EFFECTS OF WATER AVAILABILITY ON GROWTH AND PHYSIOLOGY OF *RICINUS COMMUNIS* L

by

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SUMMARY

This study is concerned with the responses of expansion growth to plant water status over various periods and the factors controlling such responses. The plant mostly used was Ricinus but Helianthus and Aesculus were also used.

Expansion growth at Y around 0 bar was studied by floating leaf discs in water. Absorption at 1°C satisfied the initial water deficits alone, but at 30°C there was a prolonged additional uptake for vacuolation which accompanied disc expansion. The growth uptake was larger the earlier the stage in leaf development. The growth uptake was initially rapid and was attributable to an accumulated growth potential (an 'unexpressed growth') caused by water deficits which accordingly increased with the severity of water stress. All levels of plant water deficits retarded growth and consequently, leaves remained 'immature' even on normal plants.

Experiments with intact leaves on normal plants showed that slight losses in water balance resulted in large reductions in Y and Y_p of the leaf cells but only slight decreases in Y_s. In response the rate of leaf expansion declined as water deficits increased, thus indicating the importance of Y_p in expansion growth. Growth of Helianthus leaves ceased at higher Y and Y_p values than those of Ricinus.

Young Ricinus leaves on normal plants possessed lower cell Y_p, resulting from a slightly lower Y but a higher Y_s than older leaves. Despite this fact, the growth rate of young intact leaves was greater and persisted despite lower water deficits than older leaves. Apparently a low Y_t and presumably a large gross extensibility (E) for young cell walls favoured more rapid growth in younger than older leaves.

Observations suggest that leaf expansion was more sensitive than stomatal responses to water stress of normal growing plants.

When water stress was allowed to develop gradually, Ricinus adjusted by osmoregulation in part, allowing Y_p to be maintained at a reduced Y. Consequently young stressed leaves grew at a lower Y than normal. However, their growth rate was only partially maintained as a result of an increased Y_t and presumably a decreased E. Expansion of older stressed leaves ceased prematurely. Following rewatering, Y readjusted quicker than Y_s, therefore Y_p was temporarily higher than in normal plants. Expansion of stressed leaves resumed readily but their capacity to expand fully was permanently reduced. However cell elongation was temporarily enhanced.

The mechanism underlying the growth-water relationships were studied using excised Ricinus leaves which were deprived of water supply in an oil bomb. Apparently growth was initiated by cell wall loosening, resulting in stress relaxation which caused a reduction in Y_p and a corresponding increase in the water potential gradient

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INTRODUCTION

The growth of a plant is a reflection of the interaction of its genotype and the environmental factors prevailing in its immediate surrounding. Among the predominant environmental factors, temperature, light and moisture, the physiology, growth and survival of many plants have often been closely correlated with the moisture conditions of its environment. For, it is a general observation that plants thrive in soils with an adequate water supply while water shortage results in a great reduction in plant physiological processes and growth. Consequently, earlier investigators of plant responses to water, attempted to relate plant growth and physiological processes to soil moisture levels (e.g Veihmeyer and Hendrickson, 1927, 1950, Wadleigh and Ayer, 1945). Attention was focused mostly on the available soil moisture range, i.e. the amount of water stored between field capacity and permanent wilting point of a soil.

Two opposing concepts arose from the interpretation of such data. The first, originated by Veihmeyer and Hendrickson (1950), claimed that plant processes are little affected by water stress within the available range. The second concept, proposed by Richard and Wadleigh (1952) was that water becomes progressively less available to plants as the total soil moisture stress increases, and so plant processes are retarded before soil moisture reaches the permanent wilting point. After an extensive survey of the

literature on available evidence bearing on these hypotheses by Stanhill (1957), the second concept was generally accepted.

However, Kramer (1959) focused attention on the internal water status of the plant, pointing out that this is the main factor directly affecting plant processes rather than the environmental moisture conditions. The degree to which these processes are limited depends on the relative internal water deficits. These deficits are not always directly related to soil moisture content but rather they are generally controlled by the relative rates of water absorption and transpiration. These relationships are fully discussed by Kramer (1959,1969), Vaadia, Raney and Hagan (1961), Slatyer (1967) and Kozlowski (1968).

As would be expected, therefore, much of this earlier data did not assess plant responses to available water adequately. Recent development of adequate techniques for measuring plant water status (e.g. Spanner, 1951; Richard and Ogata, 1958; Scholander, Hammel, Hemningsen and Bradstreet, 1964; Wiebe Brown, Daniel and Campbell, 1970) has provided means for studying and for seeking re-interpretation of many of these earlier data.

Among such studies, there has been considerable interest in the effects of water deficits on growth. It must be admitted that this relationship is very complex and little understood. For example, it is generally assumed that water stress reduces growth as a result of loss of turgor causing stomatal closure. Consequently, photosynthesis is the process most directly and

severely affected by water stress. This may be partly true for growth expressed in terms of yield and productivity. Another view is that since growth is an expression of many integrated physiological processes, it is a secondary phenomenon (Woodhams and Kozlowski, 1954; Kozlowski, 1968). Thus, with the objective of understanding why water deficits reduce growth, attention has been focussed on the study of water stress and plant processes. Thus, whilst these relationships have been widely described (see Vaadia et al, 1961; Kozlowski, 1968 a,b, 1972; Kramer, 1969) little is found in the literature on water and cell expansion growth (cf Hsiao, 1973).

This is unfortunate because, as long ago as 1874, Sachs indicated that cell enlargement is directly affected by turgor pressure. Since then this point has been emphasised and Kramer (1959, 1963) further indicated that one of the first processes to be affected by water stress and the resulting turgor reduction, is a decrease in or cessation of stem elongation and leaf enlargement. With modern techniques and careful measurements of both expansion growth and other physiological processes concurrently, his view is being proven experimentally.

Using Lolium temulentum L, Wardlaw (1969) observed that leaf and root elongation was stopped at relative water content (RWC) of 75%, whilst photosynthesis was only reduced to one third of its maximum rate. Boyer (1970) observed that as leaf water potential decreased, inhibition of leaf enlargmement of maize, soybean and sunflower was more severe than that of photosynthesis and respiration.

Similarly, Acevedo, Hsiao and Henderson (1971), observed that water stress suppressed leaf expansion of maize before photosynthesis and before any reductions in polyribosome levels.

Hsiao (1973) and Hsiao, Acevedo, Fereres and Henderson (1976) compiled a tentative table which showed the possible sequence of responses of different processes to temporary water stress on the basis of their relative sensitivities. From the table, cell enlargement was shown to be affected by a small reduction in internal water status while other plant processes remained unchecked until severe stress developed. They suggested that many metabolism might reduce during temporary water-stress as a direct result of the reduction in cell expansion leading to small leaf sizes. Presumably, because the leaf is the principal organ in which many of these processes occur. In fact, Watson (1947, 1956) pointed out that the yield of crop is related more to leaf area than to variation in photosynthesis efficiency per unit leaf area. Thus, it is essential that the role of water in expansion growth be understood. This problem has been studied since the time of Sachs (1874) and has been given some consideration over the years (Heyn 1933, 1940; Burstrom, 1961; Cleland, 1971a; Green, Erickson and Buggy 1971). Although there has been some fascinating progress, our understanding of the mechanisms is still limited, as can be seen from the following literature survey.

General aspects of plant growth

The growth of entire plants or organs is the outcome of

the growth of numerous cell assemblies, consequently the growth of a single cell can, with limitations, be used to illustrate that of the whole plant.

The initial stages of cell development are marked predominantly by cell division. Earlier experiments involving cell counts of leaves from normal and droughted plants (e.g. Maximov, 1929; Kramer, 1959) suggested that the phase is little affected by water stress. More recent data has, however, indicated that under the same condition, cell division could be very sensitive to water stress. (Gardner and Nieman, 1964; Terry, Waldron and Ulrich (1971). Nevertheless, it is still believed that the next phase of cell development, cell enlargement, is more sensitive (Meyer and Boyer, 1972).

Cell enlargement mainly involves an absorption of water into the vacuole leading to large increase in cell volume (Burström, 1951; Lockhart 1965 a,b, 1967). Ashby and Wangerman (1950) found that leaf enlargement of Ipomoea caerulea from an area of about 0.5 cm^2 to 2.0 - 3.3 cm^2 was almost entirely due to cell division; thereafter leaf enlargement was entirely due to cell enlargement and these leaves could grow to areas of about 49 cm^2. Brown and Broadbent (1950) have estimated volume increments of 20 to 30 fold of root cells during cell enlargement.

The next phase of cell growth is differentiation. The phase is marked by increases in dry weight with very little or no change in cell volume. After differentiation growth

as a whole ceases and the cell is said to be 'mature'.

In a similar manner, plant organs with limited growth, such as leaves, are considered to be 'mature'.

From the above description, it is obvious that the size which a plant can attain would depend on the extent of cell enlargement which is controlled by water uptake. Of course, the full effect of water will be expressed under conditions of normal temperatures and light. In addition, nutrients (Watson and Wilson, 1956; Baker and Ray, 1962) and growth substances (e.g. Galston and Davies, 1969) may influence cell expansion. However, these factors in some cases have been shown to have a secondary effect rather than a direct influence on growth (Ray and Ruesink 1962; Kramer, 1969).

The paramount role of water is nonetheless clearly defined. Heyn (1940), pointed out that when cells reach the stage of elongation, enlargement is dependent firstly on uptake of water, elongation being impossible if this is prevented. Burström (1951) observed that on a short term basis, cell vacuolation may occur without accompanying increments in protoplasm and organic matter. Lockhart (1965a) reported that the rate of protein synthesis is far from sufficient to keep pace with vacuolation.

Water availability and cell enlargement

That growth by cell enlargement is largely controlled by internal water status has been shown both indirectly and

directly in many plants. Thus, when water becomes less available to plants through increasing water stress in the root medium, smaller cells are produced which ultimately leads to smaller leaves and stunted growth of shoot. Much of the earlier convincing indirect evidence has been reviewed by Kramer (1959, 1963), Stocker (1960), Henckel (1964).

More recently direct evidence has been gained and experimenters have attempted to quantify the relationship. Boyer (1968, 1970) found that leaves of sunflower would only grow at water potentials (Y), higher than -4.0 bar. He further observed that leaf expansion in maize and soybean was reduced by 88 and 75% of the maximum rate respectively at Y of -4 bar; and, in fact, ceased altogether at -12.0 and -8.0 bar respectively. Leaf expansion of cotton was inhibited at Y of -8.0 bar (Jordan, (1970). Similarly, Gandar and Tanner (1976) observed that leaf growth of potato ceased at Y between -4 and -5 bar. Other experimental data has been reviewed by Hsiao (1973) Hsiao et al (1976).

Further evidence in support of the essential role of water in cell growth, was gained from the observation that, as long as plants survive drought, growth resumed immediately following rewatering (Boyer 1968, 1970). Moreover, at times growth rates exceeding control rates have been demonstrated (Gates, 1955); Owen and Watson, 1956; Hsiao et al, 1970; Gandar and Tanner, 1976). Hsiao et al (1970) and Acevedo et al (1971)

further demonstrated that in maize when water stress was mild and temporary a transitory rapid leaf elongation following rewatering, could completely make up for the reduced elongation which occurred during the stress period. They suggested that metabolic processes necessary for cell expansion were not inhibited during the stress period, and that expansion was suppressed only by the reduced cell turgor. They further considered the rapid transitory growth to represent a 'stored growth', in other words during the drought period a potential for cell enlargement accumulated which was expressed when cell turgor was restored.

A similar phenomenon has been reported during water uptake studies of excised leaf tissues. Weatherley (1965) has referred to it as a 'delayed growth', and Milburn and Weatherley (1971) called it 'arrested growth'.

Water deficits and 'stored growth'

Water deficits are defined in terms of water required by cells to reach full turgidity and it is also associated with negative water potential (Y). Of the components of Y (Section 1) turgor pressure (Y_p) has been closely associated with growth and is considered to provide the physical force for cell expansion (Heyn, 1940; Ray, Green and Cleland, 1972; Hsiao et al, (1976). Although Y_p will be a good indicator of plant water status during growth studies, there is no suitable direct method

for measuring it in higher plants. Under this condition Y has been used as an alternative for a number of reasons. Apart from its direct influence on Y_p, it can be measured easily and furthermore, plant growth under normal conditions and during mild water stress has been shown to respond to changes in Y.

Water absorption is known to occur along gradients of decreasing Y. Consequently the Y of a plant must be more negative than that of the rooting medium. These deficits increase considerably even in well watered plants when rates of transpiration exceeds absorption. (cf Slatyer, 1967; Kramer, 1969). This usually occurs during the day, when stomata open, and when evaporative demands are high. For example, Klepper, Browning and Taylor (1971), measured cotton Y above -5.0 bar in the early morning, but this valve reached below -15.0 bar by noon. Similarly Hellkvist and Parsby (1976) observed that Y of Pinus sylvestris decreased from about -5.0 bar in the morning to below -12.0 bar by midday. A Y of about -12.0 bar is known to reduce tissue growth of other pine (Miller, 1965; Kaufman, 1968.)

It therefore appears that in nature cells normally grow at negative Y which can sometimes become very large, and these cells have to change physiologically in response. For instance it has been reported that expansion growth of some species may be largely confined to the night when plant water status tends to be more favourable than by day (Loomis 1934; Reed, 1939; Boyer, 1968, 1976; Bunce, 1976).

However, in growth and water relation studies it is general practice to accept growth in control plants (i.e. plants under optimum moisture conditions) as the maximum against which growth responses at higher deficits are compared. But in view of the extreme sensitivity of cell enlargement to water deficits, it is likely that normal water deficit levels could have some degree of permanent suppressing effect on growth, and if so might a water potential around zero bar have any enhancing effect on growth, or even resuscitate growth in 'mature' leaves? It is true that such conditions are not common in land plants but it may be worth considering for the insight that might be gained into associated phenomena.

Growth by water uptake of submerged tissues

It has often been assumed that leaves mature on normal plants when they ceased to expand and as such any uptake of water may arise from water deficits alone. This assumption in the past, led to a mis-interpretation of many water uptake data. For example, Dixon (1898) and later Dixon and Barlee (1940) observed a persistent water absorption by saturated leafy branches, and young and mature leaves which were submerged in water to prevent transpiration. The uptake was enhanced at high light intensities and temperatures. They attributed it to a secretion of water by the leaves, a phenomenon termed 'subaqueous transpiration'. Other workers attributed the uptake to water deficits (Smith, Dustman and Shull, 1931) or to an

injection of the inter-cellular gas spaces by water (van der Paauv, 1935; 1950).

Recently, Potter and Milburn (1970) re-examined the 'subaqueous transpiration' concept, using shoots and leaves which were submerged in water or liquid paraffin. Their results suggested that the prolonged uptake of water was caused by growth and not by 'subaqueous transpiration'. They could not detect any water secreted into the oil, rather the samples increased in fresh weights and furthermore young leaves absorbed more water than older leaves.

A similar observation was made by Weatherley (1950) during relative turgidity (now, relative water content, RWC) measurements of leaf discs floated on water at room temperature. He observed an initial rapid uptake, phase I, which he attributed to water deficit. This was followed by a slow but persistent uptake, phase II, which he attributed to growth. Phase II was even observable in discs from 'mature' leaves, suggesting that free water at (or near) atmospheric pressure can reverse leaf maturity.

However, subsequent workers (e.g. Yemm and Willis, 1954; Catsky, 1959; Molz, Truelove and Peterson 1975) using the RWC technique considered that the rapid Phase I uptake has a growth component and consequently attempted to quantify it.

Barrs and Weatherley (1962) examined the possibility of improving the RWC technique for measuring water deficits alone. They floated leaf discs at low temperatures around freezing

point so as to inhibit growth (cf True, 1895; Barlow and Hancock, 1959; Ray and Ruesink, 1962). The treatment suppressed Phase II but a fraction of phase I was also suppressed. Although this suggested a growth uptake during Phase I, they considered resaturation to be divisible into 2 phases. Weatherley (1963) extended the study. His results suggested that there might be an apoplastic and vacuolar phase of uptake during saturation. The apoplastic uptake was rapid and insensitive to temperature but the vacuolar uptake was suppressed at low temperatures.

Millar (1966) demonstrated that leaf discs could attain different saturation levels when floated on water at different temperatures.

More recently Milburn and Weatherley (1971) re-examined the temperature-water uptake hypothesis. They demonstrated that phase I cold uptake completely satisfied water deficits, that is, it included both apoplastic and vacuolar components because the amount of water absorbed greatly exceeded the total volume of the apoplast. They also showed that the temperature sensitive phase I uptake was attributable to expansion growth. It was related to leaf age and was greatly influenced by drought, consequently they considered it to be an 'arrested growth' phenomenon. Milburn and Weatherley advocated the need for further work on the 'arrested growth' phenomenon.

Mechanism of cell growth

The plant cell is generally composed of a protoplast surrounded by a cell wall. The wall is very rigid and it offers great resistance to cell expansion. Therefore, for the cell to expand, there would not only be an increase in the volume of the vacuole but also some modification in the wall which would decrease the resistances to stretching. Present knowledge of the mechanism of growth suggests that it involves 2 closely linked steps. Initially the wall is loosened through physical or biochemical transformation. This is followed by physical extension by turgor pressure through water uptake into the cell vacuoles. (Heyn, 1940, 1970; Cleland, 1959; 1971a; Probine and Preston, 1962; Lockhart 1965 a,b, Ray *et al* 1972, Hsiao, 1976). However, the steps involved and the sequence of events are still controversial. These are briefly reviewed below. In addition to these two phases there is, of course, a continuous synthesis and incorporation of new wall material (Preston 1974).

Aspects of water regulation of cell enlargement

As discussed previously, cell expansion quantitatively implies mainly an absorption of water. Consequently conditions conducive to water absorption are essential for expansion. An earlier hypothesis on active transport of water into growing cells (e.g. Bennet-Clark, Greenwood and Barker 1936, Thimann,

1951) has been abandoned through lack of satisfactorily experimental evidence. It is now established that water absorption is a passive process occurring along a gradient of decreasing water potential (Y). It is further controlled by the resistances of the cell to water flow, (Lockhart 1965b; Slatyer, 1967, Kramer, 1969).

Experiments on the permeability of growing cells to water have been inconclusive. Experiments by Thimann and Samuel (1955) Ordin and Bonner (1956) and more recently Burstrom et al (1967) showed that water permeability is large and consequently Y of growing cells are in constant equilibrium with that of the growing medium. In contrast Bennet-Clark (1956), Ray and Ruesink (1963) and more recently Boyer (1968)demonstrated resistances to water entry into growing cells. Ray and Ruesink and Boyer have attributed the variabilities in results to inadequacies in techniques.

The water potential (Y) of a cell is composed of osmotic (Y_s) and matric (Y_m) potentials and turgor pressure (Y_p). Y, Y_s, and Y_m are negative quantities while Y_p is generally positive. Y_m is often assumed to be negligible.

Assuming that water permeability is high, i.e. in the equilibrium condition, $Y = Y_s + Y_p$ should be uniform throughout the tissue. Therefore, for any net uptake of water, the Y of the growing cells must fall below that of the medium (e.g. xylem and intercellular space water) so as to establish the necessary potential gradient (cf Burstrom 1961, 1971; Ray et al, 1972). Accordingly,

growth must be initiated by reductions in Y_s. This may occur through net increases in the osmotically active solutes in the vacuoles of the growing cells by uptake or by internal synthesis, a phenomenon termed osmoregulation or osmotic adjustment. Alternatively, Y_p may be reduced to initiate growth, through a relaxation in wall pressure since the 2 pressures counter balance each other. The 2 hypotheses have been controversial as discussed below. The established water potential gradient causes water absorption into the vacuole leading to an increase in Y_p which physically stretches the cell wall.

<u>The osmotic potential and turgor pressure hypotheses and the initiation of cell expansion</u>

The osmotic hypothesis assumes that wall extensibility remains constant during the initial stages of growth while osmoregulation continues to maintain Y. According to Commoner, Fogel and Muller (1943) it was Czaja (1935) who first proposed the hypothesis. Their own observation on water absorption of potato tuber, were explained in support of this hypothesis, but they arrived at this conclusion without measuring the Y_s of the cell.

The Y_s hypothesis was, however, criticised strongly by many experimenters. For example, Went and Thimann (1937) pointed out that the hypothesis was experimentally disproved in 1924 by Ursprung and Blum, who found no significant change in Y_s in actively growing cells. Commenting on this

criticism, Commoner et al (1943) explained that failure to observe increments of sap concentration in growing cells was due to the fact that sap dilution occurs through net water absorption. This suggests a need for a technique for the separation of the 2 processes. This need was probably reflected in results of subsequent experiments. Thus, investigators either observed increases, no change, or slight decreases in Y_s of growing cells (van Overbeek, 1944; Levitt, 1948; Hackett, 1952).

However, Burström (1961) maintained that a decrease in Y_s is not an indispensable condition and cannot cause growth. More recently, Hettiaratchi and O'Callaghan (1974) proposed a theoretical model in support of the osmotic hypothesis. Whether osmoregulation is a pre-requisite in initiating growth or not, Pfeffer (1877), Grenetz and List (1973) and Cleland (1976a) have indicated that the phenomenon is important in maintaining Y_p so as to sustain growth over a long term.

The turgor hypothesis is more favoured (Heyn, 1940; Ordin et al 1956; Burström, 1961; Ray et al, 1972). One of the earliest hypothesis suggested that the wall pressure is reduced through active cell growth by synthesis and intussusception of new wall material (see reviews by Heyn, 1940; and Burstrom, 1961; See also Ray, 1962). Earlier data on whether wall material increases during or after cell elongation was inconclusive (e.g. Bonner, 1934; Frey-Wyssling, 1948; Wardrop and Cronshaw, 1958). However, recently Rayle,

Haughton and Cleland (1970) failed to find any increment in cell wall material during the initial phase of cell elongation, rather new wall material is shown to be synthesised after wall extension (Baker and Ray, 1965; Cleland 1967).

On the other hand the hypothesis that loosening of the cell wall already present leading to a relaxation in wall pressure with a consequent reduction in Y is well established. (Heyn, 1931, 1940; Lockhart, 1965 a,b, Cleland, 1971 a). By separating the initial phase of growth from water uptake and cell enlargement, increases in wall extensibility through wall loosening have often been demonstrated, the methods employed being fully reviewedby Cleland (1971 a).

The fact that suctions are generated, has generally only been inferred from evidence that induction of wall loosening is accompanied by rapid water absorption (Heyn, 1940; Cleland 1955, 1967, Catsky, 1963). However, investigators realised the need to measure Y directly (Ordin et al, 1956; Bennet-Clark, 1956; Ray and Ruesink, 1963; Burström et al, 1967) but lack of adequate techniques has complicated results obtained from such studies. More recently, Dr. Milburn of the Botany Department, University of Glasgow has developed a technique for studying leaf growth, whereby Y generated through wall loosening could be measured directly in the absence of cell enlargement by suspending water supply. (Unpublished data).

The equipment is based on a modification of the classical nitrogen gas or air bomb (Scholander et al, 1964) in which

oil is used to submerge and pressurise the leaf so as to prevent transpiration and water loss through distillation. The oil bomb's perspex chamber is illuminated thus preventing CO_2 narcotisation produced by respiration in darkness. The set up allows leaves to be enclosed for lengthy periods in the bomb.

The growth principle is based on the fact that, if a non-growing leaf is enclosed in the oil bomb, the water content remains virtually unchanged so Y will not change. On the other hand, a growing leaf cell will continue to absorb water from the xylem by generating suction through a relaxation in wall pressure, until a threshold Y is reached. It is hypothesised that the degree of Y lowering should be proportional to the growth capacity of the leaf sample. The technique has been used in the thesis, and full description of it is given in the main text.

Cell wall loosening

Detailed account of the process of wall loosening has been given by Heyn (1940), Burström (1961) Probine and Preston (1962), Lockhart (1965 a,b), Cleland (1971a) and Preston (1974) and only certain aspects which might be relevant to the proposed study will be discussed.

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be irreversible, it is clear that plastic extensibility is more relevant to cell enlargement and wall loosening is believed to involve mostly wall plasticisation.

One of the earliest hypotheses (see review by Heyn, 1940) proposed that during that phase of elongation, the wall is passively stretched by turgor pressure (Y_p) beyond its elastic limit leading to a plastic deformation. However, it has been emphasised (Heyn, 1940; Preston, 1961) that for this physical hypothesis to hold, there should be a continuous increase in Y_p. Milthorpe and Spenser (1957) and Barrs and Weatherley (1962) observed increases in cell volume due to irreversible changes in the cell wall when tissues were floated on free water (i.e. Y near zero bar) during RWC measurements. They concluded that cell walls of fully turgid tissues can be deformed passively, a process they termed 'plastic flow'. However, as tissues generally grow at somewhat less than full turgor, plastic flow may not occur in nature. In fact, Burström (1961) has quoted several data to show that during normal cell elongation plasticity occurs in tissues with large water deficits.. Furthermore, Y_p decreased during growth (cf Burström, Uhrström and Wurscher, (1967). Experiments by Ray and Ruesink (1962) on the effect of metabolic inhibitors on growth, showed that cell expansion cannot be controlled by a physical plasticity of the cell wall. However, more recently, a theoretical model on cell enlargement

proposed by Hettiaratchi and O'Callaghan (1974) supports the physical hypothesis.

It is however, generally accepted that wall loosening is initiated by biochemical transformation in the mechanical properties of the cell wall. However, the biochemistry of the process is still inconclusive. Heyn (1931, 1940), who proposed the hypothesis, considered it to be induced by hormones. He observed greater increase in plasticity of auxin-treated than non-treated coleoptiles. His experiment was repeated and confirmed by subsequent workers (Reviews by Heyn, 1940; Burstrom, 1961; Cleland, 1971a).

Other regulatory mechanisms, among which are ribonucleic acid (RNA) and protein synthesis (Key and Ingle, 1964; Hamilton, Moore and Ramsay, 1965, Key, 1969) and enzymes such as polysaccharide hydrolases (Ray, 1962; Fan and Maclachlan, 1966; Masuda, Oi and Satamura, 1970) have been associated with wall loosening. The process is also believed to be controlled by the pH of the wall solution (Bonner, 1934; Burstrom, 1951), Rayle and Cleland (1970) and Hager, Menzel and Krauss (1971) independently proposed that wall loosening is initiated by a wall loosening factor, probably H^+ ions. This theory has been criticised by some experimenters (e.g. Penney, Dunlop, Perley and Penney, 1975). However, Cleland (1976b)has attributed these contrary results to inadequacies in experimental technique.

Respiratory inhibitors are known to suppress and even

reverse wall loosening (Cleland and Bonner, 1956; Ray and Ruesink, 1962, Black, Bullock, Chantler, Clarke, Hanson and Jolley, 1967).

In his hypothesis, Heyn (1940) indicated that wall loosening should be considered as the limiting factor in elongation and that Y_p and water uptake are generally non-limiting. However, over the years evidence has accumulated to suggest that Y_p is a pre-requisite in wall loosening because in many species the process does not occur until Y_p exceeds a critical value. This phenomenon is discussed below.

Turgor pressure, the yield point concept and the rate of cell growth

Thimann and Schneider (1938) observed that growth of Avena coleoptile tissues was directly proportional to the osmotic gradient between the cell sap and the growing mannitol medium. In other words, growth stops at Y which corresponds approximately to zero Y_p. The response has also been demonstrated for cotton leaves (Wadleigh and Gauch, 1948) and for leaves of Phaseolus vulgaris (Brouwer, 1963).

However, it has been demonstrated for many plants that growth stops before Y_p reaches zero bar. This turgor pressure, Y_t, has been referred to as threshold turgor for growth, critical turgor pressure or wall yielding threshold (Cleland, 1959; Probine and Preston, 1962; Green 1968, Boyer, 1968, 1971,

Hsiao et al, 1976).

The phenomenon was reported first by Cleland (1959). He found that wall relaxation of Avena would not occur until the Y_p of the cells exceeded about -6 bar. He postulated that wall elasticity is controlled by weak bonds which are broken during stress relaxation only when Y_p exceeds some critical value, Y_t. Ray and Ruesink (1963) also demonstrated Y_t, in Avena tissue.

Further knowledge of the concept was obtained from work with Nitella by Green, (1968) and Green et al (1971). They monitored Y and cell enlargement concurrently during shifts in the Y of the growing medium. Y_p was measured directly with an intra-cellular micro-manometer. Continuous photographs of the growing algae were taken. They found that a small drop in Y_p leads to immediate cessation of growth, though Y_p was still positive. Apparently during steady state extension, Y_t was only slightly lower than Y_p.

Further experiments showed that Y_t is adjustable. Thus they observed that a turgor step-down initially stopped growth, but growth returned to the original rate after 15 minutes. Similarly, a turgor step-up was followed by a transitory rapid growth which gradually fell to the original rate. They explained that the Y_t decreased in value during a turgor step-down but increases during turgor step-up.

The critical turgor concept for growth has been demonstrated in leaves of sunflower, soybean and maize

(Boyer, 1968, 1970) cotton (Jordan, 1970), Maize (Hsiao et al, 1970, Acevedo et al, 1971) potato (Gander and Tanner, 1976) and for leaves and stems of cotton, maize, ryegrass and bean (Lawlor, 1969). Apparently its magnitude varies with species. Seemingly the concept is a common phenomenon. Consequently, Cleland (1959) and Green et al (1971) proposed that the driving force for growth should be regarded as only that part of Y_p which exceeds Y_t.

Green et al (1971) showed that the rate of growth (r) in Nitella may be expressed by the equation -

$$r = E\ (Y_p - Y_t)$$

where E represents wall extensibility which is dependent on the biochemical processes leading to wall loosening and synthesis of new wall material. Green et al presented experimental data to show that regulation in growth rate during short term turgor changes lies more with Y_t than with changes in E. Earlier, Lockhart (1965b) had given 2 generalised but detailed growth equations. The first showed the situation for plants whose growth rate is proportional to Y_p but the second equation incorporated the turgor threshold concept.

The simplified growth rate equation of Green et al (1971) has been used to describe the growth responses of many higher plants. Apparently both Y_t and E could vary in magnitude with water stress. (Acevedo et al, 1971, Meyer and Boyer, 1972, Bunce, 1977).

Cleland (1971a) has suggested that what may be needed for cell elongation, is probably a critical amount of elastic extension rather than a critical growth turgor. However, he realised that the 2 factors are almost indistinguishable. Whilst this is the case, the Y_t concept should probably be maintained.

The question has been raised by Cleland (1971a)as to whether Y_t is a physiological or physical parameter. In other words whether the Y_t is related to stress relaxation or wall extension. He suggested that Y_t would be a physiological parameter if rapid growth ('stored growth') or increased extensibility can be demonstrated at Y_p below Y_t. Experimental data to test this have been inconclusive. (Cleland 1967, 1971a; Masuda, 1969; Ray, 1961). However, in a theoretical model Grenetz and List (1973) have considered it to be a physiological parameter.

The requirement of Y_p in cell elongation is, therefore, well established. However, this concept has been recently questioned by Burström (1964, 1971) and Burstrom et al (1967) arguing that emphasis should be placed on the water potential gradient required for water absorption. Ray et al (1972) have answered these criticisms and have re-emphasised the important role of Y_p.

Factors controlling cell enlargement during drought

The ability of plants to survive, metabolically function

and grow during periods of water stress has fascinated many investigators and researches have been conducted to elucidate the mechanisms underlying these responses. Many plant attributes, anatomical, morphological, physiological or a combination of these, have been postulated to explain the phenomenon (Parker 1956, 1968; Iljin 1957; Stocker, 1960; Oppenheimer, 1960, 1968; Hsiao, 1973).

Considering expansion growth and plant water, it has been demonstrated that during short periods of mild water stress, adjustments in the yield threshold (Y_t) and wall extensibility (E) may allow growth to continue at a reduced turgor (Green, 1968; Acevedo *et al*, 1971; Green *et al* (1971).

However, Hsiao *et al* (1974) have suggested that where turgor pressures fall to zero, partial growth could be maintained through osmoregulation. Osmoregulation reduces Y_s with a consequent increase in Y_p; thus providing the turgor required for growth while Y is reduced.

Osmotic adjustment as a possible drought resistance mechanism has long been recognised. Thus Maximov (1929) has discussed its advantages. The phenomenon is common in plants growing in saline condition or in osmotic medium (Slatyer, 1961; Lawlor, 1969; Meiri and Poljakoff-Mayber, 1967, 1969; Jennings, 1976). Earlier evidence on the phenomenon in soil grown plants has been indirect (Magness, Degman and Furr (1935) Eaton and Ergle, 1948). Direct evidence has been recently obtained thus, Graecen and Oh (1972) demonstrated that

osmoregulation by roots of germinating pea resulted in high Y_p which maintained growth during water stress. Similarly, Meyer and Boyer (1972) observed a partial growth of desiccated hypocotyls of soybean which adjusted osmotically. In contrast, growth was completely inhibited in seedlings which failed to osmoregulate. Osmoregulation has also been demonstrated in corn and sorghum (Hsiao, et al 1976) and in cotton (Brown Jordan and Thomas, 1976; Cutler and Rains, 1977).

The aim of this research has been to provide further knowledge of the phenomenon of growth and its relationship to water availability.

This thesis, therefore, studies tissue growth (particularly in response expansion growth), to available plant water. Ricinus communis L, was the main experimental species. This plant has been found suitable for many water relation studies (e.g. Barrs and Weatherley, 1962; Tinklin and Weatherley, 1966, Potter and Milburn, 1970).

For studies on growth when water was freely available, it was decided to repeat and extend the water uptake studies of Milburn and Weatherley (1971). A few water uptake experiments were conducted with discs of Helianthus annuus. This plant was chosen because its growth is known to be greatly suppressed by slight increases in water deficits (Boyer, 1968). It was also used in some of the subsequent studies.

In order to make a fair evaluation of the growth-water relationships, it was found essential to study some aspects

of the tissue water relationships of young and older leaves.

Growth of intact organs including plastochrone intervals of leaves, leaf expansion, elongation of petioles and stem internodes in relation to available water, was studied over both short and long periods. To understand how water controls these growth responses, the magnitudes of Y, Y_s and Y_p of intact leaves during growth were assessed. In this respect also, the growth capacities of young and older excised leaves when water supply was zero, was studied using an oil bomb technique as developed for the purpose.

Other experiments such as transpiration, stomatal responses and morphological modifications to water deficits were conducted to assist with the interpretation of the observed growth responses.

In an attempt to apply the knowledge gained from the above study to understanding other growth phenomenon, the relationships between leaf morphology, internal water status and growth, and tree height of Aesculus hippocastinum were studied. (Appendix 3).

SECTION 1

MATERIALS AND GENERAL METHODS

Experimental Plants

Ricinus communis L. var *Gibsoni* (castor bean) was the main experimental plant. *Helianthus annuus* L. (sunflower) was included in some of the studies. Seeds of the two species were supplied by Thompson and Morgan (Ipswich Ltd., London Road, Ipswich, Suffolk, England). Prior to use, *Ricinus* seeds were stored at 2°C in a darkened refrigerator. *Helianthus* seeds were stored at 4°C in darkness in a seed-room.

Growth conditions

(a) Growth Cabinet

Where it was desired to maintain environmental conditions constant, a Fison standard climatic cabinet model 250 was used. The cabinet was set to cycle between 2 conditions of temperature and humidity and between periods of darkness and light. Except where stated otherwise, the cabinet was set for 14 hours photoperiod (radiation 32 W/m^2, (400 - 700 nm) measured with a UDT model 40x optometer). Temperature and relative humidity (%RH) during the light period were 30°C ± 1 and 65 ± 1%. During dark periods temperature was 30°C and RH was 95%.

Preliminary tests showed that plants became slightly shorter than normal. The middle vents in the cabinet were, therefore, sealed with plastic tape to minimise the amount of air flowing through the cabinet.

(b) Greenhouse

The greenhouse is situated at the Botany Research Laboratories

at Garscube. Between the months of October and May lighting was supplemented with artificial illumination provided by Thorn 400 MBFR/4 high pressure vapour mercury lamps. The lamps were spaced 0.8 metre (m) apart and hung 1.0 m above the greenhouse benches. Illumination was for 16 hours (03.00 to 19.00 hr. G.M.T.) and light intensity at plant level measured with a Megatron light meter ranged from 4,200 to 33,000 lux. During the same months the greenhouse was heated.

The heaters were off for the rest of the year and plants were also subjected to solar illumination only. On experimental days solar radiation at plant level was measured (arbitrary units) with Casella Radiometer Model 293. The instrument was calibrated against a Kipp solarimeter (Kipp & Zonen, Holland). 3.5 arbitrary units was equivalent to 100 Watts per metre square.

Temperatures and relative humidities were recorded continuously with a thermohygrograph. The recorded ranges are:-

Season	Temperature °C		Relative Humidity %	
	Day	Night	Day	Night
Winter	15 - 25	13 - 15	60 - 66	90 - 100
Summer	20 - 35	15 - 20	40 - 60	85 - 100

(c) Field conditions

Some experiments were conducted in the experimental garden at Garscube during the summer months. Environmental conditions on experimental days have been described in the relevant section.

Germination of seeds and growth of plants

Ricinus seed was soaked for 24 hours. Afterwards, the caruncle was removed and a small cut was made into the seed-coat at the

opposite end. This treatment hastens germination (Hall, personal communication). 25 seeds were germinated in moist vermiculite (Alexander Products Ltd., Burnham-on-Sea, Somerset, U.K.) in a perspex tray (21 x 35 x 5 cm) with a closed lid. Germination took place in an incubator at 30°C, 4 to 6 days after sowing. The cotyledons were red in colour and were held together by the remnant of the endosperm. This was removed by hand. The seedlings were transferred to the greenhouse to allow the cotyledons to turn green.

When seedlings were 10 to 14 days old they were transplanted singly into 9 cm plastic pots containing John Innes potting compost I (equal volumes of fine sand, loam and peat). The soil was prepared by the gardners at Garscube.

Usually after emergence of alternate leaves 3 and 5 (or leaf 6, depending on the size of the plant) the plants were potted up into plastic pots of 12 cm and 15 cm. diameters respectively. All the pots have drainage holes.

Seedlings became infested occasionally with red spiders. These were effectively eliminated with a Morestan smoke generator (contains 20% W/W quinomethionate) supplied by Pan Britannica Industries Ltd., Britannica House, Waltham Cross, Herts., England.

Helianthus seeds were germinated in moist Fisons Levington compost contained in a 21 x 35 x 4 cm. tray (25 seeds per tray) in the greenhouse. Germination occurred in 7 to 10 days. One week after germination, the seedlings were transplanted singly into 9 cm pots containing John Innes potting compost I. They were potted up successively into 12, 15 and 21 cm pots with drainage holes when they became pot bound.

Helianthus seedlings became infested occasionally by white flies. These were controlled by spraying with Murphy Liquid Malathion

(10 ml/4.5 litres).

Prior to experimentation pot grown Ricinus and Helianthus seedlings were watered daily and were liquid fed once a week with proprietary Liquinure solution (2 ml/litre). The liquinure ensures an adequate nutrient balance. Some of the plants were transferred to the growth cabinet when required. This occurred at least 2 weeks before they were needed for experimentation, thus allowing them to adjust to the growth cabinet conditions.

Apart from pot plants, field grown Ricinus plants were used. Greenhouse and growth cabinet plants beyond alternate leaf 10 stage were planted in rows in the experimental garden in early May (1975, 1976). The rows were 60 cm apart and the distance between each plant was 45 cm. Plants were irrigated naturally by rain water.

Induction of plant water stress

The water status of a soil-grown plant is controlled by the evaporative conditions of the atmosphere, soil-moisture tensions and the control the plant itself exerts over water loss and absorption. Manipulation of one or more of these controls have been employed in order to induce different degrees of internal water deficits during the studies. These have been described in the appropriate sections.

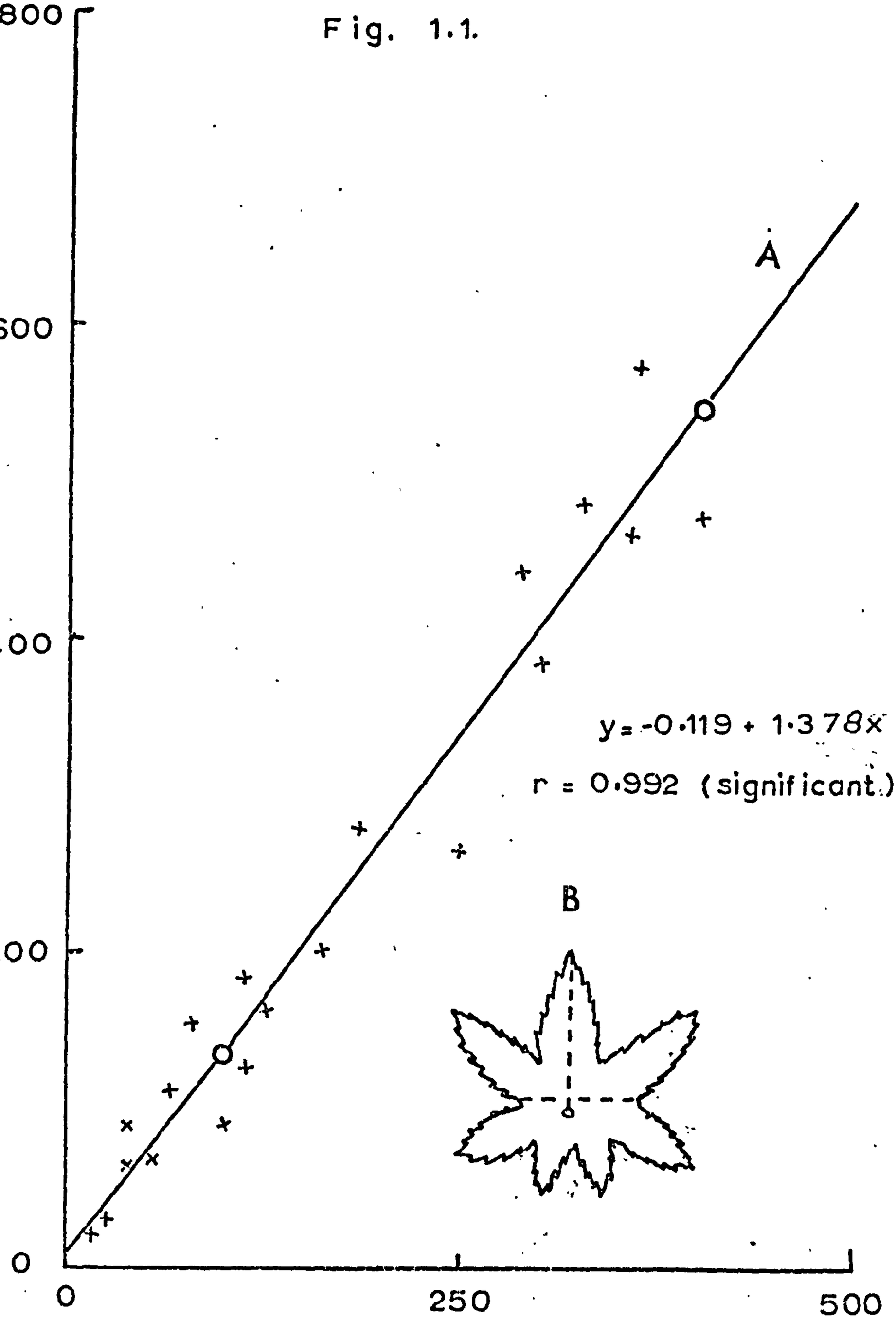
Estimation of leaf area

Methods of measuring leaf areas have been reviewed by Marshall (1968). In the present study, it was desirable to measure the leaf area while intact through time without destroying the leaf. This was achieved by first establishing length x breadth and area relationships from samples of leaves of Ricinus and Helianthus.

Figure 1.1 A: Regression of leaf area on length x breadth of greenhouse grown Ricinus plants.

B: Diagram of Ricinus leaf showing measurements made in determining area (broken lines).

Fig. 1.1.


800
600
400
200
0
Leaf area (cm2)
A
y = -0·119 + 1·378x
r = 0·992 (significant.)
B
0
250
500
Length x breadth

Apart from its importance to expansion growth studies, accurate estimation of leaf areas is also essential for transpiration-rate determination.

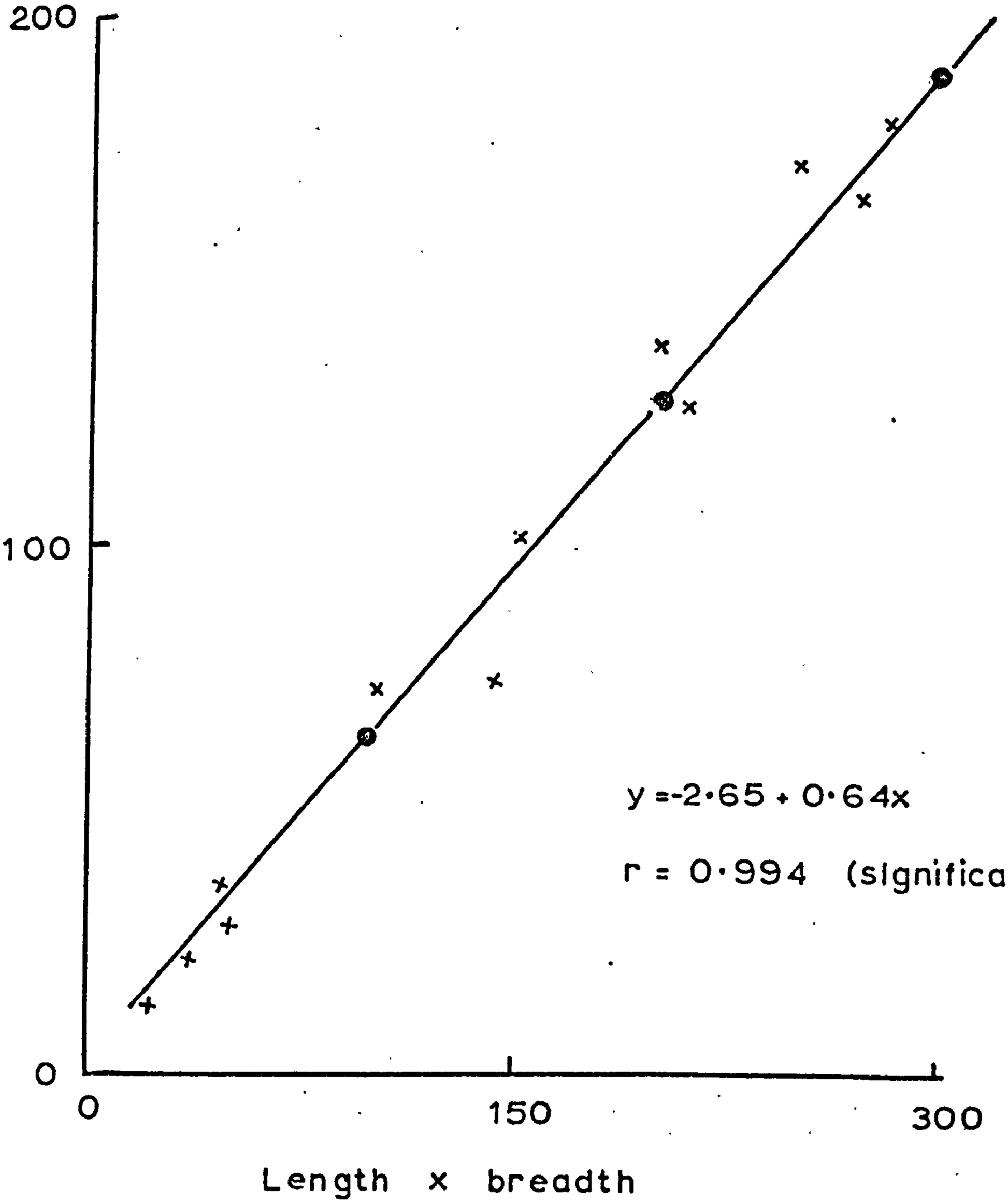
The leaf of Ricinus is orbicular in outline, peltate and deeply palmately lobed. There are generally 7 or 8 serrated lobes but exceptionally 6 - 9 or even 10 lobes. The length and breadth of about 40 leaves were measured using a ruler. The length (L) was taken as the distance from the tip of the terminal lobe to the point of attachment of the petiole. The breadth (B) was the distance between the second notches on either sides of the terminal lobe (figure 1.1). Preliminary study showed that these measurements were best correlated to the leaf area. After measuring the L and B, each leaf was traced on a hard uniform paper of known area. The paper was weighed and again a second time with the traced leaf portion carefully cut off. The ratio of traced leaf weight to total paper weight was determined, from which the area of the leaf was calculated by proportion. A graph relating leaf areas to L x B was plotted with a regression line fitted through the points (figure 1.1). The relationship was found to be suitable for both 7 and 8 lobed leaves.

The above relationship was made for well watered greenhouse plants during the winter months 1974, but it was also found suitable for growth cabinet and greenhouse plants growing under different moisture regimes. However, the relationship changed during the summer months for well watered greenhouse plants and a + 12% correction was made. The new relationship was found suitable for field grown Ricinus plants.

Helianthus has simple leaves which are easy to measure. A curve relating leaf area to leaf L x B was drawn up (figure 1.2). The length was taken as the distance from the tip to the point of attachment of the petiole to the blade. The breadth was the length

Figure 1.2 Regression of leaf area on length x breadth of greenhouse grown Helianthus plants.

Fig. 1.2.


200
100
0
0
150
300
Leaf area (cm2)
Length x breadth
y =-2·65 + 0·64x
r = 0·994 (significa

of the broadest portion of the blade.

Leaf areas of experimental plants were estimated using the appropriate regression equation after L x B measurements. Expansion growth was estimated solely by this method after an initial attempt to use a linear transducer to measure extension growth proved unsuccessful.

Determination of disc area

Discs areas were measured with the aid of a photographic enlarger (universal Alpha II Gnome). A disc with a drop of water was placed on the plate holder of the enlarger and covered with a plastic cover slip. The assembled holder was positioned in the enlarger. The image of the disc was focused with a tenfold magnification and it was exposed on a graph paper. First, measurements were made along 2 diameters, then along 2 more diameters after rotating the graph paper through about 45^{o}. The mean of the 4 diameters were determined and the disc areas calculated as πr^2.

Leaf thickness measurements

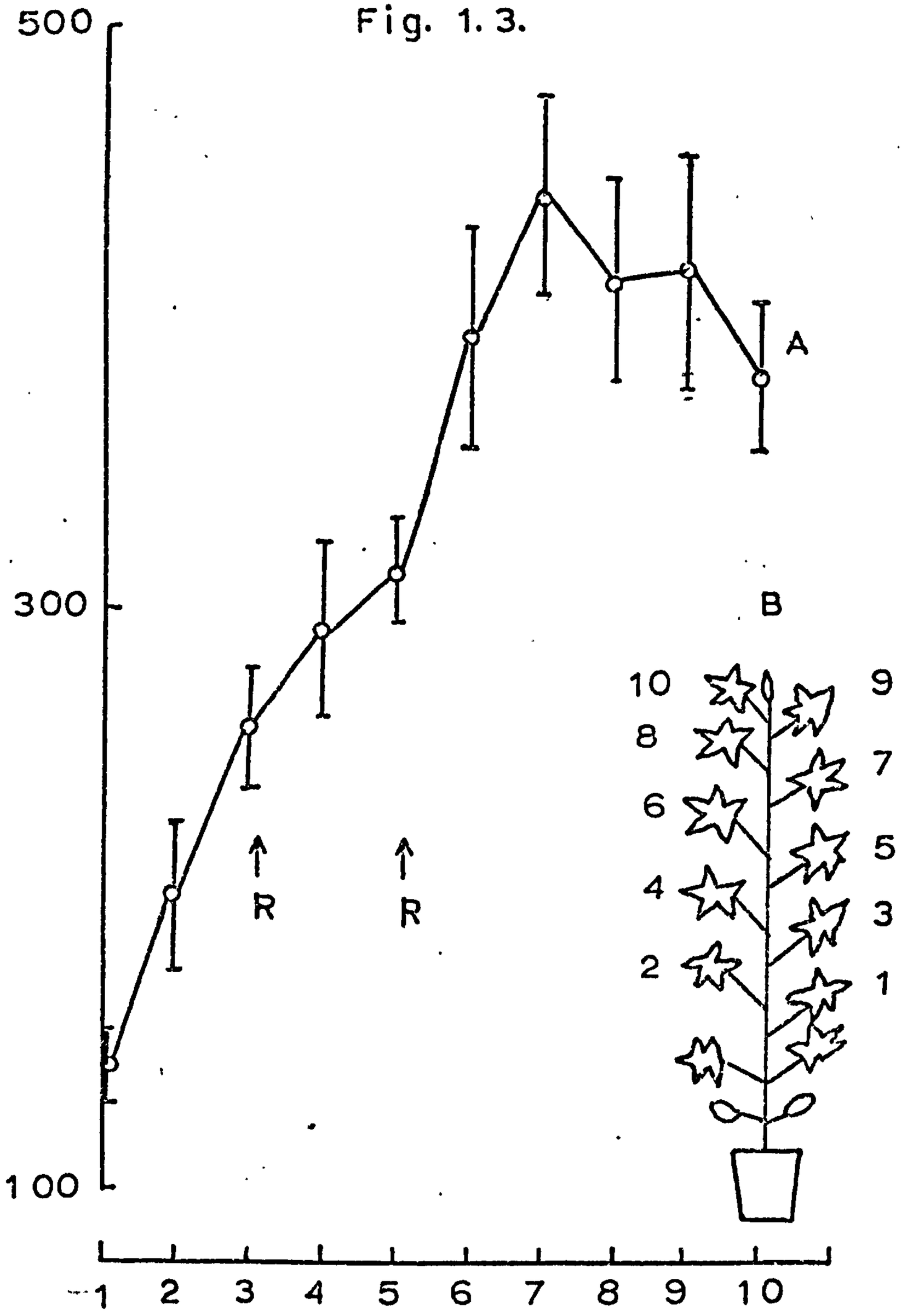
Leaf thickness was measured using a Mercer micrometer which was graduated to 0.01 millimeters. Leaf portions with less prominent veinlets were sampled and the thickness measured.

Measurement of plastochrone and leaf production

Growth potential of Ricinus leaves was also assessed by determining the plastochrone interval, and the rate of leaf production. Plastochrone, as originally defined, described the time interval between the beginning of successive leaf primordia (Askenasy, 1880; see Whaley, 1961). In the present study, it was measured as the time interval between opening of successive leaf buds. This

Figure 1.3 Mean leaf area (with standard error) of alternate leaf 1 to 10 of Ricinus (A). Diagram of Ricinus showing alternate leaf 1-10 (B).

Fig. 1.3.



R=repotting

modification was non-destructive and also convenient. It is also comparable with the modifications adopted by Milthorpe (1956). Leaf production was taken as the reciprocal of the plastochrone.

Estimation of % leaf maturity

In section 5 it was found necessary to relate leaf developmental stage and the maximum xylem tensions it develops in an oil bomb. However, the oil bomb technique has the disadvantage of requiring excised leaves. Once a growing leaf is cut, it is not possible to assess directly its overall potential growth performance. It was, therefore, necessary to establish some measure of leaf maturity and the method of Bünning (1956) was adopted.

The first 2 leaves of Ricinus seedlings are 5 lobed and are opposite to each other. These are followed by alternate leaves (7 to 8 lobed) which are the 'normal' leaves of Ricinus. The course of development of alternate leaves 1 to 10 were followed by measuring their areas at 3 or 4 days interval until they ceased to expand (i.e. they 'matured'). The plants were well watered and were initially in 9 cm pots but were potted up into 12 and 15 cm pots at 3 and 5 leaf stage respectively.

Figure 1.3 shows a summary of the results (mean with standard errors). Leaf 7 was chosen for experimentation in the subsequent study because it can reach a very large area and therefore offered a reasonable sized sample for the bomb at most stages of its development. The maturity was estimated as -

$$\text{Leaf maturity \%} = \frac{\text{Area of leaf 7 when used for experiment}}{\text{Area of leaf 6 (on same plant) when fully mature + 12\%.}} \times \frac{100}{1}$$

Anatomical Studies

Transverse sections of leaves were prepared by Mrs. D.L. Leake of the anatomy and microscopy laboratory of the Botany Department, Glasgow University. Photographs were taken using a Carl Zeiss Photomicroscope II with an automatic exposure control.

Measurement of Plant Water Status

Over the years investigators have developed various methods for measuring and expressing plant water status. These methods have been discussed and reviewed by Hewlett and Kramer (1963), Barrs (1968) Boyer (1969) and Slavik (1974). Normally the methods measure the volumetric water content which is expressed here as the relative water content (RWC 1°C), or the energy status of water in the plant expressed as the water potential.

Measurement of Relative water content (RWC 1°C)

In order to minimise injection via cut edges the ratio of leaf material to cut edge was minimised by using large disc sizes (1.75 cm or 1.45 cm diameters for Ricinus and Helianthus respectively). Discs were punched from 'mature' leaves with cork borers each fitted with a spring loaded piston. This facilitated rapid ejection of the discs into black sampling bottles with lids. The discs were sampled near the midrib thus the leaf margins and tips were avoided. The leaves were supported during sampling on a rubber bung. Two to three discs were transferred at a time from the collecting bottle into a weighing bottle of known weight. The fresh weight was measured on a Unimatic CL41 balance with ± 1 mg precision. These discs formed a sample.

After determination of the fresh weight, the sample was floated on 10 ml distilled water at 1°C (cf Milburn and Weatherley, 1971) in

4.5 cm diameter closed Petri-dish. The Petri-dish was transferred into a perspex tray $\frac{1}{6}$ filled with water and suspended on a glycol anti-freeze contained in a Gallenkamp refrigerator. The refrigerator was controlled at $-5^{\circ}C$. It was covered with a polythene sheet to reduce warm air convection. The water in the Petri-dish during experimentation remained at $1^{\circ}C$.

The sample was illuminated at around compensation point (see below). After 4 hour floatation, the discs were taken with a pair of forceps and surface dried carefully between 8 layers of absorbent tissue, and the turgid weight (TW) (assuming growth was negligible) obtained. The sample was then dried in a Gallenkamp hot box oven (OV100) at $95^{\circ}C$ for 24 hours, and reweighed to obtain the dry weight (DW). The 24 hour drying was found to be convenient. The RWC ($1^{\circ}C$) was calculated from the following equation (cf Barrs and Weatherley, 1962).

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

Injection and dry weight changes during floatation were relatively unimportant under this experimental set up.

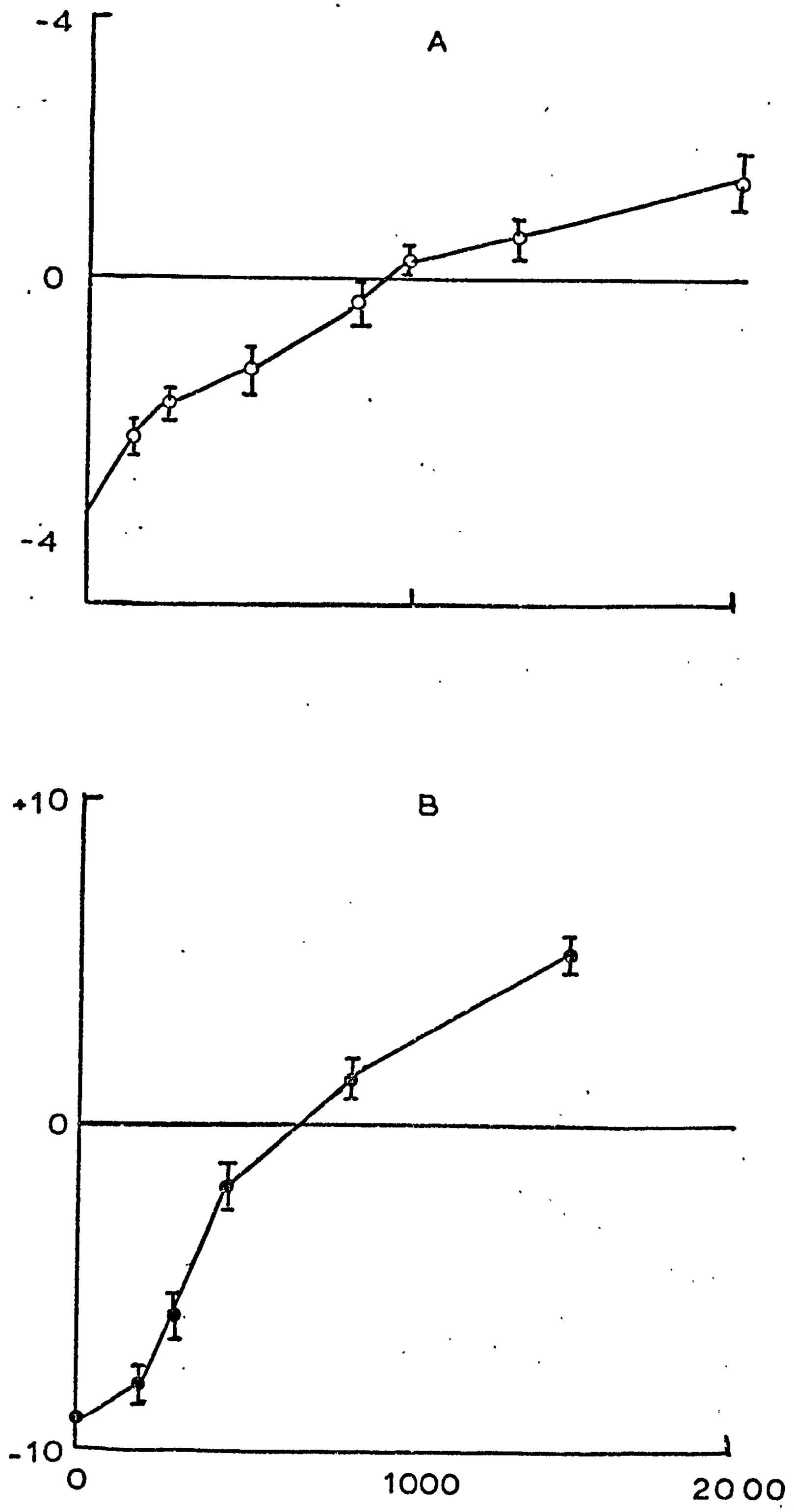
Determination of compensation point

For RWC, water uptake and oil bomb experiments it was desirable to illuminate samples around compensation point so as to minimise changes in dry weight and CO_2 narcotisation during experimentation.

Discs punched from a mature leaf were divided into 7 groups of 4 discs each. The fresh weight of the groups were similar. One group was oven dried immediately at $95^{\circ}C$ for 24 hours. The dry weight measured was taken to represent the initial dry weights of the remaining samples. The remaining 6 groups were each floated

Figure 1.4 Effect of light intensity on dry weight of leaf discs of Ricinus (A) and Helianthus (B) floated on water.

Fig. 1.4.


A
-4
0
-4
B
+10
0
-10
0
1000
2000
% change in dry weight
Light intensity (lux)

(for 48 and 24 hours for Ricinus and Helianthus respectively) on distilled water at 30°C contained in closed Petri-dishes. 5 of the Petri-dishes were placed at different distances from a 0.6M long 20 watt fluorescent tube (Ricinus) or a bank of 2.4 M long 60 watt fluorescent tube (Helianthus) in a dark room. A glass tank of water isolated the light from the Petri-dishes to minimise radiant heating of the Petri-dishes. The 6th Petri-dish was enclosed in a black paper bag and left on a bench in the dark room. The temperature of the room was maintained at 30°C by a Humex Turbo heater (Model TP/1, 1.5 k.w. capacity).

After floatation the discs were mopped dry and were oven dried at 95°C for 24 hours. Percentage changes in dry weight were calculated (% of initial dry weight) and plotted against light intensity which was measured with the Eel photometer. The experiment was repeated several times and the results for each species were similar, examples of which are shown in figure 1.4.

Measurement of plant water potentials

The energy status of water in a tissue (or its ability to do work) is expressed as its water potential (Y). This is proportional to the relative chemical potential of water, either, to the difference between chemical potential of pure water and chemical potential of water in the tissue. The water potential of pure water at atmospheric pressure is 0 and it is decreased (ve -) by addition of solutes or when attracted to surfaces. In the plant the Y has the following components.

$$Y = Y_p + Y_s + Y_m + Y_g$$

where p, s, m and g refer to pressure (turgor or tension) solute

(osmotic), matric and gravitational components, Y_s, Y_m and Y_g are negative components while Y_p is either positive or negative. Y_g is the component due to elevation and so is significant in tall trees. Y_m effects are assumed to be negligible between full turgid and tissues at incipient plasmolysis (Wiebe, 1966). Thus, in normal small plants, Y may be expressed in terms of Y_s and Y_p.

$$Y = Y_p + Y_s$$

These terms are widely used and are adopted here being expressed in bar (1.0 bar equals 10^5 Pa). Y is numerically equal but opposite in sign to the suction pressure of Stiles (1922) or the diffusion pressure deficit (DPD), of Meyer (1945). Similarly, Y_s is equivalent to but opposite in sign to osmotic pressure (cf Meyer, 1945).

Water potential

A large number of samples was handled at a time and as such an effective and yet rapid technique was required to measure water potential. The pressure bomb technique of Scholander et al (1964) was, therefore, used. The technique is based on the fact that if a leaf or stem is cut, the water columns will retreat from the exposed ends of the conduits as a result of the negative pressures in the xylem. If a pressure is applied to the leafy end, the cut end being maintained at atmospheric pressure, xylem sap will be expressed from the cut end. The pressure applied (P) increases the Y of the leaf cells adjoining the xylem to a value equal to the osmotic pressure of the xylem sap (Y_{xs}) at atmospheric pressure:-

$$Y = P + Y_{xs}$$

(Y is equivalent to leaf water potential assuming leaf and xylem

water is in equilibrium. The bomb pressure balances the xylem pressure potential or the xylem tension (Y_x). The latter term is used in this thesis, it is positive in sign. The Y_{xs} component of the water potential is usually very high (close to zero) and is often assumed to be insignificant. However, the magnitude varies with species (cf Kaufman, 1968a)and for accurate measurement of Y it is necessary to evaluate Y_{xs}.

The air bomb and measurement of Y_x

The air bomb used to measure xylem tensions of leaves is shown in plate 1.1. The chamber of the bomb is made from stainless steel and is 15.2 cm deep and of 5.7 cm diameter. The chamber has a screw cap lid with a hole for a rubber bung which contains the petiole. Pressure is supplied from a compressed air cylinder which is connected to the chamber and to a pressure guage of 300 lb./in^2 (14.5 lb/in^2 = 1 bar) capacity.

An experimental leaf was enclosed in a polythene bag while intact for at least one hour. Obviously in a transpiring leaf the Y of cells distant from the xylem would be more negative than for cells adjoining the xylem conduits. Bagging, therefore, prevents transpiration but also allows the xylem tension to equilibrate with the leaf cells water potential. After excision the petiole was fitted, with the aid of tubular cork borers, inside a hole in a 1.6 cm long number 21 rubber bung. About 0.5 cm of the cut end of the petiole was allowed to protrude for observation. When necessary, a glass rod was pushed into hollow- petioles of Ricinus to prevent them from being crushed during sealing in the rubber bung. The bung was fitted inside the lid of the bomb chamber and the lid was screwed onto the bomb. In this way the polythene bag with the

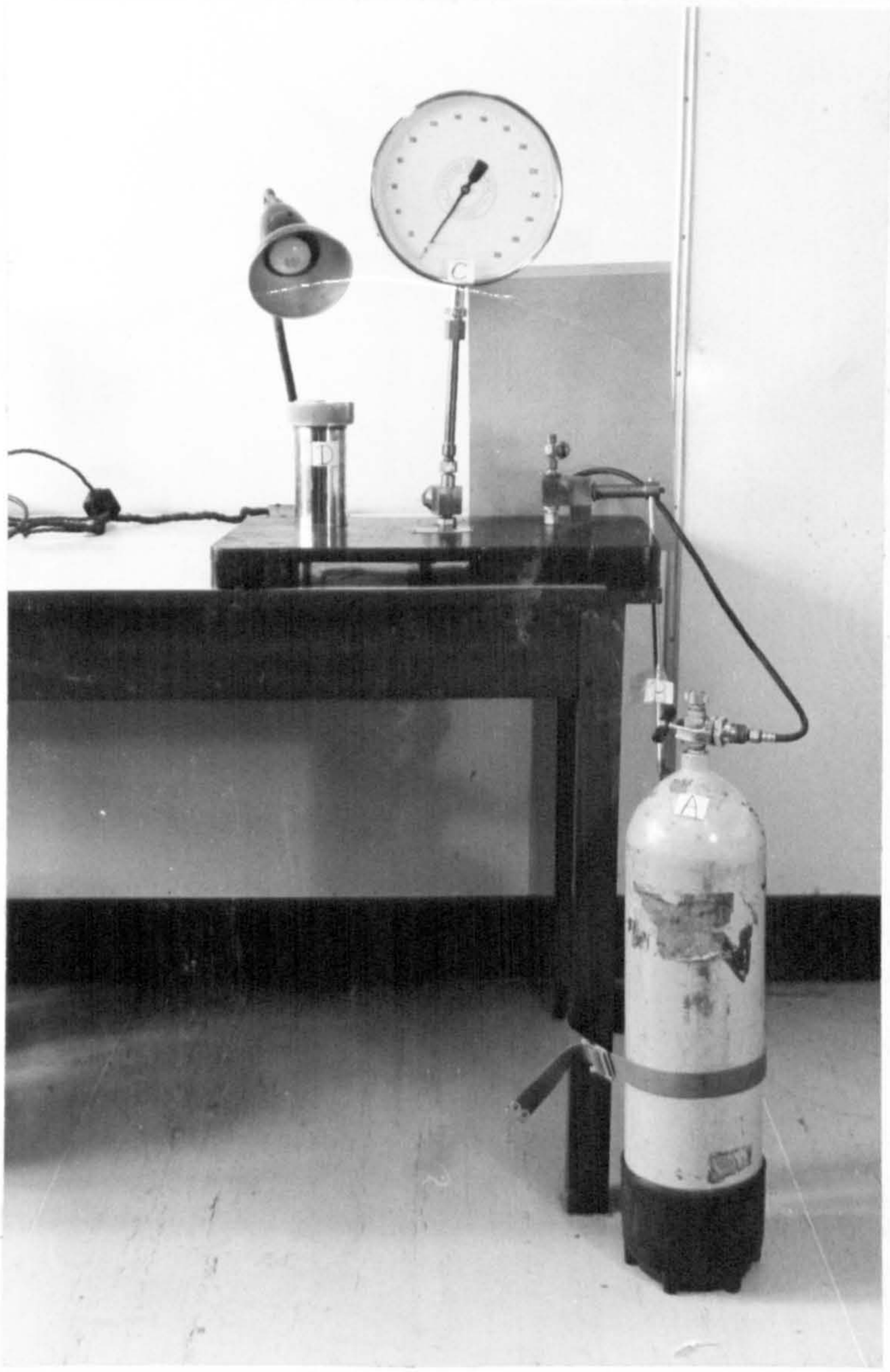


Plate 1.1 The air bomb.

(A) Cylinder with compressed air.

(B) Handle for controlling inlet/outlet valve.

(C) Pressure gauge (lb/in^2).

(D) Bomb chamber.

sample was sealed inside the bomb.

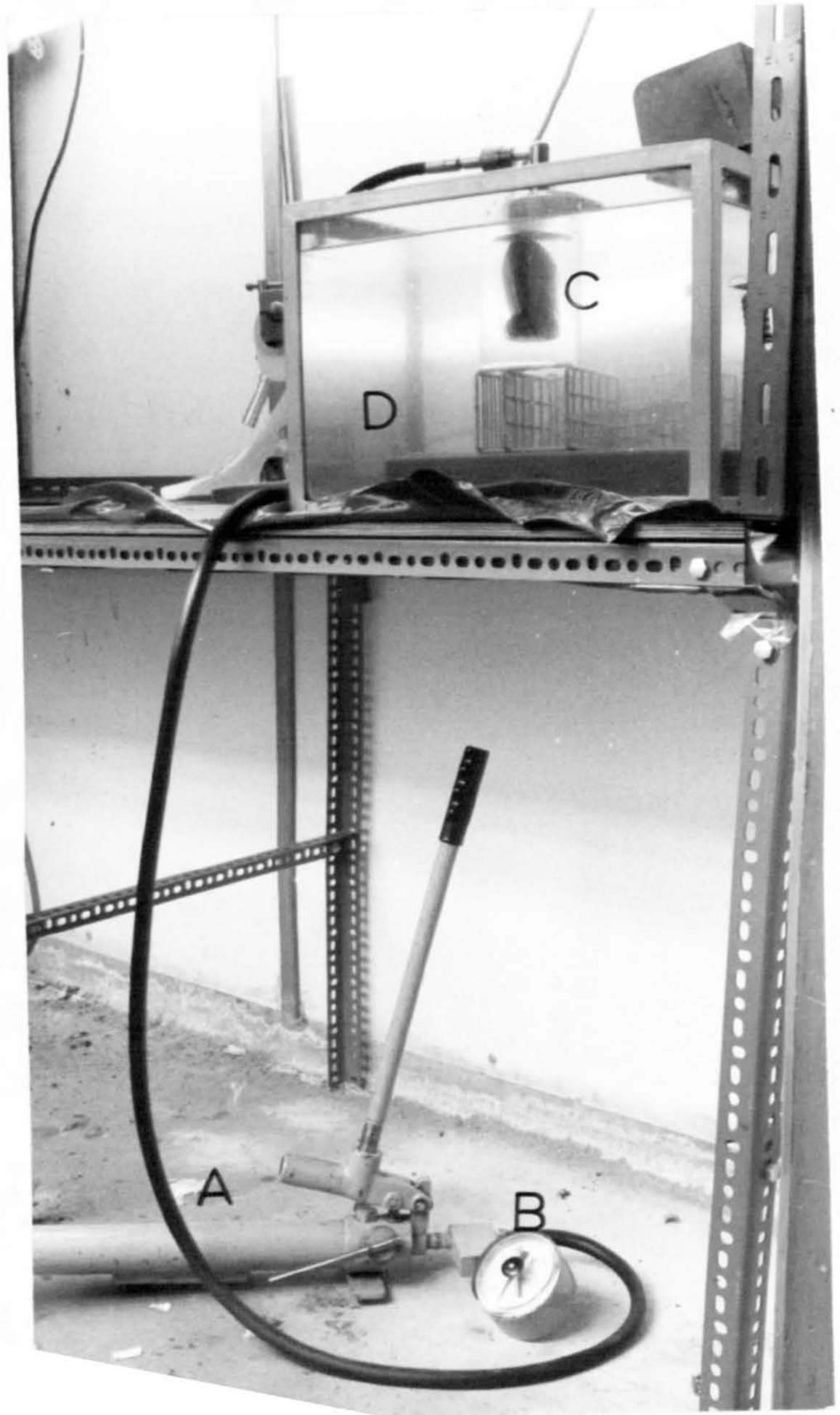
The bomb pressure was increased gradually (cf Waring and Cleary, 1967) and the pressure to produce incipient xylem sap exudation was measured. When this pressure was missed, the bomb was partially vented to return the sap to the xylem. The measurement was repeated. Similarly, a measurement was repeated, if froth instead of water was exuded in the first instance. Normally bomb measurements took less than 5 minutes.

Bagging the leaf helped to minimise water loss during bomb measurements (cf Gee et al, 1974). The petioles were not re-cut after excision in order to avoid errors due to increases in Y_p of the leaf cells (cf Scholander et al, 1964). However, the cut end was trimmed slightly to ensure that the vessels were unsealed when measurement lasted for more than 5 minutes. With all these precautions, it was assumed that the Y_x measured and the Y estimated (after corrections for Y_{xs}) were accurate (cf Boyer, 1969).

An attempt to compare the estimated Y with values measured directly with an 4R33 dew point micro-voltmeter was unsuccessful owing to a technical fault developed by the micro-voltmeter.

The oil bomb and measurement of Y_x.

A photograph of the oil bomb is given in Plate 1.2. The basic design and operation is essentially similar to the air bomb. However, the oil bomb has a transparent thick walled perspex chamber, 13.5 cm high, and 9.5 cm external diameter, with an inner volume of 260 cc. The chamber lid is made of brass and is in 2 parts, an inner circular piece (5.6 cm diameter) and an outer screw head piece. The two fit together and, with an intervening 'O' ring gasket, provided a perfect seal during operation. On the inner piece is a hollow raised pillar



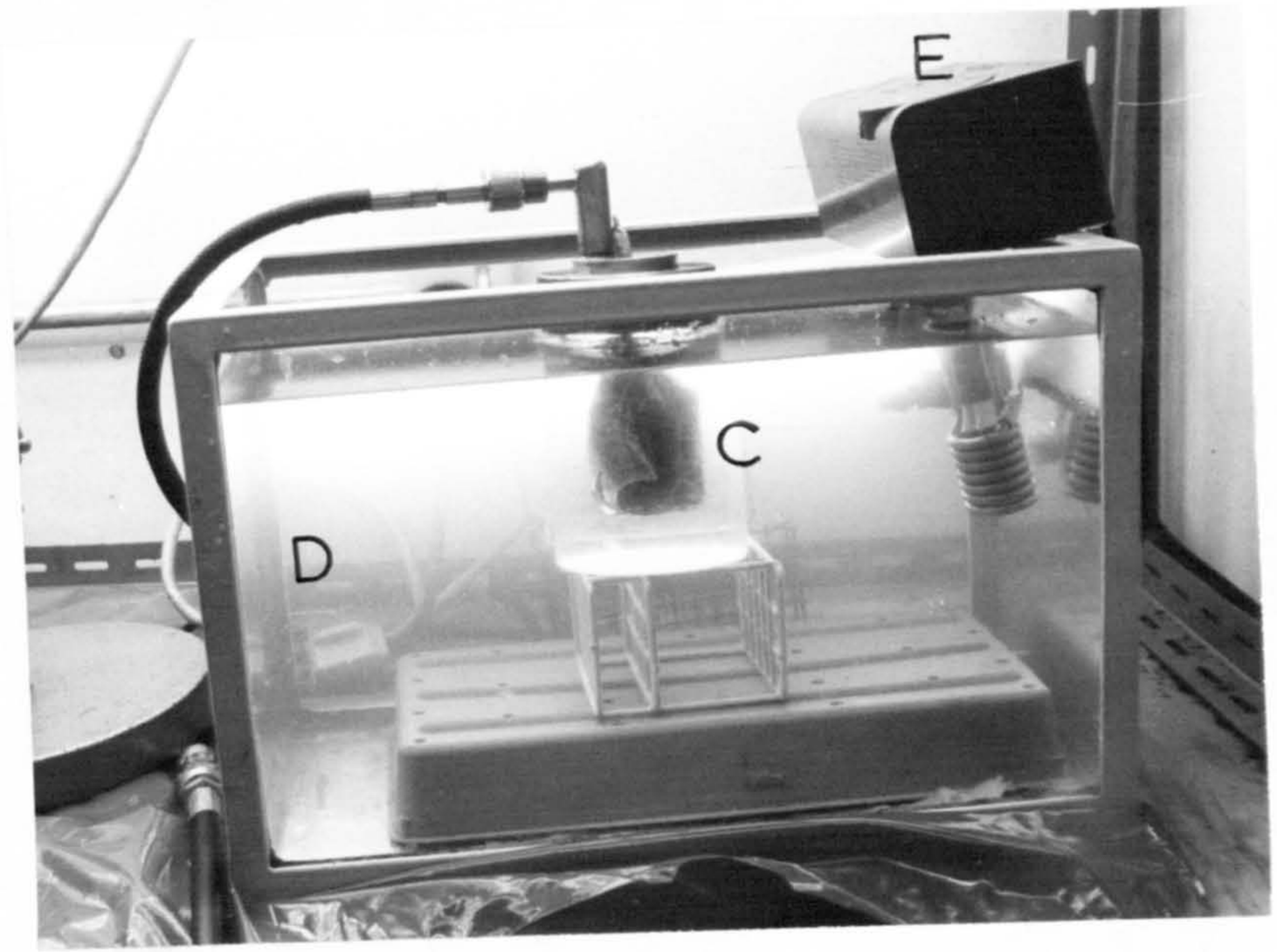


Plate 1.2 The oil bomb
- A Hydraulic pump
- B Pressure gauge
- C Bomb chamber
- D Water bath
- E Thermostat

screwed to a high pressure hose (SCUBA) to the hydraulic pump (Tangy Epco, supplied by Buck and Hickman, 49 Robertson Street, Glasgow, G.2.). The pump is 50 cm long and has a diameter of 6 cm. It has a valve for controlling pressure inflow and outflow and a handle which is operated manually to raise the bomb pressure. Centrally in the lid is a hole for accommodating a number 15 rubber bung which seals the leaf petiole.

Both the bomb and the pump were filled with paraffin oil 'light' (Medicinal liquid paraffin B, 0.83 to 0.87 g ml^{-1} at 20°C supplied by Hopkins and Williams, Asschem, Reading Industrial Estate, Falkirk, FKZ 11S, Scotland). The blade of the leaf was submerged in the bomb. The paraffin prevents transpiration and provides a non-toxic environment for the leaf (Potter and Milburn, 1970). The bomb temperature was maintained at 30 ± 1°C by submerging it in a continually stirred glass water-bath controlled at this temperature. Illumination (1000 lux) of the bath was provided by a 20 watt white fluorescent tube. The actual light intensity reaching the leaf was not measured, but was assumed tobe around compensation point.

When the bomb is pressurised, initially air bubbles are released from the leaf and the stomata become injected making the leaf translucent within seconds of pressure application. This indicates that the stomata offers least resistance to intercellular space injection of the oil.

(a) <u>Calibration checks and tests against an air bomb and checks on Y_s measurements</u>

To check the accuracy of the oil bomb, xylem tensions (Y_x) values measured with it, were compared with values obtained from the air bomb. It was also found necessary to assess changes in osmotic potential (Y_s) which might occur as a result of treating leaves

with paraffin oil.

Equilibrated (bagged) 'mature' leaves of Ricinus at various levels of water potentials were used. The xylem tensions were measured first in the air bomb. Afterwards the bag was transferred to a humid room (RH over 90%) opened and part of the leaf blade was quickly cut, resealed in another bag and frozen for Y_s measurement cyoscopically. After replacing the number 21 rubber bung on the petiole with a number 15 bung the remaining leaf portion was sealed inside the oil bomb and the Y_x measured. The time interval between air bomb and oil bomb measurements was about 5 minutes. The leaf was removed from the bomb, mopped with absorbent paper then bagged and frozen for Y_s measurement.

Table 1.1

Xylem Sap Tension Measurements: Air Bomb - Oil Bomb
Some Leaves Given Water Previously
All Leaves were Equilibrated in Humid Air

Experiment number	Xylem sap tension (bar)		Discrepancy between values
	Air Bomb	Oil Bomb	
1	2.3	2.3	0.0
2	2.3	2.4	0.1
3	2.8	3.0	0.2
4	3.1	3.2	0.1
5	3.4	3.4	0.0
6	3.7	3.7	0.0
7	4.4	4.4	0.0
8	4.8	4.9	0.1
9	5.2	5.3	0.1
10	5.5	5.6	0.1
11	7.4	7.8	0.4
12	8.3	8.7	0.3
13	8.8	8.6	0.2

Contd./

Experiment number	Xylem sap tension (bar) Air Bomb	Oil Bomb	Discrepancy between values
14	9.5	9.5	0.0
15	10.2	10.3	0.1
16	11.7	12.9	1.2

The results are given in Table 1.1 for Y_x. Differences between the values from the 2 bombs are generally within experimental error with a single exception where there was a discrepancy of 1.2 bar below air bomb reading of 11.7 bar. Such a large discrepancy can arise when the oil fails to penetrate the intercellular spaces probably owing to tightly closed stomata. This can be overcome by punching the leaf epidermis with pinpricks or razor-blade slits.

Table 1.2

Comparison of Osmotic Potential of Sap from Air-bomb and Oil-bomb leaves
Osmotic Potentials were determined by Cryoscopy of Sap Centrifuged from frozen leaf tissue

Experiment number	Cell Sap Osmotic Potential (- bar) Air Bomb	Oil Bomb	Discrepancy between values
1	9.2	0.9	0.7
2	*10.3	12.3	2.0
3	10.9	10.8	0.1
4	11.3	11.4	0.1
5	11.5	11.5	0.0
6	11.9	11.1	0.8
7	12.2	12.2	0.0
8	12.2	12.0	0.2
9	13.1	13.7	0.6
10	13.2	13.7	0.5
11	14.0	14.0	0.0
12	14.3	13.6	0.7
13	14.4	13.6	0.8
14	*14.5	13.7	0.8
15	*15.5	14.5	1.0

* Small sample volume.

Differences between Y_s of sap from leaves treated with oil and air pressures (Table 1.2) proved to be negligible (0.0 to 0.8 bar) provided the sap samples were sufficiently large to ensure minimal oil contamination.

(b) Tests of the pressurisation procedure

During the preliminary studies, the oil bomb was not completely vented between readings. The method however was abandoned because it was considered that water could be expelled from the mesophyll cells into the xylem thus lowering the Y_x to atmospheric pressure. Consequently the cells of the petiole protruding beyond the bomb might absorb some of this water. When this happens greater pressures are necessary to extract water from the mesophyll cells than should be necessary.

In subsequent studies the bomb was completely vented so as to keep the xylem sap under tension. Pressure was applied to the leaf intermittently. This procedure can however, draw gas into the xylem but the set up was considered more natural than the first approach. During venting, the pressure was released slowly in order to avoid cavitation of the xylem columns.

Measurement of cell sap osmotic potential, Y_s

Solute potentials of leaf cell sap and xylem sap were determined by cryoscopy. The technique involves the measurement of the freezing point depression of the sap. The principle is based on the fact that the addition of a non-volatile solute to pure water depresses its freezing point through lowering its relative vapour pressure. The degree of depression is proportional to the number of molecules of solute present. The freezing point depression is linearly

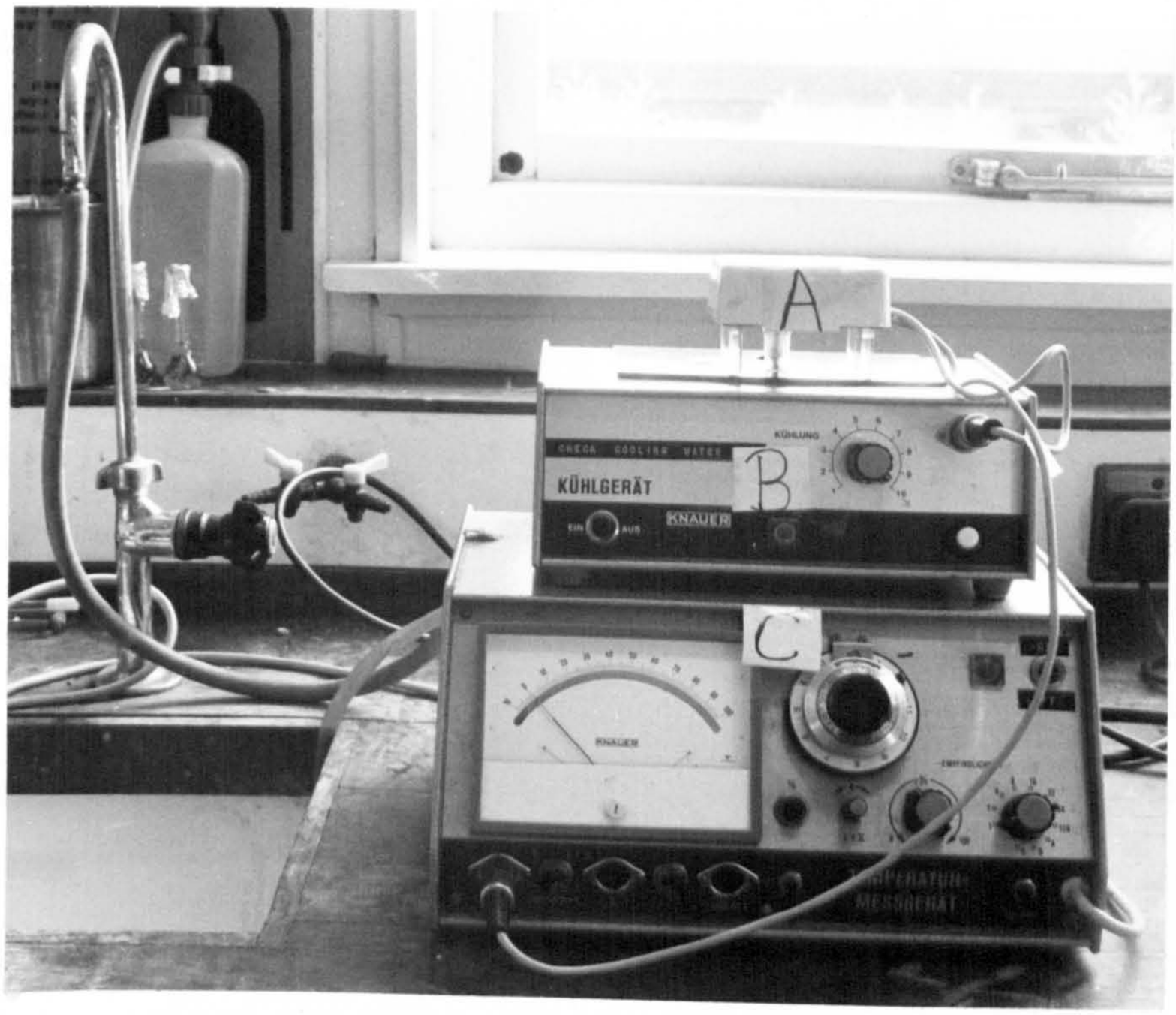


Plate 1.3 The Cryoscopic equipment.

(A) Measuring head of

(B) Thermoelectric cooling unit connected to

(C) Electronic temperature measuring instrument.

related to the osmotic potential of the solution (Slatyer, 1967). Other methods for osmotic potential measurements have been reviewed by Barrs (1968).

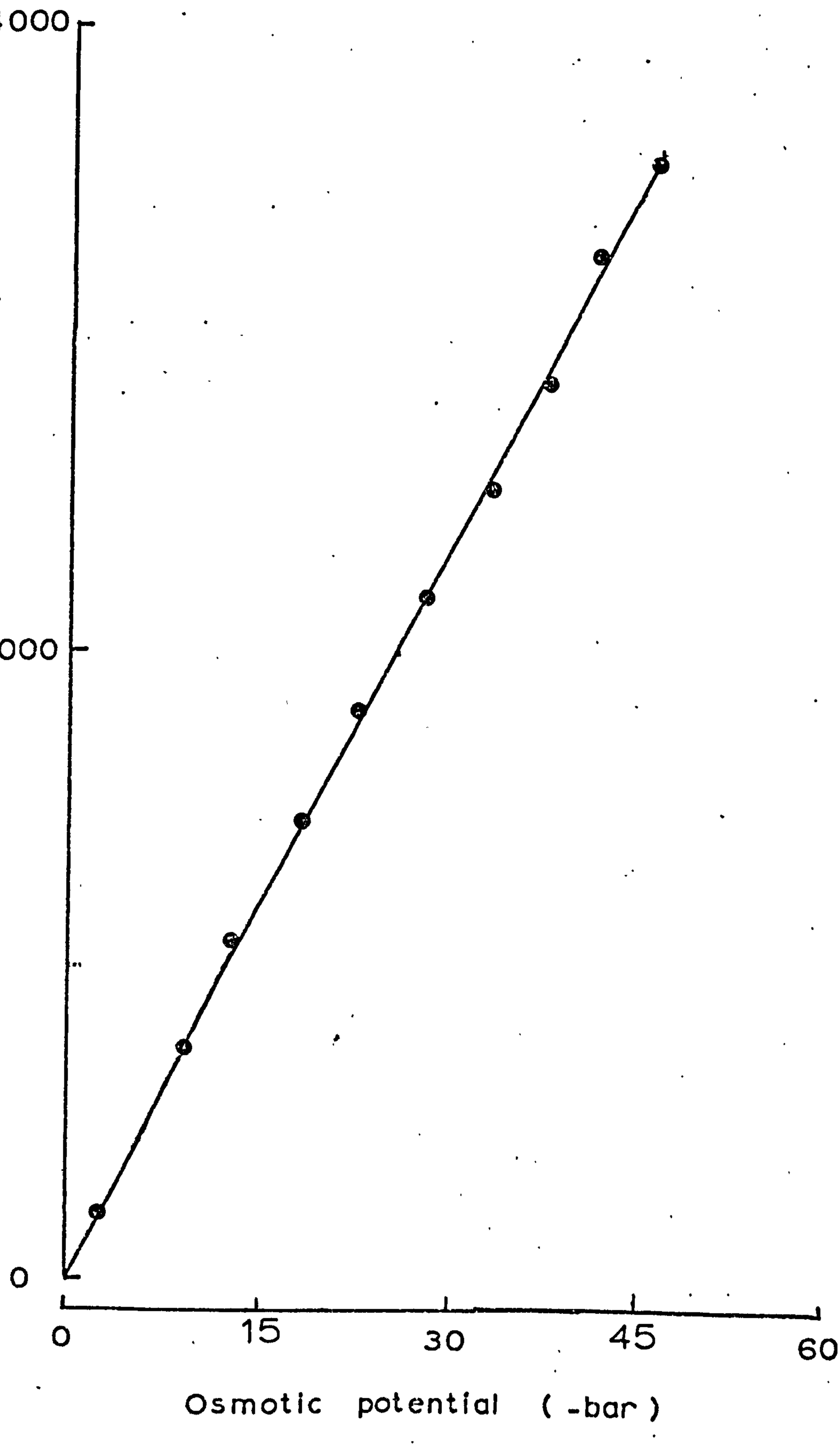
The cryoscopic unit used consisted of a thermoelectric cooling unit connected to an electronic temperature measuring instrument (Plate 1.3). The instrument was purchased from Knauer, Wissenschaftlicher Geratebau, KG Dr - Ing. Herbert Knauer and Co. GmbH, 1 Berlin 37, Holstweg 18. Ruf: 848705. The method of operation is detailed in the operation manual.

The freezing point lowering of solutions are recorded in arbitrary units (cryoscopic units). The instrument was therefore calibrated initially using deionized water and molal solutions of sodium chloride. The osmotic potentials of the molal NaCl solutions at 0° and $30^{\circ}C$ were obtained from standard tables of Lang (1967). One hundred microlitres of the solution measured accurately with a micropipette was used. Calibration was conducted with the super-cooling set at 40 scale division. The calibration curve is shown in figure 1.5. From the curve it was estimated that Y_s of -1.0 bar was equivalent to 76.27 cryoscope divisions at $30^{\circ}C$. Using this relationship, the Y_s of molal sucrose solutions measured on the cryoscopic unit were found to vary from -0.5 at lower concentrations to + 1.3 bar at higher concentrations (around 1 molal) as compared with values derived from Robinson and Stokes (1959).

After using the cryoscopic unit for two years the freezing unit lost efficiency and it became difficult to induce freezing as described. (This was not even remedied by passing the cooling water first through a cold refrigerator before circulating through the freezer unit). The instrument was, therefore, re-calibrated at a lower super-cooling temperature corresponding to 30 scale division. This allowed a wider

Figure 1.5 Graph relating cryoscopic values of molal NaCl solutions to their osmotic potentials (at 30°C). The osmotic potential values were obtained from standard tables of Lang (1967).

Fig. 1.5.


4000
2000
0
Cryoscopic division
0
15
30
45
60
Osmotic potential (-bar)

super-cooling range on the meter. The new standard curve using molal NaCl solutions gave -1.0 bar equal 62 cryoscopic divisions at 30°C.

For accurate measurements, the following precautions were taken.

1. The super-cooling temperature was kept constant at the calibration value at all measurements.
2. At the beginning of each experiment and during experimentation the instrument was re-calibrated with de-ionized water. Occasionally checks were carried out using NaCl or sucrose solutions of known Y_s.
3. 100 microlitres of solution was used for each measurement.
4. When measurement of a sample was repeated, the sap was thawed and stirred thoroughly to ensure mixing of dissolved ice and solutes.
5. The temperature sensors and the measuring vessels were rinsed and dried before each measurement.
6. During experimentation samples were kept in a cold room ($4 \pm 1°C$) and were removed in turns for measurement. The cold storage helped to minimise changes in sap composition through chemical reaction.

Measurement of Y_s and Y_{xs} by refractometry

The refractive index (RI) of a solution is proportional to its concentration. This allows the method to be used to determine osmotic potentials of solutions after initially constructing a standard curve for the relationship (cf Barrs, 1968). Apart from the ease and rapidity of the technique, refractometry requires very little solution and was, therefore, employed when samples were too small for cryoscopy.

Plate 1.4 The Abbé refractometer (C) connected to a water bath (B)

(A) Thermostat and water pump.

(D) Thermometer.

(E) Illuminator.

Figure 1.6 Regression of osmotic potential on refractive index (RI) of Ricinus leaf cell sap at 30°C. The refractive index of distilled water at 30°C is 1.3330.

Figure 1.7 Relationship between refractive index and osmotic potential of xylem sap of Ricinus at 30°C.

Fig 1.6.

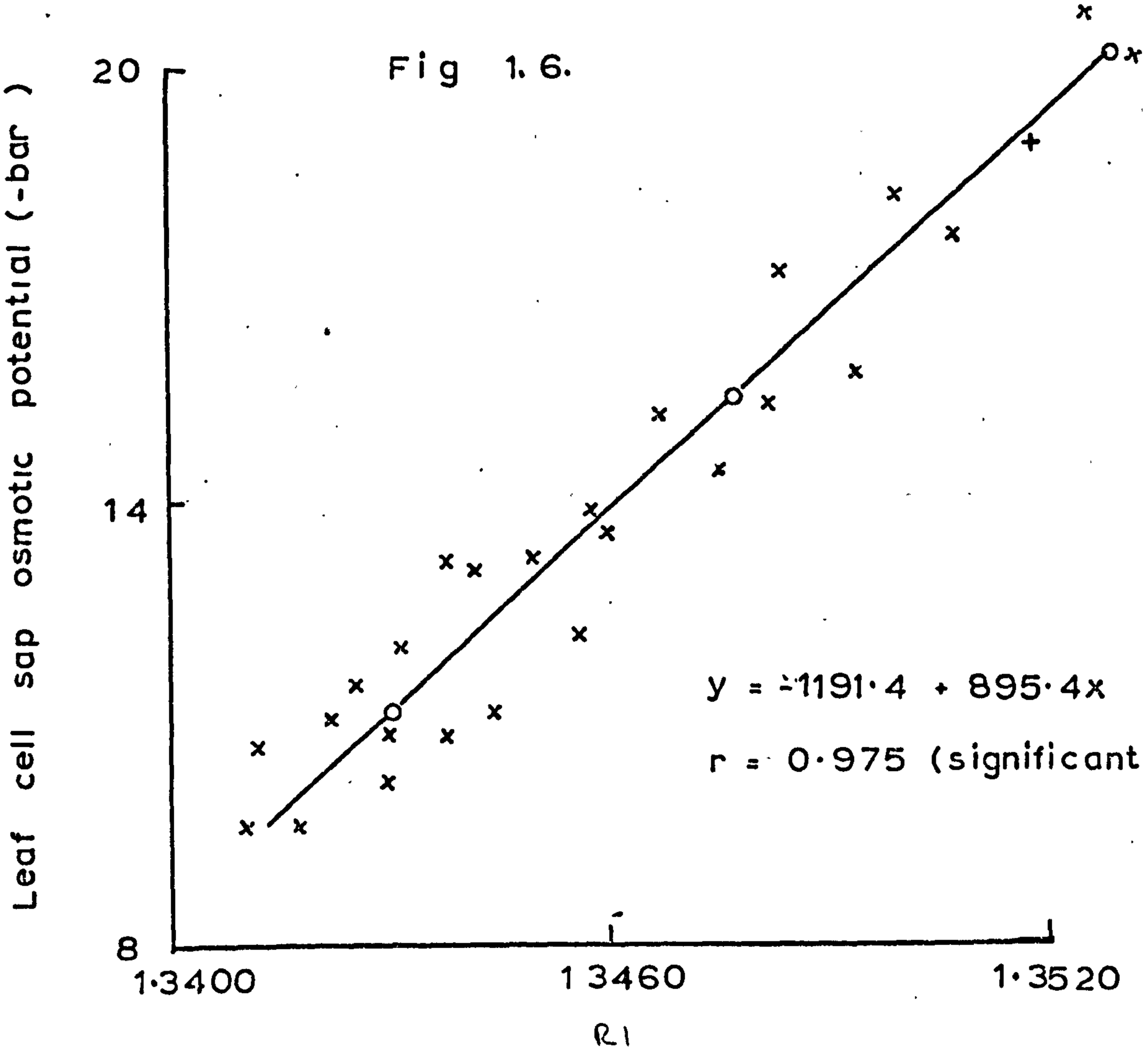
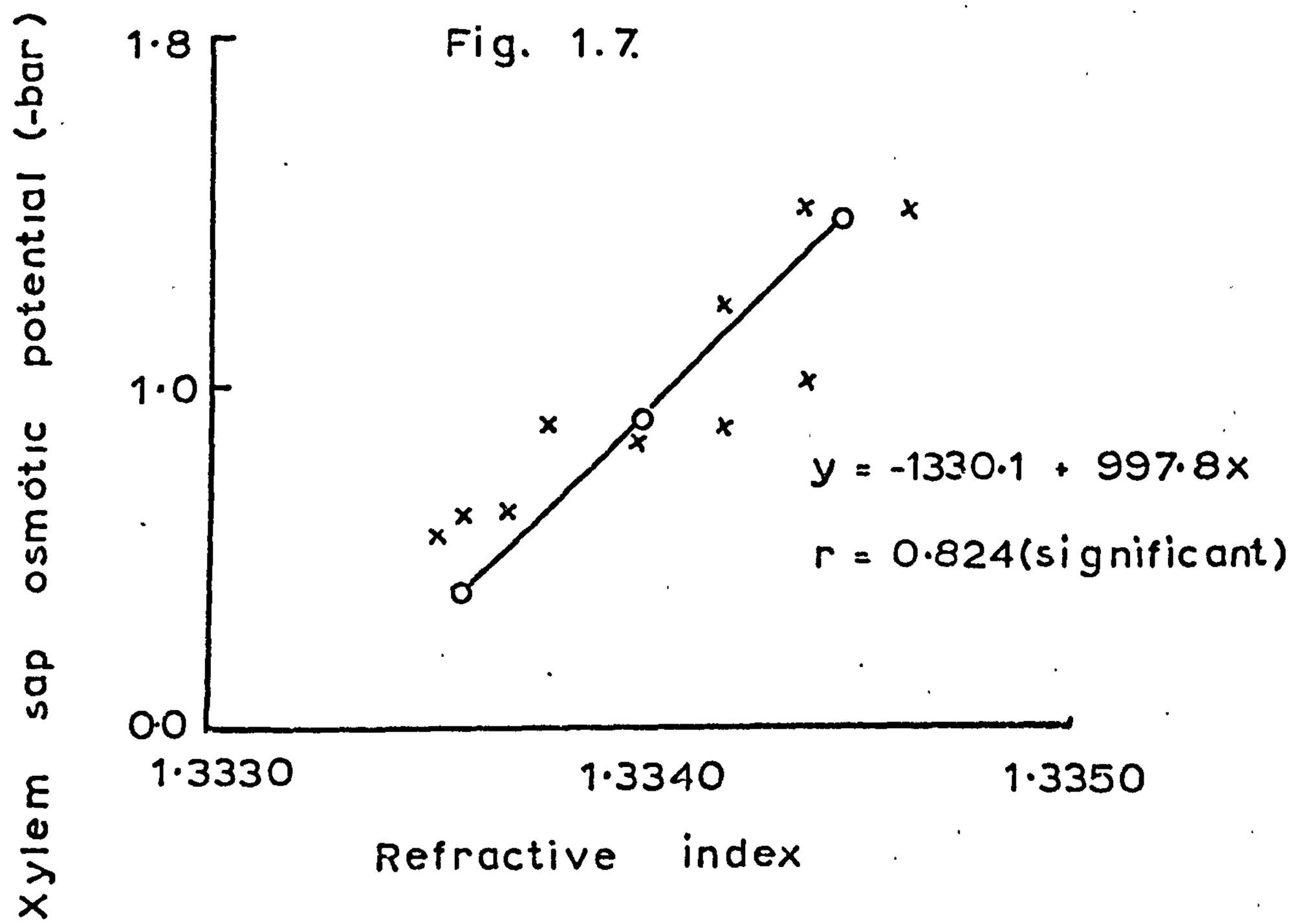

Leaf cell sap osmotic potential (-bar)
20
14
8
1·3400
1 3460
1·3520
R1
y = -1191·4 + 895·4x
r = 0·975 (significant)

Fig. 1.7.


Xylem sap osmotic potential (-bar)
1·8
1·0
0·0
1·3330
1·3340
1·3350
Refractive index
y = -1330·1 + 997·8x
r = 0·824(significant)

An Atago Abbé refractometer, number 302 with a refractive index range, ND 1.3000 to 1.7000 with a I 0.0002 accuracy was used (Plate 1.4). The prism was water jacketed by connecting the inlet and outlet by the aid of rubber tubings to a combined pump and thermostat operating in a bath at 30 $\pm$ 1°C. The operation of the instrument is given in the operation manual.

Standard curves for osmotic potential (measured cryoscopically) and RI for leaf cell sap (Figure 1.6) and xylem cell sap (Figure 1.7) were drawn. Regression lines were fitted through the point. Checks made periodically suggested that the relationship remained fairly stable.

Extraction of cell sap for cryoscopy and refractometry

Leaf cell sap was extracted by the freezing disruption method (Slatyer 1967, Barrs, 1968). Bagged leaves were frozen in a freezer at -17°C usually for 1-2 hours. They were thawed at room temperatures. Barrs (1968) has indicated that turbidity of the sap does not affect its Y_s. However, a clear sap was preferred and consequently the sap was extracted by centrifugation (cf Necas, 1965). This approach is easy and also convenient.

A Gallenkamp universal centrifuge (maximum speed 6000 RPM) was used. A 2 ml. syringe with a layer of filter paper in the base was suspended in the centrifuge tube. The syringe was $\frac{1}{2}$ - $\frac{2}{3}$ filled with a sample of the thawed leaf lamina, excluding the plain veins (see Appendix 1). Figure 1.8 shows a schematic diagram of the set up for centrifugation.

Initially, it was found necessary to select a speed for centrifugation which would extract sufficient sap from the ruptured cells. Samples were, therefore, centrifuged at intervals of 500 RPM

Figure 1.8 Diagramatic presentation of the centrifugation set up for leaf cell sap extraction.

Figure 1.9 Volume of sap extracted (% total volume) from frozen - thawed leaf tissue at different revolutions per minute.

Fig. 1.8.

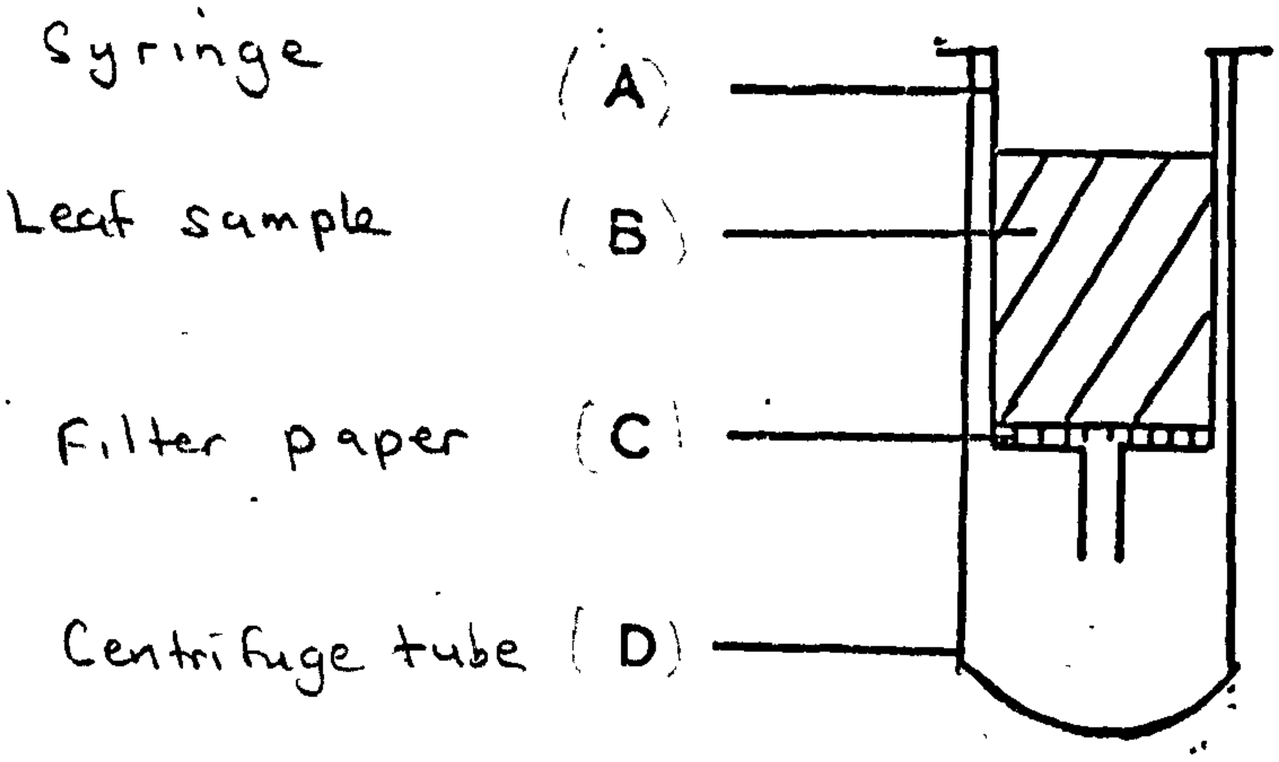
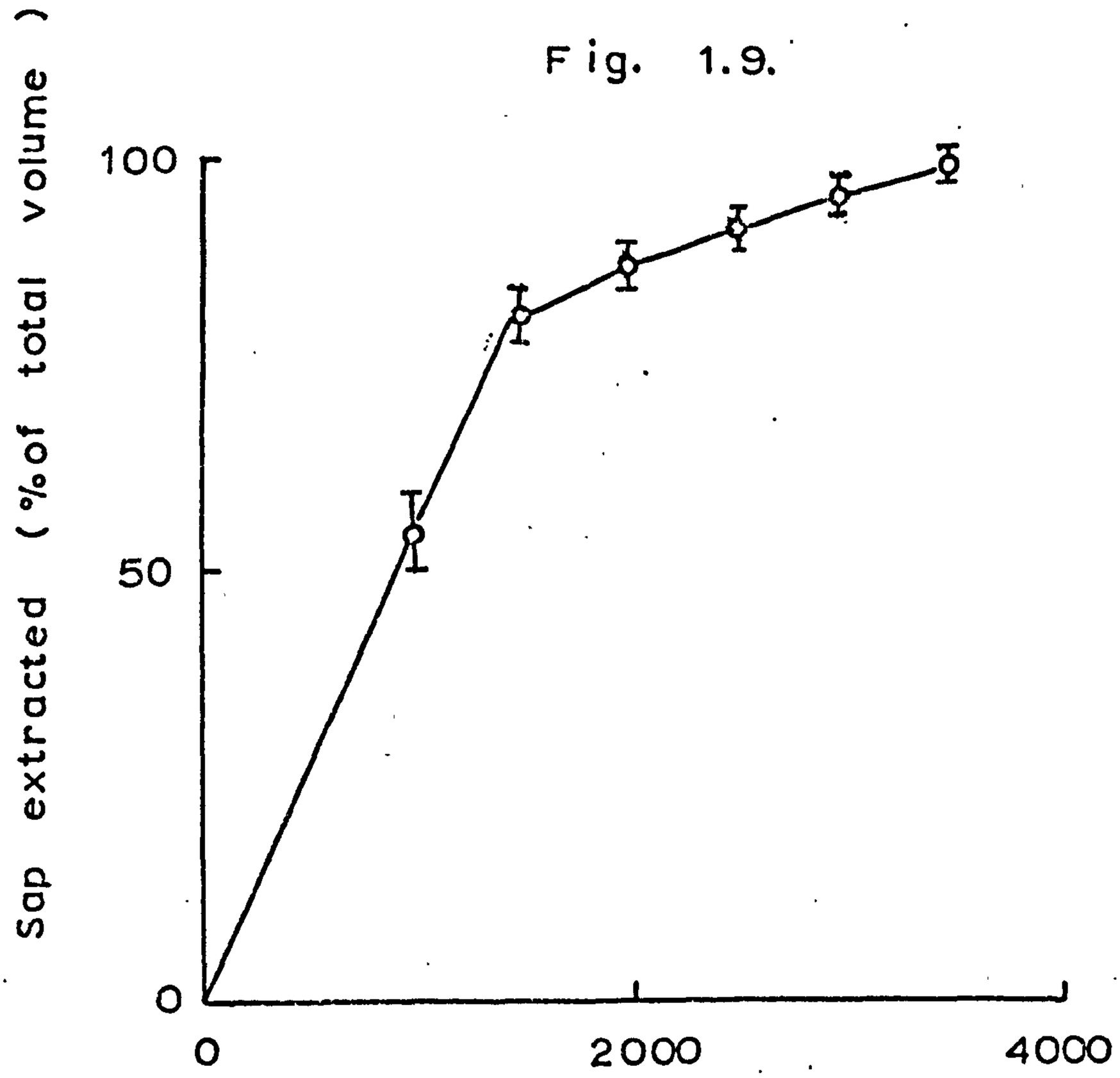

Syringe (A)
Leaf sample (B)
Filter paper (C)
Centrifuge tube (D)

Fig. 1.9.


Sap extracted (% of total volume)
100
50
0
0
2000
4000
Revolutions per minute.

for 5 to 10 minutes, starting from 1000 to 3500 RPM. The spinning time was found sufficient for yielding maximum sap at each set RPM. During spinning, cell sap was extracted, filtered and centrifuged simultaneously. The sap collected at the bottom of the centrifuge tube. Weight of cumulative sap at each successive spinning speed was measured and the values plotted as percentages of the total extracted sap against RPM (Figure 1.9). About 80% of the sap was obtained at 2000 RPM. This spinning speed was, therefore, adopted.

Xylem sap was obtained by increasing the air bomb pressure gradually beyond the 'balancing point' and collecting the sap exuded in a micropipette. Prior to this the cut end of the petiole was rinsed with distilled water to wash away sap from damaged cells. The petiole tip was then dried with tissue paper.

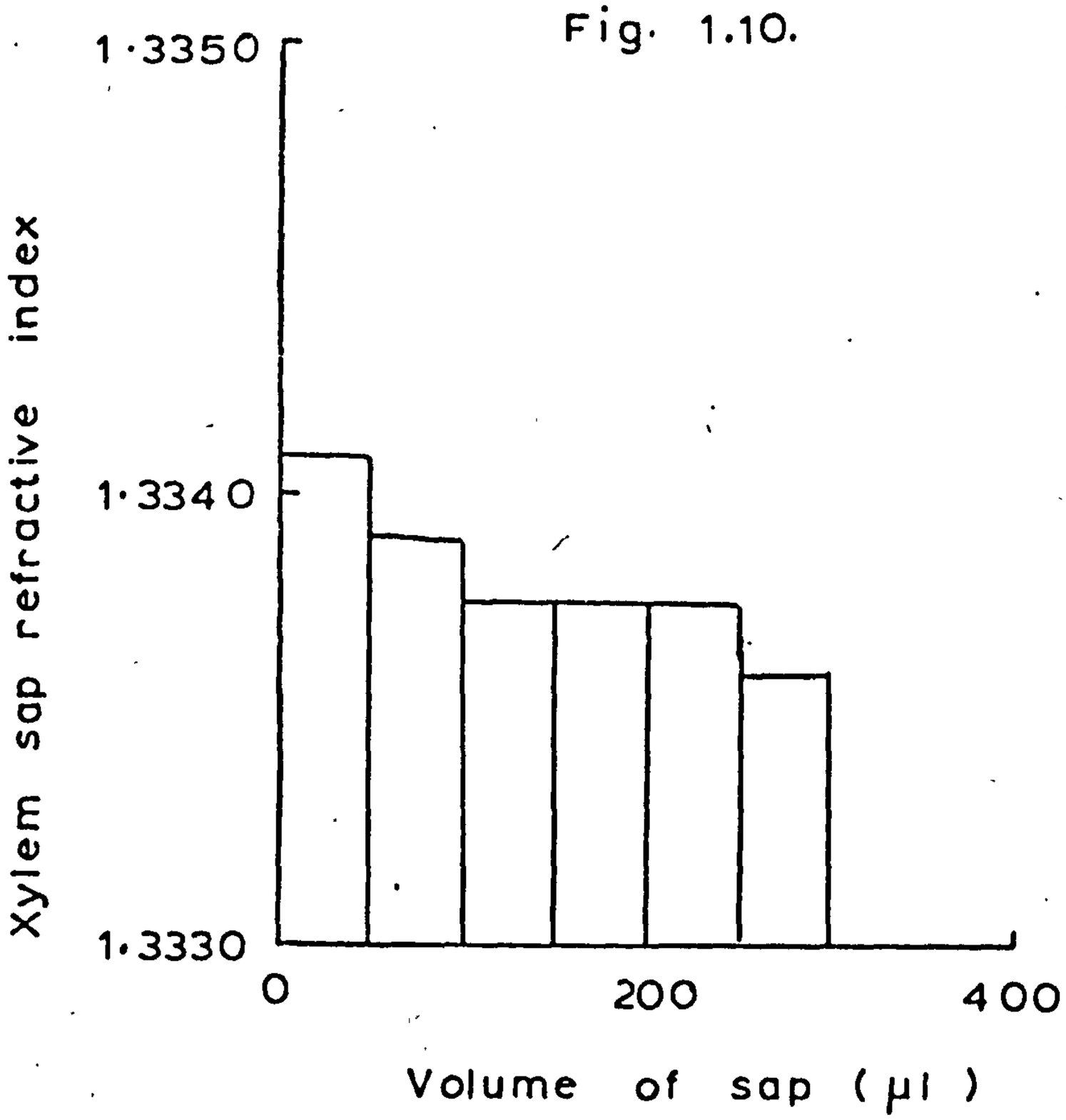
Preliminary analysis by refractometry suggested that the sap became diluted as the volume extracted increased (Figure 1.10) This probably arose from dilution by 'osmotic water' extracted from the symplasts. However for an accurate estimation of Ψ, it is essential that the Ψ_{xs} measured, should be a true representation of the xylem sap. It was, therefore, decided to measure the RI of the first 25-50 µl. of sap extracted and to estimate Ψ_s from the standard curve (Figure 1.7).

Estimation of turgor pressure (Ψ_p)

There was no available direct method for measuring Ψ_p, although this has been successfully done for the lower organisms (e.g Green and Stanton 1967; Green, 1968), and recently for some higher plant cells by Steudle et al (1975). Therefore, after both Ψ and Ψ_s were measured, Ψ_p was calculated using the conventional equation, $\Psi = \Psi_s + \Psi_p$.

Figure 1.10 Changes in xylem sap concentration with increasing volume of sap exudation during pressurization in an air bomb.

Fig. 1.10.


1·3350
1·3340
1·3330
Xylem sap refractive index
0
200
400
Volume of sap (µl)

Measurement of leaf stomatal resistances

Stomatal resistance to water vapour diffusion was measured with a stomatal diffusion porometer (Plate 1.5). The equipment was purchased from LAMBDA Instruments Corporation, 4421 Superior Street, P.O. Box 4425, Lincoln, Nebraska 68504, U.S.A. Calibration and operation procedures are fully described in the brochure. Initially, the equipment was calibrated. A water bath covered with a polythene sheet served as a calibration chamber. The temperature was controlled at 25°C ± 0.5 and RH was over 95% Calibration curves obtained are given in Appendix 1.

Stomatal resistance was calculated by the following equation, suggested by Kanemasu, Thurtell and Tanner (1969).

$$r_s = r_o + {}^{t}/S \text{ (sec cm}^{-1}\text{)}$$

where r_s is stomatal resistance, t, is transit time; S, the slope of the calibration curve; r_o the diffusion resistance of the vapour cup which is taken as the intercept of the curve and the abscissa. The slope of the calibration curve is directly proportional to the amount of water vapour that diffuses into the cup and inversely proportional to the aperture area. The S and r_o for the sensor cup used for this work are 2.60 and -2.2 respectively for Hum I and 1.23 and -2.4 respectively for Hum II.

When leaf temperature was below the calibration temperature (25°C), the transit time was multiplied by an appropriate correction factor (graph in brochure) to obtain equivalent 25°C sensor response.

Stomatal imprints

About 1 part of Silflex Catalyst was mixed thoroughly with about 9 parts Silflo rubber on a slide. A thin layer of the paste was spread over the leaf surface. When it hardened (2 minutes later)

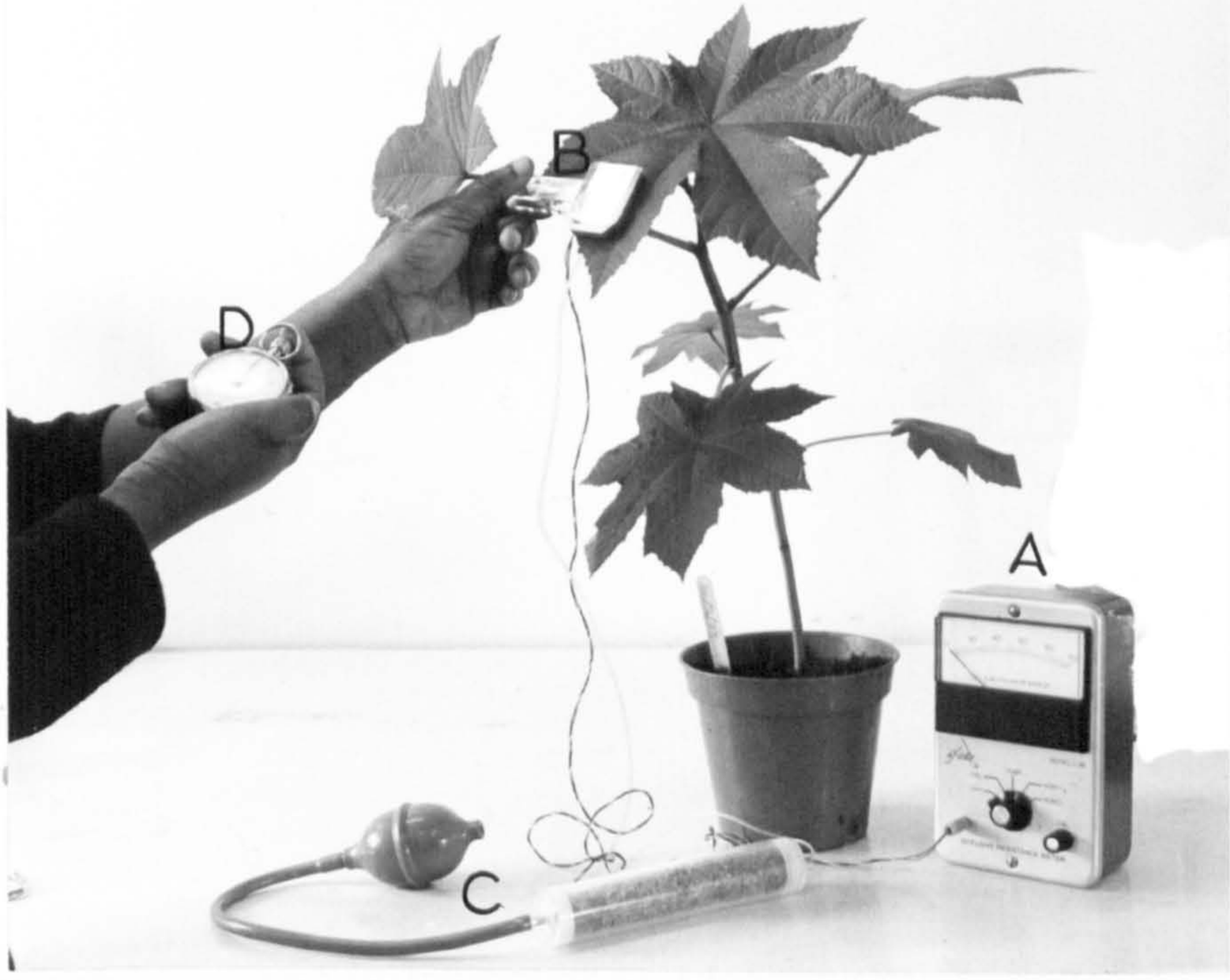


Plate 1.5 Stomatal diffusion porometer

A Li - cor diffusive resistance meter

B Sensor cup

C Drying tube assembly

D Stop watch

it was peeled off carefully from the leaf onto a slide on which was spread a thin layer of nail varnish. Another slide was laid on top to keep the imprint flat. It was left to dry for about 30 minutes in a warm place (usually on top of a radiator) to hasten evaporation of the solvent. After this the rubber was peeled off leaving the imprint.

Photographs of the stomatal imprints were taken on the Zeiss photomicroscope II.

Transpiration studies

The pot-plant weighing method (cf Jarvis and Jarvis, 1963) was used. Pots were enclosed singly in polythene bags to prevent evaporation from the soil surface. Plants in 12 cm pots were weighed using a heavy duty top-pan mettler balance (Mettler P 1000) giving weights to an accuracy of $\pm$ 0.1 gm. Plants in 15 cm pots were weighed on the Gallenkamp heavy duty solution balance. When transpiration rate was expressed as gm / cm^2 / hr., the leaf area of only one surface was used, because stomata on the upper surface remained virtually closed. Some transpiration rates were expressed as gm / plant / day.

Statistical analysis

Where the experimental results are clear and conclusions can be easily drawn no statistical tests were employed. However, where it was desirable to analyse data statistically the following tests were carried out.

(a) Standard error (SE)

Standard error of the mean value of a series of observations was calculated as follows:-

$$SE = \frac{SD}{\sqrt{n}}$$

n = number of observations.

SD = standard deviation calculated as

$$SD = \sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

The SE is shown on the graphs as vertical bars drawn symmetrically about the mean point, i.e. this line is 2 x SE in length.

(b) Correlation and Regression analysis

The interdependence of 2 quantitative variables, x and y, was investigated using correlation and regression analyses. To estimate the degree of association between x and y, the correlation coefficient r was calculated as

$$r = \frac{\Sigma (x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma (x - \bar{x})^2 \Sigma (y - \bar{y})^2}}$$

The r value was compared with the valves in the r distribution table by Pearson and Hartley, using n-2 degrees of freedom. If r is significant at 5% level or less, the 2 variables were considered associated.

Assuming that the variable y depends on x in a linear manner, a regression analysis is conducted. The best fitting regression line is estimated by the expression -

$$y = a + bx$$

where b, the regression coefficient is estimated by -

$$b = \frac{\Sigma (x - \bar{x})(y - \bar{y})}{\Sigma (x - \bar{x})^2}$$

a, the constant term in the regression is estimated as -

$$a = \bar{y} - b\bar{x}$$

In order to fit the regression line, 2 (or more) values of x were selected and the corresponding valves of y were calculated from the regression equation. A straight line was drawn through the points.

SECTION 2

GROWTH AND WATER UPTAKE OF LEAF DISCS FLOATED ON WATER

Evidence from water uptake and relative water content (RWC %) studies shows that, given free water (water potential, Y, of 0 bar) excised tissues continue to absorb water long after they have gained full turgidity (Barrs and Weatherley, 1962; Potter and Milburn, 1970; Milburn and Weatherley, 1971). The uptake, supposedly a growth phenomenon, has been demonstrated not only in young tissue, but also in 'mature' leaves (i.e. defined as cessation of growth) on well-watered plants, thus suggesting that Y around zero bar could cause 'mature' leaves to grow. The following experiments re-examine this phenomenon and also attempt to ascertain the casual factors.

As a first step it is essential that the 2 uptake mechanisms, the absorption for true water deficits and the growth uptake be separated. Earlier attempts in this respect (see for example Yemm and Willis, 1954; Barrs et al, 1962) produced conflicting interpretations. More recently Milburn and Weatherley (1971) suggested that temperatures around freezing point would suppress the growth uptake but would permit complete hydration. On the other hand both uptake processes operate at warm temperatures and consequently comparison of the cold and warm uptake potentials of a leaf or discs taken from it can differentiate between a growing and a mature leaf.

The experiments reported here adopt the Milburn and Weatherley technique. It is hoped that the results obtained will serve as a basis for improving the RWC technique whilst nonetheless: fulfilling its prime objective to provide a measurement of growth water deficits.

A great deal of data already exist on water uptake of whole leaves and shoots. In the present study leaf discs were considered suitable because they provide a large number of uniform samples from a single leaf over long periods, thus enabling comparative studies during leaf development. Moreover, Weatherley (1950) has shown that the course of water absorption of whole leaves and leaf discs is similar.

2.1 Material and methods

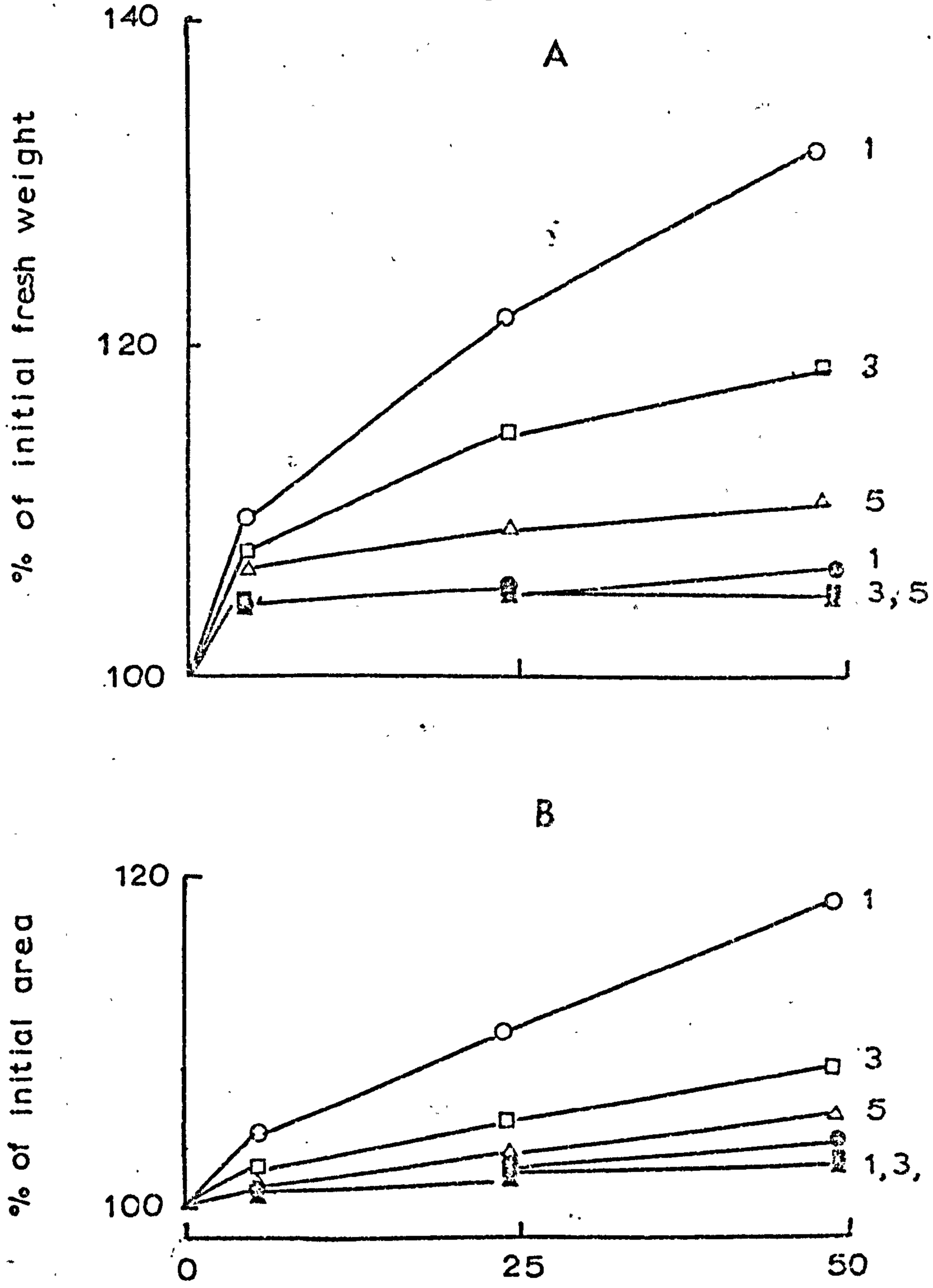
Ricinus leaf discs were obtained from greenhouse grown plants. There were 2 types of plant. The first set was at 5-7 leaf stage and were growing in well watered soil contained in 12 and 15 cm diameter pots. The second type of plant was larger and older (over 10 leaf stage). They were raised singly in boxes (36 x 36 x 32 cm). Some of these plants were allowed to dry out over long periods (about 2 months). The hydraulic capacity of the massive root medium allowed water stress to develop gradually with time, (See section 4). The plants appeared to become adjusted to their environment and young leaves did not wilt. These, here referred to as 'stressed leaves' were sampled after their expansion has been arrested by the water deficits.

Discs (1.75 cm in diameter) were punched in the early mornings from an experimental leaf into a closed sampling bottle. Two discs, one identified by a small hole made by a 10 µl. micropipette were randomly dispensed into a weighing bottle. These formed a sample. Area changes of the marked disc following water uptake were studied, and were assumed to represent expansion growth of the whole sample.

The initial fresh weight and area of each sample were

Figure 2.1 Changes with time in fresh weights and areas of floating Ricinus leaf discs at 30°C (O, □, Δ) and 1°C (O, □, ▲). The discs were punched from leaves of a well watered plant. The numbers on the curves refer to the leaf position, counting the first expanded leaf near the apex as one.

Fig. 2.1


A
140
120
100
% of initial fresh weight
1
3
5
1
3, 5
B
120
100
% of initial area
1
3
5
1, 3, 5.
0
25
50
Time (hr)

measured. Afterwards 2-3 parallel samples were floated at 1°C (cold discs) and 30°C (warm discs) for 48 hours in closed petri-dishes. The petri-dishes were placed in water baths controlled at the set temperatures. The discs were illuminated at around compensation point.

The fresh weights together with the corresponding area increments were determined at intervals. The results were plotted as percentages of the initial fresh weights or areas against time. The first part of the curves, representing changes within the first 4 hours will be referred to as phase I and the remaining part as phase II (cf Barrs and Weatherley, 1962).

Results

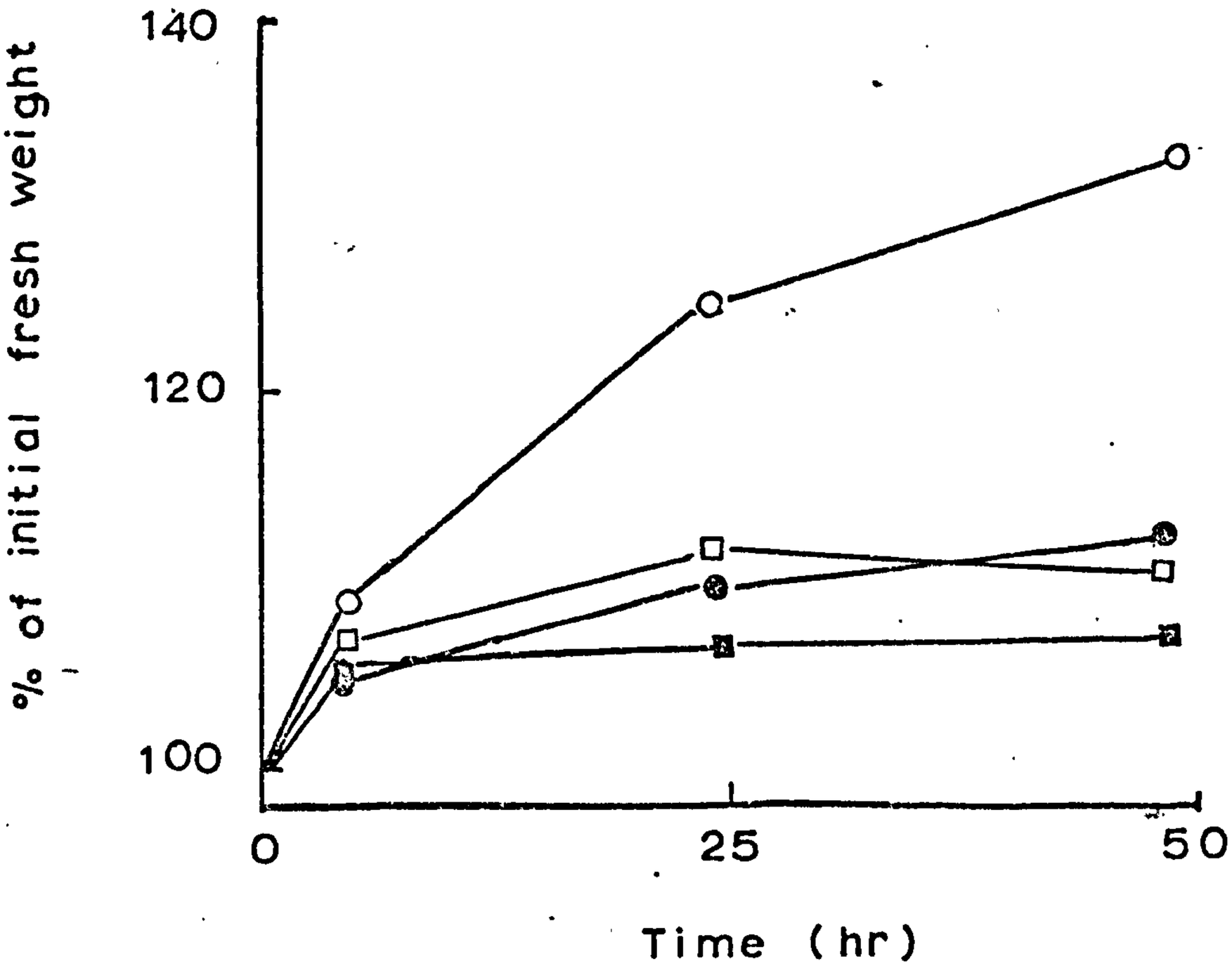
2.2 Water uptake and leaf disc expansion in relation to leaf age

Studies were first conducted to see how water absorption and expansion curves of leaf discs of different ages from well watered plants compare with each other. Samples were taken from the youngest expanded leaf (nearest apex) and the next 2 or 3 leaves below it. The oldest of the leaves (furthest from apex) was fully expanded and had ceased growing.

The curves obtained are shown in Figure 2.1. Fresh weights and area changes were rapid during phase I, this was followed by a slower but persistent uptake during phase II. Absorption was small at 1°C and the values obtained were identical for all leaves. After phase I, cold uptake virtually ceased, however, there was a tendency for the young leaves to continue absorbing at a very slow rate. Greater absorption occurred at 30°C, the rate of which was influenced by the physiological state of the leaves, younger leaves absorbing proportionately

Figure 2.2 Effect of aging on change of fresh weight of discs floated on water at 30°C (open symbols) and 1°C (closed symbols). Discs from young (○,●) and older (□, ■) leaves of a control plant.

Fig. 2.2



more water than older leaves. This suggests that a growth phenomenon was operative.

Visual comparison of cold and warm young discs showed that differences in colouration became more and more pronounced as the experiment progressed. Thus, whereas the cold discs retained the initial reddish green colour, warm discs became green, the colour associated with leaf maturity. Since growth was not expected in the cold it seems reasonable to suggest that the cold uptake represents the initial water deficits. It is thus noteworthy that leaf 5 showed a growth uptake during both phase I and II. The decline in fresh weight (Figure 2.2) suggests that the older leaf can mature fully in water at 0 bar.

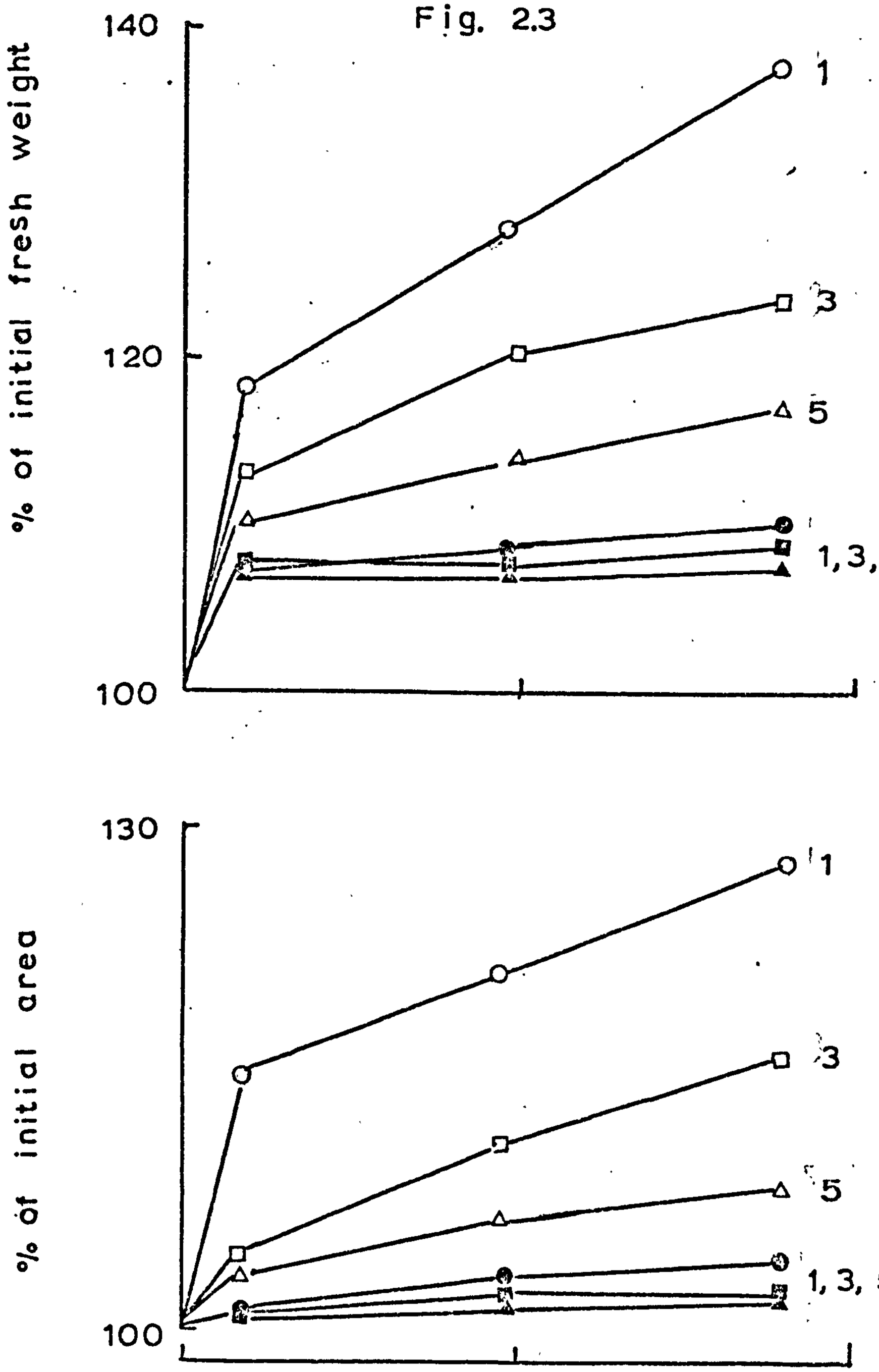
2.3 Effect of Drought on Water Uptake and Growth of Discs

It was decided to examine the cold and warm absorption potentials of discs taken from plants subjected to soil water stress, a treatment known to increase internal water deficits and to check growth. Milburn and Weatherley (1971) have suggested that the growth uptake is influenced by the water stress previously experienced by leaves.

Well watered Ricinus plants were subjected to a 3 day drying out cycle. Discs were sampled for water uptake and expansion experiments. The results are shown in Figure 3.3. Leaf 5 stopped growing before, and leaves 1 and 3 during the drought period. The samples showed greater absorption than normal leaves (see Figure 2.1). The fact that the discs grew (warm-cold values) suggests that, although their leaves stopped growing, they were physiologically young but were unable to express their growth while intact as a result of the internal water deficits.

Figure 2.3 Effect of a short period of drought on the water uptake and areas of leaf discs. Symbols are as for Figure 2.1. The numbers on the curves refer to the leaf position, counting the first expanded apical leaf as one.

Fig. 2.3


% of initial fresh weight
140
120
100
1
3
5
1, 3, 5.
% of initial area
130
100
1
3
5
1, 3, 5
0
25
50
Time (hr)

The curves (Figure 2.3) show that phase I growth uptake is massive and is increased in younger discs. Seemingly, phase I has been influenced by the drought treatment. However, phase II of the curves shows the normal slow but persistent uptake. It could be deduced that water stress causes a potential for growth to build up but this becomes rapidly expressed when favourable internal water is restored.

2.4 Growth retention capacity of a leaf during its development

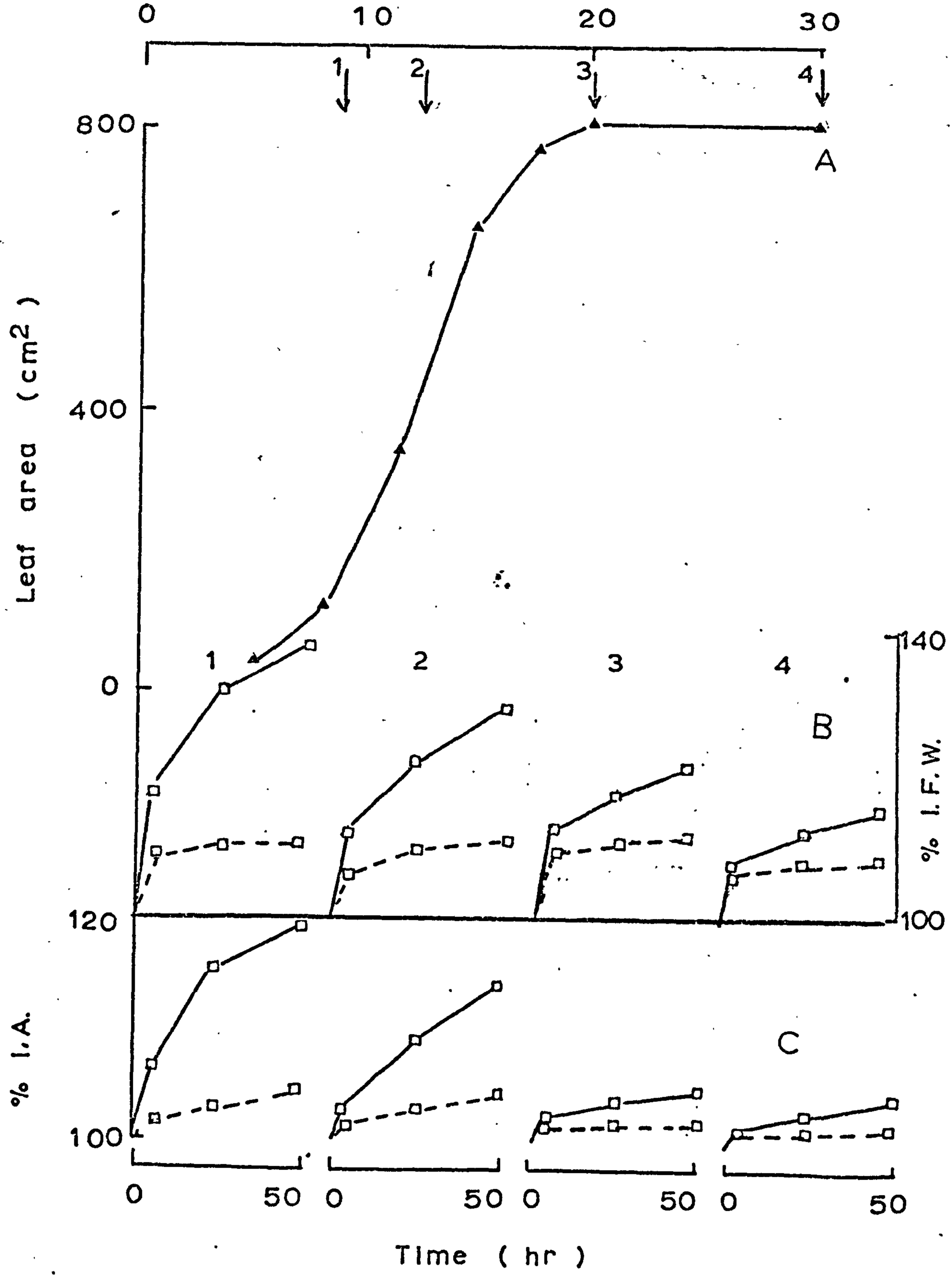
The development of leaves on well watered large plants (type 2) was followed. Leaf areas were measured at 3 or 4 days intervals. Discs were taken from the leaves while they were still expanding and sampling continued days after they had fully 'matured'. Water potentials of the experimental plants were measured periodically and these ranged from -3 to -4 bar.

Samples of fresh weights and disc area curves are shown in Figure 2.4 and also shown is the growth curve of the experimental leaf. Cold uptake was not influenced by stage of development of the leaf and the observed variations in the values attained could be reasonably attributed to fluctuations in water deficits. On the other hand, the warm uptake values progressively declined with time. Hence on day 8 after emergence of the leaf, warm uptake was 132% initial fresh weight (IFW); this decreased to 116% on day 32 on which day, curve A shows that the leaf had become fully 'mature' 10 days earlier.
If the growth potential could be expressed as % IFW of warm at 48 hours minus the % IFW of cold at 4 hours, Figure 2.6 curve A shows that the potential decreases with leaf development. However, even a 'mature' leaf retains a potential for growth which apparently becomes expressed when apoplastic water is

Figure 2.4 Growth curve of a leaf on a well watered plant and fresh weight (B) with corresponding area changes (C) of discs punched periodically from it.

The numbers on the arrows correspond to the respective fresh weight and area curves □—□ 30°C, □---□ 1°C.

Fig. 2.4.


Days after leaf emergence
0
10
20
30
1
2
3
4
800
400
0
120
100
Leaf area (cm2)
A
1
2
3
4
140
100
% I.F.W.
B
% I.A.
C
0
50
Time (hr)

around zero bar.

Since 'mature' leaves on normal plant senesce rapidly, the above experiment was repeated using stressed leaves. The aim was to investigate the extent to which the growth potential retained, changes with time, following growth arrestment by restriction of water supply.

Leaves were sampled which had emerged during the drought period. These stressed leaves were densely veined, reddish green in colour and ceased to expand after reaching smaller areas (70 to 450 cm^2) than normal leaves (over 600 cm^2). Hence the growth of these leaves may be considered to have been arrested by drought (cf Stocker, 1960).

Since it was intended to take samples from the same leaf over a long period of time, only 2 samples (2 or 1 discs each) 1 for cold and the other for warm floatation were used for each determination. However, similar uptake curves were obtained for the replicate leaves, consequently the results may be considered valid. The Y of the droughted plants was measured periodically (see Section 4, Figure 4.7).

Figure 2.5 compares typical curves of fresh weight and disc-area of a stressed leaf with those obtained a few days after water had been restored to the plant. Phase I cold uptake increased progressively throughout the drought period but was drastically reduced when favourable plant water status was restored (Curve B, number 4). This response conforms with the Y results thus providing additional evidence for the view that cold uptake satisfies water deficits. However, as previously, cold uptake continued during phase II. The nature of this uptake is investigated in a later study. There

Figure 2.5 Growth curve (A) of a stressed leaf during drought and following rewatering (big arrow). B and C are fresh weights and area curves respectively of discs punched periodically (↓) from the leaf. The numbers on the small arrows correspond to the respective curves. Floatation at 30°C (Δ——Δ), 1°C (Δ--Δ).

Fig. 2.5.

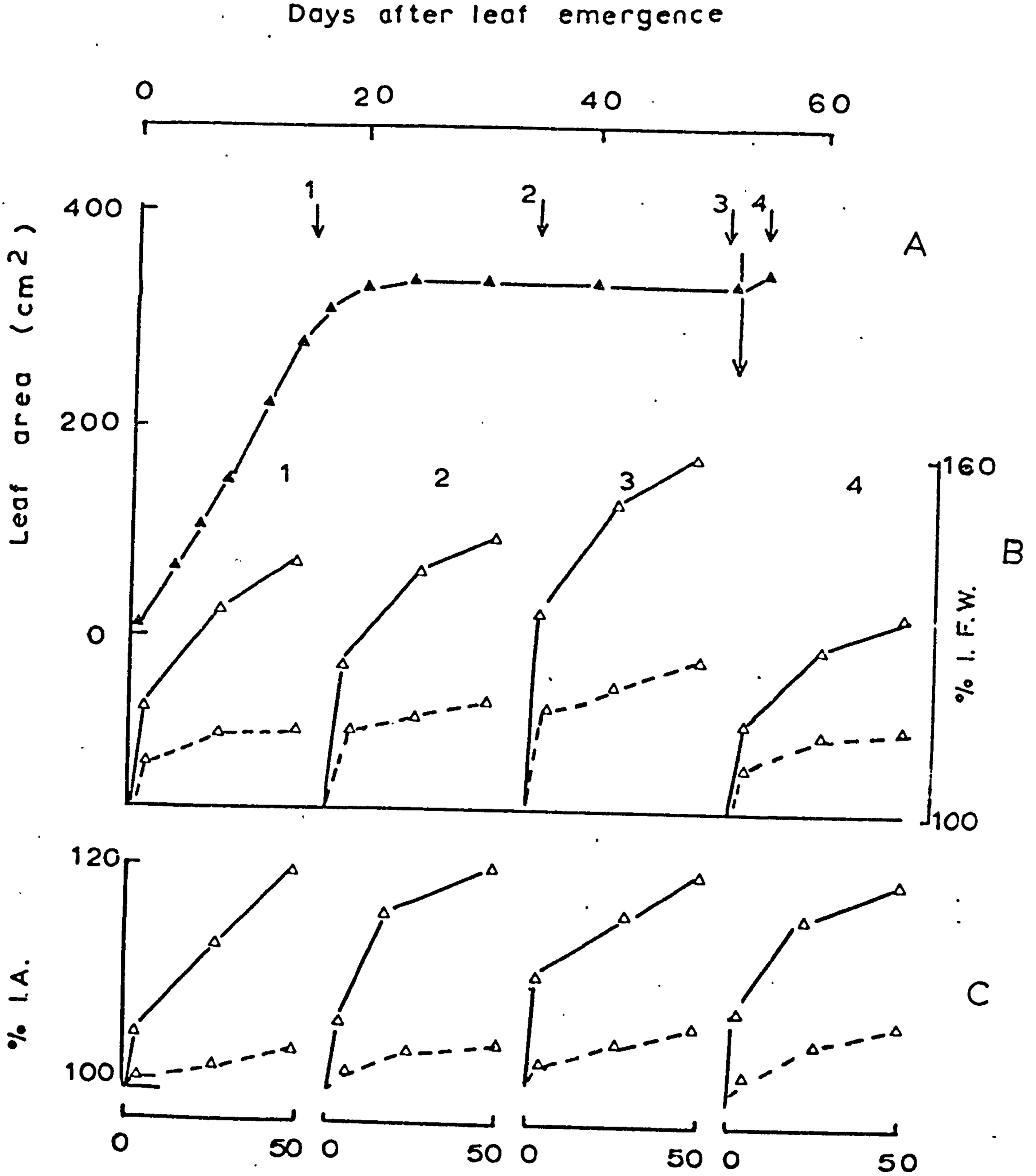
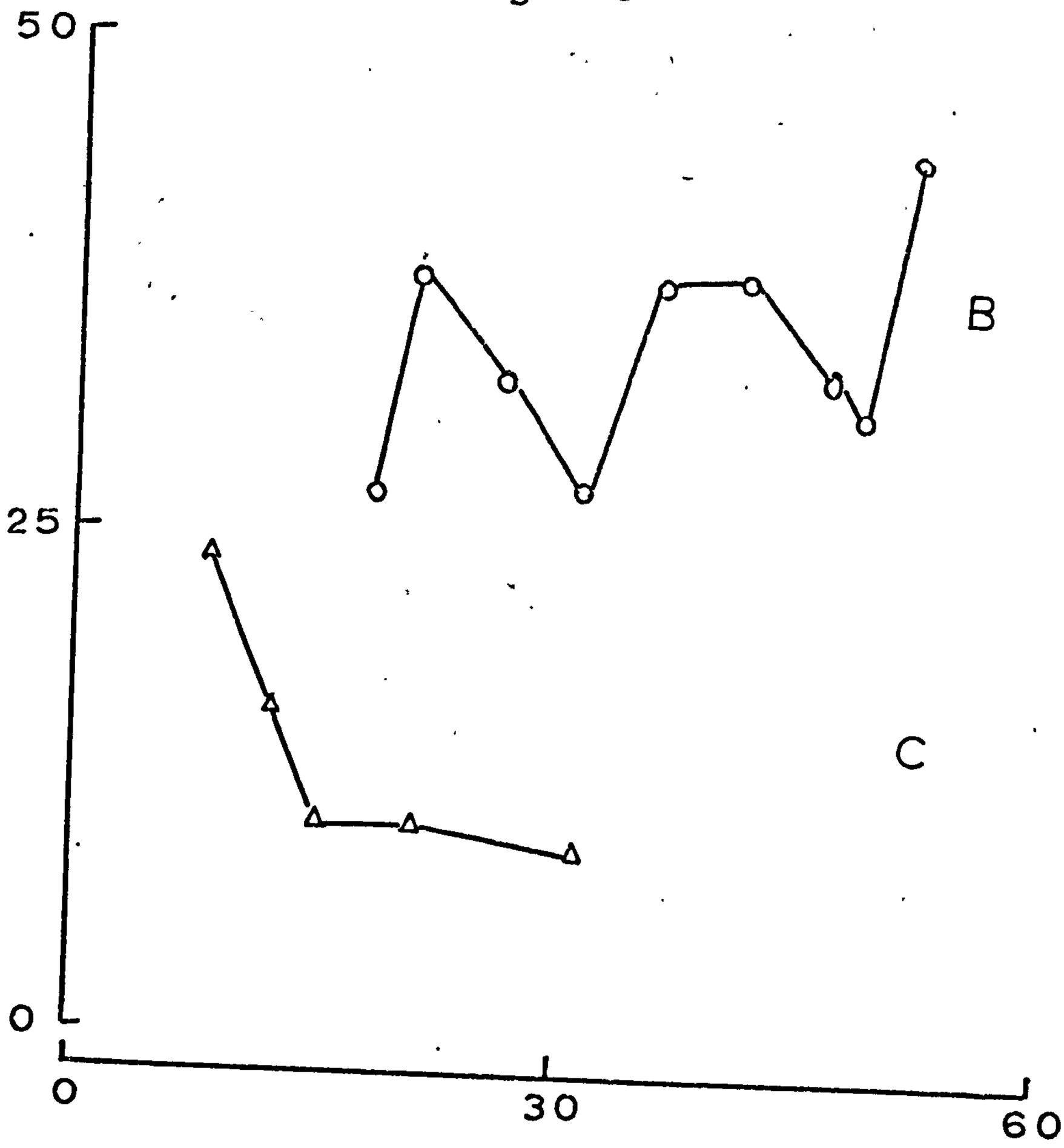

Days after leaf emergence
0
20
40
60
1
2
3
4
A
400
200
0
Leaf area (cm2)
1
2
3
4
160
100
% I.F.W.
B
120
100
% I.A.
C
0
50
0
50
0
50
0
50
Time (hr)

Figure 2.6 Growth retention capacity with time of normal Ricinus leaves (A) and leaves whose growth has been suppressed by a prolonged drought (B).

Fig. 2.6.



were only small changes in cold disc areas.

As expected there was a massive phase I warm uptake which was followed by the normal slower phase II uptake. The discs areas show a similar pattern. Apparently, the increase in water deficit uptake (cold phase I) with time, is super-imposed on the phase I growth uptake.

The scatter in the points of Figure 2.6 (probably attributable to the errors described above), complicates an assessment of the trend of changes of the growth potential. However, it is reasonable to suggest that the potential for growth which built up is retained at a constant value over long periods, probably until the leaves senesce.

2.5 Cold and Warm interchange experiments

Discs were taken from well watered and prolonged stressed plants. During the course of the experiment some of the warm and cold discs were interchanged.

Figure 2.7 clearly demonstrates that floatation at $1^{o}C$ suppressed the growth uptake and the corresponding area increment, however, transfer to warm resulted in the elimination of the suppressing effect.

Figure 2.8 gives a detailed analysis of the immediate responses of the samples which occurred after the transfer and also shows the effect of the interchanges which were carried out after phase I when water deficits of the cold samples were expected to have been completely satisfied. The rate of uptake in warm conditions by the original cold discs, increased sharply and was accompanied by a large increase in area before it gradually declined to a slower rate. The transitory rapid

Figure 2.7 Effect of high (30°C, o——o) and low (1°C o- - -o) temperatures on saturation and growth of leaf discs.

Figure 2.7a, a young normal leaf.

Figure 2.7b, a stressed leaf.

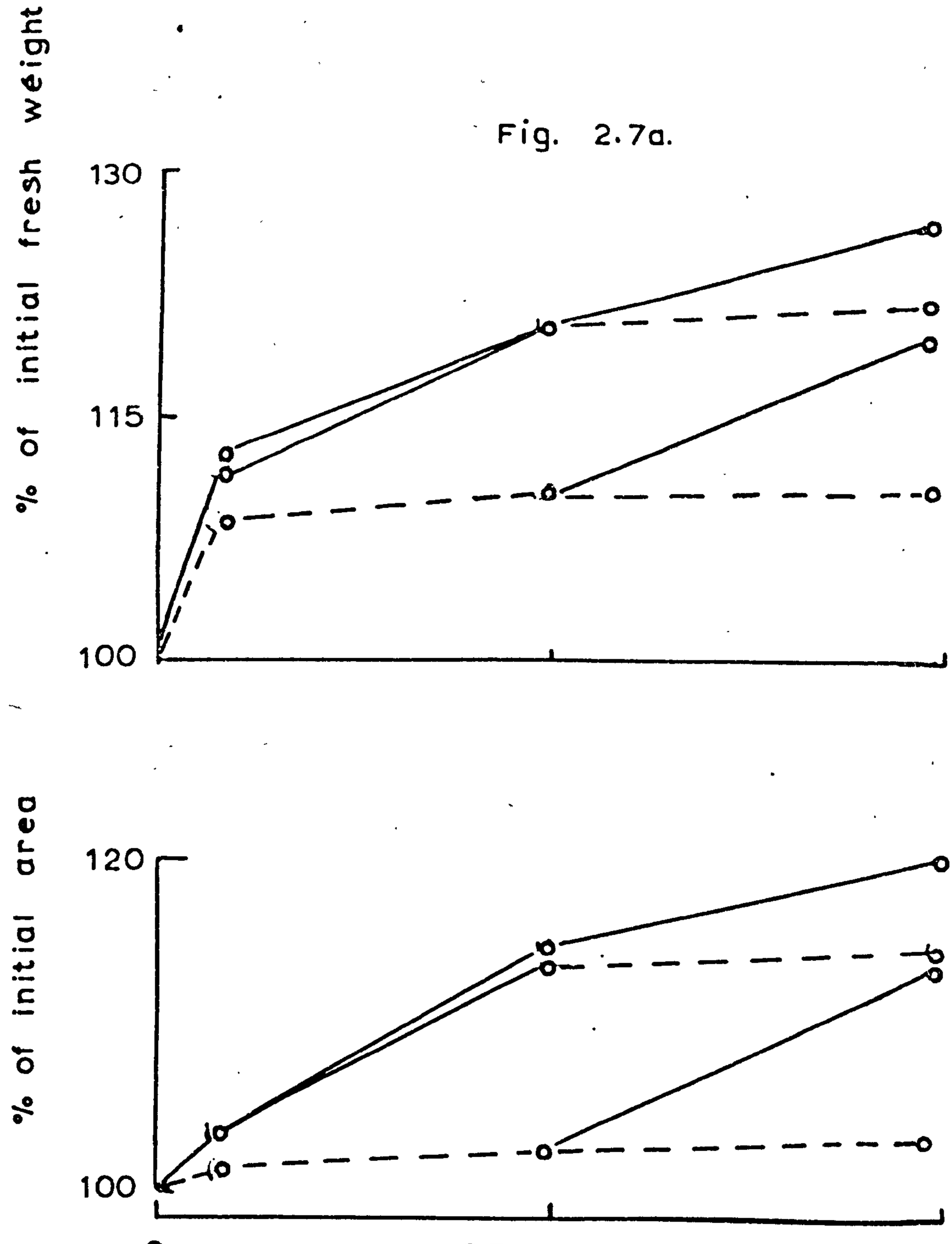

% of initial fresh weight
130
115
100
% of initial area
120
100
0
25
50
Time (hr)

Fig. 2.7a.

Fig. 2.7b.

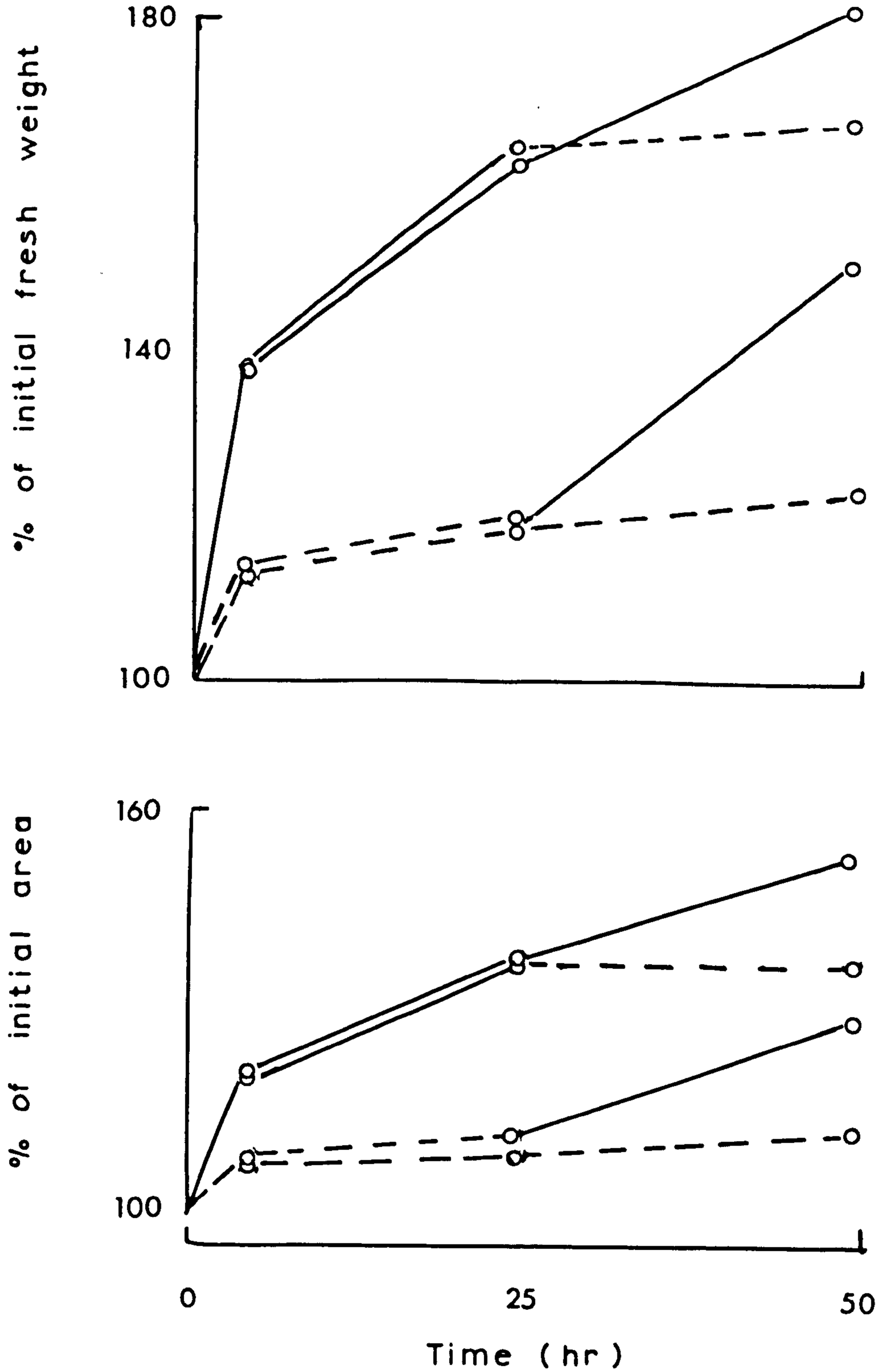
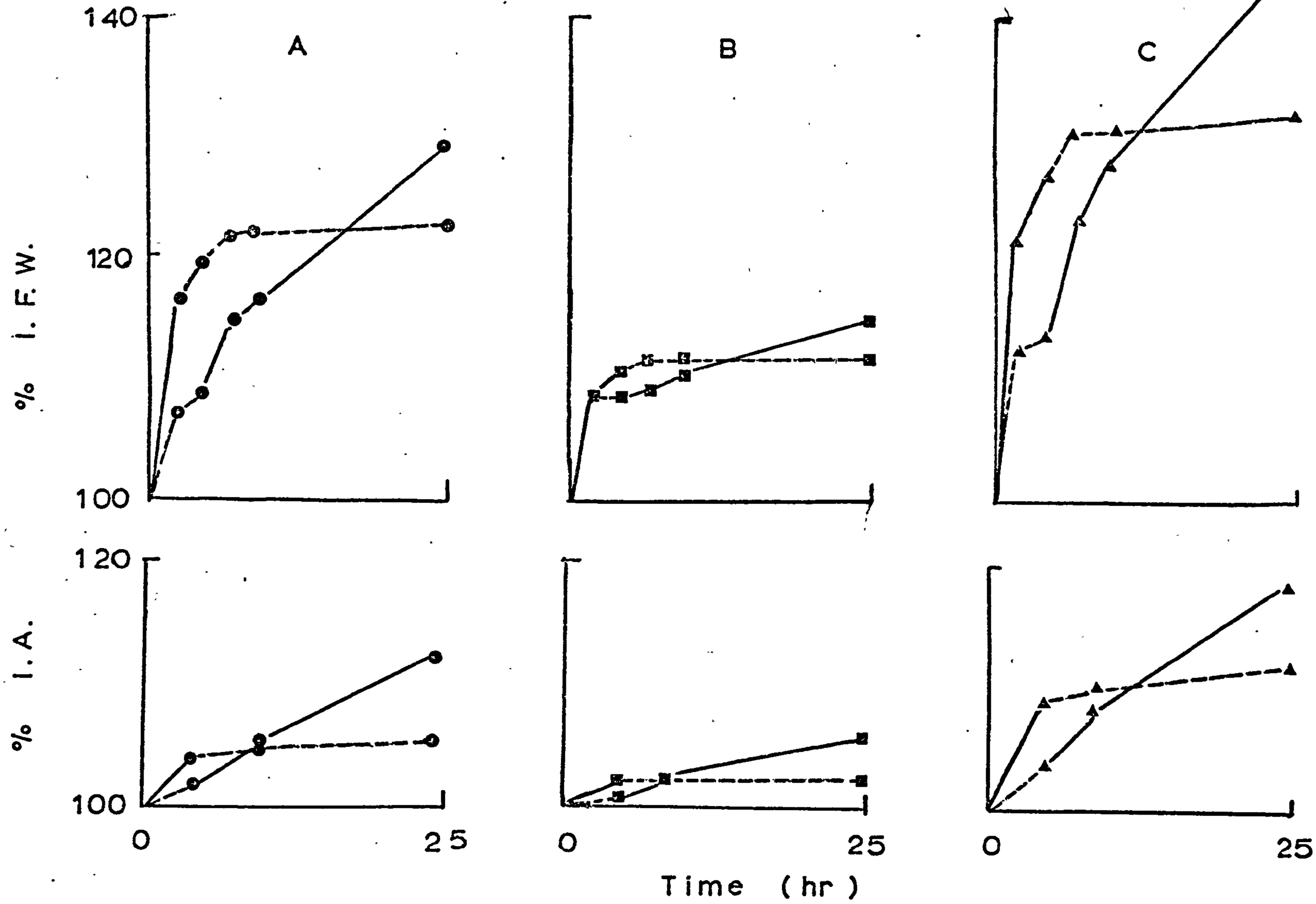

% of initial fresh weight
180
140
100
% of initial area
160
100
0
25
50
Time (hr)

Fig. 2.8.



uptake shows that the growth potential accumulated on the intact plant termed 'unexpressed growth' was not expressed during the initial cold uptake. The results further show that, when warm discs were transferred to low temperatures, only a small volume of water was absorbed, of which 80 - 100% occurred within the first 2-4 hours. This uptake may be attributed to water deficits ~~incurred during~~ still remaining from the phase I growth uptake.

2.6 Effect of diurnal water deficits and growth

The sensitivity of cell enlargement to water is so high that in some species leaf expansion is smaller by day when Y are low than by night during which time favourable internal water is restored (Boyer, 1968).

In the following experiment it was assumed that if cold phase I uptake represents the true water deficits, samples taken at the end of the day (day discs) should absorb more water in the cold than samples taken at the end of the night (night discs). Furthermore, differences in 'unexpressed growth' would be observed depending on the extent to which the growth of the intact leaf has been suppressed during the 2 periods. Differences in leaf expansion of Ricinus by day and by night were negligible (Section 4). It was, therefore, decided to compare its uptake responses with those of Helianthus whose expansion growth is known to vary greatly by day and by night (Boyer, 1968).

The Helianthus plants used were at 6-7 leaf stage and were growing in 15 cm pots in a growth cabinet which was set for 14 hour light (52 W/m^2). Temperature and relative humidity during the light period were 35°C and 60% respectively and 20°C and 95% during the dark period. The high light intensity was expected to counter any increment in respiration which may arise

from the high cabinet temperatures.

Because of the small leaf sizes, 14.5 cm diameter discs were used. Discs shrink rapidly through losing water after sampling. To reduce this error to a minimum, individual samples were punched directly into the weighing bottle and weighed immediately. Day discs were sampled from one half of the leaf and the other half was sampled for "night discs". The pattern of response of day and night discs were the same whether day or night discs were sampled first, suggesting that errors attributable to possible age differences between the discs are negligible.

Samples were illuminated around compensation point. Floatation was limited to 24 hours and during this period only a few measurements were made. This was found necessary in order to avoid damage to the delicate discs.

Transpiration of the plants (gm/plant) and expansion of the experimental leaves (% initial area) by day and night were measured. In addition 'mature' leaves were sampled for Y measurements. The results are shown in Table 2.1.

Table 2.1.

Comparative data for growth, transpiration and water deficits by night and by day of well watered Ricinus and Helianthus plants in a growth cabinet

Plant Characters	Time	
	Night	Day
Ricinus		
Growth of experimental leaf (% of initial area)		
Young	112	110
'Mature'	0	0
Transpiration (gm / plant)	47	220
Leaf water potential (bar)	-3.0	-4.6

Figure 2.9 Effect of variations in growth and water deficits by day and night on discs water uptake and area curves of Ricinus. Young (○,●) and 'mature' (△,▲) leaves. Floatation was at 30°C (open symbols) and 1°C (closed symbols).

Fig. 2.9.

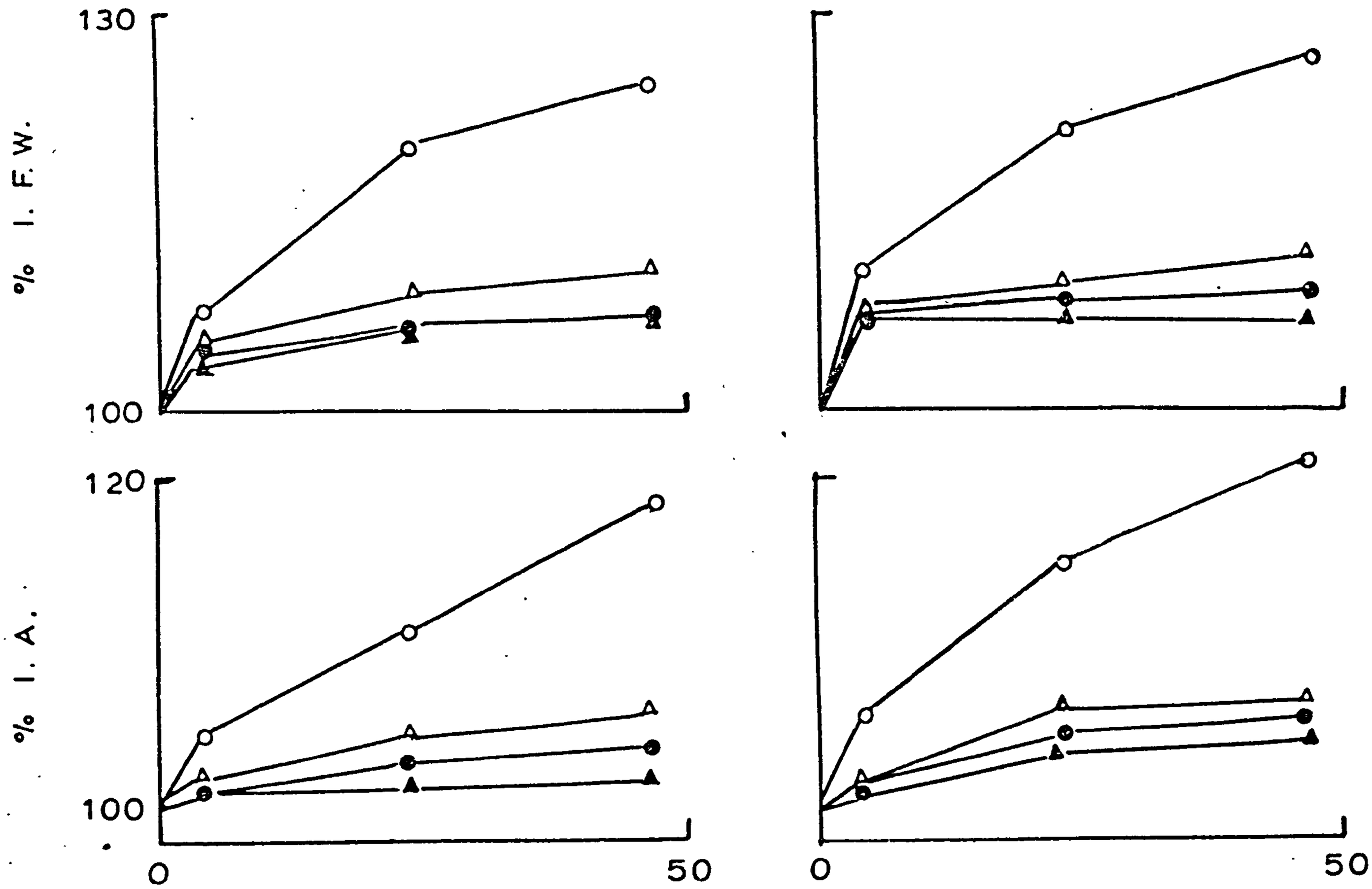


Table 2.1. Contd./

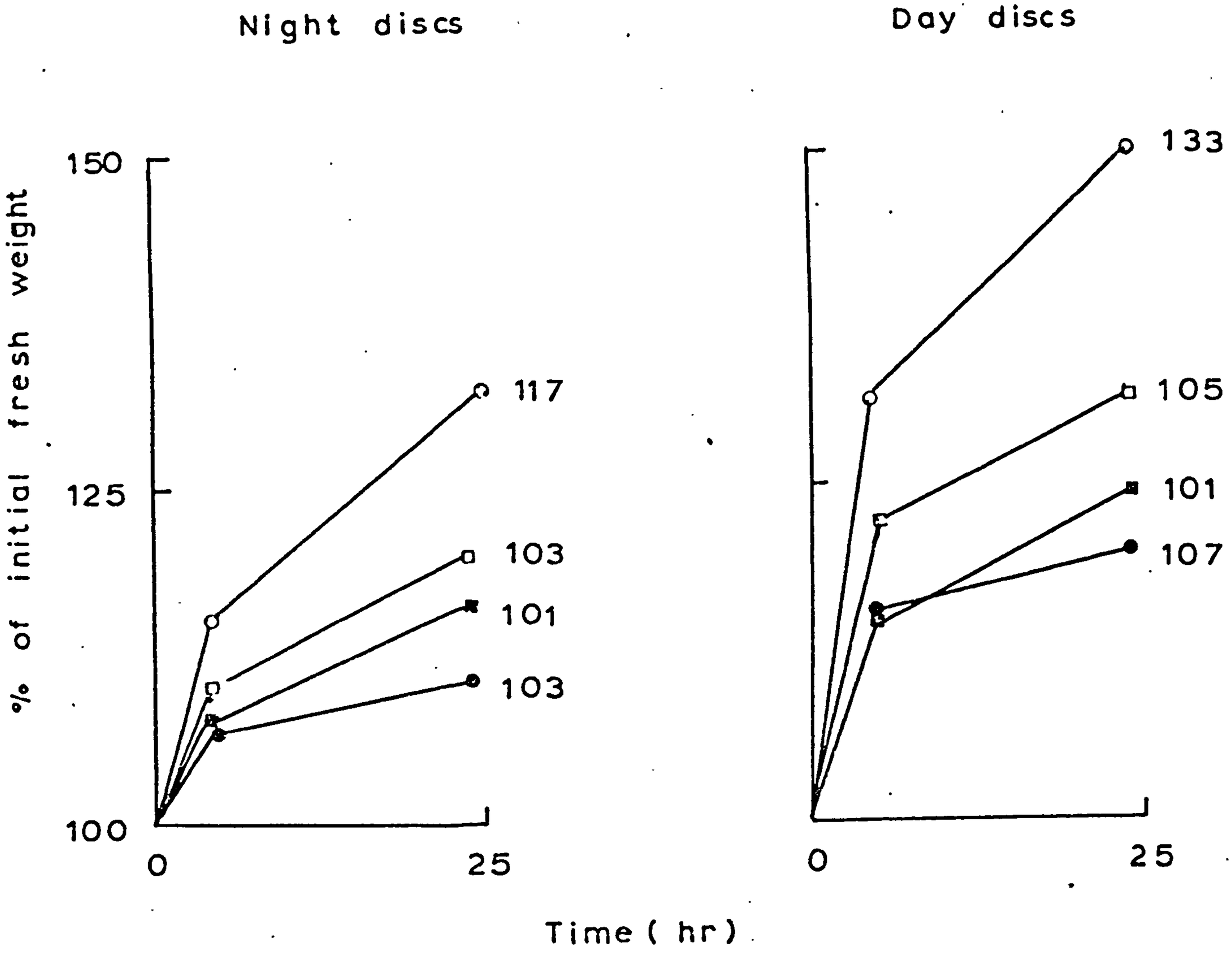
Plant Characters	Time	
	Night	Day
Helianthus		
Growth of experimental leaf (% of initial area)		
Young	122	115
Older	104	103
Transpiration (gm / plant)	28	94
Leaf water potential (bar)	- 2.1	- 3.7

The experimental conditions in the growth cabinet for Ricinus plants is the same as described in Section 1 except that lighting was reduced to 12 hours. Transpiration and water deficits were higher by day than by night and consequently leaf expansion was more reduced by day than by night. However, the differences for Helianthus observed here was less than reported previously (cf Boyer 1968, 1976).

Figures 2.9 and 2.10 show examples of the water uptake curves obtained. As postulated, phase I cold uptake values by the day discs were higher than those of night discs. The total growth of Helianthus (% 1 FW of warm discs at 24 hours - % 1 FW cold discs at 4 hours) by day discs was 35% and 15% for the young and older leaves respectively, of these 17% and 7% for the former and latter leaves were contributed by phase I growth uptake. Total growth by night discs was 25% and 12% for the younger and older leaves respectively. The contributions of the phase I growth uptake to these values were 8% and 2% respectively for the young and older leaves. Thus the results clearly show that the main difference in the day and night

Figure 2.10 Effect of variations in growth and water deficits by day and by night on discs water uptake and area curves of Helianthus. Young (o, •), mature (□, ■) leaves. Floatation was at 30°C (open symbols) and 1°C (closed symbols). Discs areas (% I.A.) at the end of the 24 hours are shown against the respective curves.

Fig. 2.10


Night discs
Day discs
% of initial fresh weight
150
125
100
0
25
117
103
101
103
133
105
101
107
Time (hr)

curves lies in the degree of the 'unexpressed growth,' the magnitude of which depended on the extent to which growth of the intact leaf has been suppressed by water stress. The results for Ricinus further support this view. Thus, differences between the 'unexpressed growth' of the day and night discs were negligible. It is significant that water stress suppresses relatively the growth of the ... rapidly growing young leaf more than older leaves. Figure 2.10 further shows that unlike young discs, the persistent phase II cold uptake by older discs was not accompanied by proportional expansion. Probably damaged cells, at the cut edges of the older discs, became injected.

2.7 The nature of the growth uptake

Fresh weight increments and expansion of discs during water absorption could be caused by reversible elastic extensions of the cell walls or irreversible changes in the cell. It is only the latter changes which are relevant to growth (Heyn 1940). The following experiments (cf Potter and Milburn, 1970) were designed to investigate the significance of the growth uptake. It also attempts to ascertain whether the slow but continued cold phase II uptake constitutes a growth uptake.

Discs were taken from normal and stressed leaves of Ricinus. After 48 hours cold and warm floatation, the samples were dehydrated at laboratory temperatures to about their initial fresh weights after which they were rehydrated at 1°C. During dehydration, discs were laid flat on a wire gauze suspended on a test tube rack. This allowed air to circulate freely around them. Shrinkage and wilting of warm discs were observed during dehydration. This was more severe the larger the previous growth uptake and the wilting observed could be

Figure 2.11 Fresh weights and area changes of young and 'mature' leaf discs (3rd (o, •) and 5th (□, ■) from apex respectively. The discs were initially floated on water at 30°C (——) and 1°C (---) followed by a brief period of dehydration in air and then rehydration at 1°C.

Figure 2.11a normal leaves.

Figure 2.11b stressed leaves.

Fig. 2.11a.

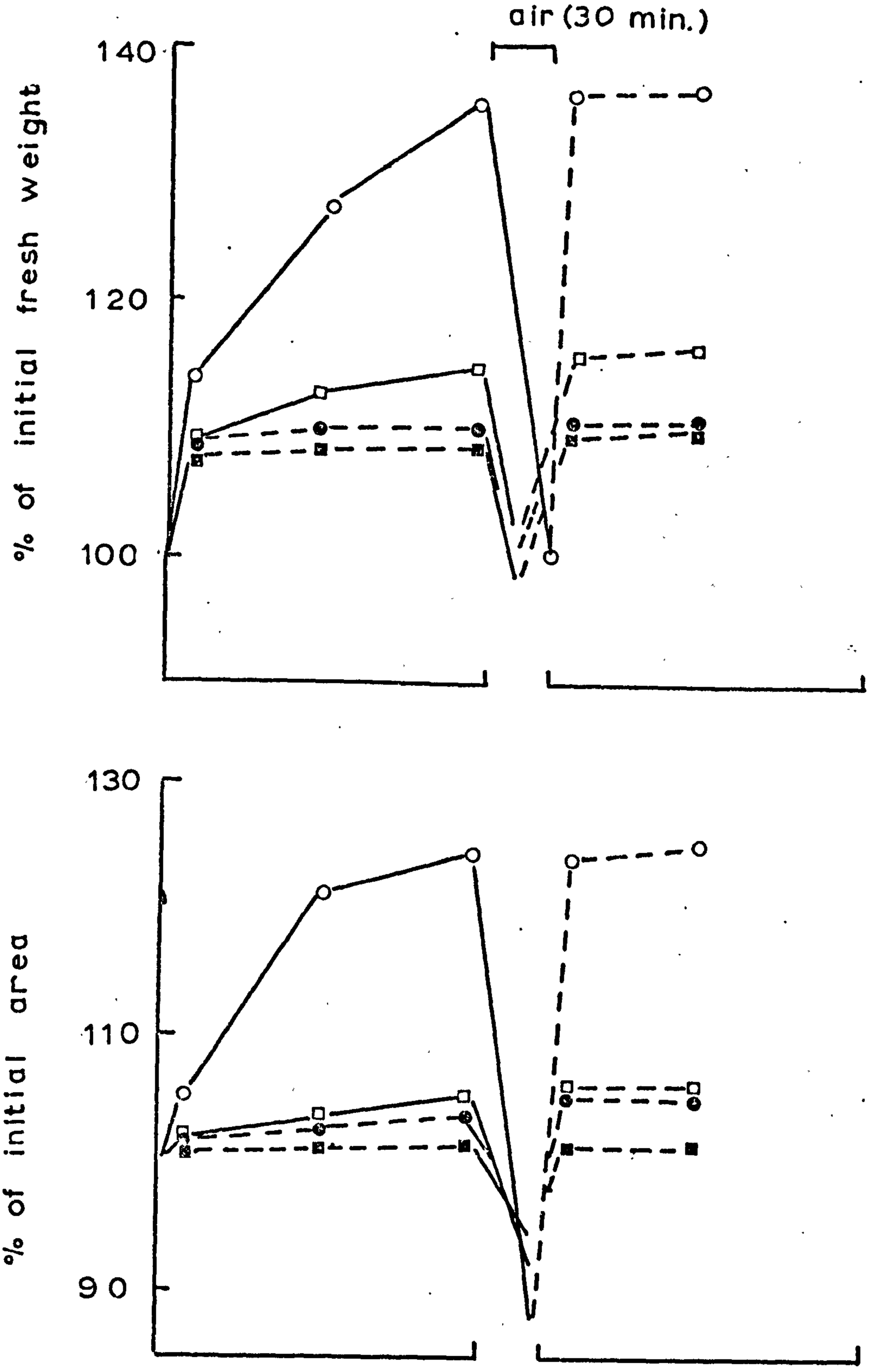
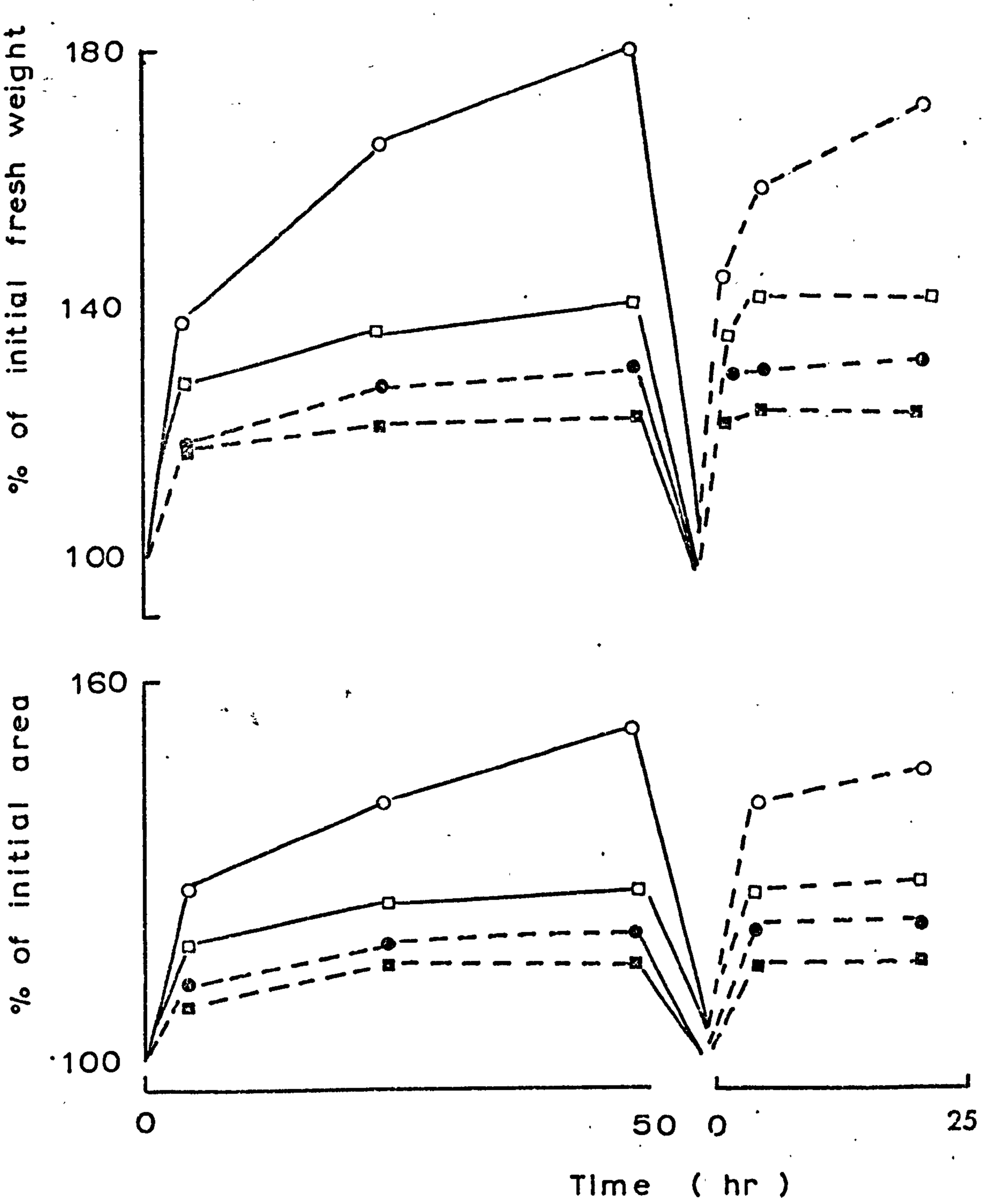

air (30 min.)
140
120
100
% of initial fresh weight
130
110
90
% of initial area
0
50
0
50
Time (hr)

Fig. 2.11b.


Air (40 min)
% of initial fresh weight
180
140
100
% of initial area
160
100
0
50
0
25
Time (hr)

attributed to the following:-

Firstly, since discs were illuminated around compensation point during hydration, there could be little or no net increment in wall material and as such the enlarging cell walls could be thinner than normal. Secondly, since these warm discs grew, it is likely that upon dehydration they were exposed to a lower RWC (%) (more severe water stress) than indicated by their initial water content.

Figure 2.11$_a$ shows that the rates of uptake and the corresponding discs area change during cold rehydration were greatly increased compared with the initial cold rates. With the exception of the young stressed leaf, all the discs completely recovered to their pre-dehydration weights and areas within 4 hours of cold rehydration. This observation supports the hypothesis that cold uptake completely satisfies water deficits. If the phase II cold uptake was in response to water deficits as a result of an incomplete saturation by cold phase I, it would have taken longer than the 4 hours to reach the pre-dehydration fresh weights and areas. It, therefore, seems that the slow but observable uptake by turgid tissues during cold floatation is also a growth phenomenon, probably "creep". This growth uptake was calculated to be about 3% and 8% (i.e. % IFW at 48 hr - % IFW at 4 hr) in normal and stressed leaves respectively.

Figure 2.11 B shows that the younger stressed discs recovered about 86% and 92% of the pre-dehydration fresh weights and areas respectively by 4 hours cold rehydration. Although this incomplete recovery (even after 24 hours floatation) could be attributed to the factors described above, it is also likely

Figure 2.12 Effect of warm (——) and cold (- - -) uptake on fresh weights and areas of discs. A pair of samples was initially floated on water at 30°C (o) or 1°C (●) for 4 hours and then briefly dehydrated in air, after which one each of the warm and cold discs were rehydrated at 1°C or of 30°C.

Figure 2.12a a normal young leaf.

Figure 2.12b a young stressed leaf (3rd from apex).

Fig. 2.12a.

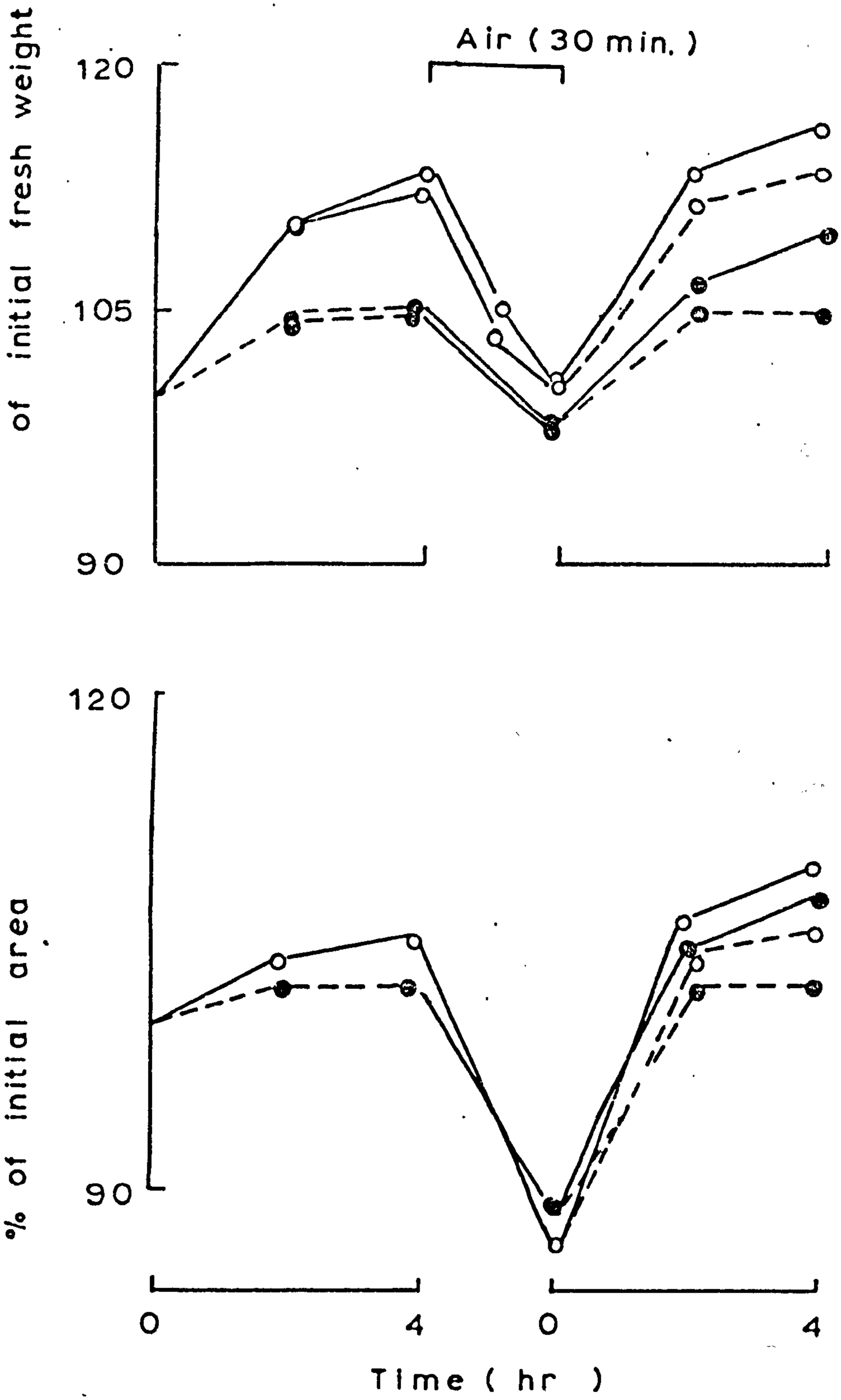
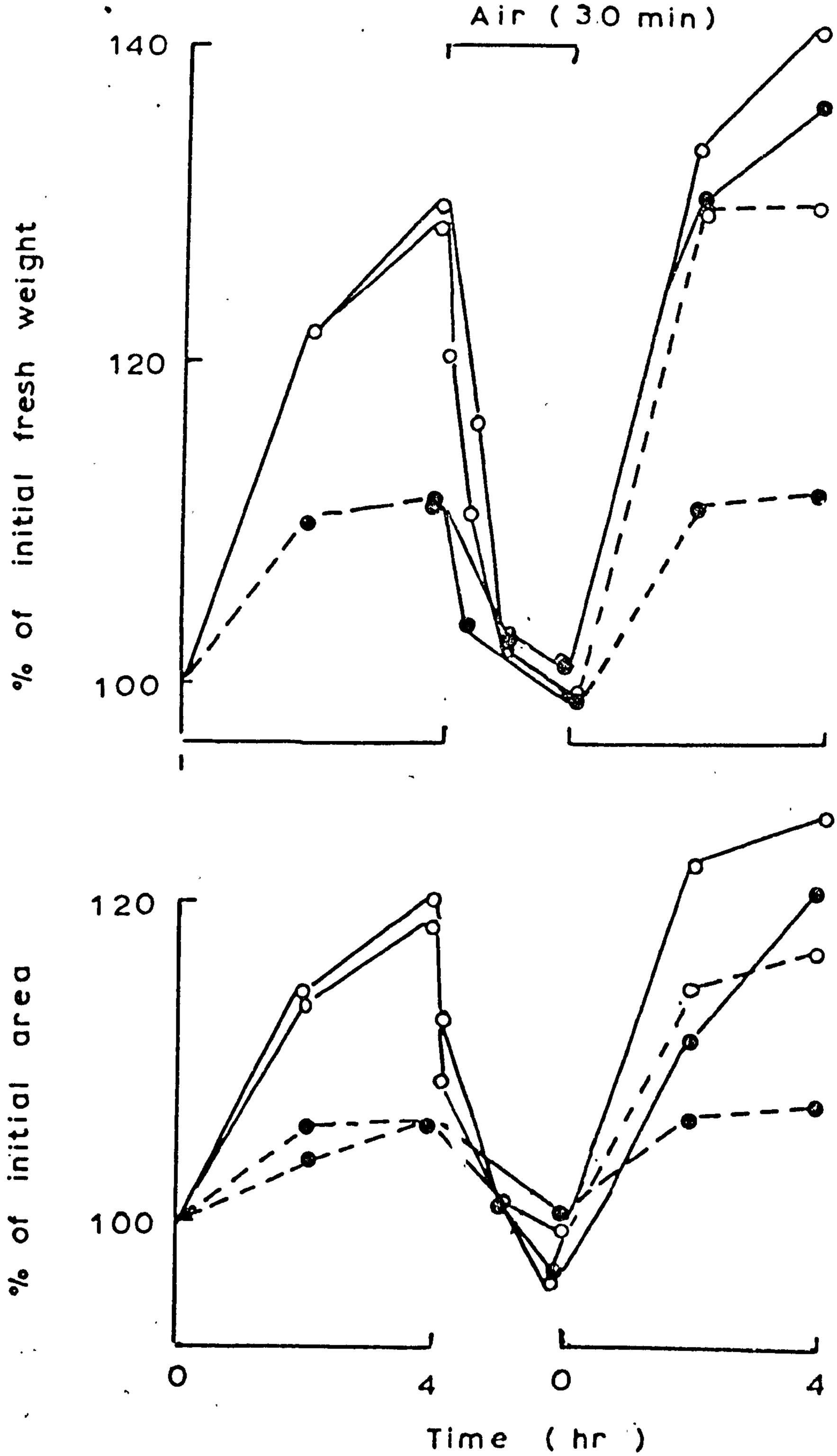

Air (30 min.)
% of initial fresh weight
120
105
90
% of initial area
120
90
0
4
0
4
Time (hr)

Fig. 2.12b


Air (30 min)
140
120
100
% of initial fresh weight
120
100
% of initial area
0
4
0
4
Time (hr)

that a small proportion of the total enlargement measured resulted from elastic extensions in the cell wall.

In Figure 2.12 a,b, the samples were dehydrated after phase I. Afterwards some of the warm and cold samples were rehydrated at 1°C and the remaining at 30°C. The figure shows that samples rehydrated in the cold completely recovered their phase I pre-dehydration weights and areas in 4 hours. Rehydration in warm conditions on the other hand, resulted in values which were greater than the pre-hydration values. The gain by the original warm discs was small representing a steady uptake, but the original cold discs increased greatly. For example, the stressed leaves showed a 3 fold increase over their previous cold values. This massive gain is significant and attributable to the growth potential which accumulated on the intact plant but which was not expressed during the initial cold uptake.

The figure further shows that the phase I extra warm uptake is entirely irreversible.

The results generally confirm and extend those of Milburn and Weatherley (1971).

2.8 Discussion

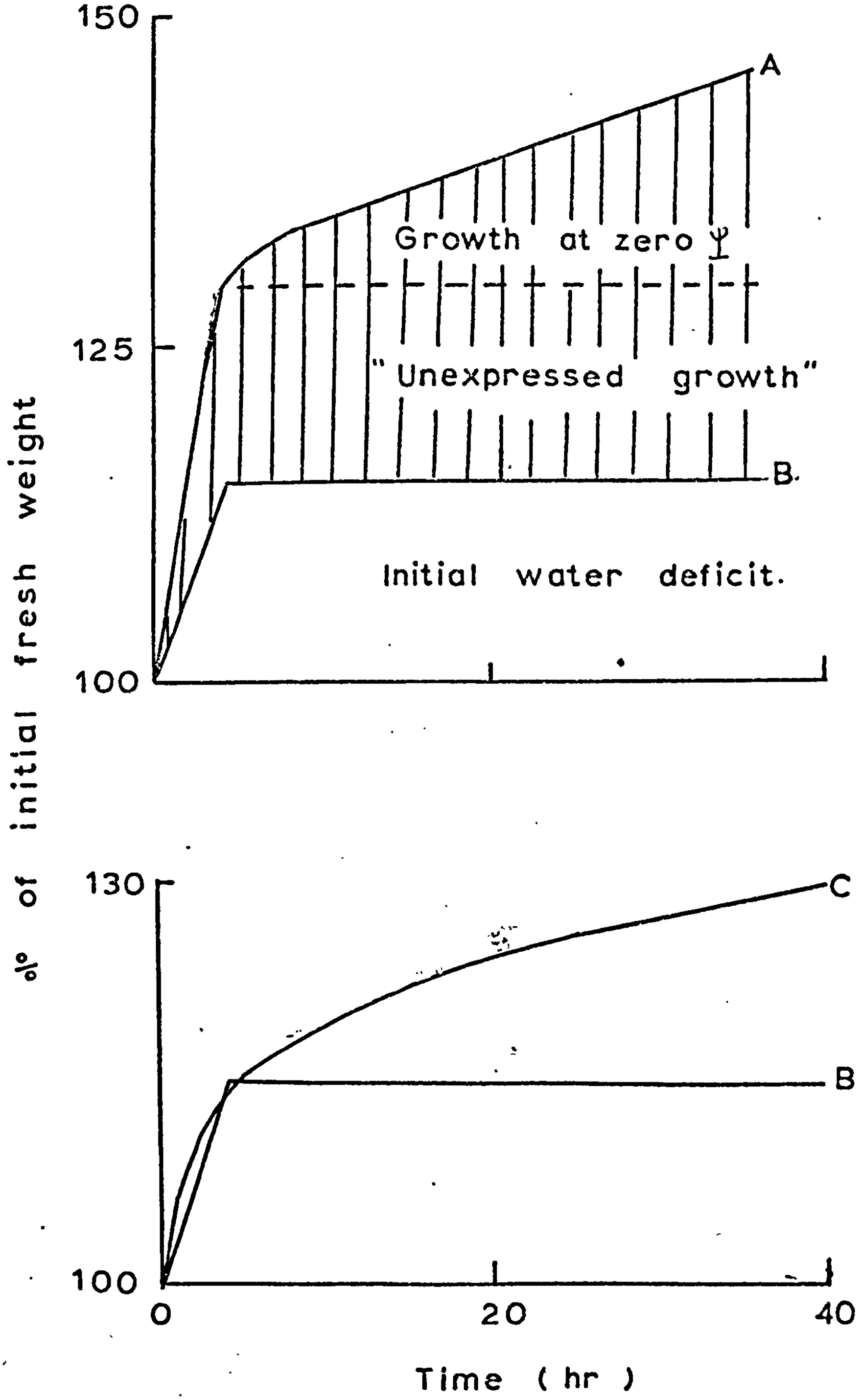
The results presented here show that the relative contributions of the water deficits and growth uptake processes are influenced by the temperature of floatation. Thus, when leaf discs were allowed to float on water at warm temperatures (30°C) there was a substantial absorption phase which, apart from satisfying the initial water deficit, consisted of a growth component the magnitude of which depended on the stage of leaf maturity and the duration of drought. Furthermore,

the results show that the growth uptake was accompanied by a proportional increase in area. On the other hand, when discs were floated at low temperatures (1°C) and over a short duration (4 hours) water is absorbed to satisfy the initial water deficits alone.

Evidence on the magnitude of resistances to water movement offered by different plant organs and tissues is still inconclusive (e.g. Tinklin and Weatherley 1966, 1968; Hoffman and Splinter, 1968; Stoker and Weatherley, 1971; Boyer, 1974). However, assuming that young and older leaves on a small plant possess equal resistances, in the absence of transpiration, their water potentials will be in equilibration through xylem conduction. Therefore, differences in RWC of the leaves would be expected to be negligible. In fact, data presented in Section 3 shows that differences in Y between younger and older leaves, although significant, are very small. Thus from the data here, the warm uptake values, even those obtained for leaves under negligible transpiration conditions do not conform to these water deficit relationships. In contrast, absorption at low temperatures, especially phase I, is fairly similar for all leaves under most conditions suggesting that this uptake represents the true water deficits.

These results support the view of Milburn and Weatherley (1971) that water absorbed in the cold represents a complete recovery of total cell water deficits. However, it should be emphasised that this is only true when uptake is limited to phase I. Consequently, the results contrast with an earlier view of Barrs and Weatherley (1962) which suggested that cold uptake only hydrated the cell walls, while phase I warm

Fig. 2.13.


150
125
100
fresh weight
% of initial
A
Growth at zero Ψ
"Unexpressed growth"
B.
Initial water deficit.
130
100
C
B
0
20
40
Time (hr)

uptake represents the true water deficits. But the results presented here, like those of, for example, Yemm and Willis (1954), Catsky (1963), Molz et al (1975), suggest that warm absorption even during phase I, has a substantial growth uptake component. Yemm et al (1954) considered this growth uptake to be steady throughout floatation. Consequently they advocated that the turgid weight could be obtained by extrapolating phase II of the water uptake curve to zero time. However, it is obvious from the experiments here that phase II is not necessarily linear. Non-linear curves have also been obtained by Catsky (1963) and Potter and Milburn (1970); Molz et al (1975) assumed that the growth uptake begins immediately during warm floatation but at a slow rate which increases to a steady maximum rate at the same time as phase I is completed.

The results here show that phase I warm uptake was in response to the initial water deficit, but super-imposed upon this uptake was an additional phase of uptake which is attributable to growth. Figure 2.13 attempts to partition the warm uptake curve into the various components. As suggested by Molz et al (1975), the growth uptake may begin immediately, initially in cells near the cut edge of the discs. However, the uptake progresses rapidly inward (probably through high conductivity by xylem vessels).

In contrast to Molz et al view, Figure 2.13 shows that the growth uptake was at its maximum rate within the first few hours (phase I) during which 'unexpressed growth' became manifested before a reduced rate was established (phase II).

All the experiments clearly show that during floatation

at warm temperatures growth played a much larger role in both rapid (phase I) and prolonged (phase II) water uptake than previously expected (cf Millar 1966) and, therefore, relative water content (RWC) must be measured near 0°C after 4 hours floatation. If measured at higher temperatures a growth water deficit is added depending on the stage of leaf maturity.

The slow growth observed in the cold during phase II is attributable to 'creep'. Probine and Preston (1962) described a similar physical growth process for Nitella when it was subjected to a constant stress. A somewhat similar, but involving a more rapid growth, 'plastic flow', was observed by Barrs and Weatherley (1962), for Ricinus leaves floated at 3°C. Although the 'passive plastic' overstretching hypothesis (see review by Heyn, 1940; Hettiaratchi and O'Callaghan, 1974) is not widely accepted, the evidence suggests that the phenomenon may be operative in fully turgid tissues.

The transitory rapid growth during phase I (Figure 2.13) was influenced by water stress (Fig. 2.3, 2.5). The indications are that a potential for cell enlargement, an 'unexpressed growth', builds up following growth suppression by water stress but this became rapidly expressed when the water deficit was satisfied. These results and the conclusions drawn accord with the 'arrested growth hypothesis' of Milburn and Weatherley (1971).

A similar phenomenon has been observed for intact plants (Gates, 1955; Owen and Watson, 1956; Hsiao et al, 1970; Acevedo et al, 1971; Barlow and Boersma, 1972). Hsiao (1973) called the phenomenon 'stored growth' and proposed that during drought, metabolites required for cell extensibility may

accumulate and will consequently enable rapid cell expansion when favourable internal water status is restored. However, since stress relaxation lowers Y, it could be argued that any 'stored growth' in the form of wall loosening (see Cleland, 1971 a) would contribute to the total initial water deficits (e.g. Figure 2.13) and hence would be obscured by the water deficit uptake (cold phase I) and would not contribute to the observed phase I growth. It is more reasonable to suggest that the 'unexpressed growth' measured consisted of both wall loosening and extension. It will be interesting to know the nature of the accumulated growth metabolites, if any.

The fact that 'unexpressed growth' was demonstrated for normal leaves, suggests that the normal water deficits which generally persist in land plants continually suppress cell enlargement.

It has been assumed that once plant organs such as leaves have grown to maturity (i.e. cease to expand) their capacity to continue growth is lost. Any water uptake would, therefore, be expected to arise from a water deficit alone (Dixon, 1898, Smith et al, 1931; Dixon and Barlee, 1940). The results here, like those of Weatherley (1950), and Potter and Milburn (1970), clearly show that 'mature' leaves retain their capacity to grow which is expressed when supplied with free water, i.e. Y near zero bar. The implications are that leaves only mature in so far as Y will allow and remain immature even on normal plants.

The persistent water absorption reported here suggests that the water potential gradient between the growing cells and the medium was maintained. Thus, if the growth uptake

is considered as a function of Y, it could be hypothesised (other factors being constant) that young leaves generate greater suction than older leaves. These assumptions are investigated in the following sections.

SECTION 3

ASPECTS OF THE WATER RELATIONS OF RICINUS AND HELIANTHUS

In the previous section attention was drawn to the relationships between rate of growth of young and older leaves when water was freely available. However, in nature the water potential (Y) of the plant is virtually never zero, and consequently cells grow at negative Y. The magnitude of this deficit is highly variable and it is known that cell expansion and other physiological processes respond accordingly (Boyer 1968, 1971; Hsiao, 1973). It is also generally known that the internal water balance is controlled by the properties of the tissues of the plant concerned. Before embarking on a detailed study of the effects of internal water deficits on growth it was decided to study first the general water relations of Ricinus leaf tissues. This appeared worthwhile to provide a basic understanding of the internal water-growth relationships.

Initially it was decided to include other species in the study for comparison. Eucalyptus and Gossypium were used for a period but because of the small leaf sizes and short petioles (especially those of Eucalyptus) of the plants it was found impossible to measure all the plant water deficit parameters employed here on single leaves. Helianthus leaves were, however, found to be more suitable.

3.1 Materials and methods

Plant water status was assessed by measuring leaf relative water content (% RWC 1°C) and leaf water potential. The water potential parameters measured are - xylem tensions (Y_x) leaf cell sap osmotic potential (Y_s) and occasionally xylem sap osmotic potential (Y_{xs}). Leaf water potential (Y) and turgor

Plate 3.1 Field grown Ricinus plants.

pressure (Y_p) were estimated from the Y and Y_s data on the assumption that xylem and leaf cells were in equilibrium.

Two types of Ricinus plants were used. The first types were greenhouse and growth cabinet plants growing in 15 cm. pots. They were at the 6 to 12 leaf stage and their stem heights ranged from 40 - 70 cms. The second types of plant used were larger and older and either field grown (Plate 3.1) or raised singly in boxes (36 X 36 X 32 cm.) in the greenhouse. These plants were over 150 cm. tall and had developed 1 or 2 side branches. Plants of Helianthus were at 8 - 16 leaf stage, 60-120 cm. high and were growing singly in 15 cm. or 21 cm. diameter pots. All the plants were growing in moist soil but during experimentation some of them were given the following 3 water stress treatments.

In the first set of experiments Ricinus plants (type 1, and pot grown type 2), and also Helianthus plants were allowed to undergo 1 or 2 drying - rewatering cycles. High summer temperatures and radiation (Summer 1976 for Ricinus and 1977 for Helianthus) caused the smaller plants to wilt 2-4 days after rewatering and larger Ricinus plants after 5-7 days. Leaves were sampled at the end of the first or second drying out cycle or during the second drying out cycle.

In the second set of stress treatments, the roots of type 1 Ricinus plants were cooled to inhibit water uptake by burying the pot in chips of ice and water. Transpiration was increased by illuminating every two plants with a 60 watts incandescent tungsten-filament bulb which was placed at a distance of 20 cm. from the upper leaves. These plants wilted

rapidly in 4 - 6 hours.

Plants used in the third treatment were prepared similarly to those in the first treatment. Pot soil was allowed to dry out until the plant showed permanent wilting (i.e. the plants remained wilted overnight) before they were rewatered. Three or more of the permanent wilting cycles served as a pre-conditioning treatment. This procedure has been used by previous workers for other species (e.g. Zavitkouski and Ferrell, 1968; Plaut, Halevy and Duskin, 1975; Brown et al, 1976). Observations showed that wilting in Ricinus was not accompanied by a measurable laminar shrinkage. In contrast the leaf area of Helianthus was reduced to about 90 - 95% of the initial area. Furthermore the time Ricinus took to wilt increased. Also, the degree of wilting became less severe with each successive cycle of water deprivation. In contrast, the severity of wilting in Helianthus did not decrease and moreover the time the plant took to wilt was not noticeably longer with each successive cycle of water deprivation. In other words, Ricinus became drought hardened but Helianthus did not.

Well watered plants, those given treatments 1, 2 and 3, will be referred to as, normal, rapidly stressed and repeatedly stressed plants respectively.

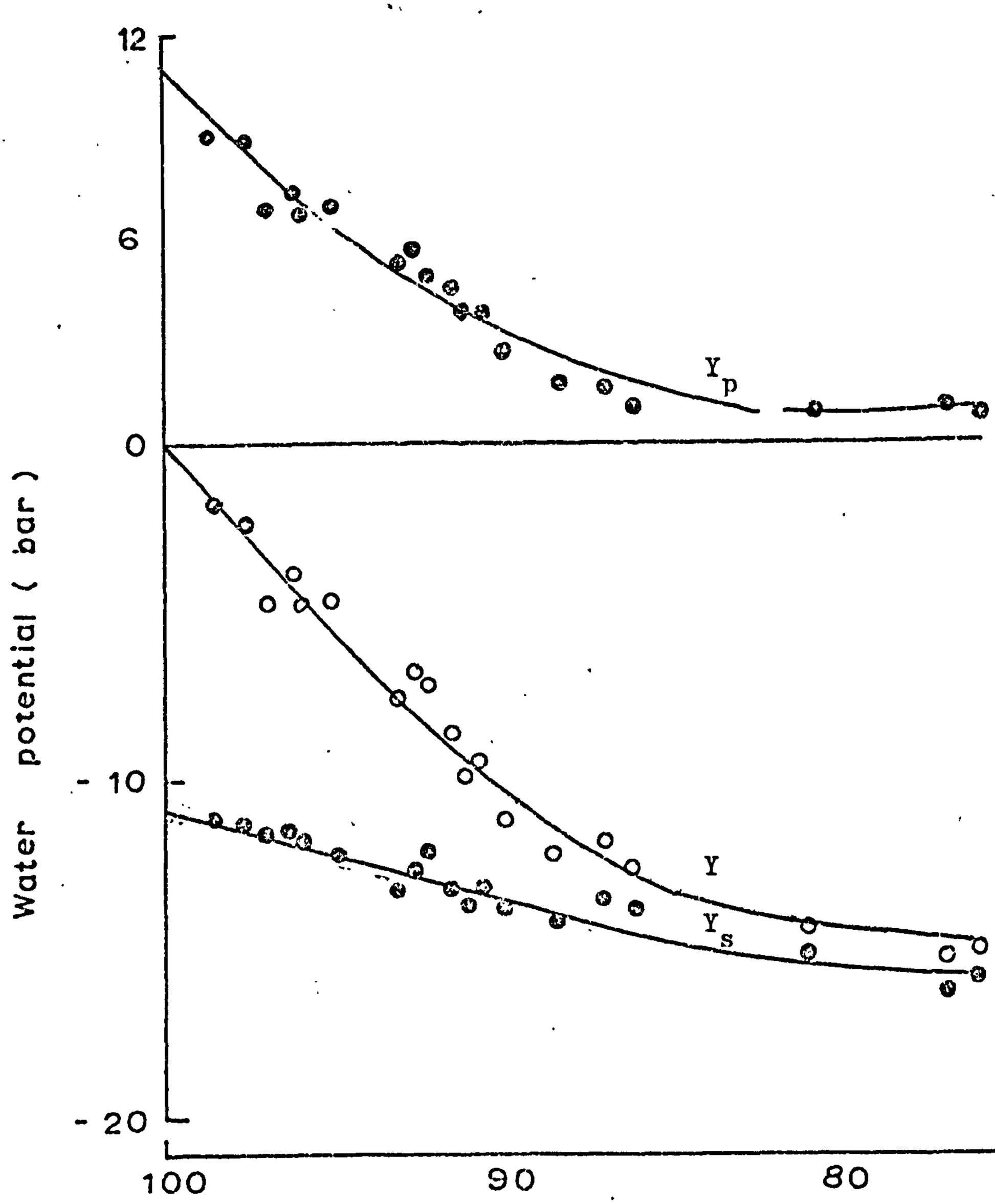
Results

3.2 Relationships between RWC (1°C), Y, Y_s and Y_p.

Initially variations in Y, Y_s and Y_p with reduction in tissue water content were closely examined. 'Mature' bagged Ricinus and Helianthus leaves were sampled from normal and

Figure 3.1 The relationship between relative water content (% RWC 1°C) and leaf water potential (Y), leaf cell sap osmotic potential (Y_s) and turgor pressure (Y_p) of Ricinus.

Fig. 3.1


12
6
0
- 10
- 20
Water potential (bar)
Y_p
Y
Y_s
100
90
80
Relative water content (% R.W.C. 1°C)

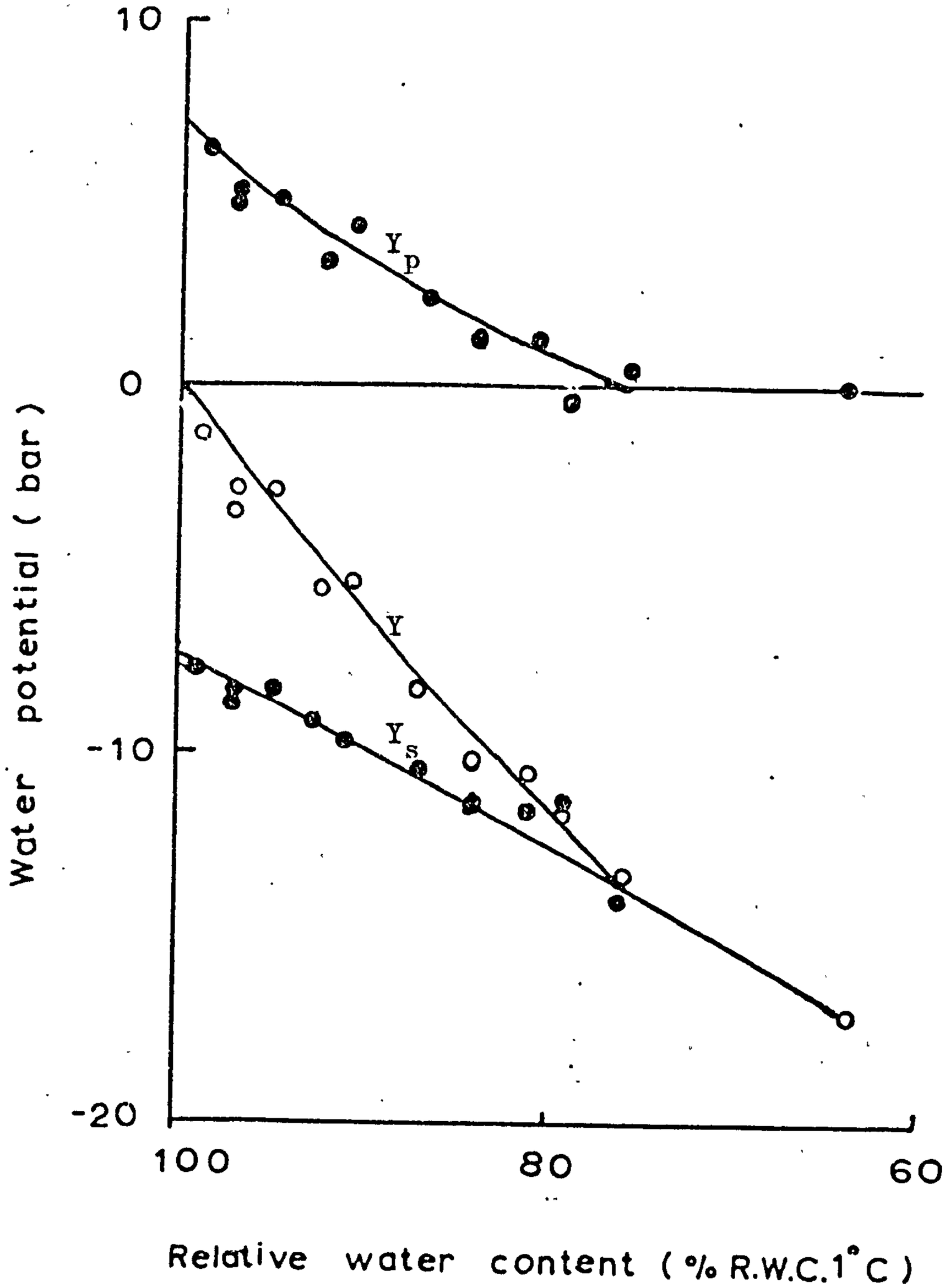
rapidly stressed plants for Y_x measurements. Afterwards the bag was quickly opened in the humid cold room (RH over 90%) and a small piece of the leaf blade was quickly cut, resealed in another bag and frozen for Y_s measurements. At the same time 4-8 discs were punched for RWC determinations. The remaining Ricinus leaf sample was bagged and resealed in the bomb and xylem sap was extracted from it for Y_{xs} measurements. However, the remaining Helianthus leaf sample was found too small to yield enough sap for Y_{xs} measurement. This was, however, studied on parallel leaves and was found to vary randomly from 0.2 to 0.7 bar between a Y_x of 0 to 10 bar and, therefore, a simple addition of a mean Y_{xs} value to Y_x values is not proposed. Consequently, bomb measurements were taken to represent Y.

It was not possible to obtain intact leaves of Ricinus and Helianthus with RWC above 98% and those of Ricinus below 83%. These samples were, therefore, obtained by allowing the respective excised leaves to take up water at 1°C for 4 hours while sealed in polythene bags or artificially stressed at laboratory temperatures. Leaves from the former treatment were assumed to be fully turgid and, therefore, to possess a RWC of 100% and a Y of 0 bar.

The results obtained are presented in a modified form of a Höfler diagram in Figures 3.1 and 3.2 for Ricinus and Helianthus respectively. Figure 3.1 shows that as tissue water content decreased Y reduced rapidly until a RWC of around 88%. (Observations made suggest that Ricinus leaf becomes flaccid at this RWC). Below RWC of 88%, Y decreased slowly reaching values of -14.0 bar at 83% to RWC.

Figure 3.2 The relationship between relative water content (% RWC 1°C) and leaf water potential (Y) leaf cell sap osmotic potential (Y_s) and turgor pressure (Y_p) of Helianthus.

Fig. 3.2


10
0
-10
-20
100
80
60
Y_p
Y
Y_s
Water potential (bar)
Relative water content (% R.W.C. 1°C)

From 100% to 88% RWC Y_s decreased gradually from -11.0 to -14.0 bar and appeared to be linearly related to RWC, thereafter the relationship became non-linear. Y_p declined more rapidly with decreasing RWC than did Y_s, falling from about 11 bar at full turgidity to 1.0 bar at 83% RWC. The results clearly show that over this range of tissue water, Y_p accounted for the major part of the decreases in Y. After this point Y_p remained fairly constant, and further lowering of Y (artificially stressed excised leaves) were accounted for by Y_s. On account of residual Y_p in wilted leaves, Y and Y_s were not identical but ran parallel to each other. At RWC of 75%, Y, Y_s and Y_p were about -14.5, -15.5 and 1.0 bar respectively. The low RWC and Y_p suggest that Y_s decreased through concentration of cell sap, resulting from tissue water-loss rather than accumulation of solutes.

Figure 3.2 shows that, like Ricinus, initial decreases in tissue water content of turgid leaves of Helianthus caused large decreases in Y. However, indications are that unlike Ricinus tissues, a significant part of the fall in Y was contributed by decreases in Y_s. For example, at a RWC of 90%, Y of Ricinus was about - 10 bar. Y_s dropped from -11.0 to -13.5 bar, thus contributing to about 25% of the fall in Y. At the same RWC, Y of Helianthus was -6 bar and Y_s has decreased from -7.5 to - 9.8 bar, the contribution made here to the Y was about 38%.

Figure 3.2 further shows that at RWC below 80%, Y_s of Helianthus tended to be less negative than Y consequently the points for Y_p lie below the line in the region corresponding to negative turgor pressure.

The experiments here further show that solute content of Helianthus tissue as found in normal growing plants is lower than that of Ricinus. However, comparatively lower Y_s of Helianthus has been recorded by Gardner and Ehlig (1965) and Boyer (1968, 1970). The differences probably reflect differences in the conditions under which the plants were grown.

3.3 Gradients of Y, Y_s and Y_p of normal, rapidly stressed and repeatedly stressed plants

The presence of decreasing water potential gradients from the root up the plant is a common phenomenon. Such a hydrostatic gradient in response to gravity is theoretically 0.1 bar per meter rise in non-transpiring plants but the gradient could be steeper during rapid transpiration.

The Y has often been assumed to be identical for all leaves on small plants, because of the low resistance to water flow offered by the xylem (see for example Tinklin and Weatherley, 1966, 1968). However, recent experimental data suggests that this is not necessarily so (Begg and Turner, 1970; Acevedo et al 1971; Cary and Fisher, 1971). Moreover, very little is known about Y_s and Y_p relations of different leaves on a plant although these parameters are important for growth. The following experiments examine the Y, Y_s and Y_p differences in young and older leaves at different known height of insertion on normal, rapidly stressed and repeatedly stressed plants of Ricinus and Helianthus.

Between 3 and 5 leaves were sampled from any one plant for Y_x and Y_s measurements. Y_x measurements of all the samples from a plant took 6-12 minutes. Preliminary study showed

Figure 3.3 The relation between Y_p, Y and Y_s for Ricinus leaves at different heights of insertion on the stem of:

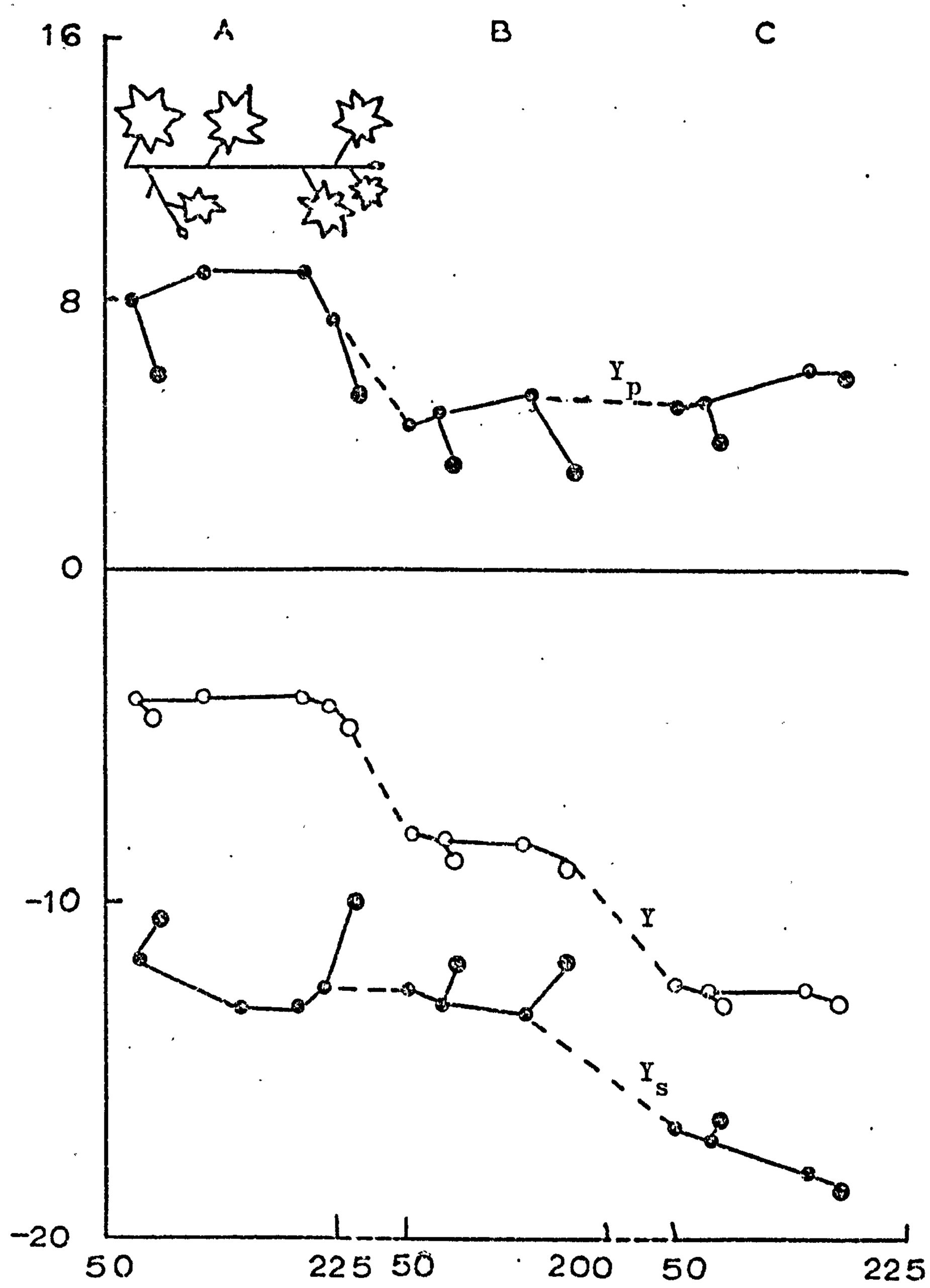
a normal plant (A)

a rapidly stressed plant (B, 3-5 day drought)

a repeatedly stressed plant (C, leaves sampled 8-12 days after water deprivation).

Bigger symbols represent young apical leaves.

Fig. 3.3


16
A
B
C
8
0
-10
-20
Water potential (bar)
Y_p
Y
Y_s
50
225
50
200
50
225
Height of leaf insertion (cm)

that Y_{xs} of Ricinus was maintained fairly constant (about -1.0 bar) over most stress levels (see also Figure 3.6). This value was, therefore, added to the Y_x values to obtain leaf water potentials (Y). No corrections were made for Helianthus for reasons already given (see page 75).

Examples of the water deficit parameters measured in leaves from different heights on the stem and in relation to plant water stress, are shown in Figure 3.3 and Table 3.1 for Ricinus and Figure 3.4 for Helianthus.

Table 3.1

Effect of Rapidly Induced Water Stress (by root cooling) on the Water Potential Gradients in Leaves Along Stems of Ricinus Plants

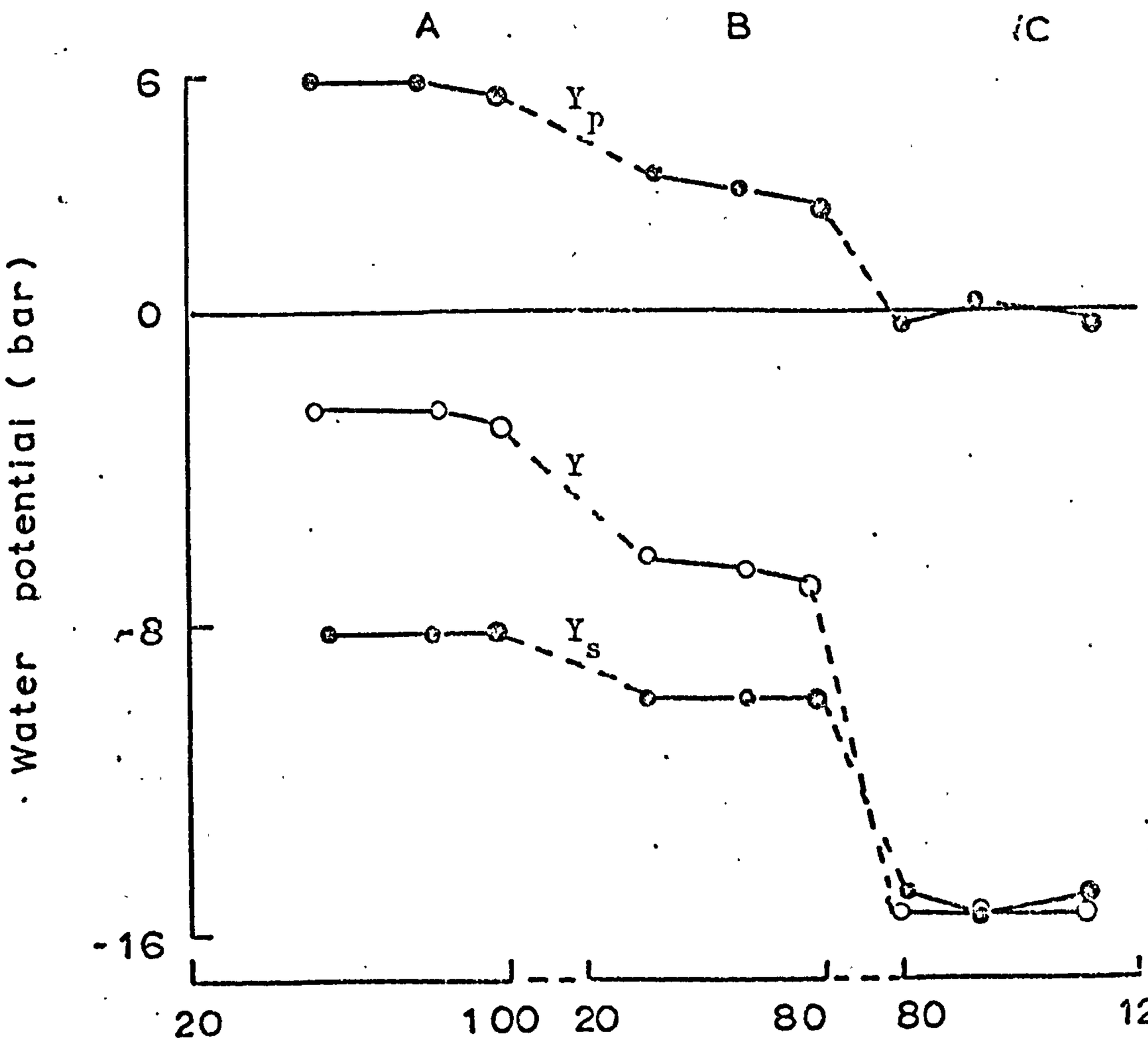
Experimental plant	Height at Sampling (cm)	Relative Water Content (% RWC 1°C)	Water potential (bar) Y	Y_s	Y_p
1	36	91	-10.1	-13.3	3.2
	39	89	-10.1	-14.0	3.9
	44	90	-10.4	-12.0	1.6
2	40	92	- 8.7	-12.6	3.9
	45	91	- 8.8	-12.3	3.5
	46	90	- 9.0	-11.2	2.2

A. Leaf water potential Y

The data for Ricinus shows that under most stress conditions Y decreased only slightly from lower 'mature' leaves to upper leaves which were approaching maturity. Differences of about 0 - 0.2 bar per 0.4 - 0.5 m rise

Figure 3.4 The relation between Y_p, Y and Y_s for Helianthus leaves at different heights of insertion on the stem of:
a normal plant (A)
a rapidly stressed plant (B, 3-5 day drought)
a repeatedly stressed plant (C, leaves sampled at the end of a drying cycle).
Bigger symbols represent young apical leaves.

Fig. 3.4


A
B
C
6
0
-8
-16
Water potential (bar)
Y_p
Y
Y_s
20
100
20
80
80
120
Height of leaf insertion (cm)

were measured. However Y were usually low in rapidly expanding young leaves, irrespective of their positions on the stem. These leaves were typically the first and occasionally the second expanded leaves from the apices of the main stem or side branches. Y in these leaves were 0.2 to 1.0 bar lower than 'mature' leaves at similar height of insertion or which were at only a few cm distant. Figure 3.4 shows that Y of young leaves (expanded leaves near main apex) of Helianthus were also slightly lower than older leaves. The differences ranged from about 0.2 to 0.6 bar when Y were higher than -10.0 bar, but became insignificant thereafter.

B. Osmotic potential (Y_s) and turgor pressure (Y_p)

Figure 3.3 and Table 3.1 shows that Y_s of normal and rapidly stressed Ricinus decreased slightly (about - 1.5 bar) from lower 'mature' leaves to upper leaves. However, young leaves had similar Y_s values if growing on side branches or apical shoots and these could be about 2.0 to 3.0 bar higher than in older leaves. Consequently, Y_p were about 2.0 to 3.0 bar lower in the former than latter leaves.

However, unlike normal and rapidly stressed plants, Y_s of the repeatedly stressed plants decreased from the bottom to the top of the main shoot. Consequently Y_s of younger leaves on the main apex was lower than in older leaves, allowing the former leaves a corresponding high Y_p despite their low Y. Figure 3.3 clearly shows that Y_p of these young

leaves could be higher than those of their counterparts on normal plants. However, Y_s of young leaves on branches of hardened plants was not reduced to the same degree as those on the main apex. The low Y_s with the consequent high Y_p suggests that repeatedly stressed plants adjust osmotically during drought by increasing net cell solutes.

Figure 3.4 shows that unlike Ricinus differences in Y_s of older and young leaves of Helianthus, for all 3 types of plant, were negligible. Consequently Y_p of young Helianthus leaves were only slightly lower than older leaves. Figure 3.4 further shows that when water stress was severe Y and Y_s became numerically identical and consequently Y_p falls to values around zero bar. Helianthus therefore, does not adjust osmotically during water stress.

Table 3.2

Recovery of Water Potential Parameters of Helianthus Leaves Following Rewatering After a Period of Water Stress

Water Potential (bar)	Repeatedly Stressed Plant	Days after Rewatering 1	2	3
Y	-14.8	-3.9	-3.3	-3.3
Y_s	-14.8	-11.1	-9.4	-9.1
Y_p	0.0	7.2	6.1	5.8

Table 3.2 shows that Y and Y_s did not recover readily upon rewatering following water stress presumably partly owing to a reduction in xylem conductance through cavitation

by water stress (cf Milburn, 1973).

C. Differences of Y, Y_s and Y_p of young and older leaves of plants in darkness

The Y gradients measured for both Ricinus and Helianthus in 3.3 A,B were markedly greater than the theoretical hydrostatic gradient of about 0.1 bar m^{-1} required to raise water against gravity. In theory the upper leaves being more exposed and also receiving greater radiation should transpire faster and so possess lower Y than the much sheltered lower leaves. However, it is obvious from the results that these two factors are unimportant here because the experimental leaves were enclosed to prevent transpiration and promote equilibrium one hour or more before measurements were made. Moreover the variations between young and 'mature' leaves of Ricinus did not obviously correlate with their position on the plant.

However, the transpiration hypothesis was tested by repeating the experiments in darkness, using normal plants. After bagging the experimental leaves, the whole plants were enclosed in polythene bags which were kept humid by moist tissue paper. This treatment was to minimise transpiration of unbagged leaves. One to two hours later, leaves were sampled for Y measurements. The results obtained (examples given Pg.82) showed that the magnitude of Y differences was low, this probably resulted because transpiration from unbagged leaves was negligible. However, the Y pattern for young and older leaves on a plant was always the same.

Ricinus				Helianthus			
Height of leaf insertion (cm)	Water potential (bar) Y	Y_s	Y_p	Height of leaf insertion (cm)	Water potential (bar) Y	Y_s	Y_p
40	-3.4	-12.0	8.6	40	-2.0	-8.5	6.5
48	-3.4	-12.6	9.2	52	-2.0	-8.6	6.6
58*	-3.9	-10.2	6.3	67*	-2.2	-8.6	6.4

*young leaves.

3.4 The diurnal variations in plant water status

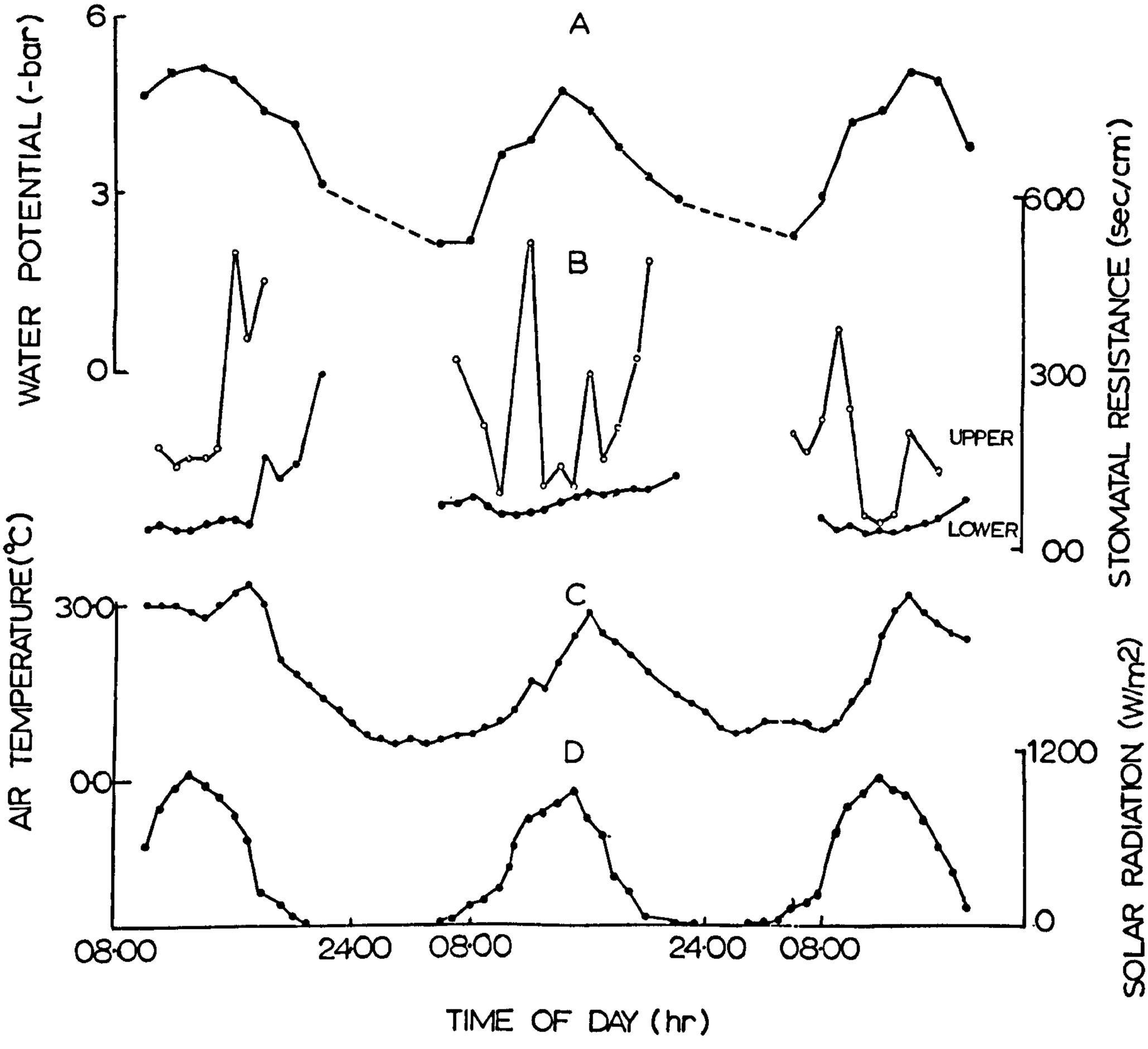
During the day, absorption lags behind transpiration because of high evaporative demands and because stomata open in light. Consequently, internal water deficits increase. At night when transpiration is negligible, the day time deficits are reduced or completely eliminated depending on the soil moisture status and the efficiency of the root system in water absorption.

In the following experiments it was considered desirable to study the diurnal variations in water status and stomatal diffusion resistances experienced by Ricinus in relation to variations in solar radiation (or artificial light) temperature and relative humidity.

Growth cabinet, greenhouse, and field - grown plants were used. All plants were grown in moist soil. The growth cabinet conditions were as described in Section 1 except that the lighting was set for 12 hours. Water potentials (Y_x, Y_s and Y_{xs}) were measured at 2 hourly intervals between 06.00 to 24.00 hrs. Initially, two upper 'mature' leaves from 1 plant was sampled. Subsequently, one leaf from each of 2 plants was sampled. Each plant provided material for at least 2 consecutive determinations. After bomb measurements, one

Figure 3.5 Diurnal patterns of: leaf water potential (A), stomatal resistances, (B, upper o ; lower ●; of field grown Ricinus plants, in relation to air temperature (C) solar radiation (D). The measurements were made on 16/8/76 to 18/8/76.

Fig. 3.5.


A
B
C
D
WATER POTENTIAL (-bar)
0
ω
6
UPPER
LOWER
60
30
0
STOMATAL RESISTANCE (sec/cm')
AIR TEMPERATURE(°C)
30
0
1200
0
SOLAR RADIATION (W/m2)
08·00
24·00
08·00
24·00
08·00
TIME OF DAY (hr)

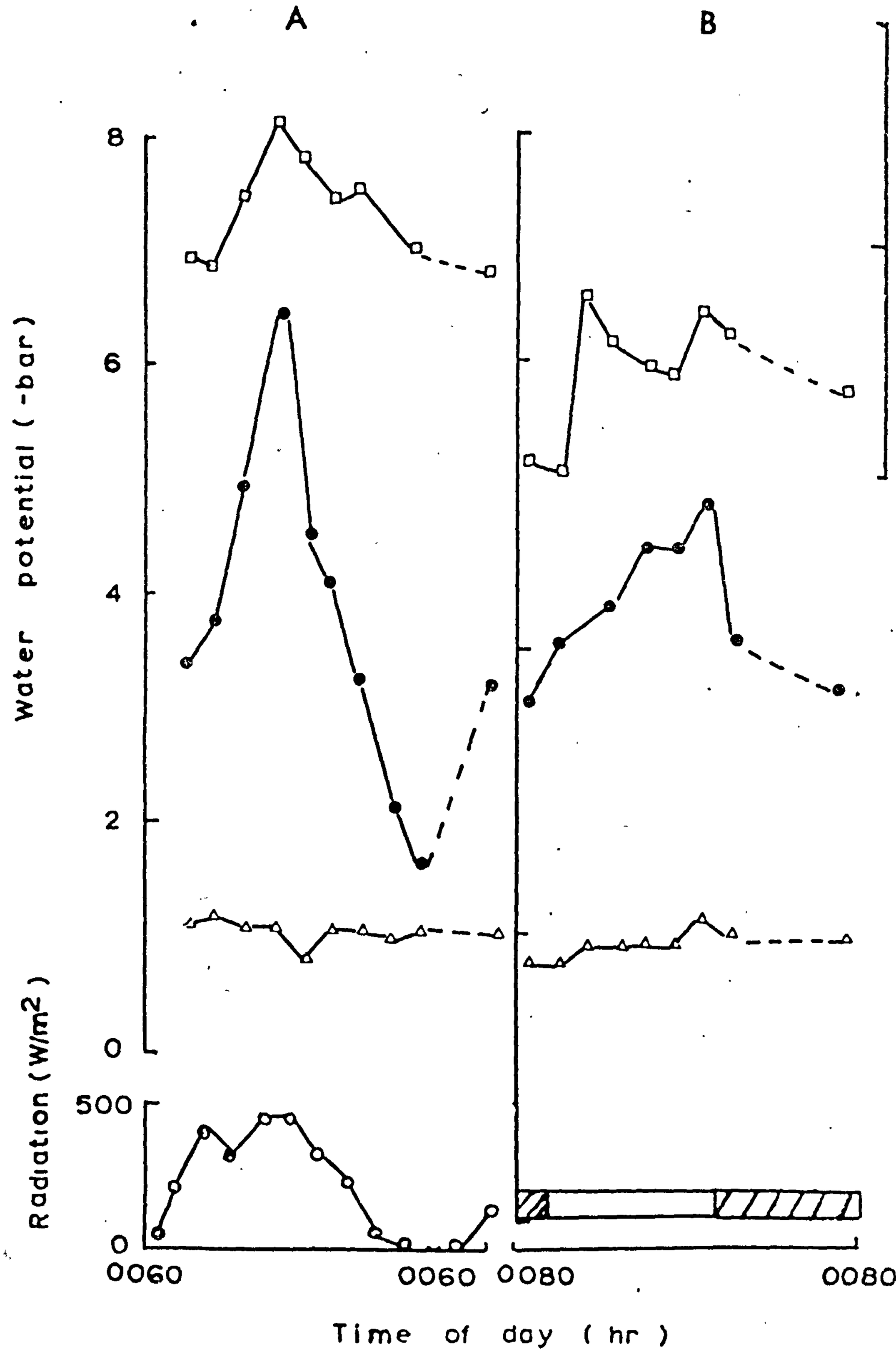
of the leaves was frozen for Y_s determination and the other leaf was prepared for xylem sap extraction for Y_{xs} measurement.

Stomatal resistances to water vapour diffusion of the upper and lower epidermes of upper expanded leaves of field grown plants were estimated at hourly intervals. The plants used were the same as those sampled for Y measurements. At each determination 2-3 measurements were taken from various parts of the leaf blade of the lower epidermis. The values were found to be almost identical. Because of the high resistances offered by the upper epidermis stomata, only one reading was taken on each occasion.

Figures 3.5 and 3.6 show examples of curves obtained for the experiments in the field and growth cabinet and greenhouse respectively. It was found that presentation of all the results in a graphical form complicated the curves for the various parameters. For this reason Y_s, Y_{xs} and relative humidity (RH%) for figure 3.5 and temperature and RH% for figure 3.6 are presented in Appendix 2 (a and b). Because of the small number of daily measurements made, only the general pattern of plant water deficits was observed. Maximum relative humidity (RH) and minimum temperature in the greenhouse and field were recorded in the early mornings and evening and at night. Minimum values of RH and maximum values of temperature and radiation were obtained between 12.00 and 14.00 hours G.M.T. Although evaporation was not recorded, it could be assumed that conditions occurring generally between 12.00 and 14.00 hours were conducive to high evaporation. The early morning, evening and night conditions conversely reflect low evaporation rates.

Figure 3.6 Diurnal pattern of leaf water potential (●), leaf cell sap osmotic potential (□), xylem sap osmotic potential (Δ) of greenhouse (A) and growth cabinet plants (B) in relation to radiation (greenhouse plants) or light (▭) and darkness (▭) periods. The measurements in the greenhouse were made on 4/3/76 and the morning of 5/3/76.

Fig. 3.6


A
B
8
6
4
2
0
Water potential (-bar)
14
10
Y_s
Radiation (W/m^2)
500
0
0060
0060
0080
0080
Time of day (hr)

As expected from theory (Slatyer, 1967) the results show that in moist soil and with variable environmental conditions, Ricinus started each day with a high water status in their leaves, Y around -3.0 bar. As evaporation increased, water deficits increased so that minimum values were attained usually between 12.00 and 14.00 hours. In the evening when climatic conditions suggest lowered evaporation, water deficits were recovered gradually becoming more rapid during the night to early morning values.

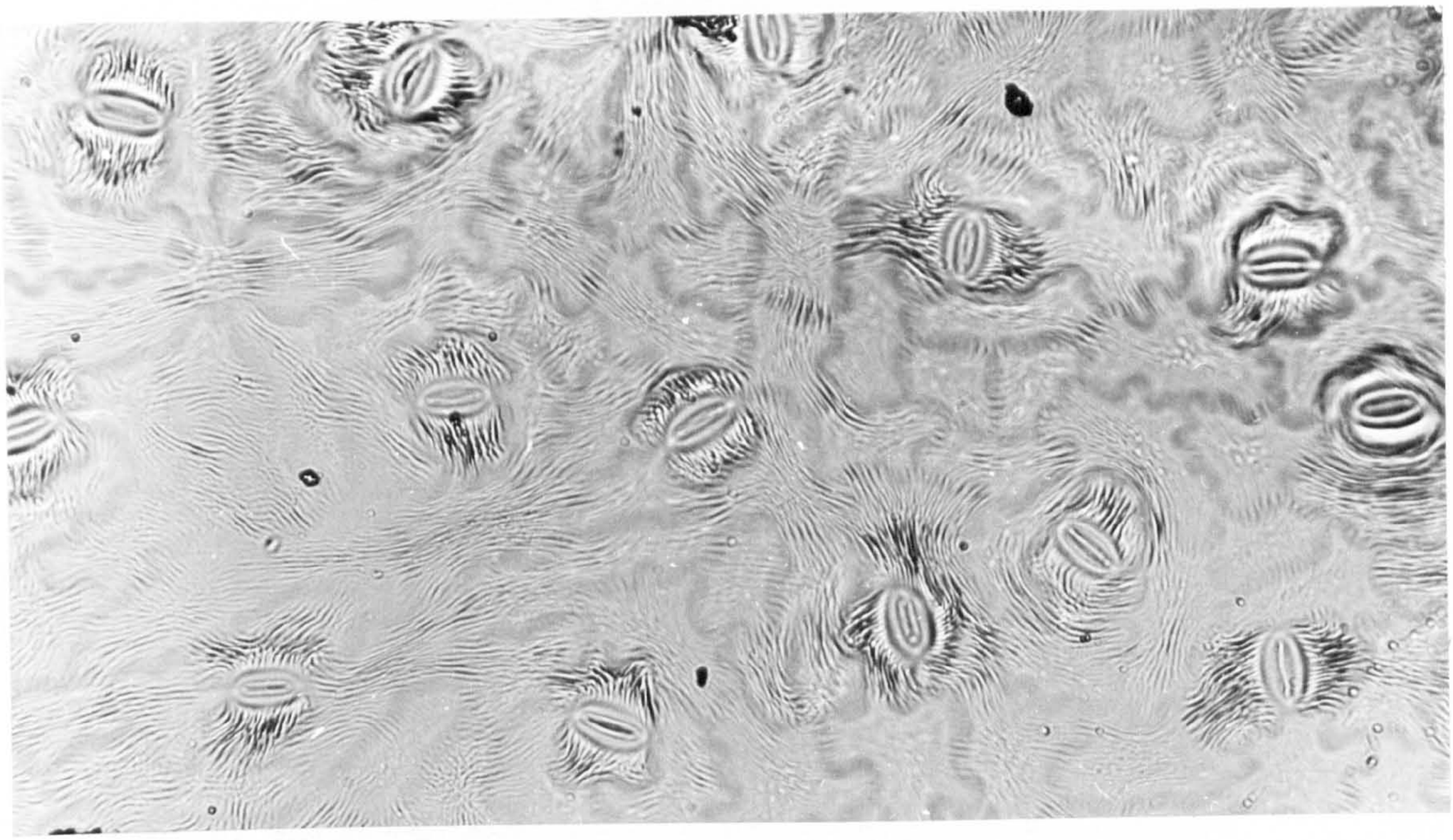
Figure 3.6 B also suggests that the diurnal pattern of growth cabinet plants is generally correlated with the evaporative demands of the environment.

Comparisons between the 3 conditions suggest that the diurnal drop in water potential was small for the controlled growth room plants. However, greenhouse and field plants subjected to much severer and highly fluctuating environmental conditions experienced greater deficits.

Xylem sap osmotic potential (Y_{xs}) in all 3 sets of plants was around -1.0 bar and showed very little diurnal fluctuation (about 0.1-0.4 bar). Leaf cell sap osmotic potential (Y_s) was slightly higher in growth cabinet than in greenhouse and field plants. The diurnal drop for all the 3 sets of plants appears to be similar.

Despite the low Y_s values by day, the estimated Y_p remained lower by day than by night. The drop in Y_s by day seemed only temporary and was more due to concentration of the original cell sap, as a consequent of water loss, than net solute accumulation. At night when transpiration was negligible and the rate of absorption increased, the cell sap became

A



B

Plate 3.2 Stomatal imprints of A Upper epidermis, B lower epidermis

diluted and Y_s increased again (Figure 3.6). Plate 3.2 shows upper and lower epidermes of Ricinus. Stomatal frequencies of the upper and lower leaf surfaces were in a ratio of about 1:2. However, the differences between the resistances to water vapour diffusion offered by the 2 sets of stomata do not reflect differences in their densities. Comparing these resistances to the plant water status, figure 3.5 shows that the upper stomata appeared to display cycling with a two-hourly periodicity during the day. This response was not correlated with the measured internal water deficits.

The lower stomata on the other hand exhibited diurnal cycling which was similar in most respects to the diurnal variations in water deficits. Both lower stomatal resistances and internal water deficits were generally correlated with radiation and temperature. For example on 16/8/76 (a bright cloudless day) when temperature and radiation were high, Y reached a minimum value of -5.0 bar and stomatal resistances were low about 5 sec. cm^{-1}. On the other hand on 17/8/76, which was sunny with cloudy intervals, when both temperature and radiation were low, Y was high, dropping to only -4.5 bar, and stomatal resistances were slightly higher during the day than values recorded on 16/8/76.

Table 3.3

Changes in leaf water potential (Y) of field growing Ricinus plants in relation to days after rain

The values are means of hourly measurements made between 11.00 and 15.00 hours

Days after rain	Leaf water potential (- bar)
2	4.9 ± 0.1
3	4.3 ± 0.1

Contd./ Table 3.3

Days after rain	Leaf water potential (- bar)
4	4.8 ± 0.1
7	5.2 ± 0.2
9	5.3 ± 0.1
13	5.5 ± 0.1

Leaf water potential measured periodically for field plants around 12.00 and 16.00 hours from 14 - 27/8/76 when there was no rainfall is shown in Table 3.3. There was very little variation in Y during this period. Early morning values which reflect more the true internal deficit were -2.1 and -2.5 bar for day 2 and 9 respectively suggesting that the roots were able to absorb water efficiently from lower moist soil and consequently the plant was able to start each day with a favourable internal water status.

The data below were obtained for normal greenhouse plants which were subjected to various soil moisture regimes. Water potentials measurements were at 06.00 to 07.00 hours (first), then at 12.00 to 14.00 hours (second) and finally at 06.00 to 07.00 hours (third) in the following morning (see Table below).

Plant water potential (bar)

Plant	First			Second			Third		
	$-Y$	$-Y_s$	Y_p	$-Y$	$-Y_s$	Y_p	$-Y$	$-Y_s$	Y_p
1	- 4.8	-12.6	7.8	- 6.8	-12.4	5.8	- 5.2	-12.0	6.8
2	- 6.3	-12.2	5.9	- 8.5	-13.0	4.4	- 8.0	-12.7	4.7
3	- 6.7	-12.0	5.3	- 8.1	-12.2	4.1	- 7.4	-12.0	4.6
4	-10.5	-13.8	3.3	-12.0	-14.4	2.4	-11.3	-14.0	2.7

The diurnal drop in Y was very small and unlike normal plants (Figure 6A) there was only a slight overnight recovery apparently due to the less soil moisture available to the roots. This

Table 3.4

Effect of short and long periods of water stress on the water relations of Ricinus leaves and the refractive indices of leaf cell sap

Relative water content (% 1°C)	Short-term water stress				Long-term water stress			
	Leaves from rapidly stressed plants				Leaves from rapidly stressed plants and * prolonged stressed plants			
	Y	Y_s	Y_p	RI	Y	Y_s	Y_p	RI
93	- 7.7	-12.2	3.5	1.3440	- 8.8	-19.5	11.1	1.3502
91	-10.6	-13.4	2.8	1.3452	-11.6	-19.7	8.1	1.3528
89	-13.0	-13.8	0.8	1.3460	- 8.1	-16.6	8.5	1.3486
88	-11.3	-13.5	2.2	1.3460	-13.5	-18.5	5.0	1.3532
85	-13.2	-14.2	1.0	1.3462	-16.0	-21.2	5.2	1.3528
83	-15.0	-17.2	2.2	1.3485	-13.6	-20.7	7.1	1.3540
81	-14.5	-15.7	1.2	1.3470	-13.9	-22.6	8.7	1.3572

* Data were drawn from Section 4.

response is in accord with theoretical expectation (cf Slatyer, 1967).

Discussion

The data presented in 3:3 suggests that young rapidly growing leaves of Ricinus and Helianthus have slightly lower water potentials than 'mature' leaves at most levels of water stress. These discrepancies were too great to be attributed to a vertical hydrostatic gradient of 0.1 bar per meter. Similarly, it has been reported that young (upper leaves) have lower Y than 'mature' (lower leaves) of other species. For example, Acevedo et al (1971) observed that young leaves of maize can possess a Y about 1.5 bar lower than older leaves. Kassam and Elston (1976) measured Y of -4.0 to -9.5 bar for older lower leaves and -9 to -12 bar for upper young leaves of Vicia faba. Other examples were demonstrated by Hartt (1967) Miller et al (1968) and Shepherd (1973).

Y gradients in vertical stems exceeding the hydrostatic gradient have often been attributed to differences in rates of transpiration and resistances to water flow (see for example Rawlins, 1963; Slatyer, 1967). Some of the results cited above had been attributed to these two factors (e.g Kassam and Elston 1976; Miller et al, 1968) on the expectation that both resistances to water flow and transpiration rates increased with height (Monteith and Bull, 1970). Other experimental work contradicts this transpiration hypothesis. For example Squire (1976) could not attribute lower Y observed in high yielding tea clones, than in low yielding ones, to differences in transpiration rate. Similarly, Hellkvist and Parsby (1976) observed lower Y in fast growing clones of Pinus sylvestris than slow growing ones under conditions favouring either rapid or negligible transpiration.

In Ricinus the Y differences between young and older leaves were not correlated with their position on the plant and moreover the pattern of response for these and also for Helianthus was the same when transpiration was negligible. Thus, under the circumstance, Y of all the leaves were expected to be in dynamic equilibrium with each other through xylem transport since resistances to water flow by the xylem are low (cf Slatyer, 1967; Boyer, 1971; Stoker and Weatherley, 1971). Although departure from this relationship could occur owing to probable differences in cell wall elasticity, it is more likely that growth is the critical factor.

It is known that during leaf enlargement Y is reduced through cell wall relaxation. This provides the water potential gradient between the growing cells and the xylem, which consequently promotes water movement into the growing cells for expansion. Presumably during rapid growth, dynamic equilibrium with xylem water remains incomplete and consequently the Y of young rapidly growing leaves may persistently lag behind those of older leaves. Similar observations were reported as long ago as 1942 by Burström (in Burström, 1961). The growth and water uptake data (Section 2) for both Ricinus and Helianthus are in harmony with this 'growth hypothesis'. Thus, although the differences between Y of young and older leaves are small they provide some support for the conclusions tentatively drawn from water uptake experiments.

The observation by Potter and Milburn (1970) that fully turgid submerged shoots at different ages developed water deficits in proportion to their growth rates, adds further support to the views expressed here.

Boyer (1968) measured persistent Y of -1.5 to -2.5 bar in growing leaves (zero transpiration) with their petioles freely

supplied with water. He attributed this, and the persistent uptake of water to resistances opposing water flow to the growing cells. But it is also likely that equilibration was not achieved owing to rapid incorporation of water by cell growth, because sufficiently large resistances to give protracted uptake, to the extent detected, cannot be demonstrated.

Although the growth water-deficits presumably occurred when the leaves were on the intact plant, it is also possible that they developed by post excision growth through cell wall relaxation, probably during enclosure in the bomb. (e.g. Catsky, 1963, Tinklin and Weatherley 1966; Tinklin, 1967; Boyer, 1968, 1970). This phenomenon has been demonstrated for Ricinus (Section 5).

Figure 3.3 and Table 3.1 show that Y_s in young Ricinus leaves of normal or rapidly stressed plants were greater than those of older leaves. Hellkvist et al (1974) also observed tendencies in upper young leaves Picea sitchensis to possess higher Y_s than lower older leaves. Figure 3.4 however, shows that both young and older leaves of Helianthus possess similar Y_s. There are other experimental data showing the trends in Y_s opposite to that observed for Ricinus. For example, Y_s has been shown to be lower in younger than older leaves of wheat and potato (Shepherd, 1973) and in V. faba (Kassam and Elston, 1976). Such variations have been attributed to differences in cell wall elasticity (cf Shepherd, 1973).

The higher Y_s observed for the rapidly growing young Ricinus leaves perhaps resulted from rapid utilization of solutes during growth. However, as pointed out by (Hsiao and Acevedo (1974)and Hellebust (1976), a high Y_s does confer a low Y_p, and it is, therefore, not beneficial for growth. Both the high Y_s and the low Y measured in young leaves resulted in Y_p about 2-3bar lower in young leaves than in older leaves.

It follows then that if young leaves were to enlarge faster than older leaves, not only must there be a high wall extensibility but also, they should have a lower critical turgor for growth (Y_t) (cf Green et al, 1971). A small Y_t value would confer the high growth turgor (Y_{gr}) necessary for rapid growth. This aspect of the work is investigated in the next two sections.

The relatively similar Y_p maintained by both young and older leaves of Helianthus (Figure 3.4) is necessary as this species already possesses low cell solutes and any further lowering in young leaves would lead to insufficient turgor for growth (see Boyer, 1968; 1970).

It has been proposed that decreases in Y without changes in tissue water content can be achieved by decreases in Y_s through the uptake of solutes, coupled with a proportionate increase in cell wall extensibility. (Warren Wilson, 1967). This relationship was suggested to be advantageous for continued growth because it maintains cell turgor and prevents wilting. (Cheung et al 1975; Hsiao et al 1974). However, the results presented in 3.2 and 3.3 show that when Ricinus and Helianthus leaves are rapidly stressed, there was no detectable increase in net cell solutes. Instead, most of the reduction in Y was attributable to a reduction in Y_p. Although Y_s of Helianthus was reduced relatively more than Y_s of

Ricinus, apparently this is attributable to reduction in cell volume because of its high wall elasticity. This did not operate to maintain cell turgor, however, and it is doubtful whether Y_p fell below zero. Negative Y_p has been recorded for some few other species (see for example Boyer, 1967; Gardner and Ehlig, 1965; Slatyer, 1957a). Boyer, (1967) attributed the negative Y_p for Helianthus to errors incurred through the mixing of protoplast and cell wall solution during freezing for Y_s measurements. Recently Tyree (1976), also questioned the existence of negative Y_p, and considered them to have arisen through errors in techniques. It is also possible that Y_m contribution to Y becomes more significant as the leaf becomes very dehydrated (Wiebe, 1966; Boyer, 1967). This phenomenon could prevent Y_p from becoming negative.

In contrast to rapidly stressed Ricinus plants, the results further show that under conditions where severe internal water deficits are allowed to develop slowly (such as in repeatedly stressed plants) Ricinus adjusted osmotically. Consequently, Y_p is maintained at a high value despite the low Y. Osmoregulation is a common phenomenon observed in osmotically stressed plants and plants in saline habitats (Slatyer, 1961, Meiri and Poljakoff-Mayber, 1969, Jennings, 1976). Recently the phenomenon has been observed in few water stressed plants. For example it has been demonstrated for roots of pea (Graecen and Oh, 1972) and for maize roots and leaves (Hsiao et al, 1976) for soybean hypocotyls Meyer and Boyer, 1972) and also for leaves of cotton (Brown et al, 1976, Cutler and Rains, 1977).

Since the permanent wilting point corresponds to zero turgor in the leaf (Slatyer, 1957b; Cheung et al, 1975) an osmoregulation with the corresponding low Y_s will delay wilting despite existing

low Y. Observations made suggested that rapidly stressed plants wilted at higher Y than repeatedly stressed plants. On the other hand, wilting of Helianthus leaves during water stress was severe and frequent, probably because these leaves failed to adjust osmotically. If osmotic adjustments offer some adaptive advantages under water stress, it is significant that in Ricinus the response was so pronounced in the main apical region (Figure 3.3) which is the centre for important physiological and developmental activities. The significance of the lack of the same degree of adjustment in other apical regions (small branches) may be viewed as a means of concentrating plant metabolites in the main apex.

Osmoregulation involves increases in synthesis or breakdown of metabolite or both with a corresponding increment in net cell solutes (Hsiao et al, 1976). Table 3.4 clearly shows that cell sap of hardened plants contained more osmotically active substances, at a given cell water content, than rapidly stressed plants. However, the mechanism responsible here is unknown.

In general, the diurnal pattern of plant water status of Ricinus is comparable to similar patterns described for other species (e.g. Hellkvist et al, 1974; 1976; Klepper, 1968; Plaut et al, 1975). However, in contrast with many species Ricinus maintains a more favourable water balance, showing a diurnal drop of about -4 bar compared with, for example, -6 bar for grape-vine (Hardie and Considine, 1976) -12 to -14 for cotton (Jordan, 1970; Klepper, Browning and Taylor, 1971) -8 for rose (Plaut et al, 1975) and for large trees, -10 for Pinus sylvestris and -7 bar for Picea sitchensis (Hellkvist et al, 1974, 1976).

It has been reported that plants show differences in their water relations when under natural and artificial conditions (Cary

and Wright, 1971; Watts, 1974; Bunce, 1977). Figures 3.5 and 3.6 demonstrate this phenomenon for Ricinus. Thus, greenhouse plants suffered the greatest water deficits and the growth cabinet plants the least. However Y_s of both greenhouse and field plants were lower than growth cabinet plants and consequently Y_p was highest in the field, followed by greenhouse and growth cabinet plants. The variations may be attributed to differences in - environmental conditions, soil mass, quantity of roots and probably also in efficiency of the root system.

SECTION 4

GROWTH OF INTACT PLANT ORGANS IN RELATION TO INTERNAL WATER STATUS

A large turgor pressure (Y_p) and hence a favourable plant water status, is required for optimum enlargement of plant cells. Large reductions in tissue growth are known to occur with only small increases in plant water stress (Boyer, 1968; 1970; Acevedo *et al*, 1971; Lawlor and Milford, 1973; Bunce, 1977). Apparently, in many species cell enlargement does not occur until the Y_p exceeds some critical value (Y_t). Consequently, growth rate is not proportional to the cell turgor as originally assumed (cf Lockhart, 1965b). The turgor available for growth (Y_{gr}) can, however, be described as;

$$Y_{gr} = Y_p - Y_t$$

The Y_t is known to correspond to the Y at which growth stops (Boyer, 1968). Seemingly, the magnitude of Y_t varies with species and it is also affected by water stress (Green *et al*, 1971, Meyer and Boyer, 1972).

Experiments presented in Section 3 showed that Y and Y_p decreased when *Ricinus* tissue lost water rapidly, but when stress developed slowly leaves osmoregulate, consequently Y_p remained high. The results further showed that contrary to the expectation that young leaves expand faster, because they have a more favourable water balance, in fact they suffer greater water deficits than older leaves. The experiments described in this section investigate the internal water status-growth relationships.

4.1 Experiment 4.1

Studies on growth, development and physiology of Ricinus plants subjected to watering and drying cycles

Greenhouse and growth-cabinet plants at alternate-leaf 3 stage were subjected to 3 pre-determined moisture regime treatments - (A),

(B) and (C) soil moisture content (SMC).

Soil moisture level was controlled by pot weighing (cf Slatyer, 1957a) using a Gallenkamp heavy duty solution balance OHAUS 119D BCJ - 800 with 1 g precision. The moisture content of the soil at field capacity, that is after standing in water to become thoroughly saturated and allowing the pots to drain for 24 hours whilst evaporation from the soil surface was prevented, was found to be 71.4 $\pm$ 4.6% of the soil dry weight. The soil weight (after making (corrections for pot and plant weights) at this point was taken to be 100% soil moisture content (SMC). Treatment A consisted of soil kept at near this point by daily addition of water. In the other treatments (B and C) the soil, initially at field capacity, was allowed to dry out to 66% and 33% respectively of the weight at field capacity before rewatering.

An attempt was made to make corrections for plant growth at the end of each drying cycle. Therefore, parallel samples of plants from each of treatments B and C (both greenhouse and growth cabinet) were harvested (a) at the beginning of the experiment (t_1), (b) during potting up (leaf 6 stage; t_2) and then the fresh weights (w_1 and w_2 respectively) were determined. An approximate growth rate per day (x_1) from germination (t_o) to t_1 (period 1) and from t_1 to t_2 (period 2, x_2) were estimated on the assumption that the rate was steady throughout the development of the plant. For example, x_2 was calculated as

$$x_2 = \frac{w_2 - w_1}{t_2 - t_1} \text{ gm/day.}$$

Plant weight (p) on, for example, day D during period 3 (from leaf 6 to 10 stage) was estimated as,

$$P = \bar{w}_2 + x_2 D \text{ gm}$$

To obtain weight of soil, P + pot weight was subtracted from the total weight. The plants were allowed to grow until leaf 10 was fully expanded. This took approximately 8-9 weeks. During this period B and C plants received overall approximately 30-35 and 16-22 watering cycles respectively. It was observed that the length of the watering to drying-out cycles differed from pot to pot within the same treatment. The SMC (%) stated for each treatment is, therefore, an average of the SMC (%) experienced by all the seedlings in the treatment before rewatering took place.

4.1a Effect on Y, Y_s and Y_p

Table 4.1 gives examples of the water potential parameters measured. 'Matured' leaves were used. As expected, Y, Y_s and Y_p decreased with increasing soil moisture stresses. Greenhouse plants have lower Y than growth cabinet plants, however, because of the low Y_s of the former plants. Y_p was higher in greenhouse than in growth cabinet plants. The results are in reasonable agreement with those in Section 3.

(Table 4.1 follows on a separate page.)

Table 4.1

Water deficits developed by Ricinus plants in soil allowed to dry repeatedly to predetermined moisture content (% S.M.C.) before rewatering. Measurements for B and C usually occurred at the end of drying but occasionally, following rewatering (*). Measurement for A was conducted periodically.

Days after experiment began	Water potential (. bar) A (100% SMC)			B (66% SMC)			C (33% SMC)		
	-Y	$-Y_s$	Y_p	-Y	$-Y_s$	Y_p	-Y	$-Y_s$	Y_p
Growth cabinet									
(5.5.76 - 30.6.76)	3.0	9.8	6.8	6.3	11.1	4.8	7.8	12.0	4.8
10 - 20				*2.8	10.2	7.4			
20 - 30	2.5	10.8	8.3	6.0	11.5	5.5	9.0	13.2	4.2
							*3.0	11.1	8.1
31 - 45	3.6	11.2	7.6	5.6	11.0	5.6	8.8	13.4	4.6
46 - 60	3.2	10.3	7.1	5.8	11.6	5.8	9.8	14.4	4.8
				*3.2	10.7	7.1	*3.2	11.0	7.8
Greenhouse									
(5.3.76 - 5.5.76)	3.8	11.8	8.0	7.6	12.4	4.8	8.6	13.2	4.6
10 - 20				*3.9	11.7	7.8	*4.0	12.8	8.8
21 - 30	4.3	11.7	7.4	6.5	12.5	6.0	7.4	13.3	5.9
31 - 40	4.0	12.6	8.6	6.9	13.3	6.4	8.0	13.0	5.0
				*3.2	13.1	10.1			
41 - 50	4.3	12.6	8.3	5.5	13.0	7.5	10.8	15.2	4.4
							*2.9	12.1	9.2
51 - 60	4.3	12.6	8.3	9.4	14.0	4.6	9.7	16.0	6.3

(Y and Y_s are negative values.)

4.1b Effect on growth

Plastochrone interval (days) leaf production and leaf development of successive leaves 3 to 10 were determined. Leaf areas were measured at 3 to 4 day intervals; in addition daily growth of leaf 7 together with its petiole and proximal internode were measured.

The growth responses of greenhouse and growth cabinet plants were essentially similar and therefore only selected results are shown here.

Table 4.2

Plastochrone (in days) for leaves 3 to 10 of Ricinus plants in well watered soil, (A), soil allowed to dry repeatedly to 66%, (B) and 33% (C) soil moisture content, before rewatering.

Greenhouse

A

Plant	Leaves 3-4	4-5	5-6	6-7	7-8	8-9	9-10	Mean	Rate of leaf production
1	5	6	6	4	4	4	4	4.7	0.21
2	5	5	5	5	4	5	4	4.8	0.21
3	4	6	6	4	3	2	3	4.0	0.25
Mean	4.7	5.7	5.7	4.3	3.7	3.7	3.7	4.5	0.22
Rate of leaf production	0.21	0.17	0.17	0.23	0.27	0.27	0.27		

B

Plant	Leaves 3-4	4-5	5-6	6-7	7-8	8-9	9-10	Mean	Rate of leaf production
1	6	5	6	5	3	5	3	4.7	0.21
2	4	5	6	4	3	5	3	4.4	0.23
3	5	4	6	5	2	5	3	4.6	0.22
Mean	5.0	4.7	6.0	4.7	2.7	5.0	3.0	4.6	0.22
Rate of leaf production	0.20	0.21	0.15	0.21	0.37	0.19	0.33		

Table 4.2 Contd./

Greenhouse

C

Plant	Leaves 3-4	4-5	5-6	6-7	7-8	8-9	9-10	Mean	Rate of leaf production
1	9	7	4	7	4	5	4	5.7	0.18
2	8	6	5	5	5	5	5	5.5	0.18
3	7	6	5	6	4	4	6	5.4	0.19
Mean	8.0	6.3	4.7	6.0	4.3	4.7	5.0	5.6	0.18
Rate of leaf production	0.12	0.16	0.21	0.17	0.23	0.21	0.20		

Growth cabinet

A

Plant	Leaves 3-4	4-5	5-6	6-7	7-8	8-9	9-10	Mean	Rate of leaf production
1	4	4	4	4	6	4	4	4.3	0.23
2	5	4	3	6	4	5	4	4.4	0.23
3	6	5	6	5	5	4	5	5.1	0.20
Mean	5.0	4.3	4.3	5.0	5.0	4.7	4.3	4.6	0.22
Rate of leaf production	0.20	0.23	0.23	0.20	0.20	0.21	0.23		

B

Plant	Leaves 3-4	4-5	5-6	6-7	7-8	8-9	9-10	Mean	Rate of leaf production
1	5	5	6	4	5	5	5	5.0	0.20
2	4	5	5	6	4	5	4	4.7	0.21
3	5	6	5	4	5	4	6	5.0	0.20
Mean	4.7	5.3	5.3	4.6	4.7	4.7	5.0	4.9	0.20
Rate of leaf production	0.21	0.19	0.2	0.21	0.21	0.21	0.20		

Table 4.2 Contd./

Growth cabinet

C

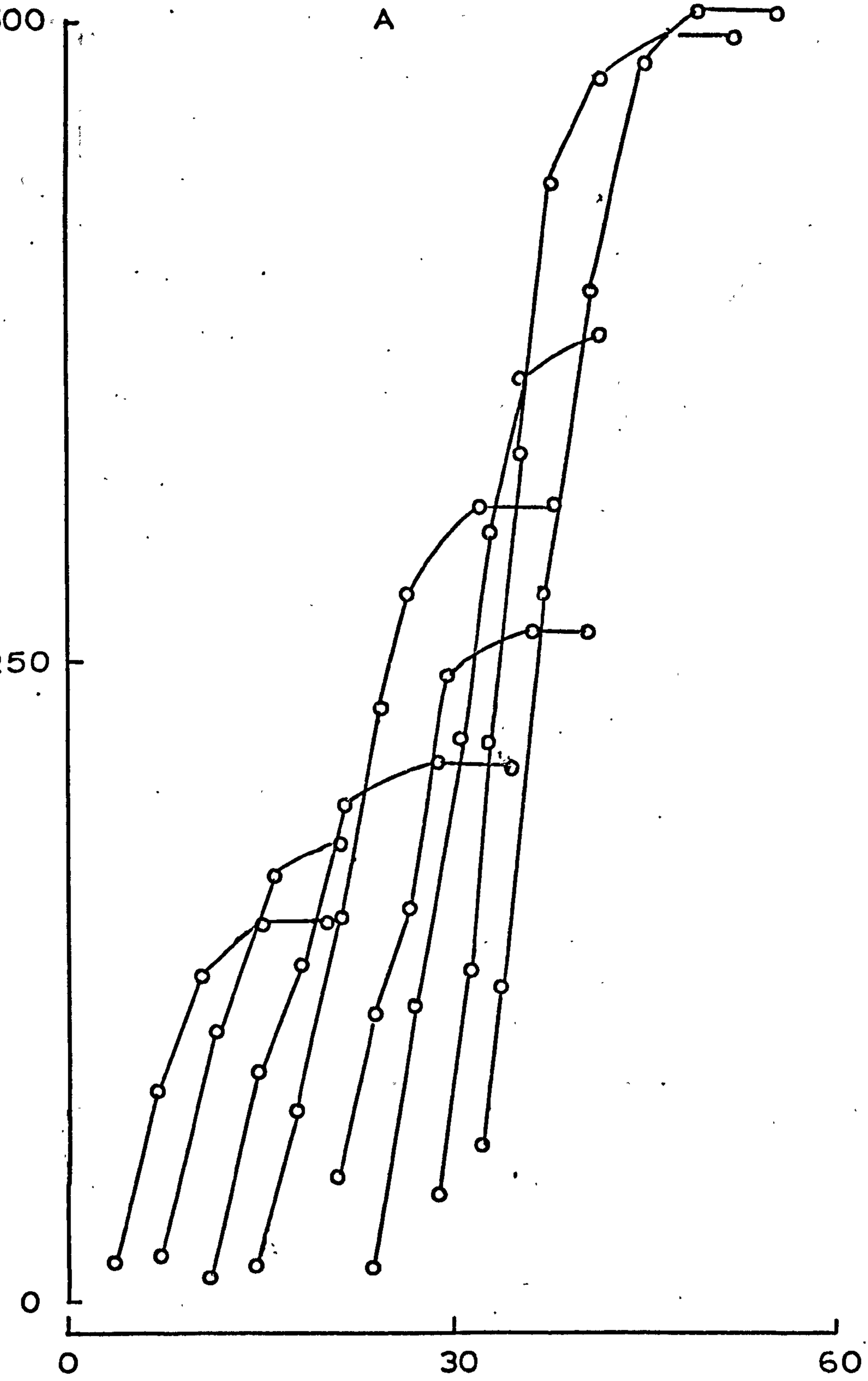
Plant	Leaves 3-4	4-5	5-6	6-7	7-8	8-9	9-10	Mean	Rate of leaf production
1	8	6	5	6	6	6	5	6.0	0.16
2	9	6	7	6	4	6	6	6.2	0.16
3	8	6	6	6	6	6	6	6.3	0.16
Mean	8.3	6.0	6.0	6.0	5.3	6.0	5.7	6.2	0.16
Rate of leaf production	0.12	0.17	0.17	0.17	0.19	0.17	0.17		

Table 4.2 shows that plastochrone interval and the rate of leaf production decreased as water stress increased. In the growth cabinet, the decreases were proportional to stress severity, however in the greenhouse treatment B did not show any significant effect on these 2 parameters. Furthermore, there was a tendency for the plastochrone interval of greenhouse plants to decrease as the number of drying cycles increased, but this was not so obvious in the growth cabinet plants.

Table 4.3 shows that leaf expansion was progressively reduced with increasing plant water deficits. Thus, leaf area was largest in treatment A and least in treatment C. Figure 4.1 further shows that water stress affected the pattern of development of successive leaves on a plant. Thus, whereas, in general, maximum leaf areas reached by successive leaves increased from the base up (curve A), this pattern became very distorted by water stress. Leaves, especially those of treatment C appeared to lose their potential to fully expand, although they had periodic access to sufficient water.

Figure 4.1 Growth curves of leaf 3-10 of growth cabinet plants in well watered soil (A) and soil allowed to dry repeatedly to 66% (B) and 33% (C) predetermined soil moisture content (%) before rewatering. The experiment was conducted 7/8/75 - 30/9/75.

Fig. 4.1



B

400

200

0

Leaf area (cm^2)

0

30

60

Time (d)

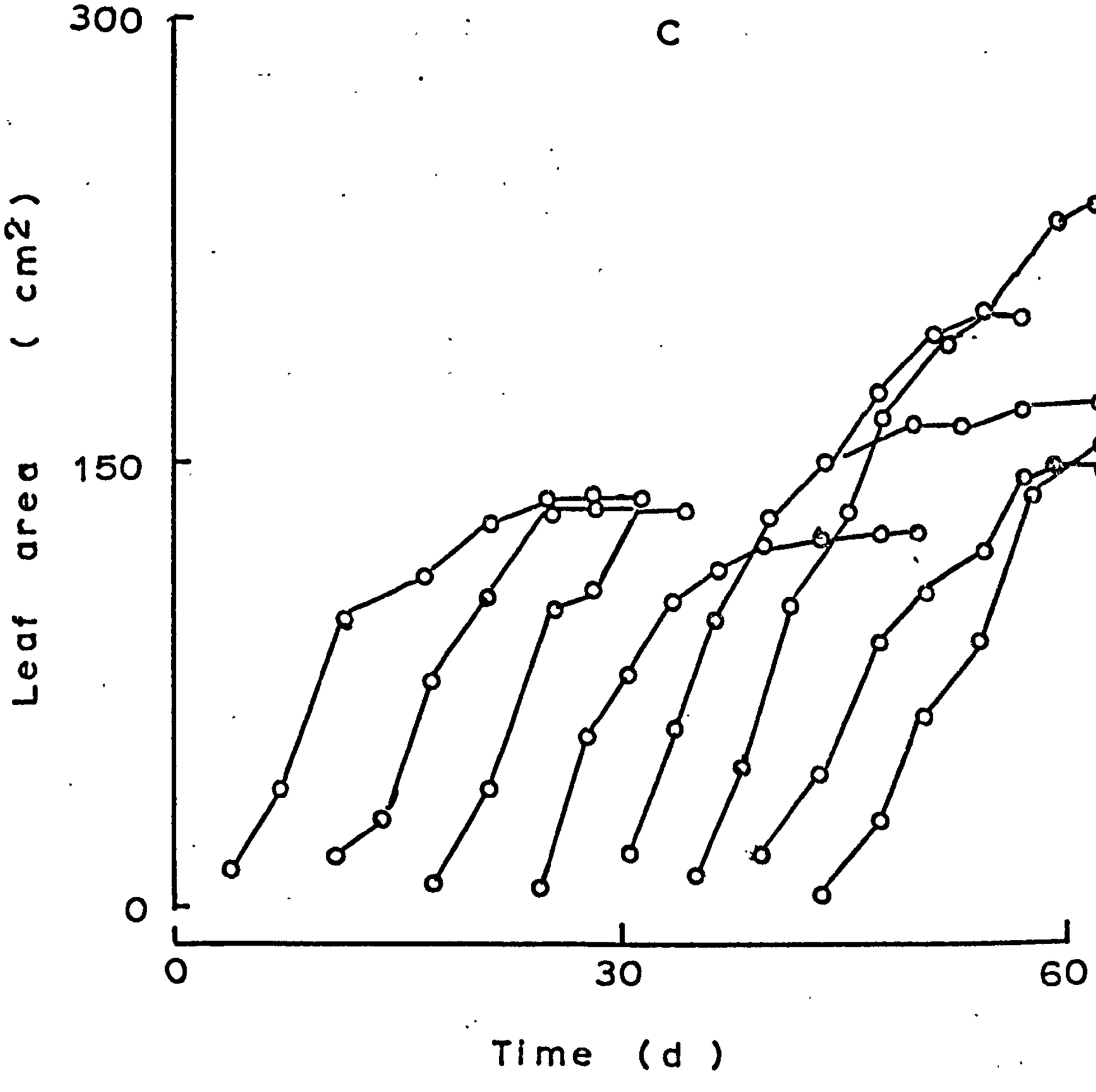

C
300
150
0
(cm2)
Leaf area
0
30
60
Time (d)

Table 4.3

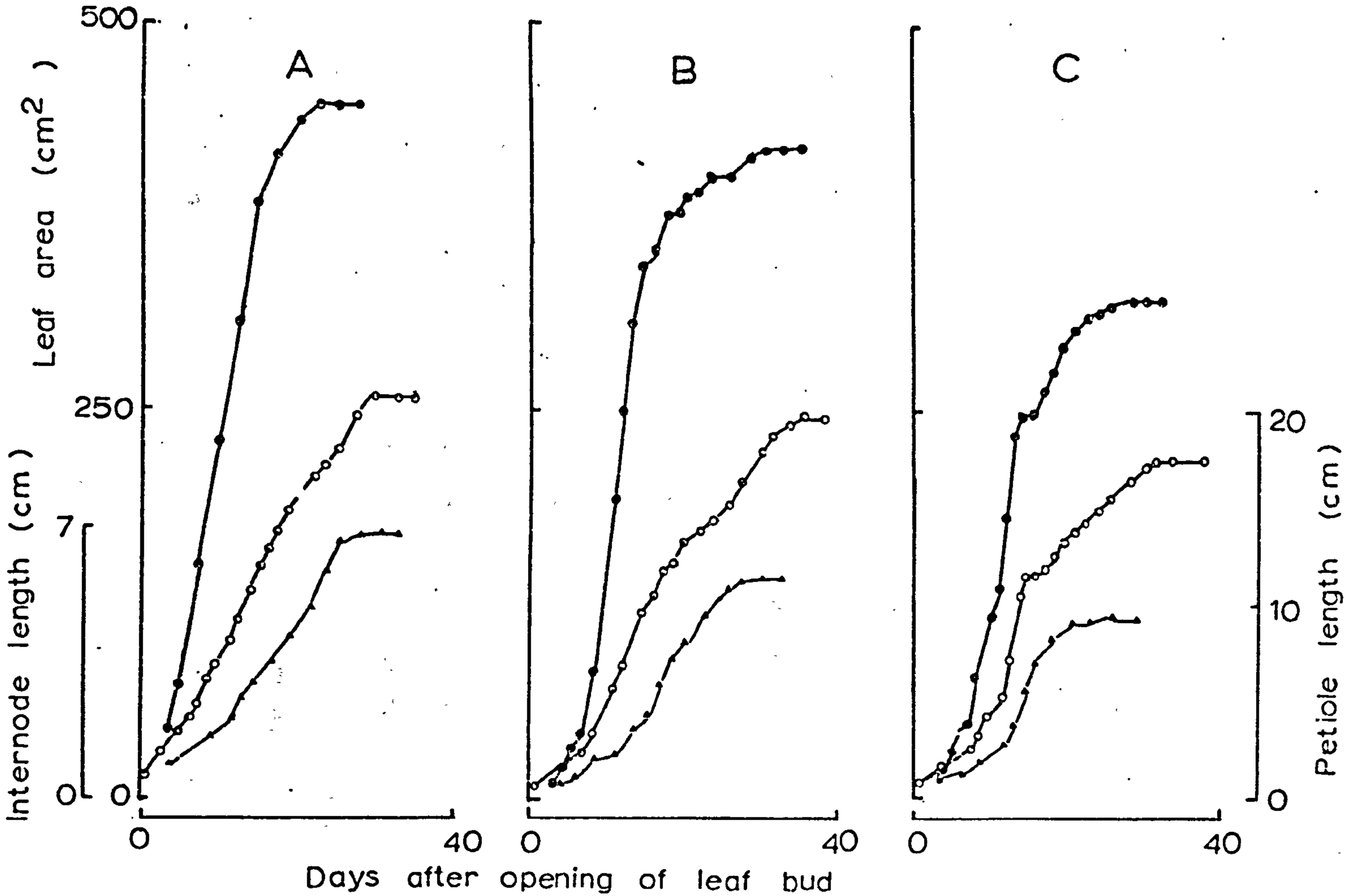
Total leaf areas of leaf 3 - 10 of growth cabinet and greenhouse plants which were subjected to various degrees of repeated watering - drying out cycles.

Date of experiment	Mean total leaf areas of plants (cm^2). Soil moisture content (% S.M.C.)		
	A - 100%	B - 66%	C - 33%
Growth cabinet			
7.8.75 - 30.9.75	2755	2135	1042
20.12.75 - 25.2.76	2240	1794	1388
1.5.76 - 30.6.76	2255	2023	1554
Total	7250	5952	3984
Mean	2416	1984	1328
% of A		82	55
Greenhouse			
1.8.75 - 30.9.75	3485	3402	3010
20.12.75 - 25.2.76	3091	2500	2385
5.3.76 - 8.5.76	3723	2996	2203
Total	10299	8298	7598
Mean	3433	2766	2532
% of A		80.5	73.7

Figure 4.2 gives a detailed picture of the effect of water stress on leaf expansion, and petiole and internode elongation. Here again smaller organs were developed in response to water stress. The figure clearly shows that the rate of growth was slowed down by water stress consequently the time interval required to reach maximum sizes was prolonged. The greater distortion in the growth curve of treatment B during the latter stages of its development seem to suggest that expansion of older growing leaves was completely inhibited at a water potential which only reduced the expansion of younger leaves. The growth curve for treatment C leaf shows that in

Figure 4.2 Growth curves of leaf 7 (Lamina - ●, petiole ○ and internode ▲) of greenhouse Ricinus plants. A (control) B and C were growing in soil repeatedly dried to 66% and 33% (S.M.C.) respectively before rewatering. The experiment was conducted 5.3.76 to 8.5.76

Fig. 4.2.


A
B
C
500
250
0
Leaf area (cm2)
7
0
Internode length (cm)
20
10
0
Petiole length (cm)
0
40
Days after opening of leaf bud

spite of the much reduced rate, growth was more steady than in B plants. This may suggest that C plants achieved some hardening probably as a result of the more severe water stress. Observations made showed that temporary wilting, which was initially frequent in C plants, became less obvious with successive drying cycles. Furthermore, Table 4.1 shows that Y developed by C plants, as the experiment progressed was accompanied by greater reductions in Y_s thus enabling a stable Y_p.

The response of leaf development to a short period of water stress is shown in figure 4.3. The treatment postponed leaf expansion and prolonged the duration of leaf development, however the leaf attained an area comparable to controls. This result generally accords with those of Acevedo *et al* (1971).

In general leaves grew bigger in the summer than winter months (Table 4.3), presumably partly as a reflection of variations in greenhouse conditions (probably light and temperature) under which the seedlings were raised, and also under which the greenhouse plants grew. Table 4.3 also shows that leaf sizes of greenhouse grown plants were larger than growth cabinet plants. Apparently this response is more related to Y_p than Y differences in the 2 plants.

4.1c Effect on stomatal resistances and transpiration rate

Reductions in growth during water stress have often been assumed to occur primarily through suppression of photosynthesis as a result of stomatal closure (Kramer, 1962). However, recent experiments (reviewed by Hsiao, 1973) suggest that growth by cell enlargement may be more sensitive to water stress than stomatal closure.

Figure 4.3 Effect of a single cycle of soil drying (Δ) on expansion growth of leaf 10 (B) compared with that on a well watered plant (A).

Plant water status was, Y, -8.5; Y_s, -12.2; and Y_p, 3.7 bar when leaf expansion was suspended. The arrows indicate suspension ($\uparrow$) and restoration ($\downarrow$) of watering.

Fig. 4.3

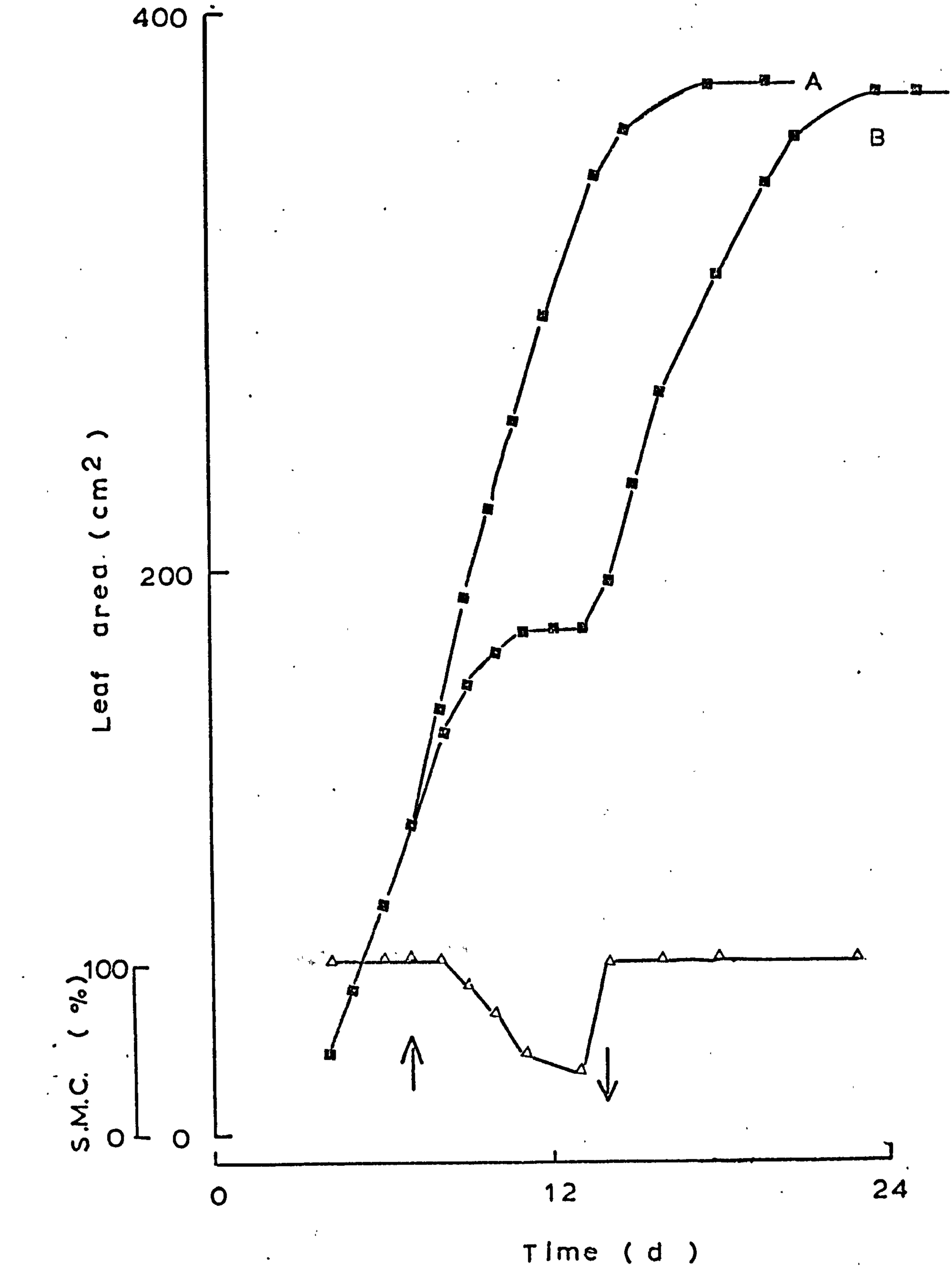


Table 4.4

Effect of repeated soil drying/rewatering cycles on stomatal diffusive resistances and transpiration compared with those of controls (A). Measurements were made between 11.00 and 14.00 hours.

Treatment	Stomatal resistance (sec. cm^{-1})		Transpiration rate ($mg/cm^2/hr$)	
	End of drying cycle	A day after rewatering	End of drying cycle	A day after rewatering
A (100% SMC)	5.0 ± 0.3	5.0 ± 0.3	14.5 ± 0.4	14.5 ± 0.4
B (66% SMC)	16.0 ± 0.5	5.0 ± 0.4	11.5 ± 0.7	14.5 ± 0.7
C (33% SMC)	70.0 ± 7.9	6.0 ± 0.7	2.5 ± 0.7	13.6 ± 1.0

Means of stomatal resistances and transpiration rates measured periodically after the 3rd week of experimentation are shown in Table 4.4. Measurements were made between 11.00 and 14.00 hours. Preliminary study showed that low stomatal resistances and high transpiration occurred during this time. The stomatal resistances of the second expanded leaf from the shoot apex was measured. Variations in resistances between this leaf and those of the remaining leaves on a plant were found to be less than 2 sec. cm^{-1}.

The Table clearly shows that leaves retained some degree of porosity during the stress period and that the applied moisture stress did not cause permanent damage to normal stomatal functioning. Thus both stomatal resistances and the rate of transpiration virtually recovered to control values when favourable internal water status was restored. This response does not correlate with the growth pattern where, for example, leaf expansion of C plants remained at a reduced rate

throughout the experiment.

Experiment ...

4.2 Growth under fluctuating and steady water potentials

4.2a Growth rates of intact leaves at different developmental stages.

Although the experiments in 4.1 suggest a very close dependence of leaf enlargement on plant water status, because water potential measurements were made less frequently, the data does not provide a precise assessment of threshold Y values for growth. The experiments described below investigate this phenomenon. They also assess the differences in sensitivity of expansion growth to Y between young and older leaves.

Potted plants at 6-7 leaf stages which were subjected to varying degrees of internal water stress were used. Growth rates (% initial area per day) were assessed at different levels of leaf insertion from first expanded leaf nearest the apex (as one) and then to leaves 2 and 3 below it of increasing maturity.

Water potentials were measured 2-3 times for each plant during the period by excising 'mature' leaves below the experimental leaves. Results obtained from Section 3 showed that differences were present between water potentials of experimental leaves (especially leaf one) and those sampled, but there was no precise way to correct for these differences. Results obtained are summarised in Table 4.5 for growth cabinet plants. Greenhouse plants gave similar results. Maximum leaf expansion occurred when Y were high (corresponding to high Y_p), but growth was greatly suppressed as the Y decreased. The results further show that growth rate varied for leaves at

Table 4.5

Growth rates (% initial area per day) of Ricinus leaves at different developmental stages in relation to plant water status.

Plant	Leaf number (counting first expanded leaf nearest shoot apex as one)	Initial leaf area (cm^2)	Growth rate (% initial area per day)	Mean water potential (bar)		
				Leaf water potential (- Y)	Leaf cell sap osmotic pressure (- Y_s)	Turgor pressure (Y_p)
1	1	43	179	-4.1	-13.9	9.8
	2	194	129			
2	1	82	154	-4.4	-10.7	6.3
	2	219	109			
3	1	32	136	-7.7	-12.7	5.0
	2	102	113			
	3	158	0			
4	1	38	142	-6.6	-13.0	6.4
	2	164	114			
5	1	46	106	-9.0	-12.0	3.0
	2	130	0			
6	1	41	0	-13.5	-15.7	2.2
	2	123	0			

different stages of development, younger leaves expanding more than older leaves. Moreover, growth persisted to much lower Y for the former than latter leaves. For both leaf types expansion ceased before Y_p falls to 0 bar suggesting that Ricinus, like other species, possesses a critical turgor for growth (cf Hsiao 1973).

4.2b Leaf expansion at constant water potentials.

It could be argued that the growth-water relationships reported above were complicated by diurnal variations in plant water status with possible corresponding fluctuations in growth rate during the period. In the following experiments the method was refined by maintaining Y at fairly constant levels, adopting the technique used by Boyer (1968, 1970) but with some modifications.

The experiment was repeated in a dark room. Evapotranspiration was reduced to a minimum by enclosing each plant in a large polythene bag which was lined with moist filter paper. The experiment was also conducted using Helianthus seedlings which were at 6 leaf-stage. Since we were interested in the effect of water stress on rapidly growing leaves, growth measurements were conducted on young leaves only. These had initial areas ranging from 80-120 cm^2 for Ricinus and 30-35 cm^2 for Helianthus.

The overall growth was much less when plants grew in complete darkness than when under alternate light and dark periods (cf Section 2 and 4.2c). Presumably, this partly reflects the lack of nutrients possibly photosynthates. However, the pattern of leaf-expansion - water relationships remained the same as reported previously. Thus, leaf

Figure 4.4 Growth rate of Ricinus leaves (% initial area, per 24 hours) in relation to leaf water potential (Y). The Y are the means of 2 measurements taken during the 24 hours. Initial leaf areas ranged from 80 to 120 cm^2. Transpiration was negligible during the period.

Figure 4.5 Growth rate of Helianthus (% initial area per 24 hours) in relation to leaf water potential (Y). The Y are the means of 2 measurements taken during the period. Initial leaf areas ranged from 30 to 35 cm^2. Transpiration was negligible.

Fig. 4.4.

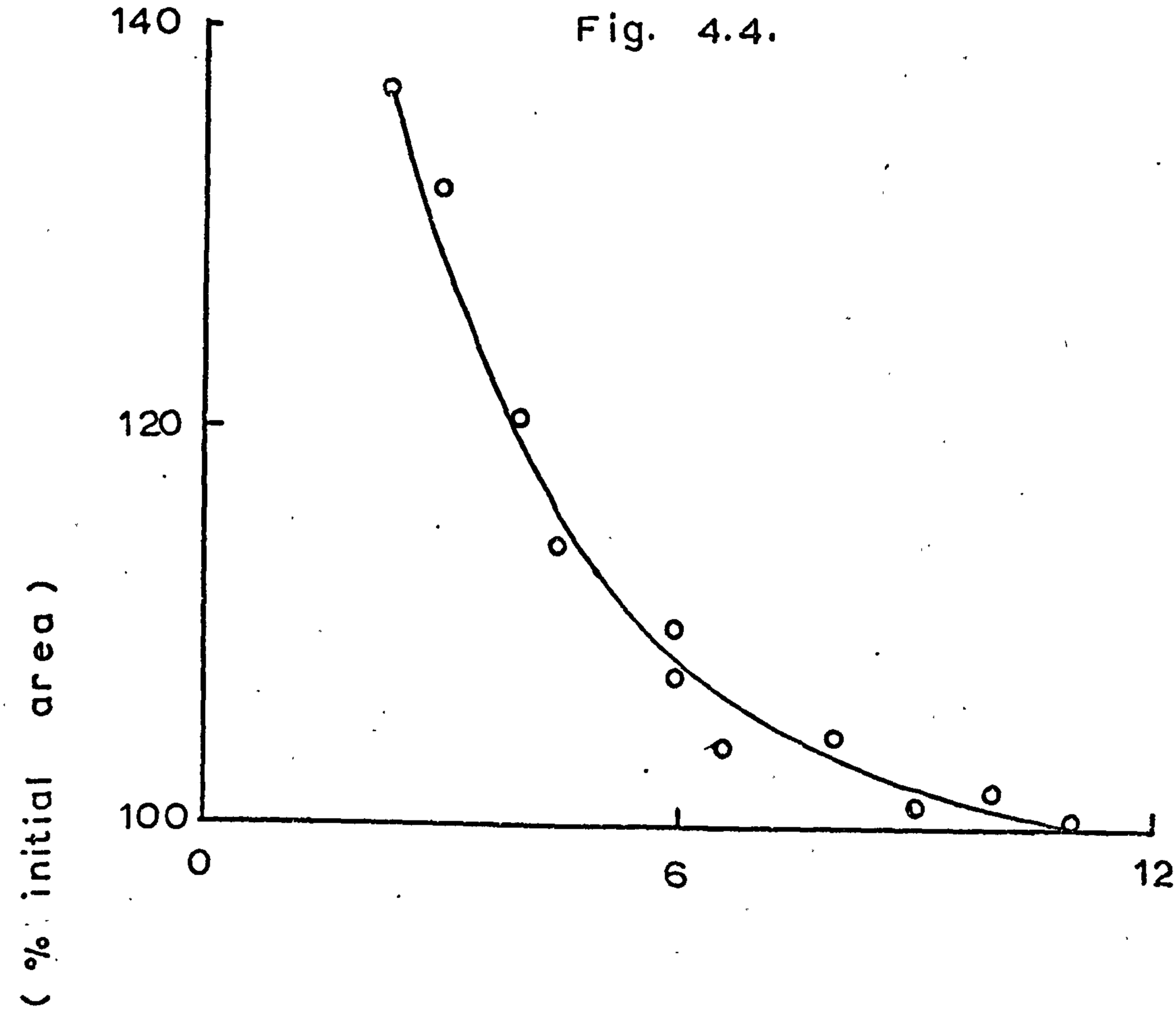
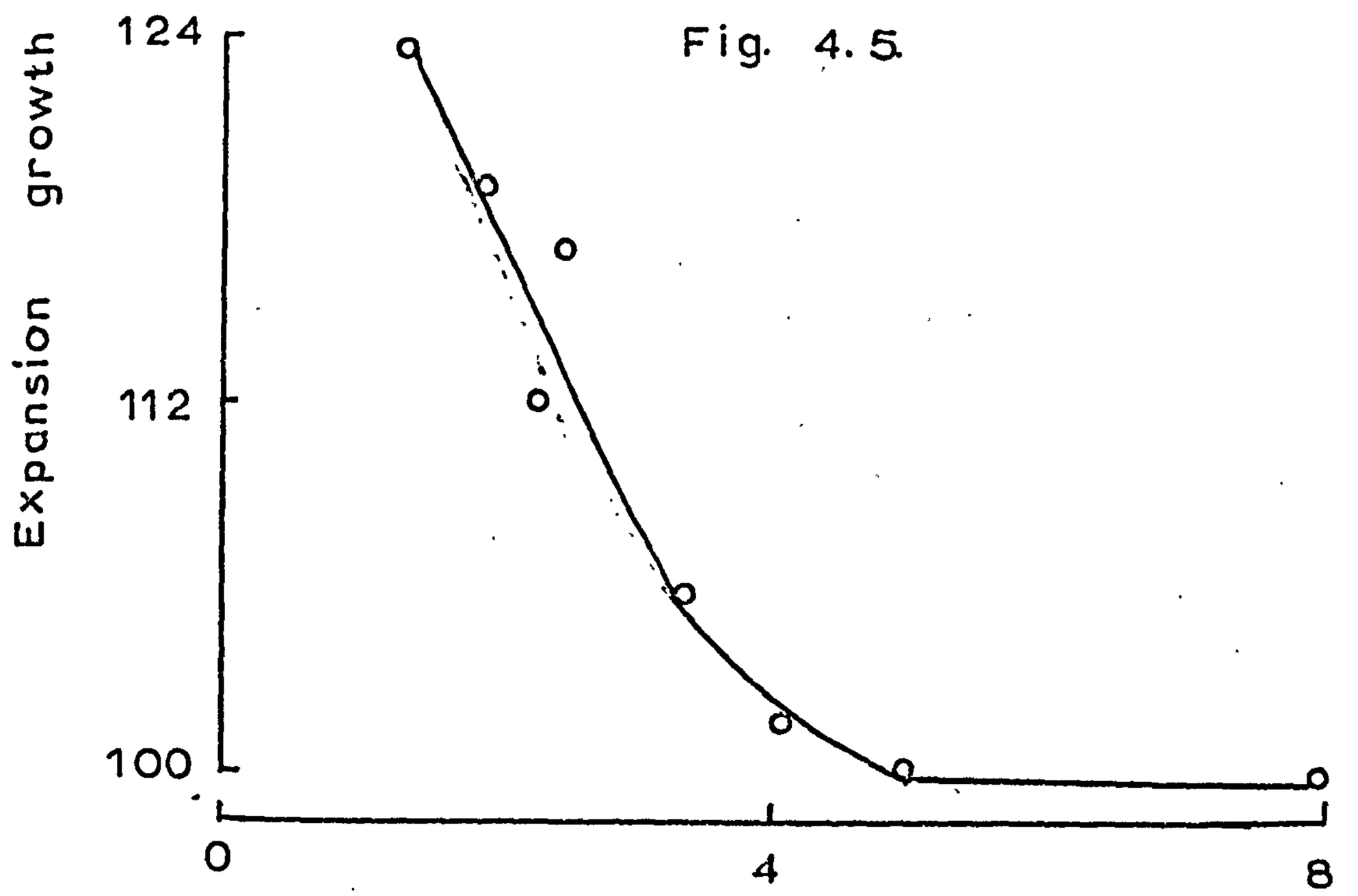

140
120
100
0
6
12
Expansion growth (% initial area)

Fig. 4.5


124
112
100
0
4
8
Expansion growth
Water potential (-bar)

expansion was rapidly reduced with decreases in Y and became completely suppressed at Y of -10.0 to -11.0 and -4.5 to -5.0 bar respectively for Ricinus and Helianthus (Figures 4.4 and 4.5). These corresponded to a Y_p of 2 to 3 for Ricinus and 3 to 4 for Helianthus. The critical Y for Helianthus was slightly lower than that reported previously (cf Boyer 1968, 1970).

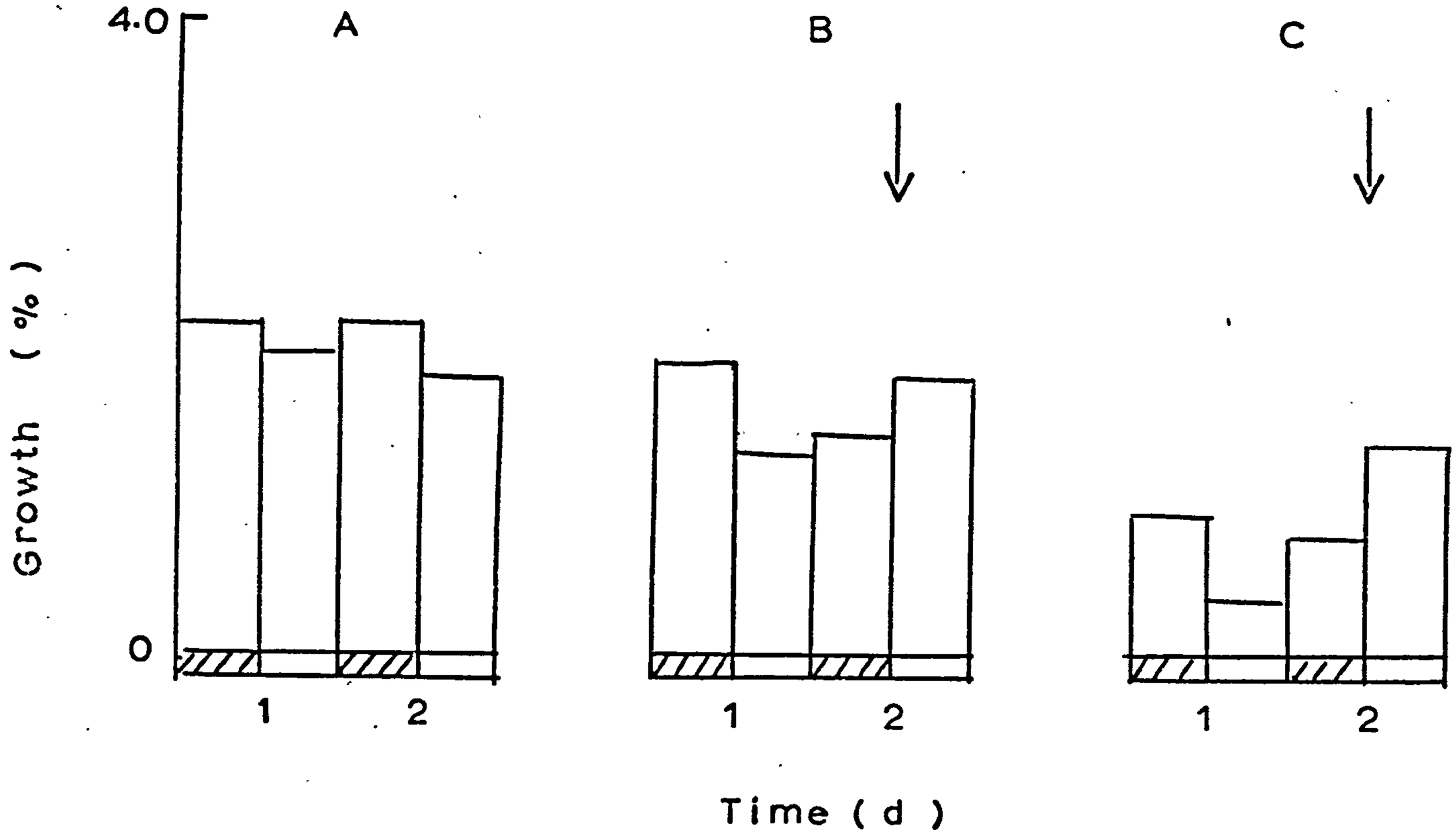
4.2c Growth of leaves by day and night in relation to increasing plant water stress.

If it is assumed that growth rate is the product of cell wall extensibility (E) and the turgor available for growth (Ygr) (Green et al, 1971) leaf expansion would be expected to be greater by night when Y_p are high than by day when Y_p is decreased (see Section 3). The following experiments investigate this hypothesis. Growth cabinet and greenhouse plants suffering from varying degrees of water stress and field plants were used. It was not convenient to measure growth precisely by day and night for greenhouse and field plants, therefore, measurements were made at 07.00, 19.00 and again at 07.00 hours the following morning. Leaf expansion of the youngest expanded leaves (originally 60 - 100 cm^2) of the potted plants were determined during 2 successive light and dark cycles. In the field, leaves at different stages of development were sampled.

Figure 4.6 shows data for growth cabinet plants. Leaf expansion of well watered plants (Curve A) was slightly higher by night when stomata were expected to close and when Y increased (cf Section 3) than by day when the reverse conditions occur. However, as internal water deficits increased, (curves B and C), growth was suppressed more during the daytime than at

Figure 4.6 Mean growth (% initial area per hour) of young leaves of growth cabinet plants during the light (▭) and dark (▥) periods. Plant A is control, B and C were growing in dry soil which induced maximum plant water stress of - Y, -6.0; Y_s -11.8; in B and Y, -8.2; Y_s, -12.5; bar in C plants. The arrows indicate watering.

Fig. 4.6



night. Consequently differences in leaf expansion between the 2 periods increased. The fact that rewatering of stressed plants enhanced leaf expansion suggests that the previously observed decline in growth rate was not solely attributable to aging but also partly to the water deficits. Similar results were obtained for greenhouse plants.

Table 4.6

Day and night growth of leaves of field grown Ricinus plants

(The data were obtained on the same plants and at the same time as the data in Figure 3.5)

Initial leaf area (cm^2)	Leaf growth (% initial area)	
	Day	Night
21 - 40	119.0	116.0
41 - 60	119.0	120.0
61 - 80	123.0	122.0
81 - 100	123.0	117.0
141 - 160	117.0	124.0
161 - 180	119.0	114.0
201 - 220	118.0	115.0
241 - 260	115.0	110.0
281 - 300	114.0	113.0
Mean % growth	118.6 $\pm$ 1.1	116.8 $\pm$ 1.6

However, contrary to the above 2 conditions, and despite the more favourable internal water status during the night (cf Figure 3.5), Table 4.6 shows that growth of field plants was slightly better during the day than at night. These differences could be attributed to the very cold night temperatures (minimum 8°C) and very warm days (maximum 35°C) interacting with plant water status.

The above hypothesis was investigated by studying leaf expansion and water potentials of well watered plants in the

growth cabinet when temperatures during the light and dark periods were 30°C and 8°C respectively. Table 4.7 shows that water deficits were ~~higher~~ lower by the day than by night despite the fact that transpiration was higher by day than by night. This suggests that absorption was suppressed by the cold temperatures probably through reduction in root permeability (cf Kramer, 1969). Table 4.7 shows also that, although critical levels of Y for inhibition of leaf growth did not develop, leaf expansion was completely suppressed during the night. This suggested that growth was affected through other effects of temperature, possibly the inhibition of growth metabolic processes (cf Ray and Ruesink, 1962). The observation accorded with experiments reported previously (Section 2) that leaf expansion require high temperatures to proceed. The results further show that low temperatures can interact with internal water deficits to suppress growth and agrees with experiments on maize reported by Watts (1972, 1974).

Experiment 4.3

4.3 Effect of osmotic adjustments on growth, development and physiology of Ricinus.

In experiments reported previously (4.1), where the period of water stress was complicated by periodic rewatering, osmotic adjustments of Ricinus failed to reveal any growth advantages in comparison with those observed for other species (cf Graecen and Oh 1972). In the experiments here, drought was more severe and its duration was prolonged. A detailed study of water potential parameters and their critical levels for leaf expansion and plant

Table 4.7

Leaf growth, water potential and transpiration of growth cabinet plants by day and night. Day and night temperatures were 30°C and 8°C respectively.

Experimental plant	Leaf number (counting leaf nearest apex as one)	Leaf growth (% initial area)		Water potential (bar)						Transpiration (gm / plant)	
		Day	Night	Day			Night			Day	Night
				Y	Y_s	Y_p	Y	Y_s	Y_p		
1	1	135	103	5.6	11.8	6.2	6.6	12.2	5.6	237	40
	2	110	0								
	3	104	0								
2	1	136	0	5.5	12.0	6.5	6.5	12.0	5.5	220	30
	2	105	0								
3	2	112	0	5.0	12.1	7.1	6.6	12.5	5.9	170	40
	3	104	0								

(Y and Y_s are negative values.)

survival together with the responses of other leaf characteristics were studied. Although severe drought, which may be detrimental to plant growth, occurs frequently in nature, little is known of key factors controlling leaf expansion during this period.

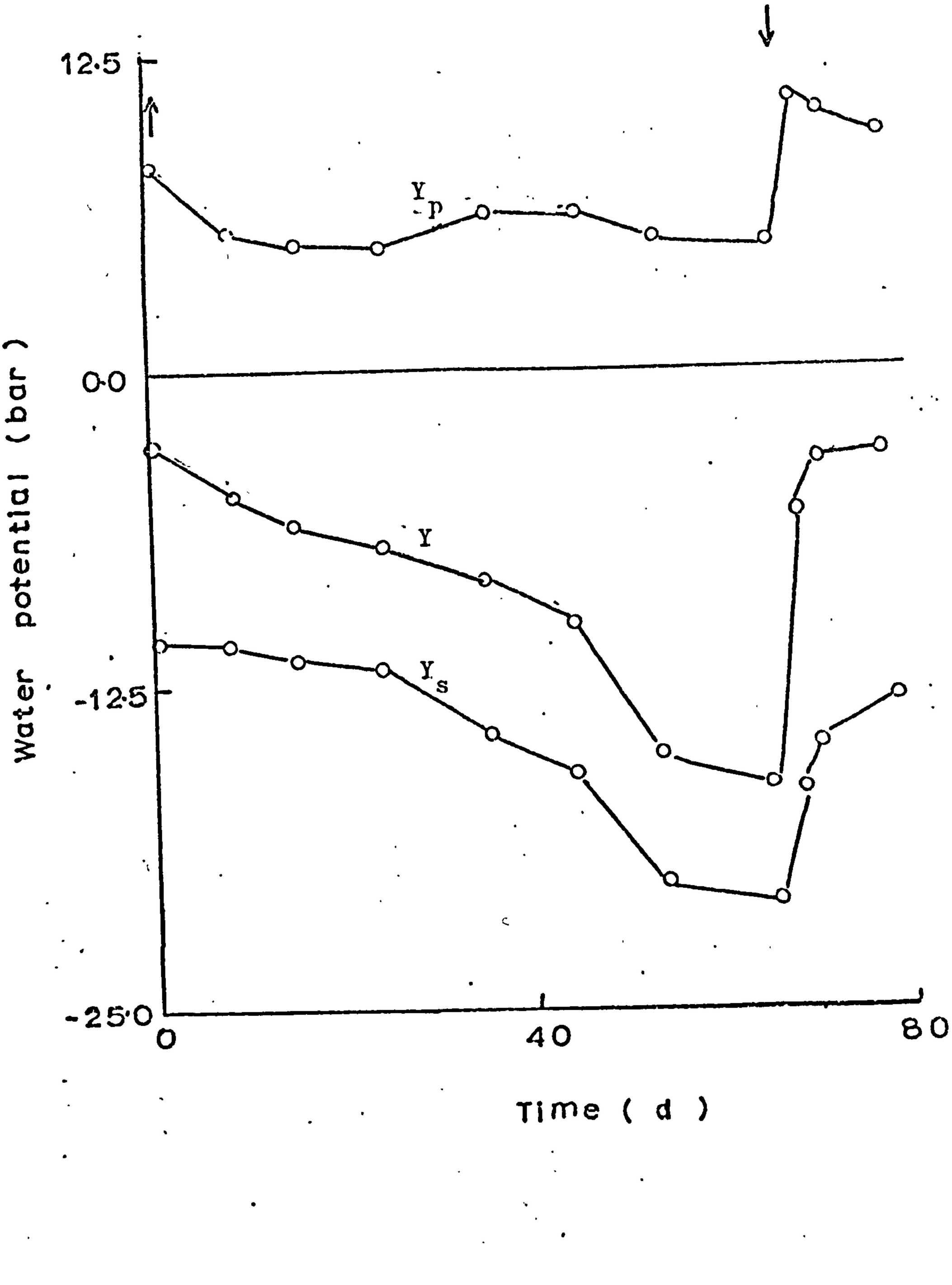
4.3a Effect of severe and prolonged water stress.

In the first set of experiments, drought was imposed by withholding water. The plants used were large as described previously (2.1). Two to three days (summer plants) or 1-2 weeks (winter plants) after watering had ceased, each experimental box was enclosed in a polythene bag to prevent evaporation. Preliminary study showed that when the boxes were enclosed immediately when watering had ceased, the onset of plant drought was very prolonged. If this treatment was delayed for some few days, the soil water was not reduced significantly, though onset of plant drought was slightly hastened initially. This experimental set-up allowed plant water stress to develop gradually with time. Most of the plants were unbranched but a few with side branches were included to increase the number of leaves available for sampling. Leaves on the experimental plants before, during and after water stress treatment were referred to as normal, stressed and post-stressed leaves respectively. Leaves on control plants were also called normal leaves. Rewatering of stressed plants occurred after 65-75 days for winter and 60 days for summer plants.

Soil moisture content (S.M.C.) dropped from 71.4 $\pm$ 4.63 to 4.67 $\pm$ 0.30% by the end of the drought period. There was no significant variation in SMC % of samples taken from

Figure 4.7 Changes in leaf water potential, (Ψ), osmotic potential (Ψ_s), and turgor pressure (Ψ_p) of winter grown plants, ($\uparrow$) on subjection to water stress, ($\downarrow$) on relieving stress by watering.

Fig. 4.7



different parts of the soil mass.

A. Effect of treatment on Y, Y_s and Y_p.

Figure 4.7 shows changes in Y, Y_s and calculated Y_p during the drought period and after water had been restored. With the exception of an initial rapid onset of plant drought, the curves for summer grown plants were essentially similar to those in the figure. As stress progressed Y decreased consistently with time. Y_p fell initially but then remained fairly stable. This period of Y_p decline coincided with only slight changes in Y_s. Thereafter Y_s decreased progressively until it reached a stable valwe of -22.0 $\pm$ 1 bar.

The figure clearly shows that initially, decreases in Y were mostly the result of decreases in Y_p, until Y_s adjustment became more significant to counter balance the reductions in Y.

Extraction of xylem sap for osmotic potential determination was more difficult using droughted leaves. Two determinations gave values of -1.0 and -1.2 bar, which were comparable to those of controls. It seems unlikely, therefore, that transport of solutes through the xylem was significantly increased.

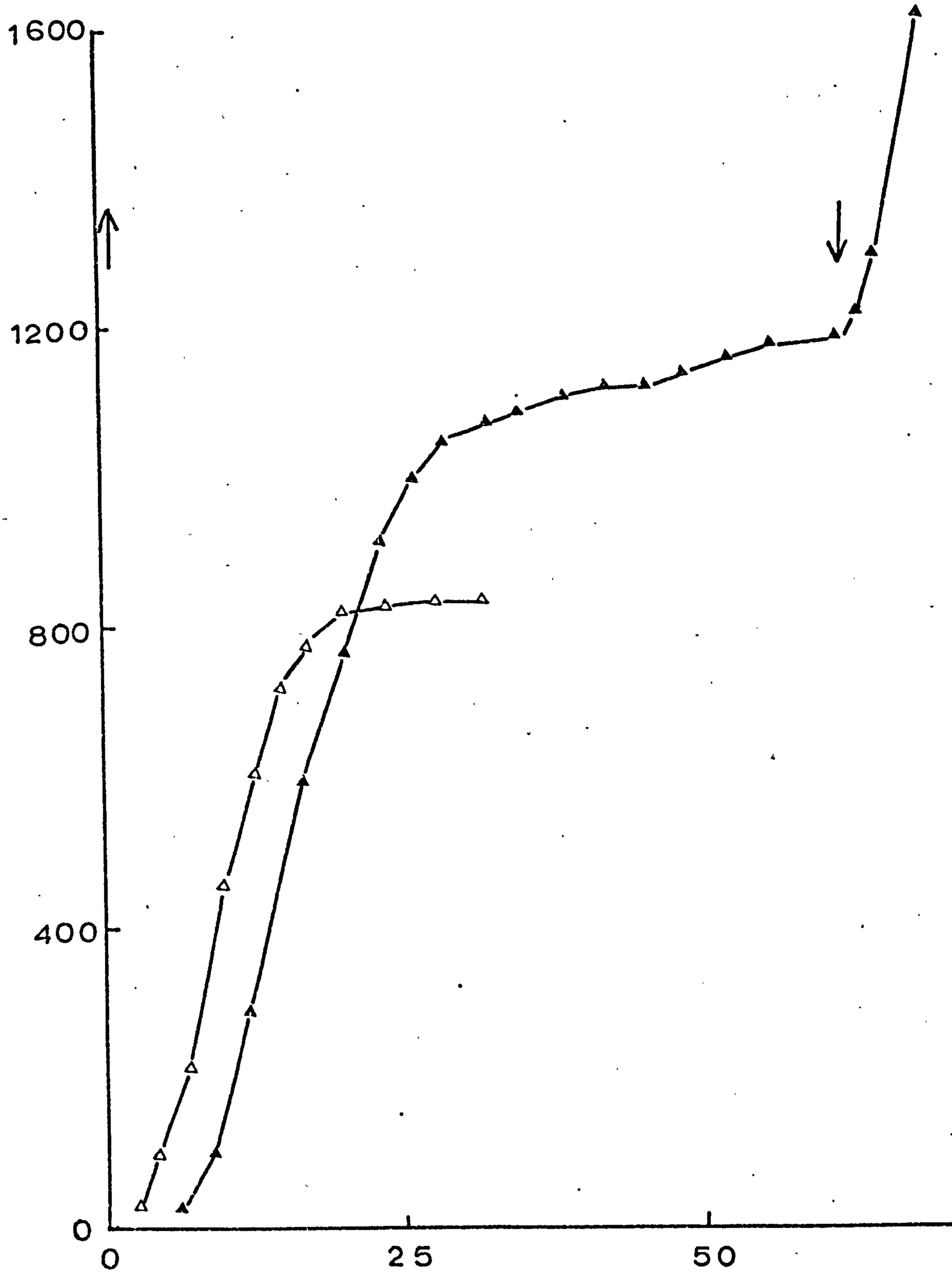
When water was restored Y recovery was more rapid than Y_s. Initially, Y_p increased sharply to high levels (over 10 bar) before falling steadily to normal levels.

B. Effect on leaf production, expansion and other morphological features.

Table 4.8 shows that the plastochrone interval gradually

Figure 4.8 Growth of a single leaf on a well watered plant (Δ) compared with the cumulative leaf area of a prolonged water stressed plant (▲). Arrows correspond to, ▲, and indicate, (↑) subjection to water stress, (↓) rewatering.

Fig. 4.8



increased from 4 to 21 days by the end of the drought period.

Table 4.8

Plastochrones (days) for stressed and post-stressed leaves of Ricinus plants subjected to prolonged and severe water stress.
(* opening of flower bud)

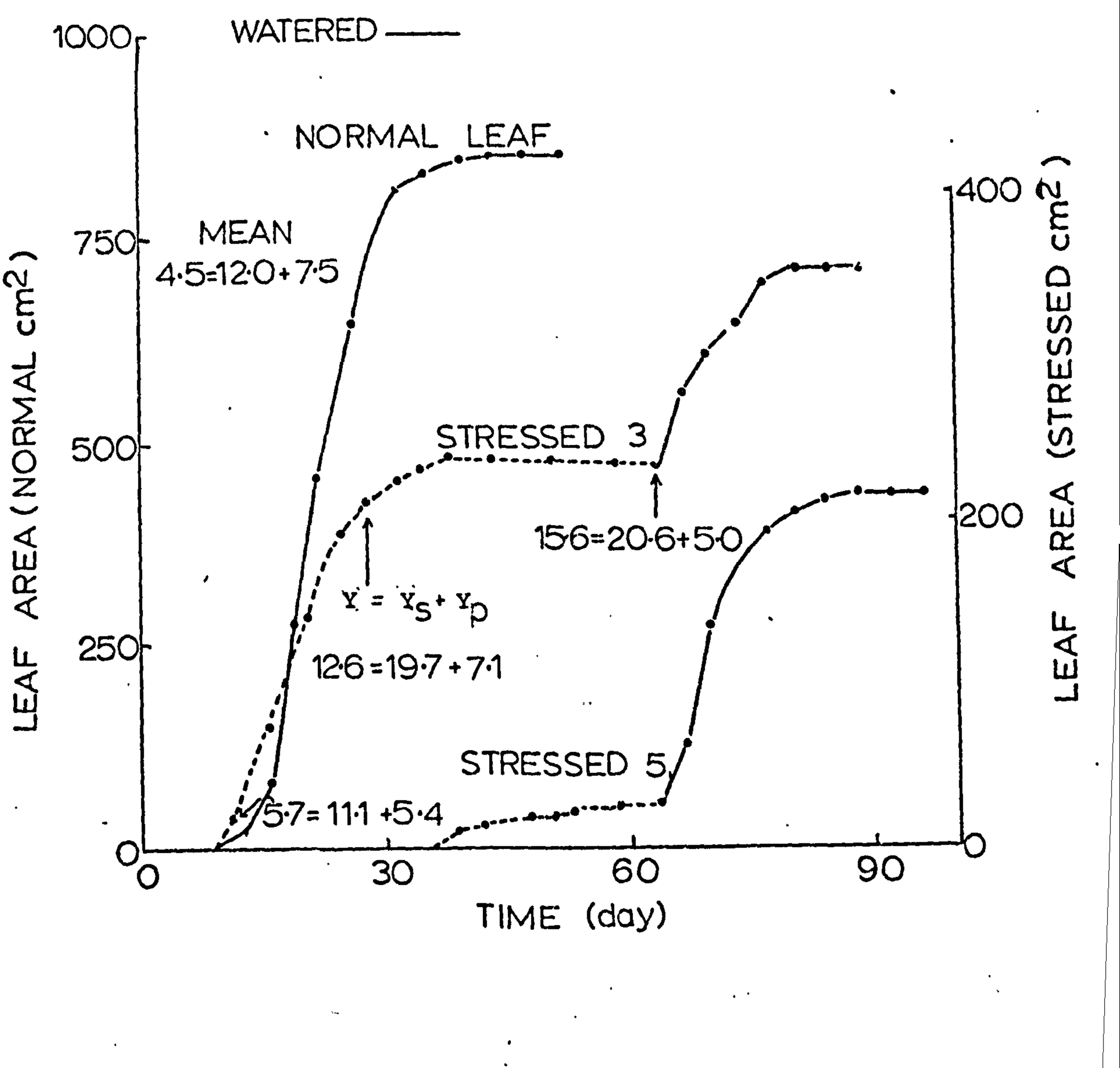
Date watering ceased	Stressed Leaves							Post-stressed leaves		
	0-1	1-2	2-3	3-4	4-5	5-6	6-7	0-1	1-2	2-3
17.1.76	3	6	10	7	7*	10	15	6	4	3
9.3.76	5	6	7	7	11	4*	18	7	3	3
5.5.76	4	4	8	9	10	15*	21	6	5	4

Figures 4.8 and 4.9 show examples of leaf growth curves for a whole stressed plant and stressed leaves respectively. Also shown for comparison are curves for single normal leaves. The rate of leaf expansion of stressed leaves decreased progressively with time. Individual leaves stopped expanding prematurely, progressively towards the stem apex. The maximum leaf areas decreased with each successive leaf (Table 4.8). Nevertheless, because leaves continued to develop (Table 4.8) and also very young leaves expanded partially, growth was measurable on the whole plant as shown in Figure 4.8.

Observations made showed that growth of side branches was very poor and many of them eventually died. On the main shoot, wilting, senescence and abscission of all older normal leaves progressed acropetally as water stress

Figure 4.9 Effect of water supply on leaf water potential (Ψ) osmotic potential (Ψ_s) and turgor pressure (Ψ_p) and growth of a normal leaf and stressed leaves of Ricinus.

Fig. 4.9.


STRESSED-----
WATERED ——
NORMAL LEAF
MEAN
4·5=12·0+7·5
STRESSED 3
15·6=20·6+5·0
Y = Ys + Yp
12·6 = 19·7 + 7·1
STRESSED 5
5·7 = 11·1 +5·4
LEAF AREA (NORMAL cm2)
LEAF AREA (STRESSED cm2)
TIME (day)
1000
750
500
250
0
400
200
0
30
60
90

increased. Petioles of young normal leaves and older stressed leaves moved epinastically to horizontal positions and thus displaced the leaf laminae from horizontal to vertical positions. The leaves became flaccid but wilting was not detectable (Plate 4.1). Apparently, this was due to their young physiological state (see Section 2 also Ludlow, 1974; Milburn and Weatherley, 1971) and also the high Y_p they maintained.

Other leaf features observed are summarised in Table 4.9. Fresh weights and dry weights per unit area were obtained from leaf discs immediately after sampling or after they had been saturated for 4 hours at 1°C. This saturation was used to minimize errors which could be attributed to shrinkage of leaf area through water loss (Mederski and Alles, 1968). Discs were punched from 'mature' leaves on control plants or older stressed leaves. Surprisingly, prolonged stress did not affect these parameters significantly.

Many of the stressed plants developed flower buds, apparently at the expense of leaf production, (see Table 4.7). Accordingly, the drought treatment was terminated 2 to 3 weeks later for lack of material for sampling.

Upon rewatering, Table 4.7 shows that the plastochrone of the 2nd - 3rd leaf returned to normal. All the leaves resumed expansion as shown in Figure 4.9 but their growth rates did not initially compare with controls. Observations made showed that 3 to 4 days later, however, young stressed leaves resumed normal rates. Stressed leaves became thicker but their leaf areas at full expansion were reduced

Plate 4.1 Ricinus plants showing (A) normal leaves, (B) wilted leaves and (C) leaves which have adjusted osmotically.

Table 4.9

Comparisons in morphological features of normal and stressed leaves of Ricinus

Leaf Character	Normal Mature Leaves	Stressed leaves (Numbered leaves developing after the imposition of stress when growth had ceased) 1 2 3 4 5 6	Stressed Leaves (Arrested growth leaves after rewatering)
Maximum leaf area (cm^2)	798 ± 46	580 460 342 254 116 69	242 ± 20
Leaf fresh weight per unit area (gm/cm^2)			
a) When sampled	0.0129 ± 0.0005	0.0121 ± 0.0004	
b) After saturation (1°C)	0.0151 ± 0.0060	0.0155 ± 0.0010	0.0248 ± 0.0009
Leaf dry weight per unit area (gm/cm^2)			
a) When sampled	0.0030 ± 0.0001	0.0035 ± 0.0001	
b) After saturation	0.0029 ± 0.0001	0.0030 ± 0.0001	0.0044 ± 0.0002
Leaf thickness (mm)	0.26 ± 0.03	0.22 ± 0.03	0.37 ± 0.06

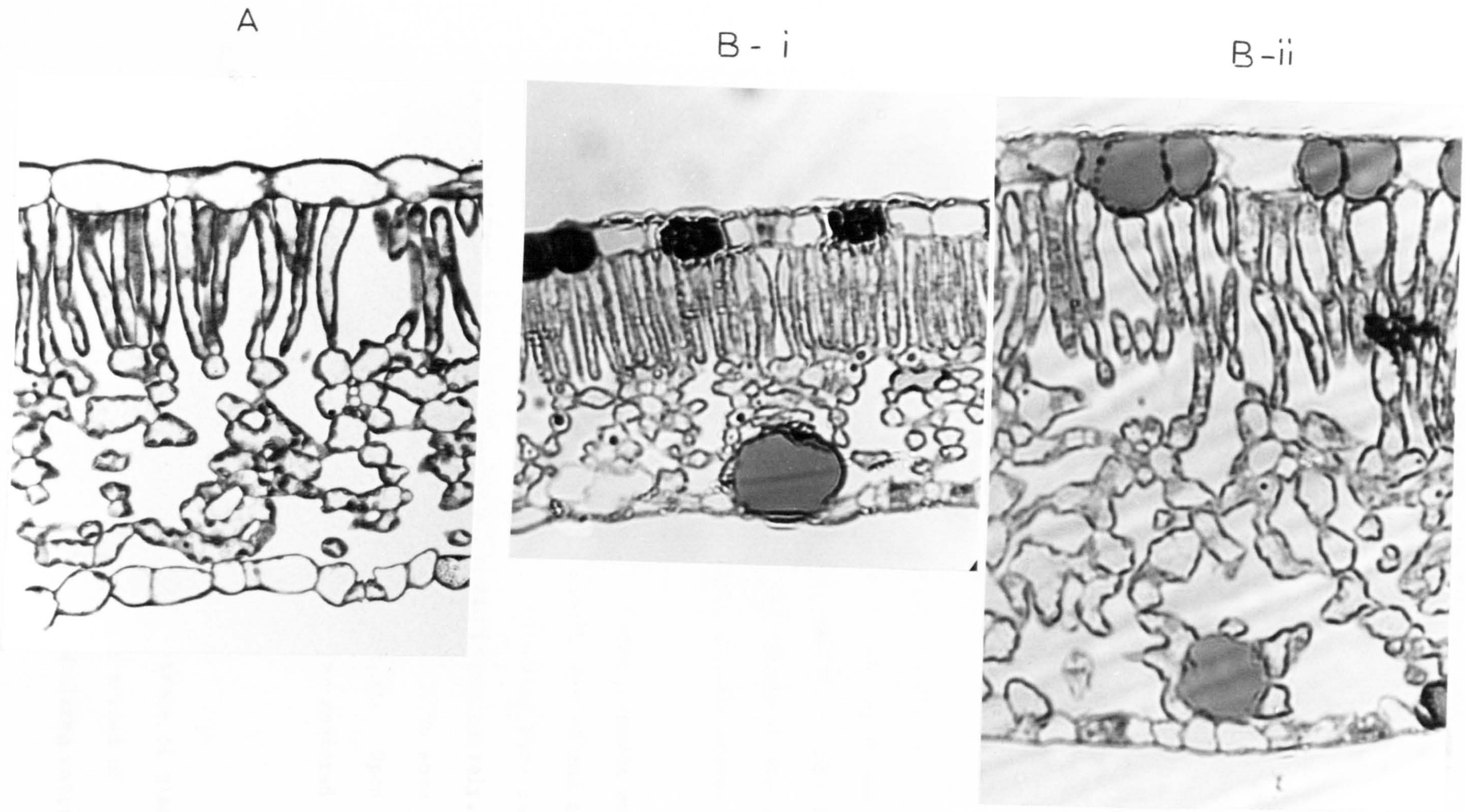


Plate 4.2 Transverse sections of leaves of Ricinus. A - normal leaf, B - stressed leaf: (i) on a plant subjected to a prolonged water stress, (ii) following the release of water stress.

below 50% of control (Table 4.9; Figure 4.9).

Post-stressed leaves also remained small, nevertheless normal leaf thickness was restored to the 3rd and 5th leaf. Plate 4.2A, Bi and ii shows T.S of normal and stressed leaves and those of stressed leaves following rewatering. Plate 4.2Bi shows that the small leaf sizes are associated with a reduction in cell enlargement. In addition, plate 4.2Bii indicates that the observed leaf morphological alteration which was induced by water stress, partly resulted because some of the cells were permanently damaged and did not enlarge following rewatering. Furthermore, lateral increase in epidermal cells was reduced while their elongation was enhanced. This fact, together with a large increase in the volume of the spongy mesophyll contributed to the observed thicker leaves than in normal plants.

Stomatal resistances of older stressed leaves were measured periodically during the drought period and after rewatering. However, they are not presented here because they were insufficient to prove a trend conclusively. However, they suggested that the leaf maintains some degree of permeability during the period of drought. Upon rewatering, normal stomatal functioning was restored in 2-3 days.

4.3b Effects of osmotic stress

It became obvious from 4.3a that lethal levels of plant water deficits could not be reached within the period of experimentation (about 65 days) by merely withholding water

supply to the rooting medium. In the second set of experiments, it was, therefore, decided to increase soil moisture tensions rapidly through reduction of soil solution osmotic potential by adding sodium chloride (Na Cl) solutions to the soil. Now if Ricinus absorbs the NaCl into its tissue as has been reported for other species (cf Slatyer, 1967) it will enable evaluation of the importance of Y_s adjustment on growth during stress to be made more fully while other factors remain unaltered. Well watered plants at alternate leaf 5 stage in 15 cm. pots were divided into 5 similar groups, A, B, C, D and E, of 4 plants each. They were subjected to watering, initially on alternate days but later, daily (big plants) with 200 mls. (to each pot) of water, and NaCl solutions of 0.02, 0.05, 0.1 and 0.3 molar respectively (approximately equal to -1.0, -2.3, -4.6 and -13.1 bar respectively). Once a week, Liquinure solution (2 ml./l) was added to the watering solutions.

To minimize evaporation from the soil surface which might cause increased concentration of soil solution, pot surfaces were covered with aluminium foil for most of the time. Soil solution osmotic potential values measured cryoscopically on solutions extracted by centrifugation gave highly variable values for samples from a single pot and also for the replicates of a sample. The reasons for the discrepancies are not known and the data are not presented.

The experiments were conducted in the greenhouse and the treatments were started from 13.6.76 to 18.8.76 during which, plant water deficits and leaf expansion were studied. After this, individual plants were selected and the soil was repeatedly

washed until the soil solution osmotic potential compared with controls. The plants were then subjected to normal watering for several days during which growth and water deficit readjustments were studied.

A. Salt tolerance of Ricinus

Injury became apparent first in older lower leaves of E plants after 6 days and progressed to other leaves and finally the stem. All the plants in E died by the 26th day. Salt was absorbed into injured leaves resulting in translucent injected areas of the leaf laminae. Prior to death of the plant Y and Y_s of uninjured leaves reached -16.8 and-23.8 bar respectively (Table 4.10). There was only 50% death in D plants and this occurred between day 40 and 48. There was no death in B and C plants.

Table 4.10a

Effect of salinity stress on internal water deficits of Ricinus plants. Measurements were made at 09.00 to 10.00 hours

Time (days)	Treatments											
	B (-1.0 bar)			C (-2.3 bar)			D (-4.6 bar)			E (-13.6 bar)		
	Water potential (bar)											
	Y	Y_s	Y_p	Y	Y_s	Y_p	Y	Y_s	Y_p	Y	Y_s	Y_p
1	4.5	11.8	7.3	5.4	11.8	6.4	7.6	12.3	4.7	7.6	11.5	3.9
3	5.5	10.2	4.7	10.5	13.0	2.5	10.1	12.4	2.3	10.8	14.6	3.8
6	5.1	13.2	8.1	9.0	14.9	5.9	13.4	15.3	1.9	15.5	16.0	0.5
13	9.0	16.5	7.5	8.9	13.2	4.3	11.1	17.6	6.5	14.1	21.2	7.1
18	6.4	14.3	7.9	10.7	18.6	7.9	11.1	16.2	5.1	15.3	21.9	6.6
20	7.6	15.5	7.9	14.2	18.3	4.1				*19.8	23.8	4.0

Table 4.10 Contd./

Time (days)	Treatments											
	B (-1.0 bar)			C (-2.3 bar)			D (-4.6 bar)			E (-13.6 bar)		
	Water potential (bar)											
	Y	$-Y_s$	Y_p	-Y	Y_s	Y_p	-Y	$-Y_s$	Y_p	-Y	$-Y_s$	Y_p
24	6.8	16.4	9.6	11.8	18.9	7.1	14.9	19.3	5.4			
32	9.7	17.9	8.2	9.7	18.9	7.2	14.9	21.2	7.3			
38	10.2	20.6	10.4	14.0	21.0	7.0	*13.1	24.4	11.3			
46				12.5	23.0	10.5	*15.7	25.2	9.5			

* Plants showed signs of injury. Y and Y_s are negative values.
Water potentials measured for normal plants (A) were,
Y, -3.5 to -5.5; Y_s, -11.0 to-13.0; Y_p, 7.0 to 9.0 bar.

B. Effect on % RWC, Y, Y_s, Y_{xs} and Y_p.

The results are shown in Figure 4.10 and Table 4.10. It is clear that all the water deficit indices shown were affected by increasing osmotic stress. Table 4.10 shows that initially Y_p was reduced with a consequent reduction in Y suggesting that initially the effect of the osmotic stress was to restrict water availability to the plant. With time, decreases in Y_s became more significant and continued to decrease with time, in contrast to Y, which showed fluctuations. The decreases in Y_s resulted in increases in Y_p despite the low Y thus showing an osmotic adjustment phenomenon.

Y_{xs} decreased from about -1.0 bar in A plants to -2.1, -3.2, -3.5 and-4.5 in B, C, D and E plants respectively. This shows that in contrast to water stress plants, greater amounts of solutes were transported up the plant than

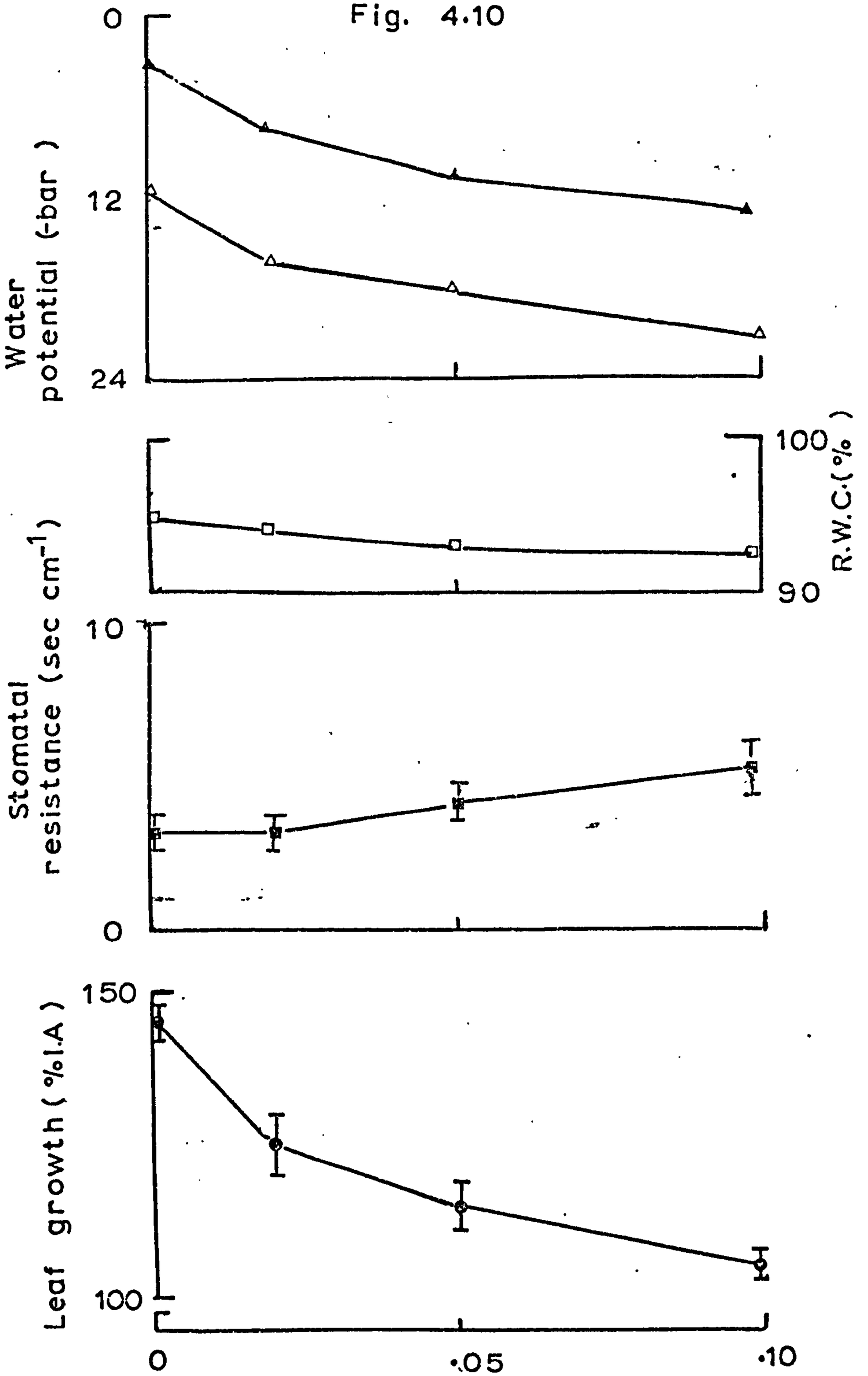
Figure 4.10 The effect of salinity stress on (from top) leaf water potential (Y) and leaf cell sap osmotic potential (Y_x).

Leaf relative water content (RWC, % $1^{o}C$)

Lower stomatal diffusion resistance (sec cm^{-1})

Leaf growth (% of initial area per day, initial leaf areas ranged from 60 to 100 cm^{2}).

Fig. 4.10


0
12
24
Water potential (-bar)
100
90
R.W.C.(%)
10
0
Stomatal resistance (sec cm-1)
150
100
Leaf growth (%I.A)
0
·05
·10
Molal Na Cl solution

normal.

Table 4.10b Examples of water potential differences between young and older leaves:

Treatment							
B				C			
Height of leaf insertion (cm)	Water potential (bar)			Height of leaf insertion (cm)	Water potential (bar)		
	$-Y$	$-Y_s$	Y_p		$-Y$	$-Y_s$	Y_p
51	-5.8	-15.8	11.0	40	-7.0	-19.5	12.5
53	-5.8	-16.0	10.2	45	-7.0	-18.5	11.5
54*	-6.0	-15.5	9.5	46*	-7.1	-18.5	11.4

* Young leaf.

These show that, in contrast to water stressed plants, osmotic adjustment was not more pronounced in younger than older leaves (cf Figure 3.3). This may be viewed as a mechanism utilized by young leaves to avoid damaging effects of excessive inorganic ions in their cytoplasm (cf Parker, 1972) which could also inhibit other metabolic processes, (cf Hellebust, 1976).

In Table 4.11 Refractive Indices (RI) from osmotic stressed plants are compared with those from water stressed plants. At a given Y_s, RI change was greater in the latter than in the former plants. This suggests that the cell sap of the osmotic stressed plants is composed mostly of inorganic salts (Na^+ and Cl^- ions) and not organic solutes such as sugars (cf Barrs, 1968). Whatever the osmoticum Table 4.10 shows that the osmotic adjustment continued gradually until a critical Y_s of -22.0 ± 1.0 bar was reached. Further lowering of Y_s caused death as was observed for D and E plants.

Table 4.11

Comparison between refractive indices and osmotic potentials (-bar) of leaf cell sap of water stressed and salinity stressed leaves.

Leaf cell sap osmotic potential (Y_s, - bar)	Refractive index				
	Water Stressed	Salinity Stressed			
		B	C	D	E
12.0 - 13.0	1.3450				
13.1 - 14.0	1.3468		1.3424		
14.1 - 15.0		1.3440	1.3454		1.3434
15.1 - 16.0	1.3498	1.3436			
16.1 - 17.0	1.3498	1.3461		1.3438	
17.1 - 18.0	1.3530	1.3476		1.3458	
18.1 - 19.0	1.3542		1.3454		
19.1 - 20.0				1.3475	1.3450
20.1 - 21.0	1.3556	1.3484	1.3476		1.3462
21.1 - 22.0	1.3572		1.3480	1.3462	

When normal watering was restored treatment D plants did not survive. Table 4.12 shows that the recovery of Y, Y_s and Y_p was proportional to time elapsed and it also depended on the degree of osmotic stress. Y_s again appeared to be the important factor, recovering at a slower rate than Y.

C. Effect on leaf growth.

Figures 4.10 and 4.11 show that B, C and D exhibited lower growth rates than A and the degree of reduction increased with increasing osmotic stress. Growth in E was zero. In Figure 4.11 the normal sigmoid curve was distorted and instead of a growth period of about 20 days (A), leaves in treatments B, C and D took 38, 42 and 50 days

Figure 4.11 Growth curves of salinity stressed plants B, C and D compared with that of a well watered plant (A). NaCl solutions of 0.02, 0.05 and 0.1 molal were added to the soil of B, C and D plants respectively.

Fig. 4.11

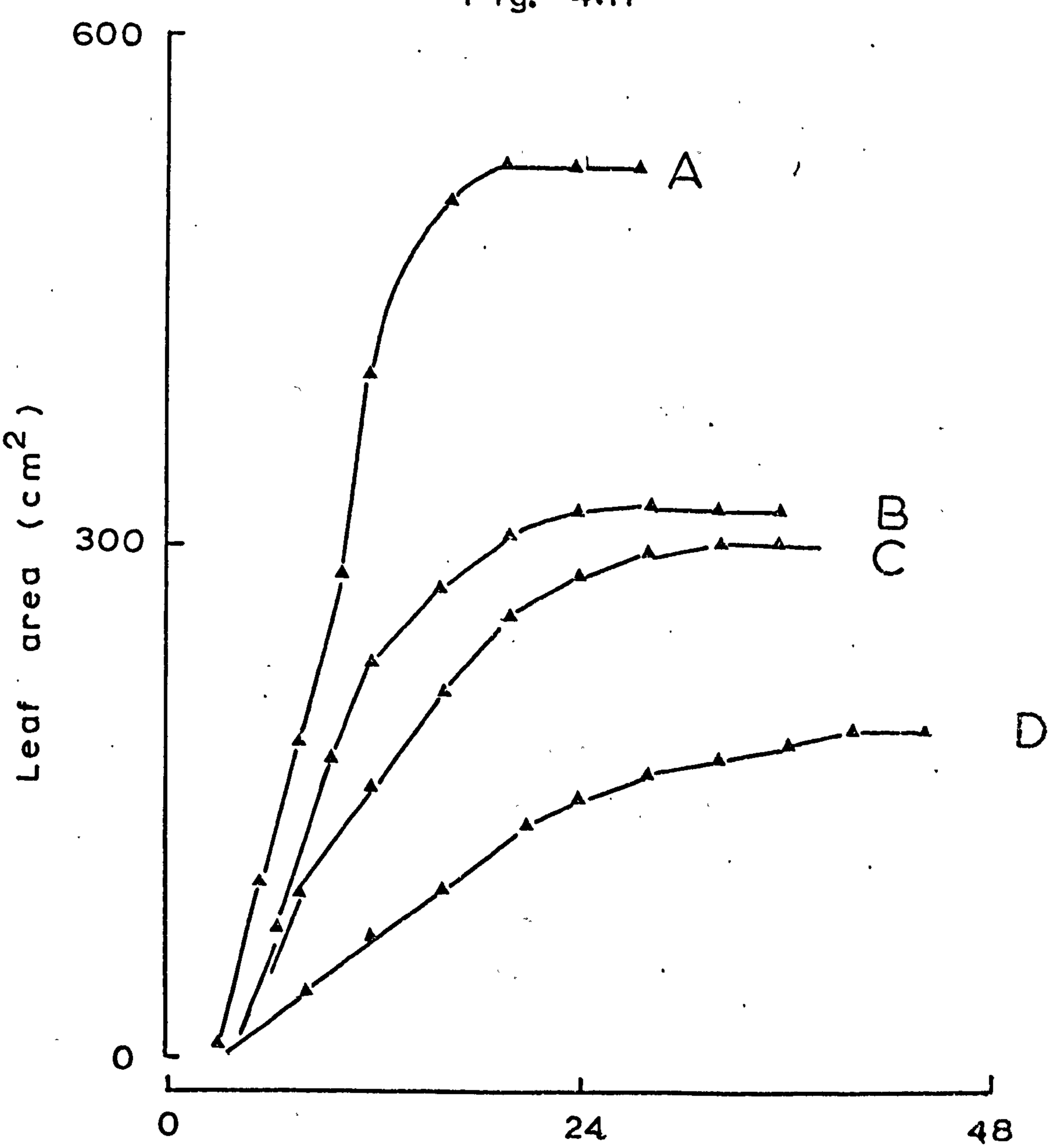


Table 4.12

Readjustment of leaf water potential (Y), osmotic potential (Y_s) and turgor pressure (Y_p) on removal of salinity stress.

Days after rewatering	Treatments					
	B (-1.0 bar)			C (-2.6 bar)		
	Water potential (bar)					
	-Y	$-Y_s$	Y_p	-Y	$-Y_s$	Y_p
1	-7.0	-20.2	13.2			
3	-6.8	-16.0	9.2	- 8.3	- 17.9	9.6
4				- 7.4	- 16.4	9.0
5	-4.5	- 14.0	9.5	- 6.0	- 16.8	10.8
10	-4.4	-13.6	9.2	-4.8	-15.8	11.0

to reach 'maturity' at areas 65, 60 and 40% respectively of that of A. Apart from suppressing leaf expansion, the treatment also reduced stem elongation (Plate 4.3).

Observations made showed that B, C and D leaves exhibited darker green colour and showed no signs of wilting. The leaves were succulent and both leaf thickness and leaf fresh weights per unit area increased with increasing stress (Table 4.13). Dry weight per unit area did not appear to have been greatly affected by the treatment, probably partly due to the accumulated solutes.

D. Effect on stomatal resistances.

Figure 4.10 shows means of stomatal resistances recorded between 11.00 and 14.00 hours. The figure clearly shows that leaf expansion was more sensitive to the treatment than stomatal resistances. For example, although the rate of leaf expansion of treatment B was reduced to 85%

Plate 4.3 Ricinus plants subjected to salt stress.

Table 4.13

Morphological responses of Ricinus leaf to salinity stress

Salt (NaCl) treatment (- bar)	Leaf thickness (mm)	Leaf fresh weight per unit area (gm/cm^2)	Leaf dry weight per unit area (gm/cm^2)	Leaf Succulency (Fresh Weight / Dry Weight)
0.0 (A)	0.26 ± 0.0004	0.0117 ± 0.0003	0.0035 ± 0.0001	3.3
1.0 (B)	0.28 ± 0.0002	0.0152 ± 0.0003	0.0033 ± 0.0007	4.6
2.3 (C)	0.31 ± 0.0005	0.0170 ± 0.0004	0.0036 ± 0.0005	4.7
4.6 (D)	0.36 ± 0.0009	0.0176 ± 0.0001	0.0034 ± 0.0007	5.2

of control (A), their stomatal resistances were similar. Also, the resistances increased only slightly with increasing stress. The indications are that the high Y_p which developed in the leaves prevented the development of high stomatal resistances.

Table 4.14

Summary of critical growth potentials in a range of plants

Plant	Water potential (-bar)	Turgor pressure (bar)	Author
Helianthus	4.0	6.0	Boyer (1968, 1970)
Soybean	12.0	1.0	Boyer (1970)
Maize	8.0	7.0 - 8.0	"
Maize	8.0 - 7.0	-	Acevedo, Hsiao and Henderson (1971)
Potato	4.0 - 5.0		Gandar & Tanner (1976)
Cotton	8.0	-	Jordan (1970)
Ricinus	11.0 - 7.7	1.0 - 5.0	(This paper)
Helianthus	5.0	4.0	(-)

Discussion

Experiments reported here add further evidence to the already generally accepted concept that favourable plant water status is essential for optimum tissue enlargement. Apparently, this builds up the physical force, Y_p, which mechanically stretches the cell wall from inside (Ray et al, 1971). The data for Ricinus clearly shows that decreasing plant water status caused retardation of cell enlargement. Consequently, leaf expansion and the elongation of petiole and stem were reduced. When severe water deficits (around Y = -11.0 bar) developed in normal Ricinus plants, leaf expansion was completely arrested (Figure 4.3). This corresponded to a critical growth turgor (Y_t) of 2-3 bar. Apparently, like many plants (see Table 4.14) expansion growth in Ricinus ceased before turgor pressures reached zero. The table further shows that species differ in sensitivity to increasing water deficits. Comparison of Figures 4.4 and 4.5 shows that growth rate of Ricinus was less reduced at the onset of water stress and furthermore persisted to a lower Y than Helianthus. The growth response of Ricinus however compares favourably with that of soybean (Boyer 1971) which also seems to be resistant to water stress until at a lower Y.

A low Y_t suggests a large Y_{gr} for growth. Probably, this fact, in conjunction with the slow build-up of a water deficit by day, (Section 3) allowed only a slightly lower growth rate of Ricinus (at normal temperatures) by day than by night (Figure 4.6). This contrasts with the large differences observed between day and night growth of other species (Boyer 1968, 1970).

The fact that water was the critical factor was shown by the dramatic resumption of leaf expansion of all water stressed Ricinus plants, including those whose growth was suspended for several days (Figures 4.3 and 4.9).

However, the rate of expansion did not return to normal immediately, following rewatering. Boyer (1968, 1970) observed similar growth response for Helianthus plants which were stressed for several days. In contrast, complete rate recovery following rewatering has been demonstrated for pine needles (Kaufman, 1968b)and faster rate than controls has been observed for tomato (Gates, 1955) and sugar beet (Owen and Watson, 1956).

Apparently, the growth arrestment and reduced growth rate, prolonged the development of Ricinus leaves. For example, in Figure 4.3 the development of the stressed leaf took 24 instead of the 18 days recorded for the normal leaf (A). The suspension of ageing and senescence of young leaves by water stress has been reported for tobacco (Petrie and Arthur, 1943) and more recently for Panicum Maximum (Ludlow, 1974). The influence of this phenomenon on the final leaf areas is variable. Petrie et al reported bigger leaf areas of the stressed leaves than the control leaves. Acevedo et al (1971) observed that a 1-2 day water stress, imposed during the development of maize, resulted in leaf areas smaller than in those of control plants. The indications are that for Ricinus leaf areas compared with those of controls when water stress lasted for a few days (Figure 4.3). On the other hand, when stress was induced repeatedly (Section 4.1) or when it was prolonged (Figure 4.9) leaves failed to expand to their maximum sizes. This suggests that prolonged water stress may have a permanent injurious effect on leaf expansion.

Table 4.5 clearly shows that growth rates were not identical for all growing leaves on a Ricinus plant. Furthermore, leaves approaching 'maturity' stopped growing first in response to slight decreases in Y, but young leaves continued growing until they were

prevented by much lower Y. This observation extends the previous finding (Section 2) that the ability of leaf cells to extract water from the xylem (for growth) decreased as the leaf aged. Further indications are that despite their lower Y_p, the low Y_t of young normal leaves coupled with their expectedly high wall extensibility, enabled them to grow at a faster rate than older leaves. So far there have only been reports of overall threshold growth pressures for higher plants (e.g. Table 4.14). Perhaps part of Boyer's data (1968 pg. 1060 Table III) showed a similar age effect on Y_t. However, he failed to comment on the phenomenon, probably because he considered the pressure differences involved to be negligible.

The responses of the water potential parameters to prolonged soil water stress and salinity stress (Figure 4.7 and Table 4.10) give further insight into the osmoregulatory mechanism previously observed (Section 3). The adjustment of Y_s was gradual requiring some days to take effect. Also it is limited in extent ranging from about -11.0 ± 1 bar in normal plants to a limit of -22.0 ± 1 bar. Presumably, this Y_s value could be considered to be critical for growth and survival of Ricinus during drought and accordingly further reduction caused death of the plants.

The fact that Y_s readjustment following rewatering was slower than that of Y provides further evidence that Ricinus adjusted osmotically during the previous water stress. The prolonged Y_s readjustment suggests that the excess solutes already accumulated required time to be diluted by new growth (cf Slatyer, 1967).
A delayed recovery of Y after water stress release, has also been demonstrated for soybean (Boyer, 1970). For Ricinus this may partly be attributed to a reduction in xylem conductance through cavitation

during the stress period (cf Milburn, 1973) and also to the low Y_s which persisted even after rewatering.

The adaptive advantages of osmoregulation lie in the fact that it maintains cell turgor for continuous growth and functions of turgor dependant processes and prevents wilting. Possibly, the unwilted conditions of Ricinus leaves (Plate 4.1C) and the large Y_p maintained, (under stress) sustained some leaf characteristics comparable with controls (Table 4.9).

The response of cell enlargement to Y_s adjustment was, however, complex. The expansion of young apical leaves was sustained at a Y of -1.0 to -4.0 bar below the minimum threshold growth value of about -11.0 bar recorded for normal plants. This accords with the suggestion of Meyer and Boyer (1972) and Cutler, Rains and Loomis (1977), that osmoregulation may reduce the capacity of cell enlargement to respond to changes in Y. Nevertheless, cells failed to enlarge fully (Plate 4.2Bi), and the rate of leaf expansion was drastically less (Figures 4.8 and 4.9) than might be expected from the comparatively high cell turgor-pressures. Leaf expansion of the saline-stressed plants behaved similarly despite the very high Y_p they maintained.

A hypothesis to explain the mechanism underlying growth adjustment of plants which adjust osmotically during short periods of water stress has been put forward by Meyer and Boyer (1972) and Hsiao (1973). Accordingly, the growth parameters, E, Y_p and Y_t may still be significant. Assuming this hypothesis is also valid for the present experiments, the observed slow growth-rate suggests an increase in Y_t or decrease in E or both (see page 24).

Both stressed and post-stressed leaves could only expand to

areas less than 50% of controls suggesting that the prolonged stress treatment caused increases in cell wall rigidity. Increase in wall thickness is a general phenomenon in plants subjected to water stress (Maximov, 1929; Shields, 1950; Stöcker, 1960). Although this is known to contribute to plant resistances to moisture stress, it obviously restrains cell enlargement because it confers a low wall extensibility.

Experiments in Section 5 suggest that Y_t increased in magnitude during drought. Probably it did not readjust to the normal value when water was restored. This suggestion is perhaps supported by the slow initial growth rate of stressed leaves (Figure 4.9) although Y_p increased to very high pressures (Figure 4.7). High cell turgor pressure (Y_p) does not benefit cell expansion automatically because Y_t may also be large and Y_{gr} is reduced accordingly.

The conclusions arrived at for Ricinus agree with those of Meyer and Boyer (1972) who proposed that although soybean hypocotyl adjusted osmotically during water stress, E was decreased and Y_t increased and consequently growth was reduced. In contrast, Graecen and Oh (1972) observed that, through osmoregulation, the growth rate of pea roots continued unimpaired during water stress; apparently through the maintenance of a constant Y_{gr}. Other means by which rate of growth can be maintained, during periods of water stress, are through decreasing Y_t and increasing E. Such a response has been demonstrated for maize (Acevedo et al 1971) rye (Green and Cumming, 1974) and soybean (Bunce, 1977) during short periods of water stress.

It must be admitted, as emphasised by Hsiao (1973), that prolonged stress could cause complications in the mechanism underlying growth responses to internal water, apparently due to the highly

integrated nature of plant processes. For example, Slatyer (1967), Flowers, Ward and Hull (1976) and Hellebust (1976) have suggested that the slow growth of osmotically adjusted plants may not be due to the low Y. But rather to an interference of the excessive solutes (especially of inorganic ions) and their possible toxic effect on metabolic processes. It would be interesting if the solutes used in osmoregulation by water stressed Ricinus plants could be identified.

Although discs from the stressed leaves expanded to areas comparable with controls (see Figure 2.5), the intact leaves from which they had been cut expanded to areas less than 50% of controls following rewatering. This may be attributable to the following mechanisms; Firstly, the discs excised and floating on water were exposed to a more favourable water status, (around 0 bar) which enhanced their expansion. Secondly, the intact leaves were exposed to correlative influences (e.g. tissue tensions) of other parts of the plant which could restrain them from expanding. Observations made showed that the discs became convex shaped during floatation suggesting that greater expansion occurred in cells near the cut edges which were obviously exposed to less tissue tension.

It is interesting to note that although leaf expansion was limited, cell elongation was greatly enhanced (Plate 4.2Bii) and consequently leaf thickness increased to about 150% of those of controls. Increases in cutinization, which generally accompanies drought (Stocker, 1960), would obviously restrict expansion growth. Thicker leaves and smaller leaf sizes were observed in salinity stressed Ricinus plants (Table 4.13) and has also been demonstrated in leaves of other plants under similar conditions (e.g. Slatyer, 1967; Meiri and Poljakoff-Mayber, 1969, 1970, Jennings, 1976).

Meiri and Poljakoff-Mayber considered it to reflect differences in the physical properties of the walls of the leaf cells. Jennings attributed the effect to hormonal imbalance of leaf cells.

It is generally assumed that the development of low water potentials in plants reduces growth as a result of loss of turgor causing stomatal closure. Although the results for Ricinus do not provide a critical comparison of stomata-cell expansion relationships with increasing water stress, it can be concluded that water stress did not suppress leaf expansion through its effect on stomata.

SECTION 5

GROWTH OF EXCISED LEAVES IN AN OIL BOMB

Cell enlargement is believed to involve 2 closely linked steps. Firstly, the wall is loosened probably through biochemical or physical transformations. This step is assumed to depend on turgor pressure and it is accompanied by a relaxation in wall pressures (consequently Y_p is reduced) with a corresponding reduction in Y below the growing medium (Cleland, 1971a). Water is absorbed from the medium in response to the water potential gradient established leading to cell volume changes during the next phase. Since the rates of stress relaxation and water uptake should be equal during steady state growth (cf Lockhart 1965b) the growth capacity of a cell could be assessed by any of these two processes.

In experiments reported previously (Section 2) the changes in fresh weights (or volume) of young and older leaf discs were studied when free water near atmospheric pressure was supplied, (i.e. xylem tensions, Y_x around zero bar). In the experiments described below the oil bomb has been used to study growth-induced changes in xylem tensions at constant volume (i.e. zero water supply). It is expected that the adjoining growing mesophyll cells will absorb water from the xylem, thus increasing Y_x, until they are prevented by greater Y_x. It is assumed that the maximum Y_x reached by a leaf may correspond to its threshold Y_x for growth. The advantage of this latter approach is that the conditions are more comparable to those encountered by a cell during normal growth, i.e. cells grow usually at negative water potentials. Moreover during drought the capacity of a plant to absorb water from the root medium is dependent on this increase in Y_x.

The oil bomb has been used to examine some of the hypothesis

developed earlier. Furthermore, because the technique allows separation of the two growth phases it has enabled the investigation of some of the processes pertaining to the initiation of cell enlargement.

5.1 Materials and methods

Except where stated, all the Ricinus plants used were grown in the standard way, (Section 1) and transpiring leaf 7 (i.e. was not equilibrated before sampling) was used. Leaves were sampled and sealed in the oil bomb. The time course of Y_x changes was followed until a plateau was reached. Periodic trimming of the petiole outside the bomb was found necessary to ensure that sealing of cut xylem vessels did not occur. Because of this it was found necessary to begin with petiole protruding about 0.5 cm.

In general, leaves were collected between 08.00 and 10.00 hours. They have an initial Y_x ranging from 2.7 to 3.5 bar. The leaves were approximately 40 to 100% maturity (%M) and had weights and areas ranging from 3.30 to 10.5 g. and 100 to 500 cm^2 respectively. Leaves under 40% M were not used because they had tender petioles which may crush when fitted in the rubber bung.

Normally after the determination of the threshold Y_x, the Y_s of the leaf was measured.

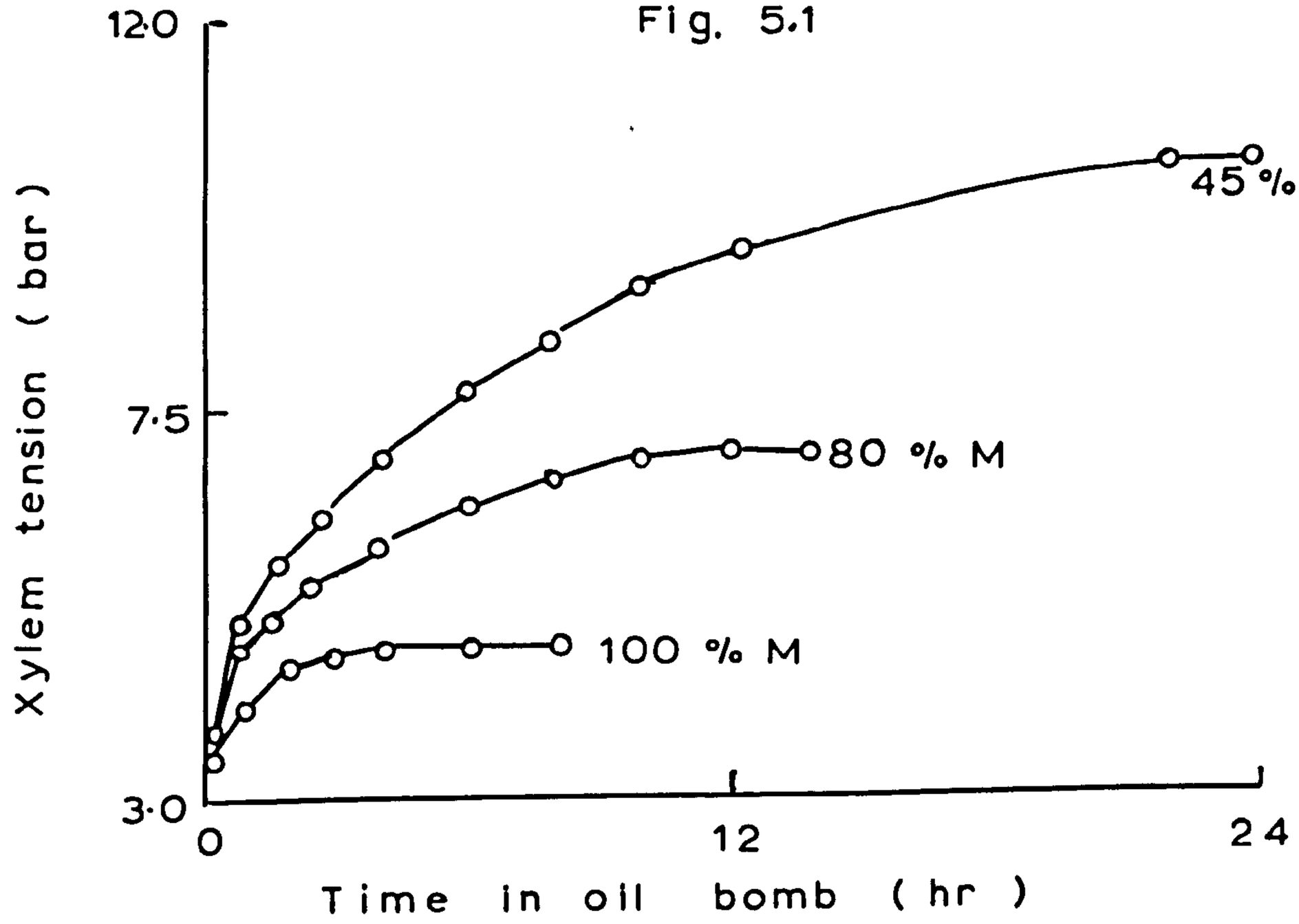
Results:

Experiment 5.2

5.2 Xylem tensions (Y_x) generated by leaves at various physiological stages of development

Figure 5.1 The time course of xylem sap tensions (Y_x) of Ricinus leaves at various stages of maturity (% M). The leaves were not equilibrated before sampling (i.e. transpiring leaves).

Fig. 5.1


12·0
7·5
3·0
0
12
24
Xylem tension (bar)
Time in oil bomb (hr)
45 %M
80 % M
100 % M

5.2a Y_x developed by normal leaves

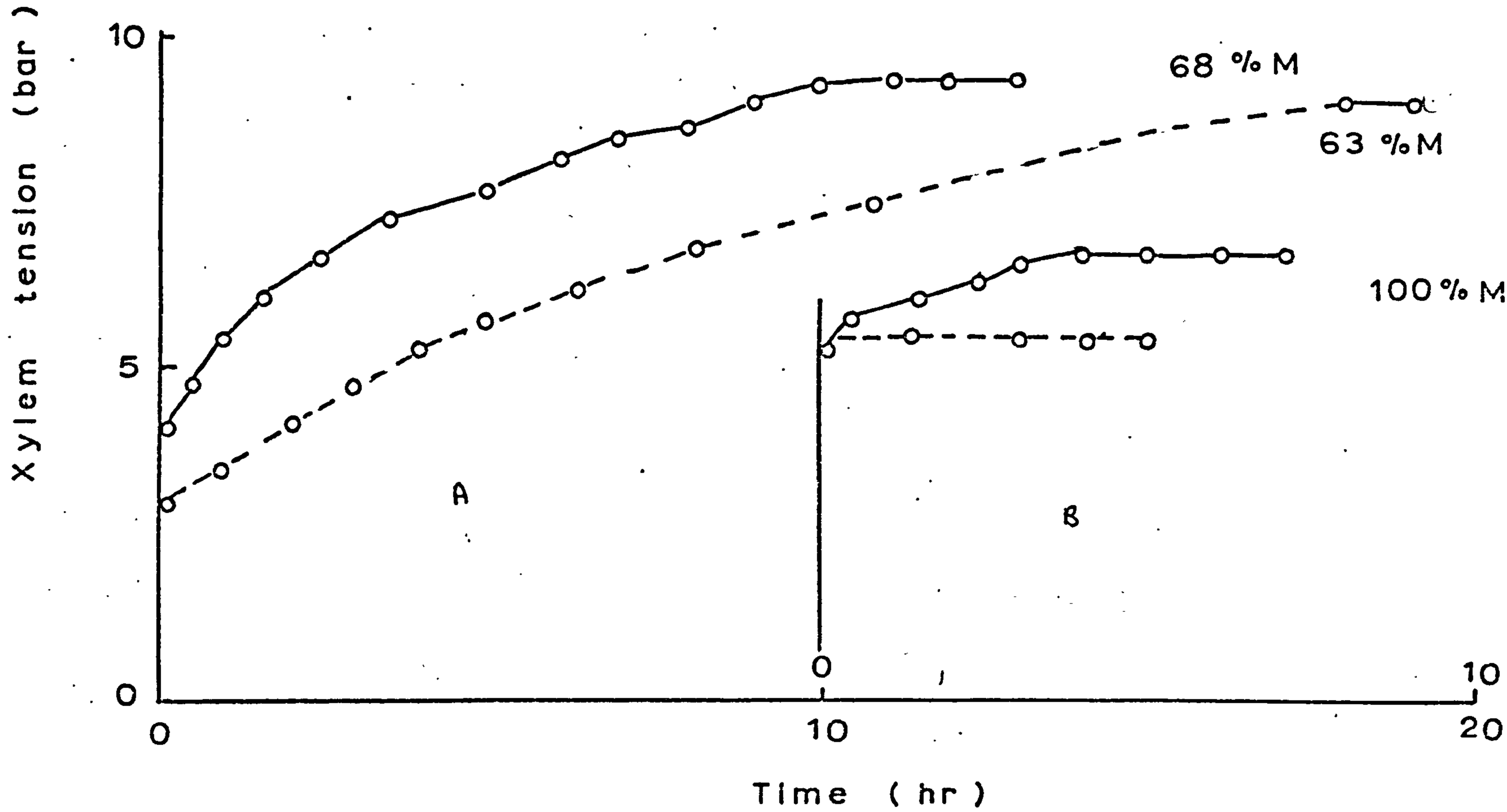
Initially, the changes in Y_x with time of young and older leaves were studied. Examples of curves obtained for Ricinus are shown in Figure 5.1. Y_x increased rapidly during the first 2 to 3 hours. This was followed by a slow, almost constant increase for several hours which then slowed down to a maximum constant value. The curves clearly show that the threshold Y_x reached and the time taken to attain this value depended on the physiological age of the leaf. Thus leaves 45 and 80% M took 22 and 12 hours respectively to reach threshold growth potentials of 10.0 and 7.0 bar respectively. Y_s measured were -12.0 and-13.5 bar for the 45% and 80% M respectively. Making corrections for Y_{xs} gave Y_t of 1.0 and 5.5 bar.

Y_x of leaves at 100% M remained virtually stable after these initial rapid changes. This probably represents water absorbed by the mesophyll cells in reaching equilibration with the xylem tension. To test this hypothesis, the time course of Y_x changes of transpiring leaves were compared with those of pre-equilibrated leaves.

Initially, the intact young and 'mature' leaves were bagged for about 2 hours to prevent transpiration and to allow equilibration of mesophyll cells and Y_x. Examples of curves obtained are shown in Figure 5.2 together with those of transpiring leaves. Unlike the transpiring young leaves the pre-equilibrated leaves assumed the steady rate of Y_x increase immediately. However, the maximum Y_x generated by each leaf and the time interval were fairly close.
On the other hand, curves B show that the Y_x of the

Figure 5.2 Xylem tensions (Ψ_x) developed by transpiring (—) or equilibrated Ricinus leaves (--). Equilibrated leaves were bagged 1-2 hours before sampling, to allow the mesophyll cells to equilibrate with the Ψ_x.

Fig. 5.2



pre-equilibrated 'mature' leaf (100% M) was virtually stable unlike the transpiring leaf. This clearly supports the above hypothesis. It is reasonable, however, to consider that the initial rapid change in Y_x of a growing transpiring leaf represents both transpiration deficit and growth. The steady uptake, however, is attributable to growth.

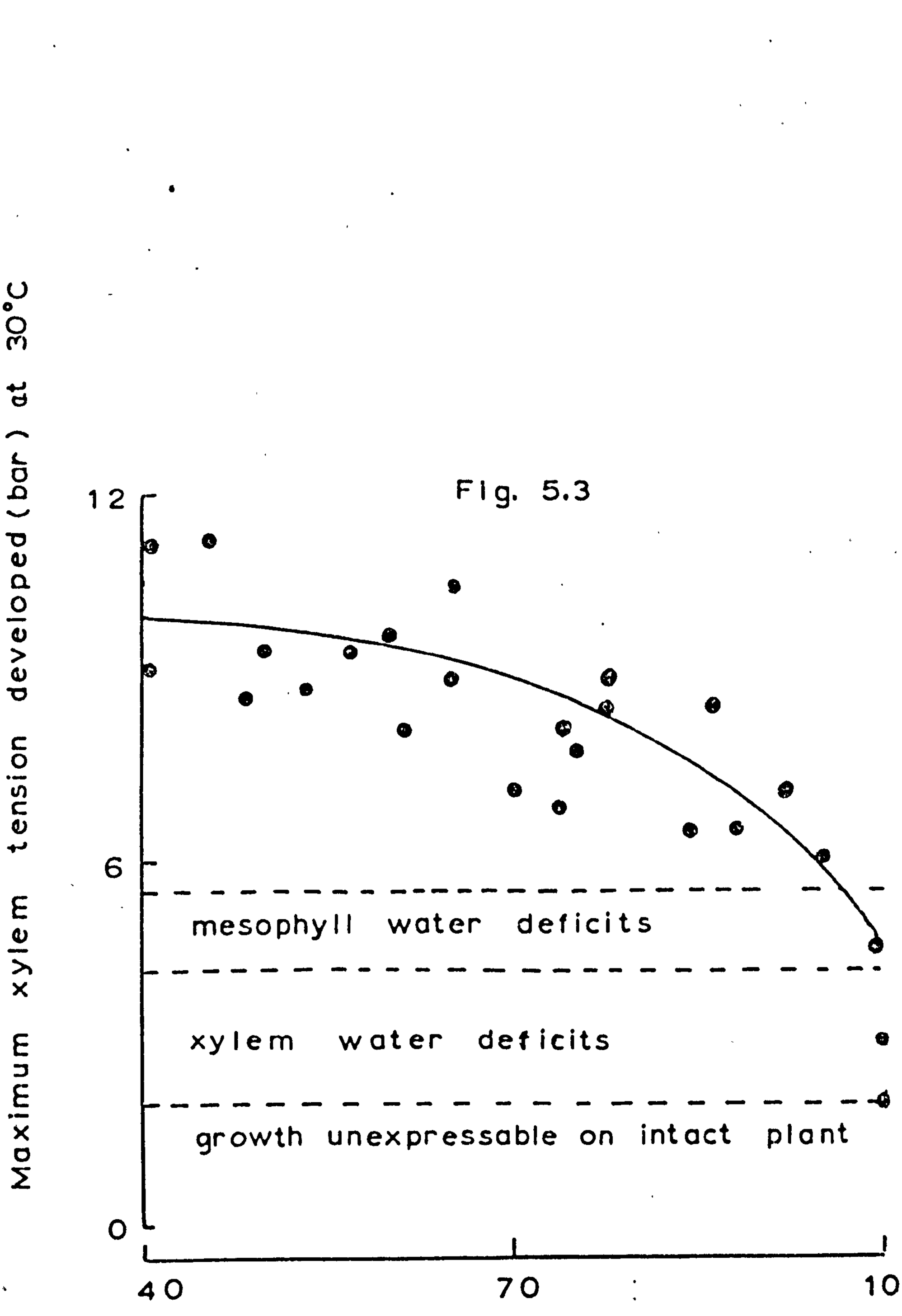
From the above experiments it seems that using pre-equilibrated leaves may simplify the growth studies. However, it was decided to use transpiring leaves for the following reasons. Firstly, the leaves became wet, probably through condensation, when they were bagged. This may be undesirable for oil bomb studies. Secondly, equilibration on the intact plant would ~~reduce~~ increase the Y of the mesophyll cells, consequently growth, especially of cells most distant from the xylem, could be enhanced.

Visual examination of the oil used in the studies did not provide evidence for water lost by leaves into the oil. This accords with results of Potter and Milburn (1970) and strongly supports the view that increases in Y_x of oil bomb leaves represent growth induced water uptake.

Figure 5.3 gives a summary of the maximum Y_x reached (i.e. maximum capacity to attract xylem water) by Ricinus leaves at various stages of maturity (%M) when enclosed in an oil bomb. It must be pointed out that the mean Y_x measured for a transpiring leaf represent both growth water uptake and deficits incurred in the following way.
The initial Y_x of an enclosed leaf represents:- (1) Deficits which exist permanently in well watered plants when even

Figure 5.3 Maximum xylem tensions developed in relation to Ricinus leaf maturity (% M).

Fig. 5.3


Maximum xylem tension developed (bar) at 30°C
12
6
0
mesophyll water deficits
xylem water deficits
growth unexpressable on intact plant
40
70
100
Maturity (% M)

transpiration is negligible (i.e. plants in darkness). This deficit is essentially water unavailable for growth and presumably causes a permanent suppression effect on expansion growth (i.e. causes 'unexpressed growth'). The xylem water content at this level may represent the upper limit of water stored for expansion growth. (2) The remaining deficits represent that incurred through transpirational losses by the leaf while intact. As previously demonstrated in the bomb, increases in Y_x are attributable to water taken up mostly for growth but superimposed upon this is a small uptake in response to the initial mesophyll water deficits.

A closer look at the figure shows that the threshold Y_x for growth of a young leaf, around 40% M is about 10.0 bar (equivalent to Y = -11.0 bar). But as a leaf grows, its capacity to generate suction decreases. Thus at 95% M the threshold growth Y_x reduces to 6.5 bar. The curve in effect represents the lower limit of xylem water available for growth of leaves at different developmental stages.

The calculated critical turgor for growth ranged from 1.0 bar for leaves at 40% M to 6.0 bar for those approaching maturity (96% M). In general, the results accord with and extend those reported previously. These showed that the volume changes of leaf discs which were supplied with free water decreased with maturity (Section 2) and also, that, the expansion of younger intact leaves persisted to a higher xylem tension than that of older leaves.

5.2b <u>Tensions developed by artificially saturated leaves</u>

Earlier results (Section 2) suggested that when leaves

Figure 5.4 The time course of xylem sap tensions (Y_x) of artificially saturated and equilibrated *Ricinus* leaves

Figure 5.5 Effect of a short period of water supply on xylem tensions (Y_x) developed by a young leaf in an oil bomb.

Fig. 5.4

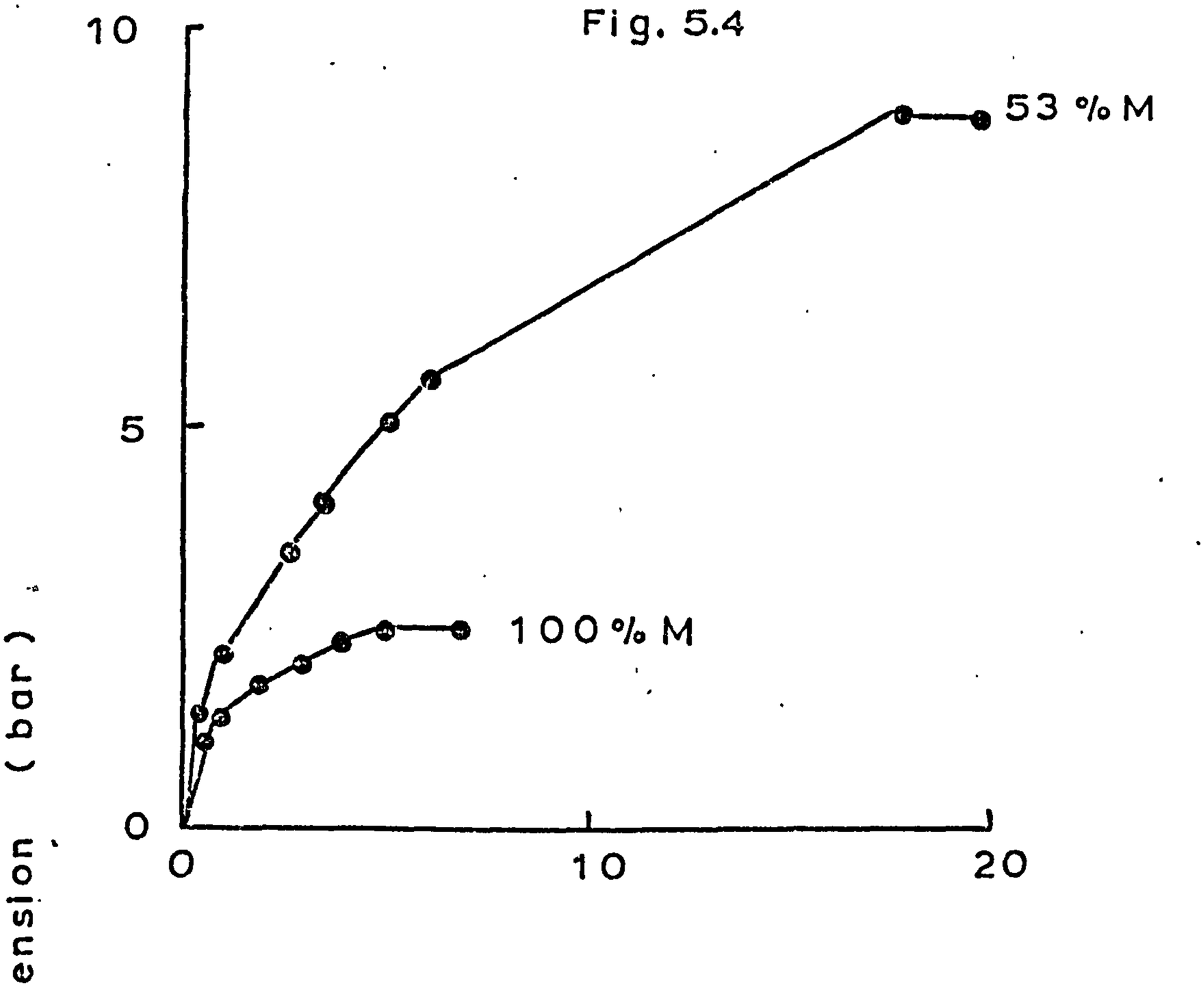
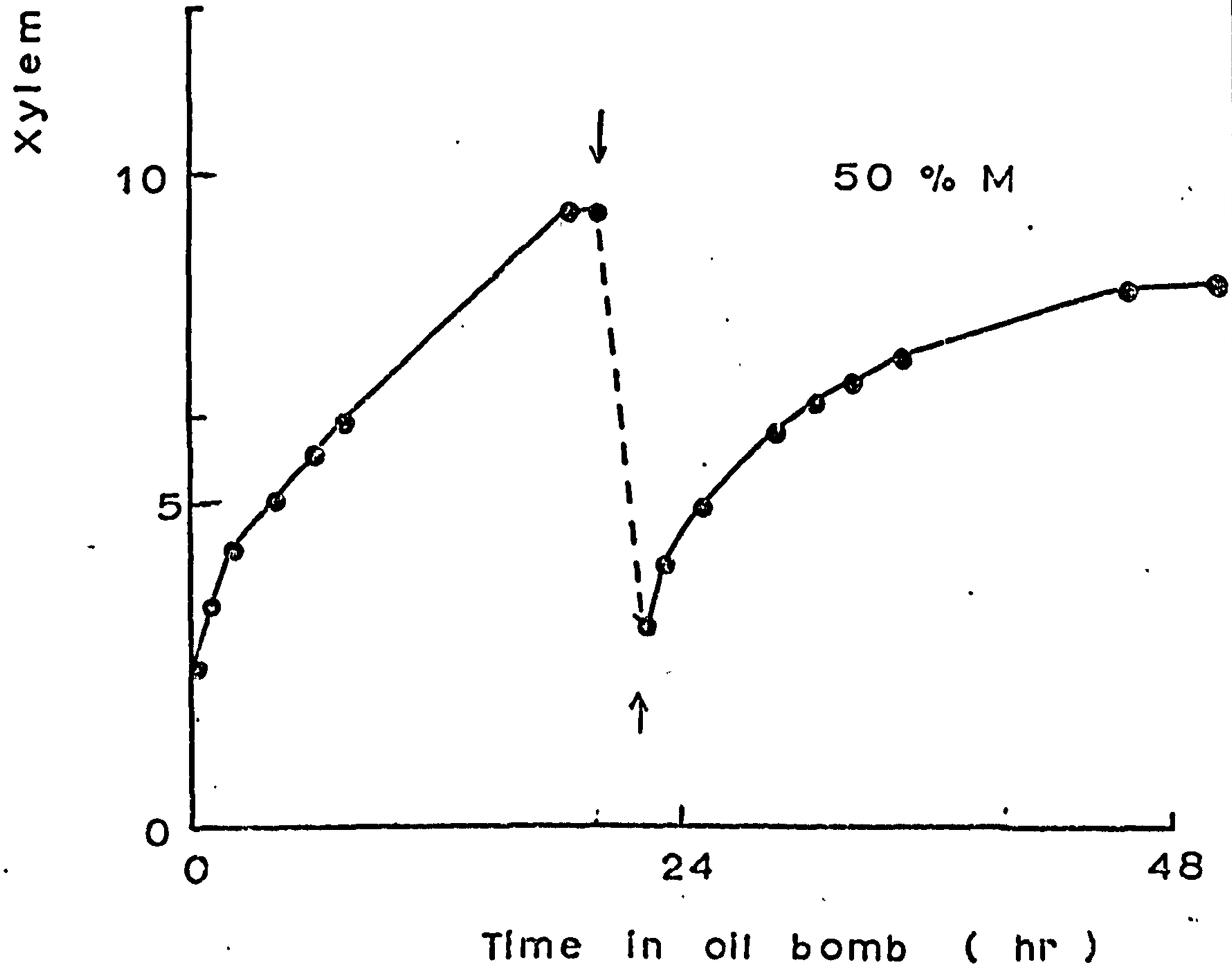

10
5
0
0
10
20
53 % M
100 % M
Xylem tension (bar)

Fig. 5.5


10
5
0
0
24
48
50 % M
Xylem tension (bar)
Time in oil bomb (hr)

were supplied with water more freely than the xylem sap (Y about zero bar) 'mature' leaves (100%M) grew. Furthermore, both young and matured leaves demonstrated 'unexpressed growth'. The phenomenon is re-examined using artificially saturated leaves.

The leaves were bagged whilst intact. They were then excised, re-cut under water and the cut end of the petiole was allowed to take up water for 2-3 hours after which the leaf was sealed in the bomb.

Figure 5.4 shows examples of curves obtained. Y_x of both the young and 'mature' leaves increased rapidly initially before slowing down to a steady state. Again, the rate of Y_x increments and the maximum value reached were greater for the former than latter leaves. Since pre-equilibrated leaves were used, the increases in Y_x must be attributable to growth. The initial rapid increases in Y_x observed here may be attributable to 'unexpressed growth' and thus may be compared with the rapid warm phase I growth uptake of leaf discs floated on water (Section 2).

Growth of the 'mature' leaf stopped at a Y_x of about 2.5 bar. Y_s measured at this Y_x was -12.5 bar and making corrections for Y_{xs} gave a Y_t of 9.0 bar. It is interesting to note that the threshold growth Y_x was comparable to the Y_x which permanently occur in normal non-transpiring plants (see Figures 3.5 and 3.6). This, therefore, supports and extends the previous hypothesis that leaves which cease to grow on normal plants still have a capacity to grow but that growth was inhibited by the permanent high xylem sap tensions.

5.2c Cell turgidity and changes in Y_x.

In Figure 5.5 a young leaf judged to have reached the plateau Y_x was supplied with water through the cut end of the petiole. The aim was to increase the turgidity of the leaf. Y_x decreased initially but with time, it increased to a new plateau. This clearly demonstrates that the turgor requirement for growth could be the critical factor curtailing cell expansion during water stress.

5.2d The effect of osmotic adjustment on leaf suction powers.

Earlier results (Section 4) had suggested that during prolonged water stress physiological adjustments occurred by which the critical Y for leaf expansion was reduced. It was also postulated that under these conditions Y_t increased in magnitude. These hypotheses are further investigated.

Because of the length of the prolonged drought period (about 65 days) it was decided to use leaves from repeatedly stressed plants. Since both plants adjusted osmotically, it was expected that repeatedly stressed plants would behave similarly to prolonged stressed plants.

Normal plants at 4 leaf stage were subjected to watering - wilting cycles until leaf 7 was suitable for experimentation. Leaves sampled were old in age (days) but young in terms of development and no satisfactory way could be found to "standardise" them.

Results obtained are shown in Table 5.1. Initial Y_x ranged from 3.7 to 9.0 bar. The table clearly shows that the maximum Y_x attained by the repeatedly stressed leaves

Table 5.1

Threshold xylem tensions (Y_x) and turgor pressure (Y_t) for growth of young transpiring leaves sampled from drought hardened plants.

(Y_t was calculated after making corrections for xylem sap osmotic potential.)

Initial xylem tension	Water potential (bar) Y_x	Y_s	Y_t	Time to reach plateau (hr)
3.7	13.5	-16.0	1.5	48
5.3	13.3	-16.0	1.8	25
6.0	14.7	-22.3	7.6	25
7.8	15.2	-21.8	5.6	29
8.2	13.7	-18.0	3.3	32
8.8	16.2	-22.2	5.0	29
9.0	13.0	-17.5	3.5	31
*9.0	9.4	-11.6	1.2	6

(* Normal leaf which was rapidly stressed).

were greater than by normal leaves (cf Figure 5.3). Furthermore, the table indicates that when normal young leaves were stressed rapidly (i.e. no osmotic adjustment, see Section 3) they did not show any substantial increase in Y_x in the oil bomb as compared with the repeatedly stressed plants. It is, therefore, reasonable to suggest that osmoregulation increased the capacity of leave tissues to extract water from the xylem for growth. The estimated critical turgor for growth (Y_t) ranged from 1.5 to 7.6 bar. These are clearly larger than those of normal growing leaves.

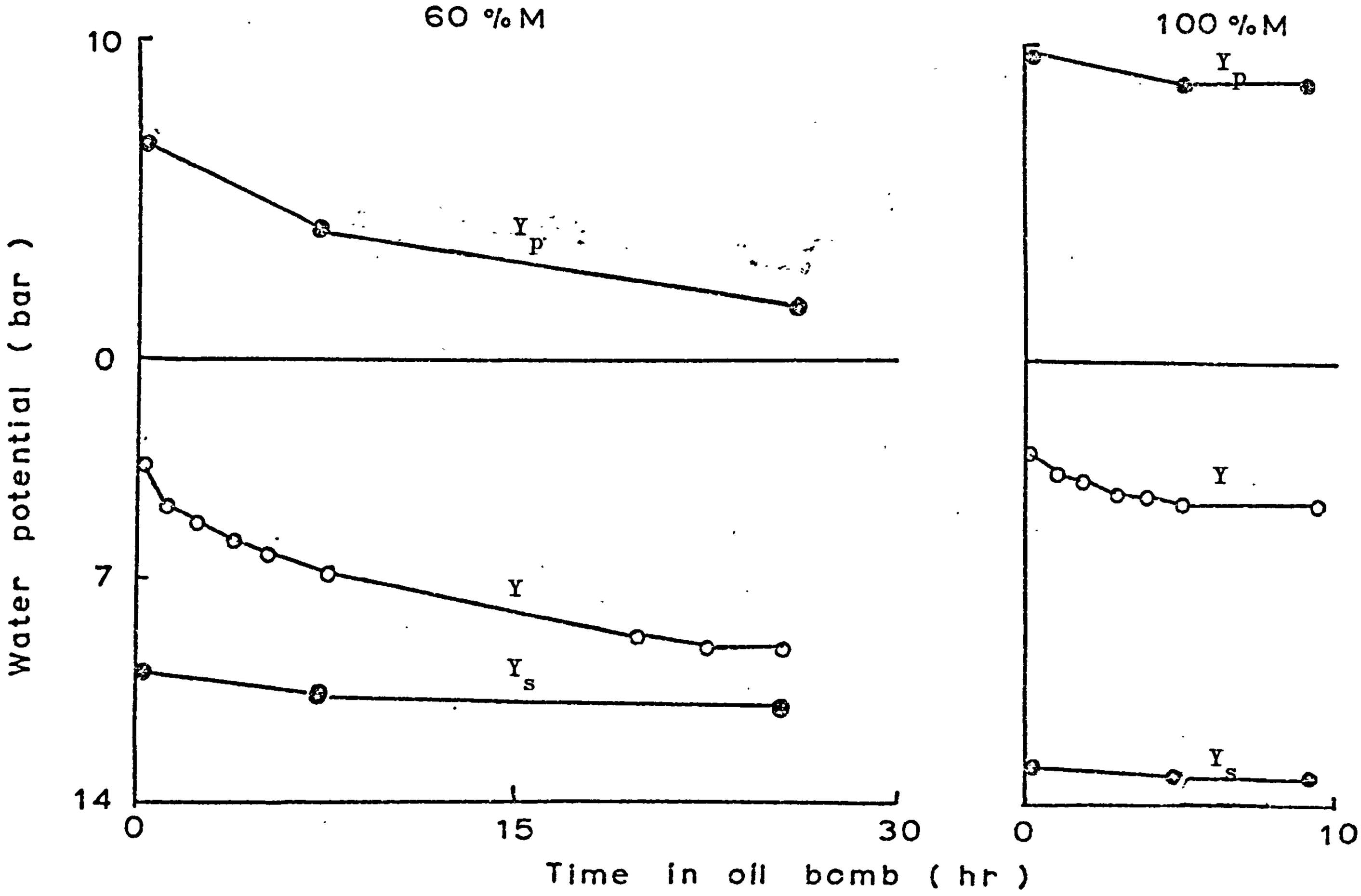
5.3 The nature of the initial processes of cell enlargement

5.3a The role of Y_s and Y_p in cell growth

Although it is now generally accepted that cell

Figure 5.6 Effect of growth on leaf water potential (Y), osmotic potential (Y_s) and turgor pressure (Y_p) of transpiring leaves enclosed in an oil bomb.

Fig. 5.6


60 % M
100 % M
10
0
7
14
0
15
30
0
10
Water potential (bar)
Time in oil bomb (hr)
Y_p
Y
Y_s

enlargement is initiated by stress relaxation (i.e. reductions in Y_p), over the years other 'growth hypotheses' have been proposed. For example it is also believed that cell growth is controlled by decreases in Y_s through increases in cell solutes (e.g Commoner et al, 1943). Of course both stress relaxation and decreases in Y_s of a growing cell could lower its Y below the medium thus increasing the driving force for growth water uptake. But the question is, which of these processes is more significant? To answer this question the magnitude and direction of change of Y_s and Y_p in relation to Y_x of oil bomb leaves were studied.

Parallel leaf samples were used. One was sealed in the oil bomb and the changes in Y_x followed until the threshold Y_x was reached. After, the Y_s of the leaf was measured. After measuring the initial Y_x of the other leaf, it was removed from the oil bomb at intervals to allow samples to be taken from it for Y_s measurements at time 0, 9-11 hours and finally when its paired leaf reached the plateau Y_x. Using data from the paired sample, the time course of the 3 water potential parameters was constructed after making corrections for Y_{xs} (Figure 5.6). Y_x and Y_p of 'mature' leaves remained fairly constant. For the young leaf Y_p and Y_s were reduced to 33% and 90% respectively of their initial values. It is, therefore, obvious that changes in Y_x were more closely related to Y_p than Y_s suggesting that stress relaxation is the primary event in cell enlargement.

5.3b The effect of metabolic inhibitors on the development of xylem sap tension.

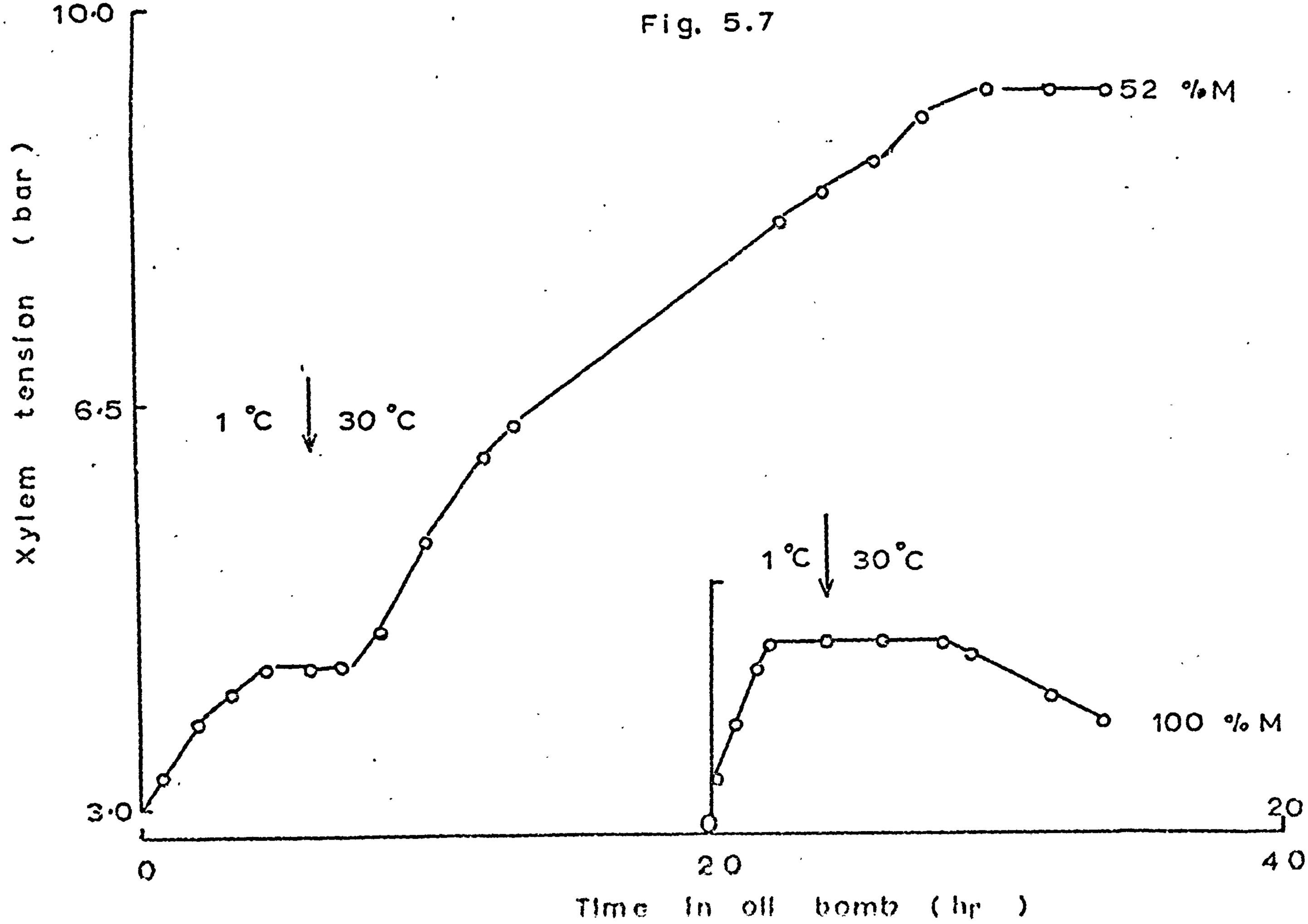
In order to determine whether the wall loosening process was initiated by biochemical or physical processes, the growth of oil bomb leaves was studied at cold temperatures and under anaerobic conditions. It was expected that if the initial processes in cell growth are basically biochemical, very little or no Y_x would be generated.

A. Effect of low temperature

The illuminated oil bomb with its enclosed leaf was placed in a cold bath controlled at about $1^{o}C$. The time course of changes in Y_x was studied. Examples of curves obtained for young and 'mature' leaves are shown in Figure 5.7. Y_x for both leaves increased for only 3 to 4 hours, presumably representing the phase for Y_x and leaf mesophyll equilibration. Observations made showed that the Y_x can remain at the plateau for days. On the other hand if the bomb is transferred to warm temperatures ($30^{o}C$) Y_x of young leaves increased to establish a new plateau after an initial lag period, presumably during which time the oil warms up (curve A). In contrast, the Y_x of 'matured' leaf did not increase but rather decreased when kept in the warm for some few hours, probably due to the death and autolysis of the cells with a subsequent translocation of water to the xylem.

Figure 5.7 Effect of low (1°C) and warm (30°C) temperatures on growth of transpiring leaves in an oil bomb.

Fig. 5.7


10·0
6·5
3·0
Xylem tension (bar)
1 °C
30 °C
52 %M
1 °C
30 °C
100 % M
0
20
40
20
Time in oil bomb (hr)

The experiment shows that in normal tissues (as opposed to fully turgid tissues, see Section 2), cell enlargement is initiated by biochemical loosening of the cell wall and not by physical processes. The results further show that high temperatures are essential for prolonged growth and thus supports the earlier observations (Sections 2 and 4) that low temperature restricts the extent of growth water uptake and cell expansion (cf Milburn and Weatherley, 1971).

B. Xylem tensions developed in darkness.

The experiment was conducted in a dark room where the lights were only switched on during Y_x measurements. The temperature of the bomb was maintained at 30°C. It was expected that with time, any O_2 dissolved in the oil would be used up and CO_2 production from respiration might be significant and thus create an anaerobic environment.

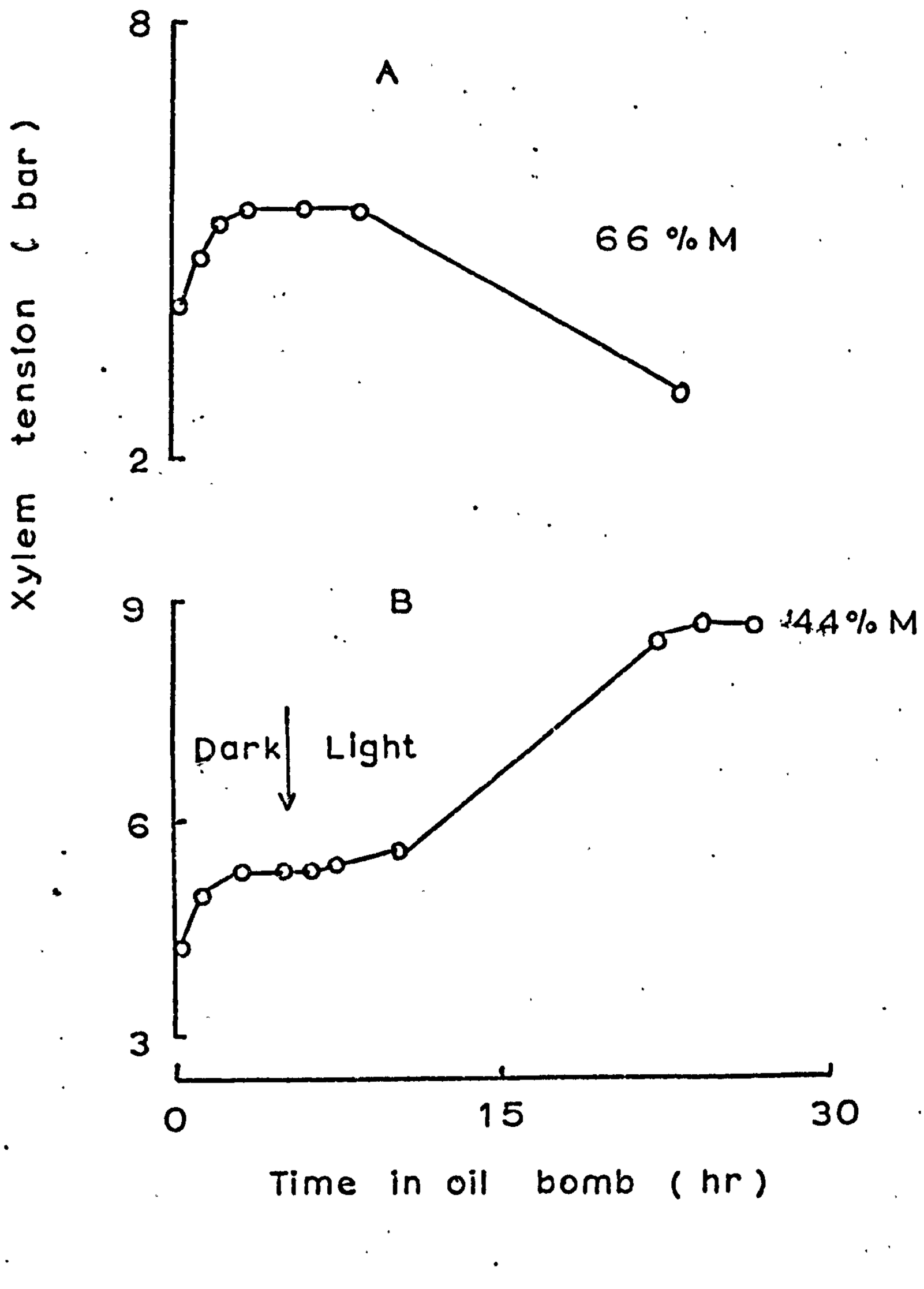
Figure 5.8 shows that an anaerobic environment suppressed the development of xylem tensions by the leaves. Consequently Y_x quickly reached a plateau within 3-4 hours. Observations made showed that when the bomb was kept in the dark for too long (after some 20 hours), the leaves became discoloured and smelled of decomposition; Furthermore, Y_x decreased and the sap exuded became green in colour. However, as shown in the figure if the leaf was re-illuminated before damage occurred, Y_x increased

Figure 5.8 Effect of anaerobic conditions in darkness on the time course of xylem tensions of young transpiring Ricinus leaves.

A - leaves in darkness

B - leaves first in dark then transferred to light.

Fig. 5.8


A
8
2
66 % M
B
9
6
3
44 % M
Dark
Light
0
15
30
Xylem tension (bar)
Time in oil bomb (hr)

to a new plateau.after an initial lag period. Apparently re-illumination allowed photosynthesis to proceed which re-established an aerobic environment. This effect on growth resembles the effect of low temperature and so may suggest an effect on wall loosening.

Discussion

The experiments have generally shown that when excised Ricinus leaves, deprived of water supply, were placed in an environment which prevented transpiration, Y_x did not remain constant but increased: This was assessed by the increases in the oil bomb pressure required to cause xylem sap exudation. There was no evidence of water secretion into the oil (cf Potter and Milburn, 1970). The possible explanation of the phenomenon was that growth of the mesophyll cells persisted in the oil bomb resulting in their absorption of water from the xylem sap, and thus raising the Y_x.

Results presented in Figure 5.1 strongly support the growth hypothesis. Apparently, given time, a leaf could develop tensions of several bar before growth stopped at a threshold Y_x. The tension developed was greater the earlier the stage in expansion growth suggesting a correlation with growth. This hypothesis was further supported by the observation that Y_x of equilibrated 'mature' leaves did not change (Figure 5.2). It is reasonable to consider that as a leaf ages its capacity to generate suction should decline. The results reported here accord with and extend experiments reported previously (Section 2) where growth water uptake of leaf discs decreased as the leaf aged.

The tendency of excised plant tissues deprived of water supply

to increase their internal water deficits, despite the fact that conditions did not permit water loss, has been reported previously. For example, Catsky (1963) observed decreases in the water content of saturated discs of young fodder cabbage which were kept in a humid container. He attributed the deficits to growth induced water uptake and termed them 'growth water saturation deficits'. Also it is generally known that growth occurs after sampling through wall relaxation leading to the development of water potentials of several bar (Tinklin and Weatherley, 1966).

However, reductions in Y during growth could also occur through decreases in Y_s (Commoner et al, 1943; Weatherley, 1950). The problem is, experiments to identify the key factor have been hampered in the past by the lack of an adequate technique for separating the processes generating the water potential gradient from the subsequent water uptake and volume changes. Using the oil bomb, it has been possible to examine the 2 hypotheses. It is obvious from Figure 5.6 that changes in Y_x are accounted for by changes in Y_p. Y_s remained virtually stable. This makes it apparent that cell wall stress relaxation is the initial stage in cell enlargement. Furthermore, it strongly supports the view that turgor stress on the wall is a pre-requisite to stress-relaxation (cf Ray et al, 1971.) However, the hypothesis that solute accumulation may be important to maintain Y_p so as to sustain growth over the long term (cf Grenetz and List, 1973; Cleland, 1976) may not be ruled out. Cell solutes are diluted through growth and Y_p is reduced accordingly, but these results clearly suggest that a large Y_p is needed for growth as a leaf matures.

Xylem water potential which baths the mesophyll cells may be

expressed as:-

$$Y \text{ xylem} = Y_p + Y_{xs} \qquad (a)$$

For a leaf at 40% M growth ceased when Y_x was about 10 bar (i.e. Y_p = - 10 bar) (Figure 5.3). Y_{xs} is generally around -1.0 bar. Therefore,

$$Y \text{ xylem} = (-10) + (-1) = -11 \text{ bar} \qquad (b)$$

The water potential of the mesophyll cells may also be expressed as:-

$$Y \text{ cell} = Y_p + Y_s \qquad (c)$$
$$Y_p = Y_t + Y_{gr}$$

substituting equation (c) becomes:

$$Y \text{ cell} = (Y_t + Y_{gr}) + Y_s \qquad (d)$$

Under the equilibrium condition in the bomb

$$Y \text{ cell} = Y \text{ xylem} = -11 \text{ bar}$$

Y_s measured at this Y was around 12.0 bar and since growth had stopped, $Y_{gr} = 0$. Therefore, $-11 = Y_t + (-12)$ and the critical turgor for growth of the cell.

$$Y_t = -1 \text{ bar}$$

Similarly, for a leaf at 96% M at zero growth,

$$Y \text{ cell} = (-6) + (-1) = -7$$

Y_s at this condition was 13.0 bar,

Therefore; $-7 = Y_t + (-13)$

Therefore, $Y_t = 6$ bar.

The results, therefore, compliment those reported previously for intact leaves (Section 4) where growth ceased at positive turgor pressures. Furthermore, the results strongly support the hypothesis that the Y_t increases in magnitude as a leaf grows and matures.

Results presented in Figure 5.1 and 5.4 suggest that leaves ceased to expand while intact on normal plants because of their inability to generate suction greater than that which normally

exists in the xylem sap. This idea is supported by the magnitude of the Y_x developed by the 'mature' Ricinus leaves when the Y_x were reduced (Figure 5.4). The observation further suggests that the assumption that leaves mature whilst intact, which has led to the misinterpretation of several water uptake experiments (e.g. Dixon, 1898; Dixon and Barlee, 1940) should be corrected. Rather, it should be considered, as suggested earlier (see Section 2), that Y_x will not allow leaves to mature fully even on normal plants, in comparison with their capacity to grow and mature when Y_x is around 0 bar.

Apparently the suction powers of Ricinus leaf cells increased when the plants adjusted osmotically during water stress. This view is supported by the maximum Y_x recorded for the repeatedly stressed leaves (Table 5.1). The highest Y_x was about 16.0 bar, considerably more severe than any previously observed. Nevertheless, Y_t increased in value. A large Y_t implies that little turgor pressure is available for growth (Y_{gr}). The results here therefore, present further evidence to suggest that Y_t increased in prolonged stressed intact Ricinus leaves leading to a slow growth rate (cf Section 4).

It has been frequently demonstrated that cell enlargement is highly sensitive to low temperatures and is also suppressed by other metabolic inhibitors (Cleland, 1955; 1971b; Barrs and Weatherley, 1962; Ray and Ruesink, 1962; Tinklin and Weatherley, 1966). Presumably, this effect on growth of these inhibitors resembles the effect of low temperatures and is due to their effect on the processes leading to stress relaxation (i.e. wall loosening) as strongly suggested by Figures 5.7 and 5.8. The results suggest

that when a cell is ready to grow (i.e. wall pressure exceeds Y_t) growth is initiated by biochemical and not physical modifications of the wall material. This accords with the growth hypothesis of Heyn (1940). The biochemical events have been hypothesised to involve either a cleavage of wall polysaccharides by some hydrolase (Fan and Maclachlan, 1967; Heyn, 1970) or rather to involve reversible breakage and reformation of some crosslinks (Cleland, 1971a). The results strongly support the view that RWC of cells should be measured at near 1°C (cf Milburn and Weatherley, 1971). If measured at higher temperatures a growth water deficit is added depending on the stage of leaf maturity.

In conclusion, therefore, it can be seen that the oil bomb is a very useful tool for studying growth under water stress and for assessing the growth potentials of leaves. The technique is especially valuable for studies of the processes initiating cell enlargement.

SECTION 6

GENERAL DISCUSSION AND CONCLUSION

It is known that cell enlargement is largely brought about by the uptake of water (Heyn, 1940; Burström, 1961). Hence plant growth is rapid when the internal water balance is favourable usually corresponding to high Y but becomes retarded at low Y.

With regard to the mechanism underlying this growth - water relationship, Sachs (1874) drew attention to turgor as a necessary factor supplying the physical force for cell enlargement. De Vries came to a similar conclusion and subsequent workers have given the mechanism some consideration over the years (see Cleland, 1971a). Although the understanding of this field is not entirely clear, the modern view is that cell growth is the result of cell wall yielding driven by turgor pressure (Ray et al, 1971). The growth variables are assumed to be gross extensibility (E_1) and turgor available for growth ($Y_{gr}=Y_p-Y_t$) where Y_p is the actual turgor pressure of the growing cells and Y_t is the lower limit of Y_p for growth to occur, (Lockhart; 1965; Green et al, 1971).

Experiments reported in this thesis add evidence to the view that cell growth depends on favourable internal water status and also provide further information about some of the proposed mechanisms underlying the growth - water status relationships. These results have been fully discussed in the various sections and are only briefly considered here.

Experiments in Section 2 showed that water deficits prevented Ricinus and Helianthus leaves from expressing their full growth

capacities. The degree of retardation was observed to be influenced by the severity of water stress and was also greater in actively growing young leaves than in older leaves. In this condition a potential for growth, here referred to as 'unexpressed growth', became accumulated.

The water deficits were completely eliminated by absorption of free water (i.e. at Y=0 bar) near freezing point but growth continued to be suppressed. On the other hand absorption of warm water not only satisfied the initial water deficits but allowed the accumulated growth to be rapidly expressed within the first 4 hours of floatation (phase 1). This suggests that warm temperatures are essential for tissue growth.

The experiments here did not give any information about the detailed nature of the accumulated growth, but the literature gives various hypotheses (Cleland, 1971a; Hsiao, 1973; Gander and Tanner, 1976) which have been considered in the light of experiments performed.

It has been shown that even what could be described as normal water deficits may be sufficient to retard expansion growth. For example Fig 2.1 and 2.10 clearly demonstrate this fact, i.e. leaves including 'mature' leaves from well-watered Ricinus and Helianthus plants displayed the 'unexpressed growth' phenomenon. That land plants generally grow at somewhat less than full turgor is a well known phenomenon. Experiments in Section 3 have estimated these permanent deficits (i.e. in the absence of transpiration) to be around Y of -3.0 and -2.0 bar for Ricinus and Helianthus respectively. These deficits though low, clearly demonstrate, that the turgor requirement for growth could be the critical factor curtailing cell expansion.

The rapid growth uptake (phase 1) was soon replaced by a slow, protracted uptake (phase 2). An interpretation of the reduced rate despite the high Y_p established could be that an upward adjustment of

Y_t, which reduced Y_{gr} occurred. A similaradjustment in Y_t following the release of water deficits has been described for Nitella (Green et al 1971). It is also likely that E decreased as the leaf discs grew and aged.

The observed persistent growth uptake and disc expansion clearly suggested a maintained water potential gradient between the growing cells and the external medium. The results indicated that the magnitude of this gradient could be proportional to the physiological age of the leaf. This hypothesis has been strongly supported by growth experiments in the oil bomb using Ricinus leaves. Furthermore the oil bomb experiments revealed the processes compensating for the persistent water potential gradient. These processes have been fully discussed in Section 5 and are summarised below.

(1) Growth is initiated by cell wall stress relaxation leading to a reduction in Y_p and a consequent increase in the water potential gradient required for water uptake and therefore growth. Y_s remained fairly constant and could therefore not initiate growth.

(2) The processes leading to stress relaxation, i.e. wall loosening, stopped at xylem tensions (Y_x) corresponding to a positive Y_p. The Y_p values at zero growth (i.e. Y_t) were comparable to those for expansion growth of intact leaves (Table 4.14). Thus suggesting that wall loosening cannot occur below Y_t and as such Y_t can be regarded as a physiological parameter. That is, it is related to stress relaxation and not to wall extension (cf Cleland 1971a).

(3) Experiments in Section 5.3b implied that the wall loosening process is biochemically controlled and consequently low temperatures and an anaerobic environment inhibited the generation of suction for growth water uptake. This clearly supports the view (cf Milburn and Weatherley,

1971) that complications caused by growth water uptake during R.W.C. measurements could be largely overcome by cold floatation.

(4) Young leaves could develop a large Y_x but as the leaves aged this capacity to generate xylem sap suction in the oil bomb decreased. The Y_x values at zero growth correspond to a large Y_t for older than for younger leaves.

(5) Figure 5.5 clearly shows that increases in cell turgidity when Y_p falls to Y_t, could allow growth of young leaves to resume.

(6) The oil bomb experiments give further insight into the phenomenon of leaf 'maturity'. The evidence suggests that the ability of intact 'mature' leaves to generate suction so attract water from the xylem, for growth could be protracted by the existence of xylem sap tensions. However Fig 5.4 like the water uptake experiment, shows that this growth potential could be expressed when Y_x is reduced to 0 bar. Apparently, under natural conditions leaves remain 'immature', but retain their growth capacity probably until they senesce (see Fig 2.6).

Since land plants generally grow under conditions with less than full turgor the view that leaves remain 'immature' could well be considered as a general phenomenon. Perhaps the only exceptions are plants which show the phenomenon of guttation. Reversal of tissue 'maturity' has often been considered in terms of hormonal regulation (cf Steward, 1971). However these experiments reported here indicate that plant water status may also be a controlling factor.

Studies on internal water stress and its effects on the growth of intact tissues are described in Sections 3 and 4. Experiments in Section 3.2 showed that slight water losses in Ricinus and Helianthus leaves growing normally resulted in large reductions in Y_p and only slight

decreases in Y_s. This phenomenon has been reported for many species (e.g. Gardner and Ehlig, 1965). It is obviously disadvantageous for cell growth and experiments in Section 4.1 and 4.2 demonstrate this fact. Leaf expansion, stem and petiole elongation were significantly reduced as plant water deficits increased. It will be noted however, that the degree of sensitivity to water stress differed in the two species. Thus, Figs 4.4 and 4.5 show that unlike Helianthus tissue growth persisted at a much lower water potential and at lower Y_p in Ricinus.

These results add evidence to the view that Y_t differed between species (see Table 4.14). They strongly suggest that for a reasonable understanding of the role of water in the growth of plant tissue, it is not enough to define the degree of cell turgidity but it is also necessary to assess the magnitude of Y_t. Presumably differences in Y_t may partly explain the more rapid growth of plants with low Y rather than those with high Y observed by Hellkvist and Parsby (1976) for pine clones and Squire (1976) for tea clones.

Apparently, Y_t does not only vary between species. It has been demonstrated, with both intact and excised leaves, that Y_t increases with increasing leaf maturity (Table 4.5 and Fig 5.3). This phenomenon is significant for Ricinus because Y_p can become about 3.0 bar lower in younger than older leaves, because of a slightly lower Ybut a higher Y_s in the former than latter leaves. However, as was clearly demonstrated in Table 4.5 young leaves grew at a greater rate and furthermore growth persisted at a much lower Y than in older leaves. This is so because in addition to their expectedly high E, young leaves also have a large Y_{gr} resulting from a small Y_t. Since stress relaxation is proportional to Y_{gr}, (e.g. Ray et al, 1971), a large Y_{gr} will ensure that greater xylem suctions are generated by water uptake for growth.

A variation in threshold turgor for stomatal functioning with leaf age is reported in the literature (Brown, 1974; Simmelsgaard, 1976). This phenomenon apparently is also operative in cell growth. This idea has been theoretically expounded by Hettiaratchi and O'Callaghan (1974) who considered that the value of Y_t will differ after each extension step, and that cell extension stops naturally when Y_t becomes identical with Y_s. Steudle, Zimmermann and Luttge (1977) have also suggested that smaller cells would require less turgor pressure to achieve their growth potential than larger cells, more turgor pressure being necessary to stretch the cell wall to initiate extension growth in larger cells.

Cell enlargement like stomatal functioning is directly controlled by cell turgidity (Slatyer, 1967). Experiments described in Section 4.1 do not allow a detailed comparison of critical turgor values for these two processes. However, the results show that with the onset of water stress, leaves of treatment C plants, retained some degree of porosity and also normal stomatal functioning was restored readily following the release of water stress. In contrast leaf expansion was completely suspended during the water stress treatment. The leaves also lost their potential to expand fully although they had access periodically to sufficient water. These results together with those for the salinity-stressed plants (Fig 4.10) suggest even greater sensitivity of cell expansion than stomata responses to water stress.

In addition to studies in growth under normal water stress, experiments were conducted to determine how adjustments in the water potential parameters and also in the growth variables may operate to control growth during prolonged and severe water stress. Because expansion - growth is very sensitive to changes in cell turgor, the maintenance of Y_p during drought is essential if growth is to be sustained.

Several mechanisms seem to maintain Y_p under stress conditions. For example Y_p could be maintained if reduction in Y is accompanied by a proportional reduction in Y_s and Y_m (refer to Y equation, Section 1). The results of several workers (Wiebe, 1966; Boyer, 1967; Shepherd, 1975) suggest that Y_m contribution to Y could be significant at low tissue water contents. Unfortunately, no satisfactory method could be found for Y_m measurement in the present study. However, this is desirable for an accurate estimate of Y_p during drought and is suggested for further work.

Experiments in Section 3 clearly showed that in contrast to Helianthus, Ricinus adjusted osmotically to maintain low Y_s and consequently high Y_p under droughted conditions. Furthermore although Helianthus leaves shrunk, the phenomenon did not allow any significant positive Y_p to be maintained (cf. Gardner and Ehlig, 1965; Cheung et al, 1975).

Interesting aspects of osmoregulation in Ricinus leaves are listed as follows

(1) Y_s of Ricinus leaves did not quickly adjust as has been reported for organs of other species (Graecen and Oh, 1972; Meyer and Boyer, 1972). Consequently it was not observed when water stress developed rapidly.

(2) Adjustment was limited in extent; that is Y_s from -11.1 in normal leaves to -22 ± 1 bar in stressed leaves. The data from the salinity-stressed plants (Section 4.3b) suggests that this Y_s value was the critical value for growth and survival. Ricinus plants died when Y_s fell below this value.

(3) The adjustment was more pronounced in younger leaves near the main apex. This allows the Y_p of these leaves to resemble those of young normal leaves (Fig 3.3). This pattern of Y_s adjustment is significant since the apical region is the main centre for important physiological

and developmental controls. Unfortunately the osmoticum was not identified by analysis.

(4) Wilting was not observed in osmotically adjusted leaves as a result of the positive Y_p maintained.

As was expected osmoregulation offered some benefits to growth. Thus the capacity of expansion growth to respond to changes in Y was reduced and young leaves grew at Y lower than the threshold value. This fact was clearly demonstrated by the observed large suctions generated by osmotically adjusted leaves when enclosed in an oil bomb (Table 5.1).

However in both salinity stressed and prolonged water stressed leaves growth rate was significantly reduced despite the large Y_p maintained. From the evidence here and the limited data reported in the literature it could be postulated that cell growth in response to osmotic adjustment may be such that;

(1) Growth is maintained at levels comparable to controls.

(2) Growth is only partially maintained.

In the former case the implications are that there is no appreciable change in both E and Y_t. This situation is generally likely to arise when water stress is mild and not prolonged (cf. Hsiao, 1973). On the other hand reductions in E and increases in Y_t may lead to partially expressed growth. Although this latter situation has been observed when stress has been limited to a few days (Meyer and Boyer, 1972), it is more likely to arise during prolonged and severe water stress, conditions known to cause increases in cell wall thickness (Stocker, 1960; Cheung et al, 1975).

In Ricinus during prolonged water stress it was estimated from measurements of leaf area that E was decreased to about 50% of controls. Y_t of osmotically adjusted leaves also increased reaching maximum values of about 7 bar (Table 5.1). This emphasises the fact that high cell turgor does not enhance cell expansion automatically (Cleland, 1959; Green et al, 1971).

It seems reasonable to assume that osmoregulation (within limits) is the critical requirement for survival during drought. Thus, the growth pattern observed i.e. growth arrestment of older leaves and reduced growth of younger leaves, are adaptions to prevent the rapid consumption of stored water. This allows protracted growth and also sustained other turgor dependent processes. Experiments with salinity-stressed Ricinus leaves (Section 4.3b) clearly demonstrated that retention of growth capacity is critical for survival. Furthermore, the reduction in cell volume (Plate 4.2 Bi) itself can be regarded as a mechanism ensuring efficient osmoregulation (cf. Cutler et al, 1977). Obviously for the survival of the plant during drought it is important for growth to be resumed when the stress is removed.

Upon rewatering stressed Ricinus plants Y_p rose to very high levels (over 11 bar) apparently as a result of the low Y_s which persisted for about 6 days before presumably becoming diluted by new growth. As expected, the rate of expansion of young stressed leaves increased. But more remarkably, growth also resumed in older stressed leaves whose growth was suspended over long periods. Figure 4.9 shows an example of such recovery.

The temporary large increase of Y_p enhanced leaf cell elongation but expansion growth was limited (Plate 4.2 Bii) This is partly attributable to possible increases in thickness of cuticle during water stress, which can conceivably restrain expansion growth. The quantitative increases in the cuticle of Ricinus during drought remain to be evaluated.

Finally it is of interest to note that when leaf thickness had returned to normal following rewatering, and even though internal water status remained favourable, leaves failed to expand fully. This suggests that prolonged water stress caused permanent damage to expansion growth.

The experiments described in this thesis have definitely provided useful information on the responses of expansion growth to various degrees of plant water status. But more importantly, it has increased and extended knowledge on the factors controlling growth over a wide range of plant-water status. Work with other species is necessary to test the general applicability of the mechanisms which have been proposed.

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ADDENDA

The following references have been cited in the text but have not been included in the Bibliography.

Burstom, H.G., Uhrstrom, I. and Wurscher, R. (1967). Growth, turgor, water potential and Young's Modulus in pea internode. Physiologia Pl. 20 213-231.

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APPENDIX I

i. Terms used throughout this text

Y Water potential i.e. $\frac{\mu w^1 - \mu w^o}{\bar{V}w}$ where μw^1 is the chemical potential of water in the cell and μw^o is that of pure water. $\bar{V}w$ is the partial molal volume of water (Slatyer 1967, p 138). Y is expressed in bar (1 bar = 0.987 atmosphere). Where there is no subscript Y represents the leaf water potential.

Y_s Potential$_{(water)}$ of solutes in leaf cell (Slayter 1967, p 139).

Y_{xs} Potential$_{(water)}$ of solutes in xylem.

Y_m Matric potential$_{(water)}$ - an effect caused by water-binding colloids and surfaces in the cell.

Y_p Water pressure potential. When positive in a plant cell this equals the turgor pressure.

Y_x Xylem tension which equals $-Y_p$. Use of the word tension is a convenience to avoid the negative sign.

Y_{gr} Turgor pressure available to cause extension growth to proceed (Green et al 1971).

Y_t The turgor pressure which must be exceeded before extension growth can proceed (Green et al 1971).

RWC — Relative water content, a measure of tissue water content (%) relative to the water content at full turgor. It is calculated as -

$$RWC\ (\%) = \frac{\text{Fresh weight - Dry weight}}{\text{Saturated weight - dry weight}} \times 100$$

It was measured at 1°C (cf. Milburn and Weatherley 1971).

E — A coefficient termed gross extensibility characterising the yielding tendency of the cell wall and incorporates the physical and biochemical aspects of the cell wall. It has the units of % vol change/hour.bar (Green et al 1971; Hsiao et al 1976).

RI — Refractive index of leaf or xylem cell sap.

%M — Leaf maturity % =

$$\frac{\text{Area of leaf 7 when used for experiment}}{\text{Area of leaf 6 when fully mature + 12\%}} \times 100$$

hr — Hour

W/m^2 — Watts per metre square.

min — Minute.

ii. Comparison of Y_s measured by cryoscopy with those by the pressure bomb.

It has been indicated that osmotic potentials determined on sap, extracted by the freeze-disruption method, are liable to inaccuracy because vacuolar sap is contaminated by apoplastic water (cf. Slatyer, 1967). Tyree (1976) maintains that pressure-volume curves (see Scholander et al 1964; Tyree and Hammel 1972; Hellkvist Richards and Jarvis 1974; Cheung et al 1975) give a more accurate value for Y_s. Accordingly pressure-volume curves were constructed for Ricinus leaves and the Y_s estimated were compared with values measured cryoscopically using the respective leaves. The conventional method of experimentation (see Tyree et al, 1972) was modified as described below.

A 'mature' leaf was bagged, excised and its petiole freely supplied with water at $1^{o}C$ for 2-4 hours, after which the saturated weight was determined. Y at this point was assumed to be 0 and the RWC ($1^{o}C$) as 100%. A small sample of known weight was removed for cryoscopy. The remaining leaf sample was laid flat (lower surface upper most) on a nylon mesh supported horizontally to allow free air circulation over the leaf to ensure even dehydration. After 5 to 40 minutes, the leaf was bagged and allowed to equilibrate for 30 to 180 minutes and the fresh weight determined. The leaf was then enclosed in a pressure bomb and the Y_x measured. This cycle of dehydration, equilibration, weighing and bomb measurement was repeated several times. Turgid leaf tissue was allowed shorter periods for dehydration and equilibrium, but this time was gradually extended as the leaf dried.

Pieces of known weights of lamina were removed periodically for Y_s measurements of cell sap cryoscopically. At the end of the experiment the Y_s of the remaining leaf was also measured. All pieces of leaf sampled were later dried at $95^{o}C$ and dry weights measured.

The dehydration method adopted here as opposed to the original over-pressurisation method has the following advantages:

1. It ensures that water is lost evenly by all the leaf cells. Clearly an over-pressurisation method would extract more water from cells adjoining the xylem than those distant from the xylem.

2. It prevents possible rupturing of leaf cells which could occur when high bomb pressures are maintained for long periods.

3. It ensures that only pure water is removed from the leaf cells.

4. The method is rapid and less tedious and also it avoids the errors incurred through evaporational losses of extracted xylem sap.

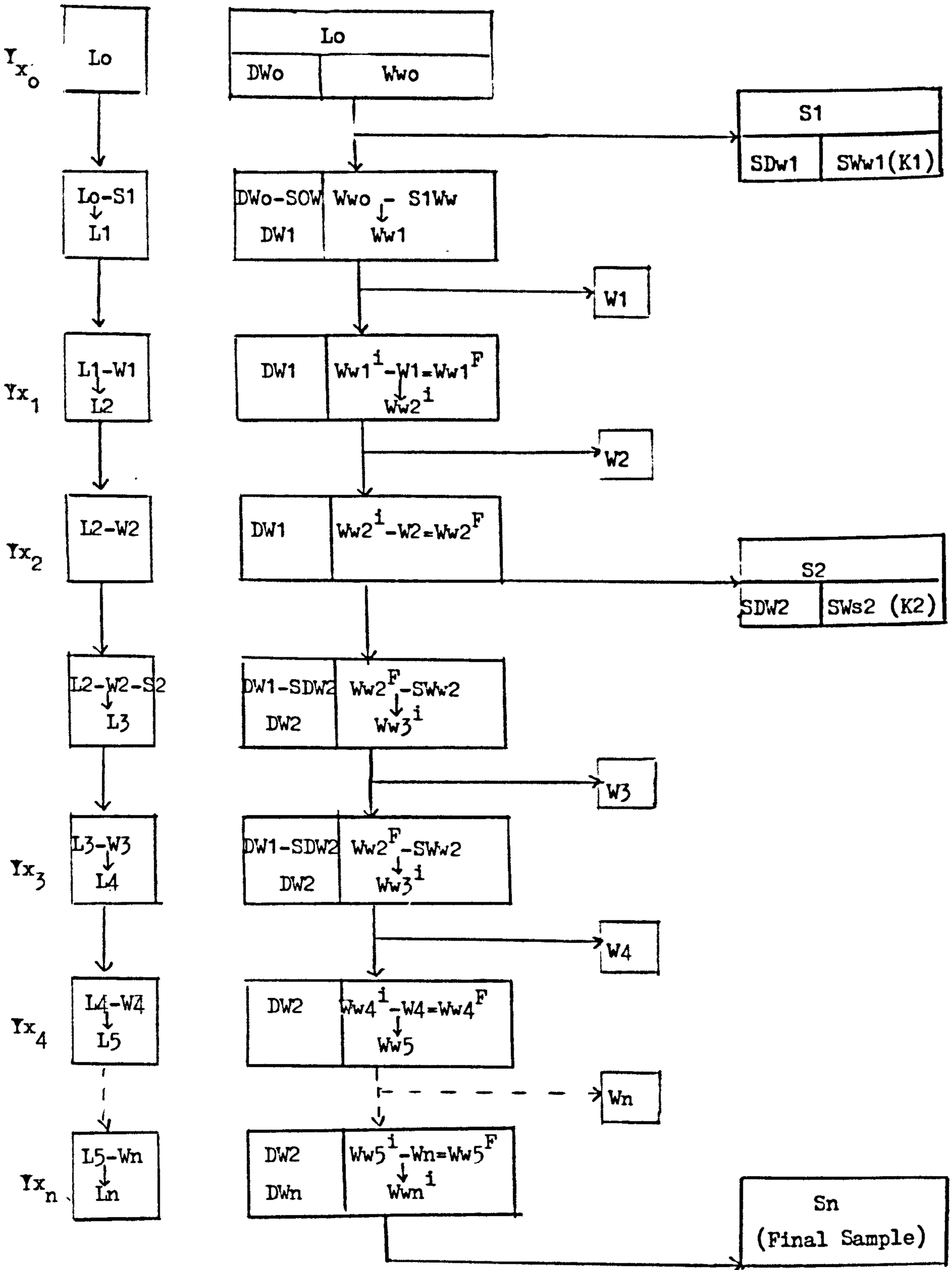
Furthermore the method adopted here whereby xylem and leaf cells were equilibrated before each bomb measurement ensured that the Y_x measured was not over estimated.

Pressure - volume curves ($\frac{1}{Y_x}$/RWC) were constructed from the data and the Y_s estimated from the curves. Differences (see Table 1A column 5) between Y_s measured by the bomb method and by cryoscopy ranged from 0.5 to 2.6 bar between RWC of 100 - 80%. However when the bomb data is corrected for Y_{xs}, the mean with standard error was observed to be 14.1 ± 0.4 (Table 1A column 6) compared with 13.8 ± 0.3 for cryoscopy. There is a good agreement between the Y_s values obtained by the two techniques, and therefore the cryoscopic method adopted in this thesis measured Y_s reasonably accurately.

Table 1A Comparison of osmotic potential of leaf cell sap by air bomb and by cryoscopy

Experiment	Sample	Osmotic potential (Y_s, -bar) Y_s cryoscopic	Y_s bomb	Discrepancy	Y_s (bomb) corrected for Y_{xs} (assumed -1.0 bar)	Discrepancy
1	1	12.5	11.9	0.6	12.9	0.4
	2	13.7	14.7	1.0	15.7	2.0
2	1	12.7	11.3	1.4	12.3	0.4
	2	14.2	13.0	1.2	14.0	0.2
	3	14.8	13.8	1.0	14.8	0.0
3	1	12.0	12.1	0.1	13.1	1.1
	2	13.6	12.7	0.9	13.7	0.1
	3	14.1	13.8	0.3	14.8	0.7
	4	14.7	14.3	0.4	15.3	0.6
4	1	13.0	10.5	2.5	11.5	1.5
	2	14.3	11.7	2.6	12.7	1.6
	3	14.8	13.8	1.0	14.8	0.0
5	1	12.8	12.7	0.8	13.7	0.2
	2	16.0	16.6	0.6	17.6	1.6
Mean ± SE		13.8±0.3			14.1± 0.4	

Fig 1A: A diagramatic presentation of the method and calculations made for constructing pressure-volume curves for Ricinus



	x					
	0	1	2	3	4	n
Yx_o						
Yx_1 x_1 =	$K_1(1-\Delta_1)$					
Yx_2 x_2 =	$K_1(1-\Delta_1)(1-\Delta_2)$					
Yx_3 x_3 =	a $K_1(1-\Delta_1)(1-\Delta_2)(1-\Delta_3)$					
	b $K_2(1-\Delta_3)$					
Yx_4 x_4 =	a $K_1(1-\Delta_1)(1-\Delta_2)(1-\Delta_3)(1-\Delta_4)$					
	b $K_2(1-\Delta_3)(10\Delta_4)$					
Yx_n x_n =	a $K_1(1-\Delta_1)(1-\Delta_2)(1-\Delta_3)(1-\Delta_4)(1-\Delta_n)$					
	b $K_2(1-\Delta_3)(1-\Delta_4)(1-\Delta_n)$					

Figure 1A shows a diagramatic presentation of the method and calculations made for constructing the pressure-volume curve of Ricinus.

The scheme assumes that no growth occurred during the operation and also that no leaf sample was removed. It is also assumed that the symplasmic volume and the apoplastic water volume remains constant.

L = Experimental leaf for Y_x measurement

DW = Dry weight

Ww = Water content

W = Water lost through dehydration

S = Leaf sample removed for cryoscopy

K = Leaf sample water content (SWw)

X = Water content of leaf sample (samples) after each hypothetical water lost

K-x = hypothetical water lost

subscript o, i, F indicate, original, initial (before dehydration)
final (after dehydration)

1, 2, 3...n indicate level of experimentation or leaf sample number.

Δ = water lost by leaf during dehydration/water content and leaf before dehydration

X for a leaf sample was calculated as

$$X = K(1-\Delta_1)(1-\Delta_2).....(1-\Delta_n)$$

Relative water content at eg $Y_{x3} = \frac{Ww_3 + X_3}{Wwo}^{F} \times 100$

iii. Effect of Freezing time on leaf cell sap osmotic potential (Y_s)

On few occasions it was not convenient to measure the Y_s immediately after freezing the samples. In such instances they were kept frozen, very often over-night, thus allowing up to about 20 hours before Y_s measurements. It was therefore found necessary to assess the degree of error if any, incurred on the Y_s values.

Method

After bomb measurements the leaf sample was unbagged in a cold humid room (RH around 90-95%) and the leaf was rapidly divided into three pieces, which were bagged separately and frozen over periods ranging from about 2 to 25 hours. For controls, the Y_s of all the 3 pieces of each leaf were measured after about 2 hours freezing. This also enabled Y_s differences, if any, between various pieces of the same leaf to be assessed. For the experimental leaves, freezing time of 1-2 hours was taken as the standard

Results and conclusions

Results for controls and experimentals are shown in Tables 1B and 1C respectively. Variations in Y_s at different portions of the same leaf and measured at the same time ranged from 0.2 to 0.7 bar (Table 1B) and is considered to be within experimental error. Therefore Y_s was measured from a leaf sample which was assumed to represent the whole leaf blade.

Table 1C shows that when freezing time is prolonged, there is a tendency for Y_s to decrease.

Table 1B Variation in osmotic potentials of samples taken from the same leaf.

Sample No.	Leaves No. and osmotic potential (-bar)					
	1	2	3	4	5	6
1	12.8	15.4	14.4	12.2	12.9	13.3
2	12.6	14.7	14.9	12.6	13.2	13.1
3	12.7	14.9	14.6	12.7	12.7	13.3
Differences between high and low Y_s	0.2	0.7	0.5	0.5	0.5	0.2
% differences (% of mean)	0.016	0.047	0.034	0.040	0.040	0.015

Table 1C Effect of various freezing times on Y_s of Ricinus leaf tissue

Freezing time (hours)	Solute Potential (-bar) Leaf No.						Mean	% error (% of Control)
	1	2	3	4	5	6		
2	13.2	14.2	11.6	13.1	14.1	16.4	13.8	-
4	-	-	-	-	-	-	-	
5	13.6	-	11.8	13.4	13.2	18.0	14.0	0.019
22	14.9	-	-	-	-	-	-	-
25	-	14.2	12.8	13.6	13.1	17.2	14.2	0.030

It is suggested that the discrepancy could have occurred through changes in oxidation during freezing. Comparison of data in Table 1B and 1C however, show that the discrepancy could be kept within experimental error, if the freezing time did not exceed 25 hours.

Figure 1B Calibration curves for Li-cor diffusive meter model Li-60 showing regression of transit time on resistance value at 25°C.

Fig. 1.B.

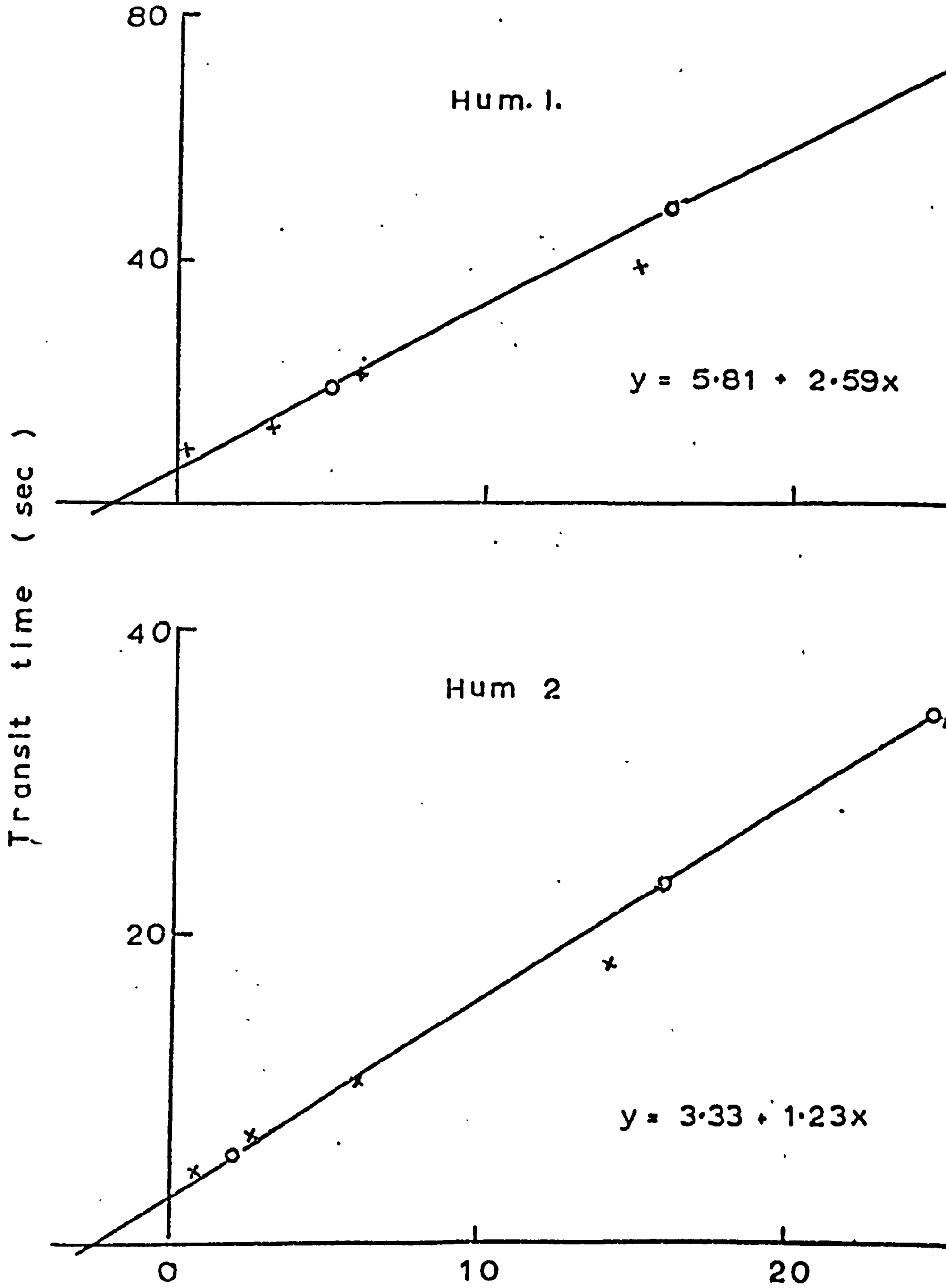

Hum. 1.
y = 5·81 + 2·59x
Hum 2
y = 3·33 + 1·23x
Transit time (sec)
80
40
40
20
0
10
20
Resistance value (sec cm⁻¹)

Table 2A Diurnal variations in leaf cell sap osmotic potential (Y_s) xylem sap osmotic potential (Y_{sx}) of field grown Ricinus and environmental relative humidity (%).

Time of day (hr)	16/8/76			17/8/76			18/8/76		
	Water potential (-bar)		Relative* humidity (%)	Water potential (-bar)		Relative humidity (%)	Water potential (-bar)		Relative humidity (%)
	Y_s	Y_{xs}		Y_s	Y_{xs}		Y_s	Y_{xs}	
06.00	-	-	-	-12.0	1.0	98	-11.4	1.2	85
08.00	-	-	-	-12.7	1.2	98	-11.4	1.1	85
10.00	-11.6	1.2	-	-12.6	1.0	98	-11.8	1.0	85
12.00	-13.7	1.0	-	-12.6	1.2	80	-12.1	1.2	67
14.00	-13.4	1.3	-	-12.8	1.0	87	-12.1	1.4	70
16.00	-12.0	1.0	-	-12.8	1.0	78	-13.0	1.0	73
18.00	-12.7	1.0	-	-11.8	1.2	78	-12.4	1.0	76
20.00	-13.0	1.0	-	-11.3	1.1	90	-	-	94
22.00	-12.4	1.2	-	-11.8	1.2	93	-	-	91

* Faulty recordings

Table 2B Diurnal changes in temperature (°C) and relative humidity of the greenhouse (4/3/76)

Time of day (hour)	Temperature (°C)	Relative humidity (%)
08.00	22	80
09.00	21	70
10.00	22	70
11.00	24	65
12.00	24	64
13.00	27	60
14.00	30	60
15.00	30	60
16.00	29	62
17.00	30	70
18.00	25	72
19.00	22	76
20.00	22	80
21.00	21	85
22.00	21	90
23.00	21	98
24.00	21	98

APPENDIX 3

RELATIONSHIP BETWEEN LEAF SIZE, INTERNAL WATER STATUS AND TREE HEIGHT OF AESCULUS HIPPOCASTIANUM (HORSE CHESTNUT)

It has frequently been observed that in many plants there is a consistent decrease in leaf sizes and increase in xeromorphy with height (Ashby 1948). This phenomenon has been attributed to several factors such as increasing water deficits up the shoot (Maximov, 1929) or unequal distribution of growth substances or inhibitors (Goodwin, 1937; Ashby, 1948). Though undoubtedly all these factors may be important, the experiments reported in this thesis (see also Stocker 1960) strongly suggest that the former factor could be most influential. The work reported below investigates whether plant water status was responsible for the fact that smaller leaves are borne at the tops of horse chestnut trees than at the base.

Materials and Method

Two horse chestnut trees growing in the grounds at Carnoubo were studied. Tree A and B were 18 m and 11 m high respectively and grew at distances of 20 m and 50 m respectively from the River Kelvin, which runs through the estate. The trees remained bare during the winter but both lower and upper leaves developed in late April. Studies were conducted between May - August 1975 and 1976. Leaf samples were obtained from two heights. Lower and upper levels for tree 1 corresponded to 2.0 and tree 2 to 18.0 m and 2.0 and 11.0 m from ground level. Samples were taken from the eastern side of the trees (nearest the river).

Larger 'mature' leaves (leaf 4 from apex) were sampled for leaf morphological studies and water potential measurements. Leaf sizes were determined by measuring the lengths of 3 middle leaflets (there were 7 leaflets per leaf) with a ruler, the mean of which represented the size of the leaf in question.

Leaf fresh weights and dry weights per unit area and leaf thickness were measured using leaf discs (0.34 cm diameter), punched from leaves which were firstly saturated in a cold room (5°C) for 3-4 hours. The discs provided uniform material and the sizes ensured that larger veins were avoided.

Water potentials were measured in the early hours of the day and in the early afternoon. The former measurements are known to reflect the true water deficits (zero transpiration) and the latter indicate the daily extremes from transpiration (cf. Slatyer 1967). Y_x and Y_{xs} were measured, but an attempt to measure Y_s of extracted mesophyll sap was unsuccessful because of its high latex content.

Results and Discussion

The results obtained for the two trees are very similar and only those for tree 1 are described below. Table 3A shows that leaf size decreased with height. Taking the lower leaves as standard, the reduction in area of upper leaves could be estimated as 26%. Table 3B shows that upper leaves tended to have more xeromorphic features than lower leaves. Comparing data taken at the beginning (early July) and later on (early September) in the season, it becomes clear that the xeromorphic features developed gradually. Observations made also showed that upper leaves were more densely veined than lower leaves.

Y data are summarised in Table 3A. Y_{xs} values for both lower and upper leaves were around -1.1 and -1.1 to -1.5 bar for non-transpiring and transpiring leaves respectively. There is a clear correlation

Table 3A Relationships between shoot height, leaf area and leaf water potential of A. hippocastanum

Shoot height (m) from ground level	Leaf length (cm)	Leaf water potential (-bar)	
		Non transpiring leaf	Transpiring leaf
2.0	222.4±5.0	2.1±0.1	3.2±0.1
18.0	165.0±9.0	3.5±0.3	5.0±0.2

Table 3B Some morphological features of lower and upper leaves of A. hippocastanum

Height m	Fresh weight/cm^2	Dry weight/unit area (g/cm^2)	Leaf thickness mm
16/6/76			
2.0	0.0161±0.0003	0.0057±0.0002	0.210±0.012
18.0	0.0151±0.0003	0.0055±0.0001	0.208±0.013
15/9/76			
2.0	0.0125±0.0002	0.0051±0.0005	0.216±0.014
18.0	0.0165±0.0003	0.0077±0.0001	0.260±0.011

of Y with height. Differences of 1.4 and 1.8 bar were observed between height 2.0 and 18.0 meters for non-transpiring and transpiring leaves respectively. The values are fairly near the theoretical hydrostatic gradient of about 0.1 bar per metre thus suggesting that resistances to water flow offered by the xylem would be negligible. Although the observed differences in Y of lower and upper leaves was slight, Y could still be a significant controlling factor in the expansion of leaves from the 2 levels (cf. Boyer 1968 and the Ricinus studies). It was expected therefore that when leaves from the 2 levels were provided with free water (ie Y = 0 bar) the growth response of upper leaves would be more pronounced than that of lower leaves (cf. Section 2). The experiments below test this hypothesis.

Twigs were sampled, enclosed in polythene bags and were supplied with water from their cut ends for 3-4 hours in the cold room (5°C). This was to satisfy the initial water deficits. After this, leaves near the shoot apices, and therefore considered young and capable of expansion, were sampled. Growth of whole leaves and discs (1.75 cm diameter) were studied at 25°C and an illumination which over a period of 24 hours caused negligible changes in dry weight. This arbitrary level, established in preliminary studies, was 1,000 lux on a Megatron light meter.

Leaves with known initial weights and areas were each stood in water contained in closed sealed graduated cylinders (0.1 ml divisions). The whole set-up was contained in a closed glass tank with water thermostatically controlled at 25°C. Growth was measured as fresh weight increments and as area changes.

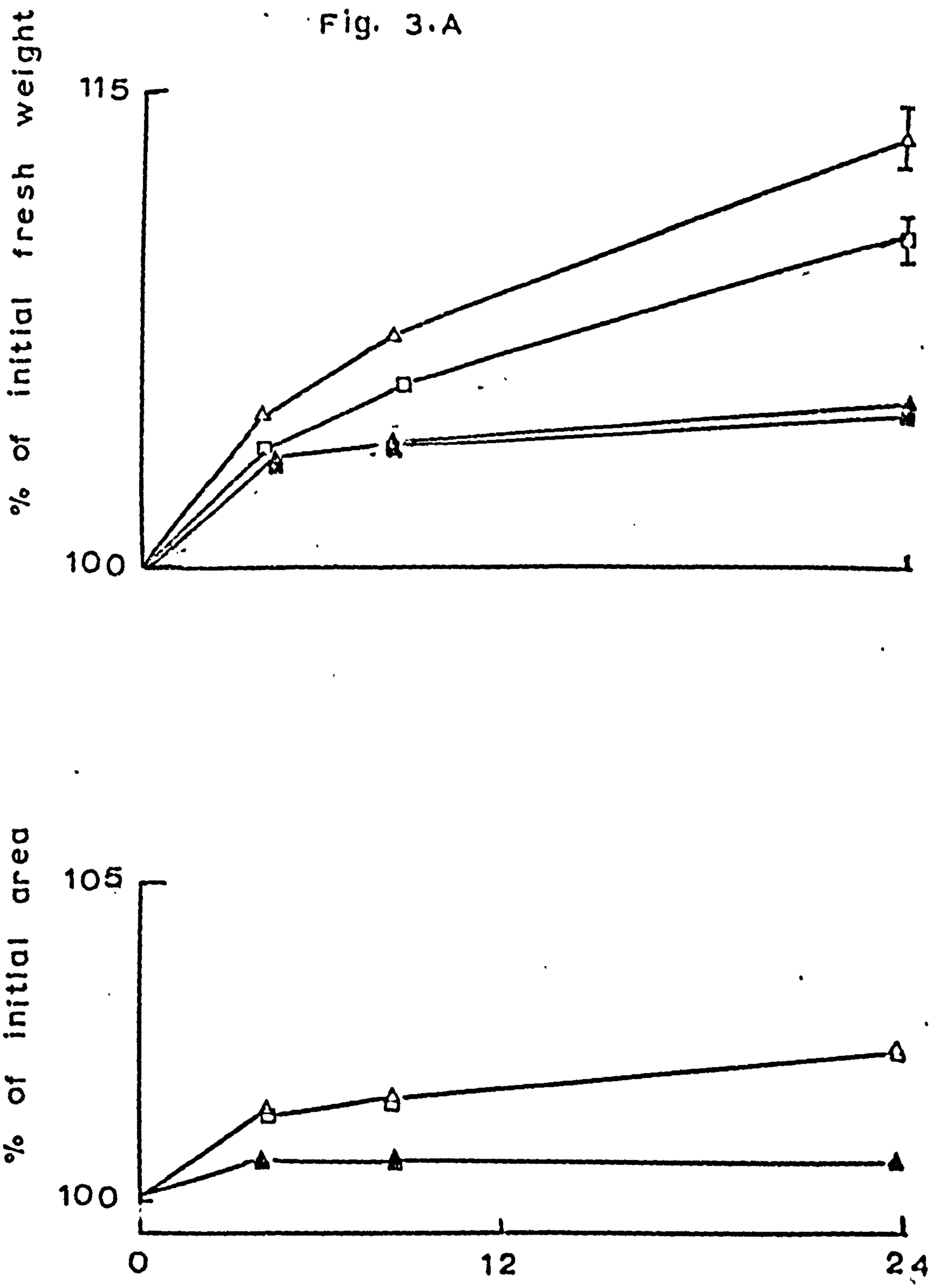
Figure 3A shows curves for changes in fresh weights (% of initial fresh weights) of discs floated at 25°C and 1°C. The 'cold' curves for upper and lower leaves reached similar values of about 105%. This

Figure 3A Changes with time in fresh weights and areas of leaf discs of the horse-chestnut.

□ lower leaves

△ upper leaves

Fig. 3.A


% of initial fresh weight
115
100
% of initial area
105
100
0
12
24
Time (hr)

probably represents deficits incurred and tensions released by discing. Comparison of cold and warm curves showed that the discs grew during floatation. The growth uptake (warm at 24 hours - cold at 4 hours) was 8.8% and 5.0% for upper and lower leaves respectively. This gives about a 3.6% (100 x 108.8/105.0%) increase in upper leaves relative to the lower leaves. Upper leaves also showed slightly more 'unexpressed growth' than lower leaves. However the accompanied disc area-change was similar for the two leaves.

Increases in whole leaf fresh weights (% of initial weight) were 103.0 $\mp$ 0.1 and 102.0 $\pm$ 0.1% respectively for upper and lower leaves. Thus the upper leaves showed a greater increase in fresh weight than the lower leaves by about 1% (ie 100 x 103/102%).

In view of the size of the water potential difference between lower and upper leaves (1.4 to 1.8 bar) the observed differences in growth water uptake though slight could be considered significant and thus suggests that on the intact plant water deficits suppress the expansion of upper more than lower leaves. The growth uptake, however, was not accompanied by measurable changes in leaves or disc sizes. This failure of upper leaves to expand more than lower leaves at Y = 0 bar, although they absorbed relatively more water, may perhaps be attributable to a greater cutinization of the top most leaves. Water stress is known to increase leaf cutinization (Stocker, 1960). This phenomenon could conceivably restrain expansion growth.

Measurements of guard cells lengths were taken as an index of vacuolation. These showed that there were only a 10.0% reduction of stomatal sizes of upper leaves relative to those of lower leaves. If other upper leaf cells were assumed to have been reduced similarly, then the stomatal data together with those from the water uptake experiments showed that the observed differences in leaf areas are

not due only to lack of vacuolation. Presumably a reduction in cell division is also significant (McCree and Davis 1974).

Nevertheless, water absorbed for vacuolation during floatation at Y = 0 bar was relatively less (1% - 3.6%) than the observed reduction in leaf cell size. This suggests that the influence of water could be small. Preliminary studies to test an alternative hypothesis, that the differences in leaf size with height is caused by differences in growth substances, were not very encouraging. Growth of horse chestnut leaf discs in a range of concentrations of kinetin, benzylamine purine and GA III, induced no greater increase in growth than water alone.

Conclusion

It seems that the control of leaf size with height in the horse chestnut may involve plant water status but there may be other unidentified controlling factors.