AN ABSTRACT OF THE THESIS OF

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Abstract approved: Larry Boersma

Laboratory experiments were used to investigate the mechanism of plant response to water stress by determining the sensitivity of leaf elongation, photosynthesis and transpiration in young corn plants to a decrease in leaf water potential.

In initial experiments, 9 day old corn plants were grown at soil water potentials of -0.35 and -2.50 bars for 6 days using the polyethylene glycol semi-permeable membrane technique of controlling soil water potential. Leaf elongation and soluble carbohydrate content were found to be more sensitive to a reduction in soil water potential than net assimilation and transpiration. Lowering the soil water potential from -0.35 to -2.50 bars resulted in a 44 percent decrease in the rate of leaf elongation and a 42 percent increase in the soluble carbohydrate content of the plant, while the rates of net assimilation and transpiration were reduced by 26 and 24 percent respectively.

The differing sensitivity of leaf elongation and photosynthesis to decreasing soil water potential was examined in detail in subsequent experiments by simultaneously monitoring the rates of net photosynthesis, transpiration, and leaf elongation and the leaf water potential of a young corn plant as it became water stressed. Leaf elongation ceased at a leaf water potential of -9 to -9.5 bars, whereas the rates of net photosynthesis and transpiration were not reduced significantly until the leaf water potential reached -12 to -13 bars. The sharp decrease in rate of net photosynthesis in the vicinity of -12 to -13 bars was due to increases in both the stomatal and mesophyll resistances to CO_2 transfer. It was concluded that the decreases in the rate of net photosynthesis due to water stress were caused by stomatal and nonstomatal effects of approximately equal magnitude. The nonstomatal or intracellular factors responsible for the decrease in the rate of net photosynthesis were not identified.

It was hypothesized that the differing sensitivity of leaf elongation and photosynthesis to water stress, may result in the accumulation of photosynthate within a mildly water stressed plant. This mechanism was demonstrated in experiments where the soluble carbohydrate content of the top 3 leaves of a mildly stressed corn plant was shown to be significantly higher than in the corresponding leaves of a nonstressed control plant after a 6 hour stress period. This increase in the soluble carbohydrate content of the mildly stressed

plant was accompanied by a significant decrease in the net photosynthetic rate of this plant. A similar response was obtained when leaf elongation rate was reduced by lowering the temperature of the apical meristem. The results were interpreted as evidence for the operation of a source-sink type control mechanism of photosynthesis in mildly stressed plants.

The effect of water potential on the elongation rate, the adenylate energy charge, ATP content and free amino acid content of the youngest unrolled leaf of a 6 leaf corn plant was examined in a final series of experiments. These experiments sought to determine whether the decrease in photosynthate utilization, observed when cell expansion was limited by water stress, was due to a simple product inhibition feedback mechanism or to the direct effect of water stress on some aspect of cell metabolism. The ATP content of the elongating cells was found to be as sensitive as leaf elongation to small changes in leaf water potential. Adenylate energy charge did decrease with leaf water potential, but was not as sensitive as the ATP content to changes in leaf water potential. The free amino acid level was found to increase at leaf water potentials lower than -10 bars, and this may indicate that the inhibition of protein synthesis during water stress may be due to the deficiency of chemical energy within the cell. It was concluded that during water stress, the biosynthetic activity of elongating cells may be limited by a low level of available energy in

the form of ATP. This decreased synthesis of ATP may be due to a direct effect of water stress on respiration and ATP formation.

The sensitivity of leaf enlargement to mild water stress and its subsequent effect on photosynthesis indicates that plant growth and production may be limited by mild stress in the field situation. The vegetative growth of plants depends on both the photosynthetic rate and the rate of increase of the photosynthetic surface area. The response of leaf enlargement to water stress in the field therefore warrants thorough further investigation.

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PHYSIOLOGICAL EFFECTS OF WATER STRESS ON YOUNG CORN PLANTS

INTRODUCTION

The overall effects of water stress on crop yield are well known, but after 50 years of research the mechanisms by which water stress reduces the growth and yield of crop plants still are not clear. It is not yet known how much of the overall growth reduction is caused by effects of decreased turgor pressure on cell enlargement and stomatal opening, or how much results from direct interference with enzyme mediated processes, brought about by dehydration of the protoplasm. The mechanism of drought tolerance also is not well understood. It is not known whether superior drought tolerance is a result of superior stomatal regulation, protoplasm stability, a combination of both or some other factor such as the ability to develop more efficient rooting systems. Most importantly the interrelationships of the above physiological mechanisms are not clear because growth and metabolism are rarely studied simultaneously. Answers to the above questions are required urgently by crop physiologists, plant breeders, and agronomists who collectively are attempting to make the most efficient use of agriculture's most precious resource, water.

A primary reason for this inadequate understanding is the failure of many workers to adequately define the degree of stress imposed

in their experiments. This has made the quantitative comparison of separate experiments difficult, if not impossible. The development of the pressure-bomb apparatus (Scholander et al., 1965) and the in situ thermocouple psychrometer (Neumann and Thurtell, 1972) has facilitated the measurement of plant water potential (ψ_c). These developments have enabled Hsiao (1973) to identify some general ranges of stress levels characterized by specific effects on different plant functions. He identified mild stress as the lowering of ψ_c by several bars from a well watered level; moderate stress as the lowering of ψ_c by more than several but less than 12 to 15 bars; and severe stress as the lowering of ψ_c by more than 15 bars. Under mildly stressed conditions the initial small decreases in water content from fully turgid tissue cause significant decreases in ψ_c. The major part of this diminution in ψ_c is due to decreases in turgor pressure (ψ_p) and not osmotic pressure (ψ_π). Consequently, turgor dependent processes such as cell expansion are the most affected by mild stress. The continued decrease of ψ_c in moderate and severe stress conditions is marked by a simultaneous decrease in osmotic potential (ψ_π) due to the increase of solute concentration within the cell. This thesis is primarily concerned with the effects of mild to moderate water stress on the physiological functions of corn in the vegetative phase of growth.

The mild to moderate phase of stress is of interest because even well watered plants commonly experience a diurnal cycle of mild to moderate water stress during the course of a clear sunny day (Turner and Begg, 1973; Carbon, 1973). In spite of the frequency of its occurrence, the effect of mild to moderate water stress on plant growth has not been investigated thoroughly. This may be so because it has not been considered important since mild stress is not visually obvious and must be detected by plant water potential measurements. Relative water content (relative turgidity) is rather insensitive as a measure of mild water stress because a very small change in water content can correspond to a decrease of several bars in the leaf water potential and a large decrease in the turgor pressure (Hsiao, 1973).

Cell expansion generally is considered to be the parameter most sensitive to a small decrease in plant water potential (Iljin, 1957; Slatyer, 1969; Kramer, 1969; Hsiao, 1973). This hypothesis was first made in 1908 by Balls and later confirmed by Loomis in 1934. More recently the availability of linear variable differential transducers (LVDT) that enable leaf elongation to be monitored on a minute by minute basis, have enabled Acevedo, Hsiao and Henderson (1971) and Barlow and Boersma (1972) to measure the extremely rapid response of leaf elongation to small changes in plant water potential. The rapid changes in the rate of cell expansion are due to the decrease in cell turgor pressure that occurs when the leaf water potential decreases

quickly (Boyer, 1968; Green, 1968; Hsiao, 1973).

Photosynthesis is believed to be less sensitive to water stress than leaf elongation, but this has not been demonstrated conclusively by concurrent measurements on the same plant (Wardlaw, 1969; Boyer, 1970a; Acevedo et al., 1971). Wardlaw (1969) measured both net photosynthesis and leaf elongation, but not water potential, on the 7th and 8th leaves of water stressed Lolium temulentum plants for 3 days. He found that leaf elongation began to decline 10 hours after the onset of mild water stress, while photosynthesis was not affected until 22 hours had elapsed. Boyer (1970a) measured leaf enlargement, net photosynthesis and leaf water potential on different corn plants subjected to the same water stress for a number of days and reported that leaf enlargement decreased sharply at a leaf water potential of -2 to -4 bars and completely stopped at -8 bars whereas net photosynthesis was not affected greatly until the leaf water potential was at least -12 bars. These measurements were made over a 24 hour dark period. The relationship between net photosynthesis and leaf enlargement may not be the same when measured in the light. Finally Acevedo et al. (1971) monitored leaf elongation and net photosynthesis simultaneously on a water stressed corn plant and found elongation to be reduced sharply before photosynthesis was noticeably affected. However Acevedo et al. did not report the leaf water potentials at which the changes in leaf elongation and net photosynthesis took place.

This summary would indicate that a clearer understanding of the relationship between leaf elongation, photosynthesis and leaf water potential may be gained by simultaneously monitoring these parameters on a plant subject to a short term water stress such as that normally encountered in the diurnal stress cycle in the field.

If mild stress causes a sharp reduction in the rate of leaf elongation, it is of interest to consider the physiological effects of this reduction on other plant functions, in particular photosynthesis. The most immediate effect of a reduction in the rate of cell expansion is a concurrent decrease in the photosynthate requirement for the biosynthesis of cell walls and protoplasm in the elongating cell. This would result in an accumulation of photosynthate in the sink region and then a decrease in the size of the growth sink for photosynthate in the elongating leaf.

In other physiological studies, where photosynthate sink size has been manipulated by chemical, environmental or excision treatments, substantial reductions in the photosynthetic rate of the source leaf have resulted (Humphries, 1963; Burt, 1966; Sweet and Wareing, 1966; King, Wardlaw and Evans, 1967). These studies have led to a revival of interest in the source-sink hypothesis (Boussingault, 1868) which postulates that the rate of photosynthate accumulation in the leaf is an internal factor controlling photosynthesis. The evidence for the source-sink hypothesis was reviewed by Neales and Incoll (1968), who

concluded that while there is a sound physiological basis for the hypothesis, its biochemical basis suffers from a lack of evidence. Subsequent to this review the photosynthetic carboxylating enzymes of both C_3 and C_4 plants have been shown to have allosteric properties (Preiss and Kosuge, 1970; Coombs, Baldry and Bucke, 1973), thereby suggesting the possibility of photosynthate regulation of these enzymes. Despite these promising discoveries the mechanism of photosynthate regulation of photosynthesis in the source leaf remains to be demonstrated.

In spite of its unproven biochemical mechanism, the source-sink hypothesis does provide a mechanism whereby mild water stress may decrease photosynthesis indirectly by causing photosynthate to accumulate in the sink leaf and ultimately in the source leaf. Although much of the direct effect of water stress on reducing photosynthesis has been ascribed to stomatal closure (Brix, 1962; Troughton, 1969; Boyer, 1970b; Kreideman and Smart, 1971), there is increasing evidence that changes in intracellular processes also may be important (Slavik, 1965; Boyer and Bowen, 1970; Redshaw and Meidner, 1972). None of the above workers measured photosynthate levels in their plants, but Redshaw and Meidner (1972) did postulate that the intracellular factor affecting photosynthesis in their experiments may have been a photosynthate accumulation. In contrast Boyer and Bowen (1970) concluded that at leaf water potentials below -11 bars the

intracellular factor affecting photosynthesis was a reduction in photochemical activity. Other workers have reported no decrease in photochemical activity of plants until water stress became very severe (Nir and Poljakoff-Mayber, 1967; Santarius, 1967). Consequently the mechanism of intracellular inhibition remains an open question with the possibility of photosynthate regulation under conditions of mild stress certainly not excluded. Finally it should be noted that the stomatal closure could result from prior changes in intracellular processes; e.g. Meidner (1962) has shown the size of the stomatal aperture in corn leaves to be very sensitive to the internal CO_2 concentration.

In addition to its possible regulation of photosynthesis, a reduction in cell expansion can also affect the relative plant growth rate, in the long term, by affecting the photosynthetic area available for light interception (Slatyer, 1973).

Because of the importance of cell enlargement to the growth of the plant and its susceptability to mild water stress a series of laboratory experiments were conducted to, firstly examine the effects of mild water stress on leaf elongation and the effects of leaf elongation on other plant functions, and secondly to examine some of the key axioms of the source-sink hypothesis as applied to water stress. The experiments included the measurement of leaf elongation, net photosynthesis, transpiration rates and soluble carbohydrate levels of

young corn plants subjected to different levels of water stress of varying duration. These experiments were followed by studies of the effect of mild water stress, sufficient to reduce the rate of leaf elongation on the metabolism of both the elongating leaf and the mature leaf supplying the elongating leaf with photosynthate.

LONG TERM RESPONSE OF LEAF ELONGATION, PHOTOSYNTHESIS, TRANSPIRATION AND SOLUBLE CARBOHYDRATE CONTENT TO MILD WATER STRESS

Introduction

The total growth of a plant over any specified time span represents the sum of the growth rates exhibited throughout the entire period. Thus, the total growth of a plant during a dry period is the sum of the growth rates at several different and decreasing soil water potentials. Research workers seeking to measure the effects of a predetermined mild water stress on such plant physiological functions as net photosynthesis, leaf elongation, transpiration and leaf water potential have long been frustrated by the lack of a suitable technique to establish and maintain a predetermined soil water potential (Kramer, 1969). If the soil water potential can be maintained at a constant level, the degree of stress imposed on the plants growing in the soil under constant environmental conditions should reach a near steady state value, at which equilibrium rates of plant physiological functions can be compared.

In the past decade three promising techniques for the control of soil water potential have been reported: (1) the hanging water column, where plants are grown on a screen over soil in which a constant water content is maintained with a hanging water column (Hsieh, 1963);

(2) the osmotic solution semi-permeable membrane method, where plants are grown in soil encased in a semi-permeable cellulose acetate membrane, which in turn is surrounded by an osmotic solution of high molecular weight such as polyethylene glycol (PEG) (Zur, 1967; Cox and Boersma, 1967); (3) the osmotic culture solution method, where plants are grown directly in osmotic solutions containing PEG (Lagerwerff, Ogata and Eagle, 1961).

Although excellent results have been obtained using the hanging water column method, the lengthy preparation and expensive equipment required for this method make it a difficult technique to use with a large number of plants. The osmotic culture solution method can also be effective in controlling plant water potential (Michel, 1970), however, its use in studies longer than a few hours is suspect because of possible phytotoxicity or interference from the PEG or chemical impurities within the material (Macklon and Weatherley, 1965; Lawlor, 1970). In contrast, the osmotic solution semi-permeable membrane method has neither of the above disadvantages and has been successfully used to maintain a constant soil water potential (Zur, 1967; Cox and Boersma, 1967; Williams and Shaykewich, 1969).

In the experiments presented here the osmotic solution semi-permeable membrane method was used to grow young corn plants at soil water potentials of -0.35 and -2.50 bars. These treatments were designed to produce a range of plant water potentials at which the

near steady state rates of net photosynthesis, leaf elongation and transpiration and the soluble carbohydrate content could be compared. In this manner, the effects of reduced cell turgor and leaf elongation on net photosynthesis and soluble carbohydrate content could be examined.

Although this method has been used previously to monitor the effect of increasing soil water potentials on transpiration (Cox and Boersma, 1967), transpiration, photosynthesis and respiration (Babalola, Boersma and Youngberg, 1968), transpiration, photosynthesis and nitrogen fixation (Kuo and Boersma, 1971) and leaf elongation (Painter, 1966) it has not been used to relate leaf elongation to leaf water potential, photosynthesis, transpiration and soluble carbohydrate content.

Materials and Methods

Two (2) pre-germinated corn (Zea mays L. var. Pride 5) seeds were planted in soil (sandy loam) contained in a lucite frame and grown in a growth chamber in a manner described for wheat by Sedgley and Boersma (1969). A light intensity of 1800 ft. c. at the top of the plants was provided by 16, 100-watt fluorescent lamps and six, 75-watt incandescent bulbs. The day and night temperatures were 27 ± 1 C and 24 ± 1 C respectively.

After 9 days, each lucite frame was enclosed in a cellulose

acetate membrane and placed in a temperature controlled osmotic chamber containing Carbowax 6000 dissolved in a Hoagland nutrient solution (Babalola, Boersma, and Youngberg, 1968). The plants then were grown for a further 6 days in a controlled environment growth room set at an air temperature of 27 ± 1 C, a relative humidity of 45 ± 2% and a light intensity of 2500 ft. c. provided at the top of the plants by a combination of fluorescent and incandescant lamps, run continuously. The plants were given a 2 day acclimatization period in the growth room, before experimental measurements were commenced to allow the soil water potential to equilibrate with the osmotic solution in the chamber.

The experiment was designed as a 2 x 2 factorial using root temperatures of 21.1 and 26.6 C and soil water potentials of -0.35 and -2.50 bars. Two (2) chambers containing 3 frames each (12 plants) were harvested from each treatment on day 0, 2, 3, 4, 5, 6. The following parameters were measured.

Net Carbon Assimilation

Net carbon assimilation was determined as the sum of root plus shoot growth. After each harvest, the roots were washed carefully, and fresh weight of both shoots and roots recorded. Subsamples of roots and shoots were then taken for drying at 60 C for 48 hours to determine water content. The remainder of each fresh sample was

stored at -15 C for carbohydrate analysis.

Transpiration

Water use from each of 4 chambers (24 plants) in each treatment was measured with a constant level device operating on the principle of the Mariotte bottle (Cox and Boersma, 1967). Transpiration rates were expressed on a dry weight basis.

Leaf Elongation

The height of the youngest unrolled leaf above the top of the plexiglass frame was measured daily with a ruler.

Leaf Water Potential (ψ_c)

On days 2 and 3 leaf discs (1 cm diameter) were taken from the second youngest unrolled leaf of 4 plants in each treatment and immediately placed in a shallow sample chamber of a Peltier thermocouple psychrometer for a psychrometric determination of ψ_c (Campbell, Zollinger and Taylor, 1966).

Soluble Carbohydrates

The plant material stored fresh at -15 C was mixed with 80% ethanol (v/v) at 50 C for 5 min in a Waring blender (high speed) at an ethanol/tissue ratio of 5:1 (v/w). After vacuum filtration, the residue

was washed 3 times with 80% ethanol at 50 C. The filtrates were combined and an aliquot was evaporated to dryness in a rotary evaporator at 70 C. The residue was taken up in 5 ml of glass distilled water and decolorized by the method according to Smith (1969). The carbohydrate concentration in the resulting extract was determined by the anthrone method (Yemm and Willis, 1954) using glucose as a standard, with the results expressed as a percentage of plant dry weight.

Results and Discussion

The increase in shoot dry weight and leaf length, plotted as a function of time (Figures 1, 2) showed a distinct linear relationship. The linear model $y = ax + b$ was fitted to the shoot growth, root growth, net assimilation and leaf length data, using the least squares method. In this equation, y is the parameter measured, b is the intercept, a is slope or rate of growth and x is the elapsed time in days. The resulting linear correlation coefficients for all treatments were significant at the 1% level indicating that increases in shoot growth, root growth, net assimilation and leaf length were linearly related to time. Thus steady state conditions of water stress were obtained in this experiment. The rate a for each of these functions is presented in Table 1.

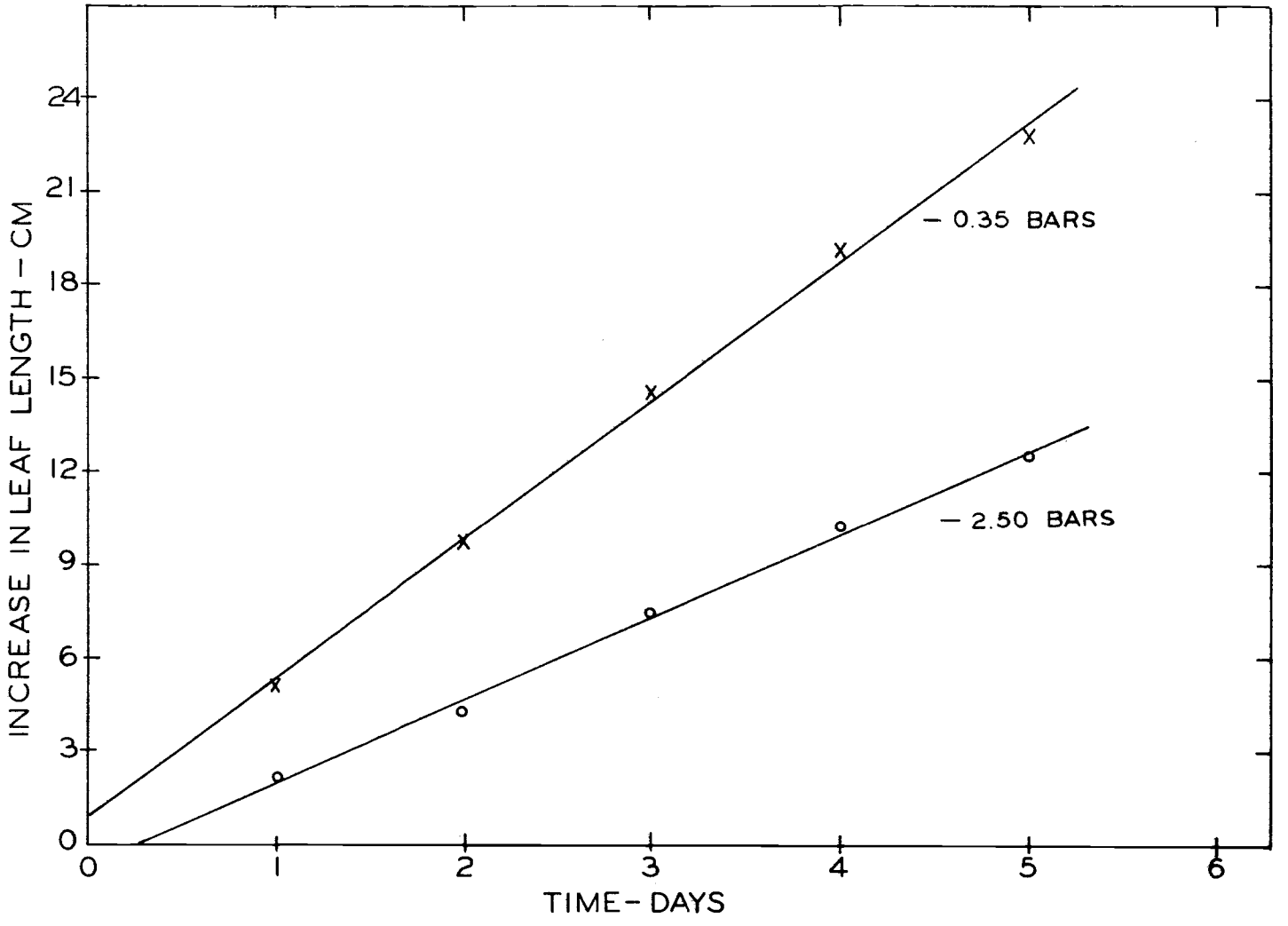

Figure 1. Increase in leaf length with time of young corn plants grown at a soil temperature of 26.6 C and soil water potentials of -0.35 and -2.50 bars.

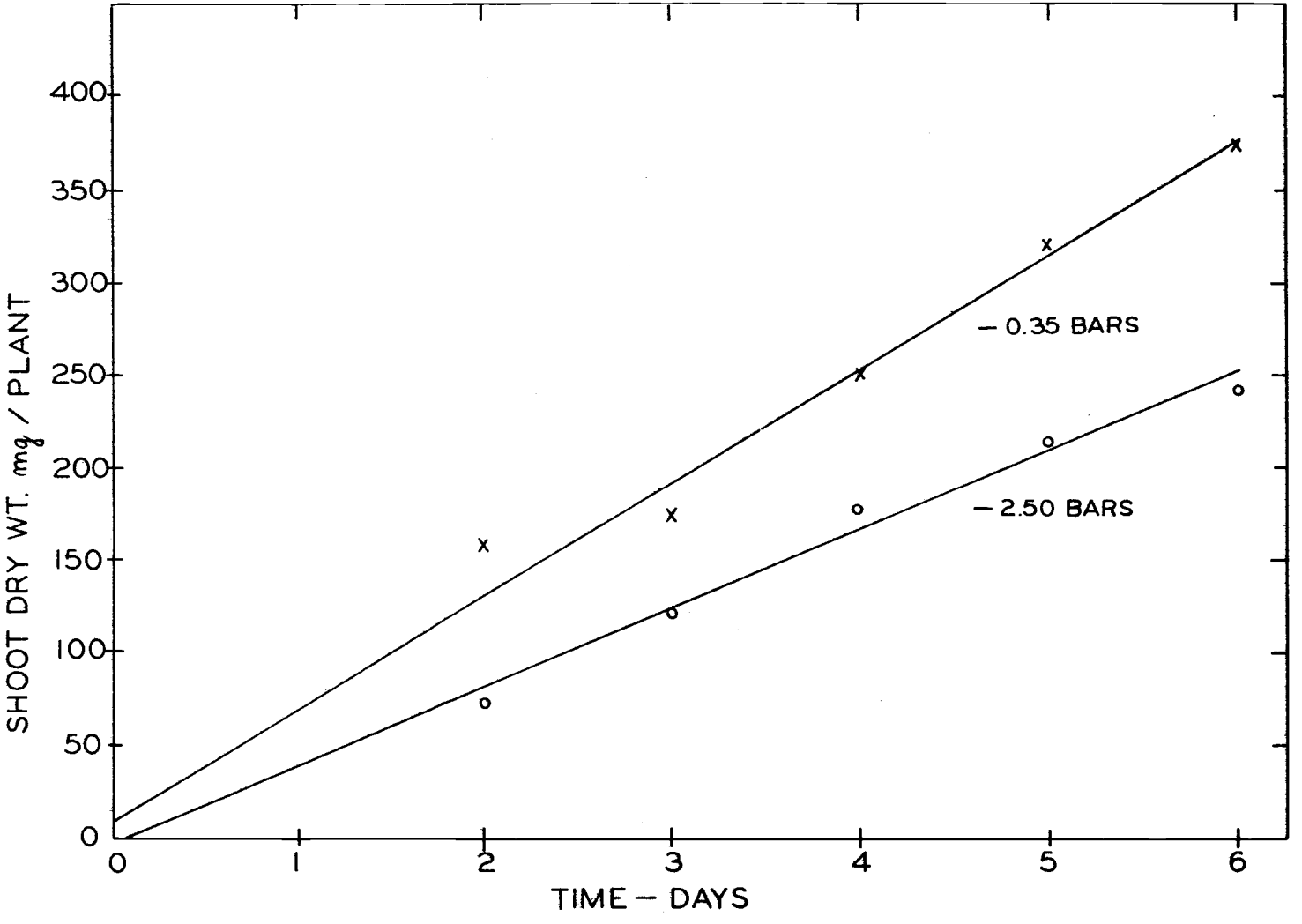

Figure 2. Increase in shoot dry weight with time of young corn plants grown at a soil temperature of 26.6 C and soil water potentials of -0.35 and -2.50 bars.

Table 1. The steady state rate of several physiological functions at root temperatures of 21.1 C and 26.6 C and soil water potentials of -0.35 and -2.50 bars.

Parameter	Soil Water Potential Bars	Root Temperature 21.1 C	Root Temperature 26.6 C
Leaf water potential (bars)	-0.35	-4.4 ± 0.3	-3.2 ± 0.4
	-2.50	-6.8 ± 0.7	-5.3 ± 0.5
Transpiration* ($cm^3 g^{-1} day^{-1}$)	-0.35	33.9 ± 2.4	45.3 ± 2.9
	-2.50	27.1 ± 3.2	32.6 ± 3.7
Shoot growth+ (mg dry wt $plant^{-1} day^{-1}$)	-0.35	53.2	61.3
	-2.50	35.0	42.0
Root growth+ (mg dry wt $plant^{-1} day^{-1}$)	-0.35	38.6	31.3
	-2.50	29.2	30.2
Net assimilation+ (mg dry wt $plant^{-1} day^{-1}$)	-0.35	91.8	92.6
	-2.50	64.2	72.1
Leaf elongation+ (cm day^{-1})	-0.35	3.74	4.50
	-2.50	1.91	2.70
Soluble carbohydrates* (% dry wt)	-0.35	14.2 ± 0.9	12.6 ± 2.1
	-2.50	20.3 ± 0.3	17.7 ± 2.4

*Mean of values recorded on days 3, 4, 5, 6.

+The value shown is the a value from the linear regression equation, the linear correlation coefficient r was significant at the 1% level for all equations fitted.

The soluble carbohydrate content of the shoots rose sharply under the continuous light regime of the growth room (Figure 3). However after 3 days a relatively constant value was reached, and the steady state soluble carbohydrate level shown in Table 1 was computed as a mean of the levels on the final 4 days of the experiment.

The leaf water potential data in Table 1 illustrate the effectiveness of the osmotic solution semi-permeable membrane method. A decrease of 2.15 bars in the water potential of the osmoticum, resulted in decreases of 2.1 and 2.4 bars in the leaf water potential of plants grown at root temperatures of 21.1 and 26.6 C respectively. This evidence together with the linear growth rates already discussed, would indicate that the osmotic solution semi-permeable membrane method functioned effectively in providing constant plant water stresses.

Although decreasing ψ_c resulted in decreases in three of the four physiological functions measured (soluble carbohydrate content increased), it affected some more severely than others (Table 1). In order to quantify these differences, the degree of change induced by lowering the soil water potential from -0.35 to -2.50 bars, is presented in Table 2 as percentage change.

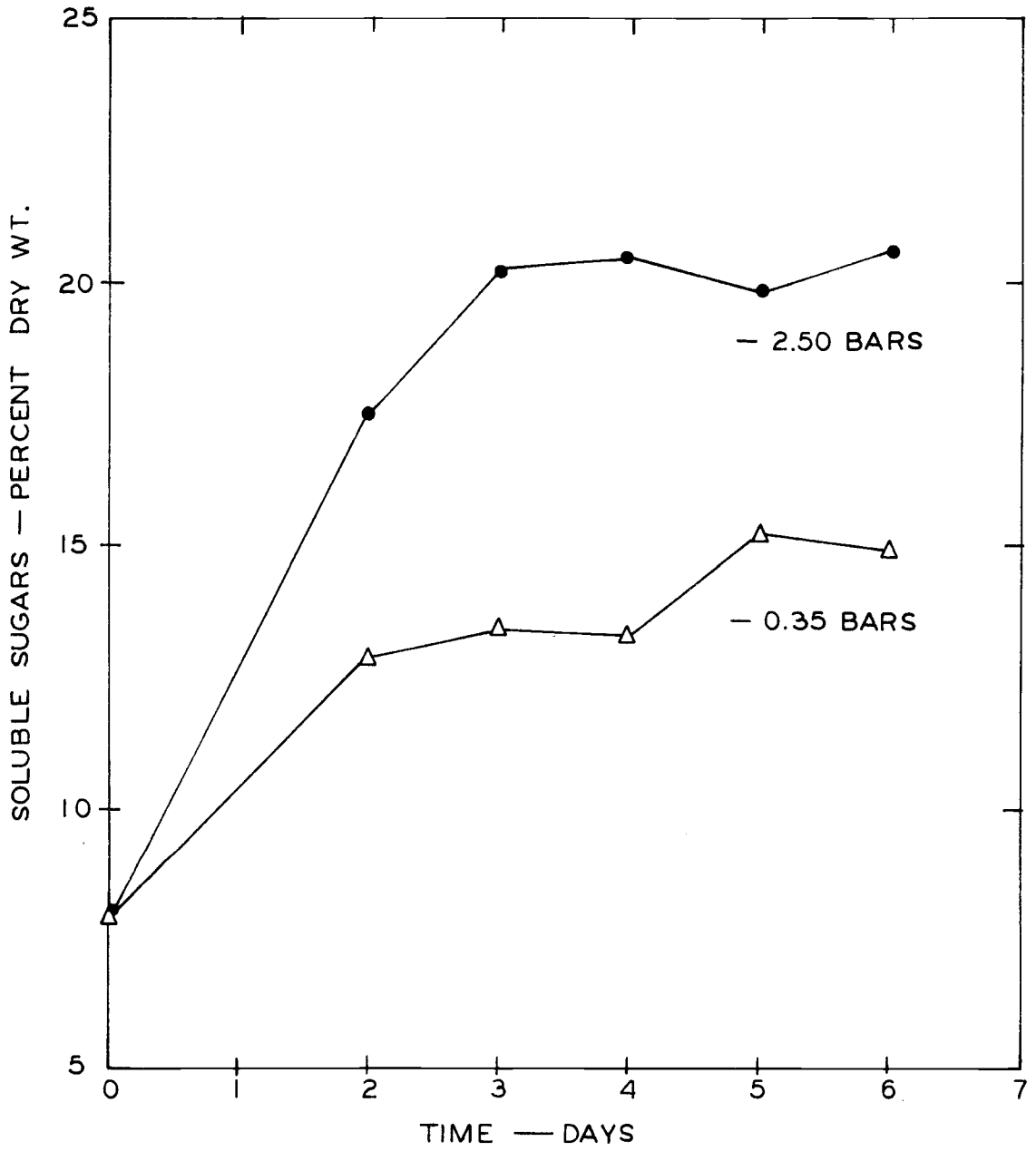

Figure 3. Change in soluble carbohydrate content with time, in young corn plants grown at a soil temperature of 26.6 C and soil water potentials of -0.35 and -2.50 bars.

Table 2. Effect of reducing the soil water potential from -0.35 to -2.50 bars on the transpiration, net assimilation and leaf elongation rates and the soluble carbohydrate content of young corn plants.

Parameter	Root Temperature 21.1 C	26.6 C	Mean
	--------- % Change* ----------		
Transpiration	-19.9	-28.0	-23.9
Net assimilation	-30.1	-22.1	-26.1
Leaf elongation	-48.9	-40.0	-44.5
Soluble carbohydrates	+42.9	+40.5	+41.7

*The change induced by increasing the soil water potential from -0.35 to -2.50 bars.

It is apparent from Table 2 that a rather small decrease in ψ_c affected leaf elongation and soluble carbohydrate content much more than transpiration or net assimilation. This trend occurred at both root temperatures. It is well known that leaf elongation is more sensitive to small decreases in ψ_c than photosynthesis and transpiration (Loomis, 1934; Kramer, 1969; Boyer, 1970a) but increases in soluble carbohydrate content have not generally been associated with decreases in leaf elongation. Both Boyer (1970a) and Acevedo, Hsiao and Henderson (1971) have proposed that carbohydrate accumulations may result from decreases in the rate of leaf elongation when photosynthesis is not strongly affected, but their reports did not demonstrate these accumulations. The work of Ordin (1960), showing that a reduction in cell turgor can decrease the rate of cellulose synthesis, also indicates that soluble carbohydrates should

accumulate when low turgor inhibits cell elongation. The functional relation of leaf elongation to ψ_c presented in Figure 4 is described by the linear equation

$$L = 0.74\psi_c + 6.86 \qquad (r = -0.994^{**}) \qquad (1)$$

where L is leaf elongation in cm day^{-1}. Extrapolation of this equation to a leaf elongation rate of zero predicts that the leaf should stop elongating when ψ_c reaches -9.2 bars. This value is slightly higher than the value of -8 bars previously reported for corn by Boyer (1970a), Acevedo et al. (1971). The difference may be due to some osmotic adjustment occurring in the long period of measurement (Meyer and Boyer, 1972) used in these experiments.

These decreases in leaf elongation were closely followed by increases in the soluble carbohydrate levels in all treatments (Figure 5). My interpretation of this observation is that the size of the growth sink for photosynthate in the young expanding leaf is a function of the rate of elongation because the accumulation of soluble carbohydrates, presumably photosynthate, was inversely related to the rate of leaf elongation. The physiological implication of the observed reduction in sink size with respect to the activity of the source (photosynthesis) is considered in Figure 6 where net assimilation is plotted as a function of soluble carbohydrate content. The negative correlation illustrated in Figure 6 suggests that the accumulation of soluble carbohydrates

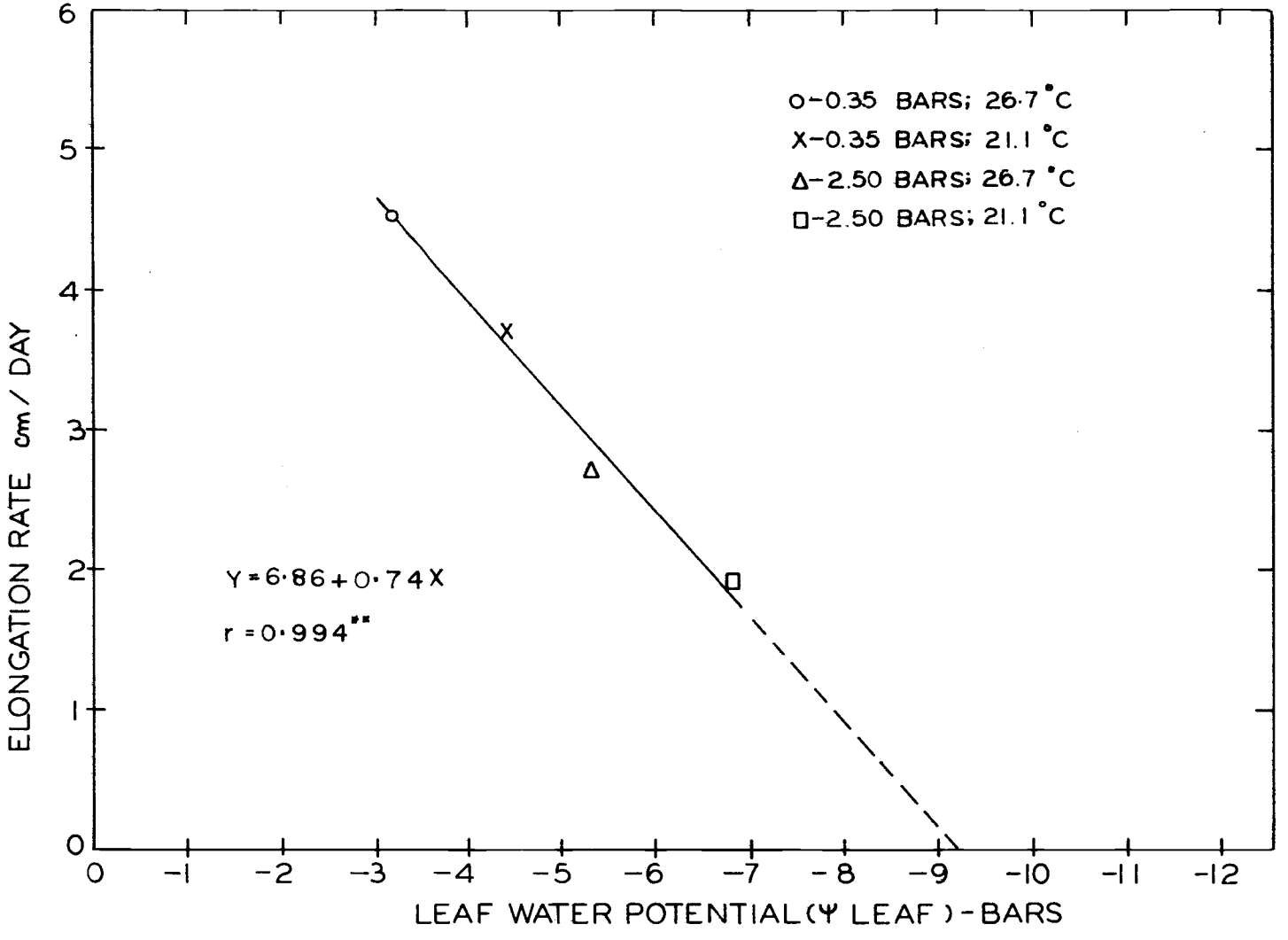

Figure 4. Functional relationship of leaf elongation to leaf water potential in young corn plants.

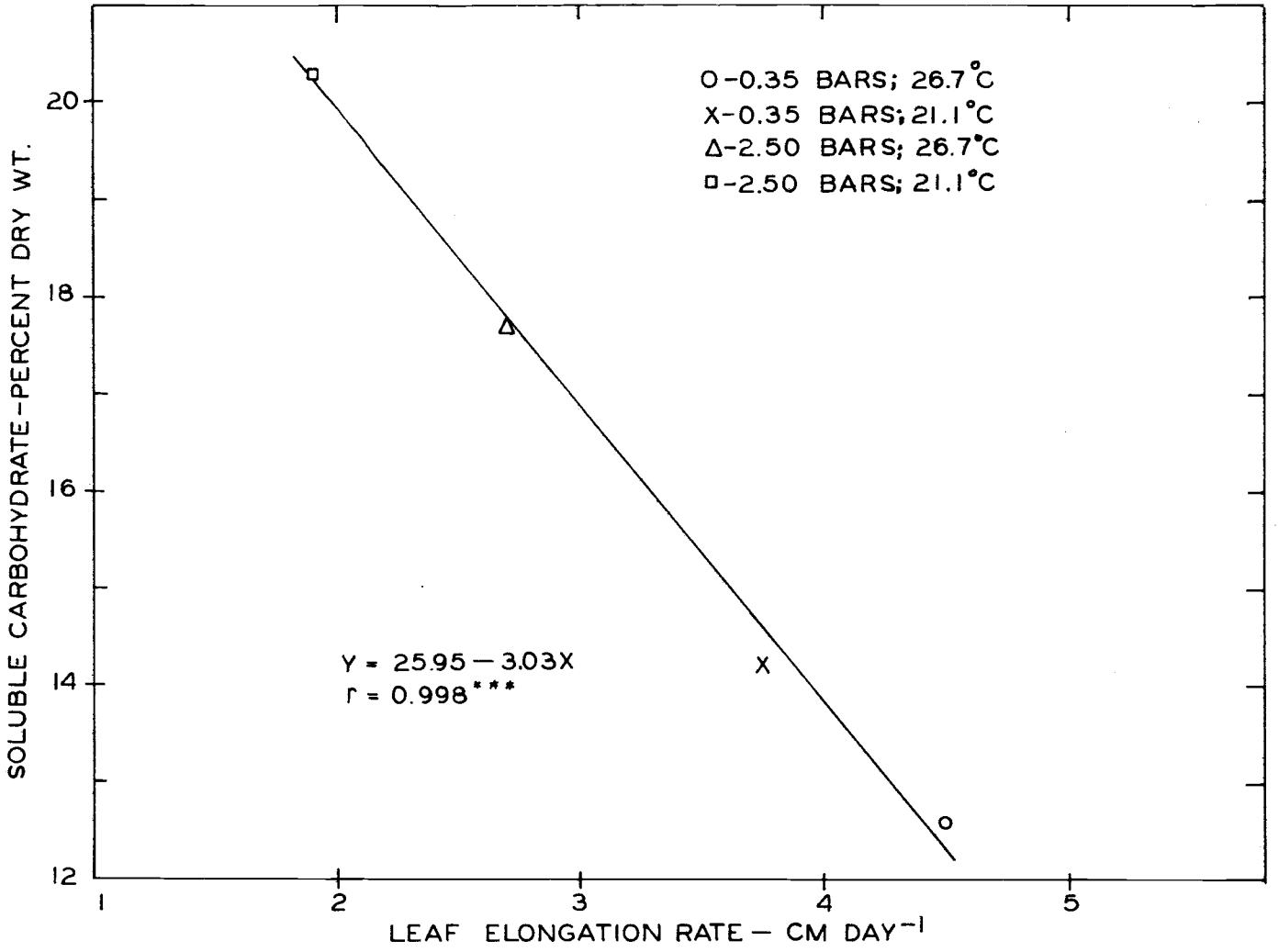

Figure 5. Functional relationship of soluble carbohydrate content to leaf elongation rate in young corn plants.

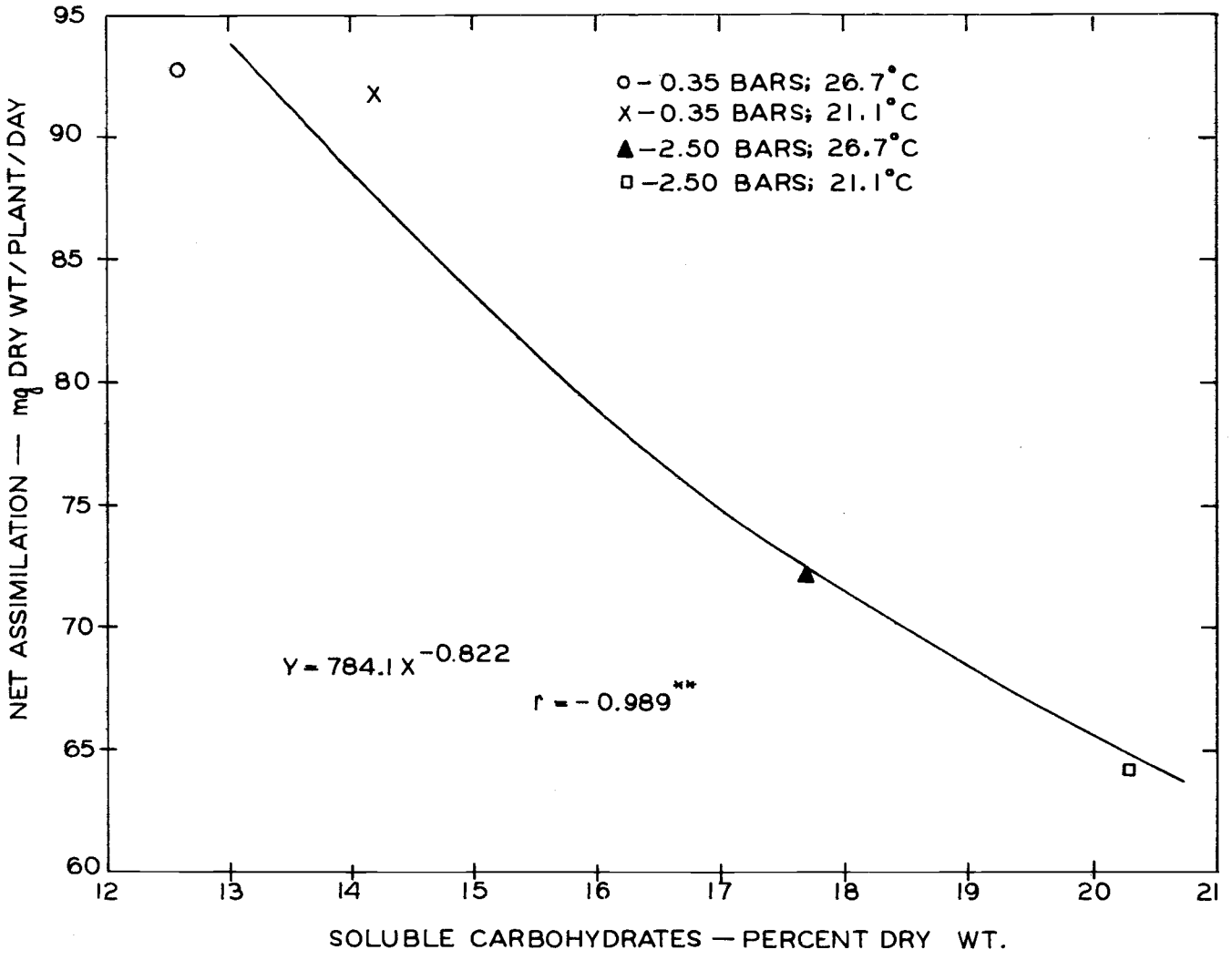

Figure 6. Functional relationship of net assimilation rate to the soluble carbohydrate content of young corn plants.

within the stressed plants decreases the rate of photosynthesis via direct or indirect feedback inhibition mechanisms. Such an inhibition of photosynthesis could cause an increase in the CO_2 concentration within the leaf, which in turn may initiate an adjustment in stomatal aperture (Meidner, 1962).

The similar magnitudes of the percent decrease in the rates of transpiration (-23.9%) and net assimilation (-26.1%) of the stressed plants observed in this experiment are consistent with the above hypothesis. The effects of mild water stress on photosynthesis generally are not considered in terms of water stress effects or source-sink relationships within the plant. But similar physiological effects have been reported when sink size has been decreased by other methods (Neales and Incoll, 1968). Moss (1962) prevented pollination of maize and found the leaf sugar content to increase while the photosynthetic rate decreased. Warren Wilson (1966) used low temperatures to decrease the rate of growth of Oxynia digyna by restricting respiration and expansion, and reported reduced net assimilation rates and increased leaf-carbohydrate content.

In whole plant studies of medium duration, such as reported here, the possibility cannot be excluded that the reported water stress effects were due to the independent effect of decreased ψ_c on each of the parameters as proposed by Wardlaw (1969). Boyer (1970b, 1973) found that net photosynthesis and chloroplast activity are not

affected much by water stresses as low as -7 bars. It would seem unlikely then that ψ_c was directly affecting photosynthesis in this experiment. It is necessary to directly measure the photosynthetic rate of the source leaf in relation to the elongation of the sink leaf and its ψ_c to demonstrate the above point.

SEQUENTIAL EFFECTS OF A SHORT TERM PLANT WATER STRESS ON LEAF ELONGATION, PHOTOSYNTHESIS AND TRANSPIRATION

Introduction

In this experiment an alternative method, to that used in the previous experiment, for testing the validity of the source-sink hypothesis of plant reaction to water stress was used. This involved the simultaneous monitoring of leaf elongation, net photosynthesis and transpiration rates at successively lower values of ψ_c during short stress periods imposed sequentially during a 12 hour period. These measurements made evaluation of the relative sensitivity of leaf elongation, photosynthesis and transpiration to water stress of increasing severity, possible.

The continuous measurement of photosynthesis and transpiration also made it possible to partition the diffusive resistances to carbon dioxide transfer into stomatal and mesophyll conponents. The theory and methodology of these calculations is described in Appendix 1.

The source-sink hypothesis postulates regulation of photosynthesis by a feed-back type mechanism operating at the molecular or organelle level. Therefore, if such an intracellular mechanism is controlling the photosynthetic rate in a water-stressed plant, any decrease in the rate of photosynthesis should be accompanied by a concurrent increase in the mesophyll resistance to carbon

dioxide transfer.

In this experiment, 2 to 3 week old corn plants were water stressed for periods up to 10 hours by rapidly lowering the root temperature (Brouwer, 1964). The simultaneous measurements of leaf elongation, photosynthesis, transpiration and leaf water potential were conducted during this short stress period. Because low root temperatures have been shown to influence the activity of the shoot meristem in young corn plants (Watts, 1972b), the influence of shoot meristem temperature on the above physiological parameters also was evaluated.

Methods and Materials

Description of Apparatus

In order to measure simultaneously the rate of net photosynthesis, transpiration and leaf elongation and the leaf water potential, it was necessary to construct a carbon assimilation system that incorporated an *in situ* leaf thermocouple psychrometer and a linear variable differential transducer (LVDT).

An open carbon assimilation system operating in the differential mode, similar to that described by Bierhuizen and Slatyer (1964) was constructed. The system is illustrated diagrammatically in Figure 7. A photograph of the equipment is shown in Figure 8. The essential

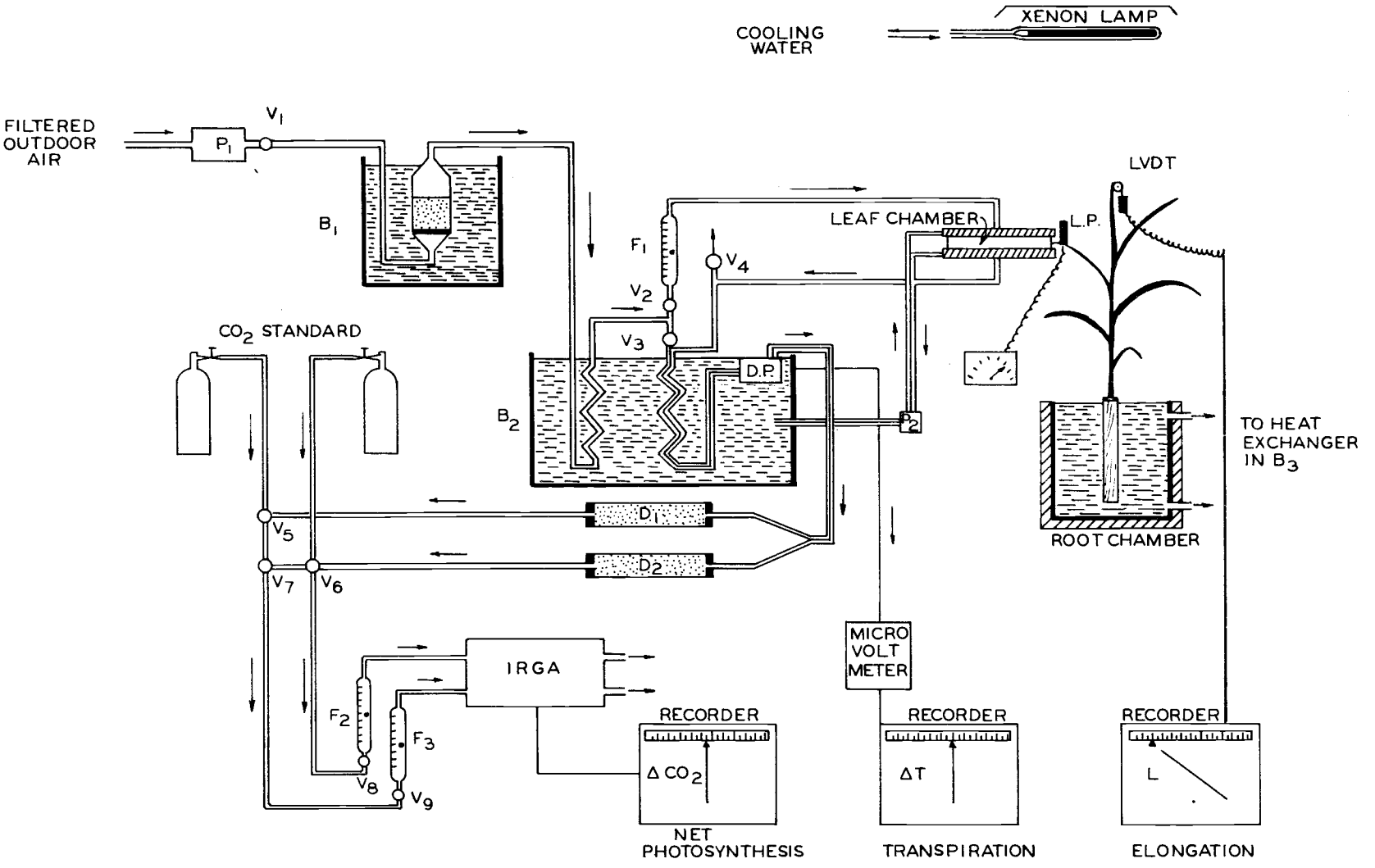

Figure 7. Schematic diagram of the carbon assimilation system used for the experiments. Symbols are explained in the text.

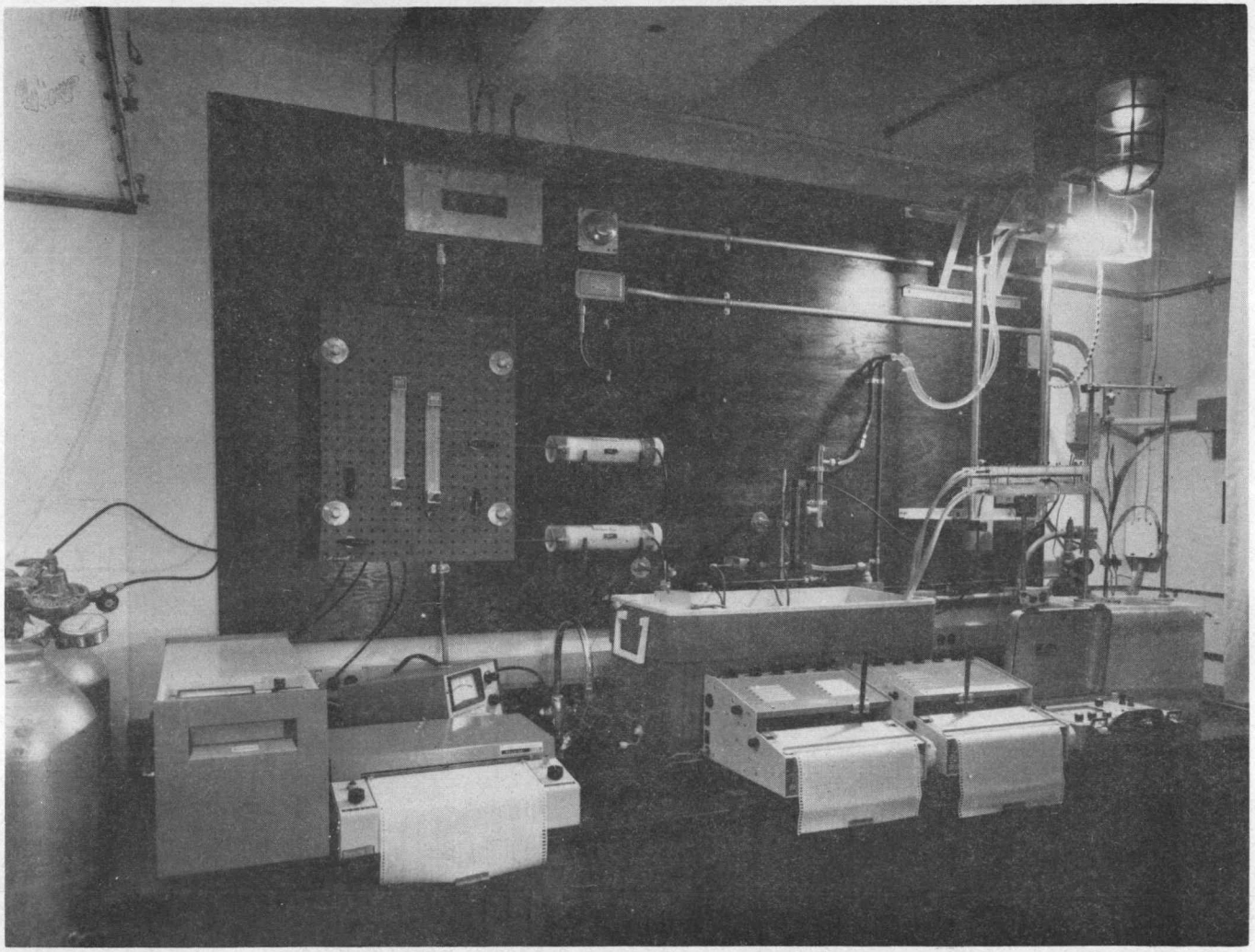

Figure 8. Photograph of the carbon assimilation system in operation.

features of this system are described below.

Air Control System. The first requirement of the system was to control the CO_2 concentration, water vapor pressure, temperature and flow rate of the air entering the leaf chamber. A near constant CO_2 concentration was attained by pumping atmospheric air through an air inlet filter on top of the building and well removed from any exhaust ducts. The CO_2 concentration of the atmospheric air was free from rapid variations, the maximum rate of change recorded being 2 ppm $hour^{-1}$. Because the infrared gas analyzer (IRGA) was operated in the differential mode using the inlet air as a reference gas, gradual variations less than 2 ppm $hour^{-1}$ did not affect the precision of the CO_2 measurement.

The flow rate of entering atmospheric air, drawn from the roof by pump P_1, was regulated by a valve V_1. The temperature and humidity were adjusted by successive passage through two temperature controlled water baths (B_1 and B_2). First the air was passed through a sintered glass diffuser, containing 500 cm^3 of distilled water, in water bath B_1, saturating it at the temperature of B_1, then it passed through a copper coil in bath B_2 set at the temperature desired in the leaf chamber. The relative humidity of the air directed to the leaf chamber was thus equal to the ratio of the saturation vapor pressure (S.V.P.) at the temperature of the bath B_1 over the S.V.P. at the temperature of the bath B_2. After passing

bath B_2 the air flow was divided. The reference air stream went directly to the differential psychrometer via regulating valve V_3 through a copper coil in bath B_2. The air stream enroute to the leaf chamber passed through regulating valve V_2 and flowmeter F_1 (1-9 L min^{-1} capacity) to regulate and measure the flow rate. The proportion of inlet air flowing through the reference and leaf chamber channels were regulated by V_3 and V_2 respectively. After passing across the leaf in the leaf chamber, the sample air flowed on through a copper coil parallel to the reference air line in bath B_2 to the differential psychrometer via exhaust valve V_4 and a copper coil in bath B_2. The sample air flow was reduced to a rate equalling that of the reference air flow entering the differential psychrometer by regulation of exhaust valve V_4.

The sample and reference air streams then followed parallel, but separate paths through the differential psychrometer, drying columns D_1, D_2, switching valves V_5, V_6, V_7, regulating valves V_8, V_9, flowmeters F_2, F_3 (0.1 to 1.5 L min^{-1} capacity) to the IRGA.

The twin drying columns were modified lucite laboratory gas drying columns in which air passed first through indicating drierite then anhydrous magnesium perchlorate.

Switching valves V_5, V_6, V_7 permitted entry of CO_2 standards into the system for calibration of the IRGA at the beginning of

each run. Regulating valves V_8 and V_9 are used to equalize the flow of the standard CO_2 gases. These valves (V_8 and V_9) did not regulate the flow of the leaf chamber and reference air, which was achieved by valves V_1, V_2, V_3, and V_4. Flowmeters F_2 and F_3 monitored the flow rate of air in the reference and leaf chamber channels, respectively, coming from the differential psychrometer to the IRGA.

Throughout the gas system 0.635 mm O.D. (1/4") copper tubing was used wherever possible. Where flexible connections were required, 0.635 mm O.D. (1/4") high density nylon tubing was used. All connections were made with Swagelock gas-tight fittings.

Because the accuracy of the measurement of CO_2 exchange is most often limited by the accuracy with which the air flow through the leaf chamber is known (Janac, Catsky and Jarvis, 1971), flowmeter F_1 was calibrated in situ with a wet test gas meter.

The Leaf Chamber. The leaf chamber was constructed of lucite with inside dimensions of 25 x 12.5 x 2.5 cm, as illustrated in Figure 9. The upper and lower surfaces of the chamber consisted of walls 1.25 cm apart forming water jackets, through which water from bath B_2 was circulated by pump P_2 (Figure 7). The leaf was held in position in the center of the chamber by nylon threads stretched across it. The air inlet and outlet on opposite sides of the chamber consisted of 24 pairs of holes (0.4 mm diameter) spaced 1 cm apart along

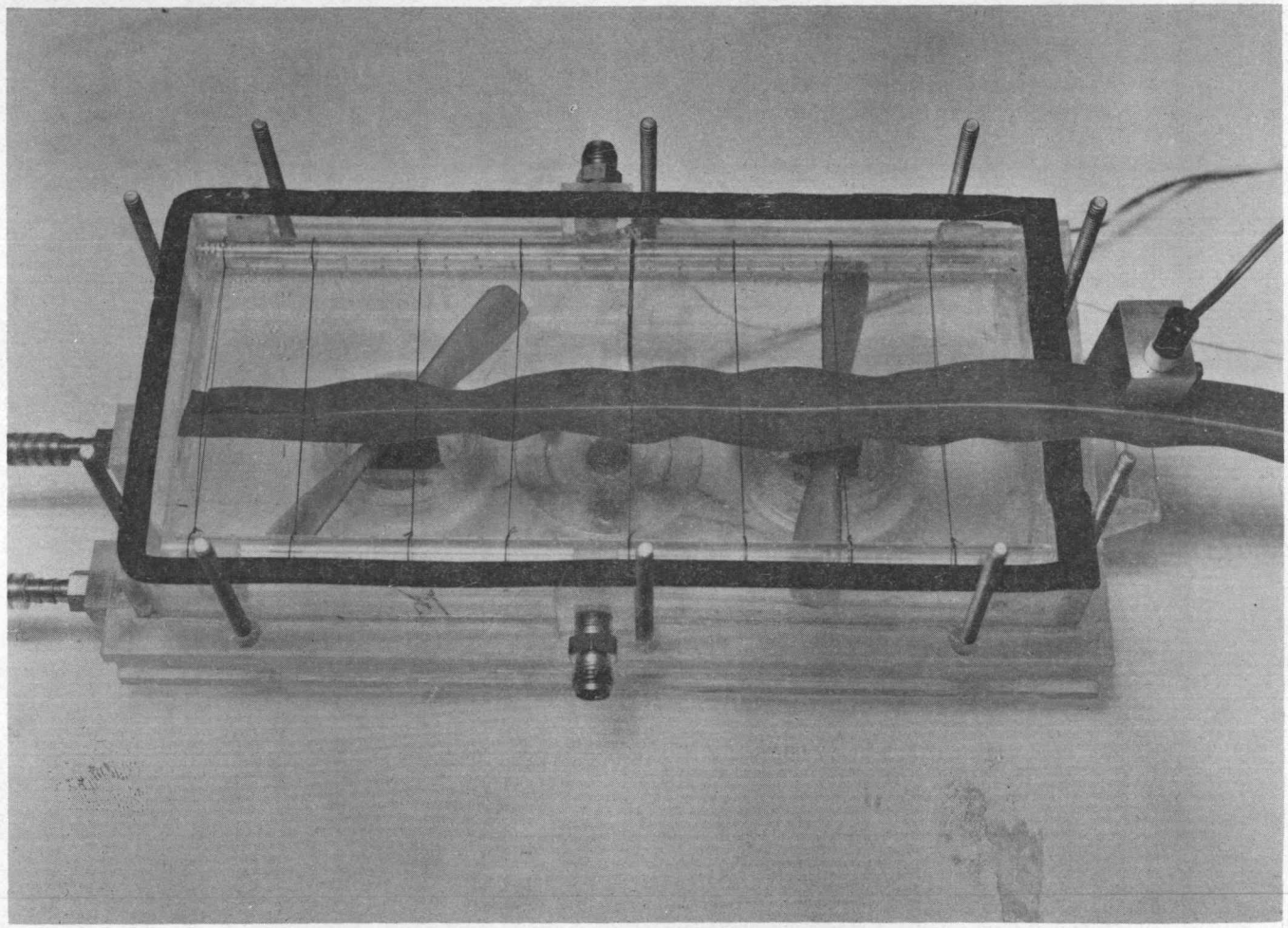

Figure 9. The leaf chamber, with the top removed, showing the experimental leaf held in place by the nylon threads, and the attachment of the in situ thermocouple psychrometer immediately outside the chamber.

a 10 mm O. D. lucite distribution tube. An equal number of holes faced downwards, and upwards at an angle of 45°, to ensure equal air flow rate across each side of the leaf. Uniform air mixing within the chamber was ensured by two electric fans in the bottom of the chamber whose speed was controlled by a variable DC power source. With the leaf in position, an air tight seal between the upper and lower half was obtained with thick (4 mm) closed-cell neoprene rubber. The chamber halves were held together with 10 lightly tightened wing nuts.

A 40 SWG Type T thermocouple was attached to the center nylon suspension thread so that it pressed against the underside of the leaf, for leaf temperature measurements.

Transpiration Measurements. The transpiration rate of the leaf in the leaf chamber was determined by measuring the difference between the water vapor pressures of the air passing over the leaf and the reference air stream with the differential psychrometer positioned in bath B_2 (Figure 7). The basic design of the differential psychrometer was similar to that described by Slatyer and Bierhuizen (1964).

The sample and reference air streams were first brought to the same temperature by passing through separate copper coils, soldered together and placed in bath B_2. The air flows were then introduced to identical wet bulb probes positioned in a 7.5 x 5 x 5 cm lucite block

also situated in bath B_2 (Figure 10). A 40 S.W.G. Type T thermocouple was placed at the tip of each lucite wet bulb probe which was covered with a small diameter cotton sleeve wick. The probes were sealed and held at the center of each inlet tube (6.75 mm diameter) by 2 O-rings on the probe bases (Figure 10). The effective cross-sectional area of the wet bulb probe was 15.5 mm^2 leaving a cross-sectional area of 20.0 mm^2 for air flow. With an air flow of 500 $cm^3 min^{-1}$, the ventilation rate was 12.5 m min^{-1}. The wick reservoirs were 25 ml scintillation vials with the caps glued to the lucite block. The seals between bottle and cap were obtained with silicone rubber gaskets.

A third thermocouple of the same type was placed in water bath B_2. The thermocouples were wired to measure the difference between the wet bulb temperatures of the air streams and the actual wet bulb temperature of the air leaving the leaf chamber. The thermocouple output was measured with a Keithley 150B Microvoltmeter and recorded on a potentiometric recorder.

For this differential psychrometer arrangement the psychrometric equations for the air streams may be written (Bierhuizen and Slatyer, 1964) as

$$(e_{wr} - e_r) = A(t - t_{wr}) , \qquad (2)$$

and

$$(e_{ws} - e_s) = A(t - t_{ws}) , \qquad (3)$$

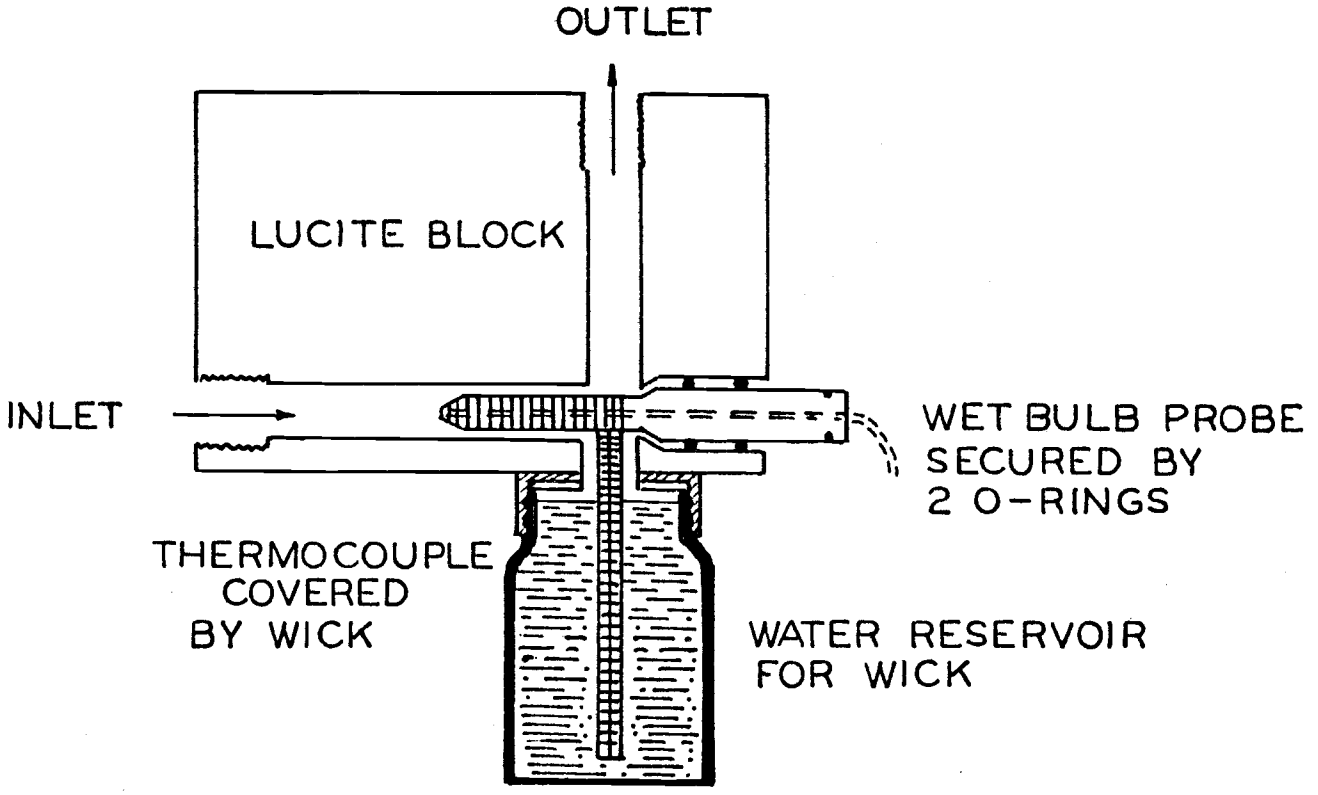

Figure 10. Cross-sectional view of one wet bulb assembly of the differential psychrometer used for transpiration measurements.

where t is the temperature of the airstreams or the temperature of water bath B_2 since both air streams have passed through copper coils in this bath, t_{wr} and t_{ws} are the wet-bulb temperatures of the reference and sample air streams respectively, e_{wr} and e_{ws} are the respective saturated water vapor pressures of the air streams at this wet bulb temperature, and e_r and e_s are the actual vapor pressures. A is the psychrometric constant.

Subtracting (2) from (3) yields

$$\Delta e = e_r - e_s = A(t_{wr} - t_{ws}) + (e_{wr} - e_{ws}). \tag{4}$$

From this equation Δe, the difference in vapor pressure of the two air streams caused by leaf transpiration can be calculated, since $(t_{wr} - t_{ws})$ is measured with the differential psychrometer and $(e_{wr} - e_{ws})$ can be obtained from tables. Δe can be converted to water vapor concentration (c) in $mg\,L^{-1}$ using the equation

$$c = p\left(\frac{\Delta e}{e_s}\right), \tag{5}$$

where e_s and p are the saturation vapor pressure and density of water vapor at the B_2 bath temperature, respectively. The transpiration rate (E) can be calculated using the equation

$$E = \frac{cF}{S}, \tag{6}$$

where S is the area of the leaf in the leaf chamber, and F is the flow rate through the leaf chamber measured with flowmeter F_1. A sample calculation of transpiration rate using the above method is presented in Appendix II.

The differential psychrometer was calibrated with a Cambridge Dew-point Hygrometer using saturated air at several temperatures. A wide range of relative humidities and air flow rates was used. A comparison between results obtained with the differential psychrometer and the Cambridge Dew-point Hygrometer is presented in Appendix III. The effect of air flow rate on psychrometer response is illustrated in Figure 11. It shows that maximum wet bulb depression occurred at flow rates as low as 300 $cm^3 min^{-1}$. The IRGA ideally requires a gas flow rate of 500 to 1000 $cm^3 min^{-1}$, so that the differential psychrometer easily satisfied the design criteria of the system.

Photosynthesis Measurements. Net photosynthesis was measured by determining the difference in CO_2 concentration (ΔCO_2) between the leaf chamber and reference air streams, with an infrared gas analyzer (IRGA). Before passing through the IRGA the air streams were dried, because water vapor interferes with the measurement of CO_2 by the IRGA.

The photosynthetic rate was then calculated with the equation

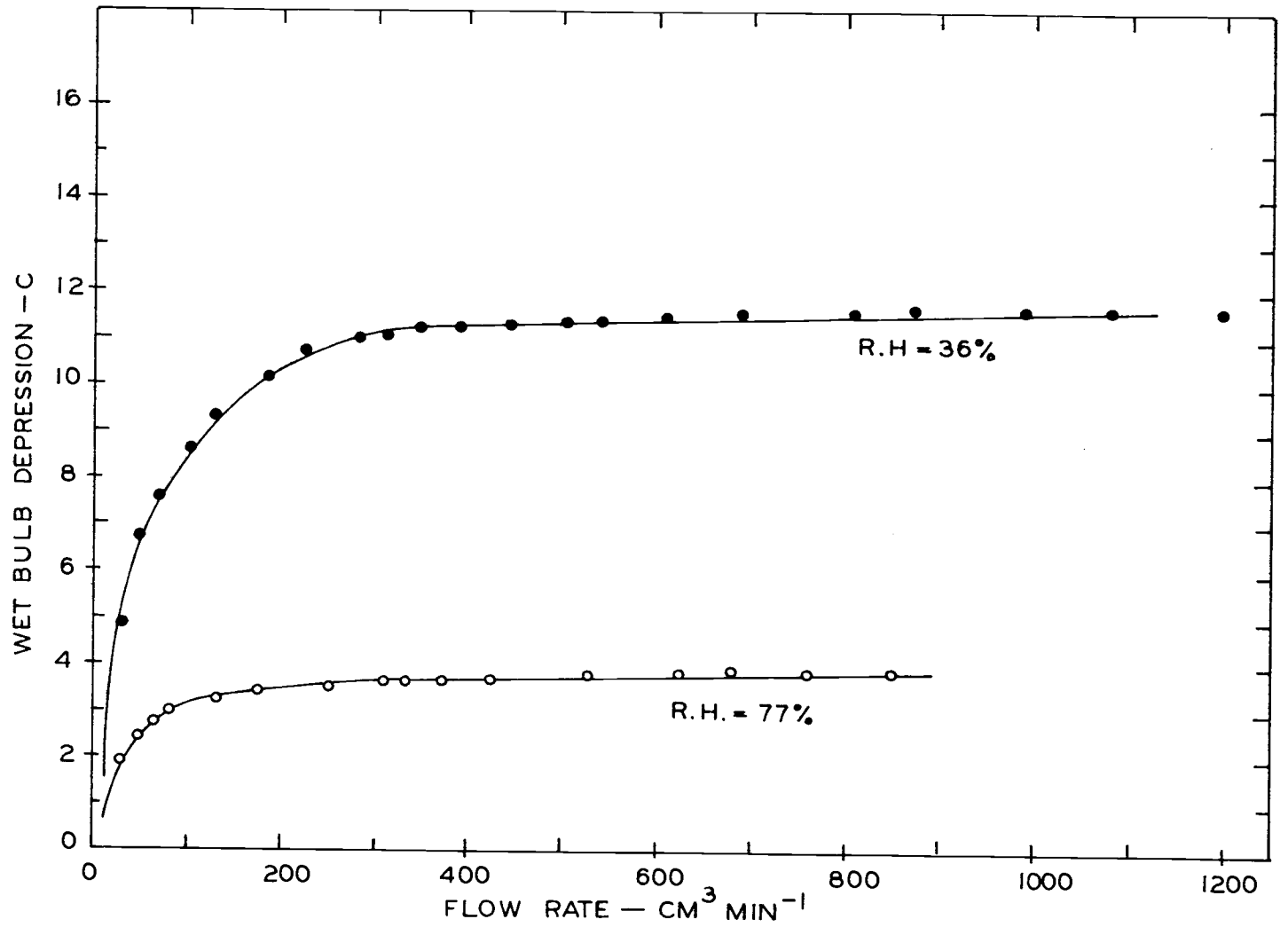

Figure 11. The effect of air flow rate on the performance of the differential psychrometer, illustrating the psychrometer to be fully ventilated at air flow rates as low as 300 cm^3 min^{-1}.

$$P_n = \frac{\Delta CO_2 \times F}{S}, \qquad (7)$$

where ΔCO_2 is in mg L^{-1}, F is the flow rate measured with flowmeter F_1 in L min^{-1}, and S is the leaf area in cm^2.

The IRGA was calibrated with standard CO_2 mixtures. Air with a calibrated CO_2 concentration approximately equal to that of the air to be used in system was passed through both sample and reference tubes, while the analyzer was zeroed. With this mixture flowing through the reference tube, a calibrated mixture with a lower concentration (50-100 ppm lower) was passed through the sample tube allowing the analyzer output to be adjusted to the sensitivity required. Within these narrow concentration ranges the analyzer output was essentially linear and no further calibration gas was required (Janac, Catsky and Jarvis, 1971). In normal operation the analyzer output was recorded with a 10 mv potentiometric strip chart recorder.

Light Source. Light was provided by a 2500 watt xenon long arc lamp mounted in such a way that its height above the plant, and thus light intensity, could be easily varied. The lamp was equipped with a quartz outer and an infrared (IR) inner filter to reduce excessive ultra-violet and IR transmission (Jarman, Barlow and Boersma, 1973). With this filter combination, the lamp produced a visual spectrum similar to natural sunlight (Figure 12). Although the lamp

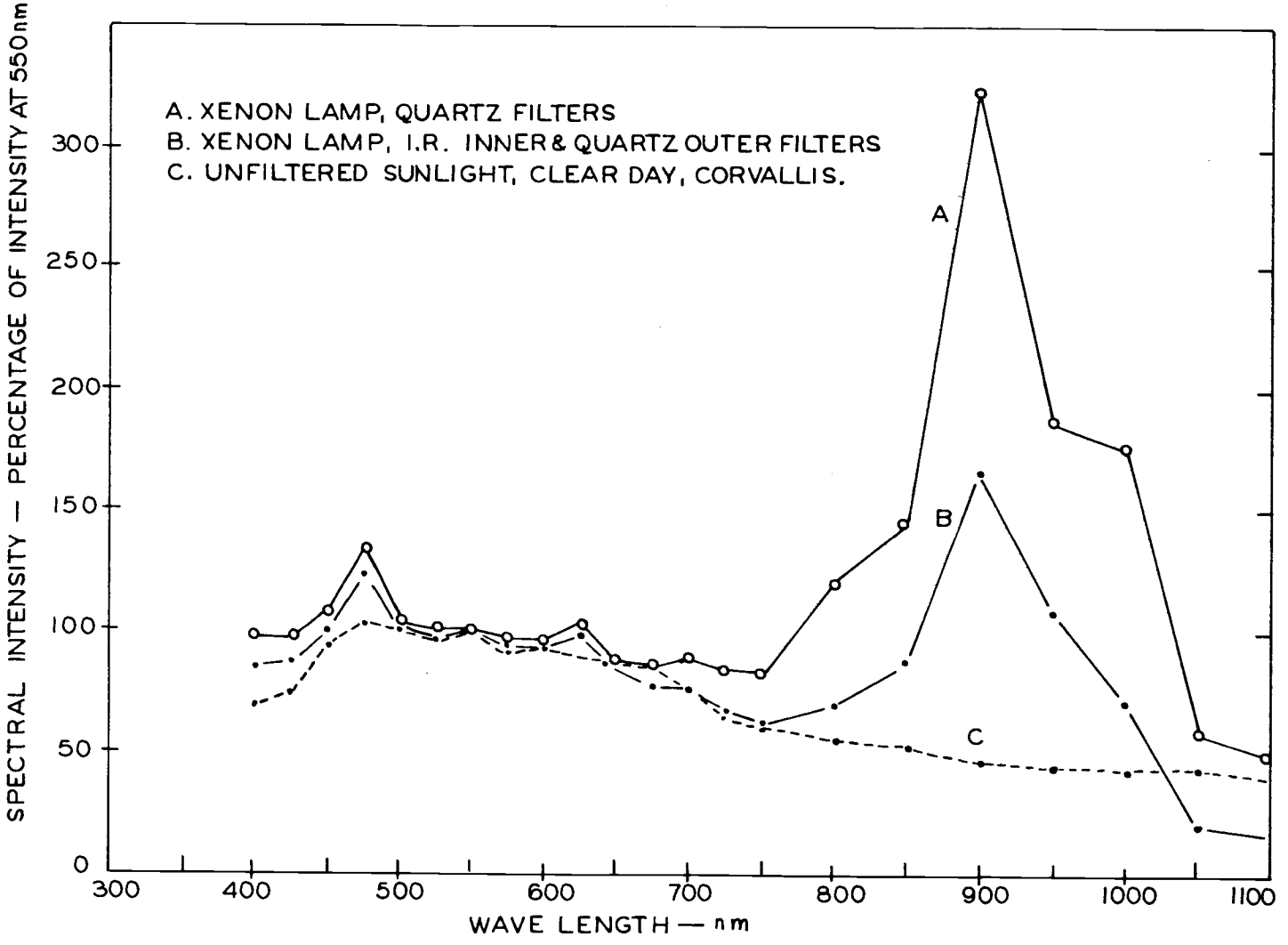

Figure 12. Spectral distribution of the radiant energy produced by the 2500 watt xenon long-arc lamp using an infrared filter, compared to the spectral distribution of unfiltered sunlight.

still exhibited a prominant infrared peak at 900 nm, the total energy emitted in the IR region was only 44% of the total radiation, which is very similar to natural daylight.

In addition to varying lamp height above the plants, the intensity of radiation could be varied by three choke taps. On the high power setting the lamp produced a radiant flux of 965 watts m^{-2} (1.38 langley min^{-1}) and a visual luminous flux of 7550 ft. c. at a distance of 38 cm from the burner. The radiant flux of the lamp on the medium and low power settings were respectively 75 and 50 percent of the high power setting.

Leaf Elongation Measurements. The leaf length was monitored continuously with a linear variable differential transducer (LVDT), connected to a potentiometric chart recorder (Hsiao, Acevedo and Henderson, 1970; Barlow and Boersma, 1972). The LVDT is an electromechanical transducer which produces an electrical output proportional to the displacement of a separate moveable iron core (Figure 13). A cotton thread taped to the tip of the youngest unrolled leaf connected it to the LVDT core via a low friction pulley. The iron core weighed less than 2 g and did not affect the rate of elongation, after an initial stretching period.

Leaf Water Potential ψ_c. An *in situ* leaf thermocouple psychrometer (Neumann and Thurtell, 1972) constructed after the design of Campbell and Campbell (1973) was used to monitor the water

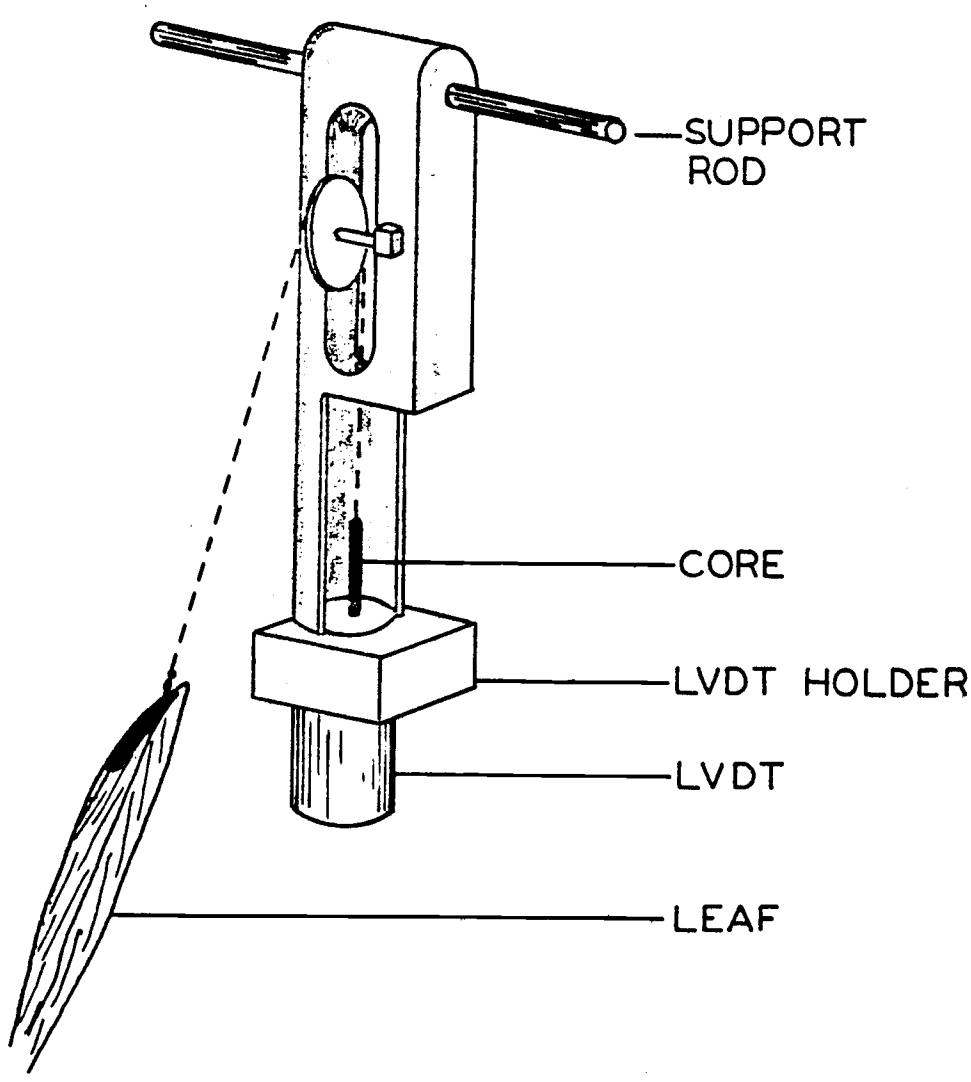

Figure 13. The LVDT in measurement position connected to the leaf via a cotton thread. The LVDT is held in position by a specially designed aluminum holder which incorporates a near frictionless pully and support rod.

potential of the leaf being used for net photosynthesis and transpiration rate measurements. It was supported by the leaf chamber, but attached to the leaf outside the chamber as illustrated in Figure 9. Before the psychrometer was attached to the leaf, the contact area was wiped with a Kimwipe moistened with xylene which removed part of the cuticle. The area was then cleaned with distilled water and thoroughly dried. The psychrometer was attached by placing the leaf in the slit of the block assembly and sealing the thermocouple assembly to the leaf with a 90% lanolin-10% bees-wax mixture (Campbell and Campbell, 1973). The psychrometer output was measured with a Wescor HR-33 Dewpoint Microvoltmeter. Prior to use, the psychrometer was calibrated using small pieces of filter paper (Whatman #1) moistened with KCl solutions of known water potential.

The initial equilibration time of the thermocouple psychrometer, after it was attached to the leaf was 1-3 hours. In practice, the in situ leaf psychrometer was always given an equilibration time of 12 hours or more, by attaching it to the leaf the night before beginning experimental measurement. After this initial equilibration period, the time necessary to adjust to changes in ψ_c was approximately 15 minutes. On corn it was necessary to facilitate vapor transfer by removing part of the cuticle with xylene to achieve the indicated equilibration times. However, with other plant species such as soybean and cocklebur cuticle removal was not necessary to obtain reasonable

equilibration times. Apparently the stomata, situated in the enclosed area of the corn leaf, closed quickly in response to the darkness imposed by the thermocouple assembly, making it necessary to obtain vapor equilibrium by the cuticular pathway.

Control of Root and Shoot Environment. A lucite root chamber measuring 21.5 x 19.5 x 31 cm and insulated with polystyrene, was used to control root temperature and soil water potential (Figure 7). The temperature of the osmotic solution in the chamber was controlled by continuously circulating the osmotic solution through a heat exchanger placed in water bath B_3 (Figure 7). The temperature of the soil contained in the cellulose acetate membranes in this chamber was monitored by a type T thermocouple placed in the soil. This thermocouple was calibrated against a Hewlett-Packard quartz thermometer.

The entire carbon assimilation system was situated in a controlled environment growth room.

Experimental Procedure

Corn (Zea mays L. var. Pride 5) plants were grown in growth chambers under the conditions described for Experiment I. When the plants reached the 7 leaf stage (2-3 weeks old), the roots were enclosed in a cellulose acetate membrane and placed in the temperature controlled osmotic root chamber located in the controlled growth

room set at 27.5 ± 1 C and a R.H. of 55 ± 1%. Plants were placed in the chamber one day before the experiments were initiated to allow acclimatization.

The bottom section of the leaf chamber together with the in situ thermocouple psychrometer was placed on the 5th leaf of the corn plant, the night before the experiment was started. The narrow distal portion of this leaf was cut off to enable a uniform broad section to be placed in the chamber (Figure 9). The cut end was sealed with a non-phytotoxic 90% lanolin-10% bees-wax mixture.

Net photosynthesis, transpiration, and ψ_c were measured on the 5th leaf because Hofstra and Nelson (1969) have shown by C^{14} translocation studies on Pride 5 corn plants of the same age, that the 5th leaf is the major source of photosynthate for the rapidly expanding 7th leaf.

On the morning of the experiment the LVDT was connected to the 7th leaf by a piece of cotton thread stuck to the tip of the leaf with a small piece of "scotch" tape. At the beginning of each experimental run the IRGA was calibrated using standard CO_2 mixtures, and the zero base lines of both IRGA and differential psychrometer were established by passing reference air through both branches of the system, by-passing the leaf chamber. The leaf chamber was then sealed by placing the upper half in position, and connected to the gas system.

The height of the xenon lamp above the leaf chamber was adjusted in each experiment so that the light intensity at the surface of the leaf in the chamber was about 381 W m^{-2} (3900 ft. c.). The quantum flux was 753 μ Einstein $m^{-2} s^{-1}$.

The temperatures of the water baths B_1 and B_2 (Figure 7) were adjusted so that the air entering the chamber was at the same temperature and relative humidity as the growth room, namely 27.5 C and 55% respectively.

Experimental measurements were commenced 1 1/2 - 2 hours after the light was turned on by determining the initial nonstress rate of net photosynthesis, transpiration and leaf elongation, at a root temperature of 27.5 C. After steady state values were achieved at this temperature, the root temperature was decreased 3.5 to 4.5 C by lowering the temperature of water bath B_3. When the thermocouple, placed in the soil with the roots, indicated that a new equilibrium had been achieved, the plant was allowed to grow for another 45 minutes at this temperature, before ψ_c, root and leaf temperatures were measured. Then root temperature was lowered again and the procedure was repeated. In this manner the root temperature was incrementally lowered to approximately 10 C to obtain a series of successively lower values of ψ_c.

In a second series of experiments the root temperature was maintained at 27.5 C while the temperature of the shoot apical

meristem was varied between 5 and 30 C. This was accomplished by placing a lucite water jacket around the meristem region of the corn stalk (Watts, 1972b). The lucite chamber, which was 5 cm high and 3.2 cm in diameter, was sealed to the corn stalk with a catalytic type silicone rubber, the day before beginning measurements. Water from bath B_3 was circulated through the chamber to control meristem temperature. A type T thermocouple was inserted in the outlet of the chamber to monitor its temperature. The experimental procedure described above was followed in these experiments. In all experiments, measurements were completed within 10 hours of exposing the plants to light.

Calculation of Transfer Resistances

The total resistance to water vapor transfer by the leaf, Σr_{H_2O} was calculated from the equation (Appendix I).

$$\Sigma r_{H_2O} = \frac{[H_2O]_c - [H_2O]_a}{E} \quad . \tag{7}$$

E was the measured rate of transpiration. The water vapor concentration within the leaf, $[H_2O]_c$ was calculated from leaf temperature data, assuming the air within the leaf to be saturated (Gaastra, 1959). The water vapor concentration $[H_2O]_a$ of the air entering the leaf chamber was measured by the reference wet bulb of the differential

psychrometer. The boundary layer resistance to CO_2 transfer r_a as measured with leaf models constructed of blotting paper, was 0.96 sec cm^{-1}.

The total resistance to CO_2 transfer was calculated from the equation

$$\Sigma r_{CO_2} = \frac{[CO_2]_a - [CO_2]_{chl}}{P_n} . \qquad (8)$$

P_n is the net photosynthetic rate, $[CO_2]_a$ is the CO_2 concentration of the air entering the leaf chamber, and $[CO_2]_{chl}$ is the CO_2 concentration at the chloroplast fixation sites, which is assumed to be zero (Gaastra, 1959). The validity of the latter assumption has recently been challenged by Whiteman and Koller (1968), who proposed that the CO_2 concentration at the compensation point provides a better estimate of $[CO_2]_{chl}$. However, it is unlikely that the assumption of $[CO_2]_{chl} = 0$ resulted in a significant error in these experiments because Forrester, Krotkov and Nelson (1966), and Moss (1971) have shown the CO_2 compensation point of Zea mays L. to be zero. Because of the above observation and the impracticality of determining the CO_2 compensation point at each level of water stress, the assumption of $[CO_2]_{chl} = 0$ was used in these calculations.

The stomatal resistance to CO_2 transfer was calculated from

$$r_s = \Sigma r_{H_2O}\left(\frac{D_{H_2O}}{D_{CO_2}}\right) - r_a \quad . \tag{9}$$

A value of 1.60 was used for the diffusitivity correction term (Fuller, Schettler and Giddings, 1966). The mesophyll resistance to CO_2 transfer was calculated from the equation

$$r_m = \Sigma r_{CO_2} - (r_a + r_s) \quad . \tag{10}$$

A sample calculation of these resistances is presented in Appendix II.

Results and Discussion

Under the constant environmental conditions of the growth room the photosynthetic rate of nonstress control plants was almost constant for a 12-hour light period (Table 3). Consequently, the effects of temperature and water stress on photosynthesis and transpiration have been reported as a percentage of the nonstress rate measured on the day of experimentation.

The net photosynthesis values shown in Table 3 are somewhat lower than those previously reported for Zea mays L. (Hesketh, 1967), because the light intensity was low (381 w m^{-2}). This low light intensity was used because higher light intensities were found to severely inhibit leaf elongation (Figure 14). The inhibition of leaf

Table 3. The diurnal pattern, in the rates of leaf elongation (leaf 7), net photosynthesis, transpiration (leaf 5), and the leaf water potential (leaf 5) of a control plant growing at a root temperature of 27.5 C and a soil water potential of -0.35 bars. The light intensity was 381 w m^{-2}.

Time	Water Potential	Elongation	Net Photosynthesis	Transpiration
hrs	Bars	$\mu m\ min^{-1}$	$mg\ CO_2\ dm^{-2} hr^{-1}$	$g\ H_2O\ dm^{-2} hr^{-1}$
900	-6.3	45.4	30.9	2.233
1100	-6.5	73.5	30.6	2.233
1300	-6.7	71.6	31.0	2.224
1500	-6.7	72.8	32.8	2.224
1700	-6.6	73.1	32.4	2.250
1900	-6.7	74.0	31.5	2.250
2100	-6.7	71.5	30.8	2.227

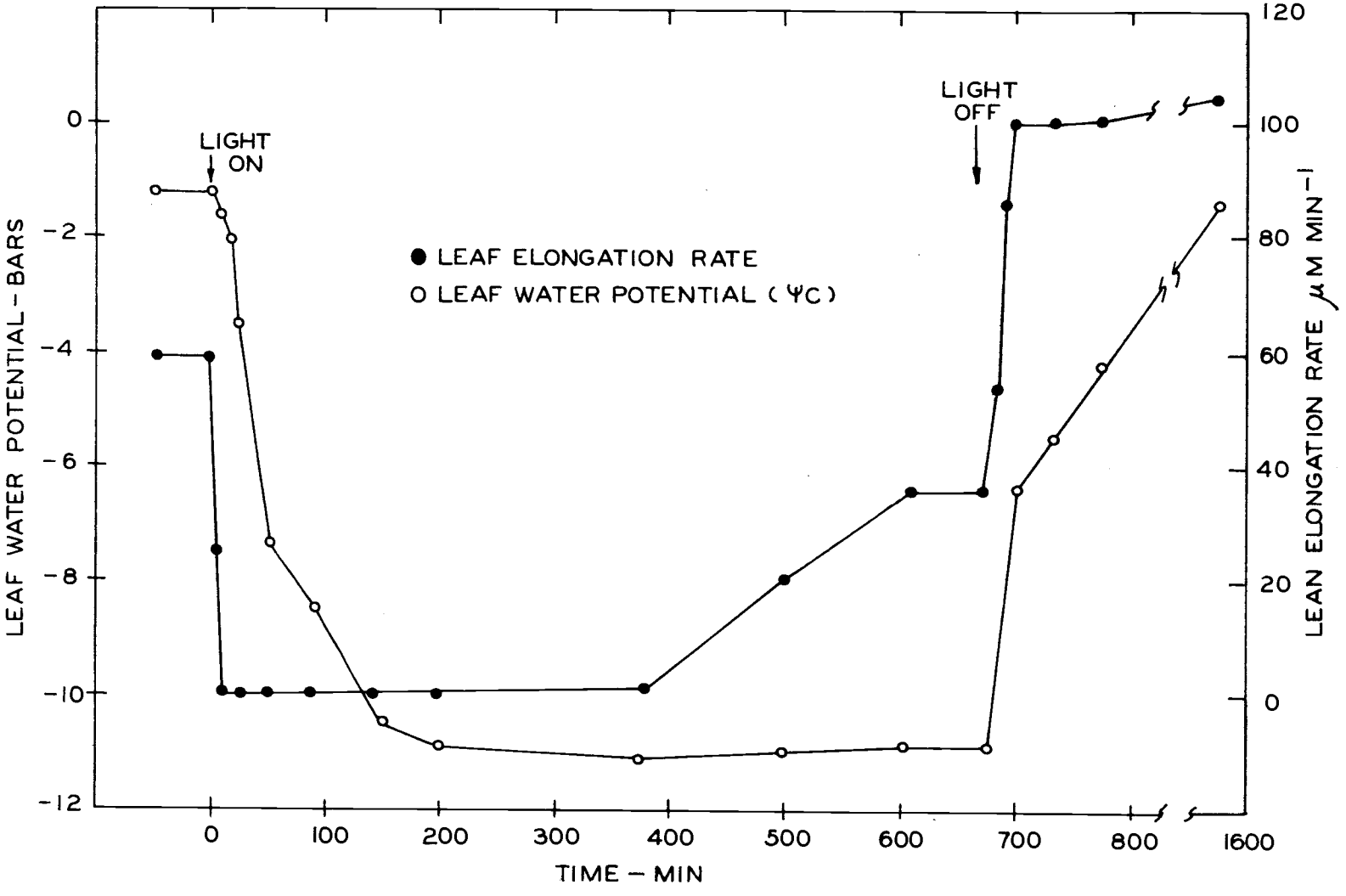

Figure 14. Changes in leaf elongation (leaf 7) and leaf water potential (leaf 5) during a 700 minute illumination period at a high light intensity (980 w m^{-2}).

elongation, by the light intensity of 980 w m^{-2} was probably due to the direct inhibitory effect of light on leaf elongation (Sachs, 1965) and lowering of the leaf water potential caused by the intense radiation (Figure 14). The inhibitory effects of high light intensities and large radiant fluxes on leaf elongation have been noted previously by Loomis (1934), and Hsiao, Acevedo and Henderson (1970). Therefore, because the aim of this experiment was to measure the response of leaf elongation to plant water stress, a lower light intensity (381 w m^{-2}) that did not severely inhibit leaf elongation (Table 3) was selected.

Lowering the root temperature from 27.5 to 10.0 C in small increments had a different effect on leaf elongation than on net photosynthesis and transpiration (Figure 15). The rate of leaf elongation decreased steadily with each decrease in root temperature until it reached zero at a root temperature of 12 C. In contrast, net photosynthesis and transpiration were still proceeding at more than 80% of the nonstress rate when the root temperature reached 12 C (Figure 15). The effects of low root temperature on leaf elongation are at least two-fold. Firstly, lowering the root temperature can decrease the rate of water adsorption by the plant roots and thereby induce water stress within the plant (Kuiper, 1964; Kleinendorst and Brouwer, 1970). In this experiment the ψ_c decreased with each decrease in root temperature below 27.5 C (Figure 16), indicating that low root

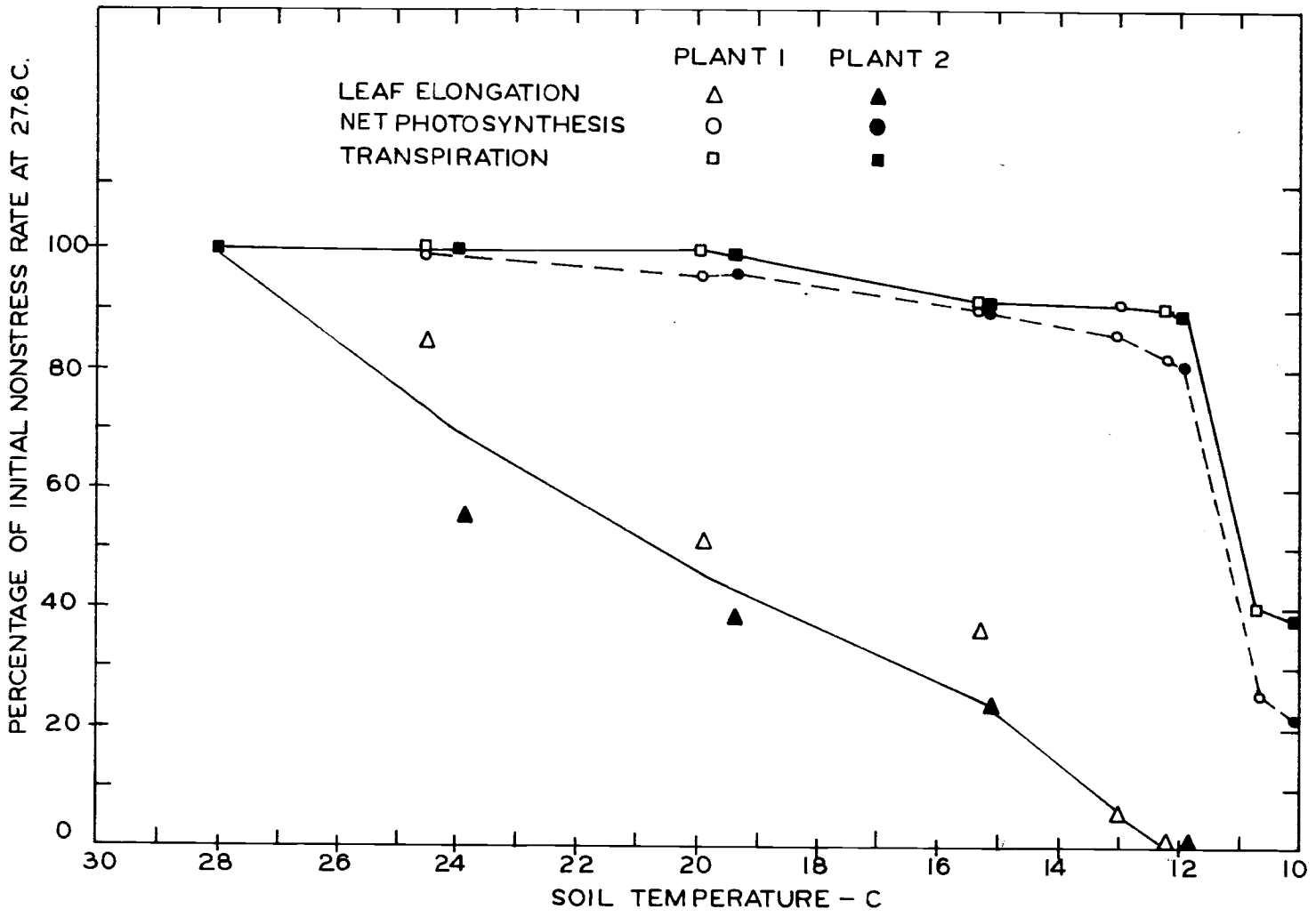

Figure 15. Steady state rates of leaf elongation (leaf 7), net photosynthesis and transpiration (leaf 5) of a corn plant with 7 unrolled leaves at soil temperatures ranging from 10 to 30 C.

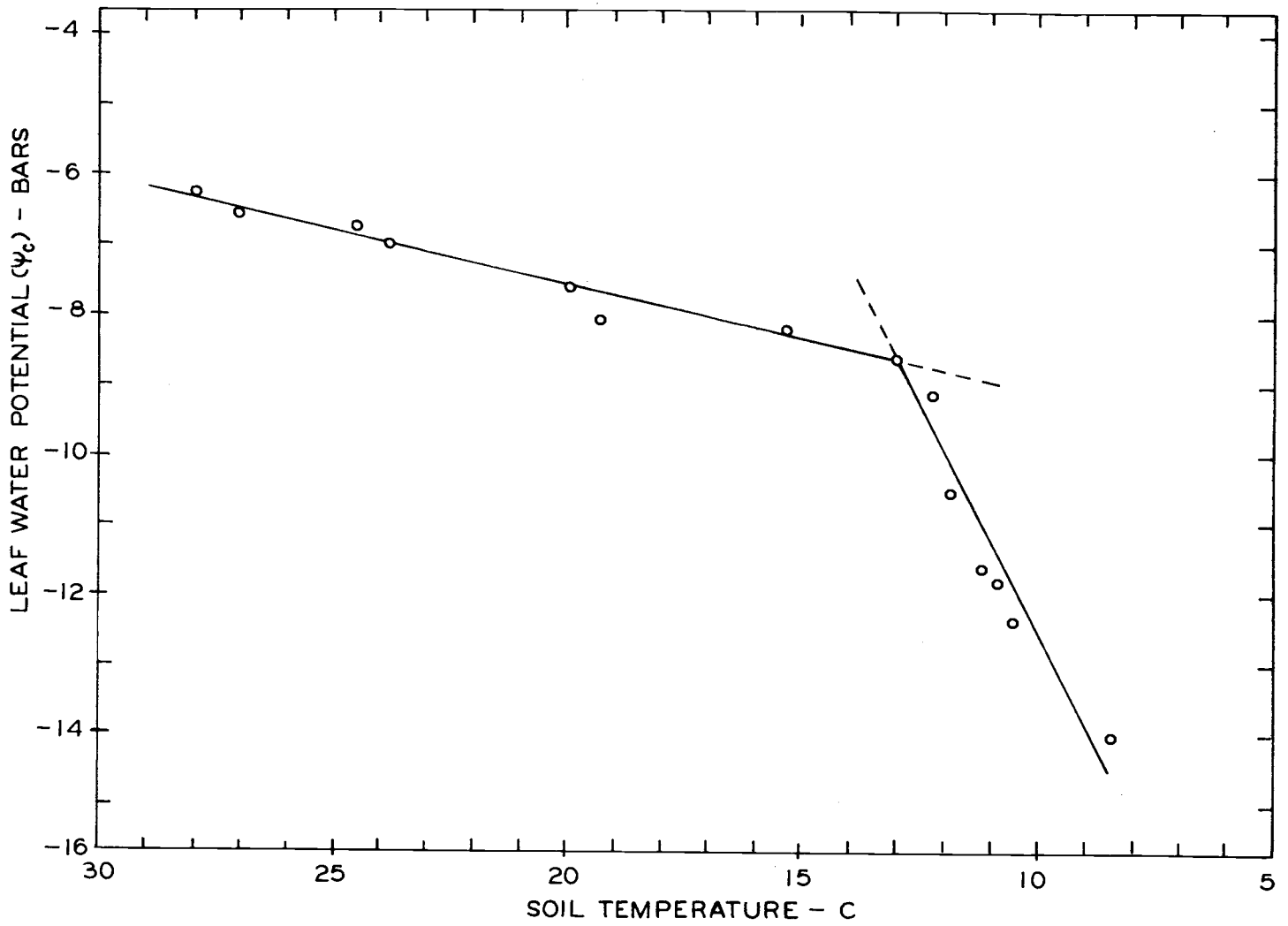

Figure 16. Steady state water potential of the 5th leaf of a 7 leaf corn plant at soil temperatures ranging from 10 to 30 C. The shoot environment was controlled at 27.5 C and 55% relative humidity.

temperatures reduced the rate of water uptake by the root system. However, the extent of this reduction was not constant with each decrease in root temperature, as there was a sharp discontinuity in the curve at a critical temperature in the vicinity of 13 C. This would indicate a change in the activation energy for water transport at this critical temperature. Discontinuous temperature response curves have been previously reported for water uptake, membrane ATPase activity, membrane permeability and mitochondrial respiration (Kuiper, 1964; Kemp, Groot and Reitsma, 1969; Hope and Aschberger, 1970; Lyons and Raison, 1970). The likely causes of these changes were recently reviewed by Kuiper (1972), who postulated that they may be correlated with changes in membrane structure. In particular, a hydrophobic melting of the membrane lipid molecules may begin at the critical temperature resulting in a transition in membrane structure from the lamellar to the globular phase, which is more permeable to water. Such a structural change may be accompanied by a collapse of the ice-like water structure to form polarized water around the lipid protein structure. It is also possible that this change from the lamellar to the globular phase could be caused by a change in the supply of metabolic energy resulting from conformational changes of key enzymes.

The second manner in which soil temperature may influence the rate of leaf elongation is by directly affecting the temperature of the

shoot apical meristem (Watts, 1972a, 1972b). When corn is in the 7 leaf stage the apical meristem is close to the soil surface and tends to follow soil temperature rather than air temperature because of conduction up the stalk and the flow of cold liquid up the xylem (Beauchamp and Torrance, 1969; Watts, 1972b). Low soil temperatures do not affect directly the temperature of the plant leaves (Beauchamp and Torrance, 1969) or their physiological function. Consequently low root temperatures influence leaf elongation directly by the temperature effect on the apical meristem and indirectly by lowering ψ_c and cell turgor but only affect net photosynthesis and transpiration indirectly by decreasing ψ_c.

In order to evaluate the effect of successively lower ψ_c values on these functions, it was necessary to quantify the direct effect of low soil temperatures on shoot apical meristem activity and leaf elongation in the absence of water stress. This was accomplished by growing corn plants at a soil temperature of 27.5 C, and independently varying the temperature of the shoot apical meristem by enclosing it in a small lucite water jacket connected to a controlled temperature recirculating water bath. In this manner, leaf elongation, net photosynthesis and transpiration were measured at shoot apical meristem temperatures varying from 5 to 30 C (Figure 17).

Lowering the shoot apical meristem temperature in small increments to 5 C did not affect net photosynthesis or transpiration.

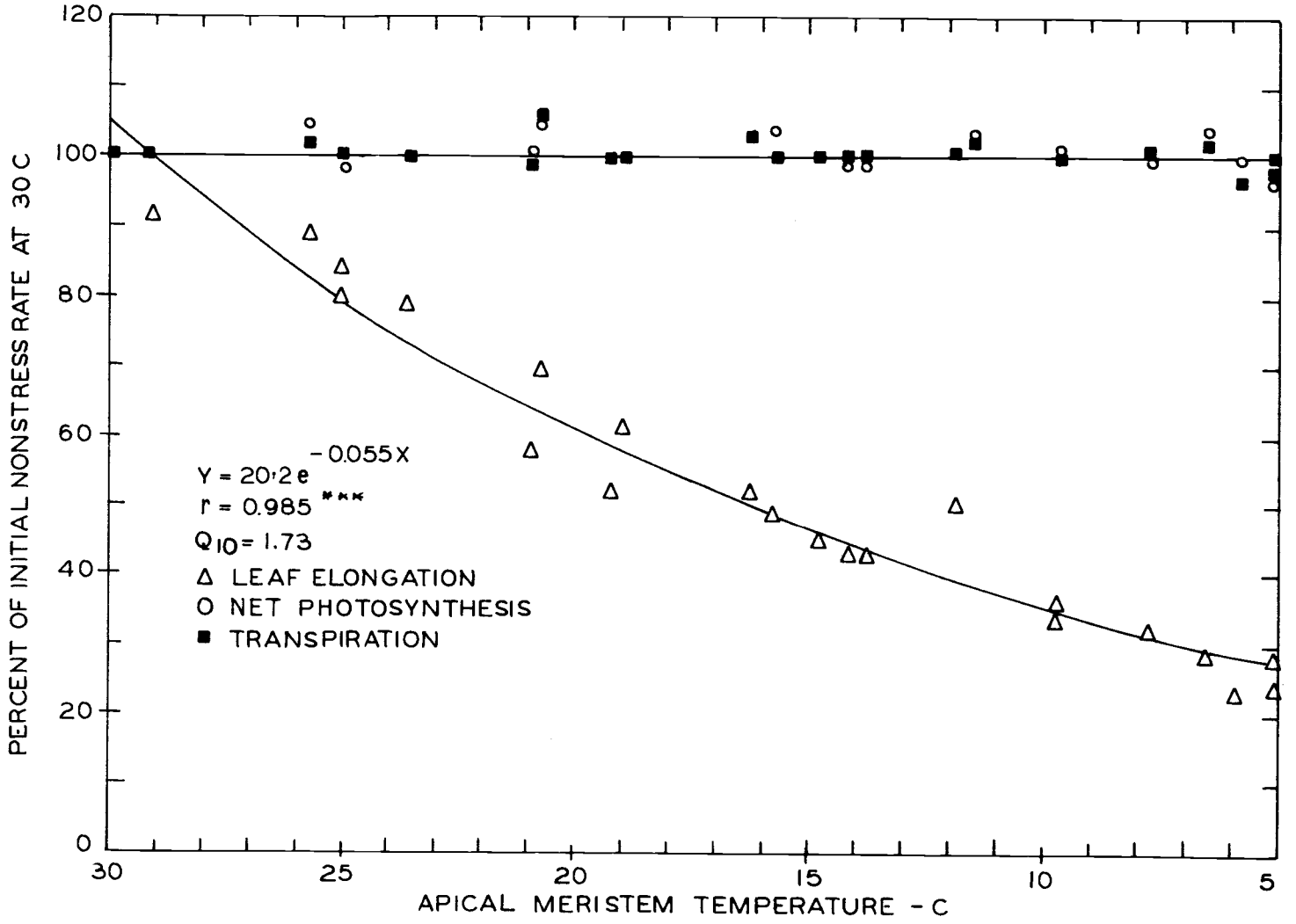

Figure 17. Steady state rates of leaf elongation (leaf 7), net photosynthesis and transpiration (leaf 5) of a corn plant with 7 unrolled leaves growing at a soil temperature of 27.5 C and shoot apical meristem temperatures ranging from 5 to 30 C.

Furthermore, the ψ_c of the experimental plant did not decrease by more than 0.7 bars in any experiment. However, the shoot meristem activity, as measured by the rate of leaf elongation was affected markedly by lowering its temperature. Unlike the effect of root temperature on ψ_c, leaf elongation was a continuous exponential function of meristem temperature down to 5 C. The Q_{10} of leaf elongation throughout the entire temperature range was constant at 1.73 which was slightly lower than the Q_{10} value of 2 for leaf growth reported by Chao and Loomis (1948) and Watts (1972b). The water jacket did not enclose all of the elongating region as well as the meristem, so that some elongation may have continued irrespective of the meristem temperature. Furthermore, the high evaporative demand in the growth room caused a large flux of warm water to continuously move through the cooled meristem, and it is unlikely that the temperature of the meristem inside the enclosing leaf sheaths was as low as that of the circulating water. Both factors would tend to decrease the observed Q_{10} value.

The lack of an effect of the low meristem temperatures on net photosynthesis, transpiration and ψ_c would indicate that these had little effect on translocation or other physiological functions, except for decreasing the apical meristem activity. This is consistent with the work of Thrower (1965) and Weatherley and Watson (1969) who found that translocation was restricted somewhat at chilling

temperatures, but that it did not stop until the temperature was below 0 C.

The important observation from the meristem collar experiment (Figure 18) was that although decreases in the temperature of the apical meristem did decrease the leaf elongation rate, meristem temperatures as low as 5 C did not stop the leaf elongation. In contrast a soil temperature of 12 C stopped leaf elongation (Figure 18). Furthermore at temperatures ranging from 15 to 30 C approximately 65% of the reduction in the rate of leaf elongation can be attributed to the lowering of the apical meristem temperature (Figure 18). Below the critical temperature of approximately 13 C the ψ_c decreased rapidly (Figure 16) and sub-threshold cell turgor pressures were probably the major factor limiting leaf elongation in this region (Figure 18).

The effect of ψ_c on net photosynthesis, transpiration and leaf elongation can now be evaluated by plotting the ψ_c values obtained in the root temperature experiment in Figure 19. Duplicate experiments are plotted separately in Figures 19a and 19b, because the ψ_c values for each plant at each soil temperature were different and a composite curve of both plants lacked clarity. It was not possible to make a quantitative correction for the effect of lowered meristem temperature on leaf elongation (Figure 18). Consequently the leaf elongation rate plots in Figure 19 show leaf elongation rate decreasing prematurely because most of the reduction in leaf elongation at high ψ_c

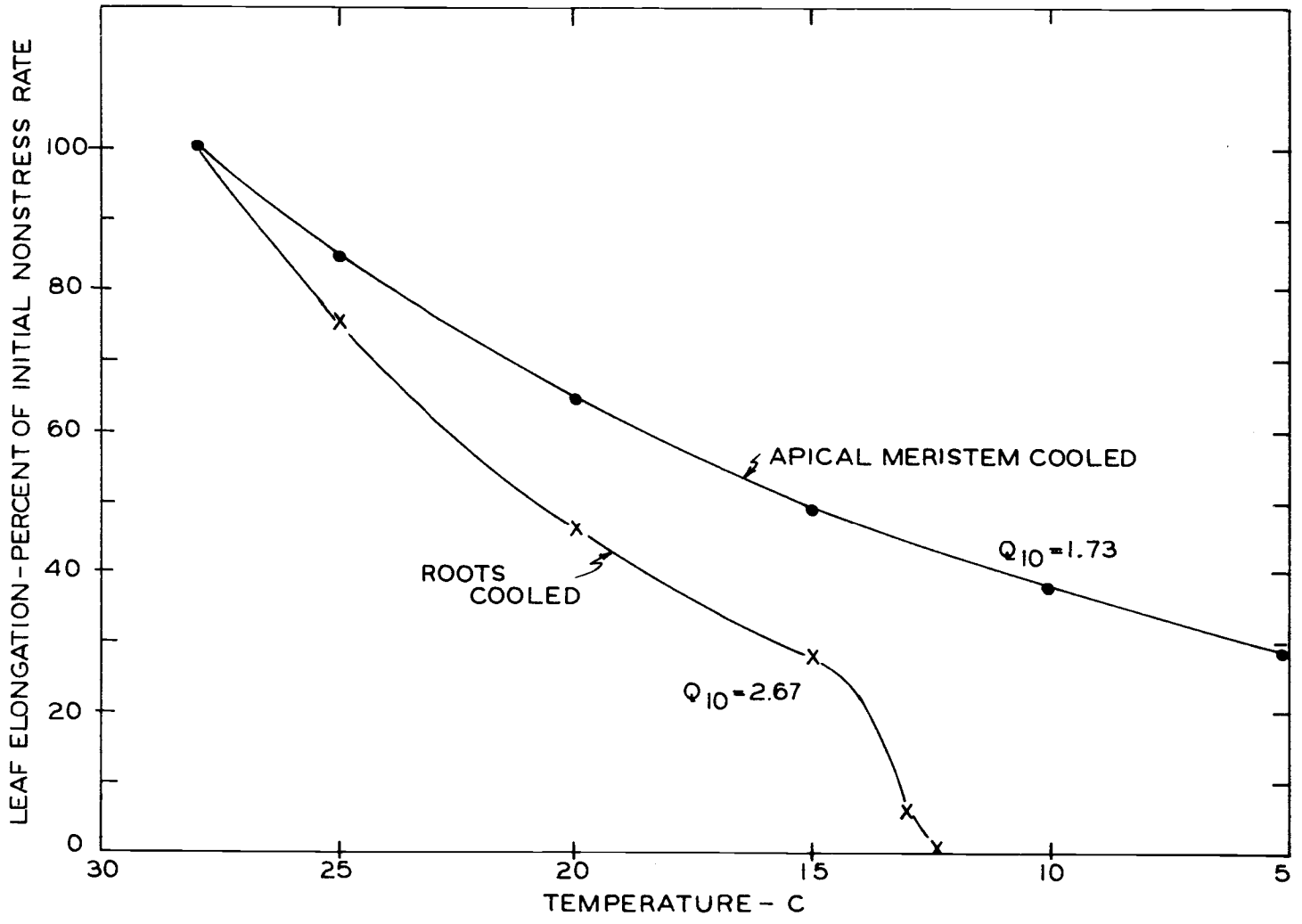

Figure 18. The elongation rate of the 7th leaf of young corn plants exposed to either soil or apical meristem temperatures ranging from 5 to 30 C.

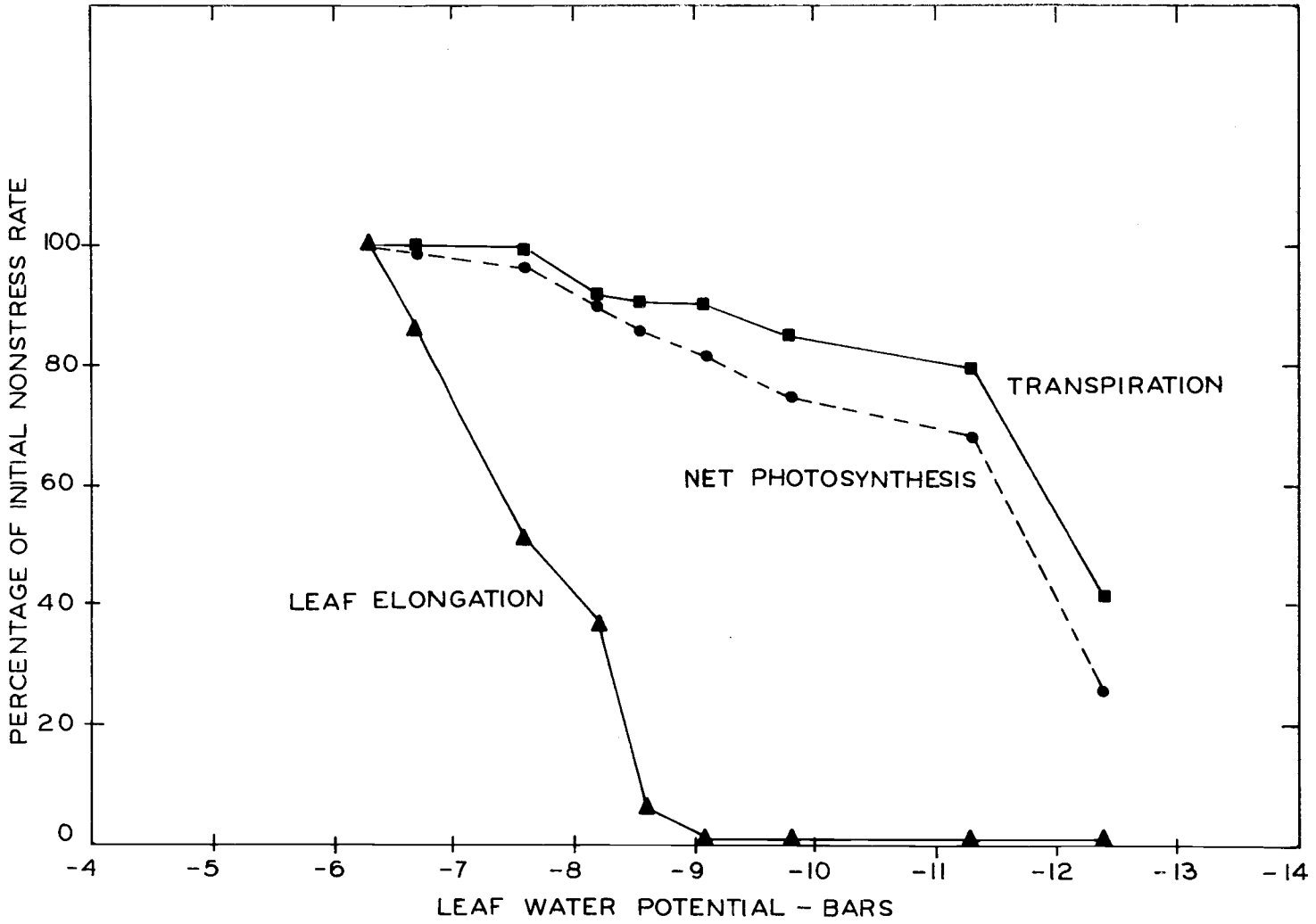

Figure 19A. Steady state rates of leaf elongation (7th leaf), net photosynthesis and transpiration (5th leaf) of a corn plant with 7 unrolled leaves as a function of the water potential of the 5th leaf. Plant 1.

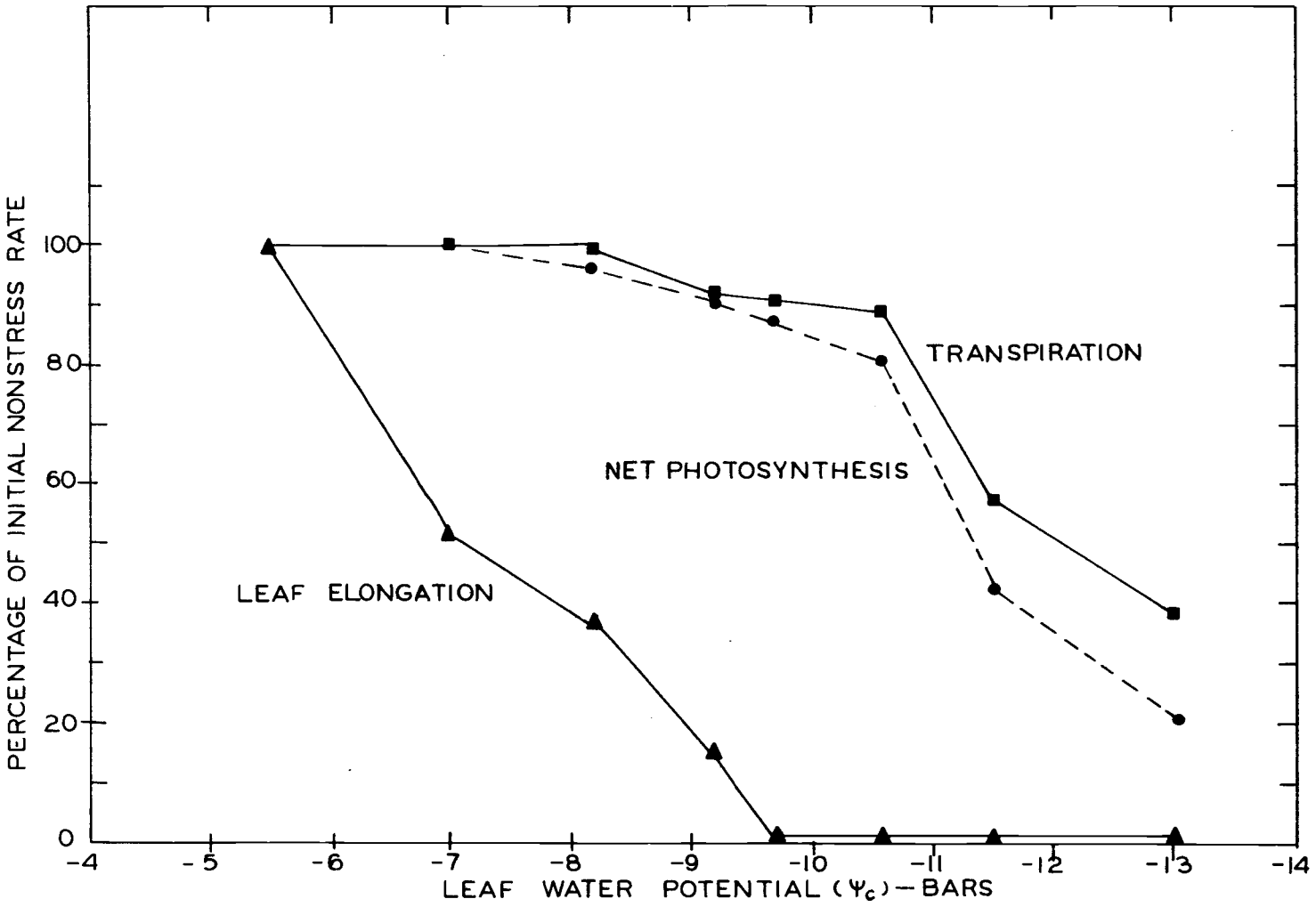

Figure 19B. Steady state rates of leaf elongation (7th leaf), net photosynthesis and transpiration (5th leaf) of a corn plant with 7 unrolled leaves as a function of the water potential of the 5th leaf. Plant 2.

values (higher soil temperatures) is due to the effect of soil temperature on apical meristem activity.

The sensitivity of leaf enlargement to small changes in ψ_c (Hsiao, 1973) was demonstrated clearly by the short term steady state measurements of this experiment. Each decrease in the ψ_c resulted in a decrease in the rate of leaf elongation (Figures 19A and 19B). Leaf elongation eventually ceased at a ψ_c of -9.1 in one plant and -9.6 bars in another, which is in good agreement with the value of -9.2 bars obtained by long term steady-state measurements. Both Boyer (1970a) and Acevedo et al. (1971) reported that corn plants of similar age stopped leaf enlargement at a ψ_c of -7 to -8 bars. The discrepancy between these estimates is probably due to differences in light intensity as Boyer made his measurements in the dark, and Acevedo et al. used a light intensity of 1100 ft.c., which is less than 1/3 of that used in this experiment. It is likely that the values of -9.0 to -9.5 bars obtained in these experiments more closely approximate the field situation.

Photosynthesis and transpiration responded similarly to decreasing ψ_c, although net photosynthesis was affected to a greater extent at any particular value of ψ_c in both plants. Both net photosynthesis and transpiration were decreased slightly by ψ_c values near -8 bars, but did not exhibit large decreases until values of -11 to -12 bars were reached. Sequential short term responses of leaf elongation, net

photosynthesis and transpiration have not been measured previously although Boyer (1970a) related photosynthesis and leaf elongation to the ψ_c of corn in a water stress study lasting several days and found a similar pattern to that illustrated in Figure 19.

Calculation of the diffusion resistances to CO_2 transfer showed that the decrease in net photosynthesis was due to increases in both the mesophyll (r_m) and stomatal resistances (r_s) (Figure 20). The increases in both r_s and r_m followed the same pattern and appeared to begin at approximately the same degree of stress (Figure 20). The mesophyll resistance increased a little more than the stomatal resistance with increasing stress, but the ratio of the resistances remained approximately the same. The increases in r_m, which indicate a degree of nonstomatal control over net photosynthesis during water stress may result from physical, photochemical, biochemical or metabolic factors affecting the rate of net photosynthesis. These can not be specifically identified because r_m was calculated as a residual term. A number of workers have recently reported similar increases in r_m under water stress conditions (Slatyer, 1973; Hansen, 1971; Redshaw and Meidner, 1972). Redshaw and Meidner (1972) concluded that the increases in r_m of water stressed tobacco plants were probably due to an increase in the rate of CO_2 evolution or an increase in the chemical resistance to CO_2 fixation. An increase in the respiration rate that increased the compensation

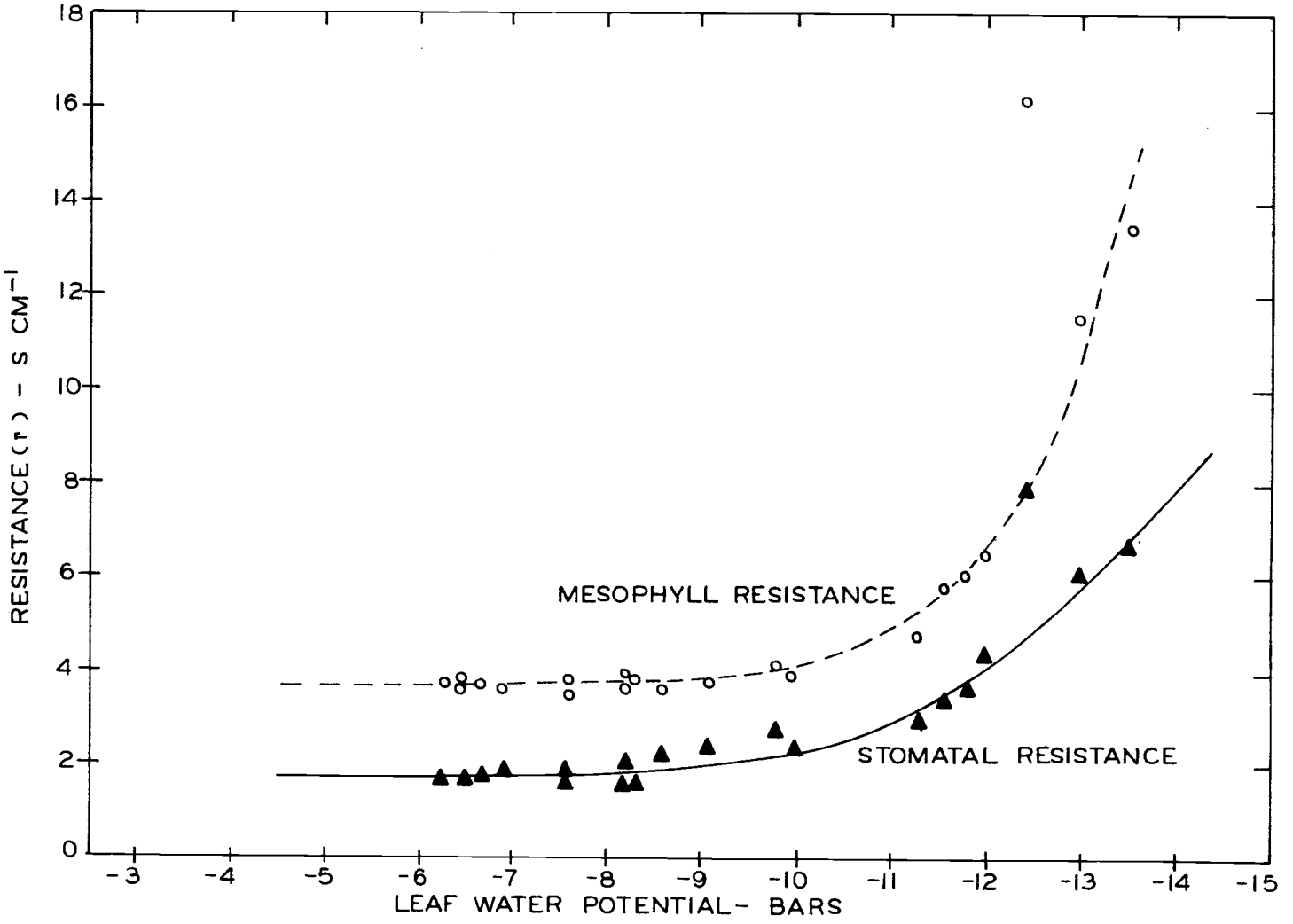

Figure 20. The stomatal and mesophyll resistances to carbon dioxide transfer of the 5th leaf of a 7 leaf corn plant, as a function of the water potential of that leaf.

point above zero, would have produced an artificial increase in r_m in this experiment as the assumption $[CO_2]_{chl} = 0$ would no longer be correct. However, Wesselius and Brouwer (1972) reported the respiration rate of water stressed corn to increase by less than 2%, and, therefore, it is unlikely that increased respiration rate was the cause of the increase in r_m in this experiment. Boyer (1971) and Wesselius and Brouwer (1972) demonstrated nonstomatal influences on photosynthesis, in water stressed sunflower and corn plants by using elevated CO_2 concentrations. Boyer and Bowen (1970) illustrated that the reduction of photosynthesis in sunflower leaves was paralleled by the loss of Hill reaction activity in vitro.

In summary it would appear that the nonstomatal factors influencing photosynthesis could be physical constrictions impeding the transfer of CO_2 to the fixation sites, a loss of photochemical activity, or biochemical inhibition of carboxylation reactions. All these mechanisms have been proposed by Neales and Incoll (1968) as possible consequences of photosynthate accumulations in the photosynthetic source leaf. Therefore the possible mechanisms of nonstomatal control of photosynthesis are at least consistent with the source-sink hypothesis under consideration. Although photosynthate levels were not measured in this experiment, the different sensitivity of leaf elongation and photosynthesis to water stress (Figure 19) would make the accumulation of photosynthate likely at moderate water stresses

(-8 to -11 bars). However, this experiment did not establish a cause and effect relationship.

The parallel responses of net photosynthesis and transpiration to increasing water stress, coupled with an increase in r_m, recorded in this study, are worthy of further comment because they illustrate, aptly, a point made by Hsiao (1973) in a recent review of water stress effects on growth. Although much of the evidence in favor of a dominant stomatal control of photosynthesis during water stress is based on studies showing a close parallel between photosynthesis and transpiration responses to water stress (Brix, 1962; Barrs, 1968; Boyer, 1970b; Hansen, 1971), this does not rule out the possibility of nonstomatal involvement particularly if the stomata are regulated by the CO_2 concentration inside the leaf.

Finally the rapid physiological responses of the young corn plants to water stress illustrated in this experiment may be indicative of plant response in the field to the diurnal cycle of water stress. The sensitivity of leaf elongation to high light intensity, soil temperature and ψ_c reported is in good agreement with earlier observations by Loomis (1934) that leaf elongation is reduced, frequently, by adverse environmental conditions in the field, and often may be restricted to dark periods. Grobbelaar (1963) in a study of the responses of young corn plants to root temperature found that leaf area, rather than net dry matter production per unit weight

(photosynthesis) was the main determinant of differences in relative growth rate. Leaf enlargement may be a very important factor limiting production in similar situations.

EFFECT OF REDUCED LEAF ELONGATION ON PHOTOSYNTHESIS

Introduction

Results of the experiments discussed above suggested that a moderate water stress (-9 to -12 bars) may cause an accumulation of photosynthate in the sink leaf and ultimately in the source leaf by decreasing the size of the growth sink within the rapidly expanding young leaf. It was proposed that this carbohydrate accumulation in the source leaf could be one of the nonstomatal factors causing the photosynthetic rate to decrease during mild water stress. Several other workers also have proposed that decreased leaf elongation (Wardlaw, 1969; Boyer, 1970a) or decreased translocation of photosynthate (Zolkevick, Drusakova and Lizandr, 1958; Hartt, 1963) could lead to accumulation of photosynthate in the source leaf thus regulating the photosynthetic rate. However none of these workers have directly measured photosynthesis in relation to photosynthate accumulation in the whole plant situation. It was pointed out above that the direct association of photosynthate accumulation with reductions in rate of photosynthesis is of prime importance in establishing the validity of the source-sink hypothesis where applied to plant reactions to water stress.

Experiments were designed seeking to establish a relationship

between sink size, photosynthate accumulation and photosynthetic rate by manipulating the rate of leaf elongation with the aid of changes in soil or apical meristem temperature. It was reasoned that if photosynthate accumulates in the plant in response to a decrease in the size of the growth sink, then the pattern of photosynthate accumulation in plants stressed by lowering the soil temperature should be different from that of plants stressed by lowering the temperature of the apical meristem. It was shown that lowering the soil temperature adversely affected the rate of elongation of the 7th leaf, by decreaseing the temperature of the apical meristem, as well as by decreasing the plant water potential. This decrease in ψ_c also would reduce the rate of cell enlargement of the 6th leaf, which is importing little photosynthate (Hofstra and Nelson, 1969) but is still expanding. Therefore if the growth sink in this leaf is reduced, photosynthate produced in this leaf may accumulate. Consequently lowering the soil temperature may result in the accumulation of photosynthate in the source leaf 5, the independent leaf 6 and the sink leaf 7.

In contrast, inhibiting leaf elongation by lowering the temperature of the apical meristem may result in the accumulation of photosynthate only in the source leaf 5 and the sink leaf 7. Photosynthate may not accumulate in the independent leaf 6 because this leaf is growing predominately by cell expansion using its own photosynthate (Sharman, 1942) and the low temperature of the meristem should not

affect its growth.

Methods and Materials

Corn plants in the 7 leaf growth stage were placed in the temperature controlled root chamber of the carbon assimilation system in an identical manner to that described in Experiment 2. An initial nonstress measurement of leaf elongation, net photosynthesis and ψ_c were taken at a soil temperature of 27.5 C. The soil temperature was then rapidly lowered to 15 C and net photosynthesis, leaf elongation and ψ_c were monitored for 6 hours at this soil temperature. At the end of the 6 hour period samples were taken from leaves 5, 6 and 7 and placed in a freezer at -15 C to be analyzed for soluble carbohydrate content. The entire leaf and sheath portions of leaves 6 and 7 were sampled, whereas only leaf chamber portion of leaf 5 was sampled. Control plants for carbohydrate analysis were grown at a soil temperature of 27.5 C for the same duration and under identical light intensities (381 w m^{-2}). Each experiment was replicated three times.

Apical meristem temperature was lowered by passing 6 C water through the lucite meristem collars described in Experiment 2. The experimental procedure was the same as described for the soil temperature experiment with the exception that control plants without meristem collars were run concurrently in this experiment.

Soluble carbohydrates were extracted and measured as described in Experiment 1. As the dry weight of the sampled leaves could not be determined, the dry weight of the 80 percent-ethanol-insoluble residue was determined. Soluble carbohydrate content then was reported as a percentage of the 80 percent-ethanol-insoluble residue.

Results and Discussion

Lowering the soil temperature to 15 C for 6 hours caused ψ_c to decrease rapidly to values between -8.5 and -9.5 bars. There was a tendency for ψ_c to continue to decrease slightly during the 6 hour stress period but these decreases never exceeded 1.0 bar and ψ_c did not fall below -10 bars in any treatment. The small decrease in the leaf elongation between 80 and 360 minutes (Figure 21), is probably a result of these small decreases in ψ_c. In Experiment 2 the net photosynthetic rate was more than 80 percent of the initial non-stress rate, when ψ_c was greater than -10 bars (Figure 19). Therefore it is unlikely that the 47 percent reduction in the rate of net photosynthesis after 360 minutes at a soil temperature of 15 C was due to a decrease in ψ_c per se (Figure 21).

The soluble carbohydrate levels of leaves 5, 6 and 7 were increased significantly by the 6 hour stress period at a soil temperature of 15 C (Table 4). Although the carbohydrate increases in leaves

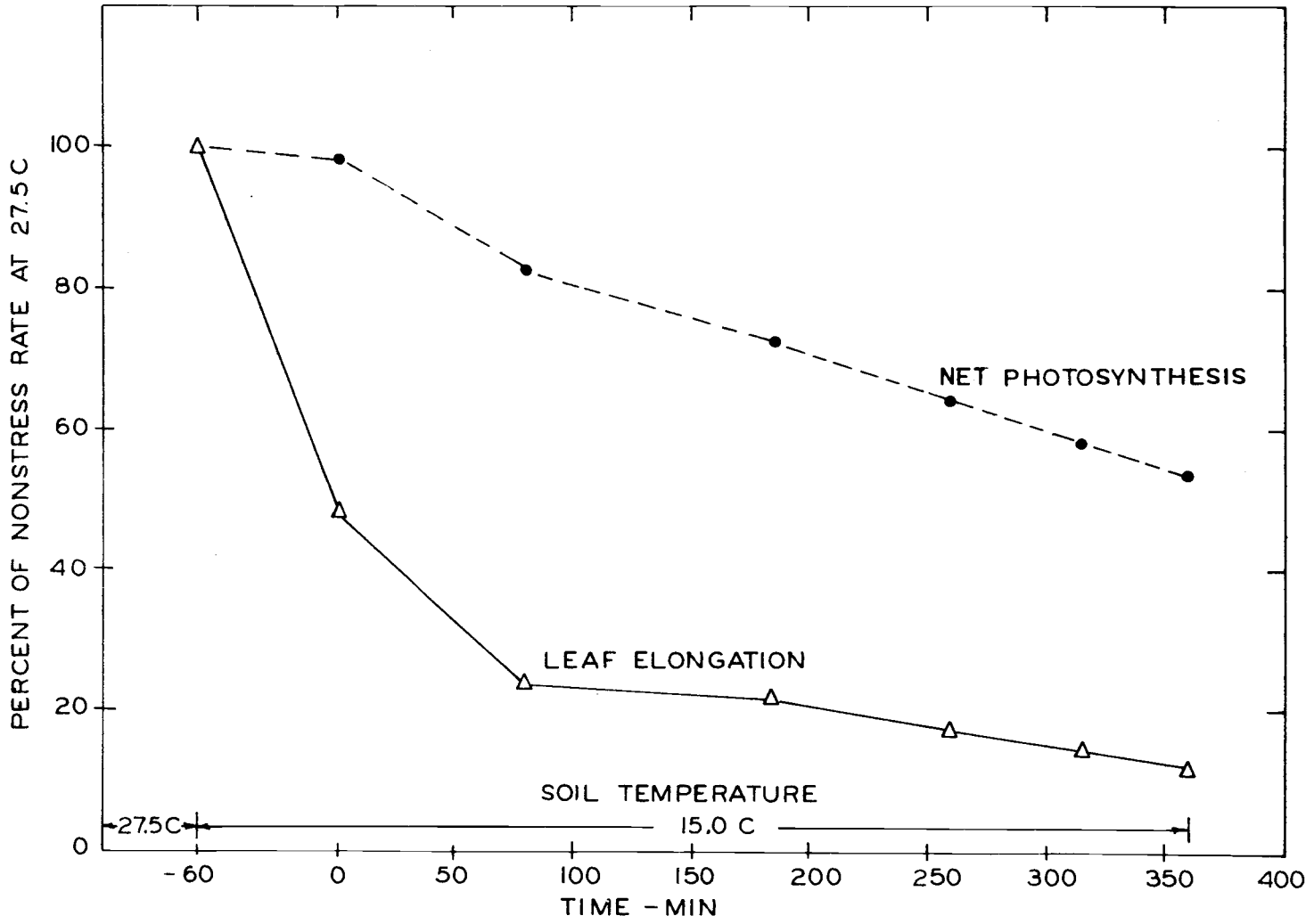

Figure 21. Effect of lowering the soil temperature from 27.5 C to 15 C on the rates of leaf elongation (leaf 7) and net photosynthesis (leaf 5) of a 7 leaf corn plant.

6 and 7 were 1 1/2 to 2 times that in leaf 5, this may not be significant because leaf 5 was more mature and only the blade portion within the leaf chamber was analyzed. These increases in soluble carbohydrates indicate that the 80-90 percent reduction in leaf elongation (leaf 7) caused by the soil temperature stress, effectively reduced the size of the leaf growth photosynthate sinks. Lowering ψ_c caused photosynthate accumulations of approximately the same magnitude in the sink leaf 7 and the independent leaf 6. This was predicted because of the general effect of decreased ψ_c on cell enlargement (Boyer, 1968; Green, 1968).

Table 4. Effect of lowering the soil temperature from 27.5 C to 15 C for 6 hours on the rates of leaf elongation (leaf 7), net photosynthesis (leaf 5) and the soluble carbohydrate content of the 7 leaf corn plants.

Parameter	Stress	Control	Significance	Difference
Soluble carbohydrates (% Res. wt.)			$LSD_{0.05}$	% of Control
Leaf 5	37.6	29.5	3.2	127.4
Leaf 6	43.3	29.4	7.2	147.3
Leaf 7	71.3	49.2	11.4	144.9
Photosynthesis ($mg\ CO_2\ dm^{-2}\ hr^{-1}$)	14.1	26.4	6.6	53.4
Leaf elongation ($\mu m\ min^{-1}$)	7.5	56.2	8.7	13.3

The decrease in the size of the photosynthate sink in leaf 7, and possibly other photosynthate sinks in the plant as well caused the soluble carbohydrate level of the source leaf 5 to increase by 27 percent. This increase was accompanied by a 47 percent decrease of the net photosynthetic rate of this leaf. The pattern of decrease in the photosynthetic rate was almost linear from 97 percent to 53 percent of the initial rate. In contrast, leaf elongation decreased rapidly to 25 percent of its pre-stress rate and declined very slowly for the remainder of the stress period (Figure 21). If photosynthesis is regarded as a measure of source activity and leaf elongation as a measure of sink size, it follows that the pattern illustrated in Figure 21 corroborates the present concept of source-sink regulation (Neales and Incoll, 1968). Namely, that a large decrease in sink size is followed by a gradual decline in source activity as photosynthate accumulates, first in the sink and then in the source.

When leaf elongation was decreased by lowering the temperature of the apical meristem region to 6 C the ψ_c remained relatively constant and did not fall below -7 bars throughout the experiment. The pattern of reaction of photosynthesis and leaf elongation to this treatment was similar to that produced by lowering the soil temperature (Figure 22). However there were some important differences.

The reduction in net photosynthetic rate of 24 percent caused by the decrease in the size of the photosynthetic sink was approximately

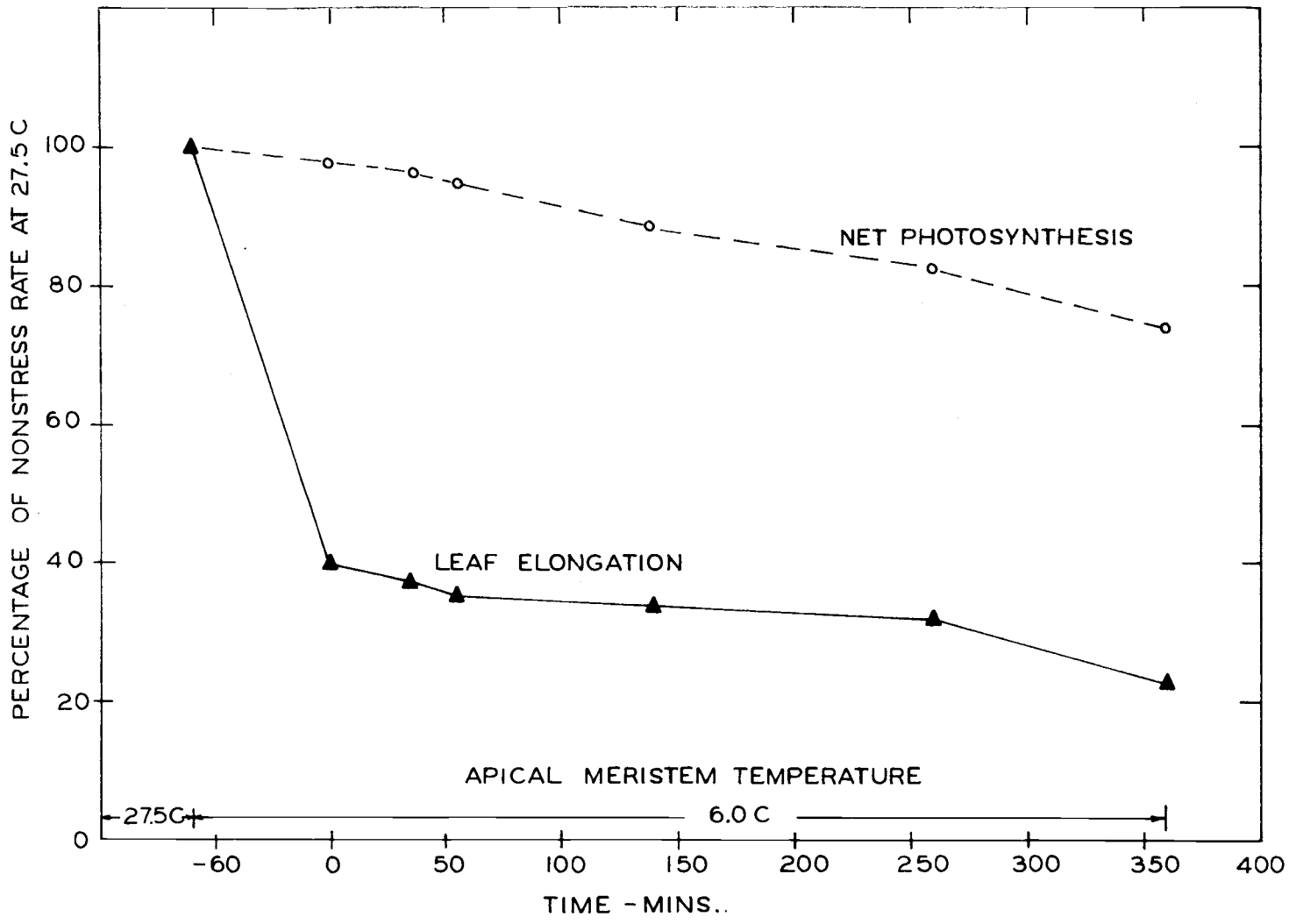

Figure 22. Effect of lowering the shoot apical meristem temperature from 27.5 C to 6.0 C on the rates of elongation (leaf 7) and net photosynthesis (leaf 5) of a 7 leaf corn plant.

one-half that caused by lowering the soil temperature to 15 C (Table 5). There are two possible explanations for this result. Firstly, the reduction in the rate of leaf elongation was smaller than that produced by lowering the soil temperature. Secondly, as predicted in the introduction, the meristem collar produced a more specific metabolic effect than the general water stress produced by the soil temperature treatment. This more specific effect would affect fewer photosynthate sinks within the plant. Although Hofstra and Nelson (1969) found leaf 7 to be the major photosynthate sink for leaf 5, the sum of all other sinks in the plant accounted for more of the photosynthate produced in leaf 5 than did leaf 7. The lack of a significant increase in the soluble carbohydrate level in leaf 6 is further evidence to support this point (Table 5).

Table 5. Effect of lowering the shoot apical meristem temperature from 27.5 C to 6 C for 6 hours on the rates of leaf elongation (leaf 7), net photosynthesis (leaf 5) and the soluble carbohydrate content of 7 leaf corn plants.

Parameter	Stress	Control	Significance	Difference
Soluble carbohydrates (% Res. wt.)			$LSD_{0.05}$	% of Control
Leaf 5	38.2	31.1	4.2	122.8
Leaf 6	30.3	31.4	n.s.	96.5
Leaf 7	73.6	52.7	7.1	139.7
Photosynthesis ($mg\ CO_2\ dm^{-2} hr^{-1}$)	19.2	25.2	1.1	76.2
Leaf elongation ($\mu m\ min^{-1}$)	10.3	48.3	8.5	21.3

The significant increase in the soluble carbohydrate levels of leaves 5 and 7, but not leaf 6, when leaf elongation is decreased by lowering the temperature of the apical meristem, is consistent with the hypothesis put forward in the introduction of this chapter. Therefore the photosynthate accumulations measured in this experiment are a result of a decrease in the size of the photosynthate sinks. More specifically, the large reductions in the rate of leaf elongation that occur under mild water stress conditions can lead to photosynthate accumulations in the source leaf and a subsequent reduction in photosynthetic rate. This indirect effect of water stress on net photosynthesis had not been previously demonstrated.

METABOLIC CHANGES ACCOMPANYING A PHYSICAL INHIBITION OF LEAF ELONGATION

Introduction

The preceding three experiments examined the effects of reduced leaf elongation on net assimilation rate, photosynthetic rate and the soluble carbohydrate content of the leaves but did not examine the effects of reduced cell expansion on the metabolism of the elongating cell. In these experiments it has been assumed that the inhibition of cell expansion by physical means will result in a slowing down of the biosynthetic reactions, which would lead to a decrease in the size of the photosynthate sink of that cell. This assumption, which is central to the functioning of the source-sink mechanism, was shown to be correct by results obtained in Experiment 3. However, the intriguing question of how the reduction in cell turgor, a physical force, is transduced into alterations in cell metabolism, remains.

Hsiao (1973) recently reviewed the mechanisms that may underly plant response to mild water stress sufficient to stop cell expansion. He concluded that mild stress does not damage directly, the biochemical components or organelles by reducing water activity, concentrating solutes in the cell, interfering with compartmentalization, or affecting macromolecular structure. The results of Experiments 2 and 3 also indicate that mild water stress had little effect on net

photosynthesis in the short term. However, it does seem unlikely that protein synthesis, cell wall synthesis, membrane assembly and other biosynthetic reactions would continue unabated when cell expansion is stopped for any length of time.

There is evidence for some overrun of these metabolic processes. When cell expansion is restricted, then released, the post-stress rate of cell enlargement is frequently higher than the pre-stress rate (Green, 1968; Acevedo et al., 1971; Barlow and Boersma, 1972). This would indicate that some accumulation of growth potential occurs when subthreshold turgor pressures limit cell expansion. However, these responses are of a short term nature and do not compensate completely for the growth lost during the stress period (Acevedo et al., 1971; Barlow and Boersma, 1972). The soluble carbohydrate accumulations in the elongating leaf, reported in Experiment 3, indicate that the rate of photosynthate usage in respiration and biosynthesis decreased when the rate of cell expansion was reduced. Furthermore, if the plant is to maintain a favorable balance of metabolites, some control mechanism(s) must modulate the biosynthesis of cell walls and protoplasm when low turgor pressures limit cell expansion.

One metabolic process that is susceptible to mild stress is protein synthesis. Hsiao (1970) has shown that mild water stresses as high as -5 bars can cause a shift in the ribosomal profiles of corn

seedlings, from polysomes to monosomes. Morilla, Boyer and Hageman (1973) also have used corn seedlings to illustrate that water stresses as high as -3 bars can reduce nitrate reductase synthesis and that this reduction is associated with a decrease in the polyribosomal content. Although considerable protein synthesis occurs during the expansion of plant cells, proteins are not the major component of plant cells as they are in animal and microbial cells (Lockhart, 1965). Consequently it is likely that these reductions in protein synthesis are the result not the cause of cellular adjustment (Hsiao, 1970). The incorporation of glucose into plant cell walls is also sensitive to small changes in ψ_c and turgor pressure (Ordin, 1960). Therefore the process of cellular adjustment may involve a change in some prerequisite common to all biosynthetic pathways. Chemical energy supply is one such prerequisite.

The availability of chemical energy in the form of high energy adenosine phosphates, has been shown by Chapman, Fall and Atkinson (1971) to regulate the growth of Escherichia coli. To quantify the amount of chemical energy available to the cell, Atkinson (1969) introduced the concept of adenylate energy charge, which is defined as

$$\text{Adenylate energy charge (EC)} = \frac{[ATP] + \frac{1}{2}[ADP]}{[ATP]+[ADP]+[AMP]}$$

[ATP], [ADP] and [AMP] are the concentrations of adenosine 5' tri, di,

and mono phosphates, respectively, in the cell. In studies of the regulation of cell wall polysaccharide synthesis, Ray (1973) has found that the hormonal activation of ADP glucose: β-1, 4-glucose glucosyltransferase (β-glucan synthetase), is dependent upon energy metabolism and presumably ATP. Ray (1973) also concluded that sugars were a necessary condition for activation of β-glucan synthetase, but they do not regulate enzyme activity. With respect to the species of nucleotide required in cell wall synthesis, Bromsel and Pradet (1968) have shown that changes in the ATP level of wheat leaves as followed closely by similar changes in the level of UTP and GTP. The requirements of energy in cell wall and protein synthesis are well established (Albersheim, 1965; Mahler and Cordes, 1971).

It follows that the availability of chemical energy within an elongating leaf cell may exert an important regulatory influence on the level of biosynthetic activity within the cell. This proposal was examined in this experiment by determining the effect of decreasing ψ_c on the rate of leaf elongation, adenylate energy charge and free amino acid content of the youngest unrolled leaf of a 6 leaf corn plant. If the availability of chemical energy is a modulator of biosynthesis within the cell, the ATP content or energy charge should be related to the rate of cell elongation.

Although this approach has not been applied to elongating leaf tissue before, the availability of chemical energy has been used

previously to describe the effects of genetic and environmental factors on plant growth. Ching (1973) has reported that the seedling vigor of several diverse species is highly correlated to the ATP content of the imbibed seeds. The ATP level decreases upon chilling cotton seedlings (Stewart and Guinn, 1969), or exposing pea roots to saline media (Hasson-Porath and Paljakoff-Mayber, 1971). The adenylate energy charge of Sycamore cells growing in cell culture, was higher when the cells were in the lag or stationary phases than when they were in the exponential growth stage (Brown and Short, 1969).

Methods and Materials

Experimental Procedure

The experiment was conducted in 2 parts. Part A consisted of a preliminary experiment to evaluate sampling techniques and a series of stop-go experiments in which the adenylate energy charge of elongating leaves was compared with that of water stress leaves that were not elongating.

Corn plants in the 6 leaf stage were prepared exactly as described in Experiment 2, and placed in a temperature controlled osmotic chamber beneath the xenon lamp, the day before experimentation began. The environmental conditions in the growth room were: air temperature 27.5 C; relative humidity 55%; light intensity

381 w m^{-2}, and remained the same for all Part A experiments.

In all four Part A experiments 2 frames each containing 2 corn plants were placed in the osmotic chamber and allowed to grow at a soil temperature of 27.5 C. On the day of experimentation LVDT's were attached to the youngest unrolled leaf of 1 plant in each frame. After a steady rate of elongation was obtained, these plants were sampled for adenylate energy charge determinations. The LVDT's then were attached to the remaining plant in each frame while the soil temperature was lowered to 10 C. When leaf elongation stopped the remaining plants were sampled for ATP and EC analysis.

In preliminary Experiment A1, leaf sampling position was evaluated. The 20-30 cm leaf (leaf 6) was divided into 3 equal lengths; one corresponding to the basal portion where cell division was dominant; a mid-section that corresponded to the elongating region (Sharman, 1942) and the protruding green tip section that was still elongating but at a much slower rate than the mid-section. The basal meristematic section was not sampled as Ching and Ching (1972) have found the EC of dividing tissues to be low and complicated with nuclear compartmentation. Both the mid-section and green tip sections were sampled for ATP and EC determinations.

In the remaining three, Part A experiments only the mid-section was sampled for ATP and EC determinations. The green tip section was used to measure leaf water potential and cell osmotic potential.

In Part B of this experiment 24 frames, each containing 2 corn plants, were placed in the temperature controlled osmotic chamber described in Experiment 1. The environmental conditions remained the same as in the preliminary experiments, except that the light intensity provided by fluorescent and incandescent lights was 2000 ft. c. at plant height. Half the plants were subjected to a soil water potential of -5.00 bars. After an overnight equilibration in the chamber, the plants were grown under continuous light for 48 hours. Four plants were sampled from each treatment for water potential, osmotic potential and ATP measurements at 0, 3, 6, 12, 24, 48 hours from the start of the light period.

Methods of Analysis

Leaf Water Potential. The ψ_c of the green tip section of the youngest unrolled leaf was measured with the Pressure-Bomb apparatus of Scholander et al. (1965).

Cell Osmotic Potential (ψ_π). The green tip section used for the measurement of ψ_c in the pressure bomb, was removed from this instrument and sealed inside a 5 cm length of 0.9 cm O. D. tygon tubing, with 2 rubber stoppers. The sample tube containing the leaf was placed in dry ice for at least 6 hours, after which it was stored at -10 C until analysis. Cell sap was extracted by allowing the sample to thaw, then placing the tygon tube in the jaws of a small vice. The

osmotic potential of the cell sap was measured by placing a small piece of filter paper, moistened with the expressed sap, in a Wescor C-51 sample changer for a psychrometric measurement of water potential.

Pressure Potential (ψ_p). Turgor pressure was calculated from the relationship $\psi_p = \psi_c - \psi_\pi$. In this determination the matrix potential of the cell wall was neglected (Boyer, 1967).

Leaf Elongation Rate. Leaf elongation rate was measured with an LVDT or by successive measurements of leaf length over time.

Determination of Adenosine Phosphates. Plant samples were taken by cutting the corn stalks just above the apical meristem. The youngest unrolled leaf (leaf 6) was separated quickly from the rest of the plant and the top third of this leaf, the green tip section, was excised for the water potential measurement. The remainder was placed immediately in liquid nitrogen. The time from plant excision to liquid nitrogen was about 15 to 20 seconds. After withdrawing the leaf from the liquid nitrogen, 7-9 cm of the mid-section was cut into 1 cm segments and placed into a mortar, prechilled to -20 C. The mortar was then returned to a polystyrene cooler containing dry ice. Small pieces of dry ice were placed with the leaf sample in the mortar.

The samples were extracted in a cold room at 4 C. Each sample was ground to a fine powder while still frozen and then extracted three times with 3 ml of 0.25M perchloric acid. After the

final centrifugation, the supernatants were combined, and neutralized with 2.5 N potassium hydroxide. The plant residue which was insoluble in 0.25 M perchloric acid was dried at 60 C for 48 hours and its dry weight noted.

The neutralized extract was treated with acid washed polyclar-AT to remove polyphenols and after centrifugation diluted 10 x with a buffer containing 0.025 M N-2-hydroxyethylpiperazine-N'-2-ethane-sulfonic acid (HEPES) and 0.025 M magnesium acetate at pH 7.5.

The ATP, ADP and AMP content of the extract was determined by the luciferin-luciferase method using an Aminco Chem-Glow photometer described by Ching and Ching (1972). In this method ADP and AMP are converted to ATP by incubating the extract with pyruvate kinase, phosphoenol pyruvate (PEP) and with adenylate kinase pyruvate kinase and PEP respectively. Since the perchlorate ion inhibits the luciferin-luciferase assay by quenching, an internal ATP standard was used for each assay. The recovery of this internal standard was 75 ± 3 percent in all determinations indicating a uniform quenching by perchlorate of 25 percent. The recoveries of known ATP, ADP and AMP through the complete procedure were 92, 88, and 83 percent respectively indicating the high efficiency of the assay.

Free Amino Acids. The free amino acid content of the neutralized perchloric acid extract was determined by the ninhydrin method of Moore and Stein (1954). L-Leucine was used as a standard.

Results and Discussion

The adenylate energy charge of the rapidly expanding leaf mid-section was more sensitive to changes in the rate of leaf elongation than the green tip of the leaf (Table 6), although water stress decreases the adenylate energy charge value of both sections. The mid-section of this youngest unrolled leaf is encased by the leaf sheath, receives little light and has few mature chloroplasts. Consequently the expanding cells of the mid-section must rely on translocated photosynthate, rather than photosynthesis within the cell, as a source of energy and substrates for biosynthesis. The cells of the mid-section are expanding rapidly, energetically less compartmentalized and respond rapidly to changes in plant water stress (Table 6). This section of the elongating leaf was therefore used for ATP and adenylate energy charge measurements throughout this experiment. The sampling procedure also enabled the tip section to be used for the measurement of leaf water potential and its components.

Table 6. Adenylate energy charge in two sections of the elongating corn leaf under water stressed and control conditions.

Treatment	Adenylate Energy Charge		Leaf Elongation Rate
	Leaf Tip	Leaf Mid-Section	
			$\mu m\ min^{-1}$
Control	0.44 ± 0.05	0.62 ± 0.08	71 ± 7
Stress	0.41 ± 0.04	0.41 ± 0.04	0

The trend for both leaf elongation and adenylate energy charge to decrease as water stress increases was examined more closely in the stop-go experiments of Part A where ψ_c was measured (Table 7). Lowering the soil temperature caused a decrease in ψ_c of almost three (3) bars which resulted in a cessation of leaf elongation and a significant decrease in the adenylate energy charge. As illustrated in Experiment 2 some of the decrease in leaf elongation could have been due to a lowering of apical meristem temperature. However the significant decrease in adenylate energy charge of the mid-section of the leaf could not have been due to a lowering of the stem temperature in this region, because Watts (1972b) illustrated that soil temperature influences do not extend more than 10 cm above the soil surface.

Table 7. Adenylate energy charge, leaf water potential and leaf elongation rate, of the 6th leaf of young corn plants under water stressed and control conditions.

	Leaf Water Potential		Leaf Elongation Rate		Adenylate Energy Charge	
	Control	Stress	Control	Stress	Control	Stress
	---- bars ----		-- $\mu m\ min^{-1}$ --			
Experiment A2	-7.1	-11.4	49.6	0	0.83	0.48
Experiment A3	-7.9	-9.3	43.9	0	0.71	0.57
Experiment A4	-6.0	-8.9	60.0	0	0.78	0.68
Mean	-7.0	-9.9	51.2	0	0.77	0.58
$LSD_{0.05}$	2.6		---		0.18	

The mean ψ_c of the stress plants was -9.9 bars. It is therefore unlikely that photosynthesis and transpiration were affected grossly (Figure 19). The sensitivity of adenylate energy charge to changes in water stress appears to be comparable to that of leaf elongation. The parallel reactions of leaf elongation and adenylate energy charge to mild water stress in these stop-go type of experiments indicates that these physiological parameters may be acting in concert.

In order to examine the relationship of adenylate energy charge and leaf elongation over a wider range of ψ_c values, with greater replication a second experiment (Experiment B) was conducted using the large osmotic root chamber described in Experiment 1. The large number of plants required for this experiment necessitated the use of a lower intensity light source (2000 ft. c.).

The soil water potential treatments of -0.2 and -5.0 bars used in this experiment resulted in ψ_c values varying from -2.6 to -17.9 bars (Table 8). The corn plants were wilting noticeably when ψ_c was -17.9 bars. The 4 bar decline in ψ_c over the 48 hour experimental period in the control treatment probably was due to the time required for the soil to come into equilibrium with the surrounding PEG solution, as most of the adjustment occurred in the first 24 hours. The plants were not given the usual 2 day equilibration period in the PEG solutions (Zur, 1967) in this experiment because a range of ψ_c

Table 8. Elongation rate, water, osmotic and pressure potentials of the 6th leaf of corn plants grown at soil water potentials of -0.2 and -5.0 bars for 48 hours.

Stress Period	Water Potential	Osmotic Potential	Turgor Pressure	Elongation Rate
hrs	---------- bars ----------			$\mu m\ min^{-1}$
Soil water potential: -0.2 bars				
0	-2.6*	-6.6*	3.9*	63.7*
3	-3.7	-7.5	3.7	62.6
6	-3.6	-7.3	3.7	61.1
12	-4.9	-7.9	3.1	56.3
24	-5.6	-8.6	3.0	60.9
48	-6.7	-10.3	3.5	63.8
Soil water potential: -5.0 bars				
0	-2.7	-6.6	3.9	55.3
3	-8.1	-8.5	0.4	6.4
6	-11.7	-11.9	0.3	2.4
12	-12.5	-12.4	-0.1	0.0
24	-16.4	-16.5	0.1	0.0
48	-17.9	-17.8	-0.1	0.0

*Mean of 4 replications.

values was desired. Despite the 4 bar decrease in ψ_c in the control treatment the leaf elongation rate of the plants remained constant through the 48 hour experiment (Table 8). The turgor pressure of the elongating leaves in the control treatment remained relatively constant throughout the experiment, because the osmotic potential decreased in concert with ψ_c (Table 8). In this experiment the steady rate of leaf elongation maintained at decreasing ψ_c, was due to osmotic adjustment as proposed by Meyer and Boyer (1972) and not adjustments in threshold turgor, or the gross extensibility (minimum yield stress) of the cell wall as reported by Green (1968), and Green, Erickson, and Buggy (1971). The functional relationship between leaf elongation and turgor pressure is illustrated in Figure 23 which is a composite of data from Parts A and B of this experiment.

The curvilinear nature of the relationship presented in Figure 23 indicates that growth is not a linear function of turgor pressure. The equation Green (1968) used to describe growth of Nitella may be applicable to higher plants if the gross extensibility of the cell wall E_g varies with turgor.

$$\text{Growth rate} = \{E_g(\psi_p)\}\{\psi_p - \psi_{pth}\} \qquad (11)$$

where ψ_p and ψ_{pth} are the turgor and threshold turgor pressures respectively.

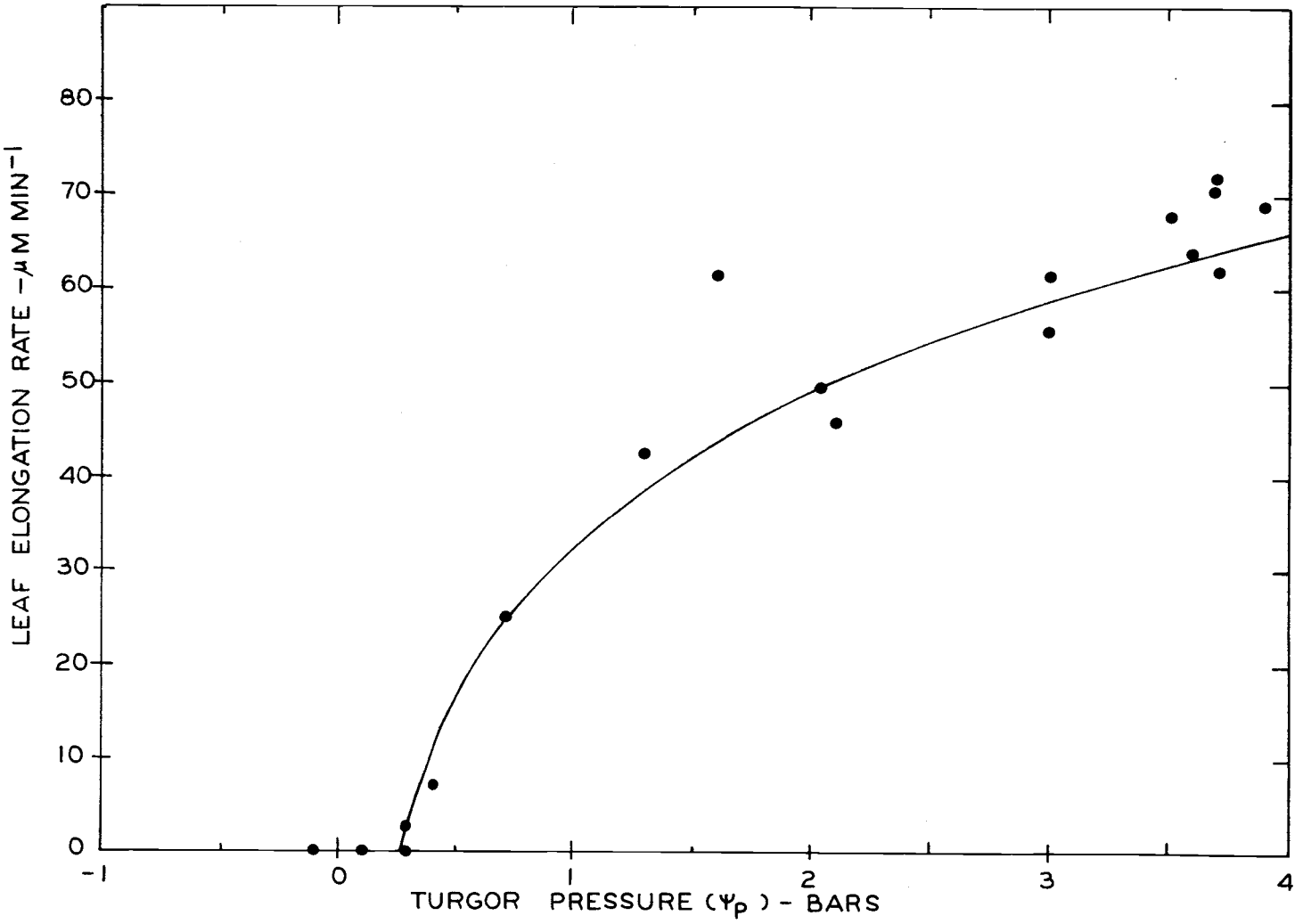

Figure 23. The rate of elongation of the 6th leaf of young corn plants as a function of the cell turgor pressure in that leaf.

The threshold turgor of approximately 0.3 bars (Figure 23) established for corn in this experiment is in agreement with the concept of a minimal turgor requirement for cell growth (Lockhart, 1965; Green, 1968; Hsiao, 1973). The turgor pressures illustrated in Figure 23 are very similar to those reported by Watts (1972a) for corn plants of the same age. However, they are much lower than the turgor pressures of 6 to 7 bars reported for Avena coleoptiles and sunflower by Cleland (1967) and Boyer (1968) respectively. It is not clear whether these differences are due to species characteristics or the method of measurement.

Both adenylate energy charge and ATP content of the elongating leaf were reduced by water stress (Table 9), but ATP content appeared to be more sensitive to changes in ψ_c than was adenylate energy charge. The ATP content of the water stressed plants was significantly lower than that of the nonstressed plants at all sampling times, whereas the adenylate energy charge of the stressed plants was significantly lower only in the 24, and 48 hours samples. It is possible that the differing sensitivity of these parameters to decreasing water stress is due to the method of the measurement. Although the value of the adenylate energy charge theoretically varies between 0 and 1 (Atkinson, 1969), the range of values obtained in this experiment was 0.40 to 0.83, which is in agreement with reported values of adenylate energy charge in plants (Chapman, Fall and Atkinson, 1971).

Furthermore the computation of adenylate energy charge requires the separate determination of ATP, ADP and AMP, and each estimate of adenylate energy charge includes the errors inherent in the determination of each component. The lack of sensitivity of adenylate energy charge to water stress in this experiment may have been due to practical difficulties of measuring ATP, ADP and AMP concentrations with sufficient precision to obtain statistical differences within a very narrow range of adenylate energy charge values.

Table 9. Adenylate energy charge and ATP content of the 6th leaf of corn plants grown at soil water potentials of -0.2 and -5.0 bars for 48 hours.

Stress Period	Aendylate Energy Charge			ATP		
	-0.2 bars	-5.0 bars	$LSD_{0.05}$	-0.2 bars	-5.0 bars	$LSD_{0.05}$
hrs				---- μ mole g^{-1} residue ----		
0	0.80*	0.80*	n.s.	1.05*	1.05*	n.s.
3	0.76	0.73	n.s.	1.65	0.99	0.33
6	0.73	0.65	n.s.	0.98	0.50	0.45
12	0.73	0.67	n.s.	1.29	0.52	0.63
24	0.78	0.60	0.15	0.78	0.34	0.23
48	0.74	0.52	0.17	0.85	0.12	0.21

*Mean of 4 replications.

Adenylate energy charge decreased linearly with decreasing ψ_c (Figure 24). There was no significant change in the adenylate energy charge values corresponding to the cessation of leaf elongation at ψ_c values of -8 to -10 bars. The decrease in adenylate energy charge as ψ_c decreases is consistent with the general observation that plant

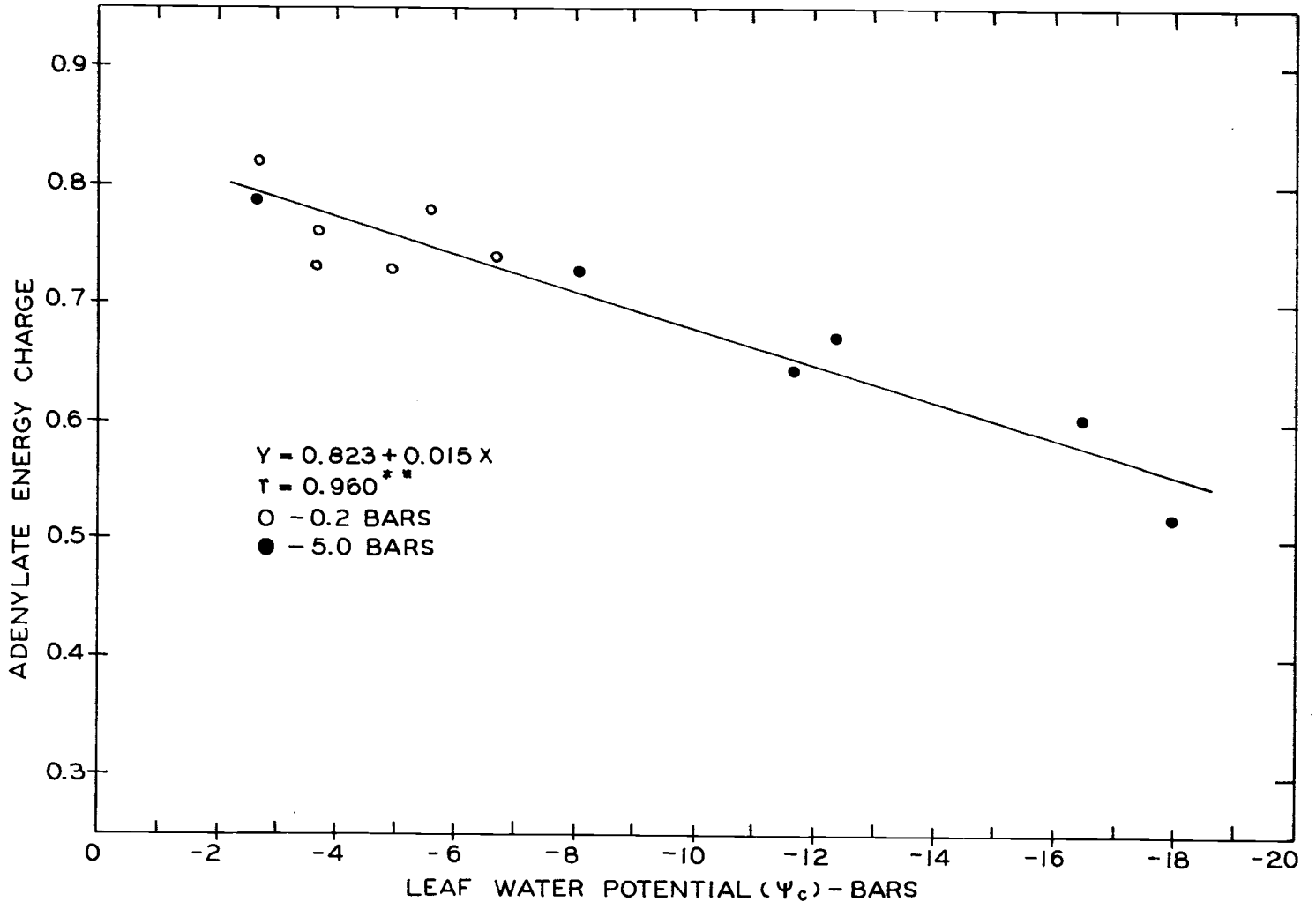

Figure 24. Adenylate energy charge of the mid-section of the 6th leaf of young corn plants as a function of the water potential of that leaf.

respiration decreases during water stress (Hsiao, 1973). Furthermore Hasson-Porath and Paljakoff-Mayber (1971) have shown the adenylate energy charge of pea roots to decrease from 0.83 to 0.73 upon exposure to saline conditions. Adenylate energy charge values for corn have not been reported previously but the values presented in Table 9 are in good agreement with adenylate energy charge values reported for a number of other species (Chapman, Fall and Atkinson, 1971).

The marked reductions in leaf elongation of the stressed plants (Table 8) were paralleled by significant decreases in the ATP content of the elongating leaf (Table 9), and a concurrent increase in the free amino acid content (Table 10). Increases in the free amino acid content of the stressed plants were significant only in the 12, 24, 48 hour samples. Increases in the free amino acid pool of water stressed plants have been shown to be primarily due to decreased protein synthesis rather than increased protein hydrolysis (Barnett and Naylor, 1966; Nir, Poljakoff-Mayber and Klein, 1970; Hsiao, 1973). The level of free amino acids in water stressed tissue is thus an indication of the rate of protein synthesis and the influx of transported metabolites. The increases in the free amino acid level of the elongating leaf in the 12, 24 and 48 hour samples of the stressed plants probably indicates a decrease in the rate of protein synthesis.

Table 10. The free amino acid content of the 6th leaf of corn plants grown at soil water potentials of -0.2 and -5.0 bars for 48 hours.

Stress Period	Free Amino Acid Content		
	-0.2 bars	-5.0 bars	$LSD_{0.05}$
hours	---------- mg g^{-1} residue ----------		
0	4.68*	4.68*	n.s.
3	4.41	5.22	n.s.
6	4.31	5.22	n.s.
12	4.83	8.10	2.83
24	4.38	9.61	3.02
48	3.98	10.41	4.24

*Mean of 4 replications

The inverse relationship of ATP and free amino acid content at decreasing ψ_c values is demonstrated in Figure 25. Although the ATP content is quite variable, it appears to be slightly more sensitive to decreasing ψ_c than is protein synthesis as measured by increases in the level of free amino acids. When the ATP content of the leaf drops below 0.8 micro moles per gram residue protein synthesis is affected and amino acids begin to accumulate.

In summary, the level of available chemical energy within the cells of the elongating region of a young corn leaf, as measured by ATP content, appears to be as sensitive to decreases in ψ_c as leaf elongation. The decrease in ATP content of the cells with decreasing ψ_c probably indicates that the decreases in protein and cell wall synthesis that follow decreases in cell turgor (Hsiao, 1970; Ordin,

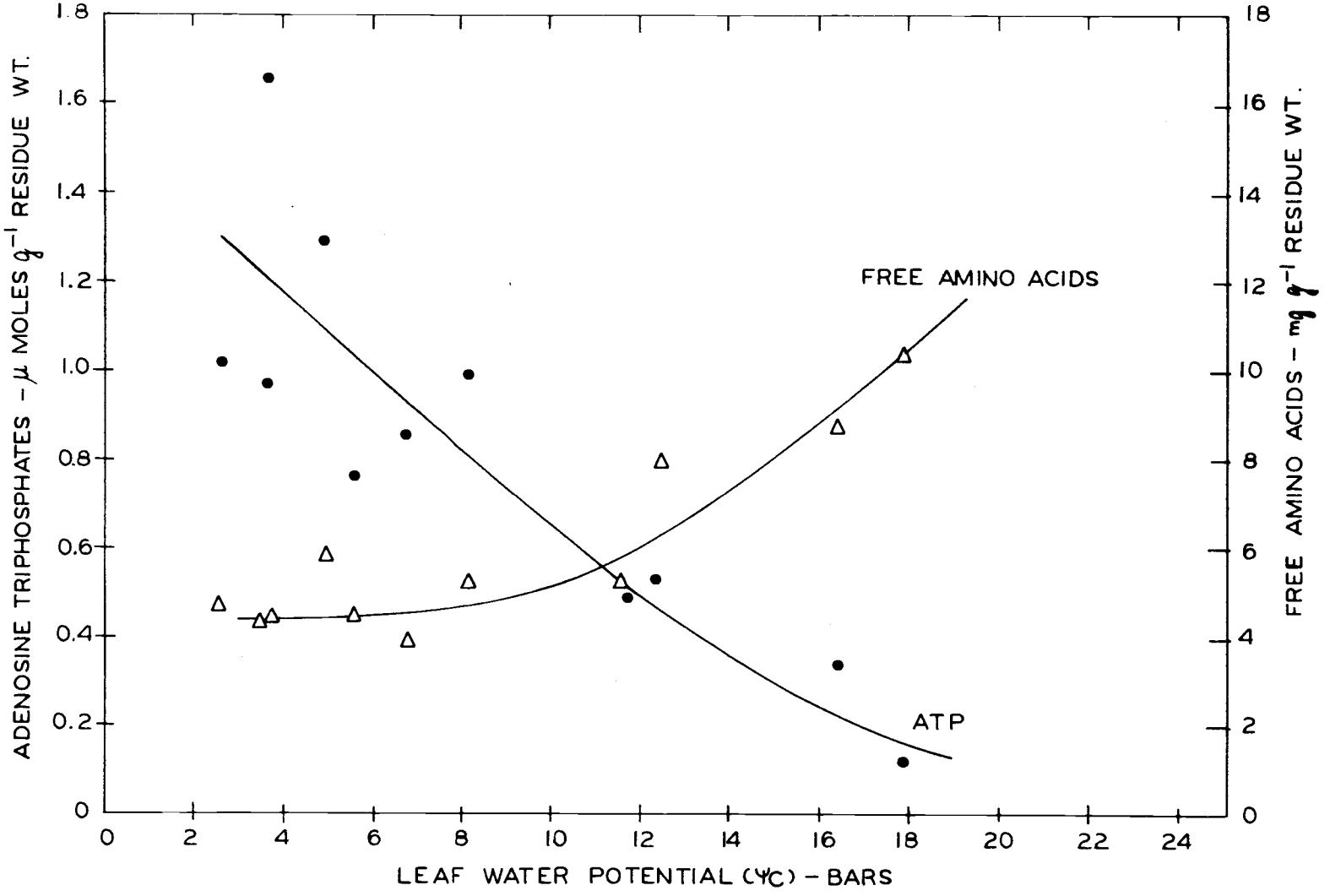

Figure 25. The effect of leaf water potential on the ATP content and free amino acid content of the mid-section of the 6th leaf of young corn plants.

1960) are not part of a simple feedback mechanism activated by a prior reduction in the rate of cell enlargement. Such a feedback mechanism would have resulted in an increase in the ATP content. The decreases in ATP content prior to the accumulation of amino acids, is also not consistent with the existence of a feedback mechanism. Therefore, it is likely that the decreases in ATP content with increasing water stress were due to a direct effect of decreased turgor pressure and ψ_c on the synthesis of ATP.

Oxidative phosphorylation is probably the major source of ATP in this enclosed mid-section of the elongating leaf which contains few chloroplasts. Respiration of corn seedlings is sensitive to water stresses as low as -5 bars (Boyer, 1970a; Koeppe, Miller and Bell, 1973). Koeppe et al. (1973) found these reductions in whole plant respiration to be paralleled by a decreased ability of mitochondria to oxidize substrates in vitro and concluded that, water stress may affect respiration by altering membranes.

This reduction in ATP content may also be due to the dehydration of some key respiratory enzymes, as Darbyshire and Steer (1973) have shown that ketose-1-phosphate aldolase to lose all activity when subjected to mild water stress (-1 bar). This loss of activity was due to fragmentation of the macromolecule into very small particles. In the same study the activities of indoleacetic acid oxidase and ribonuclease were unaffected by water stresses as high as -20 bars.

Therefore it is possible that the ATP content of an elongating cell, may play an important role in the modulation of biosynthetic reactions within the cell under water stressed conditions. The elucidation of the mechanism, whereby mild water stress decreases the ATP content of the cell requires further investigations at the organelle and molecular level.

SUMMARY AND CONCLUSIONS

The rate of elongation of the youngest unrolled leaf of 2-3 week old corn plants is very sensitive to changes in the root environment. This leaf ceased to elongate when the plant water potential was reduced from -9 to -9.5 bars by lowering the soil temperature or soil water potential. The cessation in leaf elongation was due to a decrease of cell turgor pressure below a threshold turgor of 0.3 bars. Low soil temperatures decreased leaf elongation by restricting water uptake by the plant and decreasing the activity of the apical meristem which is situated close to the soil surface in plants of this age. Soil temperature below 12 C severely decreased the leaf water potential of the corn variety Pride 5 used in this experiment.

The turgor-mediated decrease in the cell elongation of water stressed plants was paralleled by a decrease in the ATP content of the elongating cells. The adenylate energy charge of the elongating cells also decreased slowly with decreasing leaf water potential, but was not as sensitive as ATP content to decreases in the leaf water potential. An increase in the free amino acid content closely followed the decrease in leaf elongation and ATP content. It was concluded that the availability of chemical energy may modulate protein synthesis in cells of the elongating region, under conditions of water stress.

Photosynthesis and transpiration were not as sensitive to decreases in the leaf water potential, as were leaf elongation and ATP content. Photosynthesis and transpiration were reduced less than 20% by leaf water potentials of -11 to -11.5 bars. At leaf water potentials of -12 to -13 bars the rate of photosynthesis and transpiration decreased sharply to 25 and 40% of their respective non-stress rates at -6 bars, indicating a degree of stomatal closure.

An analysis of the CO_2 diffusive transfer resistances showed that the sharp decrease of photosynthesis in the -12 to -13 bar region was due to increases in both the stomatal and mesophyll resistances. The stomatal resistance increased from 1.75 s cm^{-1} at -6 bars to 6 s cm^{-1} at -13 bars. The mesophyll resistance increased from 3.7 s cm^{-1} at -6 bars,to 11.6 s cm^{-1} at -13 bars. It was concluded that the sharp decrease in photosynthesis in the -12 to -13 bar region was due to stomatal and nonstomatal influences of approximately equal magnitude.

It was shown that mild water stress, sufficient to considerably reduce leaf elongation, may significantly reduce photosynthesis by causing photosynthate to accumulate in both the source and sink leaves. Artificial inhibition of leaf elongation by lowering the temperature of the apical meristem was shown to cause a similar decrease in the rate of photosynthesis over a 6 hour period. These results were interpreted as evidence for the existence of a source-sink feedback

mechanism controlling photosynthesis in mildly water stressed corn plants. The biochemical basis of this hypothesis was not investigated.

The work indicates that too little attention has been paid to cell expansion in previous studies of water stress. This is particularly true for plants in the vegetative phase of ontogeny because the growth of a vegetative plant is dependent not only on the rate of CO_2 fixation, but also on the rate of increase in photosynthetic surface area. This point is illustrated by recent results obtained by Slatyer (1971), who compared the photosynthesis and growth of a C_3 and a C_4 species of Atriplex over a period of 23 days. Slatyer found that although the rate of leaf photosynthesis of the C_4 species always was equal to, or greater than, that of the C_3 species, overall plant growth rates were controlled primarily by leaf area. Similarly Fischer (1973) states that the major effect of water stress on wheat at the stage of ear emergence is not on photosynthetic capacity, but on the number of grains formed in each spikelet or the sink capacity.

It is possible that leaf enlargement may be a very important determinant of plant growth and production under conditions of mild to moderate stress in the field. The typical diurnal pattern of stress that occurs in the field would affect leaf enlargement to a much greater extent than photosynthesis. Finally, the ability of a plant to maintain turgor, and continue cell elongation by osmotic adjustment may be an important feature of drought tolerance, and further studies of this mechanism in the laboratory and field are warranted.

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APPENDICES

APPENDIX I

Outline of the Theory and Methodology Involved in the Computation of the Diffusive Resistances to Carbon Dioxide Flow During Photosynthesis

The ease with which CO_2 moves into a leaf in the process of photosynthesis and the ease with which water vapor moves out of the leaf in the process of transpiration, exerts a large influence on the rates of these processes. The resistances encountered by CO_2 molecules moving into the leaf to the fixation sites in the chloroplasts, may be used to quantitatively describe the physiological responses that may limit the rate of photosynthesis. The resistances encountered by water vapor molecules may be used in a similar manner. Along each diffusion pathway there are several discrete segments which may be identified by position or transfer mechanism. As several of these pathway segments are common to both CO_2 and water vapor, the simultaneous measurement of photosynthesis and transpiration enables all resistances to be either measured or calculated.

The diffusion resistances encountered by water vapor leaving the leaf are r_s the stomatal resistance and r_a the boundary layer resistance. CO_2 entering the leaf follows the same pathway and therefore encounteres the same boundary layer and stomatal resistances. However, as CO_2 must also diffuse from the substomatal

cavity to the carbon fixation sites in the mesophyll or bundle sheath cells, a further CO_2 resistance, the mesophyll resistance (r_m) is defined (Gaastra, 1959). As these resistances are in series the total resistance is equal to the sum of the individual resistances (Bange, 1953). Neglecting the negligible CO_2 and water vapor transfer through the cuticle (Jarvis, 1971) the total resistances to CO_2 and water vapor transfer may be written

$$\Sigma r_{H_2O} = r_a + r_s \tag{12}$$

and

$$\Sigma r_{CO_2} = r_a + r_s + r_m \tag{13}$$

The resistances are quantified in the following manner.

The transfer of CO_2 into and water vapor out of the leaf occurs by molecular diffusion, therefore by Fick's first law, the net flux of mass per unit area and time q_v, anywhere within the pathway if proportional to the gradient of partial pressure, or at atmospheric pressure the concentration of the property $\frac{dc}{dz}$ so that

$$q_v = -D_v \frac{dc}{dz} \tag{14}$$

where D_v is the effective molecular diffusivity of water vapor or CO_2 in air (after Jarvis, 1971). The diffusion resistance r_v is then defined by

$$r_v = \int_{z_1}^{z_2} \frac{dz}{D_v} \qquad [s\ m^{-1}]$$

and hence

$$q_v = \frac{C_1 - C_2}{r_v} \qquad [Kg\ m^{-2} s^{-1}] \tag{15}$$

and

$$r_v = \frac{C_1 - C_2}{q_v} \qquad [s\ m^{-1}] \tag{16}$$

Substituting in this equation the total resistance to water vapor transfer can be obtained.

$$\Sigma r_{H_2O} = r_a + r_s = \frac{[H_2O]_c - [H_2O]_a}{E} \qquad [s\ m^{-1}] \tag{17}$$

where E is the transpiration rate $[Kg\ m^{-2} s^{-1}]$, $[H_2O]_a$ and $[H_2O]_c$ are the water vapor concentration $[Kg\ m^{-2}]$ in the air and interior of the leaf respectively. The stomatal resistance (r_s) can be obtained by first estimating the boundary layer resistance (r_a).

$$r_s = \Sigma r_{H_2O} - r_a$$

The boundary layer resistance r_a can be measured by determining the water loss from blotting paper leaf models with similar geometry to the leaf. The total resistance to CO_2 transfer is obtained from the equation

$$\Sigma r_{CO_2} = r_a + r_s + r_{me} = \frac{[CO_2]_a - [CO_2]_{chl}}{P_N} \quad [s\ m^{-1}] \qquad (18)$$

where P_N is the net photosynthetic rate [Kg $m^{-2} s^{-1}$], $[CO_2]_a$ and $[CO_2]_{chl}$ are the CO_2 concentrations in the air and at the chloroplast fixation sites respectively. As r_a and r_s have already been calculated from the transpiration rate, the mesophyll resistance (r_m) can be calculated from the equation

$$r_m = \Sigma r_{CO_2} - (r_s + r_a)\left(\frac{D_{H_2O}}{D_{CO_2}}\right) \qquad (19)$$

where the correction term (D_{H_2O}/D_{CO_2}) is necessary to account for the difference in effective molecular diffusivities of CO_2 and water vapor.

Although the mesophyll resistance was defined as a diffusion resistance, its estimation as a residual resistance by the above method means that it contains all that is not accounted for by the stomatal and boundary layer resistances and therefore includes photochemical and biochemical processes unrelated to the transfer of CO_2 through the cell. In this study the use of mesophyll resistance as a residual term was practically expedient, because the study aimed to differentiate between stomatal and non stomatal influences reducing the photosynthetic rate during plant water stress.

APPENDIX II

Calculation of Transpiration From Differential Psychrometer Readings

Data From Chart Recorder

ΔT_1 the temperature difference between reference and sample wet bulbs was 101 μV

ΔT_2 the temperature difference between reference wet bulb and water bath B_2 (27.5 C) was -240 μV

Thermocouple calibration was 41 μV per degree C.

Therefore

$$\Delta T_1 = 2.46 \text{ C}$$

$$\Delta T_2 = -5.85 \text{ C}$$

as reference temperature was 27.5

T_r temperature of reference wet bulb is 21.65 C (27.50-5.85)

T_s temperature of sample wet bulb is 24.11 C ($T_r + \Delta T_1$)

From Equation (4)

$$\Delta e = e_s - e_r - A(t_{ws} - t_{wr}) + (e_{ws} - e_{wr})$$

where $t_{ws} - t_{wr}$ is ΔT_1 and e_{ws} and e_{wr} are the saturated vapor pressures at T_s and T_r respectively. A, the psychrometric

constant, is 0.667 mb vapor pressure per degree C

$$\Delta e = (0.667 \times 2.46) + (30.011 - 25.871)$$

$$= 5.78 \quad \text{mb}$$

The saturated vapor pressure (e_s) at 27.5 C is 36.71 mb and the density of water vapor (p) in saturated air at 27.5 C is 26.46 mg L^{-1}.

From Equation (5)

$$C = p\left(\frac{\Delta e}{e_s}\right)$$

$$C = 26.46 \times (5.78/36.71)$$

$$= 4.17 \quad \text{mg } L^{-1}$$

If the leaf area in the chamber is 40 cm^2 and the flow rate through the chamber is 3.63 L min^{-1}, the transpiration rate E is

$$E = 4.17 \times (3.63/40)$$

$$= 0.378 \quad \text{mg } cm^{-2} min^{-1}$$

$$= 2.271 \quad \text{g } dm^{-2} hr^{-1}$$

Calculation of CO_2 Transfer Resistances From Photosynthesis, Transpiration and Leaf Temperature Data

P_n = 30.9 mg CO_2 $dm^{-2}hr^{-1}$ = 0.086 μg CO_2 $cm^{-2}s^{-1}$

E = 2.407 g H_2O $dm^{-2}hr^{-1}$ = 6.686 μg H_2O $cm^{-2}s^{-1}$

T_c = 30.00 C

T_a = temperature of air entering the chamber = 27.50 C

T_r = temperature of reference wet bulb = 21.65 C

r_a = 0.96 s cm^{-1}

$[CO_2]_a$ = 345 vpm = 0.610 μg cm^{-2}

$[CO_2]_{chl}$ = 0 vpm

The saturated vapor pressure at 21.65 C is 25.87 mb and the density of water vapor in saturated air at 27.5 C is 26.46 μg cm^{-3}.

The water vapor concentration of the air entering the leaf chamber is

$$[H_2O]_a = 26.46\ (25.87/36.71)$$
$$= 18.65 \quad \mu g\ cm^{-3}$$

The water vapor concentration inside the leaf is the water vapor density of saturated air at 30.00 C, which is 30.34 μg cm^{-3}

$$[H_2O]_c = 30.34 \quad \mu g\ cm^{-3}$$

Substituting in Equation (7)

$$\Sigma r_{H_2O} = \frac{[H_2O]_c - [H_2O]_a}{E}$$

$$= \frac{30.34 - 18.65}{6.686} \quad s\ cm^{-1}$$

$$= 1.75 \quad s\ cm^{-1}$$

The total resistance to CO_2 transfer is calculated from Equation (8)

$$\Sigma r_{CO_2} = \frac{[CO_2]_a - [CO_2]_{chl}}{P_n}$$

$$= 0.610/0.086 \quad s\ cm^{-1}$$

$$= 7.09 \quad s\ cm^{-1}$$

As D_{H_2O}/D_{CO_2} is 1.60 and r_a is 0.96 s cm^{-1} the stomatal resistance to CO_2 transfer is

$$r_s = \Sigma r_{H_2O}\left(\frac{D_{H_2O}}{D_{CO_2}}\right) - r_a$$

$$= (1.75 \times 1.60) - 0.96$$

$$= 1.84 \quad s\ cm^{-1}$$

The mesophyll resistance to CO_2 transfer is

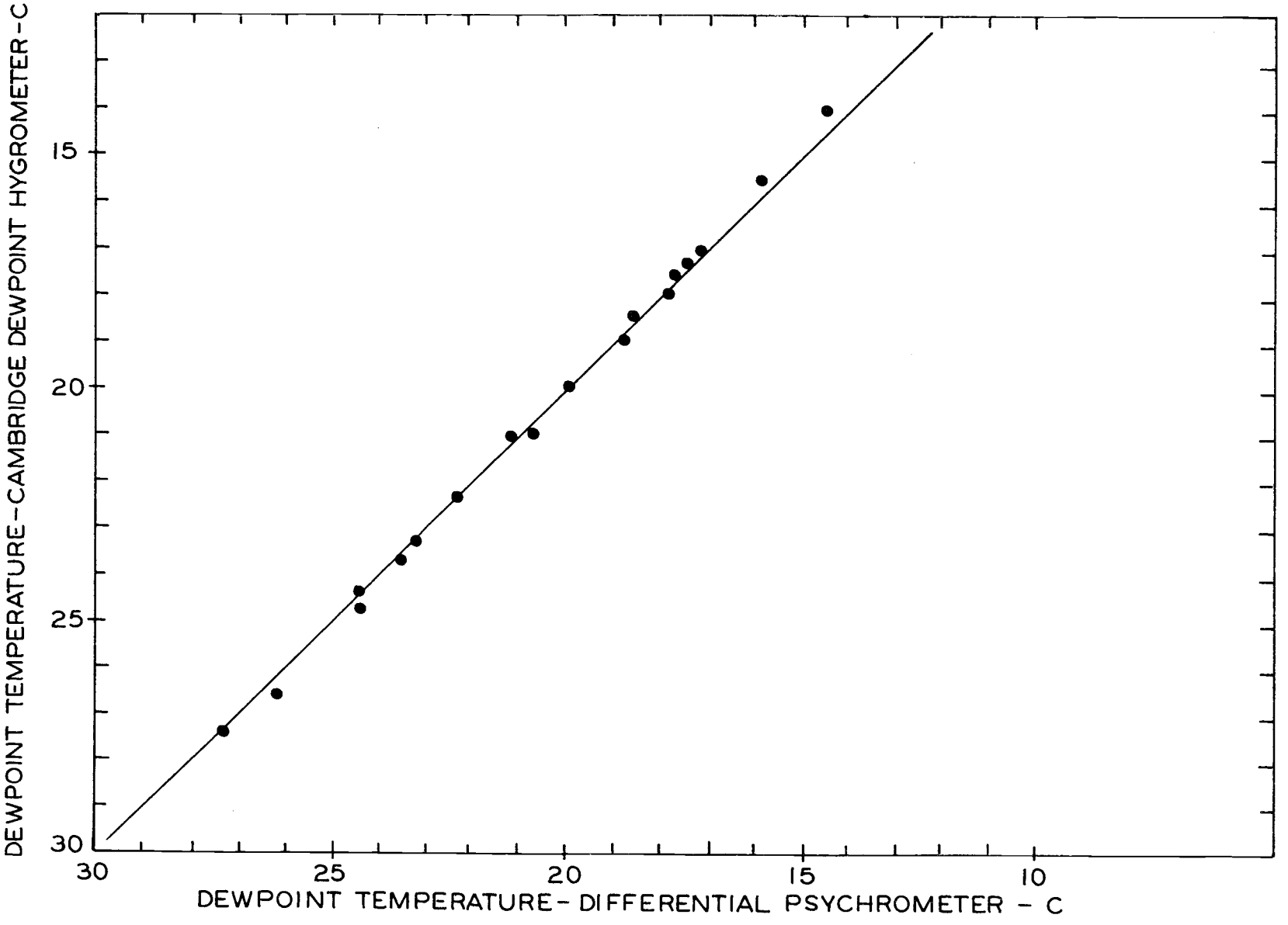
$$r_m = \Sigma r_{CO_2} - (r_a + r_s)$$

$$= 7.09 - 2.8$$

$$= 4.29 \quad s\ cm^{-1}$$

APPENDIX III

Calibration of Differential Psychrometer


DEWPOINT TEMPERATURE-CAMBRIDGE DEWPOINT HYGROMETER-C
15
20
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DEWPOINT TEMPERATURE- DIFFERENTIAL PSYCHROMETER - C

APPENDIX IV

Manufacturers of Components Used in the Construction of the Carbon Assimilation System

Regulating and Switching Valves:
Whitey Company, 5679 Landregan Street, Oakland, California 94662.

Flowmeters
1500 series E/C Rotameter, Brooks Instrument Division, Htafield, Pennsylvania.

Swagelock Gas-Tight Fittings
Crawford Fitting Company, 29500 Solon Road, Cleveland, Ohio, 44139.

Infrared Gas Analyser
Beckman IR215A equipped with 30-cm absorption tubes.

Xenon Long Arc Lamp
Osram, Berlin, Germany.

Infrared Filters for Xenon Lamp
Atlas Electric Devices Company, Chicago, Illinois 60613.

Linear Variable Differential Transducer
Model SS-102, G.L. Collins Corp., Long Beach, California.

Cooling Circuit and Microvoltmeter for Thermocouple Psychrometer
Wescor HR-33 Dewpoint Microvoltmeter
Wescor, Logan, Utah 84321.