems were then cut just above the level of the soil surface and the stumps covered with vaseline to prevent CO₂ evolution therefrom. The soil surface was then covered with a sheet of wax-backed aluminium foil to retard evaporation (see Chapter 2, section III for details).

Measurements of CO₂ evolution and evaporation were made using the CO₂ exchange-transpiration measuring equipment. Each pot remained in the plant chamber for at least three hours and the mean hourly rates were calculated in the same way as plant CO₂ exchange and transpiration rates (see Chapter 2, section VI).

(i) CO₂ evolution

The results (Table A2-I) showed that temperature and soil moisture stress had no consistent effect on the rate of CO₂ evolution from the pots.

Table A2-I Rates of CO₂ evolution (mg CO₂/hr) from pots at various temperatures and degrees of soil moisture stress.

<u>Days without water (soil moisture stress)</u>	<u>Air temperature °C</u>				
	<u>15</u>	<u>17.5</u>	<u>20</u>	<u>22.5</u>	<u>27.5</u>
0	5.0	-	5.2*	-	8.3
1	5.8	-	2.5	-	-
2	-	-	3.9	-	-
3	5.5	2.9	4.9	5.7	-
4	5.7	3.8	4.5	-	6.8
5	-	1.4	-	3.4	3.5
6	-	-	3.9	-	-
7	7.1	-	-	2.7	-
8	-	-	-	-	-
9	4.3	-	-	-	10.2
10	4.5	-	-	-	-

* Additional measurements under these conditions gave rates of 6.0, 9.1 and 10.0 mg CO₂/hr.

Because of the lack of consistency of these results the original aim of obtaining correction values for CO_2 evolution at various temperatures and soil water stress levels was abandoned, and the mean of the above results (5.25 ± 0.44 mg CO_2 /hr) taken as the correction factor for all conditions. For the purposes of the CO_2 exchange rate calculations this was simplified to 5.0 mg CO_2 /hr.

Similar determinations of CO_2 evolution from pots containing soybean roots in a pumice-peat mixture were made by Miss J. Rowley (pers. comm.). She found rates ranging from 2.3 to 6.3 mg CO_2 /hr at air temperatures between 26 and 28°C, and similar wide variations in rate at other temperatures.

The error introduced into the final results by the use of a single 'all-conditions' correction factor was small as the correction factor was added to the total photosynthetic rate (or subtracted from the total respiration rate) before the rate per unit area of leaf was calculated.

(ii) Evaporation of water from pots

The wax-backed aluminium foil pot covers provided a very effective barrier to evaporation from the soil surface for no weight loss was recorded in any of the tests. No correction for evaporation was therefore necessary and all weight loss from the pot and plant was attributed to transpiration.

APPENDIX 3

MEASUREMENT OF PLANT WATER STRESSa). Relative water content

Plant water stress was estimated by the relative water content (RWC) technique of Barrs and Weatherley (1962). Discs or segments of leaf tissue floated on distilled water take up water, the pattern of uptake with time being clearly divided into two phases. The initial, rapid phase of uptake (phase I) satisfies the water deficit in the tissue: the second phase (phase II), which is slower and prolonged, was shown by Barrs and Weatherley to be associated with growth of the tissue segments. Phase II does not commence until phase I is complete, and for accurate determination of RWC the tissue should be removed from the water at the conclusion of phase I. If this is not done, and phase II allowed to commence, the additional uptake of water results in an under-estimation of RWC.

The duration of phase I differs among species (e.g. Pinus taeda 12 hours, Harms and McGregor, 1962; wheat 4 hours, Yang and de Jong, 1968), and may also vary with the magnitude of the water deficit of the tissue (El-Sharkawy and Hesketh, 1964). It is therefore necessary to determine the duration of phase I uptake for any species with which it is intended to use the technique, and also to ascertain the constancy of

this duration at various levels of water stress.

Relative water content may also be under-estimated if there is bulk entry of water through the cut edges of the discs or segments, an effect known as injection. Before the technique was judged suitable for use with soybean leaf tissue it was therefore necessary to determine the duration of phase I uptake at various levels of water stress, and to ascertain that the results were not invalidated by the occurrence of injection.

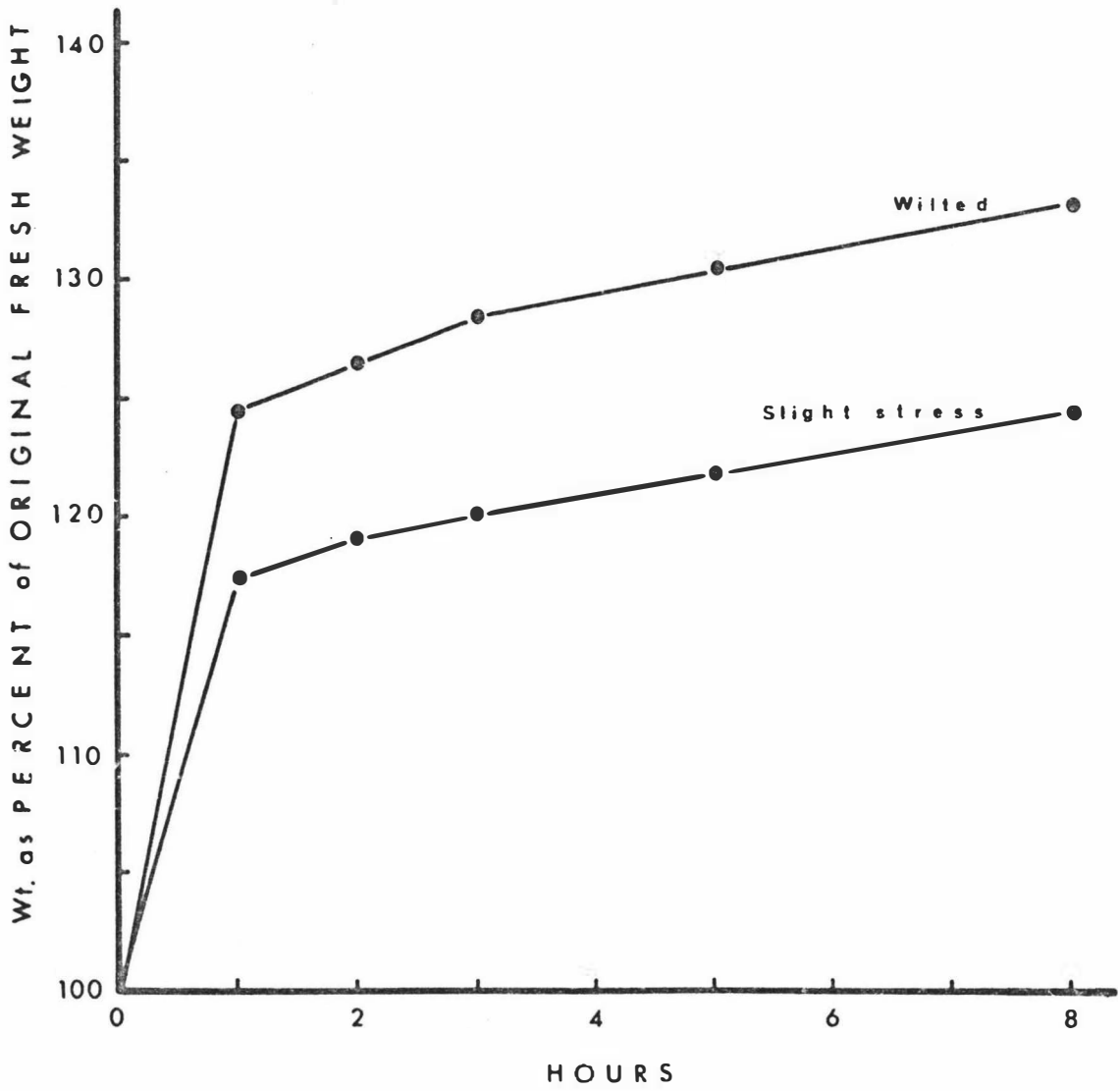
(i) Duration of phase I uptake

A sharpened cork borer was used to punch discs (each 1.3 cm. diameter) from the leaves of soybean plants that had been subjected to either a short period of water stress, or had been allowed to wilt. Care was taken to avoid the inclusion of large leaf veins in the discs. In each case the discs were bulked and separated into 10 samples, each of 6 discs. These were placed in tared, stoppered weighing bottles and the fresh weights determined. The discs were then floated on distilled water in a covered petri dish (one sample per dish) and at intervals were removed, blotted dry with filter paper, weighed in the original bottles and replaced in the dish. The weight of the samples at each weighing was expressed as a percentage of the original fresh weight, and the means of these percentage weights plotted against time (Fig. A3-1).

Figure A3-1

Change in fresh weight with time of floating
discs of soybean leaflets.

Fresh weight expressed as a percentage of
the original fresh weight.



It is apparent from this figure that phase I (the initial period of rapid water uptake) was completed within one hour for both sets of samples, and in all routine determinations of RWC one hour was therefore adopted as the time for which the discs were floated.

(ii) Determination of the presence or absence of injection

If injection does take place through the cut edges of the discs, small discs having a high circumference:area ratio will take up proportionately more water (and thus show a greater relative weight increase) than larger discs. Parallel samples of discs of two sizes (1.1 and 1.4 cm. diameter) were punched from the leaves of slightly water-stressed soybean plants, placed in tared, stoppered weighing bottles and weighed. The discs were floated on distilled water in covered petri dishes for $1\frac{1}{2}$ hours, blotted dry and reweighed. The $1\frac{1}{2}$ hour floating period was selected as being in excess of the phase I period, but not so much so that effects that would not occur during phase I would become apparent. The percentage change in weight of each sample after the floating period was determined and is given in Table A3-I.

Table A3-I Percentage change in fresh weight of discs of soybean leaf of two sizes, after $1\frac{1}{2}$ hours floating on water.

<u>Sample</u>	<u>Discs 1.1 cm diam.</u>	<u>Discs 1.4 cm diam.</u>
1	5.68	5.91
2	7.12	6.33
3	6.57	5.06
4	5.97	4.99
5	6.07	5.28
6	3.73	3.64
7	4.50	3.23
8	3.62	3.34
9	2.58	3.12
10	2.87	2.48
Mean	4.87	4.34

A t-test analysis showed that there was no significant difference between the two samples ($t = 2.567 : t_{0.02} = 2.82$). The RWC technique is thus suitable for use with soybean.

Both the duration of phase I uptake and the injection tests were repeated, the results being consistent with those given above.

b). Water potential

The most flexible tool for the measurement of water potential is the thermocouple psychrometer, which is suitable for use with plant or soil material and requires only small quantities of the sample. The technique, originally developed by Spanner (1951), has undergone considerable development at the hands of other workers, and the underlying theory has been discussed by Rawlins (1966) and Peck (1968, 1969). A search of the literature suggested that a version of the instrument described by Waister (1963) was the simplest and probably also the cheapest to construct, and a similar piece of equipment with minor modifications was built.

Essentially the apparatus consists of a temperature-controlled water bath in which are immersed a number of small air-tight sample chambers. The top of each chamber is fitted with a bung through which passes a fine-wire thermocouple. After bath and sample chambers have reached thermal equilibrium a current is passed through the thermocouple, the junction of which is thus cooled due to the Peltier effect, and a minute quantity of water condenses on the junction. The thermocouple is then switched into a galvanometer circuit, and the e.m.f. resulting from the cooling of the junction by the evaporation of the water drop measured. The galvanometer deflection is related to the relative humidity (which is translatable to

water potential) of the chamber atmosphere.

Modifications to Waister's design included the use of two water baths, one inside the other and interconnected by small holes in the inner bath, to facilitate temperature control. The outer bath, heavily lagged with insulating material and surrounded by a wooden box, was controlled to $\pm 0.5^{\circ}\text{C}$ by a commercial thermostat-regulated heater-stirrer (Tempunit). The inner bath, controlled to $\pm 0.001^{\circ}\text{C}$, was heated by a 60-watt carbon filament lamp bulb partially immersed in the water. Stirring was effected both by an electric stirrer and by the continuous bubbling of air through a perforated pipe on the floor of the bath. Temperature was sensed by a mercury contact thermometer which also activated the current to the carbon filament lamp. Temperature control was facilitated by running the outer bath at a slightly lower temperature than the inner bath, and by maintaining room temperature $3-4^{\circ}\text{C}$ below bath temperature by means of an air-conditioning unit. Temperature variation from point to point within the inner bath (in which the sample chambers were immersed) did not exceed 0.001°C .

The detailed procedures given by Box (1963) were followed in the calibration of the thermocouple psychrometers. Strips of filter paper soaked in sodium chloride solutions of known concentration were used to line the walls of the sample chambers, thus providing a known relative humidity within

the chamber. However, regardless of the strength or duration of the current passed through the thermocouples, no steady output could be obtained from any of the 14 thermocouples even after 24 hours immersion in the bath. (This exceeds the acceptable time for attainment of equilibrium: Waister (1963) found that after about 16 hours changes occurred in the sample tissue which led to a rapid rise in the galvanometer output). Additionally it was found that the signal from any given thermocouple was erratic and not related to the relative humidity of the chamber atmosphere.

Following Waister (1963) the sample chambers were of glass, but Lambert and van Schilfgaard (1965) suggested that water could be adsorbed onto the walls of glass or acrylic sample chambers thus giving rise to considerable errors, and recommended the use of metal chambers. A number of stainless steel chambers, their interiors electrolytically polished to give the smoothest possible surface, were made and tested. The results were as erratic and inconsistent as those obtained with the original glass chambers. Lack of skilled technical assistance prevented any further investigation of the problem.

APPENDIX 4

MEASUREMENT OF LEAF AREA AND DETERMINATION OF
THE LEAF AREA-LEAF DRY WEIGHT RELATIONSHIPS
OF PLANTS GROWN UNDER THE FOUR TREATMENTS

a). Measurement of leaf area

Because planimeter measurements of leaf area are very time consuming, a simple accurate method for determining leaf area that would be acceptable as a routine procedure was sought.

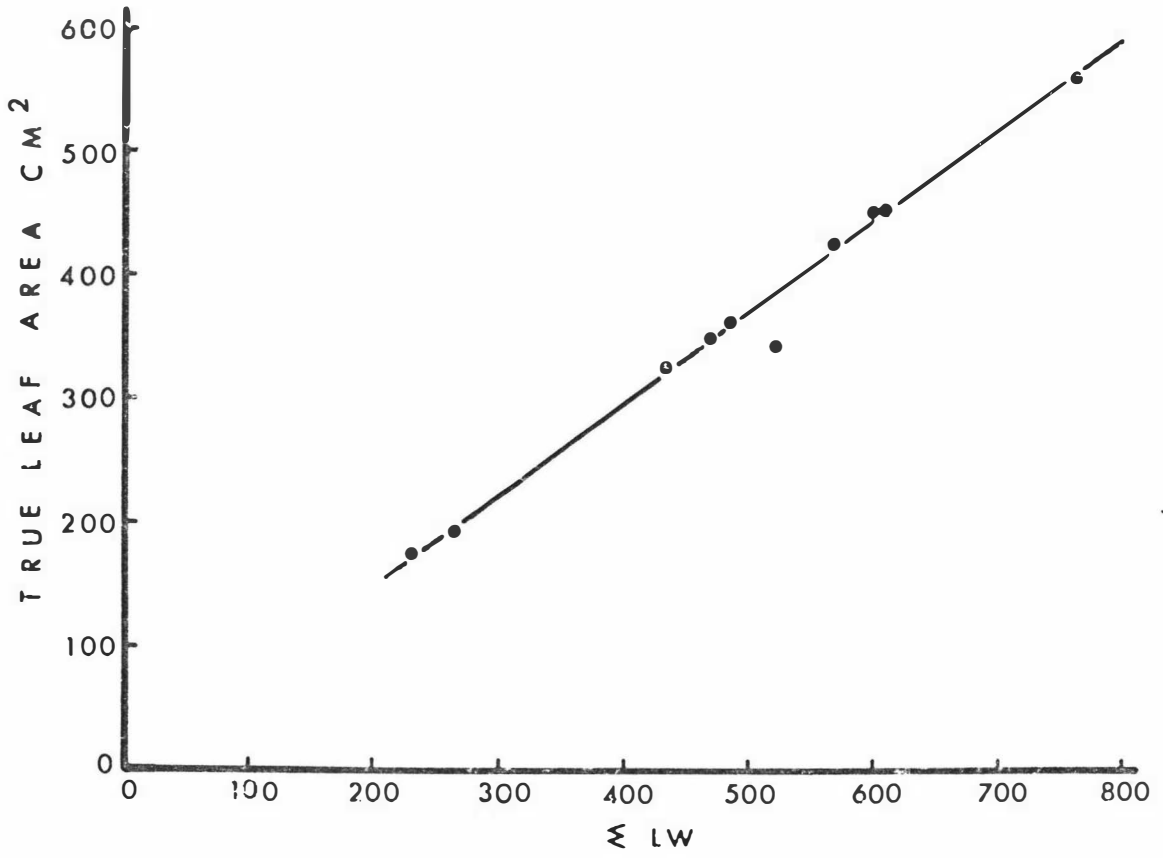
Mitchell (1953) found that for perennial ryegrass leaves the product of length and median width gave a figure that was sufficiently close to true leaf area to be satisfactory for comparative purposes. A similar approach to the problem was adopted for soybean.

Ten soybean plants that had been grown under LT conditions in a growth cabinet were selected to give a wide range of leaf areas. The leaflets were removed from each plant in turn, and the length (L) and maximum width (W) of each leaflet measured to the nearest 0.1 cm. ΣLW for each plant was calculated. The outline of each leaflet was then drawn on paper, cut out and weighed. The weight per unit area of the paper

Figure A4-1

Relationship between true leaf area and the sum of leaflet length and maximum width for the whole plant (ΣLW).

(Statistical data in text).



being known, the true leaf area was thus determined. Very small or folded leaves were excluded from the analysis.

A plot of ΣLW against true leaf area (Fig. A4-1) showed that the points fell very close to a straight line. Linear regression analysis gave the equation

$$\text{True leaf area} = 0.734 \Sigma LW + 2.48 \text{ cm}^2 \quad (1)$$

The calculated regression line is shown in Fig. A4-1. The correlation between ΣLW and true leaf area is very high ($r = 0.992$) and is highly significant at the 1% level ($p_{0.01} = 0.735$).

Leaf area was subsequently determined by this method. The relationship between ΣLW and true leaf area was found to hold good for leaves of plants grown under all four treatments.

b). Leaf area - leaf dry weight relationships

From plants grown under each of the four treatments individuals were selected to give a range of leaf areas. The total leaf area of each plant was determined as described above and the corresponding leaf dry weight was obtained by oven drying the leaves overnight at 95°C and weighing after a 10 minute cooling period.

Leaf area was plotted against leaf dry weight for each treatment (Fig. A4-2) and the linear regression equations and correlation coefficients calculated (Table A4-I).

Figure A4-2

Relationships between leaf area and leaf
dry weight of plants grown under the four
treatments.

(Statistical data in Table A4-I).

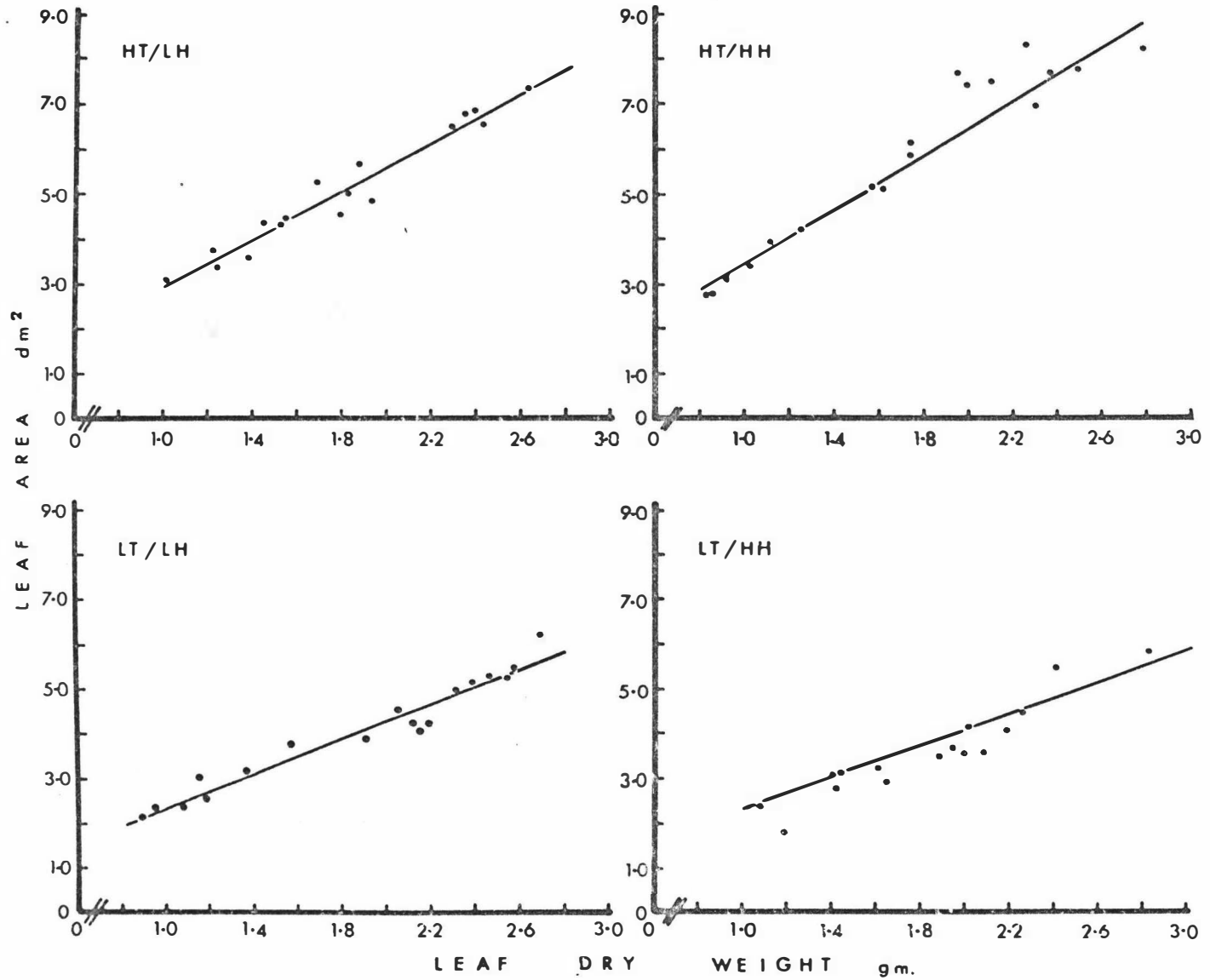


Table A4-I Regression equations and correlation coefficients (r) for the leaf area-leaf dry weight relationships of plants grown under the four treatments.

<u>Treatment</u>	<u>Leaf area (cm²) given by:</u>	<u>r</u>	<u>P_{0.01}</u>
HT/LH	269.87 x dry wt. + 21.0	0.97	0.590
HT/HH	300.06 x dry wt. + 45.8	0.95	0.590
LT/LH	192.55 x dry wt. + 41.7	0.97	0.623
LT/HH	213.40 x dry wt. - 34.7	0.94	0.606

These relationships were used to convert leaf dry weight to leaf area for the plants used in the various experiments.

APPENDIX 5

CALIBRATION OF THE CO₂-ADDITION SYSTEM

The CO₂ concentration in the plant CO₂-exchange measuring equipment was maintained within the predetermined range by the addition or extraction of CO₂, depending on whether the plant was photosynthesising or respiring. Addition of CO₂ was in the form of pulses of a CO₂/N₂ gas mixture containing 10 or 20% of CO₂. The factors determining the weight of CO₂ in each pulse were the percentage content of CO₂ in the gas mixture and the pressure at which the addition was made. For any given gas mixture the former was fixed, but the addition pressure was controlled by gas pressure regulators and a constant bleed device. The regulators permitted the pressure to be accurately determined, and maintained for as long as required. The constant bleed, from which gas escaped into the atmosphere, facilitated the setting up of the required pressure. The addition system incorporated a 3-way glass stopcock to which a take-off line was attached. Pulses of the addition gas were thus diverted for calibration rather than passing into the plant chamber system.

The required addition pressure was set up and a glass tube, fitted with "Quikfit" stopcocks at each end and filled

with carbosorb, was attached to the take-off line. The CO₂ control system was then adjusted to provide a continuous series of addition pulses, and 50-60 such pulses were passed through the carbosorb tube. The stopcocks were then closed and the tube weighed. The tube was then reattached to the take-off line, the stopcocks opened, and 100 pulses passed through. The stopcocks were then closed and the carbosorb tube reweighed. This process was repeated until a total of 500 or 600 pulses had been absorbed by the carbosorb in batches of 100 pulses. The tube was then recharged with fresh carbosorb and the process repeated. The addition pressure was held constant throughout. (The first batch of 50-60 pulses, which were not included in the pulse calibration analysis, were found to be necessary to purge the take-off line and the carbosorb tube of ambient air). The CO₂ content of each pulse was then found by plotting the gain in weight of the carbosorb tube against the number of pulses added. Additionally, the constancy of the CO₂ content per pulse was checked by calculating the gain in weight for each addition of 100 pulses. If this was constant, the CO₂ content per pulse was also constant.

The CO₂ addition system was recalibrated regularly. The gas cylinder containing the CO₂/N₂ mixture was replaced when the pressure fell to 900 p.s.i. (original pressure 2000 p.s.i.), as changes in CO₂ concentration may occur at low cylinder pressures (Brown and Rosenberg, 1968).

APPENDIX 6

DISTRIBUTION OF DRY MATTER IN THE PLANT

Soybean plants were grown under HT conditions in a growth cabinet and a number of individuals of various sizes selected for harvest.

Each plant was divided into three parts, a) root system, b) leaves, and c) stem and petioles, and the dry weight of each part determined.

The dry weights, and the percentage of the total dry weight contributed by each part are given in Table A6-I. The mean percentage of the total dry weight residing in the leaves of soybean plants grown under HT conditions is 50.08% which, for the purposes of calculating changes in leaf dry weight with time, was simplified to 50%.

A similar experiment under LT conditions showed that 53% of the total plant dry weight resided in the leaves (Miss J. Rowley, pers. comm).

Table A6-I Distribution of dry matter in soybean
plants grown under HT conditions.

Plant No.	Dry weight (gms)				Percentage of total dry weight		
	Root	Stem & Petioles	Leaves	Total	Root	Stem & Petioles	Leaves
1	1.571	1.125	2.540	5.236	30.00	21.48	48.51
2	1.064	0.844	1.727	3.635	29.27	23.21	47.51
3	1.172	1.040	2.110	4.322	27.12	24.06	48.82
4	1.289	1.307	2.542	5.138	25.09	25.44	49.47
5	1.391	0.860	1.814	4.065	34.22	21.16	44.62
6	1.973	1.426	2.493	5.892	33.48	24.20	42.31
7	1.066	0.635	1.770	3.471	30.71	18.29	50.99
8	0.859	0.584	1.475	2.918	29.44	20.01	50.55
9	0.959	0.624	1.837	3.420	28.04	18.24	53.71
10	0.745	0.433	1.169	2.347	31.74	18.45	49.81
11	0.689	0.456	1.287	2.432	28.33	18.75	52.92
12	0.666	0.522	1.473	2.661	25.03	19.62	55.35
13	1.046	0.927	2.197	4.170	25.08	22.23	52.68
14	1.244	0.812	2.114	4.170	29.83	19.47	50.69
15	1.594	0.941	2.284	4.819	33.08	19.53	47.39
16	0.790	0.566	1.507	2.863	27.59	19.77	52.64
17	0.860	0.529	1.354	2.743	31.35	19.28	49.36
18	1.081	0.646	1.603	3.330	32.46	19.40	48.14
19	0.681	0.489	1.269	2.439	27.92	20.05	52.03
20	0.730	0.554	1.435	2.719	26.85	20.37	52.77
21	0.747	0.496	1.316	2.559	29.19	19.38	51.43
Means					29.32	20.59	50.08

APPENDIX 7

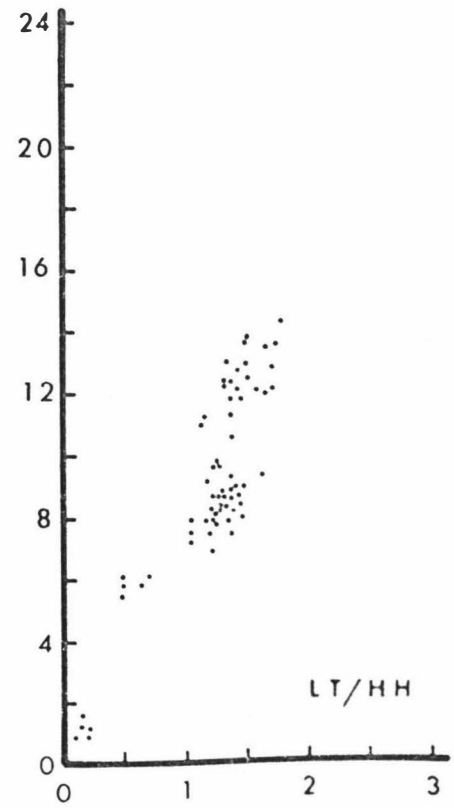
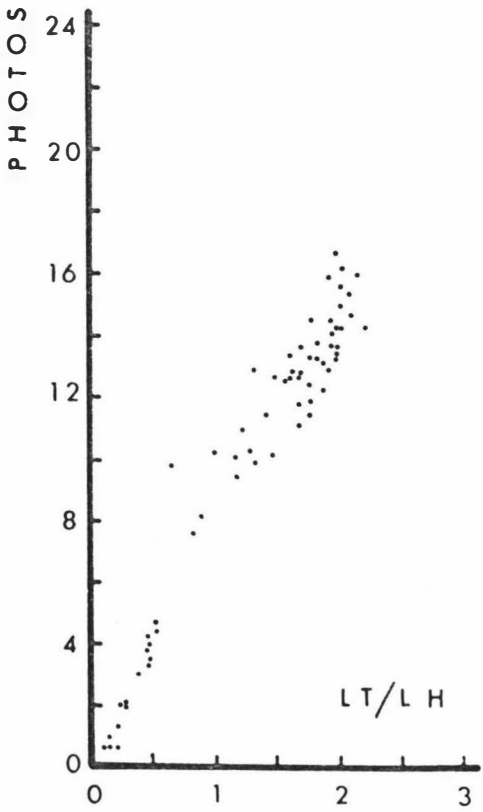
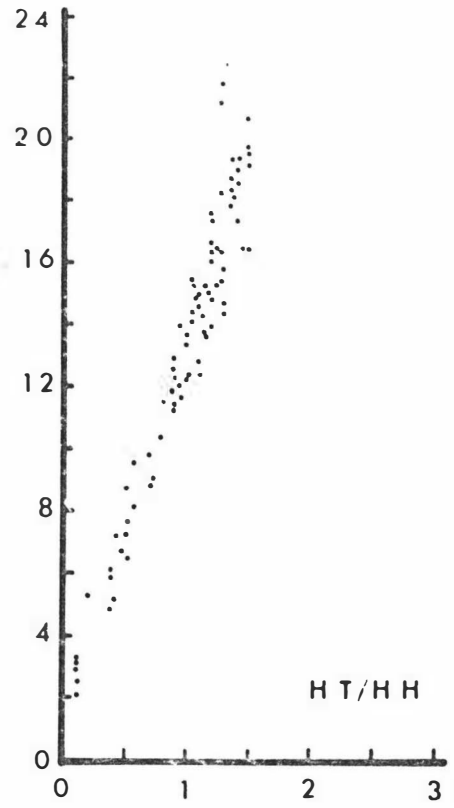
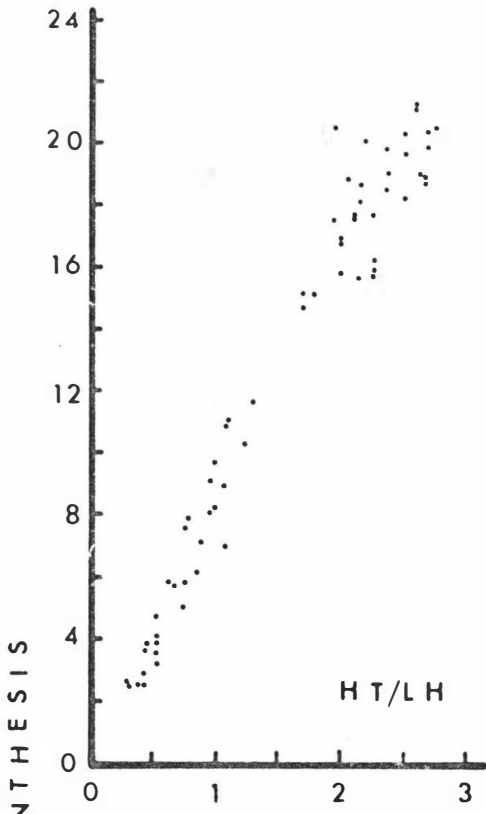
DETAIL PLOTS OF FIGURES 4-4, 4-10 AND 4-11

The figures in this appendix show the individual points from which the curves in Figs. 4-4, 4-10 and 4-11 were constructed.

Figure A7-1

Detail plots of the relationships given in
Fig. 4-4.

Relationships between the rates of photosynthesis
(mg CO₂/dm²/hr) and transpiration (gm water/dm²/hr)
for plants under the four treatments.



TRANSPIRATION

Figure A7-2

Detail plots of the relationships given in
Fig. 4-10.

Relationships between RWC and transpiration
rate ($\text{gm water/dm}^2/\text{hr}$) for plants under the
four treatments.

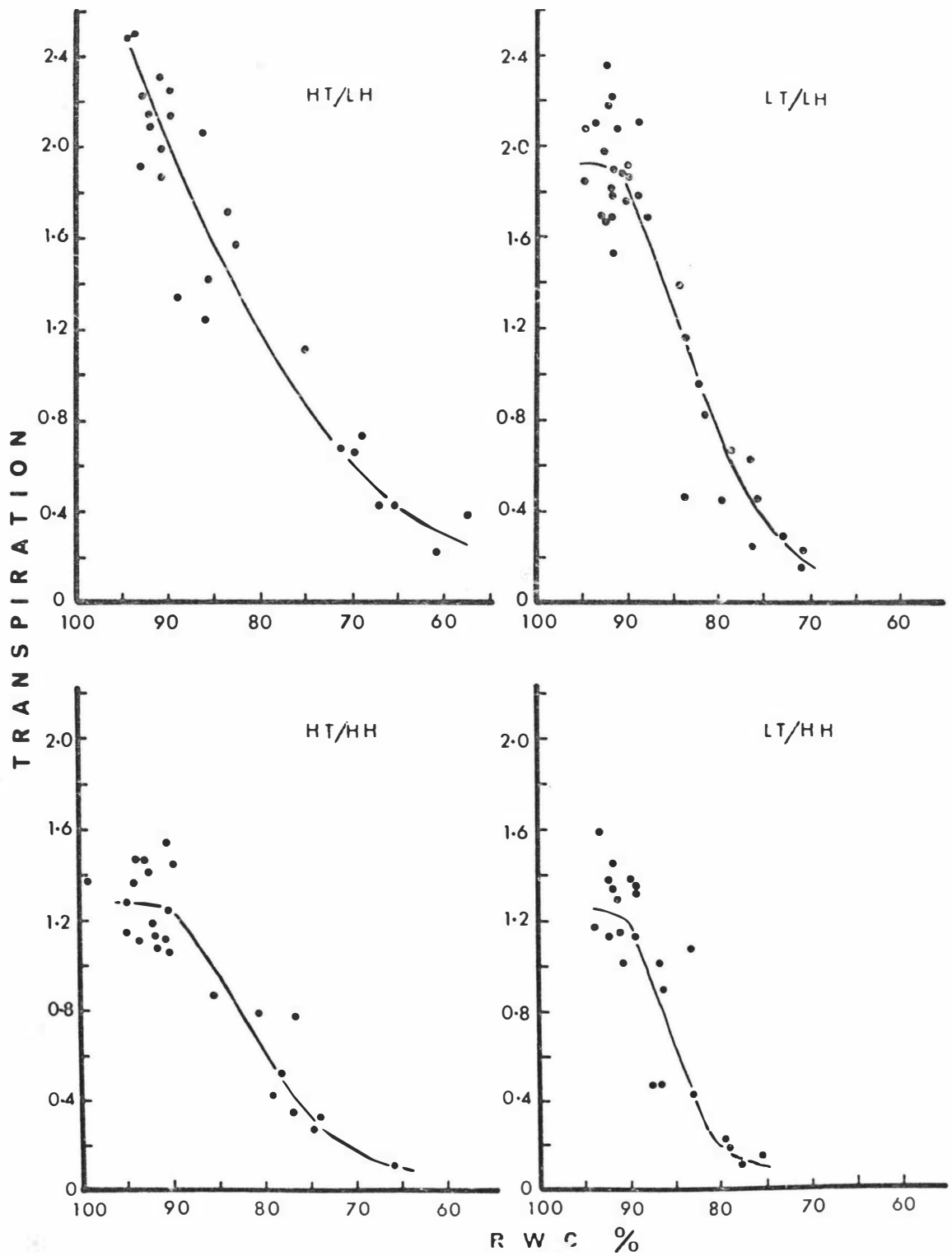
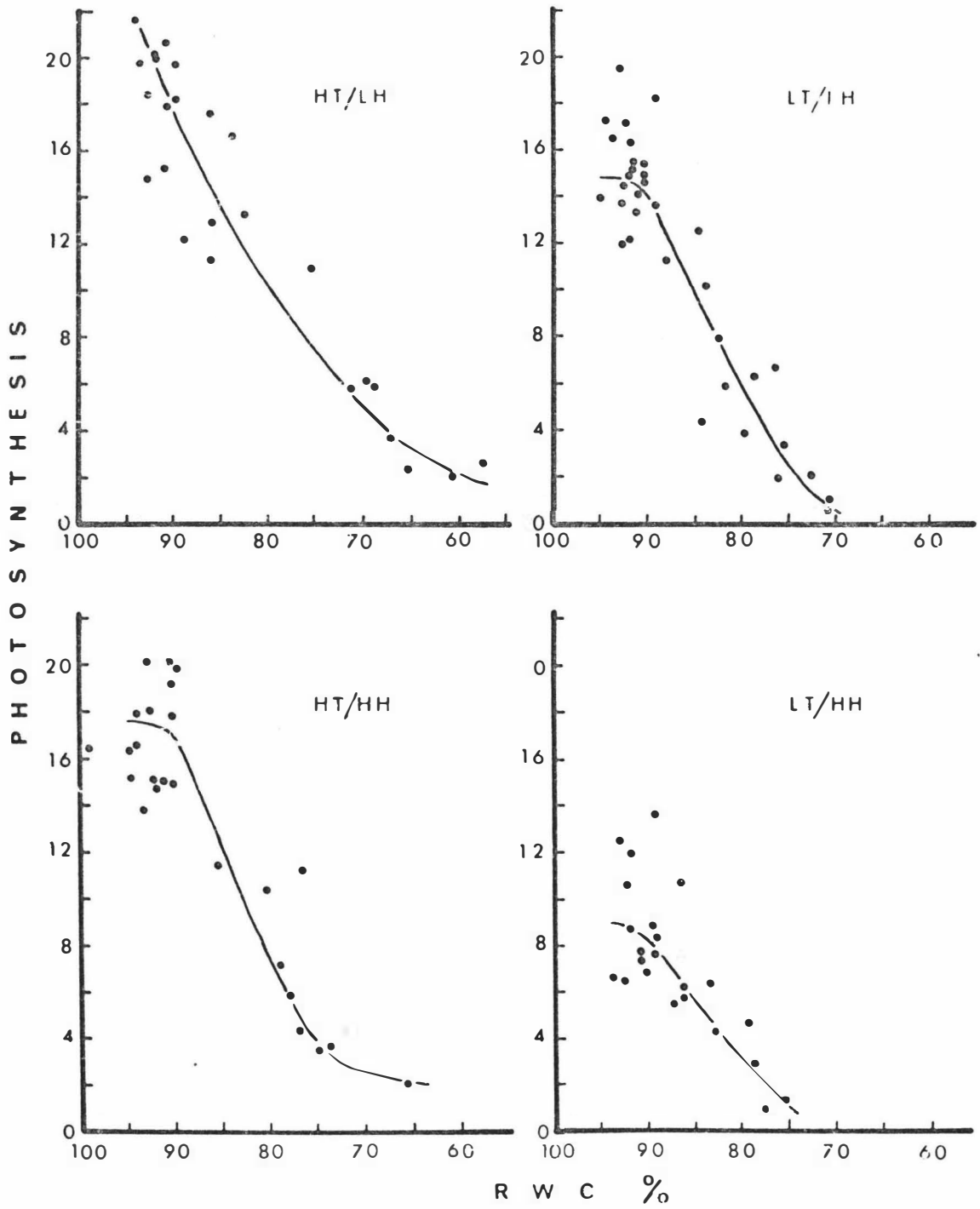


Figure A7-3

Detail plots of the relationships given
in Fig. 4-11.

Relationships between RWC and rate of
photosynthesis ($\text{mg CO}_2/\text{dm}^2/\text{hr}$) for plants
under the four treatments.



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